Metabolic suppression in thecosomatous pteropods as an effect of low temperature and hypoxia in the Eastern Tropical North Pacific

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Abstract

Many pteropod species in the eastern tropical north Pacific Ocean migrate vertically each day, transporting organic matter and respiratory carbon below the thermocline. These migrations take species into cold (15- 10º C) hypoxic water (< 20 µmol O_2 kg^{-1}) at depth. We measured the vertical distribution, oxygen consumption and ammonia excretion for seven species of pteropod, some of which migrate and some which remain in oxygenated surface waters throughout the day. Within the upper 200 meters of the water column, changes in water temperature result in a ~60-75% reduction in respiration for most species. All three species tested under hypoxic conditions responded to low O_2 with an additional ~35-50% reduction in respiratory rate. Combined, low temperature and hypoxia suppress the metabolic rate of pteropods by ~80-90%. These results shed light on the ways in which expanding regions of hypoxia and surface ocean warming may impact pelagic ecology.

Introduction

Regions of low oxygen (O_2 < 20 µmol kg^{-1}) account for ~ 7% of the volume of ocean waters (Paulmier and Ruiz-Pino). Most of this low O_2 water is found in mesopelagic features called oxygen minimum zones (OMZ) that are stable relative to the movements and life cycles of the planktonic species living there. These regions occur when high productivity in surface waters promotes an extensive export of fixed carbon to depth, as in the eastern tropical north Pacific (ETP) and the Gulf of California. Below the photic zone, midwater organisms feeding on surface-derived material respire the available O_2 at a rate faster than it can be replenished by slow deep ventilation rates (Wyrtki 1962; Fiedler and Talley 2006; Karstensen et al. 2008). These low O_2 waters influence the abundance and vertical distribution of organisms throughout the water column (Morrison et al. 1999; Wishner et al. 2000; Wishner et al. 2008).

The distribution of animals interacting with OMZs is complex, with high surface abundance and species-specific patterns of association with low O_2 waters (Wishner et al. 2008; Robinson et al. 2010). Depending upon the energetic demands of the organism and the severity of the hypoxia, OMZs can support both resident and transient (migratory) life (Seibel 2011). Species that inhabit regions of hypoxia have highly effective O_2 extraction, transport and delivery systems which allow them to function at very low O_2 partial pressures (Sanders and Childress 1990; Childress and Seibel 1998; Seibel et al. 1999). When O_2 concentrations drop below the level at which these animals can sustain their routine metabolic activity (P_{crit}), metabolism may be suppressed, by curtailing expensive physiological processes, or supplemented anaerobically (Hand and Hardewig 1996; Hochachka et al. 1996; Guppy and Withers 1999). Individuals that have surpassed their O_2 threshold must retreat to regions with O_2 levels above their P_{crit} to pay off their oxygen debt and return to routine metabolic rates.

Vertical migrators experience large variations in O_2 concentration and temperature that influence their metabolism, with consequences for species distribution, biogeochemical cycles and ecosystem dynamics (Stramma et al. 2010; Seibel 2011). Above a particular concentration, the ability of an organism to extract O_2 from seawater has evolved to match the lowest O_2 partial pressure encountered in the environment, and the metabolism of midwater animals is generally independent of O_2 (Childress and Seibel 1998). However, O_2 extraction appears to be constrained below a certain threshold, estimated near 10 µmol kg^{-1} – a value which is commonly found in the most extreme OMZs such as in the ETP (Seibel 2011). Often these hypoxic waters

are associated with deeper, cooler water masses. Colder conditions enhance the ability of migratory animals to endure hypoxia by reducing the demand for energy. Metabolism generally decreases by 2-3 fold as a result of a 10°C reduction in temperature for marine ectotherms within their natural thermal range (Hochachka and Somero 2002).

Diel vertical migratory species inhabit the surface at night and retreat into the upper oxycline and the OMZ during the daytime, putatively for niche partitioning, metabolic advantage, or predator avoidance (Hays 2003; Fernández-Álamo and Färber-Lorda 2006; Antezana 2009). Consuming fixed carbon at night, migrators transport organic matter below the thermocline where it is excreted as respiratory carbon (CO_2) and waste products (NH_3, fecal matter, DOC). This process contributes to the biological pump of carbon and nitrogen from surface waters to depth (Longhurst and Harrison 1989; Dam et al. 1995; Hays et al. 1997; Steinberg et al. 2000; Honjo et al. 2008; Robinson et al. 2010). Calculations based on these processes have been used to model the carbon flux to the deep sea. However, there are currently large imbalances in these dark ocean carbon budgets (Burd et al. 2010). Unaccounted for variation in species composition and changes in metabolic rate due to hypoxia may contribute to the uncertainty in these estimates of biogeochemical cycling (Buesseler et al. 2007; Seibel 2011). In order to accurately calculate export production, remineralization rates and carbon cycling, we must understand how the metabolic rate and depth distribution of migrators is impacted by the environmental conditions of the OMZ.

The importance of having good estimates of these cycles is made more pressing by the fact that human activities are altering temperatures and O_2 levels in the open ocean, and these physical changes interact synergistically on organisms and ecosystems (Rosa and Seibel 2008; Pörtner 2010; Seibel 2011; Vaquer-Sunyer and Duarte 2011). The warming of surface waters leads to a decrease in O_2 solubility so that as mixing occurs, less O_2 is carried to depth. Warming also increases stratification, which generally prevents mixing. Together, higher temperatures and a stable mixed layer produce conditions favorable for phytoplankton blooms, resulting in greater export of carbon out of surface waters (Sarmiento et al. 1998; Bopp et al. 2005; Behrenfeld et al. 2006). Decreases in O_2 exchange and increases in surface export contribute to the current theorized worldwide expansion of OMZs, with unknown implications for marine biota and biogeochemical cycles (Oschlies et al. 2008; Stramma et al. 2008; Stramma et al. 2010). Together hypoxia and rising temperatures could substantially alter the population distribution,

abundance and community structure of plankton throughout OMZ regions. It has been hypothesized that changes in species dynamics in response to anthropogenic forcing factors could also have a strong feedback on climate, reducing carbon sequestration in the ocean depths (Buesseler et al. 2007).

In order to understand how climate change will affect the ecology of diel vertical migrating zooplankton, we must first quantify the physiological tolerance of particular taxa to the environmental factors that they are exposed to in their environment. Here, we document the vertical distribution and abundance of pteropod species in the Eastern Tropical North Pacific and measured their O_2 consumption and NH_3 excretion rates under conditions that mimic their day- and nighttime habitats. Our results contribute to the understanding of marine zooplankton response to expanding OMZs. Thecosomatous pteropods are, in theory, unlikely inhabitants of OMZs; their aragonitic shells are believed to be vulnerable to dissolution in low pH, high CO_2 environments, such as the OMZ. However, it has been shown that some species migrate daily into the OMZ and that the metabolic rates of these species are not influenced by short term exposure to carbon dioxide (Maas et al. 2012). This study seeks to establish whether the presence of pteropods within the OMZ, despite high levels of carbon dioxide, may be facilitated by the effect of temperature and low oxygen on their physiology.

