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Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?

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Abstract. Future ocean acidification has the potential to adversely affect many marine organisms. A growing body of evidence suggests that many species could suffer from reduced fertilization success, decreases in larval- and adult growth rates, reduced calcification rates, and even mortality when being exposed to near-future levels (year 2100 scenarios) of ocean acidification. Little research focus is currently placed on those organisms/taxa that might be less vulnerable to the anticipated changes in ocean chemistry; this is unfortunate, as the comparison of more vulnerable to more tolerant physiotypes could provide us with those physiological traits that are crucial for ecological success in a future ocean. Here, we attempt to summarize some ontogenetic and lifestyle traits that lead to an increased tolerance towards high environmental pCO_2. In general, marine ectothermic metazoans with an extensive extracellular fluid volume may be less vulnerable to future acidification as their cells are already exposed to much higher pCO_2 values (0.1 to 0.4 kPa, ca. 1000 to 3900 μatm) than those of unicellular organisms and gametes, for which the ocean (0.04 kPa, ca. 400 μatm) is the extracellular space. A doubling in environmental pCO_2 therefore only represents a 10% change in extracellular pCO_2 in some marine teleosts. High extracellular pCO_2 values are to some degree related to high metabolic rates, as diffusion gradients need to be high in order to excrete an amount of CO_2 that is directly proportional to the amount of O_2 consumed. In active metazoans, such as teleost fish, cephalopods and many brachyuran crustaceans, exercise induced increases in metabolic rate require an efficient ion-regulatory machinery for CO_2 excretion and acid-base regulation, especially when anaerobic metabolism is involved and metabolic protons leak into the extracellular space. These ion-transport systems, which are located in highly developed gill epithelia, form the basis for efficient compensation of pH disturbances during exposure to elevated environmental pCO_2. Compensation of extracellular acid-base status in turn may be important in avoiding metabolic depression. So far, maintained "performance" at higher seawater pCO_2 ($>$0.3 to 0.6 kPa) has only been observed in adults/juveniles of active, high metabolic species with a powerful ion regulatory apparatus. However, while some of these taxa are adapted to cope with elevated pCO_2 during their regular embryonic development, gametes, zygotes and early embryonic stages, which lack specialized ion-regulatory epithelia, may be the true bottleneck for ecological success – even of the more tolerant taxa.

Our current understanding of which marine animal taxa will be affected adversely in their physiological and ecological fitness by projected scenarios of anthropogenic ocean acidification is quite incomplete. While a growing amount of empirical evidence from CO_2 perturbation experiments suggests that several taxa might react quite sensitively to ocean acidification, others seem to be surprisingly tolerant. However, there is little mechanistic understanding on what physiological traits are responsible for the observed differential sensitivities (see reviews of Seibel and Walsh, 2003; Pörtner et al., 2004; Fabry et al., 2008; Pörtner, 2008). This leads us to the first very basic question of how to define general CO_2 tolerance on the species level.

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1 Defining tolerance towards elevated seawater pCO_2

When trying to classify marine organisms into CO_2 sensitive and CO_2 tolerant groups, we encounter a major complication: Projected ocean acidification progresses at a rate much too slow to be simulated in the laboratory, and differences in genetic adaptation potential vary at orders of magnitude between taxa. Organisms with a high generation turnover time (e.g. bacteria, unicellular auto- and heterotrophs) will have time for thousands of generations to select for genotypes that can cope with an ocean characterized by pCO_2 values of up to ca. 0.2 kPa (ca. 2000 μatm) by the year 2300 (Caldeira and Wickett, 2003, 2005), while in long-lived species, such as the ocean quahog (*Arctica islandica; mollusca: bivalvia*), with a maximum life expectancy of ca. 400 years (Abele et al., 2008), today's genotypes may be exposed to the high pCO_2 values of the year 2300. Thus, species longevity/generation time is a crucial factor that could possibly determine future success in an – on evolutionary timescales – rapidly changing habitat.

Multi-generation experiments will be very important to understand the adaptation potential of a given species, however, such approaches are only beginning to emerge (Kurihara and Ishimatsu, 2008; Dupont and Thorndyke, 2009), especially in metazoans with long generation cycles (months, years). Thus, by reading the ideas we propose in this concept paper we should keep in mind the problems of rate of change in ocean carbonate chemistry and the genetic adaptation potential of a given species. In addition, the largely unexplored problems of species interaction and food-web feedbacks will be major factors shaping ecological performance of marine species in a future high pCO_2 ocean. For example, delayed larval development, as observed in echinoderm early life stages subjected to elevated pCO_2 (Dupont and Thorndyke, 2009), probably will increase predation related mortality in the field, even if there is no mortality difference in experimental cultures (Elkin and Marshall, 2007).

Considering the limited availability of multi-generation experiments, the best we can do at the moment to define tolerance versus sensitivity to ocean acidification, is to look at indicators for animal performance during long-term (weeks to months) CO_2 perturbation experiments. We use the term "animal performance" as the sum of the major relevant traits that ensure ecological success of a species (on a species level), i.e. among others, aerobic scope, locomotory scope, reproductive output, calcification and somatic growth, which, together, influence animal fitness. Aerobic metabolic scope (the difference between active and standard metabolic rates, see Fry, 1948, for a definition) is a parameter that can be (more or less) easily assessed in mobile animals, e.g. crustaceans, cephalopods or fish (e.g. Booth et al., 1984a; Wells and Wells, 1985; Pörtner et al., 1991; Melzner et al., 2009), whereas it can only be approximated in sessile animals, sometimes via the specific dynamic action of food (e.g. Vahl, 1984; Widdows, 1973). Aerobic metabolic scope of a species is also often directly related to locomotory scope and growth performance. While the measurement of standard metabolic rates can potentially indicate how the costs for homeostatic regulation are altered under an acute abiotic stress regime, somatic and reproductive growth performance can integrate cost re-allocation over a longer time interval. Thus, "footprints" in the energy budget in response to an abiotic stressor regime can be detected more easily in long-term growth trials. While somatic/reproductive growth may be one of the best performance indicators, it has already become clear that in order to consider possible trade-offs between single parameters all relevant indicators have to be included simultaneously to generate a meaningful assessment of a given species' vulnerability to future ocean acidification (e.g. see below, Wood et al., 2008; Kurihara et al., 2008). Unfortunately, to date there are few comprehensive performance assessments for marine metazoan species subjected to long-term elevated pCO_2. Thus, in the following text, we will place emphasis on those taxa where most information is available, hoping that future studies will focus on the simultaneous assessment of multiple performance indicators in long-term CO_2 perturbation experiments. The aim of the present review paper is thus not to compare single parameters between different species but to pool data on higher taxonomic levels to improve our understanding of major physiological characteristics that provide the basis for a high degree of CO_2 tolerance. While it is clear already now that due to the synergistic effects of a complex set of parameters CO_2 tolerance at near-future levels of ocean acidification is difficult to predict, even for closely related species (e.g. echinoderm larval stages: Dupont and Thorndyke, 2009; Widdicombe and Spicer, 2008), we will make use of those studies that have used higher pCO_2 values ($>$0.3 to 0.5 kPa) to elucidate some fundamental tolerance mechanisms that are closely related to lifestyle and metabolic rates of more active taxa.

2 Sensitive vs. tolerant physiotypes: which taxa perform best?

If we combine evidence from the few long-term CO_2 perturbation experiments (weeks to months) until now, it appears that (adult) marine ectothermic vertebrates are the most CO_2-tolerant group – various performance parameters seem not to be compromised by chronic hypercapnia at levels $>$0.3 to 0.6 kPa. Teleost species studied in long-term growth trials (wolffish, *Anarhichas minor*; salmon, *Salmo salar*) did not display reductions in somatic growth performance when exposed to pCO_2 values of up to 0.6 kPa and higher (5900 μatm; Foss et al., 2003; Fivelstad et al., 1998, 2003). In addition, recent findings indicate that long-term acclimation of Atlantic cod (*Gadus morhua*) to pCO_2 values of 0.3 and 0.6 kPa (ca. 3000 to 5900 μatm) does not seem to impact swimming performance (critical swimming speed, U_{crit}), standard and active metabolism, as well as aerobic scope

Table 1. Impact of CO_2 exposure on various physiological performance indicators like metabolic rate, acid-base regulation, growth and calcification at high seawater pCO_2 values >0.5 kPa (ca. 4900 μatm). The table gives an overview on effects assessed in marine taxa of different hypercapnia tolerance; references are noted in parenthesis. Note the scarce knowledge in specific areas (active metabolic rate under hypercapnia) and organism groups (brachyuran crabs). Active bicarbonate accumulation excludes cases where ions most probably stem from passive shell dissolution and subsequent enrichment in a closed system (e.g. in bivalves). Cited references are: (1) Fivelstad et al., 2003, (2) Foss et al., 2003, (3) Melzner et al., 2009, (4) Larsen et al., 1997, (5) Michaelidis et al., 2007, (6) Truchot, 1979, (7) Pane and Barry, 2007, (8) Spicer et al., 2007, (9) Gutowska et al., 2008, (10) Gutowska et al., submitted, 2009, (11) Siikavuopio et al., 2007, (12) Kurihara and Shirayama, 2004, (13) Dupont et al., 2008, (14) Miles et al., 2007, (15) Michaelidis et al., 2005, (16) Gazeau et al., 2007, (17) Booth et al., 1984/Lindinger et al., 1984.

