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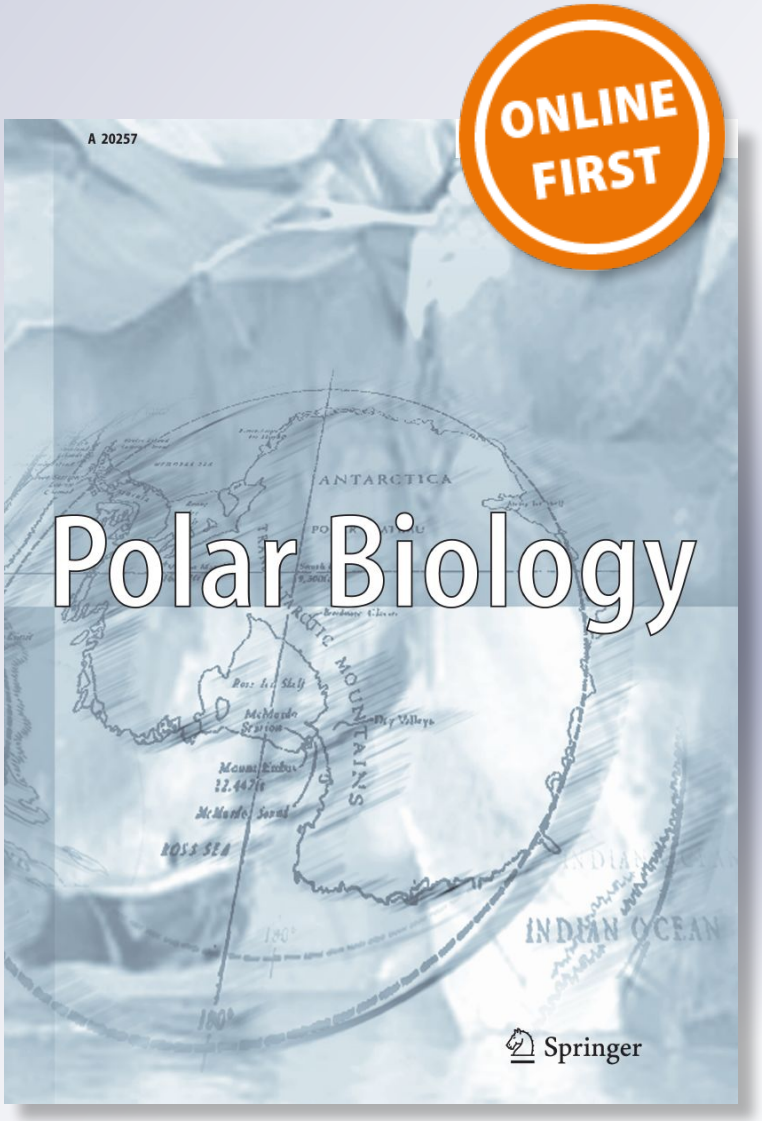

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ORIGINAL PAPER

Impact of ocean acidification on thermal tolerance and acid–base regulation of *Mytilus edulis* from the White Sea

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Abstract

Ocean warming and acidification are two important environmental drivers affecting marine organisms. Organisms living at high latitudes might be especially threatened in near future, as current environmental changes are larger and occur faster. Therefore, we investigated the effect of hypercapnia on thermal tolerance and physiological performance of sub-Arctic *Mytilus edulis* from the White Sea. Mussels were exposed (2 weeks) to 390 µatm (control) and 1120 µatm CO_2 (year 2100) before respiration rate (MO_2), anaerobic metabolite (succinate) level, haemolymph acid–base status and intracellular pH (pHi) were determined during acute warming (10–28 °C, 3 °C over night). In normocapnic mussels, warming induced MO_2 to rise exponentially until it levelled off beyond a breakpoint temperature of 20.5 °C. Concurrently, haemolymph PCO_2 rose significantly > 19 °C followed by a decrease in PO_2 indicating the pejus temperature (T_P, onset of thermal limitation). Succinate started to accumulate at 28 °C under normocapnia defining the critical temperature (T_C). pHi was maintained during warming until it dropped at 28 °C, in line with the concomitant transition to anaerobiosis. At acclimation temperature, CO_2 had only a minor impact. During warming, MO_2 was stimulated by CO_2 resulting in an elevated breakpoint of 25.8 °C. Nevertheless, alterations in haemolymph gases (> 16 °C) and the concomitant changes of pHi and succinate level (25 °C) occurred at lower temperature under hypercapnia versus normocapnia indicating a downward shift of both thermal limits T_P and T_C by CO_2. Compared to temperate conspecifics, sub-Arctic mussels showed an enhanced thermal sensitivity, exacerbated further by hypercapnia, indicating their potential vulnerability to environmental changes projected for 2100.

Keywords Global warming · Population comparison · Energy metabolism · Anaerobiosis · Extra- and intracellular acid–base status · ^{1}H-NMR spectroscopy

Introduction

Future ocean warming and acidification may adversely affect many marine organisms. Earlier studies of thermal constraints in marine ectotherms led to the concept of oxygen- and capacity-limited thermal tolerance (OCLTT; for review, see Pörtner 2010). According to the OCLTT concept, oxygen supply to tissue and thus aerobic performance of the organism is optimized in a limited temperature range supporting maximal growth, reproduction and development success. On both sides of the temperature range thermal limitation first occurs at low and high pejus temperatures (T_P) by an emerging mismatch in oxygen supply versus demand due to insufficient capacities of ventilation and circulation. Aerobic scope is further reduced at critical temperatures (T_C) when anaerobic metabolism sets in. These sublethal temperature limits may be ecologically relevant as the onset of reduced animal performance relates to the geographical distribution of species and populations (Deutsch et al. 2008).

Ocean acidification has the potential to disturb processes like growth, reproduction, calcification, energy metabolism and acid–base regulation in marine organisms. It has been hypothesised that the degree of (extracellular) acidosis and

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thus the capacity of acid–base regulation plays a key role in setting the sensitivity of marine ectotherms to elevated CO_2 tensions (Pörtner 2008). Marine invertebrates, which are characterized by poor acid–base regulation capacity (Miles et al. 2007; Melzner et al. 2009; Thomsen et al. 2010; Whiteley 2011), are considered to be adversely affected by ocean acidification. This particularly concerns calcifiers, like bivalves, due to the sensitivity of shell formation and preservation at reduced pH (Fabry et al. 2008; Doney et al. 2009; Kroeker et al. 2010).

Changes in CO_2 concentrations and temperature occur concomitantly and studying the interaction of these factors is crucial in light of ongoing climate and ecosystem changes (IPCC 2014). Elevated CO_2 concentrations (hypercapnia) may narrow the thermal window of ectotherms by a shift of sublethal thermal limits (for review, see Pörtner 2012) with projected consequences for the range of geographical distribution and associated shifts in ecosystem structure and functioning (e.g. Findlay et al. 2010; Hale et al. 2011). The role of CO_2 in shaping thermal tolerance may differ between organisms according to their regional climate and natural habitat. Recently thermal thresholds as defined by the OCLTT concept were identified in intertidal blue mussels *Mytilus edulis* from the North Sea under control and hypercapnic conditions (Zittier et al. 2015). In this population, sublethal thermal limits (T_P and T_C) remained largely unaffected by hypercapnic exposure (1120 µatm). Nevertheless, hypercapnia altered the response to warming by preventing metabolic depression in the warmth.

Vulnerability to environmental stress may differ between populations and in general may rise with increasing latitude due to adaptation to colder temperatures (e.g. Pörtner 2001; Pörtner et al. 2009; Zittier et al. 2013). Different seasonal temperature extremes and variabilities along a latitudinal cline may relate to different thermal windows of populations. The White Sea is a subpolar sea in northern Russia characterized by a colder climate than the temperate North Sea causing population specific thermal adaptations in marine invertebrates such as gastropods and lugworms (Hummel et al. 1997; Sommer et al. 1997; Tschischka et al. 2000; Sokolova and Pörtner 2003; Schröer et al. 2009). Identifying latitudinal differences in the physiological performance of populations is therefore crucial for predicting future changes in community structures and ecosystem functioning in the world's oceans.

The aim of our study was to determine the thermal window and acid–base regulation capacity of *M. edulis* from the White Sea and investigate the effects of ocean acidification under realistic CO_2 scenarios, for comparison with the respective findings in mussels from the North Sea (Zittier et al. 2015). It is hypothesised that the sub-Arctic population has a reduced tolerance to warming and elevated CO_2 levels when compared to temperate conspecifics. Mussels were incubated at oceanic control (390 µatm) CO_2 levels and those projected for the end of century (1120 µatm), for two weeks before exposure to acute warming. Oxygen consumption, acid–base status of haemolymph and extrapallial fluid as well as intracellular pH and anaerobic metabolite accumulation in mantle tissue were investigated.

Materials and methods

For comparable results mussel maintenance, incubation, experimentation as well as the experimental procedure followed those outlined in a recent study on *M. edulis* from the North Sea (Zittier et al. 2015) and are described briefly below.

