

Mar Biol (2013) 160:1995–2006  
DOI 10.1007/s00227-012-2057-8

ORIGINAL PAPER

# Impact of ocean acidification on escape performance of the king scallop, *Pecten maximus*, from Norway

Burgel Schalkhausser · Christian Bock ·  
Kristina Stemmer · Thomas Brey ·  
Hans-O Pörtner · Gisela Lannig

Received: 31 January 2012 / Accepted: 23 August 2012 / Published online: 30 September 2012  
© Springer-Verlag 2012

**Abstract** The ongoing process of ocean acidification already affects marine life, and according to the concept of oxygen and capacity limitation of thermal tolerance, these effects may be intensified at the borders of the thermal tolerance window. We studied the effects of elevated CO<sub>2</sub> concentrations on clapping performance and energy metabolism of the commercially important scallop *Pecten maximus*. Individuals were exposed for at least 30 days to 4 °C (winter) or to 10 °C (spring/summer) at either ambient (0.04 kPa, normocapnia) or predicted future PCO<sub>2</sub> levels (0.11 kPa, hypercapnia). Cold-exposed (4 °C) groups revealed thermal stress exacerbated by PCO<sub>2</sub> indicated by a high mortality overall and its increase from 55 % under normocapnia to 90 % under hypercapnia. We therefore excluded the 4 °C groups from further experimentation. Scallops at 10 °C showed impaired clapping performance following hypercapnic exposure. Force production was significantly reduced although the number of claps was unchanged between normocapnia- and hypercapnia-exposed scallops. The difference between maximal and resting metabolic rate (aerobic scope) of the hypercapnic scallops was significantly reduced compared with normocapnic animals, indicating a reduction in net aerobic

scope. Our data confirm that ocean acidification narrows the thermal tolerance range of scallops resulting in elevated vulnerability to temperature extremes and impairs the animal's performance capacity with potentially detrimental consequences for its fitness and survival in the ocean of tomorrow.

## Introduction

Atmospheric temperature and CO<sub>2</sub> concentrations have been rising dramatically over the last decades due to anthropogenic influences (IPCC 2007). In oceans, an increase in dissolved CO<sub>2</sub> results in reduced seawater pH and altered carbonate chemistry, known as ocean acidification (OA). The oceans' average pH has already declined by more than 0.1 units below the pH of pre-industrial times (Caldeira and Wickett 2003). If trends continue at current rates, pH values are predicted to decrease by 0.3–0.4 units by the end of this century (Orr 2011). These changes will affect a variety of biological processes that depend on pH and/or the components of the CO<sub>2</sub>/bicarbonate/carbonate system. Such effects are predicted to be especially significant for calcifying organisms (Fabry et al. 2008; Doney et al. 2009; Kroeker et al. 2010). Besides the obvious effect on calcification, OA exposure influences the rate of energy metabolism in invertebrates (Langenbuch and Pörtner 2004; Michaelidis et al. 2005; Lannig et al. 2010; Melatunan et al. 2011) via changes in extracellular and, possibly, intracellular pH values that cause alterations in energy partitioning. At the cellular level (muscle, liver), an extracellular acidosis causes metabolic depression by reducing the rate and costs of acid–base and ion regulation (Pörtner 1987; Pörtner et al. 2000; Pörtner and Bock 2000) and/or of protein synthesis (Langenbuch and Pörtner 2003).

Communicated by S. Dupont.

B. Schalkhausser (✉) · C. Bock · H.-O. Pörtner · G. Lannig  
Integrative Ökophysiologie, Alfred Wegener Institut für  
Polar- und Meeresforschung in der Helmholtz-Gemeinschaft,  
Am Handelshafen 12, 27570 Bremerhaven, Germany  
e-mail: Burgel.Schalkhausser@awi.de

K. Stemmer · T. Brey  
Funktionelle Ökologie, Alfred Wegener Institut für  
Polar- und Meeresforschung in der Helmholtz-Gemeinschaft,  
Columbusstrasse, 27570 Bremerhaven, Germany

At whole animal level, this is paralleled either by a decrease (Pörtner et al. 1998; Michaelidis et al. 2005) or by a stimulation in whole organism metabolic rate (Beniash et al. 2010; Stumpp et al. 2011), likely depending on whether and to what extent transepithelial mechanisms of acid–base regulation are depressed or stimulated by OA scenarios (Pörtner et al. 2000). Each of these shifts may result in trade-offs in energy allocation between different biological processes such as ion regulation, calcification, growth or development (Pörtner et al. 2000, 2004; Guderley and Pörtner 2010), possibly leading to constraints in one or more of these processes (Wood et al. 2008; Beniash et al. 2010; Stumpp et al. 2011).

The interactions between ocean acidification and other environmental factors are not well understood, and recent studies suggest a high complexity, with mainly synergistic effects. With respect to temperature, ocean acidification clearly reduced acute heat tolerance of the edible crab, *Cancer pagurus* ( $PCO_2$  of 1 kPa, Metzger et al. 2007), and narrowed the thermal tolerance range of the spider crab, *Hyas araneus*, indicated by a  $PCO_2$ -dependent lowering of the critical temperature of the animals (OA scenarios: 0.07 and 0.3 kPa, Walther et al. 2009). A temperature rise of 5 °C and a  $PCO_2$  elevated by 0.1 kPa caused respiration rates and adenylate nucleotide concentrations to fall in the snail *Littorina littorea* (Melatunan et al. 2011). An OA exposure of 0.3 kPa in combination with acute heat stress (delta 8–11 °C) impaired the locomotion capacity of the spider crab, *H. araneus* (Zittier et al. 2012). In Sydney rock oyster, *Saccostrea glomerata*, fertilization and larval development was explicitly reduced under hypercapnia (OA scenarios: 0.6, 0.7 and 1 kPa  $PCO_2$ ) above optimum temperature (delta 4 °C) (Parker et al. 2009). All of these findings indicate that thermal sensitivity is enhanced under projected OA conditions. Conversely, sensitivity to OA is presumably enhanced at thermal extremes. OA exposure also interferes synergistically with other stressors. A study on juvenile oysters *Crassostrea virginica* revealed that the combined exposure to low salinity (15 vs. 30 psu) and high  $PCO_2$  (0.07–0.08 kPa) produced greater changes in shell properties than each of the factors alone (Dickinson et al. 2012). Internal shell dissolution in *Mytilus edulis* under hypercapnic conditions was intensified in animals stressed by limited food supply, emphasizing a key role for food and thus energy availability in maximizing resistance to ocean acidification (Melzner et al. 2011).

The concept of oxygen- and capacity-limited thermal tolerance (OCLTT, Pörtner 2002) may offer a suitable approach towards a mechanistic understanding of the synergistic interaction between thermal stress and OA/pH effects (Pörtner and Farrell 2008). According to the OCLTT concept, maximal aerobic capacity and thus available metabolic power (energy use per unit time) are

limited to the organism's specific thermal tolerance window. As outlined by Guderley and Pörtner (2010), the maximal metabolic power must be partitioned between biological processes. Given that metabolic power depends on environmental parameters such as temperature or pH, environmental conditions outside the optimum range reaching the pejus (= getting worse) and critical range will impair an animal's aerobic scope, resulting in less metabolic power to sustain major fitness-related processes such as growth, reproduction, immune response or the avoidance of predators (see Pörtner 2010; Sokolova et al. 2012).

Our model organism, *Pecten maximus*, belongs to the family Pectinidae (common name scallops). Scallops are distributed worldwide and are unique among bivalves due to their swimming behaviour. To escape from predators, other bivalves close their valves tightly or use their foot for burrowing and/or somersaults. Due to a reduced foot and shells not tightly closing, scallops use a different escape strategy by fast shell closure or jet-like propulsion enabling them to swim (Wilkins 2006). Given that swimming is used to escape, for example, from predator attacks by starfish or crabs (Winter and Hamilton 1985; Ansell et al. 1991), the scallops' swimming capacity is an important performance parameter. Events of swimming activity have been shown to depend on various biotic (e.g. predator abundance or size/age (Wiborg 1963; Brand 2006) and abiotic factors such as displacement from preferred sites (Winter and Hamilton 1985), effects of currents (Gruffydd 1976) or temperature (Scheibling et al. 1995).