	Somatic Growth	Rate of calcification	Standard/routine metabolic rate (SMR/RMR)	Active metabolic rate (AMR)	active extracellular pH compensation/ (HCO_3^-) accumulation
teleost fish	o (1,2)	?	o (3)	o (3)	+ (4,5)
brachyuran crustacea	?	?	?	?	+ (6,7,8)
cephalopoda	o (9)	o/+ (9)	o (9)	?	+ (10)
echinodermata	– (11)	– (12, 13)	?	?	– (14)
bivalvia	– (15)	– (15,16)	– (15)	?	– (15,17)

o/+/–= measured values or rates remain constant /increase /decrease;
?= no data available.

(Melzner et al., 2009). In contrast, marine invertebrates generally seem less tolerant at high levels of hypercapnia. Several studies have documented decreased growth and/or calcification rates in long-term exposure studies, e.g. in mussels (Michaelidis et al., 2005), echinoderms (Siikavuopio et al., 2007), coral reef communities and individual coral species (Langdon et al., 2000; see review by Hoegh-Guldberg et al., 2007), at levels that teleost fish are not affected by. In contrast to these invertebrates, the cephalopod *Sepia officinalis* is characterized by maintained somatic growth and slightly elevated calcification rates at pCO_2 values of 0.4 and 0.6 kPa (ca. 3900 to 5900 μatm; Gutowska et al., 2008), making it the only marine invertebrate species so far that to some degree approaches adult teleost performance standards (see Table 1). We suspect that shallow water brachyuran crustaceans could be another marine invertebrate taxon likely to approach teleost CO_2 tolerance, mainly due to their high ion-regulatory capacity (Wheatly and Henry, 1992). Unfortunately, no long-term growth and calcification experiments have been conducted using this group as a model to date.

Table 1 summarizes the effects of CO_2 exposure studied so far in representatives of the different marine taxa. Interestingly, a common feature of all more CO_2 tolerant species studied so far (again, at high pCO_2 values of >0.3 to 0.6 kPa) is their ability to perform a pH compensatory reaction to protect their extracellular fluids (blood, hemolymph) from excessive acidification. This might be a crucial trait, as it has been suggested that uncompensated extracellular pH is causally linked to metabolic depression in some of the more sensitive marine invertebrates (e.g. see Reipschläger and Pörtner, 1996; Pörtner et al., 2004; Michaelidis et al., 2005; Fabry et al., 2008). Metabolic depression, while beneficial during short-term abiotic stress (e.g. Guppy and Withers, 1999), would lead to long-term reductions in growth performance, aerobic and locomotory capacity, and thus, decreased ecological fitness in general (cf. Langenbuch and Pörtner, 2004). For our line of reasoning, it is thus quite important to fully understand the mechanisms leading to extracellular pH stabilization in these more tolerant organisms. If we speak of pH, to simplify matters, all data mentioned throughout the text, tables and figures refer to the NBS scale.

3 Mechanisms of extracellular pH regulation in tolerant vs. sensitive physiotypes

Buffering of free protons builds the first line of defence against CO_2 induced acidification of body fluids: The two buffering systems that are functional in all organisms studied so far are (I) the CO_2-bicarbonate system itself and (II) the so called non-bicarbonate buffering system. Unfortunately, the CO_2-bicarbonate system is of only small efficiency for buffering in marine animals. In response to high proton concentration the chemical equilibrium between the weak carbonic acid and bicarbonate leads to a rise in aqueous CO_2. In air breathers, the resulting higher pCO_2 is typically

eliminated by means of increased ventilation. However, this process is seriously impaired by the (comparatively) low pCO_2 values in body fluids of water breathers and the resulting very small diffusion gradients between organism and the surrounding water (see Heisler, 1986, for an extended discussion). Consequently, binding of respiratory protons (originating from CO_2 hydration) by so called non-bicarbonate buffers is the first step to minimize pH changes under acidified conditions. Non-bicarbonate buffering is mainly provided by partially protonated amino acid side chains (mostly from histidine or cysteine at physiological pH values), N-terminal α-amino groups of proteins or organic/inorganic phosphate groups. As buffering can only mask protons during an acidotic pH shift and thus reduce pH changes compared to a non-buffered system, surplus protons have to be eliminated to restore the original fluid pH. This can only be achieved by means of active ion transport across specialized epithelia, such as gills, renal or digestive tissue. Although the involved ion exchange mechanisms as a whole are poorly understood and may vary between different marine taxa, the processes contributing to pH compensation are summarized as proton equivalent ion exchange. Concerning the reduction of proton activity in body fluids, it is not important if a pH change is realized by higher proton excretion rates, rising bicarbonate import from the seawater or increased retention of metabolic bicarbonate.

A useful tool to visualize the correlation of the three acid-base parameters pH, pCO_2 and bicarbonate concentration for a specific physiological environment is the so called Davenport diagram (Fig. 1a, see figure caption and Davenport, 1974). All pCO_2 isopleths in such diagrams can be calculated with the help of the Henderson-Hasselbalch Eq. (1) from fixed pH and $[HCO_3^-]$ values, if the apparent dissociation constant of carbonic acid (pK'_1) and CO_2 solubility coefficient (α_{CO2}) for the particular fluid of interest (e.g. blood, hemolymph, coelomic fluid) are known (e.g. see Truchot, 1976, Heisler, 1986, Boutilier et al., 1984). When extracellular pCO_2 rises in vivo, extracellular pH decreases, while the increment in $[HCO_3^-]$ follows the non-bicarbonate buffer line (termed "respiratory acidosis", see Fig. 1a). This is due to the production of both, protons and $[HCO_3^-]$ during the CO_2 hydration reaction in the extracellular fluid when dissociating protons are largely bound to non-bicarbonate buffers, while bicarbonate remains. Thus, a slight increase in extracellular $[HCO_3^-]$ is caused by an increase in pCO_2. The magnitude of this buffering reaction is reflected in the slope of the non-bicarbonate buffer lines. These can be constructed from in vitro measurements by equilibrating samples of extracellular fluid with known pCO_2 to subsequently measure pH and $[HCO_3^-]$ (see Fig. 1). The negative slope of the non-bicarbonate buffer line, $\Delta[HCO_3^-]/-\Delta$pH, is typically called the non-bicarbonate buffer value (β_{NB}), expressed in $m\,Eq\,l^{-1}pH^{-1}$, or slykes. In molluscs, for example, extracellular β_{NB} values range from 0.4 to 0.6 slykes in bivalves (*Mytilus edulis*; Booth et al., 1984, Lindinger et al., 1984) to values of 3 to 10 slykes in cephalopods (Pörtner et al. 1991; Gutowska et al., 2009). Thus, an acute increase in hemolymph pCO_2 would lead to a much more pronounced decrease in extracellular pH in the bivalve vs. the cephalopod. Typically, β_{NB} is directly proportional to the protein concentration in the extracellular fluid (e.g. Truchot, 1976). The red and blue non-bicarbonate buffer lines in figure 1B approximate the conditions in bivalves and cephalopods (β_{NB} blue line = ca. 3 slykes, red line = ca. 0.4 slykes).

Whether an increase in extracellular fluid $[HCO_3^-]$ is due to buffering, or whether active proton equivalent transport processes are occurring, can be easily depicted from Davenport diagrams: If, under elevated pCO_2, pH and $[HCO_3^-]$ follow the course of the non-bicarbonate buffer line in vivo, then passive buffering prevails and no active bicarbonate accumulation is contributing to the observed increase in $[HCO_3^-]$. The red symbols in Fig. 1b illustrate such a case, which may be typical for certain echinoderms, bivalves or deep-sea crustaceans under hypercapnic conditions (Miles et al., 2007; Pane and Barry, 2007; Thomsen, 2008). In this hypothetic example, environmental hypercapnia of 0.5 kPa (ca. 4900 μatm) would lead to a hemolymph pCO_2 of 0.65 kPa (ca. 6500 μatm) and extracellular pH would drop dramatically, from 7.6 to 7.0 (note: extracellular pCO_2 is always higher than seawater pCO_2, see below). Figure 1b also illustrates cases, in which active transepithelial ion-exchange processes contribute to the increase in $[HCO_3^-]$. Upon acute exposure to a pCO_2 of 0.5 kPa (ca. 4900 μatm), organisms initially follow the course of the non-bicarbonate buffer line, until ion-transport processes kick in (typically after minutes to hours) to actively elevate $[HCO_3^-]$ above the slope of non-bicarbonate buffer line (often termed "metabolic or non-respiratory alkalosis", see also Fig. 1a). Partial compensation of extracellular pH (blue dots) has been observed in sipunculids, cephalopods, some brachyuran crustaceans and some teleost fish (Heisler, 1986; Pörtner et al., 1998; Cameron, 1986; Truchot, 1975; Gutowska et al., 2009). Full compensation, i.e. restoration of the original control extracellular pH (Fig. 1b, green dots), has been demonstrated for a range of teleost fish and some brachyuran crabs tested (Heisler, 1986; Cameron, 1986; Pane and Barry, 2007; Spicer et al., 2007). The amount of bicarbonate necessary for full compensation during hypercapnic stress can easily be assessed using the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}'_1 + log([HCO_3^-]\alpha_{CO2}^{-1}pCO_2^{-1}) \quad (1)$$

with pK'_1= apparent first dissociation constant of carbonic acid, α_{CO2}=CO_2 solubility coefficient of the respective fluid (e.g. blood, hemolymph, coelomic fluid; $[mmol\,l^{-1}\,Pa^{-1}]$)

