2012

The Metabolic Response of Pteropods to Acidification Reflects Natural CO2-Exposure in Oxygen Minimum Zones

A. E. Maas
University of Rhode Island

K. F. Wishner
University of Rhode Island, kwishner@uri.edu

See next page for additional authors

Citation/Publisher Attribution

Maas, Amy E., K. F. Wishner and Brad A. Seibel. "The Metabolic Response of Pteropods to Acidification Reflects Natural CO2-Exposure in Oxygen Minimum Zones." Biogeosciences. 9(2):747-757. 2012.
Available at: http://dx.doi.org/10.5194/bg-9-747-2012

Authors
A. E. Maas, K. F. Wishner, and B. A. Seibel

Biogeosciences, 9, 747–757, 2012
www.biogeosciences.net/9/747/2012/
doi:10.5194/bg-9-747-2012

Biogeosciences

The metabolic response of pteropods to acidification reflects natural CO_2-exposure in oxygen minimum zones

A. E. Maas[1,*], **K. F. Wishner**[2], **and B. A. Seibel**[1]

[1]Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881, USA
[2]Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA
[*]now at: Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

Correspondence to: A. E. Maas (amaas@whoi.edu)

Received: 4 October 2011 – Published in Biogeosciences Discuss.: 21 October 2011
Revised: 27 January 2012 – Accepted: 30 January 2012 – Published: 15 February 2012

Abstract. Shelled pteropods (Thecosomata) are a group of holoplanktonic mollusks that are believed to be especially sensitive to ocean acidification because their aragonitic shells are highly soluble. Despite this concern, there is very little known about the physiological response of these animals to conditions of elevated carbon dioxide. This study examines the oxygen consumption and ammonia excretion of five pteropod species, collected from tropical regions of the Pacific Ocean, to elevated levels of carbon dioxide (0.10 %, 1000 ppm). Our results show that pteropods that naturally migrate into oxygen minimum zones, such as *Hyalocylis striata*, *Clio pyramidata*, *Cavolinia longirostris* and *Creseis virgula*, were not affected by carbon dioxide at the levels and duration tested. *Diacria quadridentata*, which does not migrate, responds to high carbon dioxide conditions with reduced oxygen consumption and ammonia excretion. This indicates that the natural chemical environment of individual species may influence their resilience to ocean acidification.

1 Introduction

Marine systems are a significant sink for the excess carbon produced by human activities. Since pre-industrial times, atmospheric carbon dioxide (CO_2) levels have risen from 280 ppm to the current 390 ppm (IPCC, 2007). This rise in atmospheric CO_2 concentration has grown at a slower rate than human output, a discrepancy which is due to the buffering capacity of the Earth's marine system; about 30 % of anthropogenic CO_2 ends up in the surface waters of the ocean (Sabine et al., 2004; Feely et al., 2009). As this gas dissolves and interacts with seawater, it dissociates into bicarbonate and free hydrogen ions, a process that reduces the ocean's pH and carbonate ion concentration. The pH of the ocean has already dropped by ∼0.1 units relative to preindustrial levels and is predicted to drop another 0.2 to 0.3 in the next one hundred years (Haugan and Drange, 1996; Caldeira and Wickett, 2003, 2005). Acidification has been identified as the third most pervasive human impact on the ocean (Halpern, 2008). It is therefore important to understand the physiological response of marine organisms to elevated CO_2 and reduced pH.

Elevated seawater CO_2 can be detrimental to organisms because it crosses biological membranes and reacts with intra- and extracellular fluids just as it does with ocean waters. The resulting internal acidosis influences a number of physiological processes (Seibel and Walsh, 2001, 2003; Seibel and Fabry, 2003; Pörtner et al., 2005; Miles et al., 2007; Widdicombe and Spicer, 2008). Organisms have some capacity to compensate for the pH change but at an energetic cost that may result in physiological trade-offs (Wood et al., 2008). The capacity of organisms to control acid-base balance is dependent on the rate of metabolic CO_2 production as well as exposure to natural environmental CO_2 levels. As a result, each species has a different tolerance level for environmental pH changes, with implications for growth, fecundity and survival.

Ocean acidification is also of particular concern for shell bearing organisms since calcification requires additional energy in the face of decreased carbonate ion concentration in seawater (Cohen and Holcomb, 2009). Anthropogenic CO_2 has already reduced the saturation state of calcium carbonate in the tropics. As a result, calcite precipitation has dropped by 6–11 %, and, based on climate models, this could reach 35 % in the next 100 yr (Kleypas et al., 1999). Thecosomatous pteropods have received a great deal of attention in ocean acidification discussions because they produce thin shells made of aragonite, a highly-soluble form of calcium

Table 1. Dates, locations, vessels used and species collected for physiological experiments during sampling.

Date	Location	Vessel	Species
June 2007	Gulf of California between 27° N 112° W and 111° W	R/V *New Horizon*	*H. striata* (5), *C. virgula* (8), *C. pyramidata* (17)
October–November 2007	Eastern Tropical Pacific between 9° N 90° W and 11° N 98°	R/V *Seward Johnson*	*H. striata* (30), *C. longirostris* (38), *C. virgula* (5), *C. pyramidata* (5), *D. quadridentata* (19)
December 2008–January 2009	Eastern Tropical Pacific between 9° N 90° W and 11° N 98°	R/V *Knorr*	

carbonate. These pelagic gastropods are found throughout the world, predominantly in near-surface seawater, although deep-sea species are also known to exist (Lalli and Gilmer, 1989). Studies of the potential effects of ocean acidification on pteropods have primarily focused on polar species because of their abundance and importance in regional food-webs and carbon biogeochemical cycles (Pakhomov et al., 2002; Accornero et al., 2003; Armstrong et al., 2005; Manno et al., 2010) and because the polar oceans are expected to reach undersaturation first due to the increased solubility of CO_2 in cold water (Comeau et al., 2011). The Arctic species *Limacina helicina* shows a 28 % decrease in calcification at 780 ppm CO_2, although it is capable of precipitating aragonite at low saturation states (Comeau et al., 2009, 2010a). Juveniles of *Limacina helicina* respond to elevated CO_2 (780 and 1100 ppm) with changes in shell diameter, shell increment and shell degradation (Lischka et al., 2011). The impact of CO_2 on the metabolic rate of this species, and its congener *Limacina helicina antarctica*, are less clear. It appears that environmental stressors, such as temperature and food availability, produce synergistic responses to CO_2, either increasing or decreasing metabolism (Comeau 2011; Seibel et al., 2012).