Animal collection and maintenance

Mytilus edulis (50–85 mm shell length) were collected from a small area in the shallow subtidal zone in the White Sea, Russia (12 °C, 25 PSU; 66°20′14.27″N, 33°38′12.10″E), and transported to the Alfred-Wegener-Institute for Polar and Marine Research (AWI, Bremerhaven, Germany). Mussels were cleaned from epibionts and pre-acclimated (10 °C, 32 PSU) for at least one month. They were then incubated for 2 weeks under control and elevated CO_2 concentrations (see below). As *M. edulis* can adapt to a wide range of salinities within days (e.g. Gosling 1992, 2003) the difference in salinity between habitat and laboratory conditions should be negligible in the present study and, thus, the natural North Sea water available at the institute was used. Mussels were fed daily ad libitum with freshly hatched *Artemia* larvae (Davenport et al. 2000) and a commercial living algal blend (DT's Live Marine Phytoplankton, Coralsands, Germany). Feeding was terminated three days before experimentation to avoid interference with postprandial metabolism (e.g. Bayne and Scullard 1977; Gaffney and Diehl 1986).

Incubation and experimental set up

The incubation system and experimental set up were the same as used in Zittier et al. (2015). Briefly, each incubation system was located in a temperature control room (10 °C) containing a reservoir (450 L) and header tank (210 L) both continuously bubbled (HTK gas system, Hamburg, Germany) with the control (390 µatm) or the projected oceanic CO_2 concentration for the end of century (1120 µatm). From the header tank water was supplied to the animal tanks (15 L, flow-through rate ~ 120 mL min^{-1}), was then collected in a basin (210 L, continuously bubbled with the respective air-CO_2 mixture) and re-circulated to the reservoir. Water was exchanged twice a week by disconnecting the reservoir from the circuit to subsequently refill and equilibrate it for

24 h. The experimental set up consists of two animal tanks (each with 33 animals at the start) and a reservoir (for water exchange) continuously bubbled with the respective air-CO_2 mixture (MKS Instruments Deutschland GmbH, München) and water temperature was feedback controlled by a thermostat (LAUDA RP 845, Lauda-Königshofen). Water was exchanged daily before each temperature rise.

Water chemistry in all animal tanks (incubation, experimental and reservoirs tanks for water changes) was calculated daily via the respective values of temperature (*T*), salinity (*S*), pH and total dissolved inorganic carbon (DIC). Measurements were carried out using a salinometer (WTW LF197 combination temperature and salinity probes) and a pH metre (Mettler-Toledo pH metre) calibrated at the respective temperature to NBS scale that was converted into total scale via measurements of Dickson standards. DIC was analysed with Seal Analysis SFA QuAAtro; pump Technicon trAAcs 800 TM. The partial pressure of CO_2 in seawater (PCO_2) was calculated using CO2SYS (equilibrium constants of Mehrbach et al. 1973; refitted by Dickson and Millero 1987; Pierrot et al. 2006). The physicochemical parameters of seawater are presented in Tables 1 (incubation) and 2 (experimentation).

Preparation of animals and experimental protocol

After the CO_2-incubation period, mussels ($n = 66$ per treatment) were transferred to the experimental setup. Eight mussels per treatment were used for respiration measurements. Recordings of respiration were started immediately after the mussel was placed in a respiration chamber within the experimental tank. Data for analysis were collected after recovery from handling stress (stable recordings) and after an acclimation period of at least 5 h to each temperature rise. Other mussels in the experimental tanks (for tissue and blood fluid sampling) were left undisturbed overnight.

Starting from the control temperature of 10 °C, temperature was increased by 3 °C every night until 28 °C was reached. In addition to the recording of respiration, blood and tissue samples were collected at each temperature step from mussels not subjected to analyses of respiration. Haemolymph was withdrawn from the posterior adductor muscle and extrapallial fluid was sampled from the extrapallial space and immediately analysed for acid–base parameters and gas levels. Afterwards, mantle tissue was excised, freeze-clamped immediately and stored in liquid nitrogen until further analysis of intracellular acid–base status and succinate content (see below). For a more detailed description of the procedures see also Zittier et al. (2015).

Mussels were kept unfed throughout the experimental period to avoid postprandial rise in metabolism. Reduced food supply can modulate the metabolic rate and stress resistance of animals (Melzner et al. 2011) but a pre-experiment, that followed the same experimental procedure as in the present study except that temperature was kept constant, did not affect the respiration rate indicating no effect by time or lack of food during the chosen experimental period, at least at constant temperature. However, normocapnic and hypercapnic mussels from the White Sea (present study) as well as the North Sea (Zittier et al. 2015) were all treated in the same way such that the results are comparable and differences can be attributed to the seawater CO_2 level and the ones between the two populations to the adaptation to different climate regimes of their habitats.

Determination of respiration rate

Respiration rate (MO_2) was measured as resting oxygen consumption using only readings of the lowest metabolic rates maintained for at least 40 min (see Zittier et al. 2015). Respiration rate was determined by oxygen optodes with integrated temperature compensation (TX-3, PreSens GmbH, Regensburg) using flow-through respirometry following Van

Table 1 Physicochemical parameters of seawater (mean ± SD) during control (390 µatm) and CO_2-incubation (1120 µatm) of blue mussels, *Mytilus edulis* at 10 °C

PCO_2 (µatm) (set)	*T* (°C)	pH total	PCO_2 (µatm)	*n*
390	10.2 ± 0.2	8.04 ± 0.06	413 ± 53	13
1120	10.3 ± 0.2	7.65 ± 0.04	1106 ± 111	11

Table 2 Physicochemical parameters of seawater during the acute warming protocol (3 °C over night) with blue mussels, *Mytilus edulis* under normocapnia (390 µatm) and CO_2 exposure (hypercapnia; 1120 µatm)

T °C (set)	Normocapnia			Hypercapnia		
	T (°C)	pH total	PCO_2 (µatm)	T (°C)	pH total	PCO_2 (µatm)
10	10.1	7.89	602.0	10.2	7.75	780.5
13	13.2	7.87	652.6	13.2	7.68	1006.1
16	16.1	7.94	550.3	16.1	7.64	1114.5
19	19.2	7.92	600.2	19.2	7.67	1084.5
22	22.2	7.99	505.2	22.1	7.66	1134.7
25	25.0	8.02	458.2	25.1	7.57	1384.3
28	28.0	8.00	490.0	28.0	7.64	1184.2

Dijk et al. (1999). Two-point calibration (0%, N_2 bubbled; 100%, air-saturated seawater) was performed at incubation temperature. During warming the 100%-values were checked daily and corrected when drifts became > 2% and optodes were recalibrated afterwards at the respective temperature. After experimentation, mussels were dissected and shell-free dry weight (DW) was determined. MO_2 was calculated as follows:

$$MO_2\left(\mu\text{mol } O_2\ h^{-1}\ g\ DW^{-1}\right) = \left(\Delta PO_2 \times \beta O_2 \times V_{fl}\right) \div DW,$$

where ΔPO_2 is the difference in partial pressure between in- and out-flowing water (kPa), βO_2 is the oxygen capacity of water (µmol O_2 L^{-1} kPa^{-1}), V_{fl} is the flow rate (L h^{-1}) and DW is the shell-free dry weight (g) of the mussel.

Determination of extra- and intracellular gas and acid–base status

Haemolymph and extrapallial fluid were analysed immediately after sampling as in Zittier et al. (2015). Extracellular PO_2, PCO_2 (mmHg) and pHe (NBS scale) were measured using a blood gas analyser from Eschweiler (MT 33, Germany) calibrated at the specific experimental temperature. Total CO_2 concentration (CCO_2, mmol L^{-1}, Table 3) of body fluids was analysed by gas chromatography (6890 N GC System, Agilent Technologies, USA, for reference see below). Bicarbonate concentrations [HCO_3^-] were calculated as follows:

$$\left[HCO_3^-\right](\text{mM}) = CCO_2 - \left(\alpha CO_2 \times PCO_2\right),$$

where αCO_2 is the solubility of CO_2 in the body fluid (mmol L^{-1} $mmHg^{-1}$) calculated after Heisler (1986).

Intracellular pH (pHi) and tissue concentrations of CO_2 were measured in mantle tissue samples stored in liquid nitrogen. Samples were analysed using the homogenate method developed by Pörner et al. (1990), using 160 mmol L^{-1} potassium fluoride (KF) and 0.1 mmol L^{-1} nitrilotriacetic acid (NTA) for the analysis of 200–250 mg tissue. pHi was determined at the respective experimental temperature using a pH optode (Needle-Type-Housing-pH-Microsensor, PreSens GmbH, Regensburg) and CCO_2 was determined by gas chromatography (Table 3). Intracellular PCO_2 and [HCO_3^-] concentration (Table 4) were then calculated as described above.

