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The metabolic response of thecosome pteropods from the North Atlantic and North Pacific Oceans to high CO_2 and low O_2

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Abstract. As anthropogenic activities, notably the burning of fossil fuels, increase carbon dioxide (CO_2) and result in a decrease in oxygen (O_2) concentrations in the ocean system, it becomes important to understand how different populations of marine animals will respond. Water that is naturally lower in pH, with a high concentration of carbon dioxide (hypercapnia) and a low concentration of oxygen, occurs at shallow depths (200-500 m) in the North Pacific Ocean, whereas similar conditions are absent throughout the upper water column in the North Atlantic. This contrasting hydrography provides a natural experiment to explore whether differences in environment cause populations of cosmopolitan pelagic calcifiers, specifically the aragonitic-shelled pteropods, to have a different physiological response when exposed to hypercapnia and low O_2. Using closed-chamber end-point respiration experiments, eight species of pteropods from the two ocean basins were exposed to high CO_2 (~800 µatm) while six species were also exposed to moderately low O_2 (10%, or ~130 µmol kg^{-1}) and a combined treatment of low O_2/high CO_2. None of the species tested showed a change in metabolic rate in response to high CO_2 alone. Of those species tested for an effect of O_2, only *Limacina retroversa* from the Atlantic showed a response to the combined treatment, resulting in a reduction in metabolic rate. Our results suggest that pteropods have mechanisms for coping with short-term CO_2 exposure and suggest that there can be interactive effects between stressors on the physiology of these open ocean organisms that correlate with natural exposure to low O_2 and high CO_2; these are considerations that should be taken into account in projections of organismal sensitivity to future ocean conditions.

Key Words: ocean acidification, zooplankton, respiration

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1. Introduction

Ocean acidification, a result of the dissolution of anthropogenically produced carbon dioxide (CO_2) into sea water, is increasingly considered to be one of the most pervasive human changes to the marine system (Doney et al., 2009; Gruber, 2011; Halpern et al., 2008). The pH of the ocean surface has already dropped by ~0.1 units relative to preindustrial levels and is predicted to drop another 0.3-0.4 pH units in the next one hundred years (Bopp et al., 2013; Haugan and Drange, 1996; IPCC, 2013). As CO_2 dissolves in the ocean, it causes changes in seawater carbonate chemistry, notably increasing hydrogen ion concentration and decreasing the concentration of carbonate ions. As a consequence of the changing equilibria, there is a reduction in pH and in the saturation state of calcium carbonate ($CaCO_3$), including the biogenic forms of calcite and aragonite. As ocean acidification continues, eventually the water becomes undersaturated and corrosive, meaning that, in the absence of compensating biological action, conditions will favor the dissolution of the $CaCO_3$ found in the shells and skeletons of calcifying organisms, with aragonite being more sensitive than calcite (Millero, 2007).

Ocean acidification, therefore, impacts calcifying species on multiple fronts. Changes in environmental pH can modify the acid-base balance of intra- and extracellular fluids of marine organisms, which may result in reduced fitness or outright mortality (Seibel and Fabry, 2003; Seibel and Walsh, 2001; Widdicombe and Spicer, 2008). Changes in $CaCO_3$ saturation state can also affect the ability of some calcifying animals to create and maintain calcium carbonate structures with implications for energetics, survival, competition and biogeochemical export (Fabry et al., 2008; Riebesell et al., 2000; Ries et al., 2009). Understanding the long-term effects of this increase in ocean acidity on both organisms and ecosystems has, therefore, become of great concern. Important and outstanding research goals are to understand how changing CO_2 impacts current populations and to predict whether these populations will be able to adapt to the rate and severity of the rising anthropogenic CO_2 inputs (e.g. Dam, 2013; Kelly and Hofmann, 2013; Sunday et al., 2011).

One approach to understanding the response of marine animals to increasing acidification is to examine places where animals already experience conditions of elevated CO_2 (hypercapnia). By comparing individuals that inhabit regions of high CO_2 with those that never experience high levels naturally, insight can be gained into the potential for adaptation of species to high CO_2 over evolutionary timescales. The ocean chemistry of the northwest Atlantic and the northeast Pacific Oceans provides such a natural experiment. High CO_2 concentrations are generally absent from the upper water column in the Atlantic (Wanninkhof et al., 2010). In contrast there currently are hypercapnic conditions, where the water is undersaturated with respect to aragonite, in the upper water column in parts of the Pacific.

The source of hypercapnia in the Pacific Ocean is a combined result of ocean circulation coupled with the biological processes, leading the old deep waters of the Pacific to be some of the most CO_2 rich in the ocean (Broecker et al., 1982). On top of this natural process, ocean acidification also plays a role: the pH of the upper water column in the North Pacific is decreasing by on the order of 0.001–0.002 pH units per year (Byrne et al., 2010), corresponding to a total CO_2, or dissolved inorganic carbon (DIC), increase of 1–2 μmol kg^{-1} yr^{-1} (Peng et al., 2003; Sabine et al., 2008; Sabine and Tanhua, 2010). Although the surface waters in these regions are typically well oxygenated and with a pH > 8, animals that live at or migrate to depth experience increasingly low oxygen (O_2), pH, undersaturation with respect to calcium carbonate and elevated CO_2 (Seibel, 2011). Historically these regions, which occur in many ocean basins, were in fact known more for their low O_2 than for their high CO_2 and were termed oxygen minimum zones (OMZs). These carbon maximum/oxygen minimum zones are extensive in the North Pacific Ocean, whereas similar conditions are rare in much of the Atlantic (Paulmier et al., 2011). Closely related taxa and cosmopolitan species in these two regions therefore experience very different pH levels, CO_2 and O_2 concentrations in their normal distribution. Independent from high CO_2, the reduced O_2 at depth in these OMZs has a profound impact on zooplankton distribution (i.e.: Escribano et al., 2009; Maas et al., 2014; Wishner et al., 2008) and can have important implications for the physiology of zooplankton (Childress and Seibel, 1998; Rosa and Seibel, 2008; Seibel, 2011).

Thecosome pteropods are an interesting group for investigating planktonic exposure and response to hypercapnia and low O_2. Broadly distributed throughout the open ocean, species of thecosomes found in shallow waters of temperate and polar seas can become a numerically dominant member of the zooplankton community (Bednaršek et al., 2012a; Hunt et al., 2008; van der Spoel, 1967). As such, they can be an important part of the food chain (Armstrong et al., 2005; Hunt et al., 2008; Karnovsky et al., 2008), and contribute substantially to carbon flux (Bauerfeind et al., 2009; Fabry and Deuser, 1991; Manno et al., 2010; Noji et al., 1997). Bearing thin shells of aragonite, one of the less stable forms of biogenic calcium carbonate, the

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calcification of thecosomes has been shown to be impacted by exposure to conditions replicating the projected changes in surface water pH and saturation state of the future ocean in the next 100 years (Comeau et al., 2009; Lischka et al., 2011; Manno et al., 2012). Furthermore, recent assessments have shown that their shells already bear the mark of acidification in upwelling and polar regions characterized by under-saturated conditions with respect to aragonite (Bednaršek et al., 2014a, b; Bednarsek and Ohman, 2015; Bednaršek et al., 2012b). Studies of metabolism and behavior, however, reveal a complex sensitivity to pH, dependent upon natural pre-exposure and the presence of interactive stressors (Comeau et al., 2010a; Maas et al., 2012a; Manno et al., 2012; Seibel et al., 2012).

Previous work has shown that some tropical and sub-tropical thecosome species undergo diel vertical migrations into persistent and pronounced regions of low O_2 and hypercapnia in the Eastern tropical North Pacific. These species showed no change in metabolic rate (O_2 consumption) when exposed to high CO_2 (1000 µatm), revealing the ability of some groups of thecosome to maintain aerobic metabolism in acidified waters for short periods of time. The one species in this region that does not migrate, however, responded with a suppression of metabolism when exposed to high CO_2 (Maas et al., 2012a). This work in the Eastern tropical North Pacific provides evidence that there may be the potential for environmental adaptation of thecosomes to high CO_2, but provides no insight into the combined effects of CO_2 with low O_2. Although research into this topic is underway for other calcifying organisms in coastal habitats (Gobler et al., 2014; Melzner et al., 2013), in the open ocean our understanding remains limited.

The objective of this study, therefore, was to compare the effect of high CO_2 and low O_2 on thecosome pteropods from the northwest Atlantic and the northeast Pacific Oceans. One of the benefits of this comparison is that there are a number of species of thecosomes that have cosmopolitan distributions occupying both basins and that are known to be diel vertical migrators (Table 1; Bé and Gilmer, 1977; van der Spoel, 1967). Thus populations in the Pacific would naturally experience hypercapnia and low O_2 in their daytime deep water habitat in the Pacific, while in contrast, those from the Atlantic would rarely experience the same deep water environmental stressors. Using these organisms, which are presumably adapted to their local conditions, we can test whether species exhibit a population-specific physiological response to these environmental conditions indicative of different sensitivities.

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2. Methods

Thecosome pteropods caught during cruises to the northwest Atlantic and northeast Pacific were exposed aboard ship to manipulated conditions of moderately high CO_2 and/or low O_2 for short durations (< 18 h). After this exposure their metabolic rates were measured and then compared to determine whether there were species- or region-specific responses to the treatments.

2.1 Sampling

Animals were collected on two cruises, the first on August 7th – September 1st 2011 in the northwest Atlantic aboard the R/V *Oceanus*, and the second in the northeast Pacific from August 9th – September 18th 2012 aboard the R/V *New Horizon*. The majority of the sampling in the Atlantic took place along a three-part 'z'-shaped transect running between 35°N 52°W and 50°N 42°W, as well as at sites during transit to and from port (Fig. 1). The first portion of this cruise track corresponded to a segment of the World Ocean Circulation Experiment / Climate and Ocean: Variability, Predictability and Change project (WOCE/CLIVAR) line A20. In the North Pacific the main sampling took place along a two-part transect running between 50°N 150°W and 33.5°N 135°W, corresponding to a portion of WOCE/CLIVAR line P17N, as well as at sites during transit to and from port (Fig. 1).