Little is known about the physiology of tropical pteropod species, and the impact of hypercapnia (high CO_2) has been reported only for one warm water species, *Cavolinia inflexa* (Comeau et al., 2010b). This study did not measure metabolic rate, but reported a reduction in calcification in juveniles over a 13 day exposure to high CO_2 (∼850 and 1700 ppm). There are no studies which relate the distribution of pteropods to regions of naturally occurring high carbon dioxide concentrations. In some pelagic ecosystems, such as the Eastern Tropical Pacific (ETP) and the Gulf of California, a pronounced oxygen minimum zone (OMZ) exists in which respiration below the photic layer outpaces mixing to create a region of low oxygen and elevated carbon dioxide. OMZs can therefore also be thought of as persistent carbon maximum zones (Paulmier et al., 2011). Specifically, in the ETP and Gulf of California around depths of 200 m, CO_2 levels reach higher than 1000 ppm (Fabry et al., 2008; Paulmier et al., 2011). This high CO_2 condition creates a zone of low pH, and correspondingly a decreased saturation state of calcium carbonate. Seawater is undersaturated with respect to aragonite ($\Omega_a < 1$) at 1000 ppm CO_2 in the ETP, suggesting that there would be passive dissolution of pteropod shells near 10° N by 200 m depth (Fabry et al., 2008). Testing whether these zones of low pH (<7.6) act as a barrier to pteropod distribution, and investigating the impact of hypercapnia on the metabolism of resident species will provide insight to the potential effects of anthropogenic acidification on organisms living in tropical surface waters. If pteropods are naturally found at hypercapnic conditions, it is unknown whether they will respond to laboratory exposure to CO_2 with an increase in metabolism as some metabolic processes become up-regulated to deal with acidification, with a lowered metabolic rate to withstand the energy limitation brought on by acidosis or whether they will be unaffected. Here, we report the effect of short periods of hypercapnia (6–18 h, 1000 ppm CO_2) on the routine metabolic rate (oxygen consumption) and ammonia excretion of five species of thecosomatous pteropods. We then compare these organismal responses with the natural distribution of these species in relation to the OMZ of the ETP.

2 Methods

We collected individuals representing five species of thecosomatous pteropods (*Hyalocylis striata, Clio pyramidata, Cavolinia longirostris, Creseis virgula* and *Diacria quadridentata*) from three sites in the Pacific Ocean between June 2007 and January 2009 (Table 1). Hydrographic profiles of all three regions were assembled from CTD casts made during the time of organism collection (Fig. 1). Profiles of pH in the ETP in 2008 were measured using the standard SOP for pH analysis with *m*-cresol purple (Byrne and Elliott, unpublished data). Carbonate chemistry of the region was estimated using WOCE alkalinity values (P-18 1994 and 2008), pH and profiles of salinity and temperature using the CO_2sys developed by Lewis and Wallace (1998). The system was run using the seawater pH scale, Dickson $KHSO_4$, and constants from Dickson and Millero (1987). WOCE alkalinity data from 200 m was relatively consistent at nearby latitudes between 1994 (2298 ± 9.2) and 2008 (2300.5 ± 4.5), suggesting that these values are reasonable estimates of OMZ alkalinity.

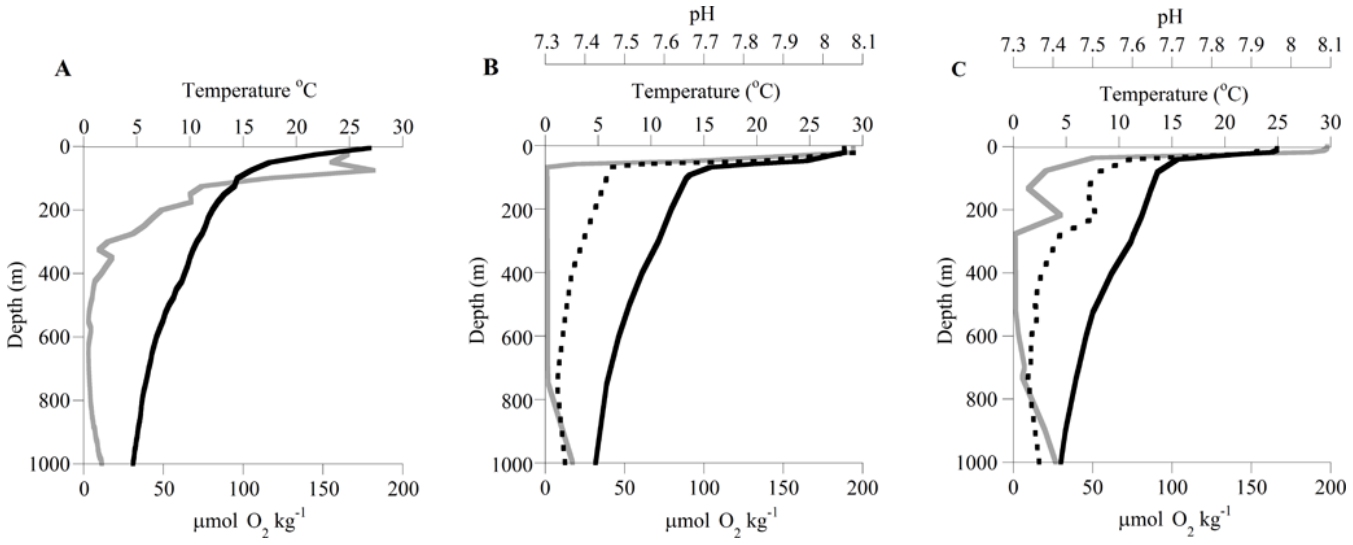

Fig. 1. A typical temperature (°C, black) and O_2 (µmol kg^{-1}, grey) profile for the Gulf of California in June 2007 **(A)**, the Tehuantepec Bowl **(B)**, and the Costa Rica Dome **(C)**. The profiles from the Tehuantepec Bowl and the Costa Rica Dome are from 2008 and also show a pH profile (dashed black).

We retrieved animals from a 61 cm-diameter 335 µm-mesh bongo net trawl, a 10 m^2 Tucker trawl with a thermally protected cod end (Childress et al., 1978), or using SCUBA down to ∼30 m (Haddock and Heine, 2005). Individuals of each species were collected using all three methods. After capture, organisms were put into 0.2 micron-filtered water at densities <10 individuals l^{-1} and left to acclimate at 20 °C for at least eight hours. This temperature was chosen to replicate the average temperature above the thermocline among the three stations. All species of pteropod in this study appear to regularly spend a portion of their day in the mixed layer, and it is here that the effects of ocean acidification will have an impact on their physiology. Acclimation was intended to allow all species to recover from the thermal shock associated with different collection methods and to provide time for gut clearance. During experiments, we put animals into glass syringe respiration chambers with a known volume (10–50 ml) of 0.2 micron-filtered seawater for no less than six hours. The water contained 25 mg of Streptomycin and 25 mg of Ampicillin l^{-1} and had been bubbled with certified gas to achieve CO_2 concentrations of ∼0.10 % (1000 ppm) or allowed to remain at standard air saturation (0.03 %, 380 ppm). To measure the pH of the water used in the hypercapnic studies, we used a flow-through water-jacketed pH electrode (Microelectrodes, Bedford NH, #16-705). The pH of hypercapnic treatments averaged 7.96 ± 0.09 ($n = 14$), whereas normocapnic water averaged pH 8.29 ± 0.04 ($n = 14$). At the beginning of each experiment, we set up a blank syringe to monitor background respiration of microbes using identically bubbled water. Organismal respiration reduced the pH of experimental chambers by ∼0.1 ($n = 13$), consistent with a respiratory quotient between 0.7 and 1.0 when compared with the O_2 consumed during the experiments. This pH end-value was achieved gradually and does not represent the value that the animal was incubated at. Most animals in the ocean can regulate oxygen independent of environmental availability down to at least 50 % saturation (Childress and Seibel, 1998; Seibel, 2011). The majority of respiration runs never fell below 70 % air saturation, and all runs which fell below 35 % (∼80µmol kg^{-1} at 20 °C) were excluded from analyses. For species where runs were included which fell below 50 % saturation, statistical tests of species specific metabolic rates show no significant differences between oxygen consumption rate above and below 50 % saturation (one-way ANCOVA: *D. quadridentata* $F_{(1,9)} = 0.81$, $p = 0.39$; *C. longirostris* $F_{(1,12)} = 1.87$, $p = 0.20$; *Hyalocylis striata* $F_{(1,17)} = 0.08$, $p = 0.79$).