The aim of our study was to investigate the impact of long-term OA exposure on the physiology of the scallop, *Pecten maximus*, at two temperatures, 4 °C (winter) versus 10 °C (spring/summer) against the background of the OCLTT concept. To analyse for energetic trade-offs and associated restrictions in performance capacities under expected OA conditions, we determined clapping performance and oxygen consumption rates under resting and exercise conditions of the commercially important scallop *P. maximus* after long-term incubation at elevated  $CO_2$  level.

## Materials and methods

### Animals and holding conditions

In February 2011, wild-cultured *Pecten maximus* (Linnaeus 1758) were obtained from a sea farm (Kvistøy Edelskjell AS) in the northern North Sea near Stavanger (58° 58' 12" N, 5° 42' 36" E). They were collected by scuba divers at a depth of approximately 20 m and a temperature of 3–5 °C. Environmental mean temperatures at a depth of 20 m vary

from 4.5 to 15.2 °C (monthly means from station Indre Utsira since 2000; data reported by the Institute of Marine Research, <http://www.imr.no/forskning/forskningsdata/stasjoner/dato.php?page=0&year=2011&stid=5869>) and ambient CO<sub>2</sub> levels of around 390 µatm (Pfeil et al. 2012).

Animals wrapped in wood wool were transported on ice to the Alfred Wegener Institute by airplane and kept in a recirculated aquarium system at 5 °C. After 2 weeks of recovery, the shells were carefully scrubbed to remove epibionts. The incubations started and all measurements were carried out in March and April 2011 to avoid possible interference with reproduction as the presumable single spawning event takes place in June as shown for *P. maximus* from Fosen (a location close to Stavanger; Strand and Nylund 1991).

Randomized groups of labelled animals were incubated in recirculating systems in temperature-control rooms at either 4 °C or at 10 °C (one system per group, comprising header, receiver and reservoir tanks and 2 experimental tanks (each equipped with 10 animals maximum) similar to the systems described by Michaelidis et al. 2005 or Findlay et al. 2010). Temperature treatments were combined with different PCO<sub>2</sub> levels, controls with ~0.039 kPa (390 µatm, normocapnia) and elevated exposures with 4 times pre-industrial PCO<sub>2</sub> at ~0.112 kPa (1120 µatm, hypercapnia). All tanks were continuously bubbled with the specific CO<sub>2</sub> concentration that was made using a gas mixing system (HTK, Hamburg, Germany). Incubation lasted a minimum of 33 days up to 60 days (see supplementary materials). According to their suspension-feeding lifestyle, scallops were drip-fed live phytoplankton 3 times per week (DT's Premium Reef Blend (*Nannochloropsis oculata*, *Phaeodactylum*, *Chlorella*, 25.3 µg/L phytoplankton dry weight; Philipp et al. 2008). Feeding lasted for ≥6 h at a concentration of at least 6.10 × 10<sup>5</sup> cells gram<sup>-1</sup> bivalve biomass hour<sup>-1</sup> (4 °C) and 9.15 × 10<sup>5</sup> cells gram<sup>-1</sup> bivalve biomass hour<sup>-1</sup> (10 °C) (water circulation was stopped during feeding times). To ensure good water quality, water of the recirculated systems was exchanged at least twice a week and animal tanks were cleaned from faeces and remaining food items at least 3 times per week. Water physicochemistry was determined at least twice a week to ensure stable conditions (see Table 1): pH was measured with a pH electrode (WTW portable pH meter ProfiLine pH 3310) that was calibrated at the respective temperature with NIST buffers and salinity with a conductivity meter (WTW conductivity meter ProfiLine Cond 1970i), and total dissolved inorganic carbon (DIC) was determined with Seal Analysis SFA QuAAtro; pump Technicon trAAcs 800 TM. The pH was converted to total scale via measurement of Dickson standards. PCO<sub>2</sub> values were calculated using CO2sys (constants of Mehrbach et al. 1973 refitted by Dickson and

**Table 1** Physicochemical conditions of seawater after long-term incubations of *P. maximus* during normocapnia and hypercapnia at different temperatures (4 and 10 °C acclimation)

Parameter	Normocapnia	Hypercapnia
4 °C		
PCO <sub>2</sub> [kPa]	0.040 ± 0.009	0.110 ± 0.028
Temperature [°C]	3.9 ± 0.8	3.5 ± 0.7
pH (NBS scale)	8.19 ± 0.06	7.76 ± 0.10
pH (total scale)	8.08 ± 0.09	7.65 ± 0.11
Salinity [psu]	31.1 ± 0.6	31.2 ± 0.8
DIC [µmol/L]	2308.5 ± 47.4	2392.4 ± 39.3
10 °C		
PCO <sub>2</sub> [kPa]	0.040 ± 0.006	0.115 ± 0.028
Temperature [°C]	9.8 ± 0.4	9.6 ± 0.7
pH (NBS scale)	8.25 ± 0.10	7.81 ± 0.07
pH (total scale)	8.08 ± 0.06	7.65 ± 0.10
Salinity [psu]	31.1 ± 0.7	31.2 ± 0.7
DIC [µmol/L]	2315.0 ± 57.9	2387.3 ± 40.8

Data are mean ± SD with *N* = 25–30 (4 °C), *N* = 23 (10 °C)

NBS National Bureau of Standards, PCO<sub>2</sub> seawater partial pressure of CO<sub>2</sub>, DIC dissolved inorganic carbon

Millero 1987; programme developed by Lewis and Wallace 1998). Water quality was monitored by measurements of ammonia and nitrite values using photometric test kits (Machery-Nagel, Nanocolor test 0–68 and test 0–03). Animals were starved for 12–24 h prior to measurements to avoid interference with postprandial metabolism and faeces excretion (Wieser and Medgyesy 1990).

Shell dimensions of the scallops in the different incubation experiments did not differ between groups prior to and after incubation resulting in overall mean ± SD of 108.52 ± 2.44 mm (height), 94.99 ± 2.63 mm (length) and 26.63 ± 1.88 mm (width) (*N* = 44–47).

#### Calculation of condition index

To obtain data about the wellbeing of the animals, we calculated the condition index CI and the muscle index MI after Shiver et al. (2002) and Pazos et al. (1997) as follows:

$$CI = \frac{tissue_{DW}}{shell_{DW}} \cdot 100 \quad (1)$$

and

$$MI = \frac{muscle_{DW}}{shell_{DW}} \cdot 100 \quad (2)$$

with DW = dry weight of the total soft tissue and of the adductor muscle, respectively, and of the shell in [g]. Dry weights were determined after drying at 75 °C for as long as no detectable changes in weight were observed.

### Measurement of haemolymph acid–base parameters

Haemolymph parameters were analysed as described in Lannig et al. (2010) using a blood gas analyser (MT 33, Eschweiler, Germany) with glass electrodes for  $PCO_2$ ,  $PO_2$  and pH that were calibrated at the respective temperature with NIST buffers or calibration gases, respectively.

Scallops were kept on ice and manually immobilized to avoid any claps while carefully sampling about 2 mL haemolymph out of the tonic adductor muscle with gas-tight, sterile syringes (0.60-mm cannula). Haemolymph samples were immediately transferred to the blood gas analyser (about 300  $\mu$ L). For the determination of total  $CO_2$  in the haemolymph ( $C_eCO_2$ ), 200  $\mu$ L of haemolymph were put into glass vials with 3 mL 0.1 M HCl via a microlitre precision syringe (Hamilton, 1700 series) and analysed by a gas chromatograph (Agilent 6890 N GC System, Agilent Technologies, USA). The rest was deep frozen in liquid nitrogen ( $N_2$ ) and stored at  $-20^\circ C$  for further analysis.

The concentration of apparent bicarbonate in the haemolymph  $[HCO_3^-]_e$  was calculated as:

$$[HCO_3^-]_e = C_eCO_2 - (\alpha CO_2 \cdot P_eCO_2) \quad (3)$$

with  $C_eCO_2$  = total  $CO_2$  concentration [mM],  $\alpha CO_2$  = solubility of  $CO_2$  in seawater (calculated from Weiss 1974:  $4^\circ C$ , 31.15 psu:  $0.5610 \text{ mmol L}^{-1} \text{ kPa}^{-1}$ ;  $10^\circ C$ , 31.15 psu:  $0.4583 \text{ mmol L}^{-1} \text{ kPa}^{-1}$ ),  $P_eCO_2$  = partial pressure of  $CO_2$  in haemolymph [kPa].

