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ORIGINAL PAPER

Future ocean acidification will be amplified by hypoxia in coastal habitats

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Abstract Ocean acidification is elicited by anthropogenic carbon dioxide emissions and resulting oceanic uptake of excess CO_2 and might constitute an abiotic stressor powerful enough to alter marine ecosystem structures. For surface waters in gas-exchange equilibrium with the atmosphere, models suggest increases in CO_2 partial pressure (pCO_2) from current values of ca. 390 µatm to ca. 700–1,000 µatm by the end of the century. However, in typically unequilibrated coastal hypoxic regions, much higher pCO_2 values can be expected, as heterotrophic degradation of organic material is necessarily related to the production of CO_2 (i.e., dissolved inorganic carbon). Here, we provide data and estimates that, even under current conditions, maximum pCO_2 values of 1,700–3,200 µatm can easily be reached when all oxygen is consumed at salinities between 35 and 20, respectively. Due to the nonlinear nature of the carbonate system, the approximate doubling of seawater pCO_2 in surface waters due to ocean acidification will most strongly affect coastal hypoxic zones as pCO_2 during hypoxia will increase proportionally: we calculate maximum pCO_2 values of ca. 4,500 µatm at a salinity of 20 ($T = 10$ °C) and ca. 3,400 µatm at a salinity of 35 ($T = 10$ °C) when all oxygen is consumed. Upwelling processes can bring these CO_2-enriched waters in contact with shallow water ecosystems and may then affect species performance there as well. We conclude that (1) combined stressor experiments (pCO_2 and pO_2) are largely missing at the moment and that (2) coastal ocean acidification experimental designs need to be closely adjusted to carbonate system variability within the specific habitat. In general, the worldwide spread of coastal hypoxic zones also simultaneously is a spread of CO_2-enriched zones. The magnitude of expected changes in pCO_2 in these regions indicates that coastal systems may be more endangered by future global climate change than previously thought.

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Introduction

Currently, large research efforts are directed toward studying anthropogenic climate change effects on marine ecosystems. Global warming, primarily caused by increasing CO_2 emissions, has the potential to restructure marine ecosystems (IPCC 2007). In highly productive coastal ecosystems, other abiotic stressors play a role as well: eutrophication largely following the human footprint has led to a spread of low-oxygen regimes where benthic metazoan communities cannot survive (Diaz and Rosenberg 2008). While the biological effects of hypoxia and anoxia on marine metazoan heterotrophic organisms are fairly well understood, it has been sparsely acknowledged that hypoxia, a consequence of respiratory gas exchange, is necessarily coupled to an increase in total dissolved

inorganic carbon and CO_2 partial pressure (pCO_2). In this paper, we will give estimates of pCO_2 values in coastal marine and estuarine habitats now and within the next 50–100 years. To do so, we will calculate how respiration in hypoxic zones interacts with the invasion of anthropogenic CO_2 into the ocean (ocean acidification). Our estimates have important repercussions for the design of experimental studies currently underway to assess the effect of ocean acidification on marine biota. We further suggest that carbonate chemistry of coastal zones may be much more sensitive with respect to future global change than previously estimated. We finally try to point the reader's attention toward key physiological systems and mechanisms that might play a role in determining marine animal vulnerability in response to the hypoxia and ocean acidification (hypercapnia) stress complex.

Anthropogenic CO_2 emissions not only are leading to global warming but also change ocean chemistry. When CO_2 diffuses into seawater, it reacts with water and carbonic acid is formed. Carbonic acid rapidly dissociates into bicarbonate $\left(HCO_3^-\right)$ and a proton and can further dissociate into the carbonate ion $\left(CO_3^{2-}\right)$ and another proton:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+ \quad (1)$$

The equilibrium constants of the inorganic carbonate system are such that under current abiotic conditions of temperature, salinity and alkalinity, roughly 90 % of the CO_2 is in the form of HCO_3^- and ~10 % in the form of CO_3^{2-}, while less than 1 % is dissolved CO_2 and carbonic acid (termed CO_2^*). The sum of the concentrations of the carbonate system species is termed total dissolved inorganic carbon (C_T):

$$C_T = \left[CO_2^*\right] + \left[HCO_3^-\right] + \left[CO_3^{2-}\right] \quad (2)$$

Any invasion of CO_2 from the atmosphere to the surface ocean leads to an increase in C_T and a shift in carbonate system speciation, namely an increase in $\left[HCO_3^-\right]$, an increase in pCO_2, a decrease in $\left[CO_3^{2-}\right]$ and a decrease in pH (Brewer 1997, see Table 1). Currently (end of 2011), atmospheric pCO_2 is at 391 ppm and increases at a rate of 2 ppm $year^{-1}$ (NOAA/ESRL, www.esrl.noaa.gov/gmd/ccgg/trends, Cao and Caldeira 2008). It has been estimated that atmospheric pCO_2 will rise to values of 700–1,000 ppm by the end of the century (IPCC 2007). It is well documented that the past time change in atmospheric pCO_2 has led to an oceanic uptake of CO_2 and associated ocean acidification (Kleypas et al. 1999). With this continuing in future, we can expect a moderate increase in $\left[HCO_3^-\right]$, while the carbonate ion concentration $\left[CO_3^{2-}\right]$ decreases starkly (by ~40 %) and pH decreases by ~0.25 units by 2100 (Table 1). Decreases in $\left[CO_3^{2-}\right]$ lead to a reduction in the calcium carbonate saturation state (Ω) for the two common polymorphs, aragonite and calcite, that marine organisms use to construct their $CaCO_3$ shells or tests:

$$\Omega = \left[CO_3^{2-}\right]\left[Ca^{2+}\right]K_{sp}^{*-1} \quad (3)$$

where K_{sp}^* = apparent stoichiometric solubility coefficient for aragonite or calcite.

In the contemporary ocean surface waters, Ω typically is >1 for both aragonite and calcite (e.g., Cao and Caldeira 2008, see Table 1). Once Ω drops <1, dissolution of $CaCO_3$ is thermodynamically favored. The carbonate ion concentration, and hence Ω, is also a function of salinity, with lower values in brackish waters (Table 1).

Coastal hypoxia is a problem of global dimensions and has been a research focus during the last decades. Diaz and Rosenberg (2008) describe severely hypoxic zones from 400 systems worldwide, covering an area of >245,000 km^2. An oxygen concentration of about 60–70 μM (~20–30 % air saturation) has been suggested as an important hypoxic threshold value (e.g., Diaz and Rosenberg 2008; Vaquer-Sunyer and Duarte 2008; Levin et al. 2009), as pronounced sublethal effects (e.g., aberrant behavior, growth

Table 1 Carbonate system speciation ($S = 20$ vs. $S = 35$) in fully air-saturated seawater for scenarios 1×, 2× and 3× CO_2, assuming constant total alkalinities (A_T)

Scenario	pCO_2 (μatm)	$C_{T,fut}$ (μmol kg^{-1})	A_T (μmol kg^{-1})	pH_{NBS}	Ω_{calc}	Ω_{arag}
$S = 20$, $[O_2] = 310.4$ μM						
1× CO_2	390	1,877	1,976	8.18	2.22	1.33
2× CO_2	780	1,948	1,976	7.90	1.23	0.74
3× CO_2	1,170	1,987	1,976	7.73	0.85	0.51
$S = 35$, $[O_2] = 282$ μM						
1× CO_2	390	2,153	2,355.5	8.17	3.57	2.27
2× CO_2	780	2,262	2,355.5	7.90	2.07	1.32
3× CO_2	1,170	2,317	2,355.5	7.74	1.47	0.93