Respiration experiments were run for a period of time which allowed for there to be a noticeable change in oxygen saturation based on the individual size and metabolic rate of the various species. At the end of each respiration incubation (6–18 h), an aliquot of water was withdrawn from both the experimental and the blank chambers using a 500 µl airtight Hamilton syringe and injected past a Clarke-type O_2 electrode (#1302) and meter (#782) in a water-jacketed injection port (#MC100, Strathkelvin Instruments, North Lanarkshire, United Kingdom; Marsh and Manahan, 1999). Experimental values were subtracted from blank values and the resulting computed O_2 consumption rates are reported in µmol g^{-1} h^{-1} (wet mass). A second sample of water was immediately drawn and frozen in a cryovial at −80 °C. These samples were later thawed and the NH_3 concentration (µmol g^{-1} h^{-1} wet mass) was measured using the indophenol blue colorimetric assay (Ivancic and Degobbis, 1984). Organisms were weighed using

Table 2. MOCNESS net parameters and average hydrographic data for each day and night net tow (tow identification, ID) at the Costa Rica Dome (CRD) and Tehuantepec Bowl (TB) during 2007 and 2008. Minimum and maximum pressures are recorded in decibars (dB) and served as a proxy for depth (1 dB ≈ 1 m). Volume of water filtered through each net was measured in m^3 (V.f.). MOCNESS data has been made available by K. Wishner.

CRD–Day								CRD–Night							
2007				2008				2007				2008			
ID	Max (dB)	Min (dB)	V.f. (m^3)	ID	Max (dB)	Min (dB)	V.f. (m^3)	ID	Max (dB)	Min (dB)	V.f. (m^3)	ID	Max (dB)	Min (dB)	V.f. (m^3)
616.4	400	350	736	637.4	400	350	751	615.4	400	350	429	641.5	400	350	808
616.5	350	300	385	637.5	350	300	867	615.5	350	300	588	641.6	350	300	935
616.6	300	250	452	637.6	300	250	785	615.6	300	250	389	641.7	300	250	764
616.7	250	200	405	637.7	250	200	815	615.7	250	200	515	641.8	250	200	834
616.8	200	150	686	637.8	200	150	645	615.8	200	150	370		200	150	–
618.1	150	100	731	635.1	150	100	552	621.1	150	100	517	638.1	150	100	484
618.2	100	80	457	635.2	100	80	267	621.2	100	80	244	638.2	100	80	288
618.3	80	60	349	635.3	80	60	334	621.3	80	60	383	638.3	80	60	329
618.4	60	50	229	635.4	60	50	211	621.4	60	50	180	638.4	60	50	186
618.5	50	40	431	635.5	50	40	168	621.5	50	40	147	638.5	50	40	214
618.6	40	30	282	635.6	40	30	98	621.6	40	30	232	638.6	40	30	188
618.7	30	20	273	635.7	30	20	248	621.7	30	20	93	638.7	30	20	238
618.8	20	0	397	635.8	20	0	330	621.8	20	0	398	638.8	20	0	238
TB–Day								TB–Night							
2007				2008				2007				2008			
ID	Max (dB)	Min (dB)	V.f. (m^3)	ID	Max (dB)	Min (dB)	V.f. (m^3)	ID	Max (dB)	Min (dB)	V.f. (m^3)	ID	Max (dB)	Min (dB)	V.f. (m^3)
611.3	400	350	378	630.4	400	350	738	609.4	550	350	1224	628.4	400	350	655
611.4	350	300	1028	630.5	350	300	659	609.5	350	150	2035		350	300	–
611.5	300	250	474	630.6	300	250	583	612.1	150	100	598	628.6	300	250	606
611.6	250	200	480	630.7	250	200	763	612.2	100	80	360	628.7	250	200	992
606.1	200	100	1282	630.8	200	150	502	612.3	80	60	392	628.8	200	150	626
606.2	100	80	200	626.1	150	100	481	612.4	60	50	327	633.1	150	100	749
606.3	80	60	233	626.2	100	80	431	612.5	50	40	256	633.2	100	80	333
606.4	60	50	81	626.3	80	60	503	612.6	40	30	400	633.3	80	60	412
606.5	50	40	118	626.4	60	50	241	612.7	30	20	291	633.4	60	50	102
606.6	40	30	108	626.5	50	40	201	612.8	20	0	535	633.5	50	40	144
606.7	30	20	78	626.6	40	30	152					633.6	40	30	211
606.8	20	0	300	626.7	30	20	164					633.7	30	20	177
				626.8	20	0	274					633.8	20	0	178

a motion-compensated shipboard balance system (Childress and Mickel, 1980), then frozen in liquid nitrogen. Upon return to shore, a subset of animals were reweighed using a Pinnacle Series Analytical Balance (±0.001 g, Denver Instruments) to verify the accuracy of the field measurements. Statistical analyses were conducted using the STATISTICA software package (StatSoft). Tests were reported as significant if $p < 0.05$.

In the ETP, diel vertical distribution of zooplankton was sampled using a vertically stratified MOCNESS (Multiple Opening/Closing Net and Environmental Sensing System; Wiebe et al., 1976; Wishner component of the ETP Program). Similar distributions are unavailable from the Gulf of California. Samples were collected from 0–400 m using 153-micron mesh nets in sampling intervals which varied from 10 m to 150 m thick during the day and night (Table 2). These samples were split using a flat-bottomed Motoda splitter and were preserved in a 4 % sodium borate-buffered formalin and sea water solution. Upon return to the laboratory, we separated each sample by size-fraction using a 64 µm-mesh sieve. Pteropods were picked out from this subsample and identified to species using a dissecting microscope. For this paper, the presence or absence of individual species of pteropods was documented for each net and assembled into a vertical profile to provide a diel vertical pattern of species specific distribution.