Methods

Pteropod distributions were sampled during the day and night during October - November 2007 and December 2008 - January 2009 at the Tehuantepec Bowl (11^o N 98^oW) and the Costa Rica Dome (9^o N 90^o W) using a vertically stratified MOCNESS (Wiebe et al. 1985) as part of the ETP Project (PI: K. Daly). This system was equipped with a Seabird SBE43 electronic sensor for O_2, as well as sensors for temperature, depth, salinity, and % light transmission. Samples for the pteropod study were collected from 0-400 meters using 153-µm mesh nets in sampling intervals which varied from 10 meters to 150 meters thick in order to capture fine scale detail at ecologically important transitions (Table 1). At sea, the contents of the nets were split using a flat-bottomed Motoda splitter, then half of the sample was preserved in 4% sodium borate-buffered formalin and sea water. In the lab these samples were poured through a 64-µm mesh sieve and washed into a Pyrex dish for sorting. Pteropods were sorted, identified and enumerated using a dissecting microscope. Population densities (individuals m^{-3}) were calculated for each

depth interval, taking into account net volume filtered and split size. Using this information, the mean weighted depth was calculated using the equation of Perry et al. (1993) as mean weighted depth =

$$\Sigma(x_i \text{ x } z_i)/ \quad \Sigma(x_i)$$

where x_i = # individuals m^{-3} and z_i = mid-depth of the net.

Using the PRIMER 6 Statistical Package (PRIMER-E, Luton UK) we created a principal component analysis of the environmental data using the mean temperature, O_2, salinity and percent light transmission values from the depth range of each net. Depth categories (0-50, 50-100, 100-350, 350-400) were assigned to each sample to allow comparisons across years despite variations in net deployment (Table 1). These values were chosen to match general hydrographic features, specifically temperature and oxygen gradients, and to provide consistency with other analyses (Wishner et al. 2008; Wishner et al. in prep). O_2 concentration data was unavailable from the MOCNESS sensor in these tows for the top 40 meters at CRD in 2007; these data were estimated using values collected by CTD casts made at the same station and year (processed by Dr. C. Flagg, Stony Brook University). Before analysis, the hydrographic data were log-transformed to achieve multivariate normality (Clarke and Gorley 2006). The principal component analysis of environmental data was paired with a resemblance matrix based on a Bray-Curtis similarity measure of pteropod presence/absence from 0-400 meters. Using the BEST BIOENV statistical analysis (Clarke 1993), we calculated which hydrographic features best predicted the distributional patterns of thecosome pteropods during the day and night.

For physiological studies, thecosomatous pteropods were collected from the Gulf of California (27^o N 112^o W) in June 2007 and from the Tehuantepec Bowl (11^o N 98^oW) and the Costa Rica Dome (9^o N 90^o W) during the ETP project (Fig. 1). Seven pteropod species were studied: *Hyalocylis striata, Creseis virgula, Clio pyramidata, Cavolinia uncinata, Cavolinia inflexa, Cavolinia longirostris* and *Diacria quadridentata*. These animals were collected with either 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). CTD casts of the water column were made just before or after collection periods to allow for comparisons with hydrography.

Post-capture, organisms were kept at either 10° or 20°C in 0.2 micron-filtered water for at least eight hours in densities < 10 individuals L^{-1} to allow for gut clearance and temperature

acclimation. Individuals were then transferred into 0.2 micron-filtered seawater in 10 mL glass syringe respiration chambers that were placed in temperature-controlled waterbaths. The experimental water was treated with 25mg L^{-1} each of Streptomycin and Ampicillin to minimize microbial respiration and remain methodologically consistent with previous studies (Childress 1971; Seibel et al. 1997; Rosa and Seibel 2010; Maas et al. 2011).

Respiration experiments investigating the effects of temperature ranged in duration from 6-18 hours to provide time for a measureable change in oxygen saturation. This variation was a function of the differences in size and metabolic rate of individuals of various species. These experiments were used to compare the oxygen consumption rates (R) of individuals over what was thought to be the temperatures of their daytime and nighttime habitats (T = 11 to 20°C respectively*)* using a temperature coefficient (Q_{10}), where

$$Q_{10} = (R2/R1)^{[(T2-T1)/10]}$$

Low oxygen experiments, run on three species for which sufficient numbers were collected (*H. striata, C. virgula* and *C. longirostris*), differed from temperature experiments in that they all were conducted at 11°C in water that had been bubbled with either ambient air (~21% O_2; 285 µmol kg^{-1}) or a certified gas mixture of 1% O_2, which achieved a mean initial O_2 concentration of 31.5 ± 8.0 µmol kg^{-1}. The duration of these experiments was shorter (2-7 hours) to prevent complete oxygen depletion of the chambers and the subsequent death of the study organisms.

At the conclusion of all experiments, water samples were tested for O_2 concentration using a Clarke-type microcathode O_2 (#1302) and meter (#782) in a water-jacketed injection port (#MC100, Strathkelvin Instruments, North Lanarkshire, United Kingdom) as described in Marsh and Manahan (1999). Water from the experimental chambers of hypoxic treatments was tested for NH_3 using the indophenol blue colorimetric assay (Ivancic and Degobbis 1984). The O_2 consumption and NH_3 excretion ratio was compared to assess the type of catabolic substrate using the estimated ratios for zooplankton metabolism of Mayzaud and Conover (1988). All specimens were weighed using a ship-board balance system (Childress and Mickel 1980), and frozen in liquid nitrogen for later examination. Using a Pinnacle Series Analytical Balance (Denver Instruments), we reweighed a subset of these animals upon return to the laboratory to verify the accuracy of the field weights (scale = ±0.001 g). The mass-specific metabolic rate (Y)

of each species of pteropod was calculated, relating to the wet mass of the organism (M) according to the power regression of

$$Y = aM^b$$

where *a* is a normalization constant and *b* is the scaling coefficient. We used species-specific scaling curves to normalize metabolic rates to a common body mass of 10 mg (*a*) for all species. T-tests were run using the STATISTICA software package (StatSoft) and were reported as significant if $p < 0.05$.

Results

The OMZ was shown to be part of the natural habitat for a number of thecosome species in the ETP (Fig 2). Of the six species found in MOCNESS nets during our expeditions, *H. striata, C. pyramidata, C. longirostris* and *C. virgula* showed a vertical distribution that included portions of the OMZ. *Diacria quadridentata* and *C. inflexa* were never found in low O_2 waters. *Cavolinia uncinata* was the only pteropod not present in MOCNESS samples; it was collected infrequently by SCUBA during both day and night dives between 0-30 m suggesting that it was never present in OMZ waters.