A_T was calculated using an A_T versus salinity relationship for the Western Baltic Sea (Beldowski et al. 2010). All scenarios were calculated from A_T and pCO_2 using CO2SYS (Lewis and Wallace 1998)

reductions, alterations in reproductive effort) often start to be apparent in many coastal invertebrates under such conditions. Many seasonal hypoxic coastal systems ('dead zones' sensu Diaz and Rosenberg 2008) are mainly located along Northern European seas and estuaries, the North American east coast and the Gulf of Mexico, as well as the coasts of Japan and China, that is, these systems follow the patterns of the human footprint. Hypoxia and anoxia have profound effects on benthic ecosystems, resulting in habitat compression, loss of fauna and biodiversity and large decreases in secondary production (see Diaz and Rosenberg 2008). Generally, eutrophication is regarded as the primary causative agent for many coastal hypoxic systems. Elevated nutrient concentrations and dissolved organic matter (DOM), typically introduced into coastal areas by means of river discharge but also by land run off, lead to enhanced rates of production of particulate organic matter (POM), partly sinking out from the surface layer to deeper waters and the benthos. The subsequent bacterial degradation of organic matter depletes the oxygen content of the water column (see Gray et al. 2002). High degrees of stratification in temperate waters and associated suppression of ventilation from above make the subsurface waters ideal nurseries for hypoxia and anoxia in the summer and autumn months.

Except for a few exceptions (e.g., Truchot and Duhameljouve 1980; Burnett 1997; Seibel and Walsh 2003; Brewer and Peltzer 2009), the biological review literature has often neglected the fact that coastal hypoxia is necessarily coupled to acidification and that the degree of acidification in hypoxic regions may represent a significant additional abiotic stressor and, occasionally, an adaptive physiological effector. Heterotrophic degradation of organic matter always results in the production of carbon dioxide and therefore acidification. There are some good examples available of simultaneous measurements of $[O_2]$ and carbonate system speciation in hypoxic coastal regions. A linear relationship between pH and $[O_2]$ has been obtained for the Charleston Harbor Estuary (east coast USA, Burnett 1997), with minimum pH_{NBS} values of <7.0 and maximum pCO_2 values in excess of 10,000 µatm (Cochran and Burnett 1996). Inverse relationships between $\Delta[O_2]$ and ΔC_T have been documented for, for example, Tampa Bay (Florida, USA), where strong diurnal patterns exist due to daytime photosynthesis versus nighttime respiration (Yates et al. 2007), the Scheldt Estuary (Belgium), where almost equimolar changes in $[O_2]$ and C_T led to extremely high pCO_2 values of >5,000 µatm (Frankignoulle et al. 1996), oxygen-minimum zones (Paulmier et al. 2011), the very large Gulf of Mexico and East China Sea hypoxic zones (Cai et al. 2011), and urban estuaries along the western Pacific coast (Feely et al. 2010).

In the following, we present data from a 10-year data set on $[O_2]$ and pH_{NBS} from the Boknis Eck Time Series Station (BE) in the Western Baltic Sea to illustrate the close relationship between both parameters in a seasonal hypoxic coastal system and calculate the carbonate system speciation at BE using C_T and A_T measurements obtained in 2009–2010. We then present calculations of pCO_2 in coastal hypoxic habitats and try to extrapolate pCO_2 values for such systems, assuming a doubling (tripling) in surface seawater pCO_2 during this century (the next centuries), with particular reference to the high sensitivity of estuarine systems. We discuss the implications of these results for ongoing efforts in hypoxia and ocean acidification research.

Methods

Time series data

Time series data from the Boknis Eck Time Series Station (BE) in Eckernfoerde Bay (greater Kiel Bay, 54°31,77N, 10°02,36E, see Fig. 1) were collected on monthly cruises as described in Hansen et al. (1999). Salinity, temperature and pH_{NBS} were measured with sensors attached to a CTD rosette, and oxygen concentrations were measured using the Winkler method (Grasshoff et al. 1983). The pH electrode was calibrated before use using pH buffers (NBS) prepared according to Grasshoff et al. (1983). Water samples for $[O_2]$ analysis were obtained from discrete depths (1, 5, 10, 15, 20, 25, 27 m). For our analysis, data were filtered for records 20 m and deeper with simultaneous measurements of oxygen, salinity, temperature and pH_{NBS}. This resulted in a data set of 280 complete records, with between 18 and 29 samples per month and spanning a time interval of 10 years between 1986 and 1995. In order to calculate seawater pCO_2 during this time interval, we estimated total alkalinity using the salinity–A_T relationship obtained by Beldowski et al. (2010) for the Western Baltic Sea. The carbonate system speciation was then calculated from estimated A_T and measured pH_{NBS} using CO2SYS (Lewis and Wallace 1998) with $KHSO_4$ dissociation constant according to Dickson (1990) and dissociation constants K_1 and K_2 according to Roy et al. (1993).

In order to obtain more precise information on seasonal changes in pCO_2 at station BE, we sampled water for accurate determinations of A_T and C_T during an entire seasonal cycle. Water samples were taken from 20 and 25 m depths on monthly cruises with the research vessel FK Littorina between January 2009 and March 2010 in the framework of BE time series measurements. Samples were stored in 500-ml Duran glass bottles, immediately poisoned using 100 µl saturated mercuric chloride solution and

