Biogeosciences Discuss., 8, 10295–10316, 2011
www.biogeosciences-discuss.net/8/10295/2011/
doi:10.5194/bgd-8-10295-2011

Biogeosciences Discussions

The metabolic response of pteropods to ocean 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

Received: 4 October 2011 – Accepted: 9 October 2011 – Published: 21 October 2011

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

Published by Copernicus Publications on behalf of the European Geosciences Union.

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 influences 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 (Feely et al., 2004). 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 (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 understanding 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; Seibel and Fabry, 2003). 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 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. 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 (780 and 1100 ppm CO_2) 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 2010a, b; Seibel et al., 2011).

Little is known about the physiology of tropical pteropod species, and the impact of hypercapnia (high CO_2) on metabolism has been reported only for one warm water species, *Cavolinia inflexa* (Comeau et al., 2010a). 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. Specifically, in the Pacific around depths of 200 m, CO_2 levels reach approximately 1000 ppm and 400 ppm at 10° N and 30° N, respectively (Fabry et al., 2008). These pHs correspond to a decreasing saturation state of calcium carbonate. Waters are undersaturated with respect to aragonite ($\Omega_a < 1$) at 1000 ppm CO_2, suggesting that there would be passive dissolution of pteropod shells near 10° N. 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 were calculated using a Multi-parameter Inorganic Carbon Analyzer (MICA) in the ETP in 2008 (Byrne and Elliott, unpublished data). 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 (Haddock and Heine, 2005).

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. 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 ± 0.10, whereas normocapnic water averaged pH 8.29 ± 0.04. At the beginning of each experiment, we set up a blank syringe to monitor background respiration of microbes using identically bubbled water.

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 (Strathkelvin Instruments, North

Lanarkshire, UK) in a water-jacketed injection port (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 dawn and frozen in a cryovial at −80 °C. These samples were later thawed and 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 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). Samples were collected from 0–400 m using 153-µm 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. Quantitative abundances are reported in Maas et al. (2011).

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 and 5; Fig. 3). When exposed to elevated CO_2, *Diacria quadridentata* responded with a significant depression in O_2 consumption rate (~ 53 %, $p = 0.033$). Similarly, *Diacria quadridentata* was the only species to respond to hypercapnic conditions with a significant reduction in NH_3 excretion (~ 63 %, $p = 0.009$, Tables 4 and 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 and 5).

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 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 most 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 diel migration of four pteropod species into 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 (~ 2350 µmol kg^{-1}, WOCE data P-18), are likely experiencing levels of CO_2 between 400–1000 ppm by a depth of 200 m, depending on latitude (Feely et al., 2004; Fabry et al., 2008; Byrne and Elliott, unpublished data). Since aragonite is thought to be undersaturated in this region, these results are surprising, suggesting a greater resiliency in some species of pteropod with respect to acidification than has been previously inferred. 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 that was never found below the mixed layer, and it exhibited a respiratory suppression and reduction of NH_3 excretion when exposed to hypercapnic conditions.

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 though internal mechanisms or the 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).

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 may not be physiologically analogous to their response to hypercapnia at depth in the OMZ.

The migratory behavior of pteropods in the ETP, which regularly exposes individuals to elevated CO_2, may have resulted in the development of physiological mechanisms by which these zooplankton can better cope with brief periods of hypercapnia (Seibel and Fabry, 2003; Seibel and Walsh, 2001, 2003). 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 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, it has also been shown that organisms capable of withstanding short periods of pronounced hypercapnia, such as the peanut worm, *Sipunculus nudus*, require time to restore extra- and intracellular pH (Reipschlager and Pörtner, 1996; Langenbuch and Pörtner, 2002, 2004). Prolonged experience of high CO_2 resulted in death for *S. nudus*. Therefore, although capable of coping with 6–12 h of elevated levels of CO_2, the perpetual acidification of surface waters may impact even the diel migratory plankton by compressing the regions of the ocean where recovery from acidosis is possible.

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.

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Table 1. Dates, locations and vessels used during sampling.