Sampling was part of a larger interdisciplinary project employing a suite of tools to explore the natural distribution and hydrographic environment of the thecosomes. The sampling design included underway measurements of hydrography, carbonate chemistry and multi-frequency acoustic backscatter. Comprehensive sampling of the water column was conducted at pre-determined stations using a depth-stratified 1-m^2 Multiple Opening/Closing Net and Environmental Sensing System with 150 µm mesh nets (MOCNESS; Wiebe et al., 1985), a towed broadband echosounder, video plankton recorder casts, and profiles with a 24 10-L Niskin bottle rosette and associated conductivity, temperature and depth (CTD) package.

Hydrographic profiles associated with this study were collected of temperature, O_2 and salinity using the CTD-Rosette-Niskin bottle package at stations along the main survey transects (Fig. 1). This CTD was equipped with dual temperature and conductivity sensors, a Digiquartz pressure sensor, a SBE43 dissolved oxygen sensor, a biospherical underwater photosynthetically active radiation (PAR) sensor with surface reference, a Wet Labs C-Star transmissometer (660 nm wavelength), and a Wet Labs ECO-AFL fluorometer. Where CTD casts were unavailable, at stations conducted during the transits to and from port, an expendable bathythermograph (XBT)

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was deployed to determine the temperature of the water column. Bottle samples of carbonate parameters, nutrients, and other parameters were collected at selected water depths using the CTD-Rosette package.

2.2 Environmental Carbonate Chemistry

Discrete pH samples were directly collected from the 10-L Niskin bottle into 10 cm cylindrical optical cells and measured within 4 h of collection (Clayton and Byrne, 1993; Dickson et al., 2007). These pH samples were analyzed spectrophotometrically on an Agilent 8453 spectrophotometer at a control temperature (25.0 ± 0.1°C) following the method detailed in Dickson (2007) and in Clayton and Byrne (1993) using m-cresol purple as the indicator. The pH results in total scale have been corrected for indicator impurity (Liu et al., 2011) and indicator perturbation to seawater samples. The pH measurements have a precision better than 0.001 and an accuracy of ~0.002.

Nutrient samples (nitrate/nitrite, phosphate, silicate, and ammonia) were collected in 20 mL plastic bottles after filtration through a 0.22um Pall capsule filter and kept frozen until analysis. Nutrient samples were analyzed either at the WHOI Nutrient Analytical Facility or the University of California, Santa Barbara, using a Lachat Instruments QuickChem 8000 four-channel continuous flow injection system, following standard colorimetric methods approved by U.S. Environmental Protection Agency.

Discrete samples were also taken for dissolved inorganic carbon (DIC) and total alkalinity (TA). These were collected in 250mL Pyrex borosilicate glass bottles after being filtered with a 0.45 µm in-line capsule filter and poisoned with saturated mercuric chloride (Dickson et al., 2007). DIC samples were analyzed on a DIC auto-analyzer (AS-C3, Apollo SciTech, Bogart, USA) via sample acidification, followed by non-dispersive infrared CO_2 detection (LiCOR 7000: Wang et al., 2013; Wang and Cai, 2004). The instrument was calibrated with certified reference material (CRM) from Dr. A.G. Dickson at the Scripps Institution of Oceanography. The DIC measurements have a precision and accuracy of ±2.0 µmol kg^{-1}. TA measurements were made with an Apollo SciTech alkalinity auto-titrator, a Ross combination pH electrode, and a pH meter (ORION 3 Star) based on a modified Gran titration method with a precision and accuracy of ±2.0 µmol kg^{-1} (Wang and Cai, 2004).

The remaining water column carbonate system parameters, including aragonite saturation state and pCO_2 were calculated from DIC-pH pairs at in situ nutrient, temperature, salinity and

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pressure using the software CO2Sys (Pierrot et al., 2006) and the dissociation constants of Mehrbach et al. (1973), refitted by Dickson and Millero (1987), and the $KHSO_4$ dissociation constant from Dickson (1990). Depths for pH=7.7, pCO_2=800 µatm and aragonite saturation state of 1 were then linearly interpolated using the closest available measurements.

Surface water pCO_2 was continuously measured throughout both cruises using an automated underway system (Model 8050, General Oceanics Inc., USA) based on headspace air-seawater equilibration followed by infrared detection (LiCOR 7000). This system was calibrated every 1-2 hours with three CO_2 gas standards traceable to World Meteorological Organization CO_2 Mole Fraction Scale. These underway pCO_2 measurements have a precision and accuracy of ~±1 µatm. Measurements made by the underway system provide insight into the carbonate chemistry parameters at stations made in transit where bottle samples were not collected.

2.3 Specimen Capture

Thecosome species were chosen for physiological study opportunistically as they appeared in net samples at successive stations. Species were targeted specifically for their abundance and the likelihood of their presence in both ocean basins. Most individuals were collected with a 1-m diameter, 150-µm mesh Reeve net with a ~25 L cod-end in the Atlantic and a similar 1-m diameter, Reeve net equipped with 330-µm mesh in the Pacific. Use of the Reeve net with its large and heavy cod-end in combination with slow haul rates (typically 5-10 m min^{-1}) allowed for a gentle collection of the delicate thecosomes, consistently supplying animals in good condition with undamaged shells and external mantle appendages. Net tows were made at night when animals were expected to congregate at shallow depths, were ~1 h in duration in an effort to minimize the handling time of the organisms, and reached a maximal depth between 100–150 m. Depths were targeted that had a high chlorophyll *a* peak during CTD casts, high acoustic backscattering on the echosounder, and/or where thecosomes had been abundantly sampled at the same station using the MOCNESS. Occasionally, individuals of less abundant species were collected from the nets of the MOCNESS for physiological study, but only if their shells were undamaged and they were swimming normally.

Post-capture, individuals were transferred to filtered water in densities of < 15 ind. L^{-1} and kept for at least 8 h in temperature controlled waterbaths to allow for gut clearance. Temperatures for experimentation (20, 15 or 10°C) were chosen to be generally representative of the waters from which the animals were sampled, based on the vertical distributions and hydrographic conditions documented with the stratified MOCNESS sampling. Chosen temperatures were typically the average of the water temperature between 25-100 m, although in the middle section of the Atlantic cruise experimental temperatures were reflective of the 25–50 m average due to the particularly shallow vertical distribution of the dominant species (*Limacina retroversa*) sampled in this region. This was to ensure that experiments were occurring at physiologically relevant and, presumably, natural temperatures for each species. After gut clearance, individuals that were in good condition (i.e., swimming and with shell intact) were used for oxygen consumption experiments.

2.4 Experimental Exposures and Oxygen Consumption Rate

Post-gut-clearance, healthy individuals were put into separate glass syringe respiration chambers with a known volume of 0.2 µm filtered seawater and 25 mg L^{-1} each of streptomycin and ampicillin. This minimized the microbial respiration effects on the measurements of carbonate chemistry and O_2 consumption rates by pteropods during the experiments. The inclusion of antibiotics, a method which has previously been used with thecosomes to prevent bacterial growth in respiration experiments (Maas et al., 2012b), was shown during the Pacific cruise to have no effect on the O_2 consumption of at least *Limacina helicina*, for the exposure durations associated with these experiments (Howes et al., 2014). The volume of water in the treatments was chosen to complement the size of the organism and temperature of the experiment and ranged between 15-50 mL in 2011 and 8-20 mL in 2012. For every 3-5 treatment chambers, a "control" respiration chamber (experimental seawater with antibiotics and without pteropods) was set up to monitor microbial activity and to provide water for characterization of the starting conditions.

Filtered seawater for experimental exposures was collected during both cruises in batches at approximately weekly intervals from the surface; experimental water thus began with chemical properties (notably including TA, DIC, pH, as well as salinity) reflective of the local environment and was then manipulated to modify CO_2 and/or O_2 concentrations. Manipulations were achieved by bubbling 1 L batches of collected seawater with gas mixes (certified accurate to ± 2%) for 45–60 min with one of two oxygen (21 and 10% O_2) levels crossed with two CO_2 (nominally 380 ppm and 800 ppm) levels. At the time of the experiment, surface air pCO_2 conditions were on average ca. 380 ppm, dictating our ambient (LC) conditions. In 2011 the

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ambient condition (~21% O_2 and 380 µatm CO_2) was achieved by bubbling with an ambient clean air line, while in 2012 it was achieved by a certified 380 ppm gas mix.

The experimentally modified concentrations mimic the CO_2 and O_2 levels that would be experienced by thecosomes within the top 400 m of the Pacific Ocean, and reflect the average projected atmospheric CO_2 level for the open ocean in the year 2100 (A2 emissions scenario, IPCC, 2007). This resulted in four total treatments: low (i.e., ambient) CO_2, high oxygen (LC/HO) representative of current ambient surface ocean conditions; high carbon, high oxygen (HC/HO), replicating what we expect average future surface oceans to resemble; low CO_2, low oxygen (LC/LO); and high carbon, low oxygen (HC/LO), which is similar to what organisms in the Pacific would experience during a diel vertical migration into the local oxygen minimum zone. The goal of this design was to allow us to compare directly the Atlantic and Pacific thecosomes to see whether exposure to 800 µatm pCO_2 and/or 10% O_2 resulted in different outcomes. The level of low O_2 chosen for this study was well above the threshold that has been designated as stressful for non-specialized metazoan life (< 2 mg O_2 L^{-1} or 60 µmol O_2 kg^{-1}; Vaquer-Sunyer and Duarte, 2008), in order to test the non-lethal effect of moderately low O_2 on individuals from the two ocean basins. Calculations based on the salinity and temperature of the water indicated that bubbling with 10% O_2 achieved conditions of 10–13% O_2 saturation at the start of experiments. Subsequent analyses (see below) also confirmed that intended CO_2 concentrations were achieved for all treatments within reasonable ranges, with the exception of the LC/LO Atlantic treatment. In this case, the gas cylinder was evidently improperly mixed by the manufacturer and analyses suggested a ca. 100 ppm CO_2 concentration. The results for this treatment are still presented but should be interpreted as a distinct treatment.