The hydrography of the CRD and the TB was consistently different between years. The thermocline was ~20 meters during both the daytime and nighttime of 2007 at both stations. In 2008 CRD had a thermocline consistently near ~20 meters, whereas TB had a deeper mixed layer of ~30 meters during the day tow and ~50 meters during the night tow (Fig. 1). This difference in the breadth of the mixed layer between day and night at TB during 2008 was likely due to an internal wave or variation in the precise position of the tows. The TB station had slightly higher temperatures in the mixed layer, of 27.8 ºC on average, whereas CRD surface temperatures averaged 25.5 ºC. Below the mixed layer, temperature dropped precipitously over a depth range of ~300 m to ~10^o C and O_2 concentration dropped from greater than 200 μmol kg^{-1} to as low as 1 μmol kg^{-1} at both stations during both seasons. There was a much sharper oxycline at TB, which led to a vertically broader OMZ. The upper oxycline region of CRD had a more gradual drop to pronounced hypoxia, although eventually O_2 levels dropped to less than 2 μmol kg^{-1} by 200-250 m compared to ~60 m at TB (Fig. 1). Individual net data and depth classifications are included in supplementary table 1.

The day and night water column abundances (# m^{-2} from 0-1000 m) at each station were rarely the same, indicating that populations were patchy (Table 2). Although many populations were found occasionally below the thermocline, such as *C. pyramidata, C. longirostris* and *C. virgula*, the mean weighted depth for each of these species was in the mixed layer. Only *H. striata* demonstrated a clear, consistent and significant difference in the day and night distribution as calculated by mean weighted depth during both years and at both stations (paired $t_3 = -8.06$, $p = 0.004$). Statistical analysis using the BEST BIOENV analysis suggested that O_2 was the best predictor of pteropod presence/absence during both the day and the night, although the correlations were not strong, likely due to the patchiness of the distribution (Day R=0.406, Night R=0.118). Net abundance data is available in supplementary table 2.

Metabolic rates for thecosomatous pteropods ranged from 2.01-12.3 O_2 g^{-1} h^{-1} at 20º C. These values are similar to those reported for other pteropods at similar temperatures (Gilmer 1974; Seibel et al. 2007). Previous work in the ETP and Gulf of California has established that there is no significant effect of location or capture type on pteropod metabolic rate (Maas et al. 2012). After scaling to a common body mass, the metabolic rates of all pteropod species ranged from an average scaled rate of 2.1 - 9.6 µmol of O_2 g^{-1} h^{-1} at 20º C and 1.2 - 3.6 µmol of O_2 g^{-1} h^{-1} at 11º C (Table 3; Fig. 3). These scaled values were used to calculate the response of metabolism to changes in temperature. Temperature coefficients for most species fell within the normal range for marine ectotherms (Q_{10} = 2-3), indicating a 2-3-fold reduction in metabolism with a 10º C reduction in temperature (Table 3; Fig. 4). *Cavolinia uncinata* showed no statistical difference in O_2 consumption between 11º and 20º C.

The three species of pteropods tested for response to low O_2, *H. striata, C. longirostris* and *C.virgula*, responded to hypoxia (~30 µmol O_2 kg^{-1}) with a decrease in O_2 consumption (Table 4, Fig. 5). This reduction in metabolic rate ranged between ~35-50% from normoxic rates at 11º C. Ammonia excretion was not influenced by hypoxia in any species. Changing O_2 consumption rates and stable NH_3 excretion resulted in a significant change in O:N ratio for *H. striata* and *C. virgula.* Generally there was a shift to a lower O:N ratio at colder temperatures indicating that a greater proportion of catabolism was fueled by protein at 11º C.

Discussion

Pteropods, like most animals, respond to decreasing temperatures with a marked reduction in metabolic rate. This is not unusual for marine ectotherms, whose temperature coefficients (Q_{10}) frequently fall between 2-3 (Smith and Teal 1973; Hochachka and Somero 2002; Seibel and Drazen 2007). The number of individuals captured in good condition and usable for respiration experiments varied among species. Post-capture, some species were more sensitive to captivity, causing them to die during experiments. As a result, there are significantly smaller datasets for *C. uncinata, D. quadridentata, C. pyramidata* and *C. inflexa*. This variability in sample size impacts the statistical power of analyses, preventing us from making any conclusions about the effect of temperature on *C. pyramidata* and possibly contributing to the unusual Q_{10} of *C. uncinata*. However, our results show that the Q_{10} of most pteropod species fell between 1.9 and 3.9 suggesting that they would use between ~55-75% less O_2 at depth, solely due to lower temperature. This response has been described in a number of animals which migrate into hypoxia and has been hypothesized to facilitate tolerance of severely O_2 depleted waters (Quetin and Childress 1976; Svetlichny et al. 2000; Rosa and Seibel 2010). Our values, which were only calculated using two temperatures, were intended to describe the response of these species at the ecologically relevant temperatures of their day and night habitat depth and we urge caution when using them to predict species specific metabolic rates at a third temperature.

The scaling coefficients describing the relationship between metabolism and body mass are remarkably negative ($-0.56 < b < -1.38$). Mass-specific O_2 consumption rates tend to scale with a factor near -0.25. In the open ocean, scaling coefficients are often much shallower (more positive) (Glazier 2005; Seibel 2007). Within pteropods, *Clione spp. and Limacina spp.* have been documented with scaling curves near quarter power (Seibel et al. 2007; Maas et al. 2011). Our extremely negative scaling coefficients may be a result of the small range in animal sizes captured in this study. Typically, a size range of at least two orders of magnitude is required for accurate measurement of scaling effects.

Species that are found below the oxycline experience periods of very low O_2 (~5-25 μmol kg^{-1}) on a daily basis. Since aerobic respiration yields the greatest energy for metabolism, strong selection exists to enhance mechanisms for oxygen extraction in species living in OMZs (Childress and Seibel 1998). In regions where O_2 saturation is below a threshold level, organisms must either respond with a reduced metabolic rate, switch to less energetically efficient anaerobic respiration, or a combination of the two (Seibel 2011). Our study indicates

that for *H. striata, C. longirostris* and *C. virgula* there is a ~30-50% reduction in O_2 consumption rate during exposure to low O_2 environments. With such substantial changes in metabolism it is likely that pteropods require time in well oxygenated water to feed, grow, and reproduce. Anaerobic responses were untested in this study, which prevents us from making any conclusions about overall metabolic depression. However, the severity of the hypoxia in the OMZ of the ETP, the less energy efficient nature of glycolysis and the decrease in pteropod O_2 consumption rate between normoxia (~285 μmol O_2) and hypoxia (~34 μmol O_2) at 11ºC suggests that suppression of total metabolism (aerobic and anaerobic pathways) is a likely tactic for pteropod survival in hypoxia in this region. Studies on other vertical migrators in the ETP such as the jumbo squid (*Dosidicus gigas*), and krill (*Euphausia eximia*), show that these species are unable to meet their metabolic needs with anaerobic metabolism alone and have to rely on metabolic suppression under hypoxic conditions (Rosa and Seibel 2010; Seibel 2011).