Date	Location	Vessel
Jun 2007	Gulf of California between 27° N 112° W and 111° W	R/V *New Horizon*
Oct–Nov 2007	Eastern Tropical Pacific between 9° N 90° W and 11° N 98° W	R/V *Seward Johnson*
Dec 2008–Jan 2009	Eastern Tropical Pacific between 9° N 90° W and 11° N 98° W	R/V *Knorr*

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

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 ± SD), and ammonia excretion (MNH_3 ± 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}) n	Mean	MNH_3 (µmol NH_3 g^{-1} h^{-1}) n	Mean	O : N 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	20	12.29 ± 7.60	20	1.21 ± 0.65	20	20.5 ± 8.6
	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

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	$F_{(1,35)} = 3.75$, $p = 0.061$	$t_{36} = 0.494$, $p = 0.624$	$t_{36} = 0.535$, $p = 0.596$
Creseis virgula	$F_{(1,10)} = 0.20$, $p = 0.660$	$t_{7} = -1.026$, $p = 0.339$	$t_{7} = 1.032$, $p = 0.336$
Clio pyramidata	$F_{(1,19)} = 1.48$, $p = 0.240$	$t_{16} = 0.415$, $p = 0.683$	$t_{17} = 2.047$, $p = 0.057$
Diacria quadridentata	$F_{(1,16)} = 5.45$, $p = \mathbf{0.033}$	$t_{15} = 2.975$, $p = \mathbf{0.009}$	$t_{15} = -0.290$, $p = 0.776$

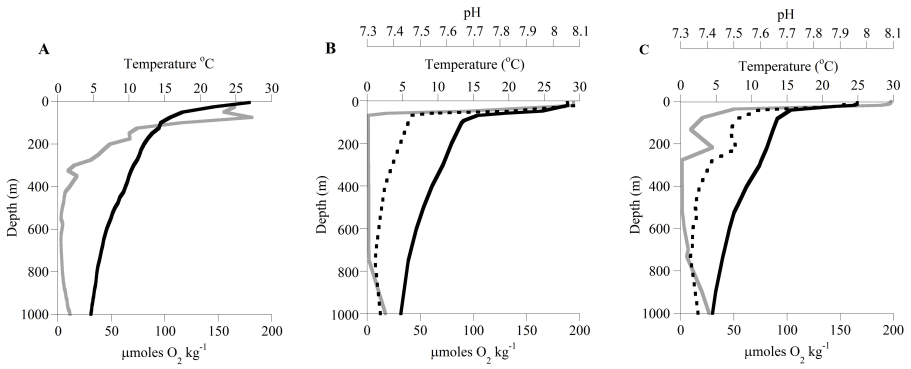

Fig. 1. A typical temperature (°C, black) and O_2 (µmol kg^{-1}, grey) profile for the Gulf of California in June 2007 **(A)**, the Tehuantepec Bowl **(B)**, and the Costa Rica Dome **(C)**.The profiles from the Tehuantepec Bowl and the Costa Rica Dome are from 2008 and also show a pH profile (dashed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

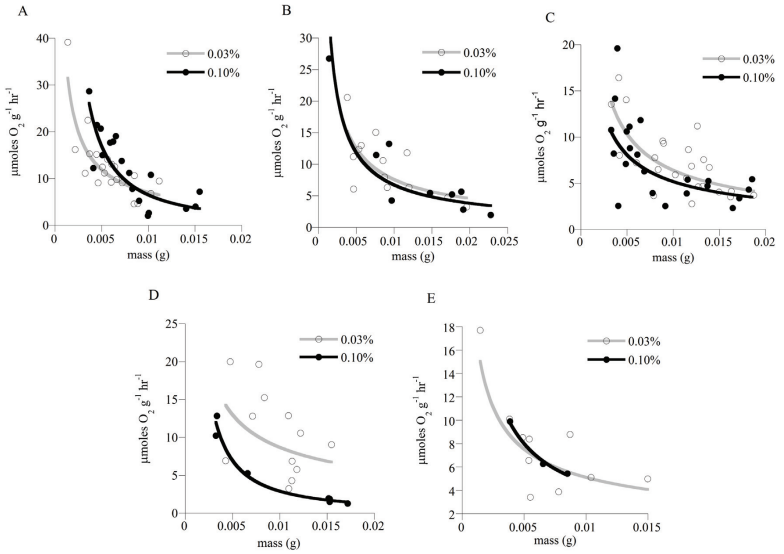

Fig. 2. Relationship between the oxygen consumption (µmol O_2 g^{-1} h^{-1}) and mass (*g*) of thecosome pteropods (Table 3): **(A)** *Cavolinia longirostris*, **(B)** *Clio pyramidata*, **(C)** *Hyalocylis striata*, **(D)** *Diacria quadridentata*, and **(E)** *Creseis virgula*. Curves indicate normocapnic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