After haemolymph sampling, animals were dissected, the wet soft tissues were weighed (to the nearest 0.1 g) and tissues samples were freeze clamped and stored in liquid  $N_2$  or at  $-80^\circ C$  for further analysis.

### Measurement of clapping performance

Experimental temperature was adjusted with a thermostat (Julabo, F32-HD), and the respective  $PCO_2$  levels of  $\sim 0.039$  kPa (normocapnia) and  $\sim 0.112$  kPa (hypercapnia) were reached by bubbling the water with either air or a mixture of  $CO_2$  and air via a multi-gas controller (MKS, PR4000). Determination of clapping performance was carried out following procedures described in previous studies (see Bailey et al. 2003; Fleury et al. 2005 and Guderley et al. 2009).

The measurements were performed using a force gauge (Mecmesin Advanced Force Gauge, 50 N). In the experimental tank, scallops were fixed on a plate using a hook-and-loop fastener (see Fig. 1 a for experimental set up). The lower valve was additionally immobilized with two clamps and stabilized with dental wax. The force gauge was placed on the front side of the scallop with a hook between its valves at an opening width, which was

observed for the undisturbed scallop (between 0.8 and 1.5 cm depending on the animal). After at least 12 h of recovery, clapping was induced by introducing aqua<sub>dest</sub> via a thin, gas-tight tube into the mantle cavity (see Bailey et al. 2005; Denny and Miller 2006). When the scallop stopped clapping, the stimulation was repeated until animals were fatigue and showed no response to further stimulation (after  $\sim 50$  min). The force of the adductor muscle during time of clapping was measured with a frequency of 10 Hz. The opening width during claps was determined using a ruler and by video analysis (Logitech, Quick Cam E2500).

Force recordings were normalized before each analysis. A “clap” was defined as a short interval of great force difference produced by the scallop with a rapid valve closing via phasic contraction as described in Fleury et al. (2005). Number of claps and force strength were determined from force per time recordings. We calculated the total force  $F_{total}$  [N] of each animal by dividing the “force impulse” (measurement force [N] multiplied by measured time [s]) by total time [s]:

$$F_{total} = \frac{\int f dt}{t_{total}} \quad (4)$$

$F_{total}$  is subdivided in a phasic part (force produced by the phasic adductor muscle) and a tonic part (force produced by the tonic muscle). The phasic force  $F_{phasic}$  [N] was calculated as sum of the clap force  $F_{clap}$  [N] (see Fig. 1 b).  $F_{clap}$  is the difference of the maximal force and the starting force during one clap. To calculate the mean phasic force  $F_{mean\ phasic}$  [N] per one clap,  $F_{phasic}$  was divided by the number of claps:

$$F_{mean\ phasic} = \frac{\sum_i^n F_{clap}}{n} = \frac{F_{phasic}}{n} \quad (5)$$

where the claps are numbered from  $i$  to  $n$ .

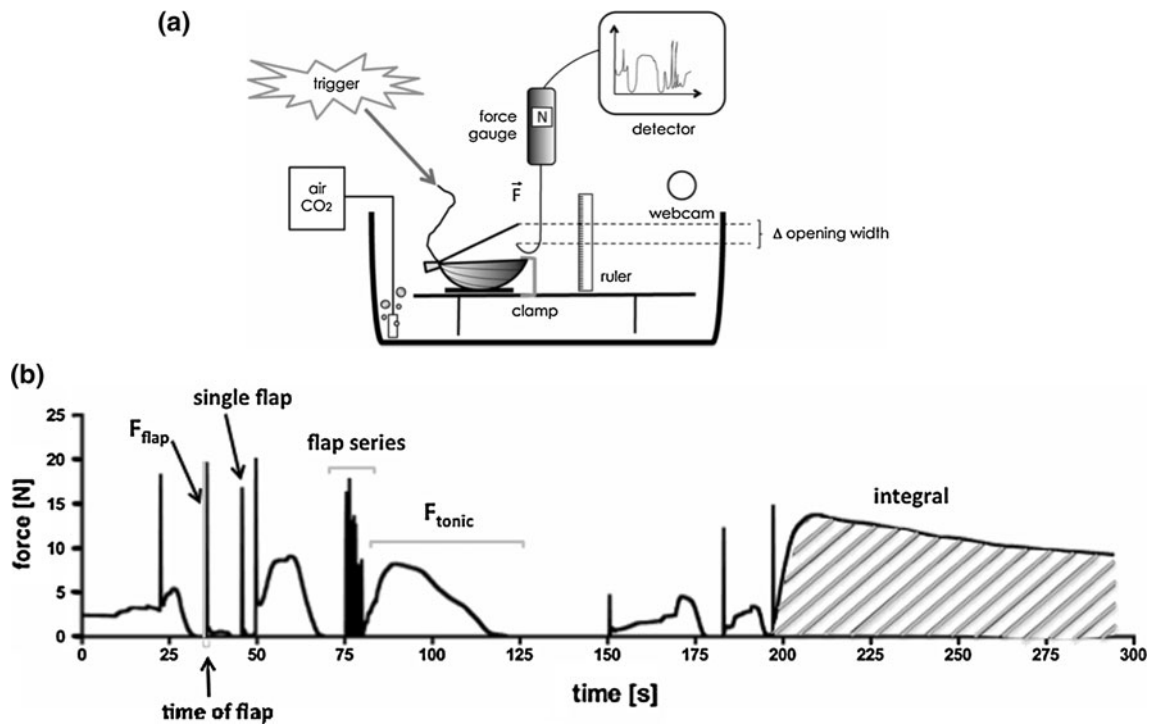
The tonic force  $F_{tonic}$  [N] was calculated from the difference of  $F_{total}$  and  $F_{phasic}$ :

$$F_{tonic} = F_{total} - F_{phasic} = F_{total} - \frac{\sum_i^n F_{clap} \cdot t_{clap}}{t_{tonic}} \quad (6)$$

where  $t_{clap}$  is the average time per clap (0.25 s) and  $t_{total}$  is the total measurement time until the animal was fatigued, while  $t_{tonic}$  is the total time of all tonic phases. We calculated with  $t_{total}$  instead of  $t_{tonic}$ , which makes a negligible difference of 0.53 %, because  $t_{tonic} = t_{total} - t_{phasic}$  and  $t_{total} \approx t_{tonic}$  (with  $t_{total} \sim 3000$  s compared with  $t_{phasic} \sim 16$  s).

### Measurement of metabolic rate

We measured respiration rates of normocapnia- and hypercapnia-exposed scallops using intermitted flow respirometry (cf. Heilmayer and Brey 2003; Tremblay et al.



**Fig. 1** Scheme of clapping performance data collection on *P. maximus*. **a** Experimental set up for force measurements. **b** Example for force recording and visualization of clap force ( $F_{clap}$ ), single

clap, clap series, tonic force ( $F_{tonic}$ ), time of a clap ( $t_{phasic}$ ), time of a tonic phase ( $t_{tonic}$ ) and part of an integral (see text for details)

2006). The scallops were placed in respiration chambers (plexiglas chambers with a volume of ca. 1.5 L; one chamber per animal) and immobilized on the bottom of the chamber using hook-and-loop fastener. The respiration chambers were placed into the experimental tank with defined temperature and  $PCO_2$  levels (see above). Oxygen saturation was measured with  $O_2$  optodes from PreSens (Microx TX2 or TX3, PreSens, Neuweiler, Germany), and continuous water circulation inside the respiratory setup was performed by a peristaltic pump (Ismatec, type Ism404B; Ismatec MCP). In order to exclude animals with potential atypical behaviour without causing disturbances during measurements (similar to Heilmayer and Brey 2003), the scallops were monitored using a web cam under continuous, shaded light. For changes between flow-through and closed system, plastic quick couplings were used in a different compartment of the experimental tank separated by a water-permeable wall in order to avoid irritations and disturbances for the scallops. Prior to measurements, scallops were allowed to recover for at least 12 h. In each experiment, 3 respiration chambers were used simultaneously. Respiration in empty chambers was measured before and after each experiment to account for potential bacterial respiration (observed values were negligible).