Tissue extraction and determination of metabolites

Tissue succinate concentrations were determined in perchloric acid (PCA) extracts from mantle tissues using ^{1}H-NMR spectroscopy as described in Zittier et al. (2015). In the North Sea mussels succinate concentration had not been affected by hypercapnia and had only increased at the highest temperature and independent of CO_2. Therefore, in the present study succinate was determined at the beginning of the warming trial and at the two highest temperatures (25° and 28 °C). Following the procedure by Zittier et al. (2015) tissue extracts were dried in a SpeedVac for spectroscopy and, prior to measurements, resolved in D_2O containing 1% trimethylsilyl propionate (TSP) as internal reference. Fully relaxed high-resolution ^{1}H-NMR spectra were recorded on a 400 MHz 9.4T WB NMR spectrometer with Avance III electronics (Bruker Biospin GmbH, Germany) as described in Zittier et al. (2015). Spectra were post-processed automatically using TopSpin 2.5 (Bruker Biospin GmbH, Germany). All data were zero filled to 64 k and processed with an exponential multiplication of 0.3 Hz prior to Fourier

Table 3 Total carbon dioxide (CCO_2, mmol L^{-1}) of haemolymph and mantle tissue in blue mussels, *Mytilus edulis* under normocapnia (390 µatm) and hypercapnia (1120 µatm) during acute warming (3 °C over night)

T (°C) (set)	Normocapnia		Hypercapnia	
	Haemolymph	Mantle	Haemolymph	Mantle
10	1.91 ± 0.11	2.15 ± 0.33	1.18 ± 0.14	1.99 ± 0.41
13	1.88 ± 0.15		1.45 ± 0.22	
16	1.23 ± 0.03	1.74 ± 0.14	1.45 ± 0.11	1.79 ± 0.30
19	1.45 ± 0.22		1.46 ± 0.16	
22	1.37 ± 0.17		1.42 ± 0.35	2.25 ± 0.65
25	1.23 ± 0.33	2.11 ± 0.14	1.48 ± 0.15	1.72 ± 0.21
28	1.67 ± 0.08	1.96 ± 0.25	2.05 ± 0.07	1.92 ± 0.29

Mean ± SD, n = 5–8

Table 4 Intracellular partial pressure of carbon dioxide (PCO_2i) and bicarbonate content ([HCO_3^-]i) of mantle tissue in blue mussels, *Mytilus edulis* under normocapnia (390 µatm) and hypercapnia (1120 µatm) during acute warming (3 °C over night)

T (°C) (set)	Normocapnia		Hypercapnia	
	PCO_{2i} (µatm)	[HCO_3^-]i (mmol L^{-1})	PCO_{2i} (µatm)	[HCO_3^-]i (mmol L^{-1})
10	6.20 ± 1.26	1.83 ± 0.29	6.68 ± 1.08	1.65 ± 0.34
16	5.76 ± 0.58	1.51 ± 0.07	6.08 ± 1.23	1.52 ± 0.26
22			8.09 ± 1.86	1.90 ± 0.52
25	9.08 ± 0.69	1.79 ± 0.13	14.24 ± 3.60	1.20 ± 0.12
28	17.24 ± 2.87	1.34 ± 0.12	19.30 ± 4.03	1.30 ± 0.27

Mean ± SD, n = 5–8

transformation. Metabolites were identified from calibrated spectra and succinate concentrations were quantified of using Chenomx 8.1 (Chenomx Inc., Edmonton, Canada).

Statistical analysis

All data were first checked for outliers using Nalimov's test and, after removal, results were further analysed using R (R Core Team 2014). After testing the data for normality and homogeneity of variance, two-way analysis of variance (ANOVA; R function: aov()) in combination with a Tukey's post hoc test (Tukey Honestly Significant Difference, R function: TukeyHSD()) was performed to analyse effects of temperature and CO_2 level and possible interactions thereof (see Online Resource 1 for an overview of ANOVA results). The breakpoint temperature of MO_2 indicates the deviation from the exponential relationship with temperature when the slope of the curve changes significantly (phase change), which is usually determined by Arrhenius breakpoint analysis (Sokal and Rohlf 1995). However, this was impossible because the number of temperatures that resulted above potential breakpoints in the warmth is insufficient to calculate linear regressions by the least-square method (Sokal and Rohlf 1995). Alternatively, the MO_2 breakpoint temperature was determined by using a sigmoidal function (R package: drc, R function: drm() with fct = LL.5(); Ritz et al. 2015), which describes biological processes that possess an exponential increase until a specific threshold when subsequently stagnation and, thus, a phase change occurs. The maximal curvature of the sigmoidal curve represents the limitation in the temperature-dependent MO_2 rise and, thus, the breakpoint temperature (cf. Zittier et al. 2015). Differences were considered significant if $p \leq 0.05$. Values are presented as mean ± standard deviation (SD), $n = 5$–8 unless stated otherwise.

Results

At control temperature (10 °C) respiration rate (MO_2) was similar under normocapnia and hypercapnia (Fig. 1). During warming, MO_2 increased progressively before it levelled off beyond a specific breakpoint temperature (phase change). Under normocapnia the course of MO_2 (Fig. 1a, black circles, solid curve) yields a calculated breakpoint temperature of 20.5 °C (Fig. 1a, vertical line). This breakpoint temperature was mirrored in patterns of haemolymph PCO_2 and PO_2 of normocapnic mussels (see below). Interestingly, the MO_2 of normocapnic mussels first showed a consistent increase (Q_{10} 10–22 °C = 2.6) before differences among individuals developed above 22 °C, which are not reflected in any other trait measured. Above 22 °C 37.5% (3 out of 8) of the mussels revealed a progressive decline in MO_2 with further warming (Fig. 1a, grey circles) while the others showed a continued increase (Fig. 1a, white circles) (these "sub-groups" were not used for statistical analyses). In contrast, all hypercapnic mussels showed similar responses during warming. The increasing MO_2 (Q_{10} 10–25 °C = 2.5) started to level off above 25 °C in all hypercapnic individuals and Q_{10} fell below 1.6 resulting in a calculated breakpoint temperature of 25.84 °C (Fig. 1b, vertical broken line). Assessing the average response of normocapnic and hypercapnic mussels, a significant main effect of temperature on MO_2 was found (2-way ANOVA, $F(6,88) = 34.27$, $p < 0.0001$). Oxygenation and acid–base status were similar in haemolymph and extrapallial fluid of all experimental

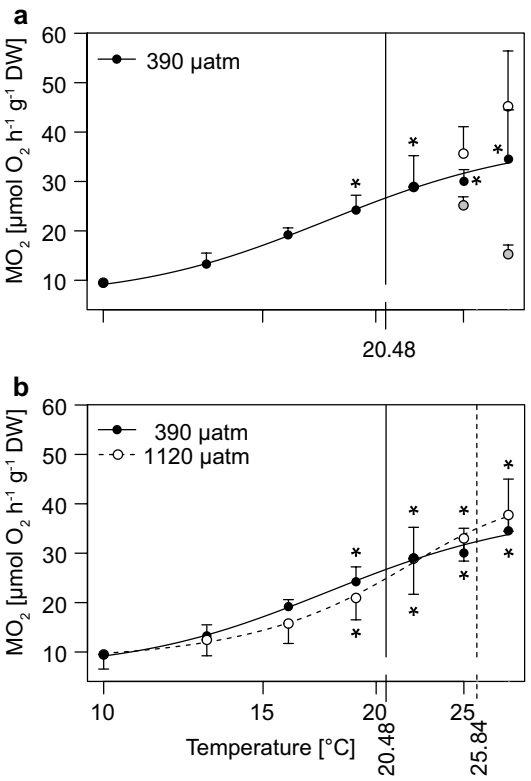

Fig. 1 Sigmoidal models for the respiration rate (MO_2) in blue mussels, *Mytilus edulis* under 390 µatm and 1120 µatm CO_2 during acute warming (3 °C over night). **a** Shows the average MO_2 course of all normocapnic mussels (black circles, solid curve; $n = 5$–8). Above 22 °C, some normocapnic individuals continued to show a progressive increase in MO_2 ($n = 5$) while others revealed a decrease in MO_2 ($n = 3$), which is indicated by the white and grey circles, respectively (data excluded for statistical analyses; for more details see text). **b** Shows the average MO_2 course of normocapnic (black circles, solid curve) and CO_2-exposed (white circles, broken curve) mussels ($n = 5$–8). Data are fitted by a five-parameter model (respective curve) and vertical lines indicate the breakpoint temperature, when a limitation in MO_2 rise occurs. Five-parameter models: $MO_2 = 6.72 + ((38.40 - 6.72)/(1 + \exp(-3.65 \times (\log(T) - 16.43)))^{1.27}$ for 390 µatm, $MO_2 = 8.12 + ((41.38 - 8.12)/(1 + \exp(-7.86 \times (\log(T) - 24.35)))^{0.44}$ for 1120 µatm. Values are given as mean ± SD. *Significantly different from the respective data at 10 °C

groups during the entire protocol in line with earlier findings (see discussion). Therefore, only haemolymph data are described in the following. Haemolymph PO_2 levels (Fig. 2a) under normocapnia started at 103.9 ± 19.7 mmHg, $n = 7$ at 10 °C and mean values remained relatively stable with warming until a decrease occurred above 22 °C towards a PO_2 of 81.2 ± 12.5 mmHg, $n = 5$ at 28 °C (ΔPO_2 −22.7). In contrast, haemolymph PO_2 under hypercapnia started at a somewhat higher level of 117.9 ± 8.7 mmHg, $n = 6$ at 10 °C, mean values remaining relatively stable just until 19 °C and decreasing rapidly thereafter, resulting in a significantly reduced PO_2 of 73.2 ± 10.7 mmHg, $n = 7$ at 28 °C (Tukey HSD, $p < 0.0001$; ΔPO_2 −44.7) compared to 10 °C. Two-way ANOVA identified a significant interaction between temperature and CO_2 treatment (2-way ANOVA, $F(6,82) = 2.24$, $p = 0.047$) and a main effect of temperature (2-way ANOVA, $F(6,82) = 8.35$, $p < 0.0001$).