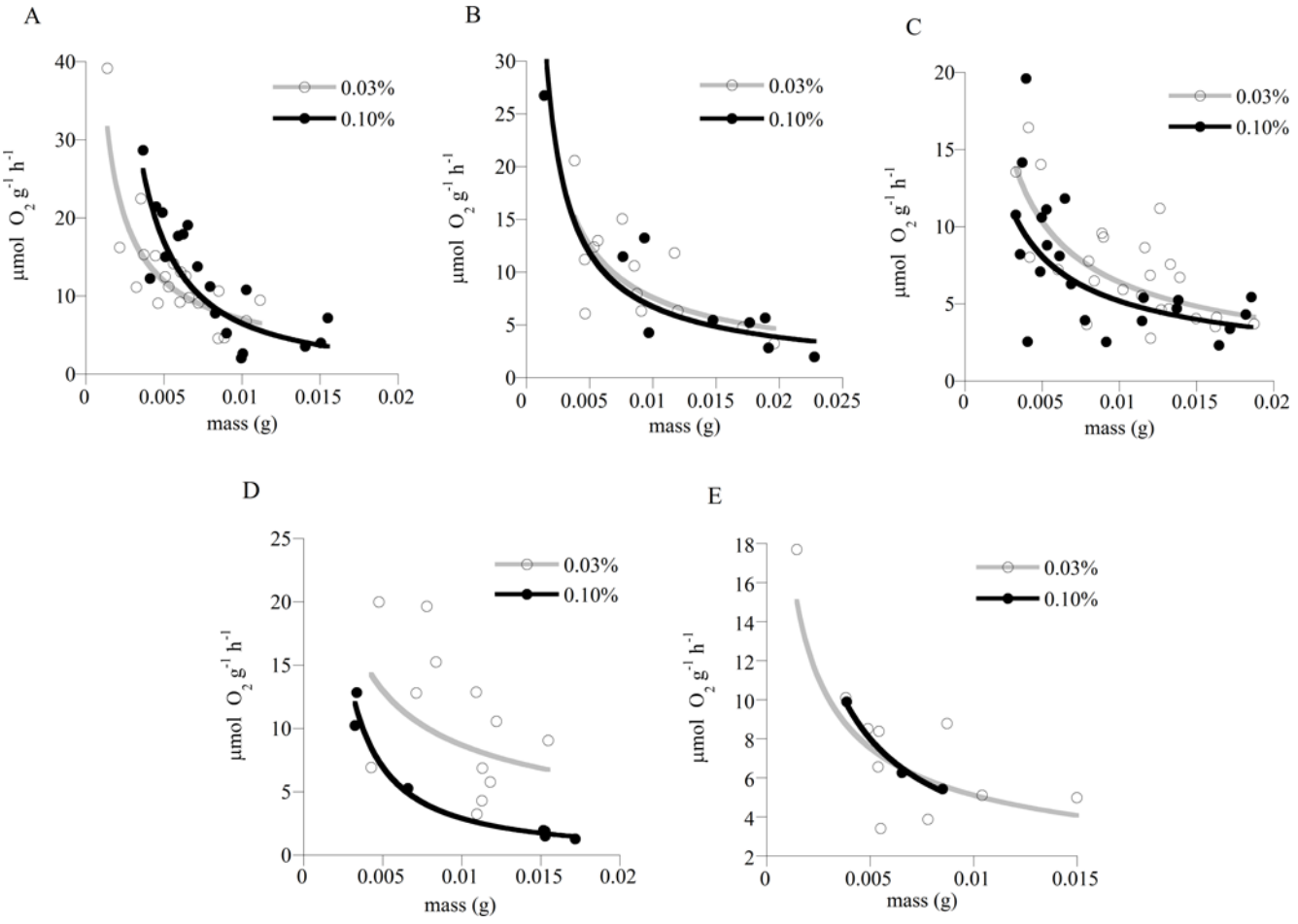

Fig. 2. Relationship between the oxygen consumption ($\mu mol\, O_2\, g^{-1}\, h^{-1}$) and mass ($g$) of thecosome pteropods (Table 3): **(A)** *Cavolinia longirostris*, **(B)** *Clio pyramidata*, **(C)** *Hyalocylis striata*, **(D)** *Diacria quadridentata*, and **(E)** *Creseis virgula*. Curves indicate normocapnic (0.03 %, grey) and hypercapnic (0.10 %, black) treatments.

3 Results

The O_2 consumption of pteropods was impacted by organismal mass (Fig. 2, Table 3). Using a one-way ANCOVA to account for the effect of mass (continuous predictor), we found that only *Diacria quadridentata* had a significant difference in the average rate of O_2 consumption between hypercapnic and normocapnic treatments (groups; Tables 4, 5; Fig. 3). When exposed to elevated CO_2, *Diacria quadridentata* responded with a significant reduction in O_2 consumption rate ($\sim$53 %, $p = 0.033$). Similarly, *Diacria quadridentata* was the only species that responded to hypercapnic conditions with a significant reduction in NH_3 excretion ($\sim$63 %, $p = 0.009$, Tables 4, 5; Fig. 4). Ratios of O_2 consumption and NH_3 excretion (O:N) were not statistically different between treatments for any of the species studied (Tables 4, 5). The duration of the experiment (between 6–18 h) did not affect the metabolic rate of pteropods (one-way ANCOVA: *H. striata* $F_{(1,37)} = 0.03$ $p = 0.86$, *C. virgula* $F_{(1,9)} = 0.17$, $p = 0.69$, *C. pyramidata* $F_{(1,15)} = 1.21$, $p = 0.29$, *C. longirostris* $F_{(1,32)} = 1.05$, $p = 0.31$, *D. quadridentata* $F_{(1,15)} = 2.69$ $p = 0.12$). Species collected at multiple sites had no significant difference in metabolic rate (students t-test: *H. striata* $p = 0.66$, *C. virgula* $p = 0.27$, *C. pyramidata* $p = 0.17$), similarly collection methods had no significant impact on metabolic rate (one-way ANOVA, $F_{(2,66)} = 0.215$, $p = 0.81$). The number of individuals captured in good condition and usable for respiration experiments varied among species, with significantly lower abundances of *C. pyramidata* and *C. virgula*. This low sample size and the large variability in oxygen consumption and ammonia excretion rates suggest that comparison between high and low CO_2 treatments should be treated with caution for *C. virgula*.

MOCNESS sampling revealed distinct differences in vertical distribution both between species and between stations (Fig. 5). *Diacria quadridentata* was the only completely non-migratory species; it was always found above the mixed

Table 3. Scaling curves describing the relationship between wet mass (M, g) and oxygen consumption rate (R, µmol O_2 g^{-1} h^{-1}) following the relationship $R = aM^b$ (Fig. 2).

	0.03 % CO_2			0.10 % CO_2		
	a	b	r^2	a	b	r^2
Cavolinia longirostris	0.23	−0.75	0.88	0.01	−1.38	0.83
Clio pyramidata	0.27	−0.72	0.67	0.16	−0.81	0.93
Hyalocylis striata	0.27	−0.69	0.53	0.27	−0.64	0.43
Diacria quadridentata	0.6	−0.58	0.41	0.01	−1.26	0.98
Creseis virgula	0.39	−0.56	0.88	0.13	−0.78	0.99

Table 4. The average size, oxygen consumption ($MO_2 \pm$ SD), and ammonia excretion ($MNH_3 \pm$ SD) of thecosome pteropods found in the Eastern Tropical Pacific at 20 °C.