Metabolic suppression is typically achieved by changes in membrane permeability which reduce ion pumping, by reductions in locomotion, and by shutting down expensive cellular processes such as ion-motive ATPases and protein synthesis (Hochachka et al. 1996; Boutilier 2001). This down regulation allows the animal to survive anaerobic periods, but generally precludes active growth and feeding. Although the ammonia excretion of pteropods exposed to hypoxia was not significantly affected, the ratio between O_2 consumed and NH_3 excreted was significantly reduced in both *H. striata* and *C. virgula* suggesting that protein was supporting a greater portion of metabolism. A similar reduction in *C. longirostris* O:N ratio was observed, although the effect was not significant ($p = 0.06$), which may be due to small sample size and a large variability in the NH_3 excretion of this species. Very little research has assessed the impact of hypoxia on protein metabolism in invertebrates (Fraser and Rogers 2007); however, results from studies of fish indicate that amino acid catabolism may be upregulated during hypoxia to maintain homeostasis (Gracey et al. 2001). The reduction of O_2 consumption by pteropods exposed in the laboratory to O_2 concentrations mimicking the OMZ reveals that migratory pteropod species are unable to meet their metabolic needs at O_2 concentrations < 30 μmol kg^{-1} without a suppression of metabolism. The specific pathways activated by hypoxia exposure in pteropods bear further investigation, particularly since one of the biochemical generalities of metabolic depression in response to hypoxia is a reduction in pH (Guppy and Withers 1999). In OMZs hypoxia and low pH occur in synchrony and may interact on the physiology of

mesopelagic species. This study reveals that metabolic suppression does appear to be an important survival tactic for pteropods living under conditions of severe hypoxia, and previous work indicates that the metabolism of migratory pteropods in this region is not impacted by a reduction in environmental pH (Maas et al. 2012). Further research investigating whether the co-occurrence of low O_2 and pH was facilitative or non-additive to metabolic suppression is warranted. The amount of suppression is likely dependent on physiological adaptation to hypoxia, the temperature at which they experience low O_2 and the energetic demand of the animal. Our results show that species specific differences in metabolic rate, size, and distribution result in different reactions to changes in temperature and hypoxia within the thecosome pteropod group.

Hyalocylis striata was the species most closely associated with hypoxic waters. Compared to the other species investigated, *H. striata* has the third lowest scaled metabolic rate (6.8 ± 2.3 µmol of O_2 g^{-1} h^{-1}). This low metabolic rate may be indicative of a less active lifestyle. This species has a relatively thin shell whose weight is reduced by the loss of the juvenile shell (protoconch). During SCUBA expeditions from 0-30 m, we observed these animals generally hovering neutrally buoyant in the water, although they responded to stimuli with a quick burst of escape swimming (personal observation). Low energetic requirements, in conjunction with metabolic suppression in response to hypoxia (~33%), allow this species to inhabit OMZs. However, their residence there is contingent on their capacity to return to regions of high O_2 as indicated by their distribution and their metabolic rate under oxygenated cold conditions.

Creseis virgula was the smallest of the pteropods studied here and it has the lowest scaled metabolic rate (4.9 ± 1.6 µmol of O_2 g^{-1} h^{-1}). Of all the species, *C. virgula* was most affected by temperature, responding to 11º C with an almost four-fold reduction in O_2 consumption. This large response to temperature can have an important influence on this species, which has the broadest consistent vertical distribution, although there may be ontogenetic differences in their distribution (personal observation). These animals have been found from 0-400 meters during both the day and night, living in waters where O_2 has dropped as low as ~1 µmol kg^{-1}. Our respiration experiments were run only on larger, adult animals, whereas the MOCNESS distribution included many size classes. This bears further investigation, as it has been shown that the energetic requirements of different life stages differ, as do the responses to environmental stressors, such as hypoxia, resulting in differences in vertical distribution for

different developmental stages within a species (Wishner et al. 2000). Beyond the effects of scaling, which predisposes smaller animals to a greater O_2 demand g^{-1}, certain life stages are engaged in highly energetic processes such as reproduction which may impact their O_2 needs. Our study shows that the distribution of *C. virgula* is not constrained by OMZ water down to ~10 µmol O_2 kg^{-1}. This species is capable of metabolic suppression of ~33% under conditions of low O_2 and is very responsive to changes in water temperature, which gives it the greatest overall change in metabolic rate at cold hypoxic conditions (~86%). However, *C. virgula* may be vulnerable to surface water warming due to their high temperature-sensitivity.

Cavolinia longirostris is found in the mixed layer during the day and night although it is also sometimes found at depth (~0-150 m at night and at 250-300 m during the day). This species has one of the highest metabolic rates of the species examined in this study and the second lowest Q_{10}. This species was found in patches both at the surface and depth at all times of day. Although less responsive to low temperatures, this species is the most affected by hypoxia; when exposed to low O_2 waters their metabolism was suppressed by 49% which caused their overall metabolic suppression to fall into a similar range (81%) as *H. striata* and *C. virgula* despite their smaller response to temperature change.

Diacria quadridentata and *C. inflexa* were the only species in this study not found in net tows below the mixed layer. *Cavolinia uncinata* was never found in MOCNESS samples, possibly due to lower abundances. They were collected on SCUBA dives during the day and night at the Costa Rica Dome, suggesting that they live only in the mixed layer. Both *C. uncinata* and *C. inflexa* have elaborate wing flaps trailing from their body which they hold fully extended while hovering in the water column, either for buoyancy or prey capture (Gilmer and Harbison 1986). These structures cause them to be much more delicate than other thecosomes and inadvertent handling or capture damage may explain the greater variation in O_2 consumption of these species.