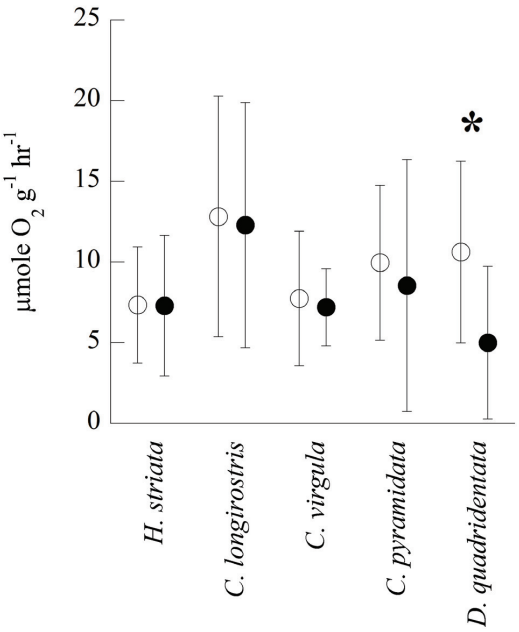

Fig. 3. The average O_2 consumption for normocapnic (0.03 %, white) and hypercapnic (0.10 %, black) treatments (Table 4). Error bars represent one standard deviation from the mean. Only *Diacria quadridentata* responded to hypercapnia with a significant change in O_2 consumption (*).

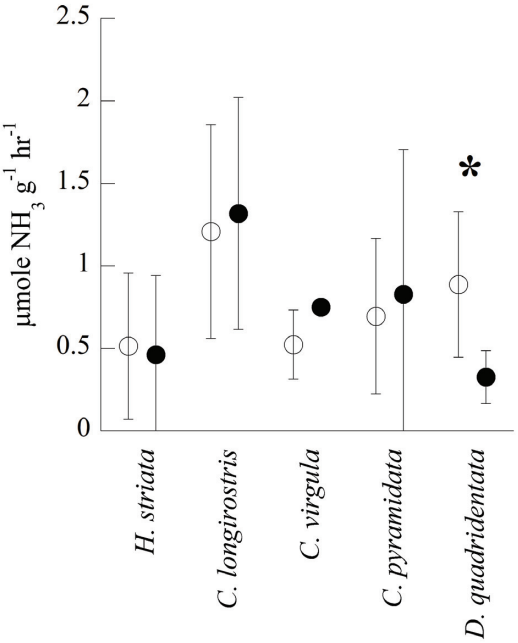

Fig. 4. The average NH_3 excretion for normocapnic (0.03 %, white) and hypercapnic (0.10 %, black) treatments (Table 4). Error bars represent one standard deviation from the mean. Only one data point was available for the species *Creseis virgula* in the hypercapnic treatment. *Diacria quadridentata*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

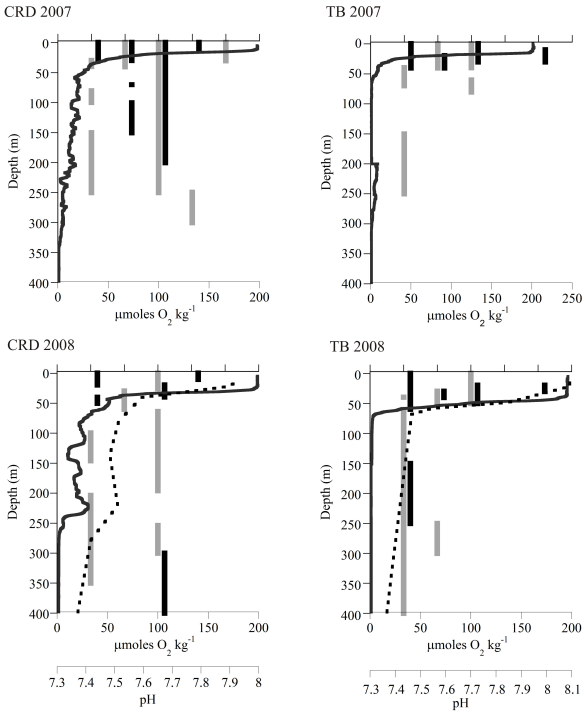

Fig. 5. The day (grey bar) and night (black bar) location of tropical pteropod species is plotted alongside the O_2 concentration at depth for 2007 and 2008 (solid dark grey line, µmol kg^{-1}) and pH in 2008 (dashed dark grey line). O_2 profiles are from MOCNESS data (assembled by R. Williams, D. Outram and K. Wishner), and pH data are from the MICA system of Byrn and Elliot (unpublished data).