In order to maintain extracellular pH constant, any factorial change in extracellular pCO_2 has to be balanced by an equivalent change in $[HCO_3^-]$ such that the ratio between the two remains constant, e.g. a 1.5-fold change in blood pCO_2 from 0.2 to 0.3 kPa (ca. 2000 to 3000 μatm) would need to

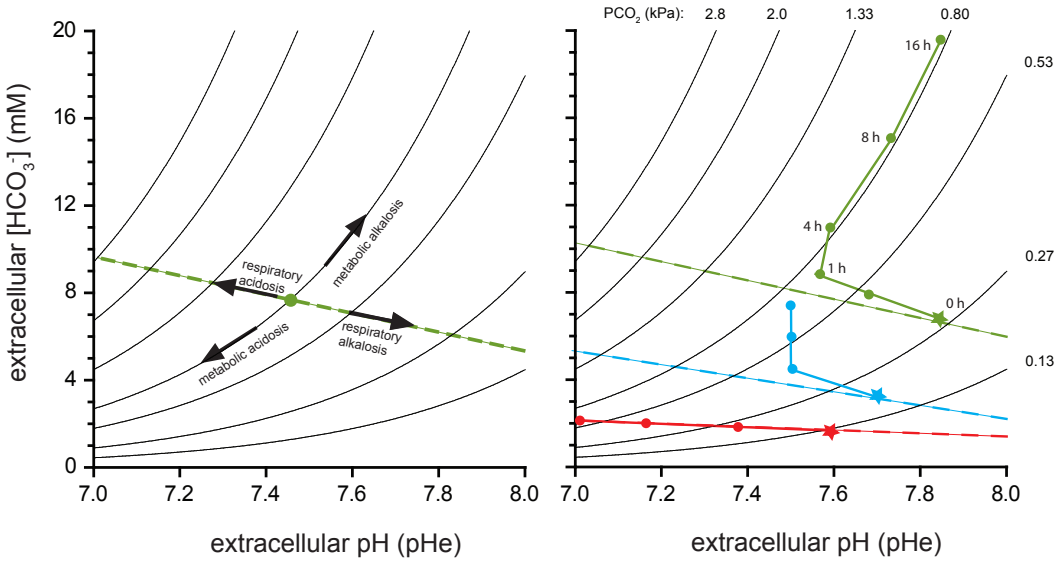

Fig. 1. Davenport diagrams. **(A)**: Schematic illustration of non-bicarbonate buffer line, dashed green line. Arrows indicate changes in pCO_2 and $[HCO_3^-]$ during respiratory acidosis/alkalosis and metabolic acidosis/alkalosis. See text for explanations. **(B)**: Three different hypothetical organisms subjected to 0.5 kPa (ca. 4900 μatm) environmental hypercapnia. Red symbols: No active accumulation of bicarbonate in the extracellular space to compensate pH, pH follows the non-bicarbonate buffer line. Blue symbols, green symbols: partial/full pH compensation through active bicarbonate accumulation. Stars indicate control parameters, numbers indicate time (h) exposed to elevated pCO_2 (hypothetical time course!). See text for a detailed discussion.

result in a 1.5-fold increase in $[HCO_3^-]$ to maintain extracellular pH at the control level.

The main prerequisite for such a rapid and efficient bicarbonate accretion are high net proton equivalent fluxes between ectothermic organisms and the surrounding seawater. Such data are currently only available for decapod crustaceans and for teleost/elasmobranch fish as well as an invertebrate (sipunculid) worm. Values of about 100 μEq kg^{-1} h^{-1} net acid efflux have been recorded for the crustacean *Carcinus maenas* exposed to a pCO_2 value of about 0.7 kPa (ca. 6900 μatm; Truchot, 1979), even higher values have been recorded in the marine teleost *Conger conger*, where exposure to 1.3 kPa CO_2 (ca. 12 800 μatm) produced a net acid efflux of 920 μEq kg^{-1} h^{-1} (Holeton et al., 1983). Rates were much lower in the sipunculid and mirrored transiently enhanced net proton release during transition to a new steady state in acid-base status under hypercapnia (Pörtner et al., 1998).

In summary, it appears that a relative degree of tolerance towards hypercapnic exposure can be found mainly in such marine ectothermic organisms that possess the ability to actively accumulate large amounts of bicarbonate ions to stabilize extracellular pH. In addition, these organisms are typically equipped with relatively high non-bicarbonate buffering capacities, which protect extracellular pH during acute CO_2 exposure. While hypercapnia typically is not a relevant stressor in the natural habitat of many marine organisms (however, see Sects. 8 and 9), high capacities for net acid extrusion directly result from an active mode of life, high metabolic rates and frequent as well as rapid metabolic rate fluctuations. We will follow this line of argument in the following paragraphs.

4 A common denominator: metabolic rate and metabolic rate fluctuations

Allowing for considerable intra-taxon variability, there are strong common ties between teleost fish, brachyuran crustaceans and cephalopod molluscs when compared with e.g. echinoderms and bivalve molluscs: All more tolerant taxa are characterized by high (specific) metabolic rates and high levels of mobility/activity. Figure 2a gives an overview of the range of metabolic rates that can be encountered in the aforementioned taxa, with standard/routine metabolic rates displayed in black, and those obtained during (exhaustive) exercise in white. For clarity sake, only subtidal and intertidal species from temperate regions were considered for this comparison. It is quite obvious that all active taxa are characterized by considerably higher metabolic rates, and, maybe even more important, higher metabolic rate fluctuations, than members from less active taxa (for references see Fig. 2). Maximum differences in oxygen consumption can be 100 to 200-fold between certain sessile echinoderms and exercising cephalopods. Even more revealing is a closer look at the flipside of the coin: Depending on the composition of their diet, marine animals have to excrete close to equimolar quantities, i.e. between 0.7 (fatty acids) and 1.0 (carbohydrates) moles