As epipelagic species, *D. quadridentata*, *C. uncinata* and *C. inflexa* experience more moderate changes in temperature and likely never inhabit hypoxic water. *Diacria quadridentata* and *C. inflexa* were some of the more sensitive pteropods to changes in temperature with a Q_{10} of 2.6 and 2.7 respectively. *Cavolinia uncinata* was the largest of the pteropods collected in the ETP, weighing 4 to 6 times more than all other species and was the only organism which was not significantly affected by temperature. All other species responded to cold temperatures with a

366 decrease in metabolic rate, whereas there was a slight increase in the average metabolic rate of *C.*
367 *uncinata* at 11 °C. This Q_{10} should be treated with caution since the standard deviation in the
368 20°C treatment, possibly a product of capture stress to these very delicate organisms, and a low
369 sample size resulted in a non-significant difference between thermal treatments. Another
370 possible explanation may be that *C. uncinata*, despite being found exclusively above the
371 thermocline, is close to its upper thermal limit when exposed to 20 °C or is below its thermal
372 limit at 11 °C. Although increases in metabolic rate with increasing temperature are the norm
373 within an animal's natural thermal conditions, above or below their thermal limit the effect of
374 temperature on metabolic demand is unpredictable as cellular thermal stress or metabolic shut-
375 down occur (Pörtner and Farrell 2008). In such circumstances a Q_{10} below 1 could indicate
376 thermal stress (Hochachka and Somero 2002).
377 The pteropods examined in this study have differences in distribution, metabolic rate, and
378 physiological response to temperature and hypoxia. As anthropogenic change causes expansion
379 of OMZs and surface warming, there will be disproportionate effects on various species. *Clio*
380 *pyramidata, C. inflexa* and *C. uncinata* were never caught in the Tehuantepec Bowl, a site where
381 hypoxic conditions occur shallowest and most severely (Fig. 1). If hypoxic waters expand to
382 match the severity of the OMZ at the Tehuantepec Bowl, these species may face physiological
383 stress from hypoxic waters directly below the thermocline.
384 Species that are found in the OMZ, such as *H. striata, C. longirostris* and *C. virgula*, are
385 living below their P_{crit}, as evidenced by their metabolic suppression under low oxygen studies.
386 Already inhabiting waters with O_2 concentrations between 1-20 μmol kg^{-1}, which penetrate
387 almost to the thermocline in some regions, it is unlikely that expanding hypoxia will impact these
388 species. However, migrators are also found in the epipelagic zone, where they must retreat to
389 recover from metabolic suppression. The warming of surface waters may impose an energetic
390 stress on species that are particularly sensitive to temperature, such as *C. virgula*, by increasing
391 their metabolic demand. This hypothesis may be corroborated by the smaller vertical range of *C.*
392 *virgula* at the Tehuantepec Bowl where surface waters are warmer and hypoxia at depth is more
393 severe. Other species, less metabolically responsive to warming, like *C. longirostris*, may be
394 unaffected.
395 The temperature effect on diel vertical migrators, such as that documented in this study,
396 has already been incorporated into analyses of carbon flux (Burd et al. 2010). However, the

reduction in O_2 consumption rate under hypoxic conditions has not been accounted for. The substantial difference in production of respiratory carbon which occurs under hypoxic conditions (~35-50%) could be a significant factor impacting calculations of DIC movement below the mixed layer. The impact on biogeochemical cycling is potentially non-trivial and warrants further investigation.

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Ethical standards and Conflict of Interest

The experiments were done in international waters, exempting them from legislation; however, experiments were conducted to comply with the current laws of the United States of America. The authors declare that they have no conflicts of interest.

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Fig. Legends:

Fig. 1: A typical temperature (ºC, black) and O_2 (µmol kg^{-1}, grey) profile for the Gulf of California (A) in 2007 and the Tehuantepec Bowl (B) and the Costa Rica Dome (C) in 2008 from CTD casts.

Fig. 2: Oxygen profiles (black line) of the CRD and TB during 2007 and 2008 with the abundance of pteropods found in vertically stratified MOCNESS tows during the daytime (light grey) and nighttime (dark grey). Note that the abundance scale differs between species and stations.

Fig. 3: The oxygen consumption rate (Y, µmol O_2 g^{-1} h^{-1}) of all pteropods declines with body mass (M, g) according to $Y = aM^b$ (Table 3).

Fig. 4: Effect of temperature on oxygen consumption. The O_2 consumption rate for different pteropod species at 11ºC is displayed as a percentage of the O_2 consumption rate at 20 ºC, both at normoxic conditions (21% O_2, Table 4). Values are scaled to the same body size by species specific constants (Table 3). *Cavolinia uncinata* was excluded because it displayed no significant difference in metabolic rate between these temperatures.

Fig. 5: Effect of hypoxia on (A) O_2 consumption and (B) O:N. The O_2 consumption rate and O:N ratio for different pteropod species under hypoxic conditions (1% O_2) is shown as a percentage of the air saturated control (21% O_2; Table 4). Significant hypoxic effects are denoted with a star (*)

Table 1: Date of collection (date) and net data for each day and night vertical profile at the Costa Rica Dome (CRD) and Tehuantepec Bowl (TB) during 2007 and 2008. Pressure was recorded in decibars (dB) and served as a proxy for depth (1 dB ≈ 1 m). Each profile is a compilation of multiple net tows (number of nets = Net #) from different dates (Date), which were grouped into a vertical series (details in supplementary table 1). The volume of water filtered through each net was summed for each profile and documented in m^3 (V.f.).

Year	Station	D/N	Date	Max dB	Net #	V.f. (m3)
2007	CRD	Night	Nov. 8, 11	400	13	4485
		Day	Nov. 8, 9	400	13	5813
	TB	Night	Oct. 29, 31	550	10	6418
		Day	Oct. 27, 30	400	12	4760
2008	CRD	Night	Dec. 30, Jan. 1	400	12	5506
		Day	Dec. 28, 29	400	13	6071
	TB	Night	Dec. 17, 20	400	12	5185
		Day	Dec. 15, 17	400	13	5692

Table 2: The calculated mean weighted depth (MWD in m, see methods) of pteropods for each year and station. The total water column abundance (# individuals 1000 m^{-2}) is from 0-400 meters for the six species of pteropods collected by the MOCNESS nets at the Tehuantepec Bowl (TB) and Costa Rica Dome (CRD).