Oxygen consumption was measured following similar techniques as described in Marsh and Manahan (1999). Briefly, at the conclusion of the experiment water was withdrawn from treatment or control chambers using an airtight 500 µL Hamilton syringe and injected past a Clarke-type microcathode (part #1302, Strathkelvin Instruments, North Lanarkshire, United Kingdom) attached to an O_2 meter (part #782) in a water-jacketed injection port (part #MC100). This was done three times, allowing the reading to stabilize for at least 30 seconds before a measurement was taken. Generally, the change in oxygen consumption was between 3–25% of the control value. In high oxygen experiments, if the oxygen level fell below 70% of air saturation they were excluded from the analysis. Animals were removed from the chamber,

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blotted dry and frozen in liquid nitrogen. These individuals were later weighed using a microbalance (± 0.0001 g) and the resulting mass specific O_2 consumption rates are reported in µmoles (g wet weight)$^{-1}$ h^{-1}. Wet weights are here used as they are more relevant for physiological understanding of animal function (Childress et al., 2008) but dry weights can be estimated from these using the wet weight to dry weight relationships developed previously for pteropods (Ikeda, 2014). To replicate the duration of exposure that would be experienced by most thecosomes in the Pacific undergoing a daily migration to depth, the experiments were targeted to last 6–12 h. In practice, experiments ranged from 6–18 h for normoxic and 3–10 h for low O_2 trials. This variation in duration resulted from balancing the need to elicit a measureable change in O_2 concentration with preventing extreme O_2 depletion of the chambers (< 6% oxygen saturation) and accounting for multiple species of variable size and metabolic rate.

2.5 Experimental Carbonate Chemistry

Carbonate chemistry of the treatments was characterized in most cases via measurements of DIC and TA of experimental seawater, unless indicated otherwise. The process of measuring the O_2 in the treatments used up a large portion of the water and then the chamber was unsealed and disturbed to remove the animal, rendering it impractical to measure the carbonate chemistry directly from the respiration chambers. DIC measurements were thus taken from control syringes within 18 h of the end of each experiment and used to represent the starting point of the carbonate chemistry conditions the animals experienced. Water samples were allowed to come to room temperature (> 6 h) before analysis. DIC was measured using the same system as that used for the hydrographic characterization (see above). Estimates of the effect of CO_2 production via respiration in treatment chambers on DIC were made using a respiratory quotient of 0.8 M of CO_2 per 1 M of O_2 consumed (Mayzaud, 1976) to characterize the ending conditions of the experiments.

Due to the small volumes of water in the experimental chambers, it was not possible to measure both DIC and TA from the control syringes. Instead, TA samples intended to be representative of the starting experimental conditions were collected via siphoning from each batch of filtered and antibiotic-treated water. These samples were subsequently measured based on the analytical method described above (Wang and Cai 2004). TA of experimental water was assumed to have been constant over the course of each experiment as water was filtered (0.2 µm) and antibiotic treated (thus microbial activities were kept at minimum), and aerobic respiration does not change TA in a significant way.

In some instances, however, measured TA from experimental water was substantially dissimilar to that of the surface measurements made from nearby in-situ surface bottle samples collected with the CTD (> 20 µmol kg^{-1}; see section 3.3). Calculated pCO_2 values in these cases were also significantly different from batches of experimental water collected from other locations, but bubbled with the same CO_2 gas tank. These differences are more than 10 times the measurement precision/accuracy and 5 times the uncertainty of duplicate sampling and measurements during the cruises. They are also beyond the likely level of TA variation due to differences in sampling location (geographic and in depth) between the in situ bottle samples and experimental water batches and rather are likely a consequence of the difficulties associated with cleanly siphoning the experimental water batches (e.g., contamination during sampling). For completeness, the carbonate chemistry system parameters for the experimental water, including aragonite saturation state and pCO_2, are reported based on calculations using DIC-TA pairs using both the in situ and experimental TA; in those cases where the TA measurements diverged substantially (> 20 µmol kg^{-1}), however, we base our interpretations on the in-situ measured TA at nearby CTD stations instead of the values of experimental water. In those circumstances where batch water was taken from test stations and CTD bottle data were unavailable, the experimental TA was checked using calculated TA values using DIC from the LC/HO treatments and pCO_2 from the underway measurements.

2.6 Statistics

Oxygen consumption rates were tested for significant differences between groups with Bonferroni pairwise post-hoc comparisons using SPSS. Univariate General Linear Models (GLM) were conducted to determine the effect of CO_2 level, O_2 level, and their interactive effect using the log transformed oxygen consumption with log transformed wet mass as a covariate separately for each species (2 factor design; "$CO_2 \times O_2$"). In the Atlantic this full factorial design was confounded by the incorrect gas mixture so each treatment was tested independently (1 factor design; "treatment"). Species that were collected during both years/basins, and experiments conducted on species at multiple temperatures, were analyzed separately so that the effect of variations in mass between seasons and the changes in metabolic rate at different temperatures would not confound the analysis.

For some species the temperature of experimentation was different among stations within a basin. For analyses with these species when comparing species between ocean basins, we applied a standard temperature coefficient (Q_{10}) to compare across temperatures. The adjusted rates (R_f) were calculated at 15°C using a Q_{10} of 2 according to the equation:

$$R_f = R_i * \left(Q_{10}^{\left(\frac{15-T_i}{10}\right)}\right)$$

where R_i is the original metabolic rate measured at the original temperature (T_i). Although previous work with thecosomes has shown that Q_{10} is species-specific (Maas et al., 2011; Maas et al., 2012b; Seibel et al., 2007), for many of the species used in this study there are no published estimates of Q_{10}. Thus, this coefficient value was chosen as it is mid-range for the published Q_{10} of non-polar thecosome species as recently compiled by Ikeda (2014; 1.3-2.7) and is consistent with estimates of average Q_{10} for marine ectotherms, which typically fall between 2-3 (Hochachka and Somero, 2002; Seibel and Drazen, 2007).

3. Results

3.1 Specimen Capture

Following currently accepted taxonomy, individuals from a total of eight species were collected over the course of the two cruises for physiological studies. The taxonomy of thecosomes has recently begun to be revisited using molecular and paleontological tools (i.e. Hunt et al., 2010; Janssen, 2012; Jennings et al., 2010; Maas et al., 2013), however, and there is growing evidence of cryptic speciation for some pteropod groups (Burridge et al., 2015; Gasca and Janssen, 2014). It thus should be noted that these inter-basin comparisons may be of cryptic congeners rather than conspecific populations.

We collected two species of thecosome pteropods exclusively from the Atlantic, *Limacina retroversa* (Fleming, 1823), a subpolar species, which is absent from the North Pacific, and *Diacria trispinosa* (Blainville, 1821), which can be found in temperate and tropical regions of the Atlantic, Pacific and Indian Oceans. Although present in both the North Atlantic and Pacific, the polar to sub-polar species *Limacina helicina* (Phipps, 1774), was only sampled in the Pacific transect. Collections of this species consisted of intermixed formae, the high spiraled *Limacina helicina helicina acuta* (van der Spoel, 1967), the lower spiraled *Limacina helicina helicina pacifica* (van der Spoel, 1967), and a forma that bore resemblance to both in a mixed

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morphology. Since both the assemblage and morphology of these formae were mixed they were tested as one population/species. In both ocean basins we collected *Styliola subula* (Quoy and Gaimard, 1827), *Cavolinia inflexa* (Lesueur, 1813) and *Clio pyramidata* (Linnaeus, 1767). There is some morphological and molecular evidence that *Cuvierina columnella* (Rang, 1827) is actually multiple distinct species, now including *Cuvierina atlantica* and *Cuvierina pacifica* (Burridge et al., 2015; Janssen, 2005), and we tested individuals of these species from their respective ocean basins.

3.2 Hydrography

Two hydrographic regimes were evident along the North Pacific study transect (Table 2; Fig. 2). The northern-most stations, including portions of the transit from port and stations from 50°N 150°W to 47 °N 144.6°W were coldest, with the temperatures between 25-100 m ranging from 5-10°C. In this area O_2 fell below 10% (~130 µmol kg^{-1}) by 250 m. In this northern part of the transect, pH fell below 7.7 by 130 m, and pCO_2 had already reached 800 µatm by ~200 m. Individuals in this area experienced an $\Omega_{Ar} = 1$ between 160-185 m, well within the typical diel vertically migratory range of both of the species found in the region (*C. pyramidata* and *L. helicina*). At stations from more southern latitudes, from 47 °N 144.6°W to 33.5°N 135°W, temperatures at depths between 25-100 m were higher, ranging between 10-15°C, representative of the transition zone into the North Pacific Gyre. Along this portion of the transect O_2 concentration consistently fell below 10% by depths between 340 and 400 m. The depth at which pH fell below 7.7 increased gradually from ~150 to 230 m as latitude decreased. Similarly, the depth at which pCO_2 in this area reached 800 µatm deepened from 330 to 440 m, and the aragonite saturation horizon transitioned from 330 m to 430 m depth. The depth at which species would experience a pH below 7.7 was within the inhabited depth range known from the literature for all of the species tested in this study region, but only the species *Clio pyramidata* likely experienced 10% O_2, 800 µatm pCO_2 and aragonite undersaturation in its typical distribution in this portion of the Pacific transect (Table 1).