Experimental runs were stopped when oxygen saturation was decreased to 75–70 % inside the chamber. Two to four

runs were measured for each animal within 12 h. During nights, chambers were flooded and connected to the flow-through system. After measuring the oxygen consumption during resting metabolism, the respiration chambers were opened and the scallops were triggered to swim until fatigue as described above. After exhaustion, chambers were closed and the oxygen consumption measurements were started immediately and recorded until animals regained resting metabolic rates. The dry mass was calculated by applying the conversion factor 0.162, which was evaluated before using 20 separate *P. maximus*.

We calculated the oxygen consumption ( $\dot{M}_{O_2}$ ) [ $\mu\text{mol } O_2 \text{ h}^{-1} \text{ gDW}^{-1}$ ] of the animal under resting and fatigue conditions as follows:

$$\dot{M}_{O_2} = \alpha_{O_2} \cdot V_{H_2O} \cdot m_{DM}^{-1} \cdot \frac{ds(O_2)}{dt} \tag{7}$$

where  $\alpha_{O_2}$  is the oxygen solubility in seawater [ $\mu\text{mol } O_2 \text{ L}^{-1} \text{ kPa}^{-1}$ ],  $V_{H_2O}$  is the water volume [L] of the chamber  $V_{chamber} - V_{animal}$ ,  $m_{DM}$  is the dry mass [g] of the animal and  $s(O_2)$  is the oxygen consumption [kPa] over time  $t$  [h].

The net aerobic scope (NAS) [ $\mu\text{mol } O_2 \text{ h}^{-1} \text{ gDW}^{-1}$ ] was calculated after Fry (1947) as

$$NAS = \dot{M}_{O_{2max}} - \dot{M}_{O_{2rest}} \tag{8}$$

where  $\dot{M}_{O_{2max}}$  is the maximal metabolic rate (MMR) after exercise and the  $\dot{M}_{O_{2rest}}$  is the resting metabolic rate (RMR).

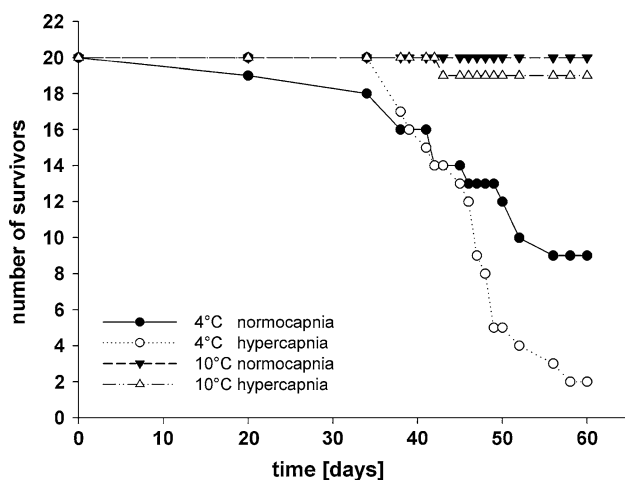
## Statistical analysis

Data sets were analysed using SigmaPlot (Version 12.0, Systat Software, Inc.) and GraphPad Prism (Version 4.0a, GraphPad Software Inc.). Interactions between effects of CO<sub>2</sub> exposure and exercise on haemolymph parameters and respiration measurements as well as differences within these groups were considered significant if the probability of Type II error was less than 0.05 using Two Way Analysis of Variance and Two Way Repeated Measures ANOVA, respectively, in combination with a Holm–Sidak Test. Unpaired *t* test (Mann–Whitney rank sum test if normality test failed) was used to identify significant differences between normocapnia- and hypercapnia-exposed scallops in net aerobic scope (NAS) and for morphological parameters and force measurements. Results are presented in box plots, and values are given as mean ± SD if not stated otherwise.

## Results

### Scallop condition and mortality

From day 20 (normocapnia) and day 38 (hypercapnia) onward, 55 % of normocapnia- and 90 % of hypercapnia-exposed scallops died at 4 °C, whereas none of the scallops incubated at 10 °C died under normocapnic and just one under hypercapnic conditions (see Fig. 2). Condition indices of scallops before and after incubation did not differ between groups with a CI of 11.38 ± 1.38 (control group at the start of the experiment, *N* = 20) and with CIs at the end of the experiment of 11.70 ± 1.94 (4 °C normocapnia, *N* = 8), 11.24 ± 0.96 (10 °C normocapnia, *N* = 19) and 11.63 ± 1.35 (10 °C hypercapnia, *N* = 19)



**Fig. 2** Survival rate of *P. maximus* after long-term incubation under normocapnia (seawater  $PCO_2 \sim 0.040$  kPa) and hypercapnia (seawater  $PCO_2 \sim 0.112$  kPa) at two temperatures (4 and 10 °C), starting with *N* = 20

(mean ± SD,  $F_{2,45} = 0.539$ ;  $p = 0.587$ ). Muscle indices of scallops after incubation did not differ between groups with  $3.66 \pm 0.44$  (4 °C normocapnia, *N* = 8),  $3.34 \pm 0.42$  (10 °C normocapnia, *N* = 19) and  $3.61 \pm 0.42$  (10 °C hypercapnia, *N* = 19) (mean ± SD,  $F_{2,45} = 2.417$ ;  $p = 0.1012$ ). Despite similar condition and muscle indices between the two temperature groups, we excluded both normocapnia- and hypercapnia-exposed scallops at 4 °C from further experimentation due to their stress level at rest as indicated by their high mortality.

### Haemolymph acid–base parameters

Following OA exposure at 10 °C, scallops displayed significantly elevated  $P_eCO_2$  ( $F_{1,37} = 84.425$ ,  $p < 0.001$ ),  $C_eCO_2$  ( $F_{1,36} = 42.403$ ,  $p < 0.001$ ) and  $[HCO_3^-]_e$  ( $F_{1,35} = 27.039$ ,  $p < 0.001$ ) and significantly lowered  $pH_e$  ( $F_{1,36} = 61.861$ ,  $p < 0.001$ ) values in both groups, resting and fatigued animals (Table 2). After exhaustive exercise, haemolymph values changed in a similar manner irrespective of ambient CO<sub>2</sub> level, resulting in significantly lowered  $P_eO_2$  ( $F_{1,36} = 28.385$ ,  $p < 0.001$ ) and  $pH_e$  ( $F_{1,36} = 15.652$ ,  $p < 0.001$ ) and significantly elevated  $P_eCO_2$  ( $F_{1,37} = 35.293$ ,  $p < 0.001$ ),  $C_eCO_2$  ( $F_{1,36} = 37.968$ ,  $p < 0.001$ ) and  $[HCO_3^-]_e$  ( $F_{1,35} = 23.730$ ,  $p < 0.001$ ) in

**Table 2** Haemolymph parameters of *P. maximus* at rest and after exercise following long-term incubation under normocapnia (seawater  $PCO_2 \sim 0.040$  kPa) and hypercapnia (seawater  $PCO_2 \sim 0.112$  kPa) at 10 °C

Parameter	Normocapnia	Hypercapnia
<b>RMR</b>		
$P_eO_2$ [kPa]	6.52 ± 1.13	7.95 ± 2.47
$P_eCO_2$ [kPa]	0.13 ± 0.02	0.26 ± 0.06*
$pH_e$ (NBS scale)	7.67 ± 0.06	7.42 ± 0.09*
$C_eCO_2$ [mM]	1.87 ± 0.15	2.11 ± 0.08*
$[HCO_3^-]_e$ [mM]	1.81 ± 0.15	1.98 ± 0.06*
<b>MMR</b>		
$P_eO_2$ [kPa]	3.71 ± 1.06 <sup>+</sup>	4.05 ± 0.58 <sup>+</sup>
$P_eCO_2$ [kPa]	0.20 ± 0.05 <sup>+</sup>	0.43 ± 0.10* <sup>+</sup>
$pH_e$ (NBS scale)	7.54 ± 0.10 <sup>+</sup>	7.32 ± 0.09* <sup>+</sup>
$C_eCO_2$ [mM]	2.10 ± 0.09 <sup>+</sup>	2.45 ± 0.16* <sup>+</sup>
$[HCO_3^-]_e$ [mM]	1.96 ± 0.05	2.25 ± 0.13* <sup>+</sup>

Data are mean ± SD with *N* = 13–15 (RMR) and *N* = 4–5 (MMR) *RMR* resting metabolic rate, *MMR* maximal metabolic rate,  $P_eO_2/P_eCO_2$  extracellular partial pressure of O<sub>2</sub>/CO<sub>2</sub>,  $pH_e$  extracellular pH, *NBS* National Bureau of Standards,  $C_eCO_2$  extracellular total dissolved inorganic carbon,  $[HCO_3^-]_e$  extracellular bicarbonate concentration

\* Significant differences between normocapnic and hypercapnic data at same metabolic rate

<sup>+</sup> Significant differences between RMR and MMR at same CO<sub>2</sub> levels

fatigued compared with resting animals in both normocapnia and hypercapnia groups (Table 2).