Depending on CO_2 treatment haemolymph PCO_2 displayed different patterns during acute warming (Fig. 2b) and was significantly affected by temperature (2-way ANOVA, $F(6,79) = 43.61$, $p < 0.0001$), CO_2 treatment (2-way ANOVA, $F(1,79) = 17.65$, $p < 0.0001$) and the interaction thereof (2-way ANOVA, $F(6,79) = 14.14$, $p < 0.0001$), $p < 0.0001$). Starting with similar values at 10 °C (390 µatm: 1.24 ± 0.55 mmHg, $n = 7$; 1120 µatm: 1.68 ± 0.08 mmHg, $n = 7$) followed by an initially stable phase, PCO_2 of normocapnic mussels showed a sudden increase above 19 °C and a second one above 25 °C resulting in the maximum level of 4.27 ± 0.74 mmHg, $n = 6$ at 28 °C (Tukey HSD, $p < 0.0001$). In contrast, haemolymph PCO_2 under hypercapnia raised already above 16 °C leading to significantly increased values that levelled off thereafter at 3.60 ± 0.63 mmHg, $n = 6$ at 28 °C (Tukey HSD, $p < 0.0001$). The earlier rise in haemolymph PCO_2 during acute warming under hypercapnia led to significantly higher levels above 16 °C when compared to normocapnia. The difference was eliminated above 22 °C when levels rose in normocapnic animals, too.

Haemolymph pH (pHe) of *M. edulis* (normocapnia: 7.65 ± 0.08, $n = 5$) was lowered somewhat by hypercapnia (7.52 ± 0.06, $n = 7$) at control temperature (10 °C) (Fig. 2c). Upon warming pHe dropped significantly during the initial temperature rise to 13 °C (390 µatm: Tukey HSD, $p < 0.0001$; 1,120 µatm: Tukey HSD, $p = 0.001$) and decreased progressively thereafter in both groups. The stronger acidosis seen under hypercapnia became significantly different from findings under normocapnia beyond 16 °C until values became similar and lowest at the highest temperature (28 °C) (390 µatm: 7.22 ± 0.09, $n = 6$; 1120 µatm: 7.18 ± 0.07, $n = 7$). Two-way ANOVA indicated a significant effect by temperature (2-way ANOVA, $F(6,79) = 33.55$, $p < 0.0001$) and CO_2 treatment (2-way ANOVA, $F(1,79) = 66.29$, $p < 0.0001$).

Albeit non-significant, an accidental leak in the MKS system in the beginning of the experiment led to increased seawater PCO_2 and CCO_2 levels during the first two days (10 and 13 °C) of normocapnic experimentation (Table 2) compared to control conditions (Table 1). This resulted in enhanced haemolymph CCO_2 level (Table 3) and, thus, calculated [HCO_3^-] concentration (Fig. 2d; grey symbols). Besides a potential, slight impact on haemolymph PCO_2 and pH values at 13 °C (cf. Figure 3), other measured traits remained unaffected by the accidental leak (including pHi). Careful evaluation of all present results and the comparative findings from the North Sea study

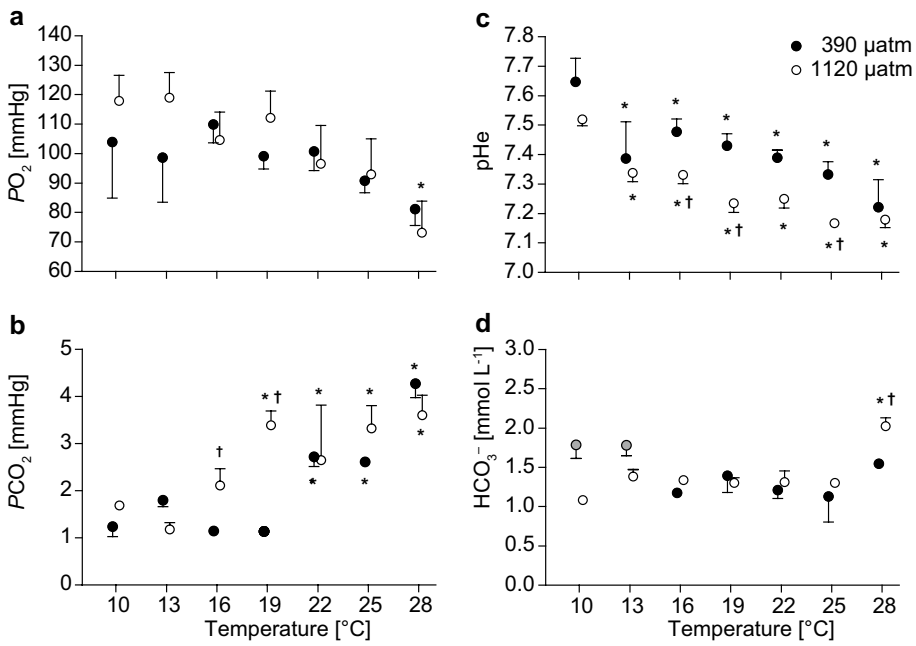

Fig. 2 Haemolymph partial pressure of oxygen (PO_2, **a**), partial pressure of carbon dioxide (PCO_2, **b**), pHe (**c**), and bicarbonate content ([HCO_3^-], **d**) in blue mussels, *Mytilus edulis* under 390 µatm and 1120 µatm CO_2 during acute warming (3 °C over night). For better viewing symbols were shifted to the left (filled circles) or right (open circles). **d** Grey symbols were excluded for statistical analyses as they indicate an acute response to an accidentally increase in seawater PCO_2 (for detailed information see text). Values are given as mean ± SD, $n = 5$–8. *Significantly different from the respective data at 10 °C, †significant difference between control and CO_2-exposed mussels at the respective temperature

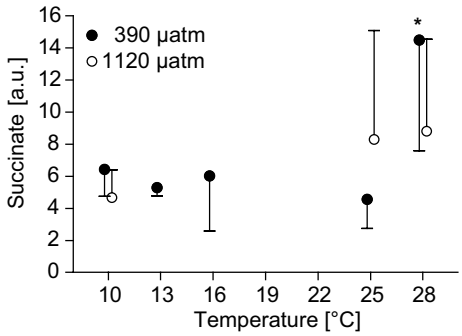

Fig. 3 Succinate content in mantle tissue of blue mussels, *Mytilus edulis* under 390 and 1120 µatm CO_2 during acute warming (3 °C over night). Succinate content is given in arbitrary units [a.u.]. For better viewing symbols were slightly shifted to the left (filled circles) or right (open circles). Values are given as mean ± SD, $n = 5–8$. *Significantly different from the respective data at 10 °C

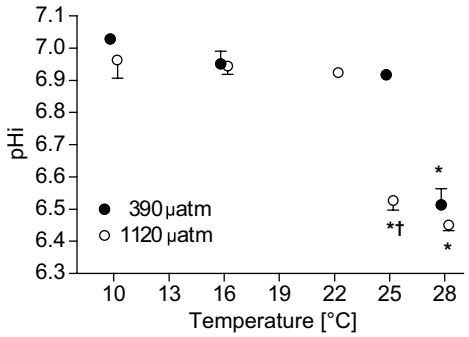

Fig. 4 Intracellular pH (pHi) of mantle tissue in blue mussels, *Mytilus edulis* under 390 and 1120 µatm CO_2 during acute warming (3 °C over night). Values are given as mean ± SD, $n = 5–8$. *Significantly different from the respective data at 10 °C, †significant difference between control and CO_2-exposed mussels at the respective temperature

(Zittier et al. 2015) suggest that the short-time alterations in seawater chemistry at the beginning of the warming trial under normocapnia did not influence the course of measured traits at higher temperatures. Considering haemolymph CCO_2 and [HCO_3^-] under normocapnia, both levels had been reduced at 16 °C, when seawater PCO_2 was restored again, and remained stable thereafter providing evidence that the elevated values at 10 and 13 °C are an acute response to the accidental elevation in CO_2 and could thus be safely excluded from statistical analysis. At 16 °C haemolymph [HCO_3^-] levels were similar in both CO_2 treatments and remained stable during warming until a sudden rise occurred at 28 °C (390 µatm: 1.17 ± 0.03 mmol L^{-1}, $n = 5$ at 16 °C vs. 1.55 ± 0.05 mmol L^{-1}, $n = 5$ at 28 °C; 1120 µatm: 1.08 ± 0.13 mmol L^{-1}, $n = 6$ at 10 °C vs. 2.03 ± 0.26 mmol L^{-1}, $n = 6$ at 28 °C, Tukey HSD, $p < 0.0001$) (Fig. 2d). Two-way ANOVA suggested a significant effect of temperature (2-way ANOVA, $F(6,62) = 12.06$, $p < 0.0001$), CO_2 treatment (2-way ANOVA, $F(1,62) = 8.13$, $p = 0.006$) and the interaction thereof (2-way ANOVA, $F(6,62) = 2.99$, $p = 0.025$). A post hoc test did not reveal any differences between CO_2 treatments until the final temperature of 28 °C was reached, where the rise in [HCO_3^-] level was stronger under hypercapnia than under normocapnia (Tukey HSD, $p = 0.02$).