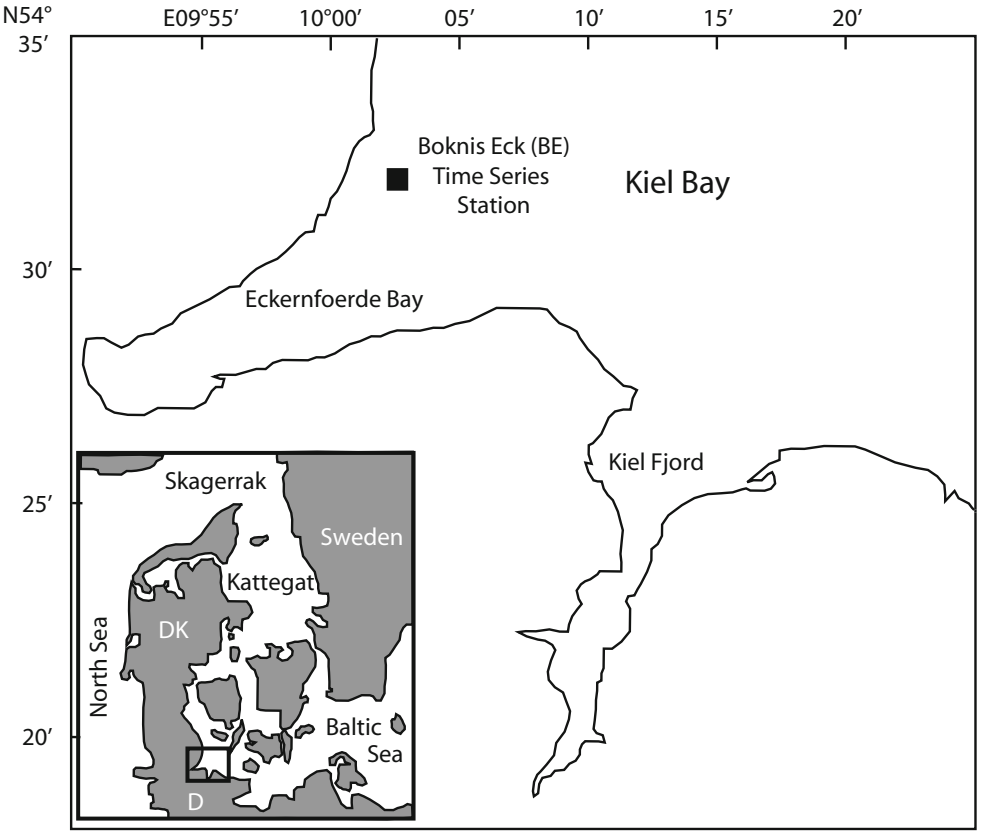

Fig. 1 Map of Kiel Bay in the Western Baltic Sea indicating the location of Boknis Eck Time Series Station (BE) on the entrance of Eckernfoerde Bay (54°31′N, 10°02′E, max. water depth 28 m)

stored at room temperature until analysis. C_T and A_T were measured by coulometric and potentiometric titration using SOMMA and VINDTA autoanalyzers, respectively (Mintrop et al. 2000; Dickson et al. 2007). A_T and C_T measurements were corrected using measurements of certified seawater standards (Dickson et al. 2007). pCO_2 and pH_{NBS} were then calculated using the CO2SYS program as described above. All other parameters (S, T, O_2) were determined as described by Hansen et al. (1999).

Surface seawater pH_{NBS} was measured on September 6, 2009, between 11:00 and 20:00 around Kiel Fjord following 2 days of strong south–westerly winds that caused upwelling of deeper water layers. Measurements were taken manually in water depths of ca. 0.5 m using a WTW pH meter equipped with a Sentix 81 pH_{NBS} electrode. More data on surface pH variability and carbonate chemistry of Kiel Fjord in 2008–2010 are given in Thomsen et al. (2010).

Carbonate system calculations

The carbonate system speciation in Table 1 and Figs. 2 and 3 was calculated using CO2SYS (Lewis and Wallace 1998), with K_1 and K_2 from Roy et al. (1993), assuming silicate and phosphate = 0 μmol kg^{-1} for present and future seawater pCO_2 values (1× CO_2 = 390 μatm, 2× CO_2 = 780 μatm, 3× CO_2 = 1,170 μatm) and total alkalinities (A_T) for salinities of 20–35 based on an A_T salinity relationship obtained by Beldowski et al. (2010). Additional generation of C_T from respiration ($C_{T,resp}$) in hypoxic regions was estimated using a $\Delta[O_2]/\Delta C_T$ conversion factor of −1.34 from Körtzinger et al. (2001; this factor is equivalent to a respiratory quotient (RQ) of 0.75) and an initial $[O_2]$ of 310.4 μM (at $S = 20$, $T = 10$ °C) and 282 μM (at $S = 35$, $T = 10$ °C), assuming 100 % air saturation. Future C_T ($C_{T,fut}$) thus was calculated as the sum of current C_T ($C_{T,curr}$), C_T from respiration processes ($C_{T,resp}$) and C_T from the invasion of anthropogenic CO_2, that is, ocean acidification ($C_{T,oa}$):

$$C_{T,fut} = C_{T,curr} + \Delta C_{T,oa} + \Delta C_{T,resp} \quad (4)$$

Seawater pCO_2, pH_{NBS} and Ω_{arag} values for the hypoxic model systems in Fig. 5 were then calculated from $C_{T,fut}$ and A_T. A_T remains constant when additional CO_2 is introduced into the system (e.g., Dickson et al. 2007). Our calculations do not include potential effects of future temperature change (which would lower oxygen solubility but increase the rate of respiratory decline in $[O_2]$, thus further increasing duration and severity of the hypoxic period). We also did not consider potential changes in A_T in a fully anoxic system. Thus, the calculations presented in Fig. 5 should be viewed as a rough indicator of magnitudes of change in pCO_2 that can be expected in coastal hypoxic systems.