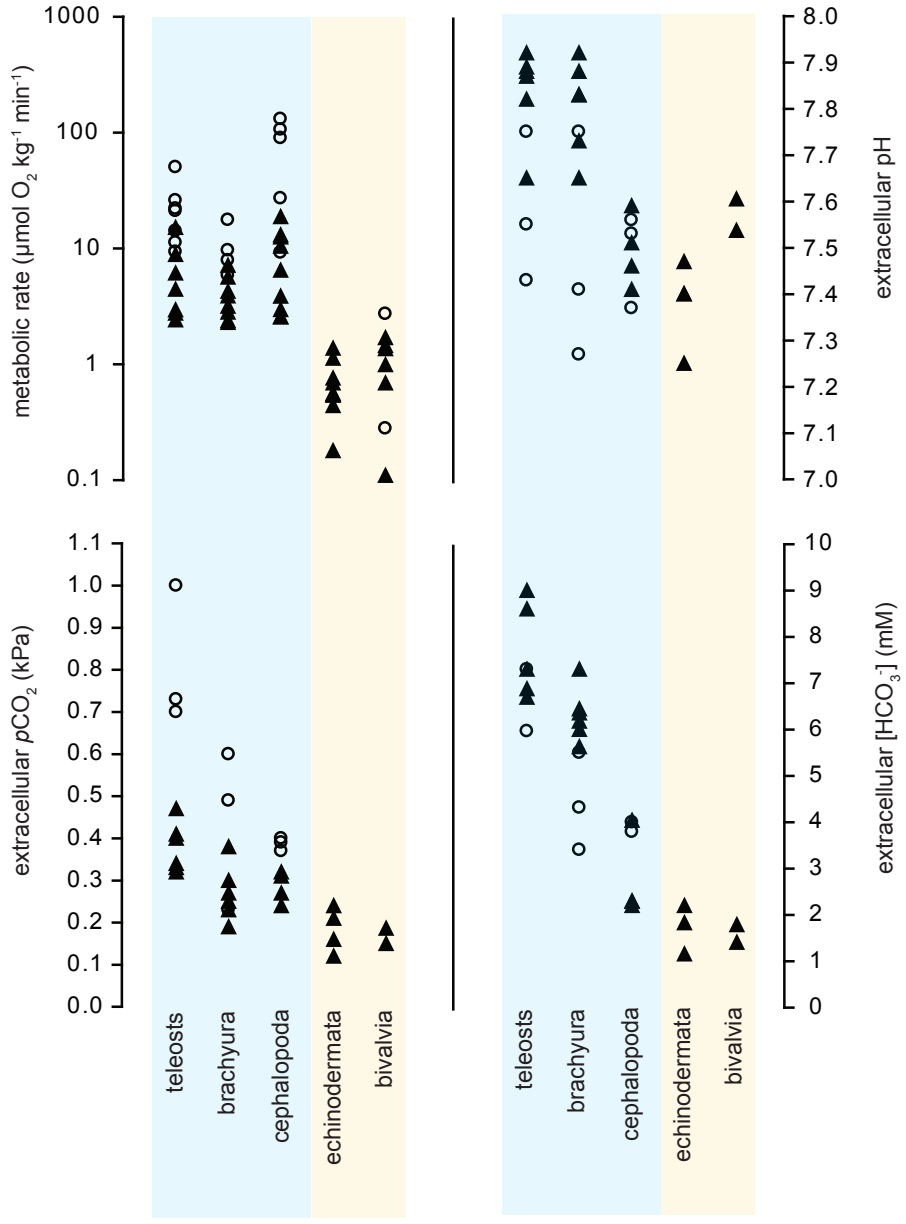

Fig. 2. (A): Routine (black symbols) and active (white symbols) metabolic rates for groups of randomly chosen marine subtidal ectothermic animals from temperate ocean regions. To ensure comparability, all metabolic rates have been scaled to an animal weight of 20 g (total body weight) at 15°C, using a Q_{10} value of 2.5 and a mass exponent of b=0.75 (see supplementary file: http://www.biogeosciences.net/6/2313/2009/bg-6-2313-2009-supplement.pdf). **(B), (C), (D)**: Acid-base parameters for groups of randomly chosen marine subtidal ectothermic animals under control (black symbols) conditions or after exercise (white symbols). (B) depicts pCO_2 values, 2C pH_{NBS} values and 2-D bicarbonate concentrations determined in extracellular fluids (blood or hemolymph) of various marine taxa. In most cases pCO_2 and bicarbonate values have been calculated from measurements of pH_{NBS} and dissolved inorganic carbon using the Henderson-Hasselbalch equation and appropriate constants (pK'_1, αCO_2). See supplementary for more detailed information and a table of references: http://www.biogeosciences.net/6/2313/2009/bg-6-2313-2009-supplement.pdf.

of CO_2 per mole of O_2 consumed. Thus, the flux of CO_2 that active vs. more inactive marine ectotherms have to channel from their mitochondria across the cell membranes into the blood space (or coelomic fluid/hemolymph) and, finally, across respiratory epithelia, also varies at the same order of magnitude. Exercise induced alterations in oxygen consumption thus are always coupled to almost equimolar changes in CO_2 flux. Such 3 to 5-fold fluctuations in O_2/CO_2 exchange in active species can occur within minutes, elicited both, by exercise and food consumption. Thus, taxa with

high metabolic rates must possess an advanced machinery for the elimination of CO_2 and associated acid-base disturbances. As a consequence, this machinery might also be helpful in coping with high pCO_2 values originating from seawater hypercapnia.

5 High extracellular pCO_2 in marine ectothermic metazoans

All marine ectothermic metazoans have one feature in common: their cells are surrounded by an extracellular fluid compartment (blood, coelomic fluid or hemolymph) that is used as a convective transport system for various substances, including dissolved gases. As with O_2, CO_2 exchange between this fluid and the external medium (seawater) is mainly realized by means of diffusion according to the following equation (Dejours, 1975):

$$MCO_2 = K_{CO2}(A\,E^{-1})(pCO_{2e} - pCO_{2sw}) \quad (2)$$

with MCO_2=CO_2 flux in moles, K_{CO2}= species (and organ) specific diffusion constant, pCO_{2e}= extracellular pCO_2, pCO_{2sw}= seawater pCO_2, A= functional diffusion area, E= thickness of the diffusion barrier.

Thus, CO_2 excretion is directly proportional to the CO_2 partial pressure gradient from the inside (extracellular fluid) to the outside (seawater). Consequently, higher marine metazoan animals are characterized by extracellular fluids with several-fold higher pCO_2 values than the surrounding seawater in order to produce a substantial net outward flow of CO_2 (see Fig. 2B), although diffusion areas also scale with metabolic rate. Minimum extracellular pCO_2 values in some marine metazoans (some echinoderms, bivalves) are little higher than 0.1 kPa (ca. 1000 μatm), most animals, however, live with extracellular pCO_2 values of 0.2 kPa (ca. 2000 μatm) and greater. Highest extracellular pCO_2 values in those water breathers are found in teleost fish (0.3 to 0.5 kPa; ca. 3000–4900 μatm). Most ectothermic marine animals maintain relatively constant extracellular pCO_2 values that go along with taxon specific extracellular $[HCO_3^-]$ and pH (under comparable abiotic conditions). Common patterns can be observed in both, brachyuran crustaceans and teleost fish: Relatively high $[HCO_3^-]$ values of 5 to 10 mM usually help support high extracellular pH values of 7.6 to 7.95 (Fig. 2c, d). On the other end of the scale, echinoderms are typically characterized by low extracellular pH (7.0 to 7.5) and low bicarbonate concentrations that are barely higher than those of seawater. Coleoid cephalopods, despite their fish like performance display relatively low extracellular pH and bicarbonate values.

Extracellular pCO_2 values may be first line indicators of an animals' susceptibility towards future ocean acidification. A simple example can illustrate this idea: any unicellular marine organism (e.g. a coccolithophorid, sperm and oocytes of broadcast spawners) today is surrounded by "extracellular" fluid (= seawater) with a pCO_2 of about 0.04 kPa (ca. 400 μatm). An increase in seawater pCO_2 by another 0.04 kPa therefore leads to a 100% increase in "extracellular" pCO_2 for that organism. A similar increase in seawater pCO_2 would probably only lead to a 40% increase in coelomic fluid pCO_2 of an echinoderm with a control coelomic fluid pCO_2 of 0.1 kPa (ca. 1000 μatm), and to a 10% increase in blood pCO_2 of a teleost fish with a control extracellular pCO_2 of 0.4 kPa (ca. 3900 μatm). In both cases, extracellular pCO_2 would need to be increased by 0.04 kPa in order to maintain a constant CO_2 diffusion gradient. Thus, the higher the pCO_2 values that cells are exposed to now, the lower the relative change that will come with future ocean acidification. Thus, fish/cephalopod/brachyuran cells will be exposed to a lower relative change in pCO_2 than cells of typical bivalves/echinoderms, while unicellular organisms (and life stages) will experience the greatest relative changes in their respective extracellular environment.

Figure 2b indicates, that following exhaustive exercise, even higher extracellular pCO_2 values can be encountered: Respiratory and metabolic acidosis result in maximum pCO_2 values between 0.4 kPa (ca. 3900 μatm, cephalopods) and $>$1.0 kPa ($>$9900 μatm, teleost fish). Thus, these taxa are adapted to cope (at least occasionally) with extracellular pCO_2 values that are up to five times higher than maximum values we might expect through ocean acidification in surface waters within the next few hundred years, i.e. 0.2 kPa (ca. 2000 μatm: Caldeira and Wickett, 2003).

Interestingly, little information is available on extracellular pCO_2 values during sub-maximal (exclusively aerobic) exercise. While one would expect that animals simply increase their extracellular pCO_2 in order to enhance CO_2 diffusion rates across gill epithelia, the few examples available for teleost fish suggest that pCO_2 is not dramatically elevated under such conditions (van den Thillart et al., 1983; Brauner et al., 2000). For other taxa (brachyuran crustaceans, cephalopods) such measurements have not been performed. It is thus quite rewarding to take a closer look at the physiological basis that enables elevated O_2/CO_2 exchange rates in teleost fish during aerobic exercise and to look at some physiological consequences of exhaustive exercise in active taxa in general. These mechanisms probably form the basis of efficient pH compensation as exploited during hypercapnia.

6 High CO_2 fluxes during (exhaustive) exercise

The capacity to live with elevated pCO_2 values in the extracellular fluid and to cope with extreme and rapid fluctuations in pCO_2 during muscular exercise is a challenge for active taxa. In order to support high metabolic rates, active groups discussed above rely on efficient circulatory systems. These do not only operate at high pressure and volume flow, but also contain intra- (fish) or extracellular (decapod crustaceans,