### Clapping performance and metabolic rate

OA exposure at 10 °C had no impact on clapping numbers ( $F_{13,11} = 2.512$ ,  $t_{1,24} = 0.0683$ ,  $p = 0.9461$ ), but a strong effect on force production resulting in significantly lowered total force, mean phasic force and tonic force values ( $F_{total}$ :  $F_{12,13} = 2.788$ ,  $t_{1,25} = 4.776$ ,  $p < 0.0001$ ;  $F_{mean\ phasic}$ :  $F_{11,11} = 1.626$ ,  $t_{1,22} = 3.976$ ,  $p = 0.0006$ ;  $F_{tonic}$ :  $F_{12,13} = 2.818$ ,  $t_{1,25} = 4.758$ ,  $p < 0.0001$ ) (Fig. 3). Recovery period and the time until scallops were fatigued were more or less similar in normocapnic and hypercapnic animals.

OA exposure at 10 °C had no effect on resting metabolic rate (RMR) with medians of  $5.10\ \mu\text{mol O}_2\ \text{h}^{-1}\ \text{gDW}^{-1}$  (normocapnia) and  $5.27\ \mu\text{mol O}_2\ \text{h}^{-1}\ \text{gDW}^{-1}$  (hypercapnia) (Fig. 4a). After exercise maximal metabolic rate (MMR) was significantly increased above that of the resting animals in both normocapnia- and hypercapnia-exposed scallops ( $F_{1,40} = 145.503$ ,  $p < 0.001$ ; Fig. 4a). However, the exercise-induced increase was lower in the hypercapnia group resulting in a significantly lower MMR ( $F_{1,40} = 4.396$ ,  $p < 0.05$ ) and net aerobic scope (NAS, Fig. 4b) in hypercapnia- than in normocapnia-exposed animals ( $F_{8,9} = 1.937$ ,  $t_{1,17} = 2.359$ ,  $p = 0.0305$ ).

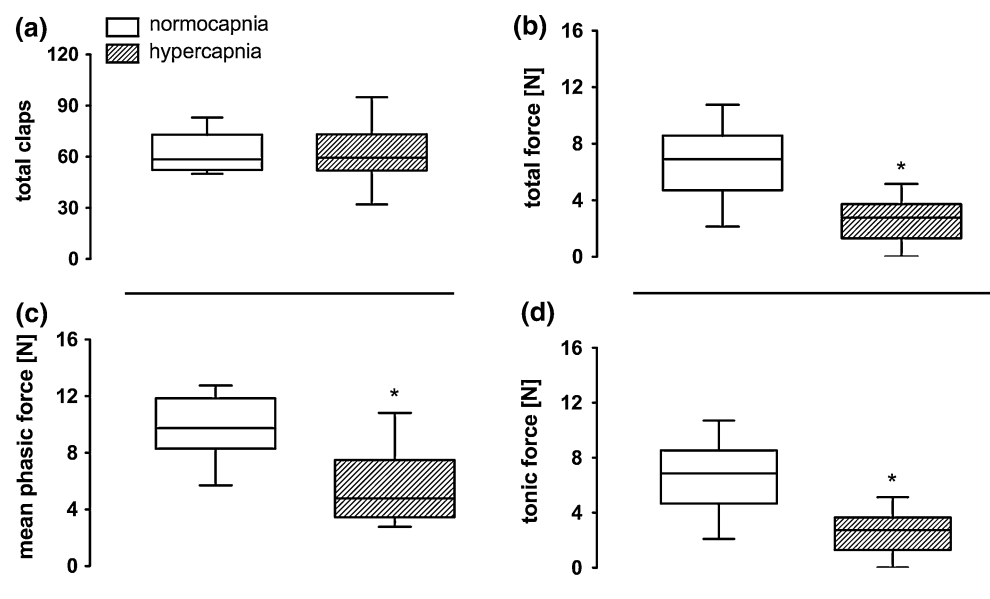
## Discussion

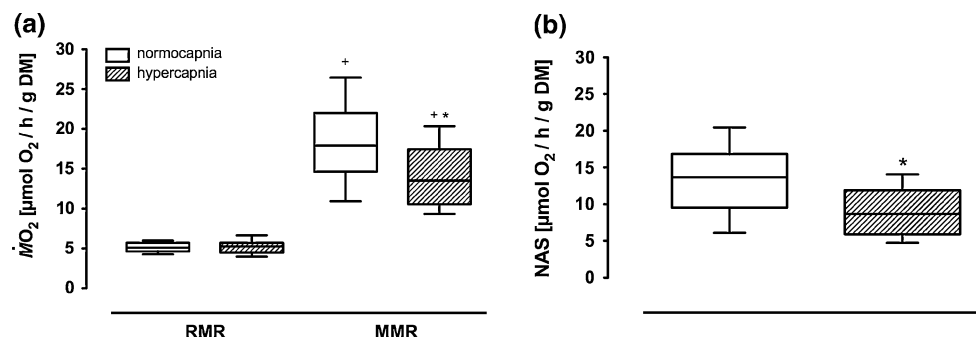
### Mortality

In contrast to incubation experiments at 10 °C, we observed a high mortality among scallops exposed to 4 °C

(55 % under normocapnia, 90 % under hypercapnia) (see Fig. 2). This was unexpected as animals had been reared at their winter environmental temperatures of 3–5 °C (see “Materials and methods”). As water quality (e.g. ammonium/nitrite levels) was similar between incubations, we assume that the high mortality at 4 °C is related to a time-dependent effect: By keeping the animals continually at 4 °C, “physiological wintertime” may have been over-extended resulting in enhanced mortality. Against the background of the OCLTT concept, these animals may have been at the lower end of their thermal window too long. Factors reflecting insufficient functional capacity that might have decreased fitness and survival rate in the cold include insufficient food uptake and digestion capacities in the cold as enzymes operate below the thermal optimum (Brock et al. 1986), and/or ciliate beat frequency may be insufficient (Riisgård and Larsen 2007). However, we observed no difference in condition or muscle indices between 4 °C- and 10 °C-exposed scallops, and our overall means of condition index ( $11.48 \pm 1.31$ ,  $N = 46$ ) and muscle index ( $3.54 \pm 0.48$ ,  $N = 46$ ) are comparable to literature data of *P. maximus* showing CIs of 6–10 and MIs of 3–6 in positively growing specimens (Pazos et al. 1997, CI was calculated from reported tissue and shell weights). Similar CI values between 10 and 25 are reported for juvenile bay scallops *Argopecten irradians* (Shriver et al. 2002). Specific reasons causing enhanced mortality and involving OCLTT capacity limitation (e.g. circulatory limitation) thus remain to be identified. In line with earlier findings on the warm side of the thermal tolerance window (see “Introduction”), our study indicates that OA exposure may have shifted the animals from pejus further to the critical range at the cold side of the thermal tolerance window.

**Fig. 3** Data for clapping performance of *P. maximus* after long-term incubation under normocapnia (seawater  $\text{PCO}_2 \sim 0.040\ \text{kPa}$ ) and hypercapnia (seawater  $\text{PCO}_2 \sim 0.112\ \text{kPa}$ ) at 10 °C. **a** Total number of claps until fatigue ( $N = 12$ –14). **b** Total force ( $N = 13$ –14). **c** Mean phasic force ( $N = 12$ ). **d** Tonic force ( $N = 13$ –14). Data are depicted in boxplots; asterisk significant differences between normocapnic and hypercapnic data





**Fig. 4** Oxygen consumption ( $\dot{M}_{O_2}$ ) of *P. maximus* after long-term incubation under normocapnia (seawater  $P_{\text{CO}_2} \sim 0.040$  kPa) and hypercapnia (seawater  $P_{\text{CO}_2} \sim 0.112$  kPa) at 10 °C. **a** Oxygen consumption at rest (RMR) and after exercise (MMR) ( $N = 9\text{--}13$ ).