		Mean wet weight (mg)	MO_2 (µmol O_2 g^{-1} h^{-1})		MNH_3 (µmol NH_3 g^{-1} h^{-1})		O:N	
			n	Mean	n	Mean	n	Mean
Hyalocylis striata	control	10.5 ± 4.2	19	7.31 ± 3.64	17	0.55 ± 0.44	17	40.3 ± 25.7
	0.10 % CO_2	8.4 ± 4.4	16	7.07 ± 3.57	14	0.33 ± 0.16	14	44.4 ± 17.9
Creseis virgula	control	6.8 ± 3.8	10	7.75 ± 4.17	8	0.52 ± 0.15	8	35.3 ± 17.7
	0.10 % CO_2	6.3 ± 2.3	3	7.20 ± 2.38	1	0.75	1	14.5
Clio pyramidata	control	9.1 ± 4.9	13	9.96 ± 4.80	10	0.70 ± 0.47	10	31.8 ± 8.3
	0.10 % CO_2	13.5 ± 6.9	9	8.55 ± 7.80	8	0.83 ± 0.89	8	25.0 ± 5.0
Cavolinia longirostris	control	8.2 ± 3.7	17	10.26 ± 6.26	17	1.02 ± 0.47	17	20.3 ± 9.3
	0.10 % CO_2	5.9 ± 2.6	18	12.82 ± 7.45	18	1.32 ± 0.70	18	22.2 ± 11.0
Diacria quadridentata	control	9.7 ± 3.3	12	10.62 ± 5.63	11	0.89 ± 0.44	11	27.5 ± 12.9
	0.10 % CO_2	10.9 ± 6.2	7	5.01 ± 4.73	6	0.33 ± 0.16	6	30.3 ± 28.6

layer (∼30 m). The distribution of all other pteropods included depths below the mixed layer into the low O_2 water at the Costa Rica Dome. Generally organisms were found at depth during the daytime and nearer the mixed layer during the evening, although portions of the *Hyalocylis striata*, *Cavolinia longirostris* and *Creseis virgula* populations were found at depth during the night. At the Tehuantepec Bowl, where the transition to the OMZ was generally more abrupt and severe, patterns of distribution were quite different. In this region we never collected *Clio pyramidata*, and *Creseis virgula* was present only at <100 m, unlike their distribution to 350–400 m during both the day and night at the Costa Rica Dome. Only *Hyalocylis striata* was found at similar depths at both stations. These distributions reveal that many pteropod species in the ETP daily inhabit regions of low O_2 (<10 µmol O_2 kg^{-1}) and low pH (Fig. 1), although the more pronounced OMZ at the Tehuantepec Bowl does appear to restrict the vertical distribution of some species.

4 Discussion

Our study of the vertical distribution of thecosomes is the first to describe the presence of four pteropod species in the pronounced OMZ of the ETP. In the hypoxic waters of the OMZ, these animals are surrounded by low pH water (7.4–7.5) and, based on the alkalinity of the region at 200 m (∼2300µmol kg^{-1}, WOCE data P-18 2007), are likely experiencing levels of CO_2 ∼ 1000 ppm by a depth of 200 m (Feely et al., 2004; Fabry et al., 2008; Byrne and Elliott, unpublished data). Assuming an average salinity of 34.7, a temperature of 10 °C, a depth of 200 m (CTD data), and incorporating the measured pH with the known alkalinity of the region, aragonite is undersaturated in the OMZ (CO_2sys: seawater pH scale, Dickson $KHSO_4$, and constants from Dickson and Millero, 1987; $\Omega Ar = 0.65$). Therefore, our distributions are surprising, suggesting a greater resiliency in some species of pteropod with respect to acidification than has been previously inferred. Since low O_2 and high CO_2 are inexorably linked in OMZs (Paulmier et al., 2011), we assume that although pH profiles were not taken in 2007 in either the Gulf of California or the ETP, the low O_2 waters found below 200 m at these locations were accompanied by similarly low pH and high CO_2 water.

Of the pteropods with habitats that include depths below 100 m, none responded to our hypercapnic treatment with a change in O_2 consumption or NH_3 excretion. *Diacria quadridentata* was the only species of tropical thecosome

Table 5. Statistical analysis comparing the difference between groups exposed to 0.03 % and 0.10 % CO_2 (Table 4). Statistical analysis for O_2 consumption (dependant variable) was conducted using a one-way ANCOVA to account for the variation due to size (continuous variable). Analysis of NH_3 excretion and O:N ratio was conducted using a two-tailed t-test.

Species	MO_2	MNH_3	O:N
Hyalocylis striata	$F_{(1,43)} = 1.03,\ p = 0.315$	$t_{42} = 0.064,\ p = 0.948$	$t_{42} = -0.853,\ p = 0.399$
Cavolinia longirostris	$F_{(1,33)} = 2.63,\ p = 0.115$	$t_{32} = -1.526,\ p = 0.137$	$t_{32} = -0.537,\ p = 0.595$
Clio pyramidata	$F_{(1,10)} = 0.20,\ p = 0.660$	$t_7 = -1.026,\ p = 0.339$	$t_7 = 1.032,\ p = 0.336$
Creseis virgula	$F_{(1,19)} = 1.48,\ p = 0.240$	–	–
Diacria quadridentata	$F_{(1,16)} = 5.45,\ \mathbf{p = 0.033}$	$t_{15} = 2.975,\ \mathbf{p = 0.009}$	$t_{15} = -0.290,\ p = 0.776$

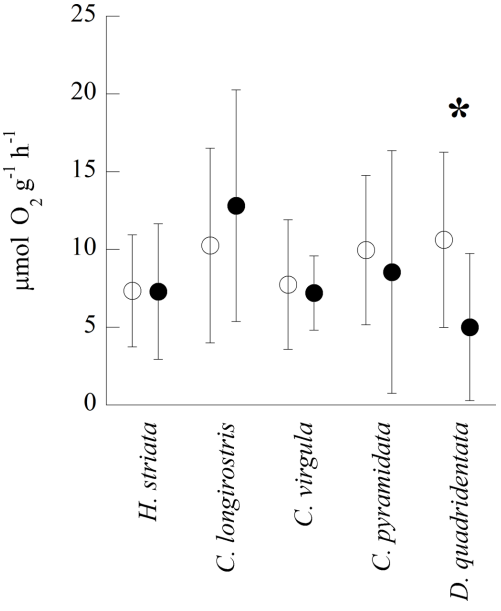

Fig. 3. The average O_2 consumption for normocapnic (0.03 %, white) and hypercapnic (0.10 %, black) treatments (Table 4). Error bars represent one standard deviation from the mean. Only *Diacria quadridentata* responded to hypercapnia with a significant change in O_2 consumption (*).