Succinate levels in mantle tissue were unaffected by CO_2 exposure at control temperature (Fig. 3). During warming, no changes in succinate levels were found under normocapnia until a sudden increase was detected at the final temperature of 28 °C. Under hypercapnia succinate levels of mantle tissue were already elevated at 25 °C and levelled off thereafter resulting in a lower final concentration than under normocapnia. Independent of the treatment, all mussels showed high inter-individual variability when succinate increased (390 µatm: 6.43 ± 1.69 arbitrary units (a.u.) at 10 °C vs. 14.50 ± 6.91 a.u. at 28 °C, Tukey HSD, $p < 0.05$; 1120 µatm: 4.67 ± 1.72 a.u. at 10 °C vs. 8.83 ± 5.73 a.u. at 28 °C). Two-way ANOVA suggested a main effect of temperature (2-way ANOVA, $F(4,41) = 4.08$, $p = 0.007$), as well as a significant interaction between temperature and CO_2 treatments (2-way ANOVA, $F(2,41) = 3.52$, $p = 0.04$).

Intracellular pH (pHi) remained unaffected by CO_2 exposure at the control temperature (Fig. 4; 390 µatm: 7.03 ± 0.01, $n = 5$; 1,120 µatm: 6.96 ± 0.14, $n = 6$) and values remained more or less unchanged during warming until a threshold temperature was reached. pHi remained unchanged under normocapnia until a sudden and significant drop occurred at the final temperature of 28 °C (6.51 ± 0.05, $n = 6$, Tukey HSD, $p < 0.0001$). Values under hypercapnia dropped significantly already at 25 °C (6.53 ± 0.07, $n = 6$, Tukey HSD, $p < 0.0001$) and decreased further to the minimum value of 6.45 ± 0.04 at 28 °C. Thus, compared to values seen under normocapnia, pHi levels of hypercapnic mussels were significantly lower at 25 °C (Tukey HSD, $p < 0.0001$) before their converged again at the next higher temperature. Accordingly, a significant effect of temperature (2-way ANOVA, $F(4,44) = 115.15$, $p < 0.0001$), CO_2 treatment (2-way ANOVA, $F(1,44) = 40.81$, $p < 0.0001$) and the interaction thereof (2-way ANOVA, $F(3,44) = 19.92$, $p < 0.0001$) were suggested.

Discussion

Thermal limits under normocapnia

Mytilus edulis from the White Sea (sub-Arctic) under normocapnia displayed an oxygen consumption rate (MO_2) of 9.43 µmol O_2 h^{-1} g DW^{-1}at the acclimation temperature of 10 °C. These rates are well in the range of those reported

previously for *M. edulis* (Schlüter and Johansen 1994; Sukhotin and Pörtner 2001; Thomsen and Melzner 2010; Zittier et al. 2015). During acute warming MO_2 increased progressively until a phase change, indicating a beginning thermal limitation, at a calculated breakpoint temperature of 20.5 °C (Fig. 1). Interestingly, beyond 22 °C the respiratory responses to warming began to vary between individuals, which was not reflected in any other measured trait. While 5 out of 8 mussels were able to increase their MO_2 until the final temperature was reached (Q_{10} (10–28 °C) = 2.4), others showed a decline in MO_2 with further warming and the Q_{10}-value (22–28 °C) fell close to zero. This partially contradicts the findings in sub-Arctic and high-Arctic *M. edulis* populations from Greenland, where MO_2 of all mussels declined above 21 °C (Thyrring et al. 2015). However, the differences in metabolic rates in the warmth found in the present study had no influence on both upper thermal threshold T_P (onset of thermal limitation) and T_C (onset of anaerobic metabolism), which were the same in all normocapnic animals (see below), at least in the short-term. Hence, they might reflect differing performance capacities to cope with temperature stress. Recent publications provide increasing evidence that inter-individual variation in basic fitness-related traits, such as activity, metabolic rates, growth performance, physiological and behavioural responses to stressors is biologically meaningful (Sukhotin et al. 2003; Tamayo et al. 2011; Calosi et al. 2013; for review see Careau et al. 2008) and should be taken into account when interpreting experimental results. Further studies are necessary to identify the cause and potential long-term effects of the inter-individual differences in metabolic responses to warming seen in the present study.

According to the OCLTT concept (see introduction), a mismatch between oxygen uptake and demand develops in the warmth beyond the pejus temperature as circulation and/or ventilation reach their capacity limits. In combination with rising oxygen demand, these functional constraints result in internal hypoxemia of the organism, which contributes to progressively reduce aerobic performance. Haemolymph PCO_2 increased significantly in all mussels during warming from 19 to 22 °C followed by the decline in haemolymph PO_2 from 22 °C onward (discussed in detail below) in line with the onset of a fall in aerobic scope and thus T_P. The average breakpoint temperature of MO_2 was found at 20.5 °C, which is close to the suggested T_P for all individuals based on the changes in haemolymph status. Thus, the phase change in MO_2 was indicative for the T_P under normocapnia.

The picture was clearer in North Sea mussels, as the onset of thermal limitation in MO_2 occurred at similar temperatures distinctly below T_C in all individuals (Zittier et al. 2015). This limitation may have involved metabolic depression induced by the observed drop in haemolymph pH, and a concomitant limitation in heart rate rise. Hence, the phase change in MO_2 in North Sea mussels is indicative for the T_P as defined by the OCLTT concept. This also supports our conclusion for the White Sea and North Sea populations that the phase change in MO_2, combined with alterations in haemolymph status, can act as indicators for the upper T_P under normocapnia, even if variable between White Sea individuals. The putative T_P of sub-Arctic *M. edulis* matches the highest habitat temperature presently experienced by mussels in the White Sea where mean summer surface temperature is 15 °C with extreme temperatures reaching 20 °C (Berger et al. 2001; Dale and Prego 2003; Sukhotin and Berger 2013; Usov et al. 2013).

The critical temperature limit is defined by the onset of anaerobic metabolism when oxygen supply becomes insufficient to cover all energy expenditure aerobically. At T_C the main anaerobic metabolite in bivalves, succinate (Zurburg and Kluytmans 1980; Sukhotin and Pörtner 1999; Hines et al. 2007) accumulated significantly in mantle tissue during warming from 25 to 28 °C in all specimens (Fig. 3). Intracellular pH (pHi) of mantle tissue remained almost constant and independent of temperature whereas extracellular pH (pHe) decreased progressively with warming (see below). pHi was maintained during warming trial until a strong acidosis set in above 25 °C. Thus, the sudden drop in pHi occurred in parallel to the onset of anaerobic metabolism, thereby co-defining T_C. These findings are in line with common results reported for marine ectotherms. For several species differences between temperature-induced pH changes in extra- and intra-cellular compartments were demonstrated (e.g. Walsh et al. 1984; Butler and Day 1993; Sommer et al. 1997). In general pHi decreases only slightly with temperature in ectothermic animals and only an extreme decrease is interpreted to indicate critical temperature limits (e.g. Van Dijk et al. 1999; Sartoris et al. 2003; Melzner et al. 2006), which is supported by our data.

In *M. edulis* from the North Sea a reduction in aerobic performance indicating T_P occurred around 25 °C and anaerobic metabolites defining T_C accumulated significantly > 28 °C (Zittier et al. 2015). In the White Sea population (present study) a reduction in aerobic performance occurred already around 20 °C and anaerobic metabolism set in > 25 °C. Both populations were studied under identical conditions during late summer after the main reproductive period. Thus, seasonal impacts should be negligible and differences between populations should mainly be due to their adaptation to different environmental temperature regimes. Thus, both upper thermal limits T_P and T_C were shifted downwards by several degrees in the sub-Arctic population. These findings are in line with evolutionary temperature adaptation to the colder environment in the sub-Arctic population. The thermal window of a species shifts with seasonal acclimatisation or latitudinal adaptation to different temperatures (Sommer et al. 1997; Chapple et al. 1998; Van Dijk et al. 1999; Sommer and Pörtner 2002; Wittmann et al.

2008; Schröer et al. 2009; Schröer et al. 2011). In general, the thermal window is shifted to low temperatures in animals from high latitudes when compared to temperate species and populations (e.g. Pörtner 2001).