			H. striata		*C. longirostris*		*C. virgula*		*C. inflexa*		*D. quadridentata*		*C. pyramidata*	
			MWD	#m^{-2}	MWD	#m^{-2}	MWD	#m^{-2}	MWD	#m^{-2}	MWD	#m^{-2}	MWD	#m^{-2}
2007	Day	TB	113	12	26	5	25	29	-	-	-	-	-	-
		CRD	107	1	24	53	39	263	-	-	21	2	275	1
	Night	TB	19	23	19	24	15	8	-	-	10	1	-	-
		CRD	18	29	26	44	19	313	25	4	11	41	17	5
2008	Day	TB	277	18	46	2	27	77	-	-	-	-	-	-
		CRD	225	9	39	11	25	235	35	8	-	-	-	-
	Night	TB	37	217	35	1	35	5	-	-	30	2	-	-
		CRD	25	21	-	-	27	49	10	1	-	-	10	1

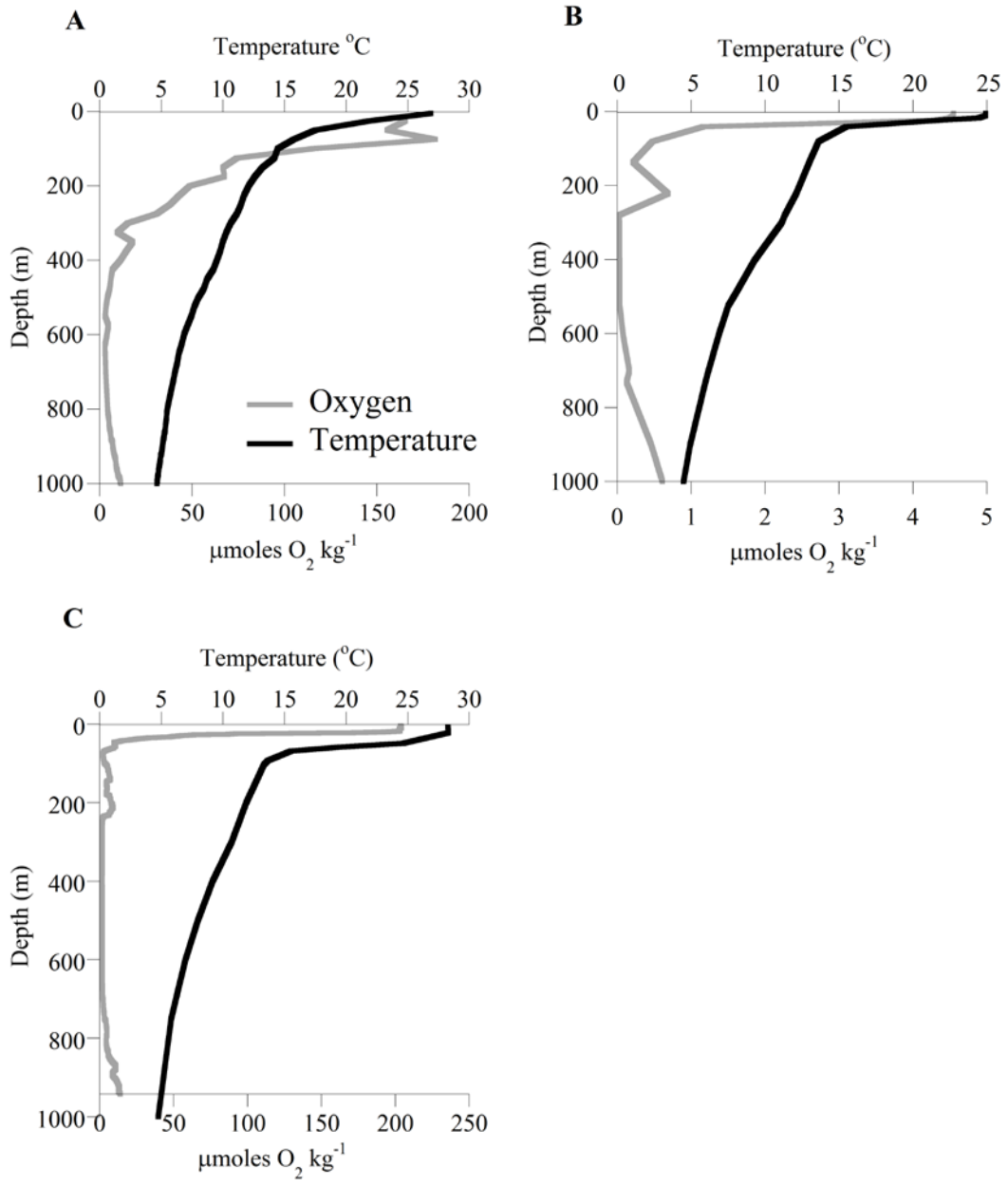
Table 3: Weights and O_2 consumption for thecosomes are reported as an average ± SD. Scaling curves for each species were plotted ($Y=aM^b$ with an r^2) to achieve a mean scaled O_2 consumption ± SD. Mean scaled values were applied to determine the temperature coefficient (Q_{10}) for each species. Student's t-tests were run to determine whether there was a significant difference (bold < 0.05) in metabolic rate between 20º C and 11º C (*p*).

		Wet weight (mg)		O_2 consumption (µmol O_2 g^{-1} h^{-1})	scaled O_2 consumption (µmol O_2 g^{-1} h^{-1})					Q_{10}
Species	ºC	Mean	n	Mean	*a*	*b*	r^2	*p*	Mean	
H. striata	20	10.5 ± 4.2	24	7.34 ± 3.59	0.27	-0.70	0.48	**>0.001**	6.8 ± 2.3	2.0
	11	16.2 ± 5.4	36	2.21 ± 0.84					3.1 ± 1.2	
C. virgula	20	6.8 ± 3.8	10	7.75 ± 4.17	0.40	-0.56	0.52	**>0.001**	4.9 ± 1.6	3.9
	11	10.7 ± 3.8	10	1.24 ± 0.52					1.2 ± 0.4	
C. longirostris	20	8.2 ± 3.7	20	12.29 ± 7.60	0.01	-1.38	0.61	**>0.001**	8.9 ± 3.8	1.9
	11	11.0 ± 2.0	11	3.18 ± 1.40					3.4 ± 1.5	
D. quadridentata	20	10.9 ± 6.2	12	10.62 ± 5.63	0.60	-0.59	0.15	**0.009**	9.6 ± 4.3	2.6
	11	12.9 ± 3.6	5	2.87 ± 0.98					3.6 ± 1.7	
C. uncinata	20	45.3 ± 30.0	6	4.01 ± 4.30	0.25	-0.73	0.78	0.078	2.1 ± 0.7	0.7
	11	75.1 ± 14.7	13	2.54 ± 0.16					3.0 ± 1.2	
C. inflexa	20	12.9 ± 9.8	4	6.47 ± 3.87	0.39	-0.59	0.69	**0.024**	6.3 ± 1.8	2.7
	11	15.2 ± 4.2	5	2.29 ± 1.33					3.0 ± 1.5	
C. pyramidata	20	9.1 ± 4.9	13	9.96 ± 4.80	0.27	-0.72	0.52	-	8.0 ± 2.7	2.2
	11	23.5	1	2.28					2.7	

Table 4: Effect of hypoxia on the average O_2 consumption, NH_3 excretion and O:N ± SD of thecosome pteropods at 11º C. Statistical significance (bold < 0.05) between treatments was reported using a Student's t-test (*p*).