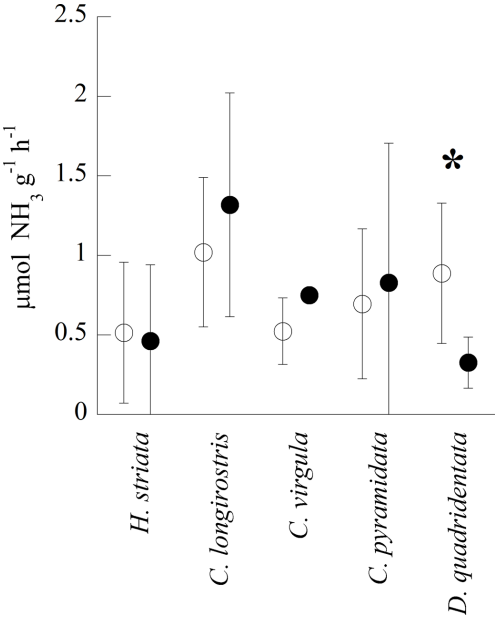

Fig. 4. The average NH_3excretion for normocapnic (0.03 %, white) and hypercapnic (0.10 %, black) treatments (Table 4). Error bars represent one standard deviation from the mean. Only one data point was available for the species *Creseis virgula* in the hypercapnic treatment. *Diacria quadridentata* was the only thecosome which responded with a significant change in NH_3 excretion (*).

that was never found below the mixed layer, and it exhibited a reduction in respiration of 53 % and a reduced NH_3 excretion of 63 % when exposed to hypercapnic conditions. For all species, the O:N ratio was not significantly different between treatments, indicating that there was no shift in metabolic substrate in use response to exposure to CO_2. An O:N ratio below a value of 16 indicates that protein is the primary fuel source for catabolism whereas a ratio of 50–60 is indicative of a diet balanced between lipid and protein catabolism (Mayzaud and Conover 1988). Tropical pteropod species O:N ranged on average between 20–40 suggesting that protein fueled a significant portion of their catabolism. In our study, O:N was highly variable, likely due to uncontrollable differences in the feeding history of captured animals.

In general, these results indicate that some species of pteropod will be able to function in the shallower, warmer, oxygenated end of their distribution under increasingly hypercapnic conditions. As calcifiers, these species may endure brief periods of acidosis by buffering their cellular pH through internal mechanisms or the partial dissolution of their aragonite shell. The mussel *Mytilus galloprovincialis* uses these tactics when exposed to elevated levels of CO_2; they survive hypercapnia through decreased protein synthesis and shell dissolution (Michaelidis et al., 2005). It has been shown that juveniles of the species *Cavolinia inflexa* are viable after being held in seawater of 7.51 pH ($\sim$1700 ppm CO_2) for 5–13 days, although under these conditions they are completely shell-less (Comeau et al., 2010b),

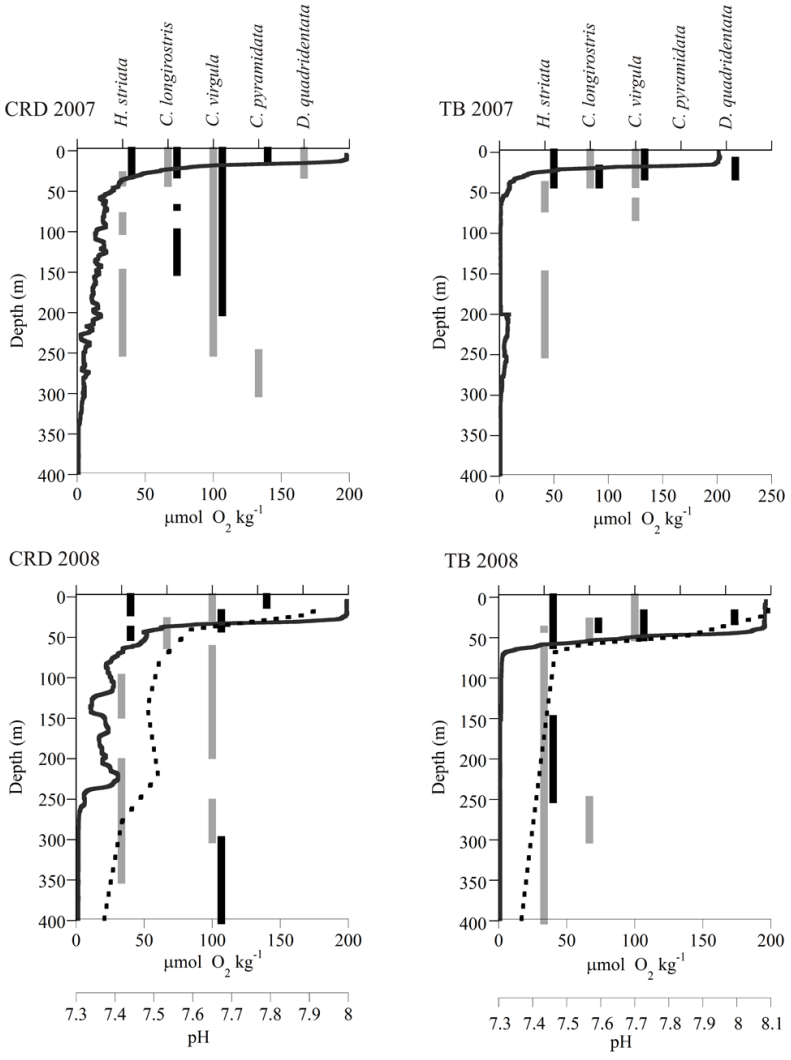

Fig. 5. The day (grey bar) and night (black bar) location of individual tropical pteropod species is plotted alongside the O_2 concentration at depth for 2007 and 2008 (solid line, µmol kg^{-1}) and pH in 2008 (dashed line). O_2 profiles are from MOCNESS data (assembled by R. Williams, D. Outram and K. Wishner), and pH was calculated by Byrn and Elliot using the standard SOP for pH analysis with *m*-cresol purple (unpublished data).

suggesting that dissolution of the shell is survivable for some species of pteropods for a brief period of time. The swimming efficiency, defensive capacity and the energetic affect of this loss of shell has yet to be quantified, and it is likely that there would be long term effects on population fitness. However, the amount of shell dissolution that would be required to buffer tissue acidification in the OMZ on a diel basis would be unlikely to cause complete loss of pteropod shells. Although their shells are delicate, the bodies of pteropods are quite small and not much dissolution would be required to attain compensation for the duration of a daily migration into the OMZ.

The natural exposure of diel vertically migratory pteropods to elevated CO_2 would be under conditions of reduced temperature and O_2 saturation, both of which have been shown to significantly depress the metabolic rate of a number of species (Seibel, 2011). This means that generally when pteropods in the ETP are experiencing elevated levels of CO_2 their metabolic rate is already suppressed due to other environmental parameters. Exposure to hypercapnia independent of these other conditions, which will occur as the surface ocean acidifies and as was the case during these experiments, may not be physiologically analogous to their response to hypercapnia at depth in the OMZ. Furthermore, this study was done exclusively at 20 °C, so animals which regularly migrate to depth may have been experiencing temperature stress from continual maintenance in mixed layer conditions. Since *D. quadridentata* appears to be found only above the thermocline, it would not be similarly impacted, potentially confounding interspecific comparisons of sensitivity to CO_2.