**b** Net aerobic scope (NAS;  $N = 9\text{--}10$ ). Data are depicted in boxplots; asterisks significant differences between normocapnic and hypercapnic data. +significant differences between RMR and MMR

The hypothesis of cold limited tolerance is further supported by observations by Strand and Brynjeldsen (2003) who reported an extraordinarily high mortality rate (52–100 %) among juvenile *P. maximus* along the coast of Norway during an abnormally cold winter (1995/6) at temperatures between 2 and 4 °C. The normal temperature profile experienced by scallops from the Stavanger region since 2000 usually yielded monthly means of 5 °C at a depth of around 20 m, and during that whole period, there was only 1 month with a mean below 5 °C (Institute of Marine Research, Norway 2012). Furthermore, scallops survived well for more than 10 months in our aquarium system at 5 °C supporting our hypothesis that *P. maximus* may have reached its lower thermal limit at around 4 °C leading to an increase in mortality.

#### Haemolymph acid–base parameters

Scallops under hypercapnia had a lower  $\text{pH}_e$ , a higher  $P_e\text{CO}_2$ , bicarbonate and  $\text{C}_e\text{CO}_2$  level than normocapnic animals, indicating that *P. maximus* did not compensate for the extracellular acidosis under long-term OA exposure. As already shown in other studies (e.g. Lindinger et al. 1984; Walsh et al. 1984; Michaelidis et al. 2005; Melzner et al. 2009), the capacity for extracellular pH regulation is low in bivalves, including scallops. Similar to oysters (Lannig et al. 2010), scallops showed a significant, albeit small elevation in bicarbonate concentrations under hypercapnia. This indicates no or only a small degree of compensation of the acid–base disturbance in the haemolymph. Passive compensation depends on the level of non-bicarbonate buffer value that was not determined. Other bivalves, for example, *Mytilus edulis*, have haemolymph non-bicarbonate buffer lines ( $\beta_{\text{NB}} = 0.4 \text{ mmol L}^{-1} \text{ pH}^{-1}$ , Booth et al. 1984) similar to seawater ( $0.3 \text{ mmol L}^{-1} \text{ pH}^{-1}$ ). It seems that in bivalves in general, a  $\text{CO}_2$  induced acidosis remains largely uncompensated, due to low buffering and limited capacity of proton equivalent ion exchange.

Exhaustive exercise at 10 °C significantly affected *P. maximus* haemolymph parameters (see Table 2). Under both normocapnic and hypercapnic conditions, values of  $\text{pH}_e$  and  $P_e\text{O}_2$  of the fatigued scallops decreased, while  $P_e\text{CO}_2$ ,  $\text{C}_e\text{CO}_2$  and bicarbonate levels increased compared with findings in resting scallops. The lack of respiratory pigments, the poor perfusion of the adductor muscle and its low mitochondrial density reflect insufficient supply and use of oxygen by the phasic adductor muscle that largely operates anaerobically during swimming (de Zwaan et al. 1980; Thompson et al. 1980). Exercising scallops mainly catabolize phospho-L-arginine and then glycogen stored in their adductor muscle (e.g. *Argopecten irradians* can use about 23–25 % of the glycogen stored in the adductor muscle, Epp et al. 1988). Glycolysis leads to octopine formation, largely during recovery after swimming (Grieshaber and Gäde 1977; Gäde et al. 1978; Chih and Ellington 1983); succinate formation may result from oxygen deficiency at mitochondrial level. In fact,  $P_e\text{O}_2$  remains low during recovery, such that scallops initially recover under hypoxic conditions, associated with a rise in  $P_e\text{CO}_2$  due to insufficient ventilation (MacDonald et al. 2006). An acidosis would thus result from respiratory  $\text{CO}_2$  accumulation and non-respiratory proton release during anaerobic end product formation in excess of the proton consumption by phospho-L-arginine degradation (Pörtner 1987). The rise in metabolic rate after exhaustive exercise in scallops (see Fig. 4) is due to increased energy demands during glycogen resynthesis and involves the oxidative degradation of anaerobic end products like D-octopine (MacDonald et al. 2006).

#### Clapping performance and metabolic rate

Present data of around 62 claps until fatigue (independent of  $\text{CO}_2$  level) fit very well with the 57 claps obtained by Bailey et al. (2003) for *P. maximus* at 12 °C. As shown by the same study, great differences in clapping performance



exist between scallop species, regardless of temperature. The authors compared the number of claps until exhaustion of three species, the Antarctic scallop, *Adamussium colbecki*, and two temperate species, *Aequipecten opercularis* and *P. maximus*. Each species was measured at its respective habitat temperature and the results varied between 24 claps (*A. opercularis*, 12 °C), 48 claps (*A. colbecki*, 0 °C) and the mentioned 57 claps (*P. maximus*, 12 °C). Fleury et al. (2005) elicited lower values of around 19 claps in the scallop *Placopecten magellanicus* at 9.5 °C, upon stimulation with starfish for 200 s. However, animals from that study were not taken to fatigue likely leading to an underestimation of the maximum possible clap numbers. Furthermore, the time of collection (season) seems to affect maximal clap numbers as shown by Guderley et al. (2009) where total clap numbers of *P. magellanicus* varied between 23 claps (spring) and 40 claps (autumn), both measured at 12 °C (close to habitat temperatures).

Our results on clapping performance and aerobic scope indicate an energetic trade-off under OA conditions at the expense of the scope of escape response. Although the number of claps was similar between normocapnic and hypercapnic animals at 10 °C, reduced force capacities in hypercapnic scallops revealed a significantly negative effect of OA exposure (see Fig. 3). The diminished activation of muscle fibres following OA exposure may indicate that ATP-consuming processes were slowed and energy demand reduced, which resulted in a weakened escape response. This effect may be mediated through the lowering of extracellular pH. In muscle tissues of marine invertebrates, extracellular acidosis slows ion exchange and acid–base regulation (Pörtner et al. 2000). The extracellular acidosis thereby mediates a slowing of myosin ATPase and the development of contractile force in muscle tissue (cf. Pörtner 2008). While oxygen consumption during rest (RMR) did not differ between normocapnia- and hypercapnia-exposed scallops at 10 °C, oxygen consumption following exhaustive exercise (MMR) was reduced in hypercapnic compared with normocapnic animals resulting in a significantly reduced net aerobic scope (NAS) by a factor of 1.49 in OA-exposed scallops (see Fig. 4).

The increase in oxygen consumption during exercise indicates enhanced aerobic energy provision on top of anaerobic metabolism. In sessile bivalves, feeding led to the highest oxygen consumption rates (Tremblay et al. 1998). Mackay and Shumway (1980) showed for the pectinid *Chlamys delicatula* that exercise (escape response) resulted in an even higher postexercise oxygen consumption rate than the rate seen during feeding. Therefore, net aerobic scope (NAS) calculated as the difference between oxygen consumption at rest and after exhaustive exercise can be assumed as the maximal NAS for scallops.

Limitations in functional capacity may be reached when cellular energy levels fall. According to Pörtner et al. (2004), OA-exposed animals would need to invest more energy into acid–base regulation. Our findings of reduced net aerobic scope indicate that OA-exposed animals at 10 °C reallocated available energy to, for example, ion regulation but at the expense of other processes, for example, activity. In the study of Wood et al. (2008), calcification rates and respiration rates of the brittlestar *Amphiura filiformis* exposed to hypercapnia were increased, but muscle mass was reduced instead. This “muscle wastage” was seen as a fast and time limited trade-off between structure (morphological integrity) and function (arm movement). In salmon and oysters, exposure to low pH or elevated  $PCO_2$  led to a partial depletion of tissue energy reserves such as glycogen and lipids (Haya et al. 1985; Lannig et al. 2010; Dickinson et al. 2012).