In contrast to the Pacific, along the entire Atlantic transect O_2 concentration was above ~200 µmol kg^{-1} in the top 500 m, while pCO_2 never reached 800 µatm and aragonite undersaturation never occurred throughout the top 1000 m. There were three dominant hydrographic regimes in the Atlantic (Table 2; Fig. 2). In the northeastern part of the sampling region (50°N 42°W to 44.9 °N 42°W), where the Gulf Stream meets the Labrador Current, average temperatures at 25-100 m were near 15°C and pH only fell below 7.7 at depths exceeding 400 m. Similarly, in the southwest part of the sampling region (from 42°N 52°W to 36°N 52°W), corresponding to the Sargasso Sea and through the Gulf Stream, pH only fell below 7.7 at depths exceeding 450 m, although the upper water column was warmer, with average temperatures being 20°C. There was a third water mass type, typical of colder fresher shelf waters, at station 32 and in an intrusion off the Grand Banks at stations 17 and 19. This water was typified by a temperature and salinity anomaly with average temperatures falling below 5°C from 25-100 m and a salinity signature < 33, contrasting significantly with the surface salinities of the northern portion (~34) and southern portion (~36) of the Atlantic transect. As a consequence, these stations contained water of the lowest pH, with surface waters reaching 7.7 at depths shallower than 200 m. Based on previous knowledge of the vertical distributions of the thecosomes used in this study, only the species *Clio pyramidata* would ever experience a pH below 7.7 in this overall Atlantic study region and none of the thecosomes studied would experience 800 µatm pCO_2 or under-saturation within their vertical range (Table 1).

3.3 Carbonate Chemistry of Experiments

Bubbling with CO_2 levels of ~380 and ~800 ppm resulted in a distinct separation of carbonate chemistry between treatments during the experiments in both oceans (Table 3). Due to pre-existing differences in the carbonate chemistry of the seawater collected in each ocean, TA concentrations were different between the two basin treatments. In the Atlantic the DIC of the ambient CO_2 treatments ranged from 2030-2090 µmol kg^{-1} and the high CO_2 treatments from 2140-2220 µmol kg^{-1}, with an average difference between treatments of similar temperature and salinity of 132 µmol kg^{-1}. Surface TA in the region decreased from ~2370 µmol kg^{-1} in the southern part of the transect to 2300 µmol kg^{-1} in the northern latitudes. In the Pacific the DIC of the ambient CO_2 treatment ranged from 1930-2020 µmol kg^{-1} and the high CO_2 treatment from 2030-2110 µmol kg^{-1}, with an average difference of 90.7 µmol kg^{-1} between the treatments. Surface TA in this basin was 2150 µmol kg^{-1} in the most northern collection and had decreased to 2200 µmol kg^{-1} by the transect mid-point.

Calculations of pCO_2 based on these measurements of DIC and TA suggested that target pCO_2 levels were generally attained and were consistent between the two cruises, with the exception of the LC/LO treatment in the Atlantic. In this case, there was a substantial deviation from the intended pCO_2, suggesting values ranging from 99-139 µatm in contrast to a range of

311-391 µatm for the LC/HO in the Atlantic and 283-409 µatm for LC/HO and 295-397 µatm in the LC/LO in the Pacific. Evidently, this indicates improper mixing of the gas concentration in the Atlantic LC/LO gas cylinder by the manufacturer. The calculations for the high CO_2 treatments were more consistent between cruises, with the HC/HO being 585-868 µatm for pCO_2 and the HC/LO being 755-783 in the Atlantic, while in the Pacific the HC/HO treatment was between 520-740 µatm and the HC/LO 546-766 µatm. The variability in calculated pCO_2 values likely represents variations in bubbling time, temperature, and the degree to which the water reached saturation relative to the gas mixtures. The variability within each distinct treatment may also reflect, to some degree, what pteropods may experience under that particular mean condition, i.e. low vs. high CO_2.

As a consequence of the natural differences in seawater carbonate chemistry, in particular the TA differences between two ocean basins, there were inherent differences in the aragonite saturation state between the Pacific and Atlantic treatments (Table 3). In the Atlantic Ω_{Ar} of the ambient CO_2 treatment ranged from 2.4-3.5, except for the LC/LO treatment (Ω_{Ar} 4.0-5.5), which was bubbled with an incorrect gas mixtures as discussed above. Comparatively, in the Pacific the ambient CO_2 condition had a lower range of Ω_{Ar} (2.2-2.4) for both the LC/HO and the LC/LO treatments. The experimental conditions of the high CO_2 treatments in the Atlantic only approached under-saturation in the middle part of the transect (Ω_{Ar} = 1.2 at mid-latitudes; Table 3), where cold northern waters of low salinity were encountered and Ω_{Ar} had a range of 1.5-2.0 for the rest of the transect in the Atlantic. The values of Ω_{Ar} were lower overall in the Pacific, although the high CO_2 treatments also never reached under-saturation (Ω_{Ar} 1.3-1.8). The manipulation of carbonate chemistry in general successfully created two distinct ranges for both pCO_2 and aragonite saturation state (Ω_{Ar}) in this study.

It is important to acknowledge that the production of CO_2 via respiration of the organisms within the chambers would modify the carbonate chemistry of the treatments over the duration of the experiments. Based on the average respiration rate, and using a respiratory quotient of 0.8 (Mayzaud, 1976), we estimate an average DIC production of ~18.0 µmol kg^{-1} by the end of an experiment. Applying such a change to the experimental conditions in the northeast Pacific, where seawater is more sensitive to changes in DIC due to a lower buffering capacity compared to the Atlantic (i.e., a worst case scenario), Ω_{Ar} would only change by <0.1 in both the LC and HC experimental chambers over the course of the respiration experiments. Although this is an

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appreciable effect, we nonetheless retain a wide separation between the ambient and high CO_2 treatments and in no cases would the treatments reach under-saturation as a consequence of this biological activity. As such, for simplicity the results reported in Table 3 do not include this variability.

3.4 Oxygen Consumption Rate

3.4.1 Effect of CO_2

Varying availability and abundances of the different thecosome pteropod species in the net samples precluded all species being exposed to the full factorial design but individuals of all species were tested under the low CO_2, high oxygen (LC/HO) and high carbon, high oxygen (HC/HO) treatments (Fig. 3, Table 4). To explore differences in metabolism attributable to a response to CO_2, the log transformed wet mass was used in a GLM as a covariate comparing the log transformed oxygen consumption (response variable) under low and high CO_2 conditions; each population within a species that was sampled in both basins or run at multiple experimental temperatures, was examined separately. There was no significant effect of CO_2 for any species in either basin.

3.4.2 Effect of basin

Following this assessment, we were interested in determining whether there were between basin differences in metabolic rate. As such we ran a GLM using log transformed metabolic rates for the three species that were found in both basins, normalized to 15 °C to account for differences in experimental temperature by applying a standard temperature coefficient. With the log-transformed wet mass as a covariate, we tested for an effect of basin, CO_2 and an interactive term. *Clio pyramidata* had a similar metabolic rate between basins. In contrast, *Cavolinia inflexa* ($F_{1,20}$=10.358, p=0.004) and *Styliola subula* ($F_{1,23}$=11.817, p=0.002) both had a significantly lower metabolic rate in the Pacific, although no interactive effect of CO_2.

3.4.2 Effect of O_2

For the species where enough individuals were collected to provide experimental replicates to explore the interactive effects of CO_2 and O_2 we also ran a species and basin specific GLM exploring the effect of treatment (Fig. 3, Table 5). *Clio pyramidata*, the only species we were able to test in both basins showed no significant effect of high CO_2, low O_2 or the interactive treatment in either basin. In the Pacific, *L. helicina* and *C. inflexa* similarly showed no significant change in metabolic rate as a consequence of any of the treatments. In contrast, in the Atlantic, there was a significant effect of treatment for *L. retroversa* and a Bonferroni post-hoc analysis comparing the treatments found that the high CO_2, low O_2 (HC/LO) treatment was significantly lower than all other treatments (Fig. 4A; $F_{3,38}$=17.836, p<0.001; a ~60% reduction in the average mass specific metabolic rate in comparison with the LC/HO treatment; Table 4). *Cuvierina atlantica* was tested at both 15 and 20 °C in the Atlantic, so to make comparisons among these experiments a temperature coefficient was applied and rates were normalized to 15 °C, after which no significant effect of any treatment was found for this species.

4. Discussion

This study reveals that short term exposure to low O_2 and high CO_2, similar to what would be experienced by individuals in the Pacific during diel vertical migration, does not influence the oxygen consumption of most of the thecosome pteropod species examined from either the Atlantic or Pacific. The only species which had a significant change in respiration in response to any of the treatments was *Limacina retroversa* from the Atlantic, which responded to the combined effect of low O_2 and high CO_2 with a reduction in oxygen consumption rate.

4.1 Experimental Design

A factor that should be considered when interpreting our results is the dynamic hydrographic conditions that the animals experience naturally between and within the ocean basins. Thecosomes of multiple species were found at a range of temperatures, salinities and carbonate chemistries, meaning that they experienced a range of pH and aragonite saturation states in their natural habitat. When comparing animals from multiple locations, we chose to use local water in order to replicate these natural conditions and to manipulate exclusively the CO_2 concentration, as this is the factor that is changing due to anthropogenic activity. This approach, however, does not control for the other parameters of the carbonate chemistry system, which will vary between regions. Despite this fact, there was a clean distinction between treatments, notably in terms of aragonite saturation state as well as CO_2 concentration, that provides insight into the effect of moderate short duration exposure to CO_2.

It is also important to note that the individuals of *L. helicina* from the Pacific experiments did occasionally have very high mortality during the period prior to experimentation (>80% at transit station T2 and T5, decreasing substantially to the northwest and along the main Pacific

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transect). These individuals, which are polar/sub-polar organisms and are typically found between -2 to 10 °C (Lalli and Gilmer, 1989), were collected from water that was likely near the upper limit of their optimal temperatures. Animals collected from these sites that were used in subsequent respiration experiments may therefore have been taken from an already stressed population of individuals and should be recognized as such.

4.2 Carbon Dioxide Effect

Hydrographic profiles collected in the Pacific coincident to sampling of thecosomes, indicate that organisms in the northern portion of the study region would experience conditions of high CO_2 and low O_2 in the upper ~450 m of their distribution (Chu et al., in review), unlike in the Atlantic. Despite these environmental differences, we found no significant effect of increasing CO_2 alone on the respiration rates of any of the species from either ocean basin. These results increase the published evidence that short term (6-18 h) exposure to enhanced CO_2 without synergistic stressors has no significant effect on the metabolic rate of many species of thecosome pteropods. Thus far, there are only two species that have been documented to show a change in metabolism based on exposure to manipulated CO_2 alone: *Limacina antarctica* (789-1000 µatm, 24 h: Seibel et al., 2012) and *Diacria quadridentata* (1000 µatm, 6-18 h: Maas et al., 2012a). The metabolic rates of all other species yet studied, including *Hyalocylis striata, Clio pyramidata, Diacavolinia longirostris, Creseis virgula* (6-18 h: Maas et al., 2012a), and *Limacina helicina* (24 h: Comeau et al., 2010a), were not significantly affected by short term exposure to high CO_2, although the latter species showed an increase in metabolic rate when high CO_2 was combined with high temperatures. Our results, which increase the geographic coverage for *L. helicina* and *C. pyramidata* and provide the first data about the species *C. pacifica, C. atlantica, L.retroversa, D. trispinosa, C.inflexa* and *S. subula,* corrobrate these earlier findings.