Gas and acid–base status of haemolymph and extrapallial fluid were similar in all experimental groups as previously found in the *M. edulis* populations from the North Sea (Zittier et al. 2015) and the Baltic Sea (Thomsen et al. 2010) as well as in *Mytilus galloprovincialis* from the Mediterranean Sea (Gazeau et al. 2014), emphasizing that both fluids are characterized by a similar carbonate system. Therefore, only haemolymph data will be discussed here. As demonstrated for several marine taxa including bivalves, a thermally induced onset of anaerobic metabolism is caused by the reduction in blood PO_2 below levels sufficient to sustain oxygen diffusion to mitochondria at elevated demand (Frederich and Pörtner 2000; Peck et al. 2002; Lannig et al. 2004). For example, in the Antarctic bivalve *Laternula elliptica* acute warming led to a sudden and drastic drop in haemolymph PO_2 by > 70% from 78.4 (control) to 20.3 mmHg when T_C was reached (Peck et al. 2002). In fact, the decreasing haemolymph PO_2 of mussels in the present study indicates that oxygen uptake from haemolymph is not fully compensated anymore by increased respiratory and/or circulatory performance. However, the decrease was less drastic and resulted only in a reduction of around 20% from 103.9 (control) to 81.2 mmHg at T_C (see Fig. 2a), which reflects the finding in North Sea population (Zittier et al. 2015). As discussed by Zittier et al. (2015), the difference to the blood PO_2 pattern reported by Peck et al. (2002) might be related to where the analysed haemolymph was drawn from. In contrast to sampling from the pericardium (Peck et al. 2002), haemolymph was sampled from the posterior adductor muscle in both *Mytilus* studies and is assumed to contain a mixture of pre- and post-branchial haemolymph (Booth et al. 1984; Walsh et al. 1984) that might disguise an oxygen limitation on the venous side and at the cellular level.

In parallel, a sharp increase in haemolymph PCO_2 occurred above 19 °C. Highly elevated haemolymph PCO_2 can be related to insufficient CO_2 release at high metabolic rates or may result from depressed ventilatory and circulatory activities due to cellular metabolic depression. Both suggest a reduction in aerobic scope during temperature stress indicting a T_P of > 19 °C. A second sharp increase in haemolymph PCO_2 occurred in parallel to the onset of anaerobic metabolism and, thus, at T_C. Here, extracellular acidosis may exacerbate a rise in PCO_2 through titration of carbonates, e.g. from the shell. This pattern of PCO_2 had not previously been reported.

In contrast to pHi, pHe decreased progressively during the warming protocol and no active regulation, indicated by bicarbonate [HCO_3^-] accumulation, was found until the final temperature of 28 °C when extracellular [HCO_3^-] increased slightly (Fig. 2d). Some reduction of pHe would be expected from the alphastat principle (− 0.017 units °C^{-1}; Reeves 1972). If pHe is reduced more strongly, as seen in the present study, this can elicit metabolic depression (e.g. Reipschläger and Pörtner 1996; Pörtner and Bock 2000). The phase change in MO_2 coincided with the fall in pHe from 7.43 at 19 °C to 7.39 at 22 °C and the subsequent reduced MO_2 of some animals at 25 °C coincided with a pH of 7.33. The strong reduction in MO_2 of some animals suggests that a pHe of < 7.4 can induce metabolic depression in sub-Arctic *M. edulis*. In the North Sea study (Zittier et al. 2015), pHe did not decrease progressively but stepwise during warming. A strong drop in pHe from 7.38 at 22 °C to 7.01 at 25 °C occurred in parallel to the limitation in MO_2 rise of all individuals. This led to the suggestion that a pHe of at least < 7.3 is necessary to elicit a depression of aerobic metabolism. The present findings support this conjecture; however, the pH threshold inducing metabolic depression may not only differ between species but also between populations and might be influenced by temperature and adaptation to high CO_2 environments (cf. Michaelidis et al. 2005; Thomsen and Melzner 2010). For example, blue mussels from the western Baltic Sea showed control metabolic rates in laboratory experiments even under 4000 µatm resulting in a pHe of 7.1 (Thomsen and Melzner 2010). However, this population regularly faces extreme environmental PCO_2 values up to 2300 µatm during summer and autumn (Thomsen et al. 2010).

In summary, our results indicate a downward shift in both upper thermal limits pejus and critical at high latitudes (White Sea vs. North Sea: T_P ~ 20 °C vs. 25 °C, T_C ~ 28 °C vs. 31 °C). The reduced heat tolerance in White Sea mussels is likely the result of evolutionary adaptation to the sub-Arctic climate. Considering that current environmental change develops more rapidly and strongly at high latitudes future warming may pose a high risk for the sub-Arctic population. According to IPCC scenarios, the projected temperature rise for the year 2100 of this region is + 6 °C (IPCC 2007) suggesting that future summer temperatures may exceed the pejus limit of the White Sea population. This, in turn, might result in reduced abundance of blue mussels in the intertidal or shallow subtidal zones in the White Sea with implications for the structure, function and services of this ecosystem.

CO_2 effects on thermal limits

Moderate hypercapnic exposure (1120 µatm) of *M. edulis* from the White Sea had no impact on MO_2 compared to normocapnia (Fig. 1b), a finding in line with results reported for other *Mytilus* populations (Thomsen et al. 2010; Zittier et al. 2015). During acute warming under hypercapnia, MO_2 rose progressively in all individuals until the final temperature of

28 °C. The calculated breakpoint temperature indicating the onset of the limitation in MO_2 rise was 25.8 °C. Succinate accumulated in mantle tissue already at 25 °C defining the critical temperature T_C. Hence, the phase change in MO_2 under hypercapnia was shifted to the critical limit while the one under normocapnia occurred at the pejus limit (see "Discussion" section; Fig. 1). As the temperature response in MO_2 changed with CO_2 exposure the breakpoint cannot be used as a general indicator for a specific thermal threshold. This matches the findings of the North Sea study (Zittier et al. 2015) where hypercapnic animals during warming remained in a more active mode and the limitation in MO_2 indicated T_C while mussels under normocapnia showed a limitation around T_P likely due to an onset of metabolic depression. Mild hypercapnia can apparently prevent metabolic depression in *M. edulis* (Zittier et al. 2015), however, the exact mechanism remains obscure. A higher metabolic rate stimulated by hypercapnia (1500 µatm) during acute warming has also been reported for oysters (Lannig et al. 2010). In crustaceans CO_2 stimulated cardiac performance through adenosine release (Stegen and Grieshaber 2001) but led to an early breakdown of heart rate when combined with acute warming (Walther et al. 2009). However, heart rate of *M. edulis* from the North Sea stagnated during warming, independent of seawater CO_2 concentration (Zittier et al. 2015). Long-term CO_2 exposure of *M. edulis* (8 weeks, ~480 to 3800 µatm) without a thermal challenge showed that aerobic metabolism is first increasing and then declining with rising CO_2 (Thomsen and Melzner 2010). A study on the metabolic profile of *M. edulis* revealed that long-term CO_2 exposure (90 days at ~2400 and ~3800 µatm) reduced succinate content in mantle tissue and thus (anaerobic) energy turnover, whereas succinate was strongly increased under the extreme levels of ~24,000 µatm (Ellis 2013). In these studies it was assumed that moderate hypercapnia increases aerobic metabolism most probably to compensate for an increased cellular energy demand while higher CO_2 levels lead to a limitation in aerobic metabolic rate. This is also in line with findings in *M. galloprovincialis* where exposure to ~5000 µatm at unchanged temperature led to metabolic depression (Michaelidis et al. 2005), which did not occur at lower levels of ~1100 µatm (Gazeau et al. 2014). However, the mechanisms resulting in metabolic stimulation by moderate hypercapnia are not yet clear and require further study.

The accumulation in succinate defining the critical limit was already seen at 25 °C compared to 28 °C under normocapnia. However, derived from succinate accumulation anaerobic metabolic rate remained lower under hypercapnia even when T_C was exceeded (Fig. 3), which may imply that anaerobic energy turnover is suppressed by moderately elevated CO_2. Mantle pHi was maintained under hypercapnia at moderate temperatures. The strong decrease in pHi was already seen at 25 °C compared to 28 °C under normocapnia in accordance with the respective onset of anaerobiosis supporting a downward shift in T_C by around 3 °C under hypercapnia. This may reflect a reduction of energy-dependent processes under combined hypercapnia and warming and may be useful to withstand temperature extremes. However, tolerating the exceedance of T_C is only possible in the short-term but will result in death during longer exposure periods.

An ecologically more important thermal limit is the pejus temperature as it may define the distribution range of the population according to OCLTT (see "Introduction" section). Our findings on oxygen and acid–base status of haemolymph provide evidence that the T_P is also shifted downwards by CO_2 as reported for other marine ectotherms (e.g. Metzger et al. 2007; Walther et al. 2009; Dissanayake and Ishimatsu 2011; Schalkhausser et al. 2012; Schiffer et al. 2014; for review see Pörtner 2012). Haemolymph PO_2 was initially slightly increased under hypercapnia but decreased progressively above 19 °C a pattern that only occurred above 22 °C under normocapnia. Accordingly, a significant increase in haemolymph PCO_2 during warming under hypercapnia was found at 19 °C rather than at 22 °C as under normocapnia. These results indicate a thermal limitation in aerobic performance at lower temperatures and a downward shift of the T_P in parallel to T_C by around 3 °C under hypercapnia. Thus, ocean acidification projected for the end of the century (~700–1100 µatm; Meehl et al. 2007) has the potential to reduce the thermal envelope as well as ecologically relevant thermal limits of sub-Arctic *M. edulis*.

Hypercapnic exposure caused a decrease in haemolymph pH (pHe) when compared to normocapnia. This reduction was similar across the warming protocol, which induced a general decrease in pHe. Upon warming this lowered pHe reaches a specific threshold at lower temperatures, which might hamper the further rise in MO_2 (see "Discussion" section) but can be compensated for by CO_2 stimulation. In hypercapnic mussels pHe fell indeed below 7.4 (the suggested pH threshold under normocapnia) at a clearly lower temperature and decreased progressively thereafter, but had no implications on MO_2 rise. This is in line with the findings in the North Sea population (Zittier et al. 2015) and emphasizes the stimulating effect of moderate hypercapnia on metabolism (see above).