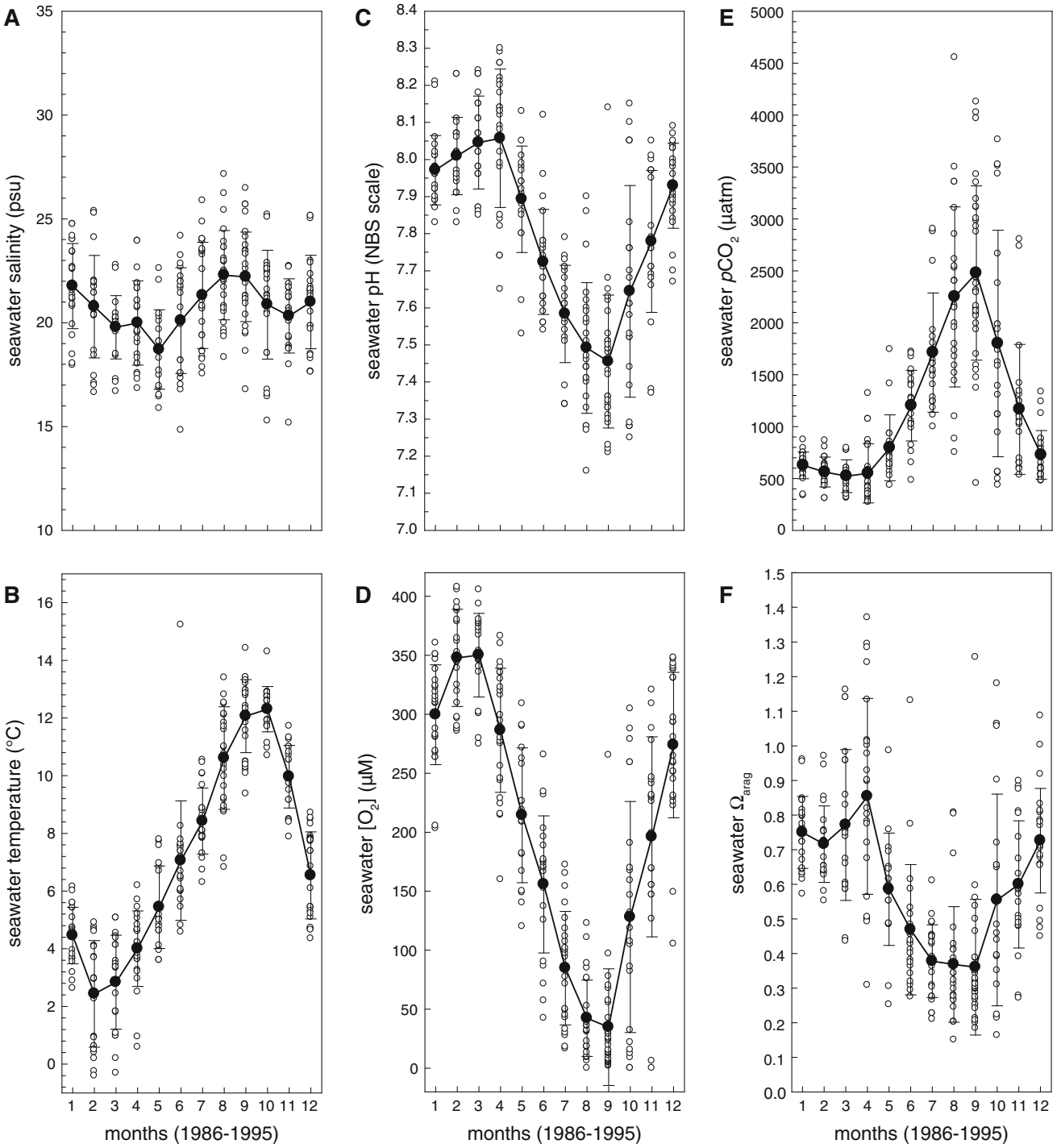

Fig. 2 Seasonal variations in salinity, temperature, $[O_2]$, pH_{NBS} (measured), pCO_2 and Ω_{arag} (estimated) between 1986 and 1995 at Boknis Eck Time Series Station (BE). Open symbols in **a**, **b** indicate individual measurements, *filled symbols* are monthly mean values, and *error bars* are standard deviations. Carbonate system calculations (pCO_2, Ω_{arag}) are based on measured pH_{NBS} and calculated A_T using an A_T–salinity relationship for the Western Baltic Sea (Beldowski et al. 2010). $[O_2]$, salinity and temperature at BETS have previously been published by Hansen et al. (1999) for the period 1957–1997

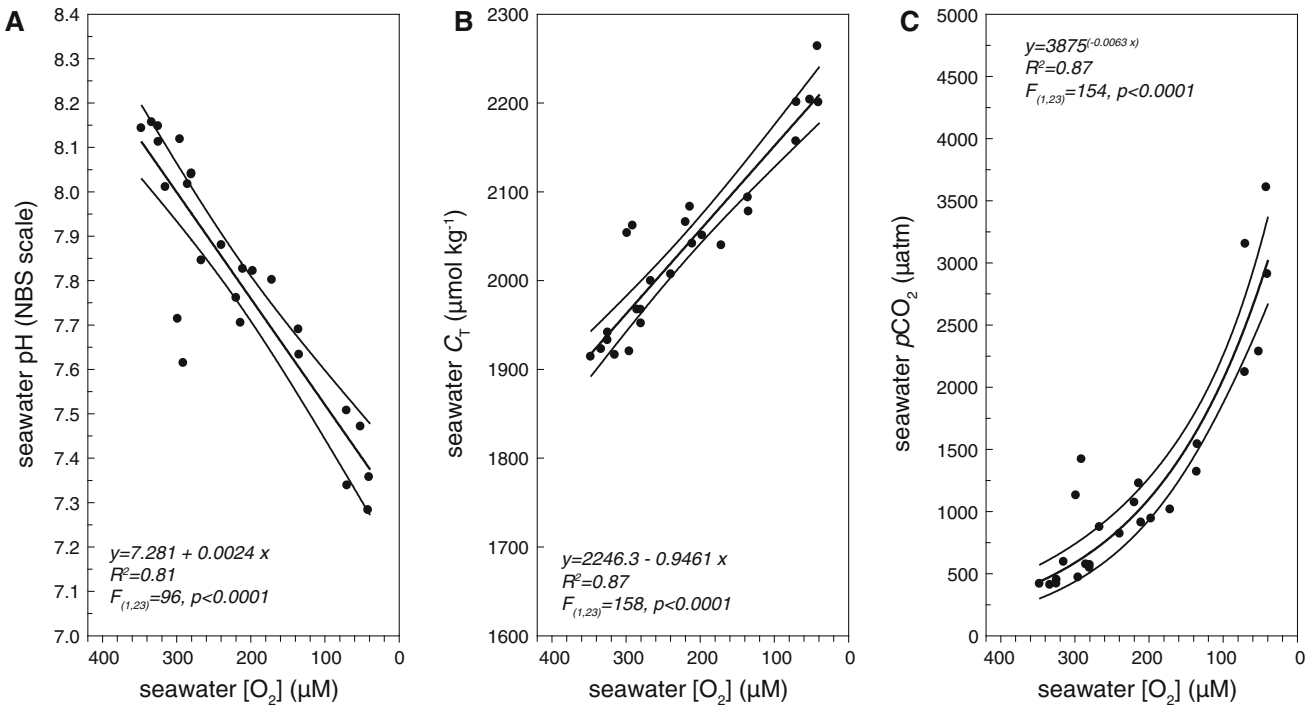

Fig. 3 Seasonal variation in carbonate chemistry at Boknis Eck Time Series Station (BE) between 2009 and 2010 in relation to $[O_2]$. Linear (**a**, **b**) and nonlinear (**c**) regressions of pH_{NBS}, dissolved inorganic carbon (C_T) and pCO_2 versus seawater $[O_2]$. pHNBS and pCO_2 were calculated from A_T and C_T measurements. Measurements were taken at the same location as those represented in Fig. 2

Results

Time series data

At the Boknis Eck Time Series (BE) Station in Kiel Bay (Fig. 1), Western Baltic Sea, stratification during the summer months results in the formation of a deeper water body at depths greater than 15 m that is shielded by a thermohaline pycnocline and therefore cannot equilibrate with the atmosphere until storms aid convective mixing in late autumn. From March–April onwards, $[O_2]$ in the bottom layers (>20 m) decreases linearly due to respiratory processes at a rate of 1.5–2 µM day^{-1}, typically reaching values of 0–50 µM in August and September (Hansen et al. 1999). $[O_2]$ and pH_{NBS} were measured simultaneously at the BE Time Series Station between 1986 and 1995 (Hansen et al. 1999). Monthly averages of pH_{NBS} followed the pattern in $[O_2]$ (Fig. 2c, d), with an average minimum pH_{NBS} of <7.5 observed in August and September. Calculating seawater pCO_2 and Ω_{arag} from pH_{NBS} and (estimated) A_T illustrates that most likely, average pCO_2 >1,000 µatm was encountered during June–November and pCO_2 >2,000 µatm during August and September (Fig. 2e). Peak pCO_2 values exceeded 3,000 µatm in autumn. Average Ω_{arag} mirrored pCO_2, reaching average minimum values of <0.5 during June–September and never rising above 1 during the entire seasonal cycle. Our accurate carbonate system determinations at BE Time Series Station during 2009–2010 obtained from the same depth horizon (Fig. 3) essentially confirm the rough estimates of pCO_2 obtained using the 1986–1995 data set: seawater pH_{NBS} declined linearly (Fig. 3a), C_T increased linearly (Fig. 3b) and pCO_2 increased exponentially (Fig. 3c) with declining $[O_2]$. Peak pCO_2 values of 2,500–3,500 µatm were observed when $[O_2]$ fell below 50 µM. While it could be argued that such stressful conditions are only encountered by benthic communities below thermohaline pycnoclines, upwelling processes can bring shallow coastal ecosystems in contact with the 'corrosive' subsurface waters (sensu Feely et al. 2008) that contain large amounts of respiratory CO_2. Figure 4 gives such an example: surface water pH_{NBS} values <7.5 were measured in September 2009 in the inner regions of Kiel Fjord following 2 days of strong southwesterly winds, while the outer parts of the Fjord retained a pH_{NBS} of 7.9-8.1. Due to the long chemical equilibration time scale, upwelled water retains high pCO_2 for long times, while physically dissolved oxygen equilibrates more rapidly with the atmosphere. Thomsen et al. (2010) demonstrated that surface water (<1 m depth) pCO_2 in Kiel Fjord follows the seasonal cycle observed in deeper layers of Kiel Bay (Fig. 2), albeit with a higher variability and lower pCO_2.