One interpretation of these results is that physiological responses may have occurred, but involved the reallocation of resources to different tissues or metabolic pathways; this redistribution could serve to maintain the thecosome total energy budget, and subsequently would not significantly change the metabolic rate of the individuals. A transcriptomic study done with individuals of *Clio pyramidata* as a companion project to the present work in fact suggested that expression of some genes was influenced by CO_2 exposure even though metabolic rate is not (Maas et al., 2015), perhaps suggesting some re-allocation among energetic demands. If this is the case it indicates that, to some degree, the short-term exposure to high CO_2 concentration is

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within the physiological tolerance of the tested species. Alternative hypotheses are that the duration of exposure was too short or the severity of the CO_2 treatment too minimal to elicit a measurable response. It is possible, for example, that some processes, like biomineralization, may be influenced by high CO_2, but only after a longer exposure duration. Finally, it may be that changes in respiration rate were subtle, requiring a much greater sample size to identify in light of biological variability, but exploration of this hypothesis would require a dedicated experiment to collect more individuals and likely a smaller number of species.

This possible tolerance to short term CO_2 exposure may be due to the fact that within their distribution or diel migrational range there are conditions, or perhaps seasons, where the natural hydrography causes many species of thecosome to experience conditions of high CO_2/low pH, and the species are therefore adapted to this range of exposure. The Arctic species *L. helicina* and subarctic species *L. retroversa*, for instance, are thought to inhabit waters which have been shown to reach a concentration of >950 µatm CO_2 and to be undersaturated with respect to aragonite during the winter season in Kongsfjord, Svalbard (Lischka and Riebesell, 2012). These conditions are pervasive throughout the upper water column, meaning that *L. helicina* and *L. retroversa*, which are not strong diel migrators, would experience seasonal under-saturation in these polar regions. The more temperate and tropical open ocean thecosomes, including *C. pyramidata, C. inflexa* and *S. subula* are all currently believed to be circumglobal and most, to varying degrees, diel migratory (Table 1; Bé and Gilmer, 1977; van der Spoel, 1967). Populations are therefore likely to encounter high CO_2 in sub-surface waters in regions associated with OMZs, including much of the North Pacific and off the coast of Northern Africa. The ability to cope with high CO_2 for short durations may have been selected for over time as a natural consequence of the types of unavoidable environmental variability experienced by these planktonic populations.

4.3 Low O_2 and Combined Effects

In the Pacific Ocean, none of the species for which we had enough individuals to perform the low O_2 study (*L. helicina, C. pyramidata,* and *C. inflexa*) had a significant change in metabolic rate under low (10%) O_2, even when combined with enhanced CO_2. These results indicate that the O_2 levels were above the concentration below which these species can no longer sustain their routine metabolic activity (Pcrit; Hochachka and Somero, 2002) and that any changes in physiology associated with the treatments required no increased energetic expenditure or metabolic reduction. As subsurface waters throughout the cruise were frequently below 10% O_2 (< ~130 µmol kg^{-1}), this indicates that these species may be naturally adapted to coping with low O_2 conditions.

In the Atlantic, examination of the effects of low O_2 is confound by an unfortunate and accidentally low level of CO_2 (~130 µatm) in the LC/HO treatment (Table 3). Tests of the effect of high CO_2 (HC/HO) and the interactive (HC/LO) treatments nonetheless remain valid, and for *L. retroversa*, exposure to HC/LO caused a large and significant reduction in metabolic rate. Suppression in metabolic rate is a common tactic for surviving unfavorable conditions (Guppy and Withers, 1999; Seibel, 2011). Although metabolic depression is generally survivable in the short term, over longer time scales there are often implications for growth, reproduction and survival (reviewed in: Pörtner, 2010; Seibel, 2011). In the Atlantic, our measured in situ O_2 levels were never below 15% (~200 µmol kg^{-1}). In contrast with the other species studied, which in at least some portions of their geographic range are occasionally found in association with subsurface low O_2 combined with hypercapnia, *L. retroversa* lives exclusively in the sub-polar North Atlantic Ocean and the Southern Circumpolar Current. As such this is the only species in this study in which no population is likely to experience conditions of low O_2 and high CO_2 together naturally anywhere in its distribution. Its inability to maintain metabolic rate during this interactive exposure may be a short-term metabolic response to environmental conditions that are unsustainable over longer time periods. As a consequence of the very low CO_2 in the LC/LO treatment, it is impossible to determine whether the metabolic suppression for *L. retroversa* in the HC/LO was in response to reduced O_2 availability alone or to the interactive effect of low O_2 with high CO_2. In the LC/LO treatment any change in respiration due to low O_2 could have been masked by a change in the energy budget as a response to the low (equivalent to pre-industrial atmospheric conditions) levels of CO_2. The results suggest that further work in the Atlantic is warranted to disentangle these stressors and to determine whether the observed change in metabolic rate was solely a consequence of O_2 availability or truly a synergistic effect.

Interestingly, although the temperature coefficients were not species-specific and may not, therefore, perfectly normalize the dataset, one trend revealed by their use was a significant difference in the normalized metabolic rates between species such as *S. subula* and *C. inflexa* from the Atlantic and Pacific Oceans. The comparatively lower metabolic rates from the Pacific may be a real response to the lower availability of O_2 for aerobic metabolism. Having a slower routine rate of O_2 consumption may be the result of a more efficient respiratory mechanism or an adaptation for coping with occasional exposures to the relatively high CO_2 and low O_2 conditions found in the northeast Pacific Ocean.

5. Conclusions

Thecosomes pteropods are thought to be some of the most sensitive of the oceanic zooplankton species to acidification. The responses we documented in the face of short-term CO_2 exposure and low O_2 reveal interesting patterns about basin scale differences in sensitivity, possibly associated with adaptation to local environmental conditions. Importantly, our results indicate that short-term exposure to high CO_2 does not have an effect on the respiration rate of multiple species of temperate and sub-polar thecosome species from both the North Atlantic and Pacific Oceans, irrespective of recent likely environmental exposure. The lack of effect of CO_2 as a single-stressor on metabolic rate in adult organisms of various species has been seen in a number of studies (reviewed in: Dupont et al., 2010; Kroeker et al., 2013), although there are many other metrics that have been shown to be more consistently affected. As such, thecosomes may have physiological coping mechanisms that allow them to maintain their energy budget for short periods of time in the face of high CO_2 via the re-allocation of their energetic resources. Over longer time periods, however, this could reduce their scope for growth and reproduction, negatively impacting the fitness of the population as has been demonstrated with other marine calcifiers (i.e.: Dupont et al., 2013; Melzner et al., 2013; Stumpp et al., 2011). Testing these hypotheses remains difficult as thecosomes are hard to maintain in captivity and there are no published studies of individuals kept fed and exposed to CO_2 in laboratory conditions for long durations (reviewed in: Howes et al., 2014; Thabet et al., 2015). Keeping individuals well fed is a critical factor since high food availability has been suggested to modulate the effect of high CO_2 exposure in both thecosomes (Seibel et al., 2012) and in other calcifying species (Thomsen et al., 2013). Comparative short-term studies of wild caught animals such as the present experiments, therefore, currently give us the best insight into the sensitivity of these open-ocean populations, and the ability to predict how they will respond to the expected changes in the ocean environment.

These findings also draw attention to the consequences of the high degree of vertical variability in the open ocean environment, with animals in the Pacific found migrating between

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deep waters, undersaturated with respect to aragonite, and the surface (Lawson, unpublished data; Chu et al., in review; Maas et al., 2012a). Recent studies in the California Current system indicate that thecosome shells show signs of in situ dissolution when associated with water masses that are undersaturated with respect to aragonite (Bednaršek et al., 2014b; Bednarsek and Ohman, 2015). Although our short duration experiments do not directly address the effect of longer-term exposure to high CO_2, it does remind us that as open ocean environments respond to anthropogenic change there may be vertical refugia from OA stress as well as regions where animals may already experience high CO_2. As surface waters acidify, the ability to endure short-duration exposure and to migrate in both the Atlantic and Pacific populations may provide mechanisms for mitigating detrimental effects of acidification exposure. The potential compression of vertical habitat associated with the shoaling of the aragonite compensation depth, however, may have implications for predator/prey interactions, carbon pumping and other ecosystem functions (Bednarsek and Ohman, 2015; Seibel, 2011). Furthermore, it is clear that thecosome shells are highly sensitive to dissolution (Comeau et al., 2012; Lischka and Riebesell, 2012; Manno et al., 2012) and there could be fitness and ecological consequences of dissolution in regions with vertical variation in carbonate chemistry.

Finally, as concerns about increasing CO_2 drive further explorations of comparative organismal physiology in the marine system, it is important to recognize that often the exposure of animals to increased CO_2 will occur in concert with expanding regions of low O_2. This has been explored in the coastal environment where the interaction of acidification with eutrophication and associated low O_2 is comparatively well studied (Cai et al., 2011; Melzner et al., 2013), and in theoretical frameworks (Gruber, 2011; Pörtner, 2010; Sokolova, 2013). Experiments in the open ocean environment, however, are only beginning to be conducted and their implications explored. This study suggests that to make accurate predictions about how populations will respond to climate change and adequately understand the factors affecting organismal response, further investigations of the interactive effects of low O_2 and hypercapnia should consider natural environmental variability, population biogeography and phylogenetic sensitivity.