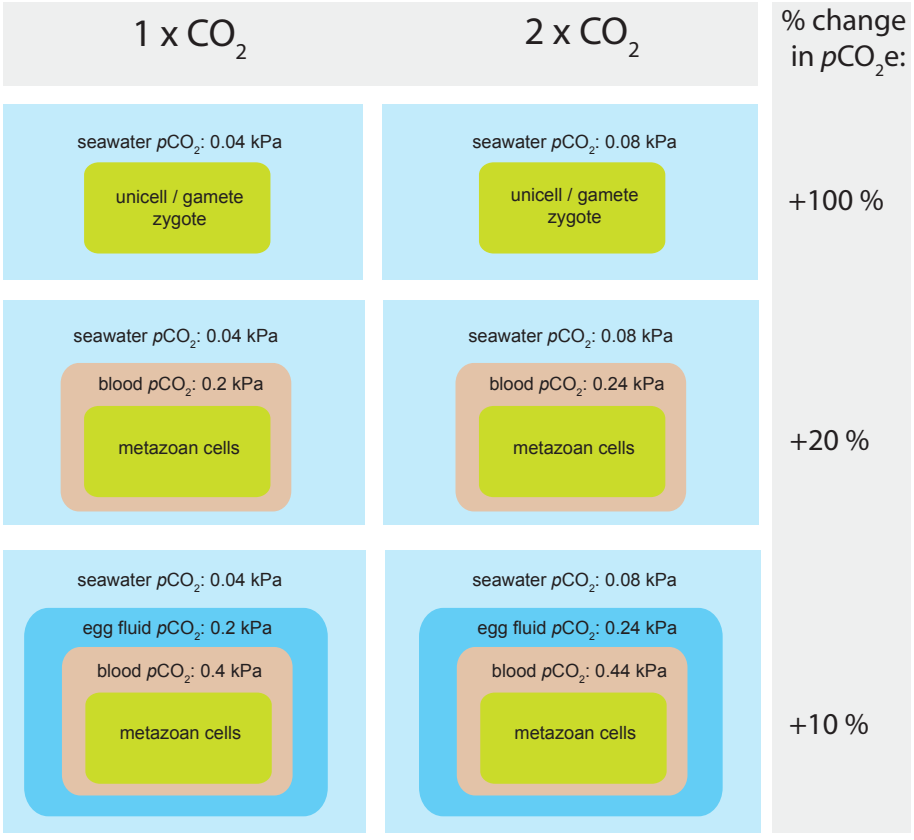

Fig. 3. Schematic illustration of relative changes in pCO_2 that a cell experiences upon doubling of ocean pCO_2 from 0.04 to 0.08 kPa (ca. 400 to 800 μatm). Unicellular organisms experience the greatest relative change in pCO_2, as their extracellular environment is the ocean. Metazoan cells are surrounded by extracellular fluid, which typically is characterized by pCO_2 values between 0.1 and 0.4 kPa (ca. 1000 to 3900 μatm). An elevation of ocean pCO_2 to 0.08 kPa (ca. 800 μatm) would probably only lead to a 20% increase in a metazoan with a control extracellular pCO_2 of 0.2 kPa. Cells of metazoan embryos, like those of cuttlefish, have to overcome yet another diffusion barrier, thus probably are exposed to even higher extracellular pCO_2 values. An equivalent change in pCO_2 by 0.04 kPa would possibly lead to an only 10% change in extracellular pCO_2. The lower relative degree of change in extracellular pCO_2 might render juvenile/adult metazoans less susceptible to future ocean acidification; however, their gametes might be the most sensitive stages.

cephalopods) respiratory pigments that greatly increase the oxygen carrying capacity of the blood. Typical active crustacean and cephalopod hemolymph can contain 70 to 200 g of respiratory protein per litre of blood, providing oxygen carrying capacities of 0.7 to 3 mM (e.g. Brix et al., 1989; Truchot, 1976; Johansen et al., 1982; Zielinski et al., 2001). Thus, in comparison to a mussel without a respiratory pigment, 3 to 8 times less blood has to be circulated per unit oxygen consumed. However, some respiratory pigments evolved to react quite sensitively to disturbances in blood homeostasis, especially in pH, to allow for fine controlled oxygen and CO_2 transport (e.g. Mangum, 1990; Melzner et al., 2007). It is thus not surprising that there have been high evolutionary pressures on the selection for physiotypes that on the one hand are able to cope with highly variable CO_2 fluxes, but on the other hand simultaneously "protect" extracellular pH within acceptable limits.

Excretory CO_2 is mainly transported in the form of bicarbonate in the extracellular fluid, as the capacity for transport of physically dissolved CO_2 is quite limited. In all active animal taxa investigated so far, this process is greatly dependent on the ubiquitous enzyme carbonic anhydrase. Currently, most information on CO_2 excretion in aquatic ectothermic animals is available for teleost fish: CO_2 diffuses from the metabolically active tissues into capillaries and into red blood cells, where bicarbonate ions are formed via carbonic anhydrase catalyzed hydration (as teleost fish lack plasma carbonic anhydrase). Protons generated during this reaction are bound to the respiratory pigment and thereby aid in the release of oxygen (Bohr shift). Bicarbonate is then transported into the plasma in exchange for Cl^- via electroneutral anion exchangers. In the gill vasculature the reverse process takes place: Transport of bicarbonate into the red blood cells and carbonic anhydrase catalyzed dehydration enable rapid diffusion of molecular CO_2 across the thin

gill epithelium and release into the surrounding water (see Tufts and Perry, 1998, for a review). During the short transit time through the gill vasculature (0.5 to 2.5 s; Cameron and Polhemus, 1974) approximately 12 to 35% of blood [HCO_3^-] is transformed and excreted (Perry, 1986). While sufficient capacities of carbonic anhydrase are necessary within the red blood cells to enable a rapid dehydration of bicarbonate during the gill passage (Henry and Swenson, 2000), the rate limiting step in CO_2 excretion in teleosts is thought to be the transfer of plasma bicarbonate into the red blood cell via the band 3 anion exchanger (e.g. Perry and Gilmour, 1993; Wood and Munger, 1994). Recent experimental evidence could convincingly establish that the rate of CO_2 excretion across gill epithelia is diffusion limited (e.g. Perry and Gilmour, 2006). Each anaemia (i.e. a low content of red blood cells in the blood) and elevated blood flow were observed to lead to elevated blood pCO_2, an effect, that could be reversed by experimentally making carbonic anhydrase available in fish plasma (Desforges et al., 2002; Gilmour and MacNeill, 2003).

During aerobic exercise, provision of oxygen to the working muscles becomes paramount and increases in metabolic rate are compensated for by elevated rates of blood convection (cardiac output). Other changes in the gill vasculature enable more efficient gas exchange, helping to maintain pCO_2, extracellular pH and [HCO_3^-] at control levels. Most important are increases in the perfused gill area (A in Eq. 2) and decreases in the gill epithelial thickness (E in Eq. 2), which are caused by increases in ventral aortic blood pressure (e.g. Kiceniuk and Jones, 1977; Randall and Daxboeck, 1984). However, elevated cardiac output can reduce gill transit time by a factor of three (Randall, 1982). As the CO_2 excretion system is already limited by the capacity of the red blood cell HCO_3^-/Cl^- exchange system, higher swimming velocities can result in slightly elevated blood pCO_2, a respiratory acidosis may develop (e.g. Brauner et al., 2000). Brauner et al. (2000) could also demonstrate that when their experimental fish (sea water acclimated rainbow trout, *Oncorhynchus mykiss*) were approaching their critical swimming speed (shortly before exhaustion), arterial pH was protected from acidification by rapid active accumulation of HCO_3^-. Extremely high blood pCO_2 values (>0.6 kPa; ca. 5900 μatm) and low extracellular pH values <7.5 are only encountered during and following exhaustive exercise (Fig. 2b, white symbols) in brachyuran crustaceans and teleost fish. These are mainly caused by anaerobic metabolism ("metabolic acidosis"): Force production by aerobic swimming muscles is complemented by the recruitment of anaerobic ("white") fibers; lactate and protons originate as metabolic end products. Both are eventually released into the extracellular fluid, where the protons can titrate plasma [HCO_3^-], thus decreasing extracellular pH (see Figs. 1a, 2c). However, rapid compensation processes are occurring during exhaustive exercise and particularly during the recovery phase. Gill ion-regulatory epithelia produce enormous net proton equivalent fluxes from the organism into the surrounding seawater, ranging in magnitude between 1200 μEq kg^{-1} h^{-1} (rainbow trout, *O. mykiss*; Holeton et al., 1983) and 4800 μEq kg^{-1} h^{-1} (blue crab, *Callinectes sapidus*; Booth et al., 1984) to restore the original acid-base status. Clearly, a powerful ion regulatory machinery can be made visible under conditions of extreme physical stress, the very same machinery that will then enable active organisms to compensate extracellular pH during hypercapnic disturbances (see above). It thus makes sense to take a closer look at ion-regulatory epithelia in the more active taxa.

7 The acid-base regulatory machinery and its main motor

Species specific mechanisms of transepithelial ion exchange have been reviewed, e.g. in Boron (2004), Claiborne et al. (2002), Perry and Gilmour (2006), and Wheatly and Henry (1992). However, we are far from exactly understanding the whole system of ion exchange mechanisms, especially in the invertebrate taxa. Interestingly, similar molecular components have been conserved in different marine animal groups. Gills are the primary sites of acid-base regulatory processes in all high metabolic rate marine taxa discussed in this text, in fish (Perry and Gilmour, 2006), crustaceans (Wheatly and Henry, 1992) and probably also in cephalopods (Schipp et al., 1979). In fish, specialized epithelial cells, the mitochondria rich cells, contain a set of ion transporting proteins and channels that are important for acid-base regulation. Cells that are active in acid secretion contain electroneutral Na^+/H^+ exchangers or V-type H^+ ATPases, coupled energetically to apical Na^+ channels. While the latter system is thought to be more important for freshwater organisms which have to absorb Na^+ (e.g. Wilson et al., 2000), the former can operate on the favourable Na^+ gradient between seawater and cytosol, shuttling one H^+ out of the cell for each Na^+ imported. While Na^+/H^+ exchangers do not directly consume energy (there is no ATPase directly linked to these proteins), they essentially operate on the energy spent by the ATP consuming sodium pump (Na^+/K^+ ATPase). Basolateral Na^+/K^+ ATPase is thus commonly considered the motor of the ion-regulatory machinery in marine animal gills. Pumping two K^+ into the cell while simultaneously removing three Na^+, it creates the low intracellular [Na^+] typical for all animal cells and thus is partly responsible for the cell's membrane potential. One potential mechanism for the removal of acid during a respiratory acidosis could be the following (established from results of studies in teleost fish; see Fig. 4): Excess CO_2 diffuses into the mitochondria rich cells and is instantly hydrated by cytosolic carbonic anhydrase into protons and bicarbonate ions. While the protons are exported via the Na^+/H^+ exchanger, bicarbonate could be released into the plasma by means of basolateral Cl^-/HCO_3^- exchangers or Na^+/HCO_3^- co-transporters.