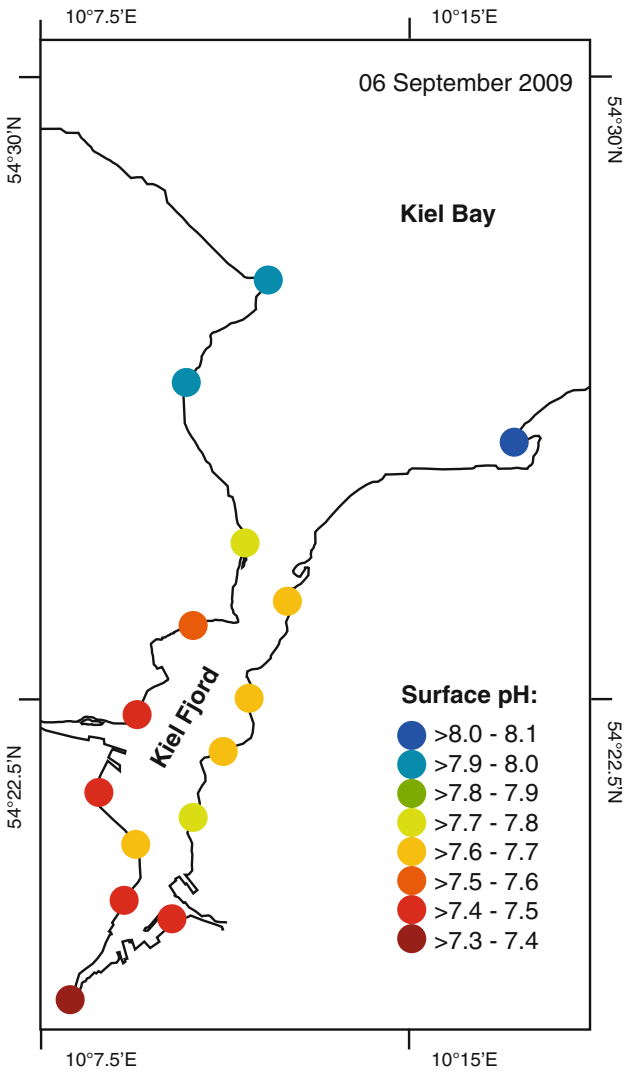

Fig. 4 Upwelling of low-pH water in Kiel Fjord. Surface seawater pH_{NBS} measured on September 6, 2009, between 11:00 and 20:00 around Kiel Fjord in water depths of ca. 0.5 m

Model calculations

Figure 5a–c gives a simulation of the changes in carbonate system speciation that can be expected during a typical seasonal hypoxic event for a salinity of 20 at a temperature of 10 °C for current pCO_2 (1× CO_2 = 390 µatm) and two future scenarios (2× CO_2 = 780 µatm, 3× CO_2 = 1,170 µatm; Table 1). Under current conditions (1× CO_2), patterns calculated resemble the measurements obtained at BE time series during 2009–2010 (Fig. 3), with pCO_2 increasing exponentially (Fig. 5a), pH decreasing linearly (Fig. 5b) and Ω_{arag} decreasing exponentially (Fig. 5c) with decreasing $[O_2]$. Maximum pCO_2 (ca. 3,200 µatm), minimum pH_{NBS} (ca. 7.3) and Ω_{arag} (ca. 0.2) at $[O_2] = 0$ µM are close to values observed or estimated for the field at Time Series Station BE (Figs. 2, 3). Increased C_T in the future ($C_{T,\,fut}$) due to ocean acidification ($\Delta C_{T,\,OA}$) leads to an enhanced sensitivity of the system during hypoxia: a doubling of surface pCO_2 to 780 µatm results in an increase in pCO_2 to ca. 4,500 µatm when all oxygen is consumed, thus a relative increase by ca. 1,300 µatm in comparison with the 1× CO_2 scenario. The 3× CO_2 scenario would lead to maximum pCO_2 values in excess of 5,000 µatm at $[O_2] = 0$ µM. As pCO_2 is the primary driver affecting animal biology, these large changes are of particular relevance. Correspondingly, Ω_{arag} is affected very strongly by 2× and 3× CO_2 scenarios, with undersaturation occurring at full air saturation already and very low Ω_{arag} (<0.5) being reached at relatively high oxygen content of the seawater (250–300 µM). Minimum pH_{NBS} values between 7.1 and 7.2 are reached in 2× and 3× CO_2 scenarios when all oxygen is consumed.

Figure 5d–f gives a simulation of the effects of salinity on carbonate system speciation when $[O_2] = 0$ µM. The figures clearly illustrate that increases in pCO_2 are more severe in brackish scenarios, which is related to the lower A_T and hence buffer capacity. Highest pCO_2 values of ca. 3,200 µatm at $S = 20$ contrast with maximum pCO_2 values of ca. 1,700 µatm at $S = 35$ (1× CO_2). Future scenarios indicate that the influence of salinity on maximum pCO_2 decreases, which leads to relatively higher increases in pCO_2 in the high-salinity scenarios, with maximum pCO_2 values ca. 4,500 µatm at 3× CO_2 and $S = 35$ (Fig. 5d). Similarly, the relative sensitivity of seawater pH_{NBS} and Ω_{arag} to salinity changes decreases from 1× to 2 × and 3× CO_2 scenarios (Fig. 5e, f).

Discussion

Our field data and simulations indicate exponential increases in pCO_2 with declining $[O_2]$ and a very high sensitivity, particularly of estuarine habitats, to the combined impacts of C_T increases due to ocean acidification and due to respiration. Magnitudes of pCO_2 reached now and in future scenarios in such systems have been shown to strongly impact animal biology. In the following sections, we will briefly discuss biological impacts of ocean acidification (hypercapnia) and of hypoxia, as well as of those of hypoxic hypercapnia, on animal physiology and fitness. We argue that it is important in experimental research to consider both factors simultaneously, as both probably significantly impact the organisms' energy budgets and, ultimately, fitness.

Ocean acidification (hypercapnia)

Biological effects of ocean acidification on heterotrophic metazoans are not understood well at present, but it is clear that at least three effects play a role: (1) direct effects of