## Conclusion

Our study revealed that ocean acidification narrows the scope for exercise performance of the active calcifier, *Pecten maximus*. At elevated  $CO_2$  levels, clapping force was reduced indicating that this species might become vulnerable to predators as their escape response is weakened following exposure to predicted OA scenarios. Our results seen together with those of other studies indicate that OA-induced energetic trade-offs exist, reducing the energy available for fitness-related processes such as locomotion, growth and/or stress resistance. The increased mortality among scallops exposed to 4 °C and elevated  $CO_2$  levels implies an OA-induced exacerbation of cold stress, in line with a narrowing of the thermal tolerance range at the cold side. Further investigations at the cellular level are necessary to examine the allocation of metabolic power to different processes and to fully unravel the picture of OA-induced impacts on energy metabolism and associated energy reallocations.

**Acknowledgments** We would like to thank Øivind Strand and the aquaculture Kvitsøy Edelskjell AS for their support in animal supply. We also gratefully acknowledge the support of M. Bullwinkel, N. Klassen and C. Otten, who assisted in animal care and water analysis during the incubation experiments. We thank O. Heilmayer for helpful discussion, Are Olsen for supporting information on  $PCO_2$  values around Stavanger and E. Schaum for language check. We thank the two anonymous reviewers and the editor, Sam Dupont, for their constructive comments on the manuscript. Burgel Schalkhauser was funded by the Bundesministerium für Bildung und Forschung (BMBF)-funded project “Biological Impacts of Ocean Acidification” (BIOACID, FKZ 03F0608B). The study is part of the “Polar regions and coasts in a changing Earth system” (PACES) research programme of the Alfred Wegener Institute for Polar and Marine Research.

## References

- Ansell A, Dao JC, Manson J (1991) Three European scallops: *Pecten maximus*, *Chlamys (Aequipecten) opercularis* and *C. (Chlamys) varia*. In: Shumway SE (ed) *Scallops: biology, ecology and aquaculture*, 1st edn. Elsevier, Amsterdam, pp 715–752
- Bailey DM, Peck LS, Bock C, Pörtner HO (2003) High-energy phosphate metabolism during exercise and recovery in temperate and Antarctic scallops: an in vivo  $^{31}\text{P}$ -NMR study. *Physiol Biochem Zool* 76(5):622–633. doi:10.1086/376920
- Bailey DM, Johnston IA, Peck LS (2005) Invertebrate muscle performance at high latitude: swimming activity in the Antarctic scallop, *Adamussium colbecki*. *Polar Biol* 28:464–469. doi:10.1007/s00300-004-0699-9
- Beniash E, Ivanina A, Lieb NS, Kurochkin I, Sokolova IM (2010) Elevated levels of carbon dioxide affect metabolism and shell formation in oysters *Crassostrea virginica*. *Mar Ecol Prog Ser* 419:95–108
- Booth CE, 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
- Brand AR (2006) Scallop ecology: distributions and behaviour. In: Shumway SE, Parson GJ (eds) *Scallops: biology ecology and aquaculture*, 1st edn. Elsevier, Amsterdam, pp 651–744. doi:10.1016/S0167-9309(06)80034-7
- Brock V, Kennedy VS, Brock A (1986) Temperature dependency of carbohydrase activity in the hepatopancreas of thirteen estuarine and coastal bivalve species from the North American east coast. *J Exp Mar Biol Ecol* 103:87–101
- Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. *Nature* 425:365
- Chih CP, Ellington WR (1983) Energy metabolism during contractile activity and environmental hypoxia in the adductor muscle of the Bay Scallop *Argopecten irradians concentricus*. *Physiol Zool* 56(4):623–631
- de Zwaan A, Thompson RJ, Livingston DR (1980) Physiological and biochemical aspects of the valve snap and valve closure responses in the giant scallop *Placopecten magellanicus* II. *Biochemistry*. *J Comp Physiol B* 137:105–114
- Denny M, Miller L (2006) Jet propulsion in the cold: mechanics of swimming in the Antarctic scallop *Adamussium colbecki*. *J Exp Biol* 209:4503–4514. doi:10.1242/jeb.02538
- Dickinson GH, Ivanina AV, Matoo OB, Pörtner HO, Lannig G, Bock C, Beniash E, Sokolova IM (2012) Interactive effects of salinity and elevated  $\text{CO}_2$  levels on juvenile eastern oysters, *Crassostrea virginica*. *J Exp Biol* 215:29–43. doi:10.1242/jeb.061481
- Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Res* 34(11):1733–1743
- Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other  $\text{CO}_2$  problem. *Annu Rev Mar Sci* 1:169–192
- Epp J, Bricelj VM, Malouf RE (1988) Seasonal partitioning and utilization of energy reserves in two age classes of the bay scallop *Argopecten irradians irradians* (Lamarck). *J Exp Mar Biol Ecol* 121:113–136
- 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, Kendall MA, Spicer JI, Widdicombe S (2010) Relative influence of ocean acidification and temperature on intertidal post-larvae at the northern edge of their geographic distribution. *Estuar Coast Shelf Sci* 86:675–682
- Fleury P-G, Janssoone X, Nadeau M, Guderley H (2005) Force production during escape responses: sequential recruitment of the phasic and tonic portions of the adductor muscle in juvenile sea scallop, *Placopecten magellanicus* (Gmelin). *J Shellfish Res* 24(4):905–911. doi:10.2983/0730-8000(2005)24[905:FPDERS]2.0.CO;2
- Fry FE (1947) Effects of the environment on animal activity. *Univ Toronto Biol Ser* 55. *Pub Ont Fish Res Lab* 68:1–62
- Gäde G, Weeda E, Gabbott PA (1978) Changes in the level of octopine during the escape responses of the scallop, *Pecten maximus* (L.). *J Comp Physiol* 124:121–127
- Grieshaber M, Gäde G (1977) Energy supply and the formation of octopine in the adductor muscle of the scallop, *Pecten jacobaeus* (Lamarck). *Comp Biochem Physiol B* 58:249–252
- Gruffydd LD (1976) Swimming in *Chlamys islandica* in relation to current speed and an investigation of hydrodynamic lift in this and other scallops. *Nor J Zool* 24:365–378
- Guderley H, Pörtner HO (2010) Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. *Can J Zool* 88:753–763
- Guderley H, Labbé-Giguere S, Janssoone X, Bourgeois M, Pérez HM, Tremblay I (2009) Thermal sensitivity of escape response performance by the scallop *Placopecten magellanicus*: impact of environmental history. *J Exp Mar Biol Ecol* 377:113–119
- Haya K, Waiwood BA, van Eeckhaute L (1985) Disruption of energy metabolism and smoltification during exposure of juvenile Atlantic salmon (*Salmo salar*) to low pH. *Comp Biochem Physiol C* 82(2):323–329
- Heilmayer O, Brey T (2003) Saving by freezing? Metabolic rates of *Adamussium colbecki* in a latitudinal context. *Mar Biol* 143:477–484. doi:10.1007/s00227-003-1079-7
- IPCC (2007) *Climate Change 2007: the physical science basis. Summary for policymakers. Contribution of working group I to the fourth assessment report. The intergovernmental panel on climate change.* [www.ipcc.ch/SPM2feb07.pdf](http://www.ipcc.ch/SPM2feb07.pdf)
- 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
- Langenbuch M, Pörtner HO (2003) Energy budget of hepatocytes from Antarctic fish (*Pachycara brachycephalum* and *Lepidonotothen kempfi*) as a function of ambient  $\text{CO}_2$ : pH-dependent limitations of cellular protein biosynthesis? *J Exp Biol* 206:3895–3903
- Langenbuch M, Pörtner HO (2004) High sensitivity to chronically elevated  $\text{CO}_2$  levels in a eurybathic marine sipunculid. *Aquat Toxicol* 70:55–61. doi:10.1016/j.aquatox.2004.07.006
- 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. doi:10.3390/md8082318
- Lewis E, Wallace DWR (1998) *CO2SYS*-Program developed for the  $\text{CO}_2$  system calculations. Carbon dioxide information analysis center; Report ORNL/CDIAC-105, Oak Ridge, Tenn, USA
- Lindinger MI, Lawren DJ, McDonald DG (1984) Acid-base balance in the sea mussel *Mytilus edulis*. Effects of environmental hypercapnia on intra and extracellular acid-base balance. *Mar Biol Lett* 5:371–381
- MacDonald BA, Bricelj VM, Shumway SE (2006) Physiologie: energy acquisition and utilisation. In: Shumway SE, Parson GJ (eds) *Scallops: biology, ecology and aquaculture*, 1st edn. Elsevier, Amsterdam, pp 417–492. doi:10.1016/S0167-9309(06)80034-7
- Mackay J, Shumway SE (1980) Factors affecting oxygen consumption in the scallop *Chlamys deliculata* (Hutton). *Ophelia* 19:19–26
- Mehrbach C, Culbertson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic

- acid in seawater at atmospheric pressure. *Limnol Oceanogr* 18(6):897–907
- Melatunan S, Calosi P, Rundle SD, Moody AJ, Widdicombe S (2011) Exposure to elevated temperature and PCO<sub>2</sub> reduces respiration rate and energy status in the Periwinkle *Littorina littorea*. *Physiol Biochem Zool* 84(6):583–594. doi:10.1086/662680
- Melzner F, Gutowska MA, Langenbruch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner HO (2009) Physiological basis for high CO<sub>2</sub> 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 A (2011) Food supply and seawater pCO<sub>2</sub> impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS ONE* 6(9):e24223. doi:10.1371/journal.pone.0024223
- Metzger R, Sartoris F, Langenbuch M, Pörtner HO (2007) Influence of elevated CO<sub>2</sub> concentrations on thermal tolerance of the edible crab *Cancer pagurus*. *J Therm Biol* 32(3):144–151. doi:10.1016/j.jtherbio.2007.01.010
- Michaelidis B, Ouzounis C, Palaras 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
- Orr JC (2011) Recent and future changes in ocean carbonate chemistry. In: Gattuso J-P, Hansson L (eds) *Ocean acidification*. Oxford University Press, Oxford, pp 41–66
- Parker LM, Ross PM, O'Connor WA (2009) The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). *Glob Change Biol* 15:2123–2136. doi:10.1111/j.1365-2486.2009.01895.x
- Pazos AJ, Román G, Acosta CP, Abad M, Sánchez JL (1997) Seasonal changes in condition and biochemical composition of the scallop *Pecten maximus* L. from suspended culture in the Ria de Arousa (Galicia, N.W., Spain) in relation to environmental conditions. *J Exp Mar Biol Ecol* 211:169–193
- Pfeil B, Olsen A, Bakker DC et al. (2012) A uniform, quality controlled, Surface Ocean CO<sub>2</sub> Atlas (SOCAT). Earth system science data (in preparation). <http://www.socat.info/>. Accessed 29 Mar 2012
- Philipp EE, Schmidt M, Gsottbauer C, Sängler AM, Abele D (2008) Size- and age-dependent changes in adductor muscle swimming physiology of the scallop *Aequipecten opercularis*. *Mar Ecol Prog Ser* 389:193–202. doi:10.3354/meps08141
- Pörtner HO (1987) Contributions of anaerobic metabolism to pH regulation in animal tissues: theory. *J Exp Biol* 131:69–87
- Pörtner HO (2002) Environmental and functional limits to muscular exercise and body size in marine invertebrate athletes. *Comp Biochem Physiol A* 133:303–321
- Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Mar Ecol Prog Ser* 373:203–217. doi:10.3354/meps07768
- 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. doi:10.1242/jeb.037523
- 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*, 1st edn. Springer, Berlin, pp 443–458
- Pörtner HO, Farrell AP (2008) Physiology and climate change. *Science* 322:690–692
- Pörtner HO, Reipschläger A, Heisler N (1998) Metabolism and acid-base regulation in *Sipunculus nudus* as a function of ambient carbon dioxide. *J Exp Biol* 201:43–55
- Pörtner HO, Bock C, Reipschläger A (2000) Modulation of the cost of pH<sub>i</sub> regulation during metabolic depression: a <sup>31</sup>P-NMR study in invertebrate (*Sipunculus nudus*) isolated muscle. *J Exp Biol* 203:2417–2428
- Pörtner HO, Langenbuch M, Reipschläger A (2004) Biological impact of elevated ocean CO<sub>2</sub> concentrations: lessons from animal physiology and earth history. *J Oceanogr* 60:705–718
- Riisgård HU, Larsen PS (2007) Viscosity of seawater controls beat frequency of water-pumping cilia and filtration rate of mussels *Mytilus edulis*. *Mar Ecol Prog Ser* 343:141–150. doi:10.3354/meps06930
- Scheibling RE, Hatcher BG, Taylor L, Barbeau MA (1995) Seeding trial of the giant scallop (*Placopecten magellanicus*) in Nova Scotia. In: Lubet P, Barret J, Dao J-C (eds) *Fisheries, biology and aquaculture of Pectinids*. 8th International Pectinid Workshop, Cherbourg, France, 22nd–29th May, 1991, IFREMER, Actes de Colloques 17:123–129
- Shriver AC, Carmichael RH, Valiela I (2002) Growth, condition, reproductive potential, and mortality of bay scallops, *Argopecten irradians*, in response to eutrophic-driven changes in food resources. *J Exp Mar Biol Ecol* 279:21–40
- Sokolova IM, Frederick F, Bagwe R, Lannig G, Sukhotin AA (2012) Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar Environ Res* 79:1–15. doi:10.1016/j.marenvres.2012.04.003
- Strand Ø, Brynjeldsen E (2003) On the relationship between low winter temperatures and mortality of juvenile scallops, *Pecten maximus* L., cultured in western Norway. *Aquacult Res* 34:1417–1422
- Strand Ø, Nylund A (1991) The reproductive cycle of the scallop *Pecten maximus* (L.) from two populations in Western-Norway, 60 N and 64 N. In: Shumway SE (ed) *An international compendium of scallop biology and culture*. Special Publication, World Aquaculture Society, Baton Rouge, pp 95–105
- Stumpp M, Wren J, Melzner F, Thorndyke MC, Dupont S (2011) CO<sub>2</sub> induced acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. *Comp Biochem Physiol A* 160:331–340. doi:10.1016/j.cbpa.2011.06.022
- Thompson RJ, Livingstone DR, de Zwaan A (1980) Physiological and biochemical aspects of the valve snap and valve closure responses in the giant scallop *Placopecten magellanicus* I. *Physiology*. *J Comp Physiol* 137:97–104
- Tremblay R, Myrand B, Guderley H (1998) Thermal sensitivity of organismal and mitochondrial oxygen consumption in relation to susceptibility of blue mussels, *Mytilus edulis* (L.), to summer mortality. *J Shellfish Res* 17:141–152
- Tremblay I, Guderley HE, Fréchette M (2006) Swimming performance, metabolic rates, and their correlates in the Iceland scallop *Chlamys islandica*. *Physiol Biochem Zool* 79(6):1046–1057
- Walsh PJ, McDonald G, Booth CE (1984) Acid-base balance in the sea mussel, *Mytilus edulis*. II. Effects of hypoxia and air-exposure on the 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
- Weiss RF (1974) Carbon dioxide in water and seawater: the solubility of a non-ideal gas. *Mar Chem* 2:203–215
- Wiborg KF (1963) Some observations on the Iceland scallop *Chlamys islandica* (Müller) in Norwegian waters. *Fiskeridirektoratets Skrifter. Serie Havundersøkelser* 13:38–53
- Wieser W, Medgyesy N (1990) Cost and efficiency of growth in the larvae of two species of fish with widely differing metabolic rates. *Proc Biol Sci* 242(1303):51–56
- Wilkens LA (2006) Neurobiology and behaviour of the scallop. In: Shumway SE, Parson GJ (eds) *Scallops: biology, ecology and*

- aquaculture, 1st edn. Elsevier, Amsterdam, pp 317–356. doi: [10.1016/S0167-9309\(06\)80034-7](https://doi.org/10.1016/S0167-9309(06)80034-7)
- Winter MA, Hamilton PV (1985) Factors influencing swimming in Bay Scallops, *Argopecten irradians* (Lamarck, 1819). J Exp Mar Biol Ecol 88:227–242
- Wood H, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. Proc R Soc Lond B 275:1767–1773
- Zittier ZM, Hirse T, Pörtner HO (2012) The synergistic effects of increasing temperature and CO<sub>2</sub> levels on activity capacity and acid-base balance in the spider crab, *Hyas araneus*. Mar Biol. doi:[10.1007/s00227-012-2073-8](https://doi.org/10.1007/s00227-012-2073-8)