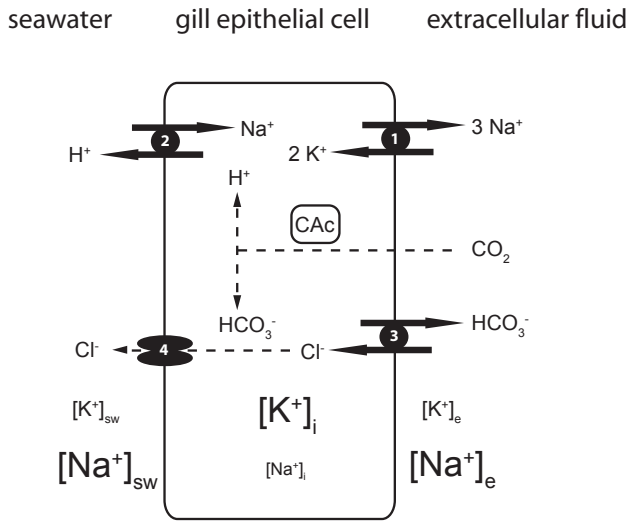

Fig. 4. Simplified schematic depiction of an epithelial gill cell (ionocyte) of a teleost fish (adapted from Perry and Gilmour, 2006). Decapod crustacean and cephalopod gill epithelia are equipped with similar proteins. **(1)**=Na^+/K^+ ATPase, **(2)**=Na^+/H^+ exchanger, **(3)**=Cl^-/HCO_3^- exchanger, **(4)**=Cl^- channel (e.g. CFTR), CAc = cytoplasmatic carbonic anhydrase. Na^+/K^+ ATPase is responsible for the low intracellular Na^+ and high K^+ concentration. Secondary active transporters, such as Na^+/H^+ exchanger can utilize the sodium gradient to export H^+. H^+ are produced when CO_2 is hydrated by CAc. The resulting HCO_3^- can be transferred into the extracellular fluid (blood, hemolymph), while Cl^- is exported to the seawater through chloride channels to maintain electroneutrality.

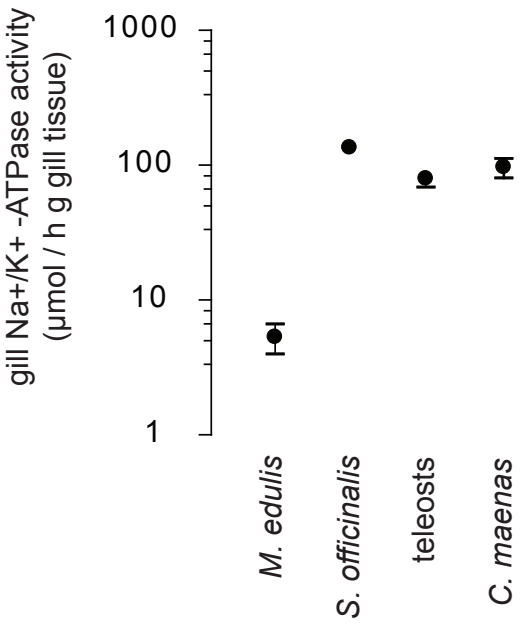

Fig. 5. Gill Na^+/K^+-ATPase activity measure in crude gill homogenates in two molluscs, the cephalopod *S. officinalis*, the bivalve *M. edulis* and the crustacean *Carcinus maenas*, acclimated and measured at 14 to 15°C vs. similar measurements on fish gill homogenates measured at 10°C. The teleost value represented in the figure is the mean of six species of shallow water teleosts from Gibbs and Somero (1990, their Table 1) and the eelpout *Z. viviparus* from Deigweiher et al. (2008). The mussel, cephalopod and crustacean measurements (Melzner and Lucassen, unpublished) were performed according to the protocol outlined in Melzner et al. (2009; see supplementary file for details: http://www.biogeosciences.net/6/2313/2009/bg-6-2313-2009-supplement.pdf).

This plasma bicarbonate may then undergo further protonation/dehydration/hydration cycles leading to a net proton extrusion via the gills. In order to maintain electroneutrality in the plasma, Cl^- is typically excreted, possibly via apical Cl^- channels (e.g. CFTR; see Perry and Gilmour, 2006; Deigweiher et al., 2008, for an extended discussion). However, the true mechanisms may be more complicated owing to the large number of transporters and channels present in gill epithelia (see also Deigweiher et al., 2008). However, basic processes can be suspected similar for decapod crustaceans and cephalopods as well; it is known by now that similar ion exchange proteins are also expressed in gills of these invertebrates (e.g. Schipp et al., 1979; Piermarini et al., 2007; Virkki et al., 2003; Henry and Swenson, 2000; Wheatly and Henry, 1992; Hu, Lucassen and Melzner, unpublished).

As Na^+/K^+ ATPase activity is the main energy sink and driving force for gill ion exchange processes in marine ectothermic animals, it can serve as a useful indicator for the overall capacity in ion and acid-base regulation. Consequently, gill Na^+/K^+ ATPase activity has been shown to correlate with metabolic rate in marine teleost species: Gibbs and Somero (1990) found highest Na^+/K^+ ATPase activities in shallow water, active species, while more inactive, deep-sea species activities were an order of magnitude lower. These relationships correspond with lower metabolic rates (e.g. Torres et al., 1979), lower gill surface areas (Hughes and Iwai, 1978) and lower muscle glycolytic enzyme capacities (Somero and Childress, 1980) in deep-sea vs. shallow water teleost species. The latter feature suggests that deep-sea fish rely less on aerobic as well as high-intensity, anaerobic "burst" swimming, thus likely would experience metabolic acidosis less often than shallow water species. Based on similar considerations, it has already been suggested that deep-sea marine animals might be significantly more vulnerable with respect to ocean acidification than shallow living species (Seibel and Walsh, 2001, 2003).

The gills of hypercapnia tolerant, shallow water marine taxa are characterized by surprisingly similar activities of Na^+/K^+ ATPase, an order of magnitude higher than those of sessile, hypometabolic species such as the blue mussel (see Fig. 5). While the comparison between high-power taxa and bivalves is confounded by the fact that the mussel gill primarily serves as a feeding organ, the lack of a true ion-regulatory organ in bivalves itself illustrates a key point: The evolution

of high metabolic rate physiotypes is closely connected to the development of extremely specialized organ structures to promote respiration and ion regulation that are very similar in their ultrastructural design (e.g. Evans et al., 2005: fish; Budelmann et al., 1999: cephalopoda; Taylor and Taylor, 1999: decapod crustacea).

Na^+/K^+ ATPase activities are modulated in vivo during metabolic rate transitions (e.g. exercise, specific dynamic action) on a short term basis by several second messenger pathways finally leading to a change in protein phoshorylation (e.g. Ramnanan and Storey, 2006). The most impressive example is the beta adrenergic stimulation of the enzyme in skeletal muscle which compensates the large K^+ efflux during exercise. Also changes in cytosolic ion composition, namely Na^+ and H^+ concentrations are involved in the regulation of Na^+/K^+-ATPase activity. In addition interaction with the cytoskeleton and membrane trafficking of the pump are regulatory mechanisms acutely controlling its function and availability (Bertorello and Katz, 1993). Long term regulation of Na^+/K^+-ATPase is under control of nuclear hormones. They trigger transcription of the subunits by binding to nuclear hormone responsive elements on the respective genes (Féraille and Doucet, 2001).

However, as has been shown that phosphorylation/dephosphorylation can activate or deactivate the enzyme, high-power animals may operate with a functional reserve that can be activated upon demand. Whether such a reserve is important for the rapid extracellular HCO_3^- accumulatory reaction observed upon acute hypercapnic exposure (see above, Fig. 1b) remains to be investigated. It has been recently shown in two marine teleost fish species, that gill Na^+/K^+ ATPase activity increases during acclimation to higher levels of hypercapnia (Deigweiher et al., 2008; Melzner et al., 2009). Rapid increases in activity in the eelpout *Zoarces viviparus* upon exposure to 1 kPa of CO_2 (ca. 9900 μatm) within two days have been observed to be related to elevated Na^+/K^+ ATPase mRNA and protein levels, suggesting that the enzyme is under tight transcriptional control. Longer acclimation (6 weeks) led to a ~80% increase in Na^+/K^+ ATPase activity. In cod (*Gadus morhua*) long term acclimation (4–12 months) led to increases in Na^+/K^+ ATPase activity and protein concentration at a pCO_2 of 0.6 kPa (ca. 5900 μatm), whereas no significant changes were observed at 0.3 kPa (ca. 3000 μatm; Melzner et al., 2009). Although this occurred in specimens from two distinct populations, it could nevertheless indicate that the control fitting of the gill ion regulatory machinery in many teleosts has high enough of an excess capacity to cope with the additional ion-regulatory challenge due to hypercapnia under more realistic scenarios of ocean acidification (i.e. 0.1 to 0.2 kPa; Caldeira and Wickett, 2003). Clearly, further studies need to address this exciting possibility.