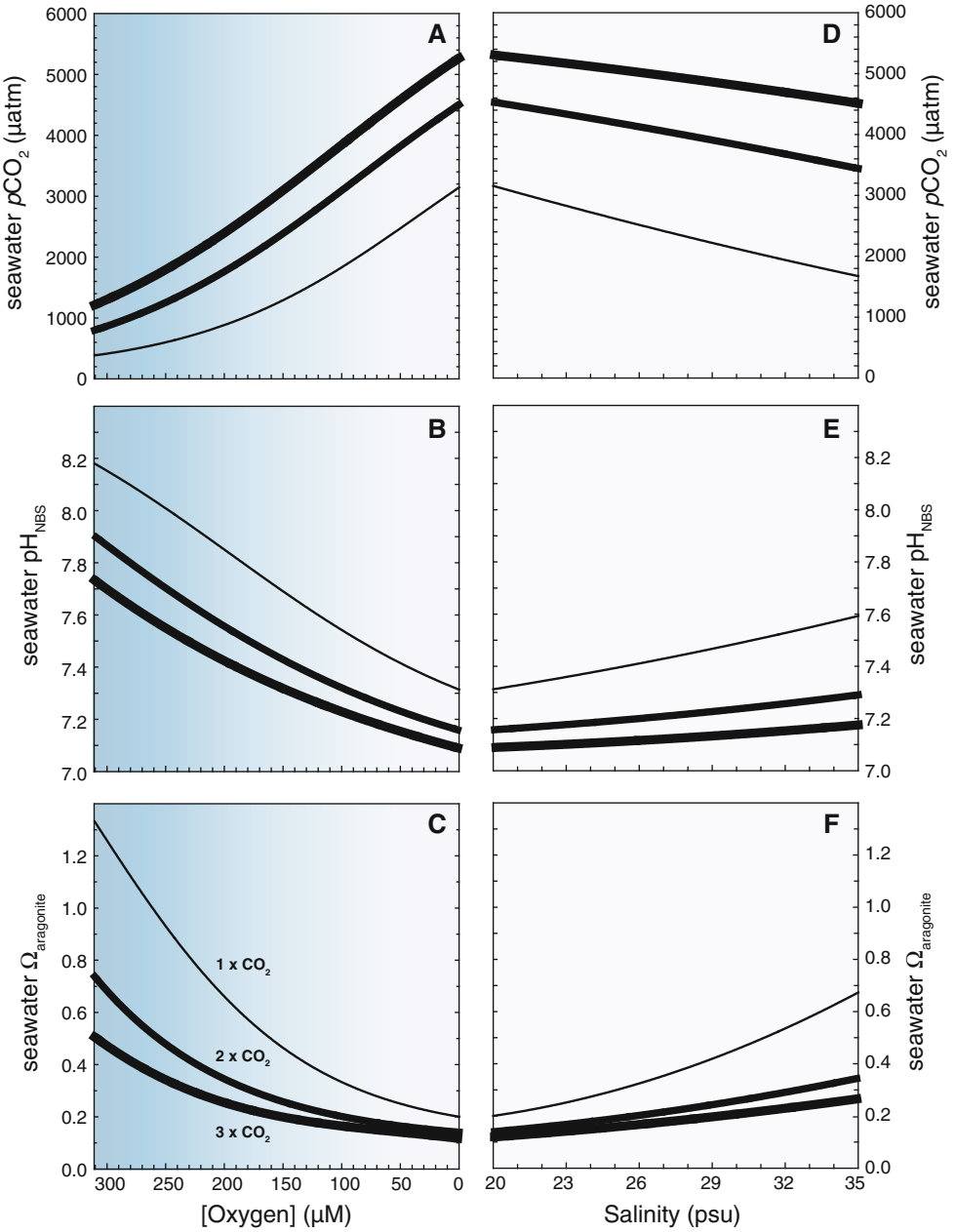

Fig. 5 Seawater pCO_2, pH_{NBS} and Ω_{arag} calculated for $S = 20$, $T = 10$ °C in response to changes in $[O_2]$ (**a**, **b**, **c**) and in response to salinity changes between $S = 20$–35, $T = 10$ °C for $[O_2] = 0$ µM (**d**, **e**, **f**). All calculations were made using CO2SYS software (Lewis and Wallace 1998). *Thin line*: 1× CO_2, intermediately *thick line*: 2× CO_2, *thick line*: 3× CO_2

altered carbonate chemistry on external shell dissolution in calcifiers, (2) pathological effects of an altered acid–base status on biomineralization and physiological processes and (3) impacts of an altered acid–base status on species' energy budgets. (1) Direct effects of low $\left[CO_3^{2-}\right]$ have been observed in some invertebrates: shell dissolution was observed at $\Omega < 1$ in pelagic snails with only thin (or no) organic coating protecting their shells (pteropods, e.g., Lischka et al. 2011). However, recent studies indicate that net calcification of exoskeletons proceeds at seawater $\Omega \ll 1$ in many marine metazoans (e.g., Ries et al. 2009; Thomsen et al. 2010; Dupont et al. 2010), even in those that experience external shell dissolution at the same time (Lischka et al. 2011). The organic matrix that is a crucial component of all skeletons and tests of marine organisms, as well as organic shell covers, probably is the chief reason that prevents external dissolution at $\Omega < 1$ (see Ries et al. 2009; Tunnicliffe et al. 2009; Thomsen et al. 2010). (2) Indirect effects of ocean acidification can result from disturbances in extra- and intracellular acid–base balance. All heterotrophic metazoans studied maintain positive diffusion gradients of CO_2 toward the surrounding medium, resulting in relatively high body fluid (extracellular) pCO_2 values of ca. 1,000–4,000 µatm (Melzner et al. 2009, Fig. 6). Thus, pCO_2 is the main effect or that impacts heterotrophic metazoans during ocean acidification as it

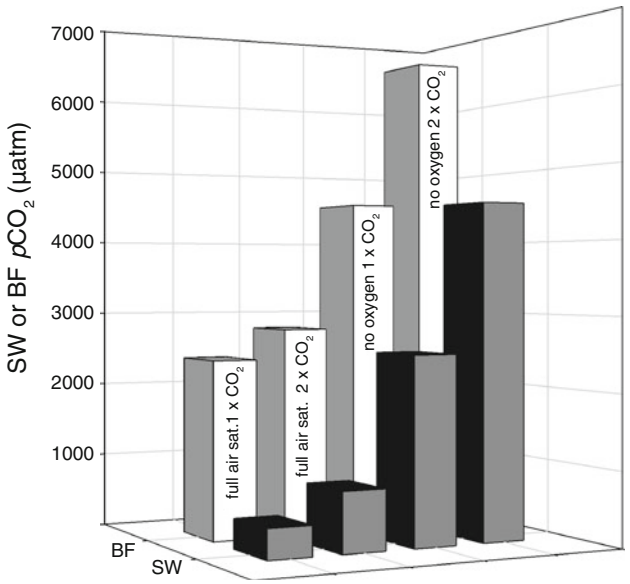

Fig. 6 Schematic illustration depicting the effects of seawater pCO_2 on extracellular (blood, hemolymph or coelomic fluid) pCO_2 in marine animals: typically, a gradient in pCO_2 of 1,000–4,000 µatm (here: 2,000 µatm) is maintained between extracellular fluids and seawater in order to drive diffusive excretion of metabolic CO_2. It is evident from this diagram that the exponential increases in pCO_2 that go along with hypoxic hypercapnia lead to extreme extracellular acid–base disturbance. Increased extracellular pCO_2 will either result in very high extracellular $[HCO_3^-]$ in extracellular pH-regulating animals or in very low pH and Ω_{arag} in animals that do not regulate extracellular pH. Both scenarios can lead to pathologies and disturbances of the energy budget (see text)