		Wet weight (mg)		O_2 consumption (µmol O_2 g^{-1} h^{-1})			NH_3 excretion (µmol NH_3 g^{-1} h^{-1})			O:N	
Species	treatments	Mean	N	Mean	p	n	Mean	p	n	Mean	p
H. striata	21% O_2	16.2 ± 5.4	36	2.21 ± 0.84	**<0.01**	26	0.079 ± 0.042	0.95	26	69.7 ± 31.2	**<0.01**
	1% O_2	11.6 ± 4.1	41	1.47 ± 0.84		13	0.078 ± 0.045		13	33.3 ± 23.9	
C. virgula	21% O_2	10.7 ± 3.8	10	1.24 ± 0.52	**<0.01**	8	0.054 ± 0.026	0.08	8	57.4 ± 33.8	**0.03**
	1% O_2	8.16 ± 1.60	19	0.82 ± 0.54		6	0.086 ± 0.036		6	20.3 ± 15.6	
C. longirostris	21% O_2	11.0 ± 2.0	11	3.18 ± 1.40	**<0.01**	8	0.204 ± 0.151	0.12	8	43.8 ± 21.2	0.06
	1% O_2	10.3 ± 2.1	7	1.63 ± 0.50		6	0.176 ± 0.064		6	21.2 ± 9.5	

Fig. 1:


A
Temperature °C
0 5 10 15 20 25 30
Depth (m)
0 200 400 600 800 1000
Oxygen
Temperature
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µmoles O2 kg-1
B
Temperature (°C)
0 5 10 15 20 25
Depth (m)
0 200 400 600 800 1000
0 1 2 3 4 5
µmoles O2 kg-1
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Depth (m)
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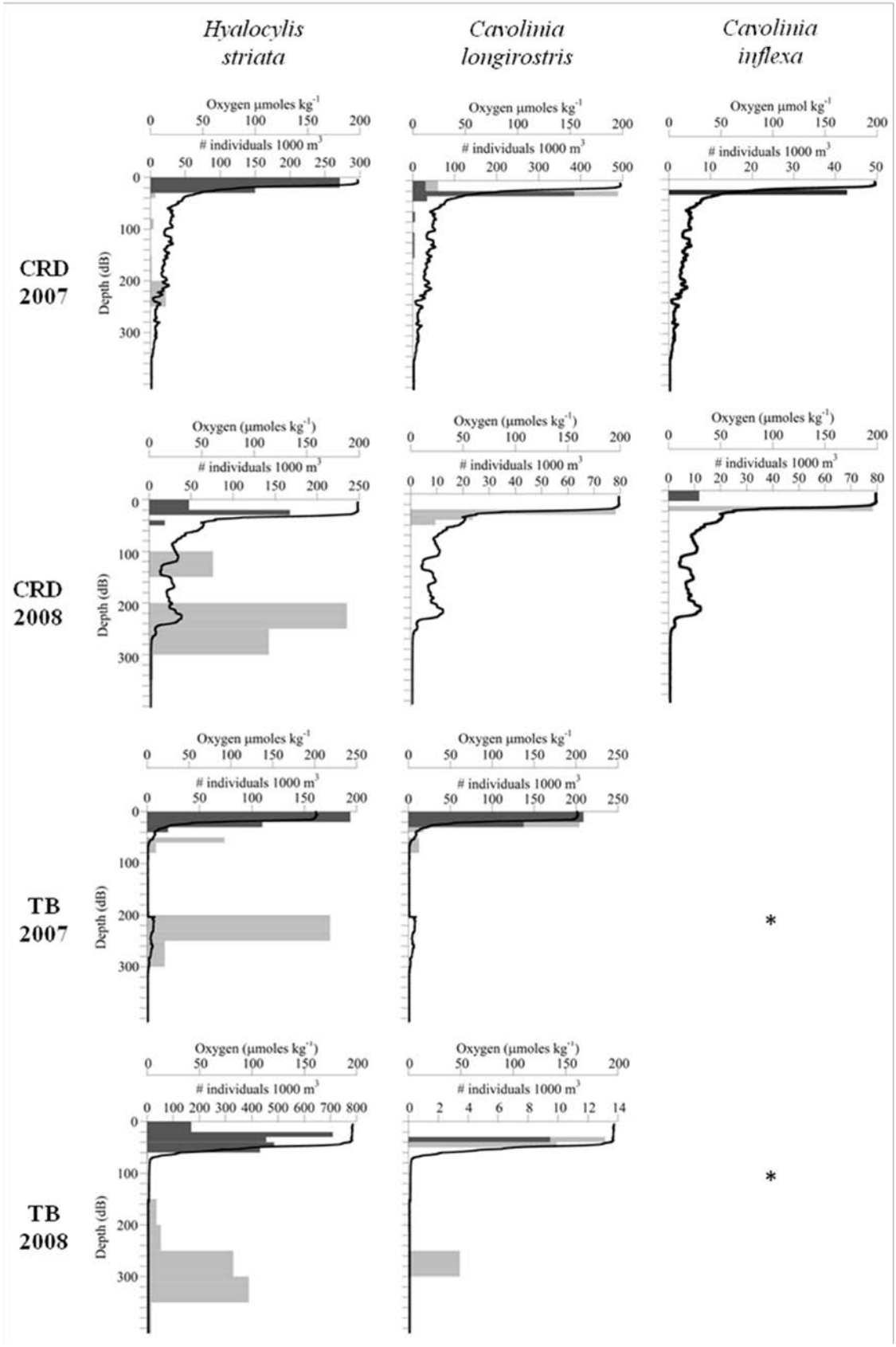
0 50 100 150 200 250
µmoles O2 kg-1

Fig. 2:


Hyalocylis striata
Cavolinia longirostris
Cavolinia inflexa
CRD 2007
CRD 2008
TB 2007
TB 2008
Depth (dB)
Oxygen µmoles kg-1
Oxygen µmol kg-1
Oxygen (µmoles kg-1)
individuals 1000 m3
*
*