Data availability

Cruise data for the project is available via BCO-DMO under the project "Horizontal and Vertical Distribution of Thecosome Pteropods in Relation to Carbonate Chemistry in the Northwest Atlantic and Northeast Pacific" (http://www.bco-dmo.org/project/2154). The raw data for the respiration experiments are included in this deposition (DOI: 10.1575/1912/6421).

Author contributions

A. Maas and G. Lawson designed the experiments. All co-authors participated in oceanographic cruises and collection of samples. A. Maas conducted all of the experiments and statistical analyses. Z.A. Wang advised on the design of the carbonate chemistry analysis and provided the measurements of both the hydrographic and experimental conditions. A. Maas prepared the manuscript with contributions from all co-authors.

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Table 1: Environmental preferences and diel vertical migratory patterns for the species used in this study based on previously published data (Bé and Gilmer, 1977; Lalli and Gilmer, 1989). Data includes published full ranges at which organisms have been found, as well as previous authors' estimates of the prefered (optimal) ranges of each species when available. Note that these are based on relatively sparse observations of broadly distributed speceis, many of which may be cryptic congeners, and thus should be treated as estimates.

Species	(optimal) temp (°C)	(optimal), depth (m)	migrator?
Cuvierina atlantica	18 to 26	100-250	possible
Cuvierina pacifica	Only recently established as a separate species, the habits are assumed to be similar to the Atlantic congener.		
Cavolinia inflexa	16 to 28	0-250	no
Clio pyramidata	7 to 27	(0-500), <1500	yes
Limacina helicina	(-2 to 10)	(50-100), <300	possible
Limacina retroversa	(7 to 12)	(20-30), < 150	possible
Styliola subula	(18 to 22)	50-300	yes
Diacria trispinosa	9 to 28	30-200	no

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Table 2: The hydrography and location for each station where animals for experiments were collected. At stations along the main transect the depth (m) at which O_2 decreased below 130 µmol O_2 kg^{-1} (~10%) and the average temperature from 25-100 m (°C) were derived from CTD casts. At a few stations (denoted via *[a])* in the Atlantic there was warm water at the surface and cold water below. The only species in this region, *Limacina retroversa*, has an optimum temperature between 7-12 °C (Bigelow, 1924) and was generally found above 50 m (Lawson, unpublished data). At these sites the average temperature is reported first for between 25-100 m and then also for 25-50 m to reflect the conditions likely experienced by the pteropods. pCO_2 and Ω_{Ar} were calculated from measured pH and DIC bottle samples. We interpolated linearly the depths (m) at which the pH decreased below 7.7, pCO_2 reached 800 µatm, and aragonite saturation (Ω_{Ar}) reached 1 from the discrete measurements at adjacent depths. At stations conducted while in transit to the main study transects (denoted by prefix T) the average temperature from 25-100 m (°C) was documented from XBT casts. At these transit stations no O_2 or carbonate chemistry data were available (noted with a dash). The species caught at each station and used in this study are demarcated with a star (*).

Year	Station	Latitude (°N)	Longitude (°W)	average temp 25-100 m	depth of 130 µmol O_2 kg^{-1}	depth of pH 7.7	depth of 800 µatm	depth of $\Omega_{Ar} = 1$	*C. atlantica*	*C. pacifica*	*C. inflexa*	*C. pyramidata*	*L. helicina*	*L. retroversa*	*S. subula*	*D. trispinosa*
2011	32	49.1	-44.3	5.3, 9.0	NA	74.1	NA	NA						*		
Atlantic	31	50.0	-42.0	14	NA	385.4	NA	NA								*
	30	49.6	-41.9	14.1	NA	452.8	NA	NA	*							*
	26	47.5	-42.0	13.3	NA	644.9	NA	NA	*			*				
	24	46.5	-42.0	14.5	NA	453.9	NA	NA	*			*				
	21	44.9	-42.0	16.5	NA	501.1	NA	NA				*				*
	19	44.0	-44.9	4.9, 11.2	NA	181.0	NA	NA						*		
	17	43.0	-47.8	1.8, 8.1	NA	143.1	NA	NA						*		
	13	40.9	-52.0	20.7	NA	756.7	NA	NA	*		*				*	
	10	47.5	-52.0	19.4	NA	466.9	NA	NA	*		*				*	
	8	38.5	-52.0	22.8	NA	805.7	NA	NA	*		*				*	
	3	36.0	-52.0	21.4	NA	937.7	NA	NA	*							
2012	T2	45.6	-128.5	-	-	-	-	-				*	*			
Pacific	T3	46.6	-133.5	-	-	-	-	-					*			
	T4	47.7	-138.5	6.4	-	-	-	-				*				
	T5	45.7	-129.8	10.0	-	-	-	-					*			
	T6	46.6	-134.9	9.5	-	-	-	-					*			
	T7	47.6	-140.2	8.6	-	-	-	-				*				
	3	49.0	-148.2	6.2	209	128.9	193.7	168.5								
	6	47.5	-145.6	7.1	235	108.3	199.2	159.1				*	*			
	7	47.0	-144.6	7.8	256	131.0	214.0	185.1				*				
	15	43.1	-138.1	10.9	363	199.5	368.2	334.8				*				
	18	41.5	-135.8	13.7	340	147.3	331.7	380.6				*				
	21	39.9	-135.0	12.7	348	162.0	332.2	302.8		*						
	24	38.6	-135.0	14.7	402	222.8	411.8	372.7		*		*				
	30	35.6	-135.0	16.2	349	200.7	437.8	425.1		*	*	*				
	32	34.4	-135.1	16.5	348	202.9	439.2	432.0		*	*	*				
	34	33.6	-135.0	17.4	368	233.3	370.1	352.4			*	*			*	
	T9	33.7	-133.6	17.0	-	-	-	-		*	*	*				
	T10	33.8	-133.2	15.9	-	-	-	-		*	*	*				

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Table 3: Carbonate chemistry during manipulation experiments. The manipulation experiments were conducted at multiple temperatures (T.) and salinities (S.) based on the conditions the organisms were caught in. As described in more detail in the text, DIC measurements were made of water drawn from the control chambers while TA was measured for batches of experimental water (denoted as xpt. TA). In situ TA (i.s. TA), based on nearby CTD bottle sampling at the surface, is also shown. At test stations, where bottle samples of in situ TA were unavailable, underway pCO_2 values and the LC/HO DIC were used to calculate in situ TA (denoted with *). In some instances, measurements of experimental TA differed by >20 µmol kg^{-1} from nearby in situ measurements of surface TA. This difference greatly exceeds expected variability based on measurement uncertainty and spatial (geographic and vertical) offsets in the locations of experimental water collection relative to the nearest CTD cast; in these circumstances, the experimental TA was likely erroneous due to sampling errors (e.g., contamination). For completeness, and to aid in identification of erroneous experimental TA values, calculations of carbonate chemisty parameters, including aragonite saturation state (Ω_{Ar}) and pCO_2 were made based on DIC and both experimental TA and in situ TA. In further data analysis and interpretation, calculations based on experimental TA are given preference except those few instances where experimental TA differed from in situ by >20 µmol kg^{-1} (bold denotes preferred calculations). Calculated saturation state and pCO_2 are reported as the average and standard deviation per batch of water. Note that the LC/LO gas tank in 2011 (in italics) appears to have been improperly mixed by the manufacturer as calculations suggested it contained a much lower CO_2 level than the intended 380 µatm; it should consequently be considered an entirely separate treatment from the 2011 LC/HO (were CO_2 levels were based on bubbling with an ambient air line).

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	Treatment	T. °C	S.	i.s. TA (µmol kg^{-1})	xpt. TA (µmol kg^{-1})	DIC (µmol kg^{-1})	i.s. ΩAr	i.s. pCO_2 (µatm)	xpt. ΩAr	xpt. pCO_2 (µatm)
2011	380 µatm CO_2/	10	33	2300.3	**2307.3**	2094.4	2.3 ± 0.2	336.2 ± 37.7	**2.4 ± 0.2**	**324.8 ± 35.8**
Atlantic	21% O_2	15	33	2300.3	**2307.3**	2066.5	2.6 ± 0.7	404.5 ± 172.7	**2.7 ± 0.7**	**390.8 ± 164.5**
		15	35	**2296.4**	2354.5	2066.4	**2.5 ± 0.1**	**382.3 ± 20.4**	3.1 ± 0.1	297.7 ± 14.3
		20	34	2353.4*	**2345.8**	2028.6	3.6 ± 0.2	302.8 ± 31.6	**3.5 ± 0.2**	**311.6 ± 32.9**
		20	34	2366.0	**2367.2**	2077.5	3.3 ± 0.1	363.1 ± 23.2	**3.3 ± 0.1**	**361.4 ± 23.1**
	380 µatm CO_2/	10	33	2300.3	**2307.3**	1919.7	4.0	139.0	**4.1**	**135.5**
	10% O_2	15	33	2300.3	**2307.3**	1774.8	5.5 ± 0.6	101.2 ± 23.9	**5.6 ± 0.6**	**99.0 ± 23.3**
		15	35	**2296.4**	2354.5	1852.7	**4.6**	**139.2**	5.3	116.1
	800 µatm CO_2/	10	33	2300.3	**2307.3**	2219.7	1.2 ± 0.2	779.9 ± 114.0	**1.2 ± 0.2**	**742.4 ± 106.8**
	21% O_2	15	33	2300.3	**2307.3**	2208.0	1.3	908.7	**1.4**	**867.8**
		15	35	**2296.4**	2354.5	2139.5	**1.9**	**585.2**	2.4	434.4
		20	34	2353.4*	**2345.8**	2176.9	2.1 ± 0.1	651.8 ± 23.4	**2.1 ± 0.1**	**678.2 ± 24.8**
		20	34	2366.0	**2367.2**	2212.7	1.9 ± 0.4	786.0± 196.0	**1.9 ± 0.4**	**780.9 ± 194.2**
	800 µatm CO_2/	15	33	2300.3	**2307.3**	2186.2	1.5 ± 0.2	788.7 ± 157.6	**1.5 ± 0.2**	**754.9 ± 148.3**
	10% O_2	15	35	**2296.4**	2354.5	2179.6	**1.5 ± 0.3**	**782.9 ± 164.6**	2.0 ± 0.3	558.2 ± 103.9
2012	380 µatm CO_2/	10	32.1	2151.9*	**2142.8**	1934.8	2.2 ± 0.1	285.2 ± 21.4	**2.3 ± 0.1**	**283.0 ± 21.2**
Pacific	21% O_2	10	33.5	2208.0	**2222.7**	2001.9	2.4 ± 0.6	302.2 ± 100.9	**2.4 ± 0.6**	**303.3 ± 101.4**
		15	32.5	**2182.6***	2095.7	1983.4	**2.2 ± 0.0**	**388.1 ± 5.5**	1.4 ± 0.0	646.7 ± 11.5
		15	33.5	2208.0	**2222.7**	2020.8	2.3 ± 0.2	407.7 ± 52.1	**2.3 ± 0.2**	**409.1 ± 52.4**
	380 µatm CO_2/	10	32.5	**2182.6***	2095.7	1973.9	**2.3 ± 0.1**	**295.5 ± 20.0**	1.4 ± 0.1	489.2 ± 41.2
	10% O_2	15	33.5	2208.0	**2222.7**	2017.5	2.3	3956.0	**2.3**	**397.4**
	800 µatm CO_2/	10	32.1	2151.9*	**2142.8**	2026.3	1.4 ± 0.1	525.0 ± 35.0	**1.4 ± 0.1**	**519.7 ± 34.5**
	21% O_2	10	33.5	2208.0	**2222.7**	2120.6	1.3	628.2	**1.3**	**631.2**
		15	32.5	**2182.6***	2095.7	2031.7	**1.8 ± 0.1**	**527.6 ± 50.9**	1.0 ± 0.1	952.4 ± 115.1
		15	33.5	2208.0	**2222.7**	2112.2	1.4 ± 0.2	736.0 ± 96.0	**1.4 ± 0.2**	**739.4 ± 96.6**
	800 µatm CO_2/	10	32.5	**2182.6***	2095.7	2066.5	**1.4 ± 0.1**	**545.5 ± 65.1**	0.8 ± 0.1	1056.0 ± 151.6
	10% O_2	15	33.5	2208.0	**2222.7**	2118.3	1.4	762.4	**1.4**	**766.0**