results in elevated body fluid pCO_2 so respiratory diffusion gradients can be maintained. Some organisms compensate this disturbance by actively altering their extracellular carbonate system speciation (i.e., net accumulation of HCO_3^-) to stabilize extracellular pH (e.g., fish), whereas others do not (e.g., bivalves; see Seibel and Walsh 2003; Melzner et al. 2009 for review). Either way, CO_2-induced changes in extracellular acid–base equilibria can have repercussions on functional processes: an altered blood carbonate system speciation may be responsible for pathologies such as hypercalcification observed in cephalopods, crustaceans and teleost fish (Gutowska et al. 2010) and has been demonstrated to lead to pathological behavioral disturbances in teleost fish (Munday et al. 2009; Dixson et al. 2010; Nilsson et al. 2012). Very high extracellular pCO_2 in animals that do not regulate extracellular pH can also lead to internal shell dissolution (Melzner et al. 2011) and might induce oxidative stress (Tomanek et al. 2011). (3) On the other hand, an unaltered carbonate system speciation may induce metabolic depression (Pörtner et al. 2005) or increase the energetic costs for homeostasis and calcification (Wood et al. 2008; Thomsen and Melzner 2010, Stumpp et al. 2011, Deigweiher et al. 2009, Widdicombe and Spicer 2008). In many calcifying marine animals, the initial step of calcification probably is the intracellular formation of an amorphous calcium carbonate (ACC) precursor phase (see Weiner and Addadi 2011 for a review). Thus, cell pH and ion homeostasis are tightly linked to biomineralization. As intracellular pH is maintained relatively constant in most marine animals even under hypercapnic conditions (e.g., Zange et al. 1990; Ellington 1993), hypercapnia-induced changes in extracellular pCO_2 and the dependent reductions in pH in weak pH regulators can adversely affect the energetics of ion exchange between intra- and extracellular compartments, primarily by increasing the driving force for protons to enter the cytosol. Thus, changes in seawater pCO_2 can potentially impact a range of cellular processes that translate into elevated energetic costs and cause energy budget reallocation (e.g., Dupont et al. 2010; Stumpp et al. 2011).

Hypoxia

Several recent contributions describe responses of marine organisms and communities to low oxygen concentrations (e.g., Grieshaber et al. 1994; Diaz and Rosenberg 1995, 2008; Burnett 1997; Boutilier 2001; Gray et al. 2002; Rabalais et al. 2002; Levin et al. 2009; Seibel 2011). While it is evident that common definitions on the use of units and sensitivity thresholds are missing in the literature (however, see Hofmann et al. 2011), two principle types of responses of heterotrophic marine metazoans with respect to declining oxygen concentrations can be distinguished: oxyregulators maintain rates of oxygen consumption with declining water pO_2, typically by increasing diffusion gradients across their gas-exchange epithelia. This can be achieved via (energy intensive) increases in ventilatory and or circulatory convection effort, increases in perfused gas-exchange areas and decreases in diffusion distances (e.g., see Grieshaber et al. 1994; Burnett 1997; Farrell and Richards 2009; Richards 2009 for biochemical and molecular adaptation mechanisms). However, once a critical oxygen partial pressure ($pO_{2,crit}$) is reached, oxygen consumption and demand are downregulated. Eventually, anaerobic metabolism is needed to support the organism's energetic requirements (see Pörtner and Grieshaber 1993; Grieshaber et al. 1994). Oxyconforming animals do not maintain oxygen consumption rates when ambient pO_2 declines. However, these animals have to rely on anaerobic energy generation once $pO_{2,crit}$ is reached as well. Onset of anaerobic metabolism marks time-limited survival. The depletion of fermentable substrate (e.g., glycogen reserves) eventually results in catastrophic drops in cellular [ATP], which induces a cascade of events that lead to depolarization of membranes, Ca^{2+} entry into the cytoplasm and cell death (see Boutilier 2001 for review). Critical pO_2

values for heterotrophic metazoans are species specific and typically reached at an air saturation between 5 and 40 % (ca. 1–8 kPa; e.g., Grieshaber et al. 1994; Cochran and Burnett 1996; Zielinski et al. 2000; Nilsson et al. 2007; Mandic et al. 2009). However, effects of hypoxia on energy allocation and energy budgets (e.g., growth performance, reproductive effort) can usually be observed well before $pO_{2,crit}$ is reached and may be more important in defining ecological fitness (see Gray et al. 2002 for review).

Few benthic marine animals can survive prolonged periods of severe hypoxia or anoxia. Among the hypoxia tolerant, many annelids and mollusks are characterized by specific physiological adaptations that enable them to use limited energy stores more efficiently during anoxia. While the final cause of death is due to thermodynamic imbalances in cellular energy metabolism in both hypoxia-tolerant and hypoxia-intolerant species (see above), the former are adapted to downregulate cellular energy demand ('metabolic depression', Hochachka 1986; Guppy and Withers 1999; Guppy 2004) and to use anaerobic metabolic pathways that are more energy-yielding (Grieshaber et al. 1994; Hochachka and Lutz 2001) via downregulation of cellular ATP and thus oxygen demand by a factor of 10–100 and increases in ATP yield by factors of 2–3 through the utilization of, for example, succinate and propionate as anaerobic end products, such species can survive anoxia on limited glycogen stores for several weeks (e.g., Dries and Theede 1974; Oeschger 1990; DeZwaan et al. 1991; Grieshaber et al. 1994; Boutilier 2001). In the deeper regions of Kiel Bay, for example, benthic communities are dominated by bivalves (*Astarte* spp. and *Arctica islandica*) that can survive weeks of anoxia by utilizing these key biochemical mechanisms: during a 50-day anoxic incubation, *A. islandica* was shown to accumulate high tissue concentrations of succinate (ca. 200 μmol g^{-1} dry mass) while simultaneously reducing aerobic metabolic rate by more than 99 % (Oeschger 1990).

Hypercapnic hypoxia

As indicated above, the majority of laboratory experimental work on hypoxia tolerance of marine organisms has been carried out without the simultaneous manipulation of pCO_2 (see references discussed in Grieshaber et al. 1994; Gray et al. 2002; Levin et al. 2009). While it seems clear that hypoxia and hypercapnia both can impact animal energy budgets and thus might act additively or synergistically (see above), few studies have independently quantified hypoxia versus hypercapnia effects. Cochran and Burnett (1996) found that hypercapnia does not alter $pO_{2,crit}$ in two estuarine fish and a crustacean (shrimp) species. Similarly, Rosa and Seibel (2008) found pO_2 and temperature, but not pCO_2, to be the dominating factors determining metabolic rate reductions in a pelagic squid species that undergoes vertical migrations into oxygen-minimum zones. Other studies suggest that elevated pCO_2 and hypoxia may act synergistically in reducing oyster fitness by stimulating parasitism and decreasing defense mechanisms: Boyd and Burnett (1999) measured a decreased production of reactive oxygen species (ROS) in oyster blood cells (hemocytes). ROS are used by hemocytes in the defense against foreign cells, such as pathogenic bacteria. In addition, Dwyer and Burnett (1996) suggested that acidic conditions favor the growth of a protozoan parasite in oysters. Further work from Burnett's working group suggests an impairment of immune functions in estuarine organisms when hypoxia is combined with hypercapnia (e.g., Boyd and Burnett 1999; Mikulski et al. 2000). On the other hand, CO_2 also serves as an important adaptive signal during hypoxia: increases in the affinity of respiratory pigments (hemocyanins) toward oxygen in crustacean species are caused by elevated pCO_2 (Booth et al. 1982; Mangum and Burnett 1986). In fish, elevated pCO_2 elicits a ventilatory response that is thought to be mediated through branchial gas receptors (e.g., Gilmour 2001; Vulesevic et al. 2006). Finally, hypercapnia has also been suggested to aid in eliciting beneficial metabolic depression by altering extra- and or intracellular acid–base status (see Guppy and Withers 1999).