The temperature-induced extracellular acidosis remained uncompensated throughout the warming protocol until a sudden and significant $[HCO_3^-]$ accumulation was found at the final temperature of 28 °C, when T_C was already surpassed. The interpretation of bicarbonate accumulation in response to temperature extremes in *M. edulis* must remain speculative at present. The drop in pHi in hypercapnic mussels might indicate bicarbonate release from intracellular sources (c.f. Table 4) beyond the acidosis caused by anaerobic metabolism. Under normocapnia a trend towards increasing haemolymph $[HCO_3^-]$ concentration was indicated when

T_C was reached. Mussels from the North Sea might have responded similarly. Normocapnic mussels experienced a significant increase and hypercapnic mussels a slight increase in haemolymph [HCO_3^-] when T_C was reached (Zittier et al. 2015). However, data under further warming and on pHi status are not available for this population. The authors assumed that residual acid–base regulation capacity may be used as a last defence under normocapnia, which was not possible anymore under hypercapnia in the warmth. The foregoing discussion provides evidence for a higher sensitivity to thermal stress and hypercapnia in sub-Arctic mussels than in temperate ones. Nevertheless, in the more sensitive White Sea mussels bicarbonate was significantly increased under hypercapnia at extreme temperatures. It needs to be clarified in further studies where the bicarbonate comes from and whether this is an additional sign of stress rather than a regulatory response.

Conclusion

In conclusion, the thermal tolerance thresholds found in this study are in line with those defined by the OCLTT concept. In line with the general picture (e.g. Pörtner et al. 2009, 2010, 2012; Peck et al. 2014) the thermal window of the sub-Arctic White Sea population is shifted to lower temperatures when compared to the temperate North Sea population. In contrast to the North Sea population, CO_2 exposure caused enhanced thermal sensitivity in White Sea mussels indicated by an earlier increase in haemolymph PCO_2 as well as an earlier onset of anaerobic conditions visible in the accumulation of succinate and the accompanying drop of intracellular pH, and possibly, cellular bicarbonate release. Obviously, tolerance to thermal stress and hypercapnia in *Mytilus sp.* (e.g. Gosling 1992) is constrained at high latitudes as a result of cold adaptation, possibly making this population vulnerable to future climate change. Studies of long-term CO_2 exposure in *Mytilus* species (Berger et al. 2001; Michaelidis et al. 2005; Beesley et al. 2008; Thomsen and Melzner 2010; Thomsen et al. 2010) indicate a reduction in whole organism performance capacity by CO_2 suggesting that longer exposure periods may exacerbate vulnerability. The larger degree of projected warming of the White Sea area until 2100 by itself (+6 °C vs. +3 °C in the North Sea, IPCC 2007) but especially in combination with the projected rise in oceanic CO_2 levels (~1000 µatm, IPCC 2007, 2013) may induce physiological stress with implications for the fitness and associated distribution range of this blue mussel population.

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Compliance with Ethical Standards

Conflicts of interest All authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent The study did not involve any human or vertebrate animal subjects. Therefore, no informed consent was required.

References

Bayne BL, Scullard C (1977) An apparent specific dynamic action in *Mytilus edulis* L. J Mar Biol Assoc UK 57:371–378

Beesley A, Lowe DM, Pascoe CK, Widdicombe S (2008) Effects of CO_2-induced seawater acidification on the health of *Mytilus edulis*. Clim Res 37:215–225. https://doi.org/10.3354/cr00765

Berger VJ, Dahle S, Galaktionov K, Kosobokova X, Naumov A, Rat'kova T, Savinov V, Savinova T (2001) White sea ecology and environment. Derzavets Publisher, St-Petersburg

Booth CH, McDonald DG, Walsh PJ (1984) Acid-base balance in the sea mussel *Mytilus edulis* I. Effects of hypoxia and air-exposure on hemolymph acid-base status Mar. Biol Lett 5:347–358

Butler PJ, Day N (1993) The relationship between intracellular pH and swimming performance of brown trout exposed to neutral and sublethal pH. J Exp Biol 176:271–284

Calosi P, Turner LM, Hawkins M, Bertolini C, Nightingale G, Truebano M, Spicer JI (2013) Multiple physiological responses to multiple environmental challenges: an individual approach. Integr Comp Biol 53:660–670

Careau V, Thomas D, Humphries MM, Reale D (2008) Energy metabolism and animal personality. Oikos 117:641–653

Chapple JP, Smerdon GR, Berry R, Hawkins AJ (1998) Seasonal changes in stress-70 protein levels reflect thermal tolerance in the marine bivalve *Mytilus edulis* L. J Exp Mar Biol Ecol 229:53–68

Dale AW, Prego R (2003) Tidal and seasonal nutrient dynamics and budget of the Chupa Estuary White Sea (Russia). Estuar Coast Shelf S 56:377–389. https://doi.org/10.1016/S0272-7714(02)00190-7

Davenport J, Smith RJJW, Packer M (2000) Mussels *Mytilus edulis*: significant consumers and destroyers of mesozooplankton. Mar Ecol Prog Ser 198:131–137

Deutsch C, Ferrel A, Seibel B, Pörtner H-O, Huey RB (2008) Climate change tightens a metabolic constraint on marine habitats. Science 348:1132–1135. https://doi.org/10.1126/scienceaaa1605

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res 34:1733–1743

Dissanayake A, Ishimatsu A (2011) Synergistic effects of elevated CO_2 and temperature on the metabolic scope and activity in a shallow-water coastal decapod (*Metapenaeus joyneri*;

Crustacea: Penaeidae). ICES J Mar Sci 68:1147–1154. https://doi.org/10.1093/icesjms/fsq188

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO_2 problem. Annu Rev Mar Sci 1:169–192

Ellis RP (2013) The impact of ocean acidification increased seawater temperature and a bacterial challenge on the immune response and physiology of the blue mussel *Mytilus edulis*. Dissertation, University of Plymouth

Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J Mar Sci 65:414–432

Findlay HS, Burrows MT, Kendall MA, Spicer JI, Widdicombe S (2010) Can ocean acidification affect population dynamics of the barnacle *Semibalanus balanoides* at its southern range edge? Ecology 91:2931–2940

Frederich M, Pörtner HO (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab *Maja squinado*. Am J Physiol Regul Integr Comp Physiol 279:R1531–R1538

Gaffney PM, Diehl WJ (1986) Growth condition and specific dynamic action in the mussel *Mytilus edulis* recovering from starvation. Mar Biol 93:401–409

Gazeau F, Alliouane S, Bock C, Bramanti L, López Correa M, Gentile M, Hirse T, Pörtner HO, Ziveri P (2014) Impact of ocean acidification and warming on the Mediterranean mussel (*Mytilus galloprovincialis*). Front Mar Sci 1:62. https://doi.org/10.3389/fmars201400062

Gosling E (1992) The mussel *Mytilus*: ecology physiology genetics and culture. Elsevier, New York

Gosling E (2003) Bivalve mollusks: biology, ecology and culture. Fishing News books, Oxford

Hale R, Calosi P, McNeill L, Mieszkowska N, Widdicombe S (2011) Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. Oikos 120:661–674. https://doi.org/10.1111/j1600-0706201019469x

Heisler N (1986) Buffering and transmembrane ion transfer processes. In: Heisler N (ed) Acid-base regulation in animals. Elsevier, Amsterdam, pp 3–48

Hines A, Oladiran GS, Bingell JP, Stentiford GD, Viant MR (2007) Direct sampling of organisms from the field and knowledge of their phenotype: key recommendations for environmental metabolomics. Environ Sci Technol 41:3375–3381

Hummel H, Sommer A, Bogaards RH, Pörtner HO (1997) Variation in genetic traits of the lugworm *Arenicola marina*: temperature related expression of mitochondrial allozymes? Mar Ecol Prog Ser 159:189–195

IPCC (2007) Climate change 2007: the physical science basis contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge and New York

IPCC (2013) 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

IPCC (2014) Climate Change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge and New York

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13:1419–1434

Lannig G, Bock C, Sartoris FJ, Pörtner HO (2004) Oxygen limitation of thermal tolerance in cod *Gadus morhua* L. studied by magnetic resonance imaging and on-line venous oxygen monitoring. Am J Physiol Regul Integr Comp Physiol 287:R902–R910. https://doi.org/10.1152/ajpregu007002003

Lannig G, Eilers S, Pörtner HO, Sokolova IM, Bock C (2010) Impact of ocean acidification on energy metabolism of oyster *Crassostrea gigas*—changes in metabolic pathways and thermal response. Mar Drugs 8:2318–2339. https://doi.org/10.3390/md8082318

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao Z-C (2007) Global climate projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, pp 747–846

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907

Melzner F, Bock C, Pörtner HO (2006) Temperature-dependent oxygen extraction from the ventilatory current and the costs of ventilation in the cephalopod *Sepia officinalis*. J Comp Physiol B 176:607–621

Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner HO (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6:2313–2331

Melzner F, Stange P, Trübenbach K, Thomsen J, Casties I, Panknin U, Gorb SN, Gutowska MA (2011) Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. PLoS ONE 6:e24223

Metzger R, Sartoris F, Langenbuch M, Pörtner HO (2007) Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*. J Therm Biol 32:144–151. https://doi.org/10.1016/jjtherbio200701010

Michaelidis B, Ouzounis C, Paleras A, Pörtner HO (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. Mar Ecol Prog Ser 293:109–118

Miles H, Widdicombe S, Spicer JI, Hall-Spencer J (2007) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. Mar Pollut Bull 54:89–96. https://doi.org/10.1016/jmarpolbul200609021

Peck LS, Pörtner HO, Hardewig I (2002) Metabolic demand oxygen supply and critical temperatures in the Antarctic bivalve *Laternula elliptica*. Physiol Biochem Zool 75:123–133

Peck LS, Morley SA, Richard J, Clark MS (2014) Acclimation and thermal tolerance in Antarctic marine ectotherms. J Exp Biol 217:16–22. https://doi.org/10.1242/jeb089946

Pierrot D, Lewis E, Wallace DWR (2006) CO2SYS DOS program developed for CO_2 system calculations. ORNL/CDIAC-105 Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory US Department of Energy, Oak Ridge

Pörner HO, Boutilier RG, Tang Y, Toews DP (1990) Determination of intracellular pH and PCO_2 after metabolic inhibition by fluoride and nitrilotriacetic acid. Resp Physiol 81:255–274

Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88:137–146. https://doi.org/10.1007/s001140100216

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373:203–217

Pörtner HO (2010) Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J Exp Biol 213:881–893. https://doi.org/10.1242/jeb037523

Pörtner HO (2012) Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Mar Ecol Prog Ser 470:273–290. https://doi.org/10.3354/meps10123

Pörtner HO, Bock C (2000) A contribution of acid-base regulation to metabolic depression in marine ectotherms. In: Heldmaier G, Klingenspor M (eds) Life in the cold. Springer Verlag, Berlin, pp 443–458

Pörtner HO, Farrell AP, Knust R, Lannig G, Mark FC, Storch D (2009) Adapting to climate change-response. Science 323:876–877

R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org

Reeves RB (1972) An imidazole alphastat hypothesis for vertebrate acid-base regulation: tissue carbon dioxide content and body temperature in bullfrogs. Respir Physiol 14:219–236

Reipschläger A, Pörtner HO (1996) Metabolic depression during environmental stress: the role of extracellular versus intracellular pH in *Sipunculus nudus*. J Exp Biol 199:1801–1807

Ritz C, Baty F, Streibig JS, Gerhard D (2015) Dose-Response analysis using R. PLoS ONE 10:e0146021. https://doi.org/10.1371/journal.pone.0146021

Sartoris FJ, Bock C, Pörtner HO (2003) Temperature-dependent pH regulation in eurythermal and stenothermal marine fish: an interspecies comparison using ^{31}P-NMR. J Therm Biol 28:363–371. https://doi.org/10.1016/S0306-4565(03)00012-3

Schalkhausser B, Bock C, Stemmer K, Brey T, Pörtner HO, Lannig G (2012) Impact of ocean acidification on escape performance of the king scallop *Pecten maximus* from Norway. Mar Biol 160:1995–2006. https://doi.org/10.1007/s00227-012-2057-8

Schiffer M, Harms L, Lucassen M, Mark FC, Pörtner HO, Storch D (2014) Temperature tolerance of different larval stages of the spider crab *Hyas araneus* exposed to elevated seawater PCO_2. Front Zool 11:87. https://doi.org/10.1186/s12983-014-0087-4

Schlüter L, Johansen SB (1994) Annual variation in condition respiration and remineralisation of *Mytilus edulis* L in the Sound Denmark. Helgol Meeresunters 48:419–430

Schröer M, Wittmann AC, Grüner M, Steeger HU, Bock C, Paul P, Pörtner HO (2009) Oxygen limited thermal tolerance and performance in the lugworm *Arenicola marina*: a latitudinal comparison. J Exp Mar Biol Ecol 372:22–30

Schröer M, Saphörster J, Bock C, Pörtner HO (2011) Oxygen and capacity limited thermal tolerance of the lugworm *Arenicola marina*: a seasonal comparison. J Exp Mar Biol Ecol 409:300–309

Sokal RR, Rohlf FJ (1995) Biometry. W. H. Freeman & Co., New York

Sokolova IM, Pörtner HO (2003) Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis* Gastropoda: Littorinidae) from different latitudes. J Exp Biol 206:195–207. https://doi.org/10.1242/jeb00054

Sommer AM, Pörtner HO (2002) Metabolic cold adaptation in the lugworm *Arenicola marina*: comparison of a North Sea and a White Sea population. Mar Ecol Prog Ser 240:171–182

Sommer A, Klein B, Pörtner HO (1997) Temperature induced anaerobiosis in two populations of the polychaete worm *Arenicola marina* (L). J Comp Physiol B 167:25–35

Stegen E, Grieshaber MK (2001) Adenosine increases ventilation rate cardiac performance and haemolymph velocity in the American lobster *Homarus americanus*. J Exp Biol 204:947–957

Sukhotin AA, Berger V (2013) Long-term monitoring studies as a powerful tool in marine ecosystem research. Hydrobiologia 706:1–9

Sukhotin AA, Pörtner HO (1999) Habitat as a factor involved in the physiological response to environmental anaerobiosis of White Sea *Mytilus edulis*. Mar Ecol Prog Ser 184:149–160

Sukhotin AA, Pörtner HO (2001) Age-dependence of metabolism in mussels *Mytilus edulis* (L) from the White Sea. J Exp Biol Ecol 257:53–72

Sukhotin AA, Lajus DL, Lesin PA (2003) Influence of age and size on pumping activity and stress resistance in the marine bivalve *Mytilus edulis* L. J Exp Mar Biol Ecol 284:129–144

Tamayo D, Ibarrola I, Urrutia MB, Navarro E (2011) The physiological basis for inter-individual growth variability in the spat of clams (*Ruditapes philippinarum*). Aquaculture 321:113–120

Thomsen J, Melzner F (2010) Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis*. Mar Biol 157:2667–2676. https://doi.org/10.1007/s00227-010-1527-0

Thomsen J, Gutowska MA, Saphörster J, Heinemann A, Trübenbach K, Fietzke J, Hiebenthal C, Eisenhauer A, Körtzinger A, Wahl M, Melzner F (2010) Calcifying invertebrates succeed in a naturally CO_2 enriched coastal habitat but are threatened by high levels of future acidification. Biogeosciences 7:5119–5156. https://doi.org/10.5194/bgd-7-5119-2010

Thyrring J, Rysgaard S, Blicher ME, Sejr MK (2015) Metabolic cold adaptation and aerobic performance of blue mussels (*Mytilus edulis*) along a temperature gradient into the High Arctic region. Mar Biol 162:235–243. https://doi.org/10.1007/s00227-014-2575-7

Tschischka K, Abele D, Pörtner HO (2000) Mitochondrial oxyconformity and cold adaptation in the polychaete *Nereis pelagica* and the bivalve *Arctica islandica* from Baltic and White Sea. J Exp Biol 203:3355–3368

Usov N, Kutcheva I, Primakov I, Martynova D (2013) Every species is good in its season: do the shifts in the annual temperature dynamics affect the phenology of the zooplankton species in the White Sea? Hydrobiologia 706:11–33

Van Dijk PLM, Tesch C, Hardewig I, Pörtner HO (1999) Physiological disturbances at critically high temperatures: a comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae). J Exp Biol 202:3611–3621

Walsh PJ, McDonald DG, Booth CH (1984) Acid-base balance in the sea mussel *Mytilus edulis* II Effects of hypoxia and air-exposure on intracellular acid-base status. Mar Biol Lett 5:359–369

Walther K, Sartoris FJ, Bock C, Pörtner HO (2009) Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus*. Biogeosciences 6:2207–2215

Whiteley NM (2011) Physiological and ecological responses of crustaceans to ocean acidification. Mar Ecol Prog Ser 430:257–271. https://doi.org/10.3354/meps09185

Wittmann AC, Schröer M, Bock C, Steeger H, Paul RJ, Pörtner HO (2008) Indicators of oxygen-and capacity-limited thermal tolerance in the lugworm *Arenicola marina*. Clim Res 37:227–240

Zittier ZMC, Hirse T, Pörtner HO (2013) The synergistic effects of increasing temperature and CO_2 levels on activity capacity and acid-base balance in the spider crab *Hyas araneus*. Mar Biol 160:2049–2062. https://doi.org/10.1007/s00227-012-2073-8

Zittier ZMC, Bock C, Lannig G, Pörtner HO (2015) Impact of ocean acidification on thermal tolerance and acid-base regulation of *Mytilus edulis* (L) from the North Sea. J Ex Mar Biol Ecol 473:16–25. https://doi.org/10.1016/jjembe201508001

Zurburg W, Kluytmans JH (1980) Organ specific changes in energy metabolism due to anaerobiosis in the sea mussel *Mytilus edulis* (L). Comp Biochem Physiol B 67:317–322