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Table 4: The average wet mass (mass; g) and mass-specific oxygen consumption rate (MO_2; µmol O_2 g^{-1} h^{-1}) ± the standard errror (SE) for each treatment (Treat.) and species. The number of individuals (N) per treatment are reported and the species are arranged by temperature (Temp; °C) as well as the year and basin of collection.

Year	Temp.	Species	Treat.	N	mass	±SE	MO_2	±SE
2011	10	*Limacina retroversa*	LC/HO	12	.00281	0.00037	10.33	1.17
Atlantic			HC/HO	13	.00284	0.00031	10.10	0.56
			LC/LO	9	.00274	0.00026	8.12	0.66
			HC/LO	9	.00377	0.00053	4.21	0.55
	15	*Clio pyramidata*	LC/HO	10	.01944	0.00408	7.81	0.71
			HC/HO	8	.01410	0.00435	8.55	1.48
			LC/LO	9	.02363	0.00867	6.63	1.21
			HC/LO	8	.03945	0.00467	6.99	0.45
		Cuvierina atlantica	LC/HO	8	.04493	0.00264	5.05	0.63
			LC/LO	10	.04636	0.00252	3.25	0.28
			HC/LO	10	.05040	0.00219	4.29	0.37
		Diacria trispinosa	LC/HO	8	.03718	0.00316	4.44	0.56
			HC/HO	10	.03589	0.0027	4.09	0.51
	20	*Cuvierina atlantica*	LC/HO	9	.01876	0.00396	4.31	0.85
			HC/HO	9	.01683	0.00284	4.53	1.13
		Cavolinia inflexa	LC/HO	8	.00626	0.00104	14.30	1.48
			HC/HO	4	.00508	0.00049	13.81	1.39
		Styliola subula	LC/HO	10	.00400	0.00038	13.96	1.80
			HC/HO	8	.00289	0.00035	15.95	0.87
2012	10	*Limacina helicina*	LC/HO	7	.00140	0.00026	5.26	1.17
Pacific			HC/HO	8	.00149	0.00021	5.51	0.69
			LC/LO	6	.00300	0.00058	4.91	0.69
			HC/LO	10	.00296	0.00038	7.18	1.45
		Clio pyramidata	LC/HO	9	.02646	0.00258	5.43	0.45
			HC/HO	8	.02355	0.00369	4.39	0.60
			LC/LO	14	.01459	0.00185	5.58	0.81
			HC/LO	12	.01250	0.00245	5.72	1.14
	15	*Cuvierina pacifica*	LC/HO	4	.01829	0.00563	3.41	0.56
			HC/HO	7	.02130	0.00636	3.53	0.57
		Cavolinia inflexa	LC/HO	5	.01330	0.00062	3.53	0.44
			HC/HO	8	.01556	0.00149	3.34	0.41
			LC/LO	4	.01405	0.00185	2.41	0.33
			HC/LO	2	.01855		3.98	
		Styliola subula	LC/HO	6	.00360	0.00044	5.30	1.20
			HC/HO	4	.00220	0.00029	7.73	2.14
		Clio pyramidata	LC/HO	4	.03020	0.0037	3.82	0.66
			HC/HO	5	.02904	0.00329	3.21	0.27

Table 5: Statistical results of the univariate general linear models (GLM) for each species were analyzed separately by year and are listed by the temperature of the experiment (Temp.; °C). For species studied at multiple temperatures (denoted by *), the metabolic rates were adjusted to 15°C using a $Q_{10} = 2$ to allow for direct comparison. The effect of the independent factors of CO_2 level (CO_2), O_2 level (O_2), their interactive effect (Int.) and the covariate of mass were analyzed in regards to the metabolic rate and reported as *p*-values for the Pacific (mean mass specific metabolic rate values found in Table 4). For the Atlantic, each treatment was tested as independent (Treat.) due to the accidentally low CO_2 condition in the LC/LO gas mixture.

			Effect on metabolic rate				
Year	Temp.	Species	CO_2	O_2	Int.	Treat.	Mass
2011	10	*Limacina retroversa*				**<0.001**	<0.001
Atlantic	15	*Clio pyramidata*				0.295	<0.001
		*Cuvierina atlantica**				0.174	<0.001
		Diacria trispinosa	.731				<0.001
		Cavolinia inflexa	.677				.008
		Styliola subula	.791				.040
2012	10	*Limacina helicina*	.464	.323	.914		.007
Pacific	15	*Clio pyramidata**	.255	.156	.726		.018
		Cuvierina pacifica	.709				<0.001
		Cavolinia inflexa	.309	.717	.219		.113
		Styliola subula	.763				.668

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Figure legends

Figure 1: Cruise tracks and animal sampling. Thecosomes were collected during the night at stations along the main survey transect (solid line) and at stations during transit (dashed line) during cruises to the northwest Atlantic in 2011 and northeast Pacific in 2012. The shapes correspond to the species caught at each station and used in this study. Blue (10 °C), grey (15 °C) and red (20 °C) boxes around the station numbers (#) correspond to the temperature that was representative of 25-100 m at each station (Table 2) and used in the experiments with animals from that station.

Figure 2: Hydrography of sampling regions. Hydrographic profiles of stations representative of the specific water mass types from the northern (P-T5, P-6, A-26), middle (P-18, A-19) and southern (P-32, A-8) portions of the Pacific (P) and Atlantic (A) study transects (station locations: Fig. 1). At station P-T5, the temperature profile (grey) was from an XBT cast because no CTDs were conducted during transits. For all stations along the main transects, left-hand plots show temperature (grey), salinity (black) and oxygen (black dotted) measured via sensors on the CTD and binned to 1 m depth intervals. Middle plots show TA (black) and DIC (grey) from from discrete bottle samples (dots show depths of bottle samples). Right-hand plots show pCO_2 (black) and aragonite saturation state (Ω; grey) calculated based on TA and DIC measurements.

Figure 3: Thecosome respirometry. Mean metabolic rate and standard error (μmol O_2 g^{-1} h^{-1}) of thecosomes exposed to low (i.e., ambient) CO_2 and normal levels of O_2 (light blue; LC/HO), high CO_2 and normal O_2 levels (dark blue; HC/HO), low CO_2 and low O_2 (light red; LC/LO), or high CO_2 and low O_2 (dark red; HC/LO). The species and temperature of the experiment are reported below the x-axis. Significance is reported based on a basin, species, and temperature specific GLM which tested for the effect of treatment on O_2 consumption with a Bonferroni post-hoc analysis. In the Atlantic analysis each treatment was tested independently, while in the Pacific CO_2 and O_2 were treated as factors. For each species and temperature, treatments are reported as non-significant (N.S.) or, in the case of significance, by letters that indicate which treatments are statistically similar (same letter) or different (different letter) at a p-value < 0.05.

Note that for *C. atlantica* the metabolic rates of individuals respired at 20° C were converted to 15°C using a temperature coefficient of 2 (see methods) for this GLM analysis.

Figure 4: Log transformed metabolic rates (μmol O_2 h^{-1}) for *L. retroversa* at 10 °C, not normalized to mass, plotted against the log transformed wet mass (mg) of individuals exposed to low CO_2 and normal levels of O_2 (black circles; LC/HO), high CO_2 and normal O_2 levels (dark grey diamonds; HC/HO), low CO_2 and low O_2 (white circles; LC/LO), or high CO_2 and low O_2 (light grey diamonds; HC/LO).