Clearly, the magnitude of acidification that can currently be expected in hypoxic coastal areas (i.e., pCO_2 >1,700–3,200 μatm, Fig. 5d, see also Feely et al. 2010: Pacific urban estuary, Cai et al. 2011: Gulf of Mexico, East China Sea) is greater than what is predicted for the next few 100 years due to ocean acidification in the oxygenated surface ocean (Caldeira and Wickett 2003, 2005). Linear increases in C_T due to respiration lead to exponential increases in pCO_2, the main physiological effector for heterotrophic metazoans (Fig. 6). Wind-driven upwelling can bring hypoxic and hypercapnic waters from below pycnoclines in contact with productive shallow water ecosystems. Figure 4 illustrates wind-driven upwelling of low-pH water in Kiel Fjord, and Thomsen et al. (2010) showed that the general pattern of seasonal pH and pCO_2 found in Kiel Fjord surface water, although more variable, resembles that of the bottom waters at Boknis Eck Time Series Station (Figs. 2, 3). Maximum surface pCO_2 values around subtidal mussel beds in Kiel Fjord surpassed 2,300 μatm in September 2008, and average values in July and August (the main reproductive season for many calcifying invertebrates) averaged ca. 1,000 μatm (Thomsen et al. 2010). As the Baltic Sea is characterized by many seasonal and permanent hypoxic zones (HELCOM 2009) and wind-driven upwelling is common (e.g., Lehmann

et al. 2002; Myrberg and Andrejev 2003), it can be expected that a similar variability in carbonate system speciation is typical across the Baltic. Similar hypoxia-induced increases in pCO_2 will be characteristic for a multitude of coastal habitats worldwide (e.g., Diaz and Rosenberg 2008; see Cai et al. 2011 for a recent description of carbonate system speciation in East China Sea and Gulf of Mexico hypoxic zones).

Future ocean acidification and hypoxia

While the magnitude of acidification associated with low-oxygen conditions that can be attained under current conditions is already sizable, progressing invasion of anthropogenic CO_2, that is, ocean acidification, will further elevate seawater pCO_2 in hypoxic regions in a nonlinear fashion (Fig. 5). This has recently also been pointed out by Brewer and Peltzer (2009) who estimated future changes in pCO_2 in oxygen-minimum zones that correspond to magnitudes of change simulated here (Fig. 3). Our model calculations for the 2× CO_2 scenario show that coastal hypoxic zones may easily encounter pCO_2 values of 3,400–4,500 µatm when most oxygen is consumed (Fig. 5d). Other habitats enriched in C_T already now, for example oceanic oxygen-minimum zones (Keeling et al. 2010; Paulmier et al. 2011), continental upwelling systems (Feely et al. 2008), estuaries (Borges et al. 2004; Feely et al. 2010; Waldbusser et al. 2011) and estuarine salt marsh systems (Wang and Cai 2004), will likely be impacted in a similar manner by future ocean acidification. It is important to stress that in oceanic oxygen-minimum zones, ocean acidification–enhanced pCO_2 will be encountered with a significant time delay, which is related to transit times of decades to a century (Karstensen et al. 2008), between last contact with the atmosphere and arrival in the OMZ. In coastal waters with seasonal hypoxia, this time delay is on the order of months to 1 year. Hence, such regions will feel the combined effect of ocean acidification and hypoxia almost instantaneously.

High pCO_2 values >3,400–4,500 µatm have been shown to negatively affect many heterotrophic metazoans in carbonate system perturbation experiments (see, e.g., Fabry et al. 2008; Ries et al. 2009; Dupont et al. 2010; Hofmann et al. 2010 for review). In order to maintain outward CO_2 diffusion gradients, animals will be challenged by severe acid–base disturbances due to proportionally higher extracellular pCO_2 values in hypoxic hypercapnic regions (Fig. 6). These acid–base disturbances may then significantly increase costs for cellular homeostasis, which would impact energy stores needed for survival under hypoxic or anoxic conditions (see above). Extremely low Ω_{arag} will be encountered in the future as well (Fig. 5c, f). This will represent an additional stressor, particularly for many mollusks that dominate coastal benthic systems, as organic covers (periostraca) are typically thin or fractured in older specimens (Harper 1997; Thomsen et al. 2010). This will lead to enhanced rates of external shell dissolution. Future warming may exacerbate the problem by intensifying stratification, lowering saturating oxygen concentrations and elevating respiration rates, which individually and in combination could lead to a prolongation of hypoxic hypercapnic periods in seasonally hypoxic systems. This could then deplete the resistance capacity of even the most stress-tolerant species: during a severe hypercapnic hypoxic event in Kiel Bay in 1981, a 90 % decline in benthic biomass could be witnessed, with only few, highly anoxia-tolerant species surviving this period (the bivalves *Arctica islandica*, *Astarte* spp. and a few other species, Weigelt and Rumohr 1986). Additionally, it has been previously shown that elevated temperature results in reduced survival times under anoxia even in anoxia-tolerant bivalve species (Dries and Theede 1974). Clearly, the combined action of temperature, hypoxia and hypercapnia will constitute a severe challenge for benthic coastal ecosystems of the future (e.g., Pörtner et al. 2005).

Conclusions and implications for future research

Our basic calculations illustrate that hypoxic coastal areas are already characterized by pCO_2 values that will probably not be reached by ocean acidification in the surface ocean in the next few 100 years (i.e., depending on salinity, >1,700–3,200 µatm), with the potential for a 50–100 % increase within this century. Particularly high pCO_2 values and very low Ω_{arag} can be expected in highly productive estuarine habitats. Yet, little attention has been paid to the magnitudes and rates of change in pCO_2 in these systems. Studies that investigate the effects of hypoxia on benthic ecosystems and species should focus energy and resources toward:

1. Studying the carbonate system speciation in hypoxic habitats in relation to $[O_2]$ and temperature variation.
2. Studying potential upwelling events and seasonal dynamics of variability in carbonate system speciation.
3. Studying species and community responses under simultaneous application of realistic $[O_2]$, $[CO_2]$ and temperature combinations. This recommendation refers to both rate and magnitude of stress application. CO_2 can act both as a stressor and as an adaptive modulator of physiological function.

In ocean acidification research, it is often recommended to primarily utilize pCO_2 values between current and up to ca 1,000 µatm in experimental CO_2 perturbation experiments (based on IPCC scenarios, e.g., IPCC 2007).

However, respiration processes can significantly elevate pCO_2 in coastal waters, and upwelling of severely hypercapnic and hypoxic water may further elevate pCO_2. Additional CO_2 through ocean acidification can produce much higher pCO_2 values than predicted by the global surface ocean model predictions (e.g., Caldeira and Wickett 2005). Thus, experimental pCO_2 levels should be assigned in a case-sensitive manner after the specific carbonate system variability of the habitat in question has been studied. In a next step, future developments can be roughly estimated using standard carbonate system speciation software and applied to perturbation experiments.

It also should be kept in mind that hypoxic hypercapnic systems and upwelling systems as described here can serve as interesting model regions where responses of adapted marine ecosystems toward high levels of acidification can already be studied today (c.f. Thomsen et al. 2010). These systems may tell us much about animal tolerance limits in response to an altered carbonate system speciation. Whether these ecosystems also can cope with the high levels of acidification estimated here to occur within this century remains to be established.

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