8 Environmental hypercapnia

Further above it was stated that typical marine ectothermic animals are seldom exposed to environmental hypercapnia. This applies for large areas of the pelagic open ocean, however there are some special habitats that do provide elevated pCO_2 values to its inhabitants: intertidal regions, estuaries, oxygen-minimum zones, upwelling coastal regions or deep-sea vent systems (see e.g. Frankignoulle et al., 1996, 1998; Weigelt and Rumohr, 1986; Dwyer and Burnett, 1996; Feely et al., 2008; Wotton et al., 2008). While present mean surface ocean pCO_2 values average around 0.04 kPa (ca. 400 μatm) much higher values are reached in the above mentioned habitats. For example, pCO_2 values in Kiel Fjord, home to numerous calcifying organisms, can rise above 0.1 to 0.2 kPa (ca. 1000 to 2000 μatm) for prolonged times during summer and autumn (Thomsen, 2008). Similarly, upwelling processes lead to elevated near-shore pCO_2 values of up to 0.1 kPa (about 1000 μatm) in continental shelf areas off the Californian coast (Feely et al., 2008). Animals living in intertidal rockpools experience even stronger short-term fluctuations (Truchot and Duhamel-Jouve, 1980): depending on respective light conditions pCO_2 values during low tide emersion periods can range between about 0.35 kPa (3500 μatm, due to extensive nighttime respiration) and almost zero (due to high photosynthetic activity). High pCO_2 values (around 1200 μatm) have also been observed in oceanic oxygen minimum layers of intermediate depths (200–1000 m) where high community respiration rates cause hypoxia and associated hypercapnia (Brewer and Peltzer, 2009). However, special physiological and biochemical adaptations enable various animal groups to populate even the most extreme habitats with respect to hypercapnic, temperature and other chemical conditions – the deep-sea hydrothermal vent ecosystems. Amongst other things, this inhospitable environment challenges its inhabitants with pCO_2 conditions as high as 7 kPa (about 69 000 μatm). Nevertheless, the vent mussel *Bathymodiolus brevior* has been found able to precipitate shells under such high pCO_2/low-pH conditions (Tunnicliffe et al., 2009). Again, following our rationale from above, organisms already living under elevated pCO_2 in their particular habitats may encounter less of a relative change in pCO_2 than e.g. oceanic species, thus may be better adapted to future acidification (however, pCO_2 in CO_2 enriched habitats may not necessarily increase at the same rate as projected for the open ocean; see calculations in Brewer and Peltzer, 2009).

9 Ontogenetic hypercapnia: the hostile environment within egg capsules

Of large evolutionary relevance might be a special "ontogenetic habitat": the egg case and egg masses of many marine ectothermic animals. Recent determinations of pH, pO_2 and pCO_2 in the fluid surrounding the cephalopod

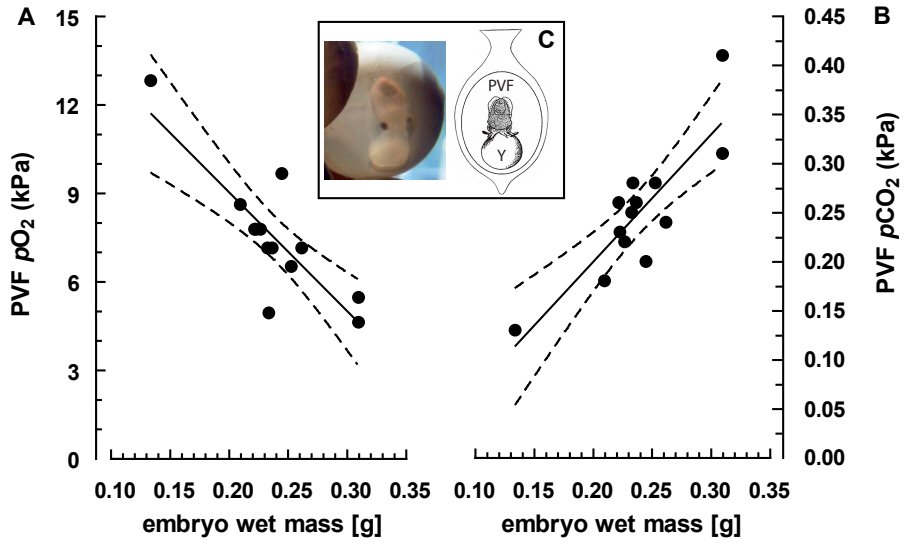

Fig. 6. Abiotic conditions in cuttlefish (cephalopod) eggs (modified, from Gutowska and Melzner, 2009). **(A)**: pO_2 in the fluid surrounding the embryo (perivitelline fluid) graphed against embryo wet mass. **(B)**: Perivitelline fluid pCO_2. Cuttlefish experience hypoxic and hypercapnic conditions towards the end of their embryonic development as the egg case serves as a diffusion barrier. **(C)**: Schematic illustration and photo of a late embryonic stage cuttlefish in its egg. These eggs can reach a diameter of almost 2 cm (see also supplementary video supplied by Gutowska and Melzner (2009) on Marine Biology homepage).

Sepia officinalis in its egg casing (perivitelline fluid) suggest that the egg case serves as a significant barrier to diffusion, of both CO_2 and O_2 (Gutowska and Melzner, 2009). Thus, embryos become progressively exposed to hypercapnic and hypoxic conditions the larger they grow within their capsules. Figure 4a shows linear relationships between wet mass and perivitelline fluid pO_2, pCO_2 and pH in late stage embryos shortly before hatching (stage 29, 30; Lemaire, 1970). Oxygen partial pressure declined to values around 4.6 kPa (from >12 kPa), while pCO_2 increased from 0.13 to 0.41 kPa (ca. 1300 to 4000 μatm); embryos thus were surrounded by about tenfold higher CO_2 values than those of ambient seawater (0.04 kPa; ca. 400 μatm) and pH values as low as 7.2 for 1 to 2 weeks at the end of their embryonic development. As extracellular pCO_2 values are always significantly elevated above ambient (see above), we can expect blood pCO_2 values of at least 0.6 to 0.7 kPa (ca. 5900 to 6900 μatm) in late *S. officinalis* hatchlings. These tolerated values have been shown to cause significant physiological disturbances in other, even adult, but more sensitive organisms such as echinoderms or bivalves. During their development within the egg case, cuttlefish even start to form their calcium carbonate (aragonite) shell, as well as their statoliths (Nixon and Mangold, 1998). Thus, obviously, hypercapnic stress is an integral part of the life cycle of *S. officinalis*. Coupled with special physiological adaptations (e.g. embryonic hemocyanins; Decleir et al., 1971), powerful net proton excretion mechanisms can be expected to be present already in these early life stages to cope with high perivitelline fluid pCO_2. Whether these high perivitelline fluid pCO_2 values render late embryonic stages of *S. officinalis* more vulnerable to additional hypercapnic stress in a progressively acidic ocean, needs to be determined. In order to maintain diffusion rates of CO_2 excretion elevated pCO_2 values would be additive to the already high perivitelline fluid pCO_2 values (see Fig. 3).

Unfortunately, no comparable data are available for egg fluid pCO_2 or pH of other marine ectothermic animals. However, assuming similar perivitelline fluid pO_2 to pCO_2 ratios, it seems likely that embryos from other marine taxa will also be surrounded by fluids of high pCO_2 and low pH. Decreased oxygen partial pressures have been measured for instance in shark eggs (Dietz and Davenport, 1987) and decapod crustacean egg masses (Fernandez et al., 2000, 2002). In this context, it is a striking analogy that embryos from most of the more CO_2 tolerant marine taxa like fish, cephalopods or brachyuran crustaceans all share a common ontogenetic characteristic, namely their relatively long developmental period and their growth to comparatively large size within the protecting egg shell. In contrast, development of many other marine invertebrate taxa (cnidarians, echinoderms, bivalves) is characterized by external fertilization, early hatching and the succession of various small, free larval stages within the water column: 55 to 85% of all benthic invertebrate species produce long-lived planktotrophic larvae spending weeks to months in the plankton, 5% produce short-lived planktotrophic larvae (spending hours to days in plankton), and about 10% produce lecithotrophic larvae (Thorson, 1950, 1966).

It is thus tempting to speculate, that the demand to cope with high pCO_2/low pH conditions during embryogenesis in *S. officinalis* and potentially, other species with lecithotrophic larval stages, selects for hypoxia- and hypercapnia-tolerant physiotypes with a highly developed ion regulatory apparatus that can efficiently export proton equivalents. The flip side of the coin may be that species like *S. officinalis* only can afford a direct mode of development and growth to comparatively large sizes (200 to 300 mg wet mass) within their egg capsules because the species is equipped with a capable gill ion-regulatory machinery to begin with. Clearly, this question of whether the life-history strategy selects for a certain physiological machinery, or vice versa, must remain unanswered at the moment.