Organismal response to acidified conditions has increasingly been shown to be highly species specific, with a great deal of variation within the mollusk group in particular. High CO_2 decreases the metabolic rate in the mussel *Mytilus galloprovincialis* (Michaelidis et al., 2005), the snail *Littorina littorea* (Melatunan et al., 2011), the thecosome pteropod *Limacina helicina antarctica* (Seibel et al., 2012) and the Humbolt squid *Dosidicus gigas* (Rosa and Seibel, 2008). Other research found an increase in metabolic rate of the pteropod *Limacina helicina* (Comeau et al., 2009, 2010a) and in the bivalve *Laternula elliptica* (Cummings et al., 2011), whereas there was no change in the metabolic rate of the cuttlefish *Sepia officianlis* (Gutowska et al., 2008) and the mussel *Mytilus edulis* (Thomsen and Melzner, 2010). The cause of these variations in response have been theorized to be a result of differences in the natural levels of organismal CO_2 production, respiratory pigment type, and ionoregulatory ability (Pörtner et al., 2005; Gutowska et al., 2008; Widdicomb and Spicer, 2008; Hendriks et al., 2010). The migratory behavior of pteropods in the ETP, which regularly exposes individuals to elevated CO_2, may therefore predispose them to be resilient with respect to surface acidification through the use of the same physiological mechanisms by which these zooplankton cope with brief periods of hypercapnia. However, these distributional patterns and physiological studies do not rule out the possibility that ocean acidification may have severe effects on pteropods. In fact, our results indicate that some species of non-migratory species such as *Diacria quadridentata* could, in the absence of acclimation and adaptation, be significantly impacted by even brief periods of exposure to CO_2 with unknown implications for species fitness, biogeography and survival. Furthermore, non-migratory pteropods, although capable of coping with the brief 6–18 h of elevated levels of CO_2 of these experiments, may still respond negatively to chronic exposure to CO_2.

The mechanisms of compensation for changes in acid-base imbalance in marine species involve an up-regulation of active ion-transportation, the production of bicarbonate and other buffers and changes in metabolic substrate use (Walsh and Milligan, 1989; Seibel and Walsh 2001; Portner et al., 2005). Full compensation for these changes often takes up to 48–72 h (Seibel and Walsh, 2003). The response measured in our experiments, being after only 6–18 h of exposure, may not be one of a final stable steady state and cannot be interpreted as indicative of a response to chronic acidification, only to the type pteropods would experience during short migrations into regions of hypercapnia. It has been shown that some organisms that are capable of withstanding short periods of pronounced hypercapnia, such as the peanut worm, *Sipunculus nudus*, require time to restore extra- and intracellular pH and cannot survive chronic high CO_2 exposure (Reipschlager and Pörtner, 1996; Langenbuch and Pörtner, 2002, 2004). Our results do not therefore imply that the perpetual acidification of surface waters would have no impact on diel migratory pteropods, as expanding regions of hypercapnia could compress the portion of the ocean where recovery from acidosis is possible, forcing pteropods in the ETP to experience acidification on a more chronic scale.

Acknowledgements. We would like to acknowledge the hard work and dedication of the captain and crew of the R/V *New Horizon*, the R/V *Seward Johnson* and the R/V *Knorr* and to thank K. Daly for her excellent organization of the ETP Project expeditions as Chief Scientist. We would like to thank everyone who helped with the MOCNESS deployment and D. Outram in particular for her insight and mentorship while compiling the distributional data. We are grateful for the hard work of our divers R. Rosa, L. Elder, B. Phillips, C. Cass, and P. Suprenand, who unfailingly provided us with research organisms. This work would not have taken place without the funding of the National Science Foundation (grant OCE-0526502 to Wishner and Seibel, OCE – 0526545 to Daly, and OCE – 0851043 to Seibel), the University of Rhode Island, and the Rhode Island Experimental Program to Stimulate Competitive Research Fellowship program.

Edited by: S. W. A. Naqvi

References

Accornero, A., Manno, C., Esposito, F., and Gambi, M. C.: The vertical flux of particulate matter in the polynya of Terra Nova Bay Part II: Biological components, Antarctic Science, 15, 175–188, 2003.

Armstrong, J. L., Boldt, J. L., Cross, A. D., Moss, J. H., Davis, N. D., Myers, K. W., Walker, R. V., Beauchamp, D. A., and Haldorson, L. J.: Distribution, size, and interannual, seasonal and diel food habits of northern Gulf of Alaska juvenile pink salmon, *Oncorhynchus gorbuscha*, Deep-Sea Res. Pt. II, 52, 247–265, 2005.

Caldeira, K. and Wickett, M. E.: Anthropogenic carbon and ocean pH, Nature, 425, 365–365, 2003.

Caldeira, K. and Wickett, M. E.: Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean, J. Geophys. Res., 110, 1–12, 2005.

Childress, J. J. and Mickel, T. J.: A motion compensated shipboard precision balance system, Deep-Sea Res., 27, 965–970, 1980.

Childress, J. J. and Seibel, B. A.: Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers, J. Experiment. Biol., 201, 1223–1232, 1998.

Childress, J. J., Barnes, A. T., Quetin, L. B., and Robison, B. H.: Thermally protecting cod ends for the recovery of living deep-sea animals, Deep-Sea Res., 25, 419–422, 1978.

Cohen, A. L. and Holcomb, M.: Why corals care about ocean acidification: Uncovering the mechanism, Oceanography, 22, 118–127, 2009.

Comeau, S., Gorsky, G., Jeffree, R., Teyssié, J.-L., and Gattuso, J.-P.: Impact of ocean acidification on a key Arctic pelagic mollusc (*Limacina helicina*), Biogeosciences, 6, 1877–1882, doi:10.5194/bg-6-1877-2009, 2009.

Comeau, S., Jeffree, R., Teyssié, J. L., and Gattuso, J. P.: Response of the Arctic pteropod *Limacina helicina* to projected future environmental conditions, PLoS One, 5, e11362, 2010a.

Comeau, S., Gorsky, G., Alliouane, S., and Gattuso, J. P.: Larvae of the pteropod *Cavolinia inflexa* exposed to aragonite undersaturation are viable but shell-less, Mar. Biol., 157, 2341–2345, 2010b.

Comeau, S., Gattuso, J. P., Nisumaa, A. M., and Orr, J.: Impact of aragonite saturation state changes on migratory pteropods, Proc. Roy. Soc. B, Biological Sciences, 279, 732–738, 2012.

Cummings, V., Hewitt, J., Van Rooyen, A., Currie, K., Beard, S., Thrush, S., Norkko, J., Barr, N., Heath, P., and Halliday, N. J.: Ocean acidification at high latitudes: Potential effects on functioning of the Antarctic bivalve *Laternula elliptica*, PloS one, 6, e16069, 2011.