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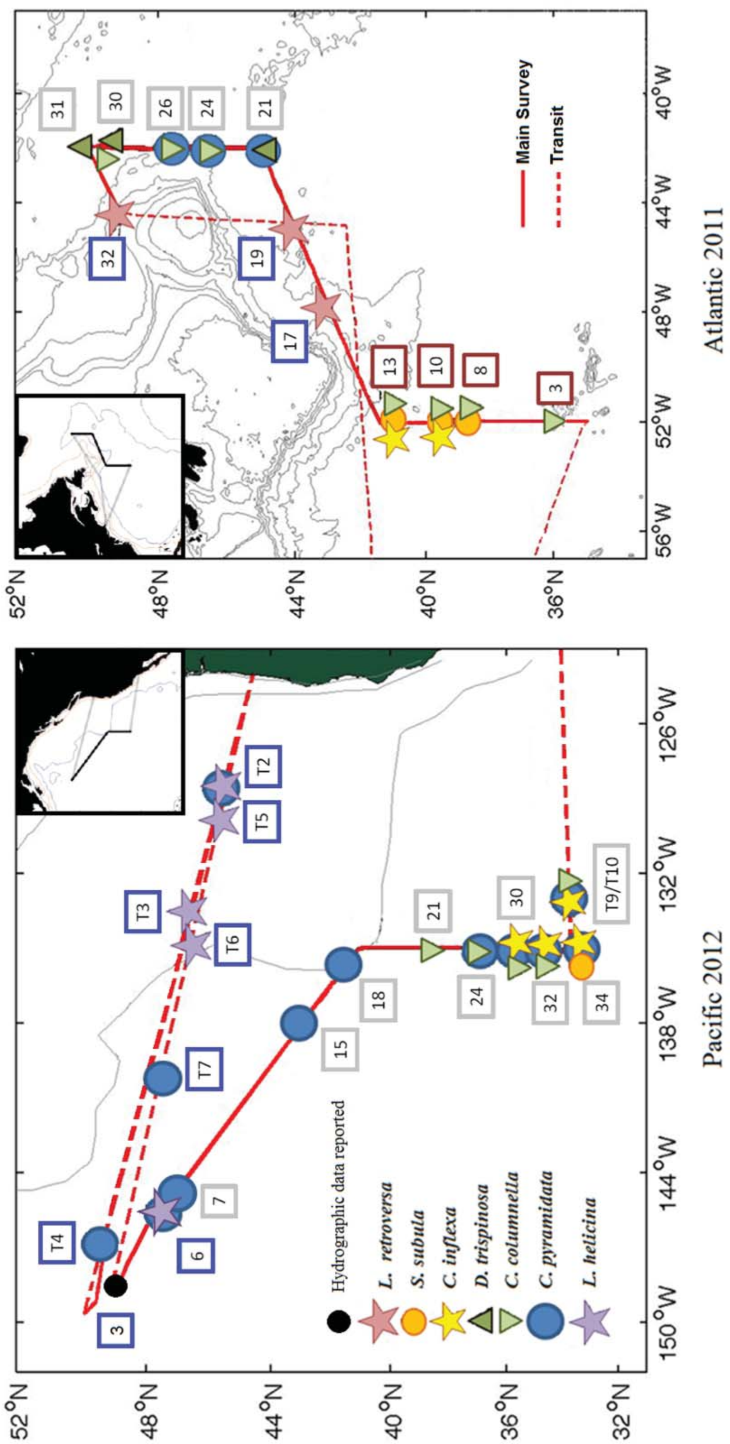

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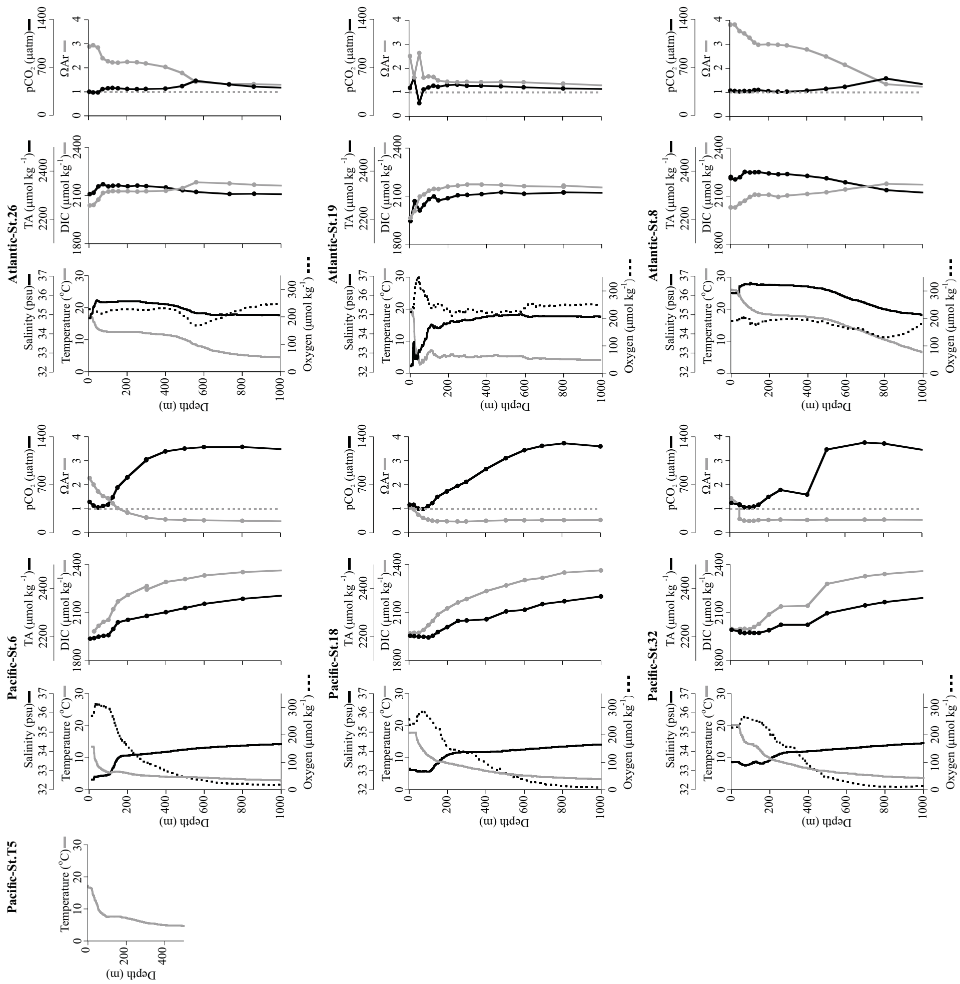

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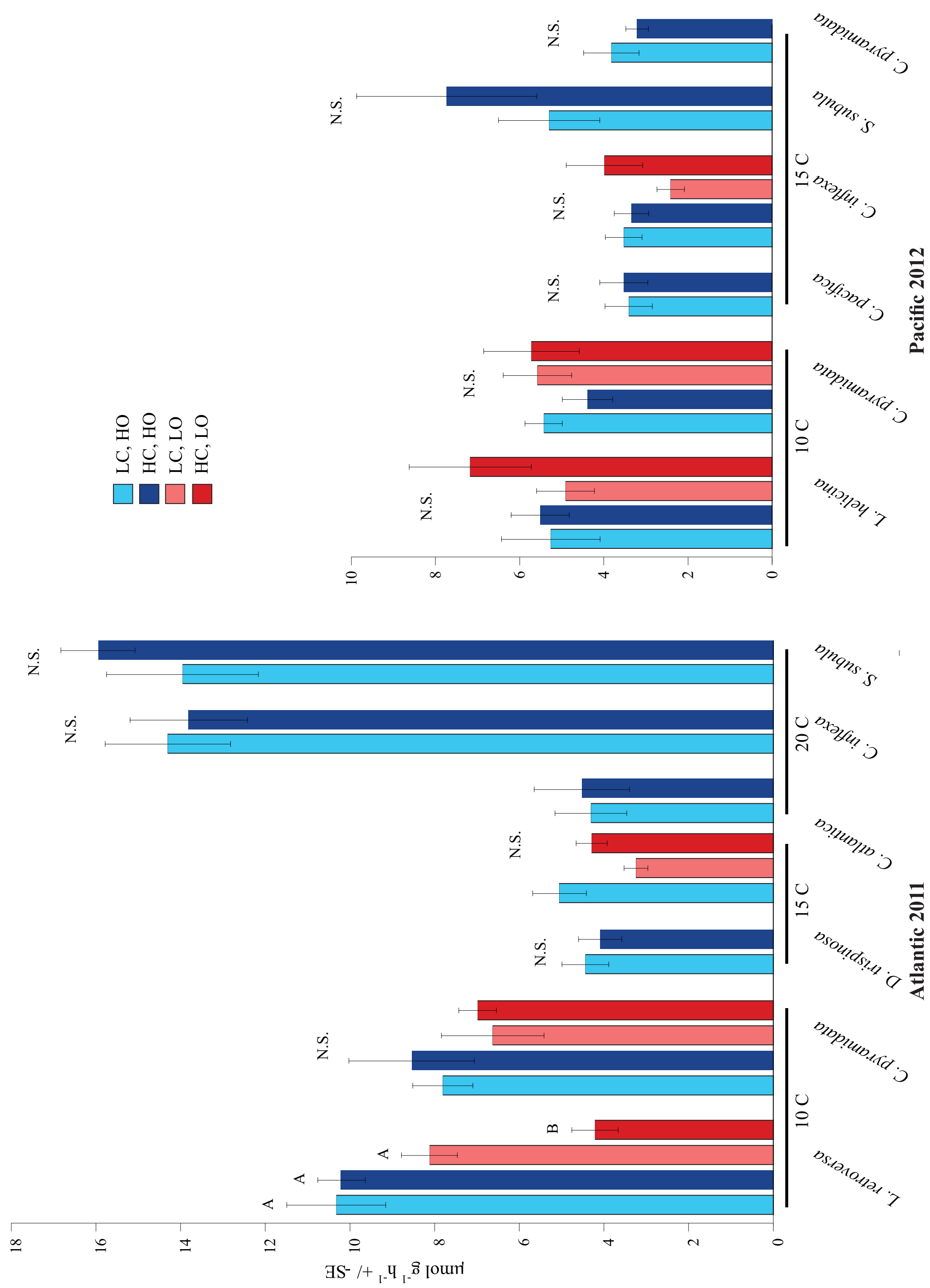

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