Following our argumentation from above (see Sect. 5) we would expect embryonic stages of *S. officinalis* to be more tolerant towards future ocean acidification than larval stages of species that develop in open seawater from early on, as any changes in ocean pCO_2 result in relatively smaller changes in perivitelline fluid pCO_2 (Fig. 3). Unfortunately, at present we lack more data for fish, decapod crustaceans and cephalopods to follow this hypothesis further. However, several studies have shown that echinoderm and bivalve larval stages sometimes can react extremely sensitive towards hypercapnia (Kurihara, 2008; Dupont et al., 2008). A slight decrease in pH can have dramatic effects, inducing 100% mortality in only 8 days post fertilization in calcifying pelagic larvae of the brittlestar *Ophiothrix fragilis* due to larval and skeletal malformations (Dupont et al., 2008). An increased mortality is also observed in many other calcifying species such as some crustaceans, molluscs and echinoderms (see Dupont and Thorndyke, 2009). Nevertheless, available data on early developmental stages reveal contradictory results and apparent paradox. In the same phyla, different species are not, or sometimes positively, affected by near-future levels of ocean acidification, e.g. by decreases in mortality. For example, at low pH a significantly higher proportion of larvae successfully reached metamorphosis in the sea urchin *Strongylocentrotus droebachiensis* (see Dupont and Thorndyke, 2009).

Little information is available on the differential sensitivities towards hypercapnia in adult vs. embryonic/larval stages. Data presented by Kikkawa et al. (2003) on CO_2 tolerance of early life stages of marine teleosts indicate that the earliest stages (cleavage) were characterized by 2 to 3-fold lower values of lethal CO_2 concentration (LC_{50}=50% of test animals die within 24 h) than later stage embryos, larval stages and juveniles. The increase of this lethal concentration from the cleavage to the embryo stages may reflect the development of ion-regulatory chloride cells on the yolk sac membrane (cf. Ishimatsu et al., 2004, 2005). These results fit our concept of ion-regulatory ability defining hypercapnia tolerance in marine animals and indicates, that even in those organisms that display a high tolerance as juveniles/adults, the true bottleneck might be the earliest stages: gametes, zygotes and cleavage stages. Essentially, at the level of gametes and zygotes, broadcast-spawning teleosts (e.g. herring) do not differ much from the echinoderm situation, i.e. both groups release cells that are directly exposed to seawater and would experience large relative changes in pCO_2 during future ocean acidification. Thus, we would expect these stages to be similarly impacted by hypercapnia. However, while Havenhand et al. (2008) could demonstrate reductions in sperm mobility and fertilization success in an echinoderm species at relatively low seawater pCO_2 values (0.1 kPa, ca. 1000 μatm), no reductions in fertilization success, embryonic growth, mortality and hatching rate could be observed in herring (*Clupea harengus*) exposed to pCO_2 values between 0.05 and 0.4 kPa (ca. 500 to 3900 μatm; Franke, 2008). The latter results go along with an alternative hypothesis: Hamdoun and Epel (2007) have argued that embryos and larvae have an inherent set of cellular defense mechanisms that provide robustness to "buffer" environmental variability. However, these authors also agree that there may be some level of external (anthropogenic) stress that "depletes" the buffering reserve given to early life stages. Clearly, many more studies are needed that titrate sensitivities of these early life stages to elevated pCO_2 to create meaningful comparisons.

In summary, recent measurements of pCO_2 in marine animal eggs showed that significant levels of hypercapnia up to 3900 μatm can be part of the normal life cycle of cephalopods (Gutowska and Melzner, 2009) and, judging from oxygen partial pressures in eggs of other taxa, probably also of decapod crustaceans and teleost/elasmobranch fish. Thus, the defence machinery against hypercapnia might already be developed (and challenged) at the beginning of the life cycle, probably in many of the more hypercapnia tolerant species.

10 Synthesis and conclusions

Hypometabolism has previously been suggested to render animals more sensitive to ocean acidification. Seibel and Walsh (2001, 2003) convincingly argued that hypometabolic deep-sea animals might be significantly more vulnerable to future ocean acidification. Knoll et al. (2007) suggested that mass extinction of primarily hypometabolic marine genera at the Permian-Triassic boundary was mainly triggered by high levels of seawater hypercapnia.

In this paper, we tried to summarize some of those physiological traits that distinguish temperate, subtidal high-power marine ectothermic taxa (brachyuran crustaceans, teleost fish, cephalopods) from more hypometabolic ones (bivalves, echinodermata) and outlined their possible relevance for an increased tolerance towards environmental hypercapnia. We explained why the evolution of high metabolic physiotypes simultaneously led to the co-evolution of a physiotype that might be pre-adapted to cope with future ocean acidification, as it has acquired an ion-regulatory machinery that can

protect body fluids from excessive acidification and, potentially, metabolic depression. Here we will briefly summarize these adaptations and point at the crucial role of further studying early life stages, as they might be most vulnerable.

High-power physiotypes, such as teleosts, cephalopods and many brachyuran crustaceans, need advanced blood oxygen and CO_2 transport mechanisms to support their active lifestyles. The invention of blood oxygen binding proteins facilitated oxygen (and CO_2) transport, but also constrained the taxa, as blood pigment pH sensitivity rendered the animals more vulnerable to extracellular pH disturbances. While the high amount of respiratory protein provided them with a high pH buffering capacity, an efficient acid-base and ion-regulatory machinery was nonetheless needed to balance shifts in extracellular pH. For apparent reasons (high rates of sea water perfusion), this apparatus was incorporated into the primary gas exchange organs, the gills. High metabolic rates called for almost equimolar rates of CO_2 excretion, which were causally linked to an efficient carbonate system manipulation machinery (carbonic anhydrase, anion exchangers in fish) in order to transport the necessary amounts of CO_2/dissolved inorganic carbon. High extracellular pCO_2 values of 0.1 to 0.4 kPa (ca. 1000 to 3900 μatm) under control conditions provided the diffusion gradient for CO_2 excretion across gill epithelia. These high extracellular pCO_2 values might be another key correlate and contribute to why marine metazoans with an extracellular convection system should be more tolerant towards future ocean acidification than unicellular organisms, as any future change in seawater pCO_2 is less of a relative change for organisms, whose cells are already surrounded by a high pCO_2 fluid (see Fig. 3). Numerous studies have shown that during extensive exercise many of the high-power taxa experience high pCO_2 oscillations due to combined respiratory and metabolic acidosis, illustrating that cells are occasionally exposed to very high pCO_2 values. In addition, the proton excretion machinery of the gills needs surplus capacity to eliminate the metabolic protons generated during aerobic and, especially, anaerobic exercise. This machinery scales with metabolic rate and the magnitude of metabolic rate fluctuations and may be extremely important under future conditions, as ocean acidification can be seen as a long-lasting respiratory acidosis. Comparable to rebalancing acid-base status after bursts of exercise, important extracellular pH compensation processes during hypercapnia rely on efficient (net) proton excretion. Thus, the more CO_2 tolerant physiotype that can more or less manipulate its extracellular environment is a direct consequence of an active, high metabolic life style.

It is quite likely that these physiological traits already play a very important role during embryonic development, when high pCO_2 values develop in and around egg capsules owing to the egg case serving as a diffusion barrier (cephalopods, fish) or due to perfusion problems (crustacean egg masses). Thus hypercapnia may be an important natural stressor during the course of ontogeny in many marine animal taxa.

An active high-metabolic lifestyle and natural hypercapnia during the course of embryonic development, as well as within the natural habitat constitute factors that pre-adapt animals to cope better with hypercapnia/future ocean acidification. Of course it needs to be emphasized that we are discussing probabilities rather than certainties; biological diversity is too great to make universal statements on the potential vulnerability on the species/genus level. An analogy from the past exemplifies this point: Knoll et al. (2007) state that selectively, more hypo-metabolic, heavily calcified taxa suffered higher species losses during the Permian-Triassic mass extinction than high-metabolic taxa with more advanced circulatory systems and respiratory surfaces. While 81% of hypometabolic genera (calcareous sponges, corals, brachiopods, bryozoans and most echinodermata) were lost during this time period, only 38% of the more hypermetabolic genera (molluscs, arthropods, chordates) suffered this fate. Multiple transition stages will most likely be observed between "tolerant" and "sensitive" groups, however, the general physiological principles outlined in this paper will, on average, define the trends.

The true bottleneck, even for the seemingly hypercapnia tolerant organisms might, however, be located in very early ontogeny. Gametes of broadcast spawners, the fertilization reaction in open seawater, the zygote and early cleavage stages might be especially vulnerable in all taxa, as for these stages, any change in ocean pCO_2 constitutes a much higher relative change in external pCO_2 than for cells of the later ontogenetic stages (see Fig. 3; however, the alternative views of Hamdoun and Epel, 2007, also should be kept in mind). In addition, specialized ion-regulatory cells develop well after cleavage. However, these stages are the most difficult to study and only little information is available at present. The generation boundary thus clearly should be a major area of research activity in the near future.

We see this paper as an attempt to highlight some common features of certain animal groups that have a high capacity to perform well in physiological terms on the short to medium scale despite high environmental pCO_2. As was stated in the beginning, multi generation experiments are largely missing at the moment and crucial life stages have not been considered. In addition, a large research effort is currently focusing on those taxa that are seemingly most sensitive to ocean acidification, while for a mechanistic understanding it is quite important to study the (apparently) more tolerant ones as well. We therefore are only beginning to see the "patterns" that define tolerance vs. sensitivity to future ocean acidification. However, past extinction events give us a good idea where to look for potential survivors in a more acidified ocean.

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