Dickson, A. G., and Millero, F. J.: A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, Deep-Sea Res. Pt. A, 34, 1733–1743, 1987.

Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C.: Impacts of ocean acidification on marine fauna and ecosystem processes, ICES J. Mar. Sci., 65, 414–432, 2008.

Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J.: Impact of anthropogenic CO_2 on the $CaCO_3$ system in the Oceans, Science, 305, 362–366, 2004.

Feely, R. A., Doney, S. C., and Cooley, S. R.: Ocean acidification: Present conditions and future changes in a high-CO_2 world, Oceanography, 22, 36–47, 2009.

Gutowska, M. A., Prtner, H. O., and Melzner, F.: Growth and calcification in the cephalopod Sepia officinalis under elevated seawater pCO_2, Mar. Ecol. Prog. Ser., 373, 303–309, 2008.

Haddock, S. H. D. and Heine, J. N.: Scientific blue-water diving, California Sea Grant College Program, 2005.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E., Fujita, R., Heinemann, D., Lenihan, H. S., Madin, E. M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., and Watson, R.: A global map of human impact on marine ecosystems, Science, 319, 948–952, 2008.

Haugan, P. M. and Drange, H.: Effects of CO_2 on the ocean environment, Energ. Convers. Manage., 37, 1019–1022, 1996.

Hendriks, I. E., Duarte, C. M., and Álvarez, M.: Vulnerability of marine biodiversity to ocean acidification: A meta-analysis, Estuarine, Coast. Shelf Sci., 86, 157–164, 2010.

IPCC: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge Univ Press, Cambridge, UK, 2007.

Ivancic, I. and Degobbis, D.: An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method, Water Res., 18, 1143–1147, 1984.

Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J. P., Langdon, C., and Opdyke, B. N.: Geochemical consequences of increased atmospheric carbon dioxide on coral reefs, Science, 284, 118–120, 1999.

Lalli, C. M. and Gilmer, R. W.: Pelagic Snails: The Biology of Holoplanktonic Gastropod Mollusks, Stanford University Press, 1989.

Langenbuch, M. and Pörtner, H. O.: Changes in metabolic rate and N excretion in the marine invertebrate *Sipunculus nudus* under conditions of environmental hypercapnia: identifying effective acid–base variables, J. Experiment. Biol., 205, 1153–1160, 2002.

Langenbuch, M. and Pörtner, H. O.: High sensitivity to chronically elevated CO_2 levels in a eurybathic marine sipunculid, Aqua. Toxicol., 70, 55–61, 2004.

Lewis, E. and Wallace, D. W. R.: Program developed for CO_2 system calculations, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, 1998.

Lischka, S., Büdenbender, J., Boxhammer, T., and Riebesell, U.: Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth, Biogeosciences, 8, 919–932, doi:10.5194/bg-8-919-2011, 2011.

Manno, C., Tirelli, V., Accornero, A., and Fonda Umani, S.: Importance of the contribution of *Limacina helicina* faecal pellets to the carbon pump in Terra Nova Bay (Antarctica), J. Plankton Res., 32, 145–152, 2010.

Marsh, A. G. and Manahan, D. T.: A method for accurate measurements of the respiration rates of marine invertebrate embryos and larvae, Mar. Ecol. Prog. Ser., 184, 1–10, 1999.

Mayzaud, P. and Conover, R. J.: O:N atomic ratio as a tool to describe zooplankton metabolism, Mar. Ecol. Prog. Ser., 45, 289–302, 1988.

Melatunan, S., Calosi, P., Rundle, S. D., Moody, A. J., and Widdicombe, S.: Exposure to elevated temperature and PCO_2 reduces respiration rate and energy status in the periwinkle *Littorina littorea*, Physiol. Biochem. Zoo., 84, 583–594, 2011.

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

Miles, H., Widdicombe, S., Spicer, J. I., and Hall-Spencer, J.: Effects of anthropogenic seawater acidification on acid–base balance in the sea urchin *Psammechinus miliaris*, Mar. Pollut. Bull., 54, 89–96, 2007.

Pakhomov, E. A., Froneman, P. W., Wassmann, P., Ratkova, T., and Arashkevich, E.: Contribution of algal sinking and zooplankton grazing to downward flux in the Lazarev Sea (Southern Ocean) during the onset of phytoplankton bloom: a lagrangian study, Mar. Ecol. Prog. Ser., 233, 73–88, 2002.

Paulmier, A., Ruiz-Pino, D., and Garçon, V.: CO_2 maximum in the oxygen minimum zone (OMZ), Biogeosciences, 8, 239–252, doi:10.5194/bg-8-239-2011, 2011.

Pörtner, H. O., Langenbuch, M., and Michaelidis, B.: Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: From Earth history to global change, J. Geophys. Res, 110, C09S10, doi:10.1029/2004JC002561, 2005.

Reipschlager, A. and Pörtner, H. O.: Metabolic depression during environmental stress: the role of extracellular versus intracellular pH in *Sipunculus nudus*, J. Experiment. Biol., 199, 1801–1807, 1996.

Rosa, R. and Seibel, B. A.: Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator, Proc. Nat. Aca. Sci., 105, 20776–20780, 2008.

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C. S., Wallace, D. W. R., and Tilbrook, B.: The Oceanic Sink for Anthropogenic CO_2, Science, 305, 367–371, 2004.

Seibel, B. A.: Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones, J. Experiment. Biol., 214, 326–336, 2011.

Seibel, B. A. and Fabry, V. J.: Marine biotic response to elevated

carbon dioxide, Advances in Applied Biodiversity Science, 4, 59–67, 2003.

Seibel, B. A. and Walsh, P. J.: Potential impacts of CO_2 injection on deep-sea biota, Science, 294, 319–320, 2001.

Seibel, B. A. and Walsh, P. J.: Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance, J. Experiment. Biol., 206, 641–650, 2003.

Seibel, B. A., Maas, A. E., and Dierssen, H. M.: Energetic plasticity underlies a variable response to ocean acidification in the pteropod, *Limacina helicina antarctica.* , PLoS One, in press, 2012.

Thomsen, J. and Melzner, F.: Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel Mytilus edulis, Mar. Biol., 157, 2667–2676, 2010.

Walsh, P. J. and Milligan, C. L.: Coordination of metabolism and intracellular acid-base status: ionic regulation and metabolic consequences, Can. J. Zoolog., 67, 2994–3004, 1989.

Wiebe, P. H., Burt, K. H., Boyd, S. H., and Morton, A. W.: A multiple opening/closing net and environmental sensing system for sampling zooplankton, J. Mar. Res., 34, 313–326, 1976.

Widdicombe, S. and Spicer, J. I.: Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us?, J. Experiment. Mar. Biol. Ecol., 366, 187–197, 2008.

Wood, H. L., Spicer, J. I., and Widdicombe, S.: Ocean acidification may increase calcification rates, but at a cost, Proc. Roy. Soc. B, 275, 1767–1773, 2008.