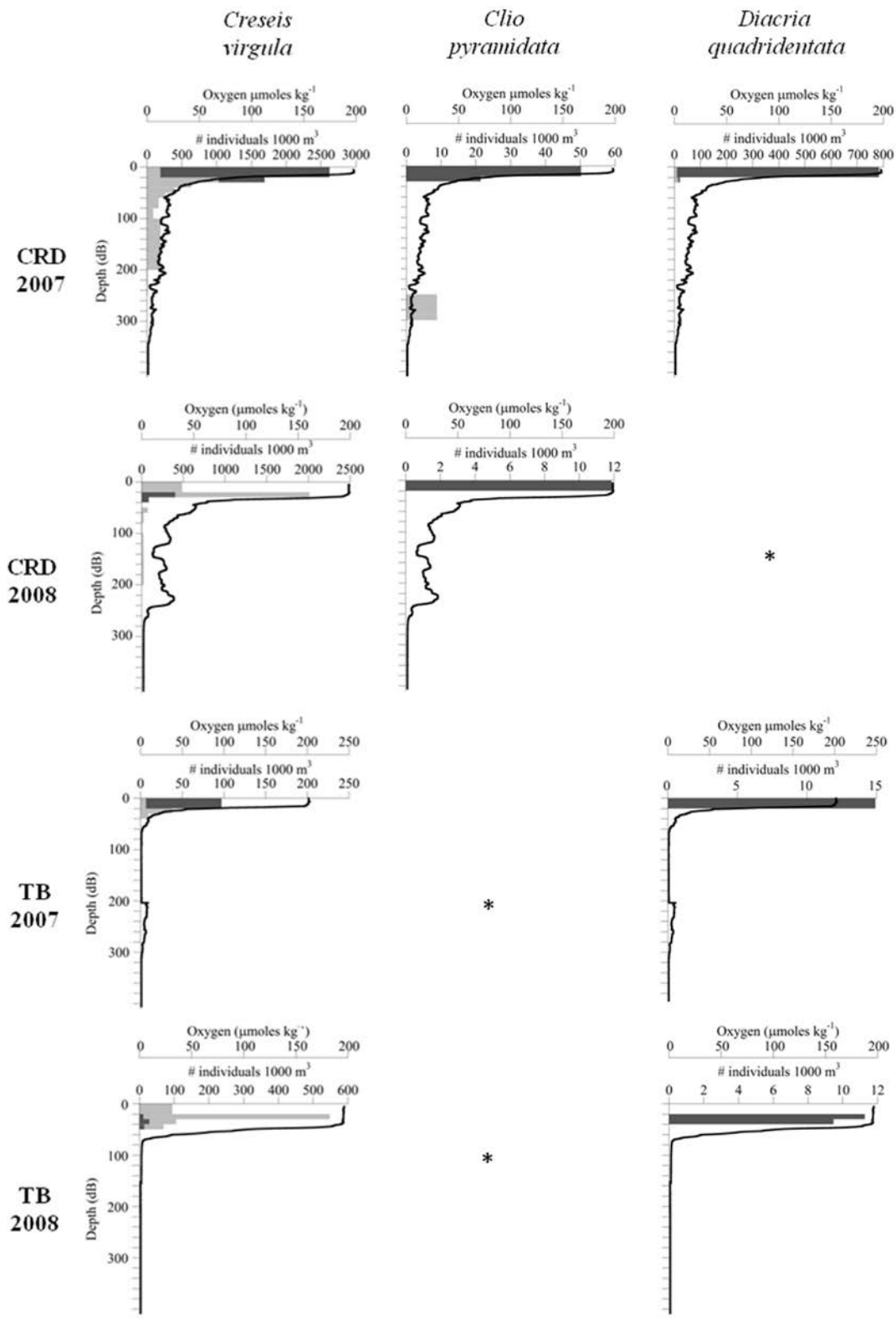

Creseis virgula
Clio pyramidata
Diacria quadridentata
CRD 2007
CRD 2008
TB 2007
TB 2008
Oxygen µmoles kg-1
Oxygen (µmoles kg-1)
individuals 1000 m3
Depth (dB)
*

Fig 3:

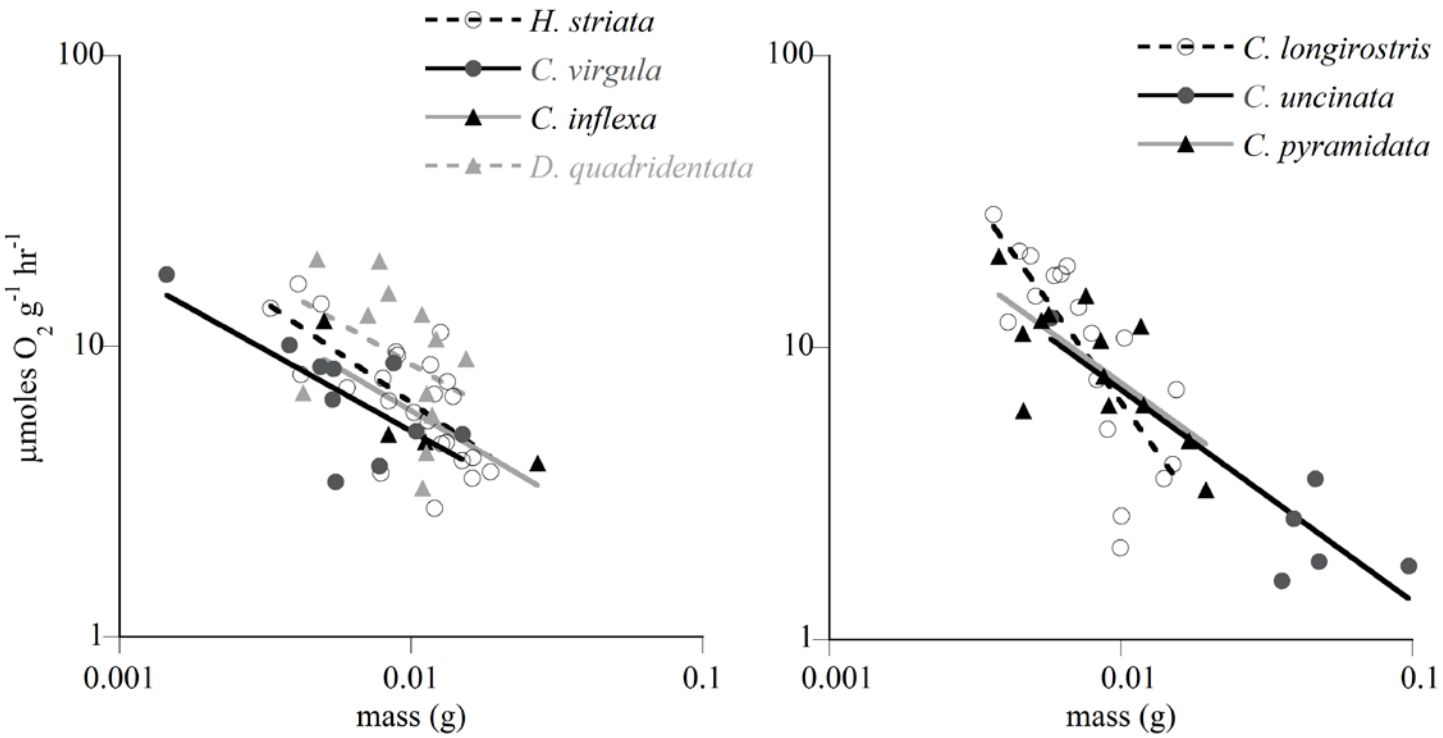

H. striata
C. virgula
C. inflexa
D. quadridentata
C. longirostris
C. uncinata
C. pyramidata
µmoles O2 g-1 hr-1
100
10
1
0.001
0.01
0.1
mass (g)

Fig. 4:

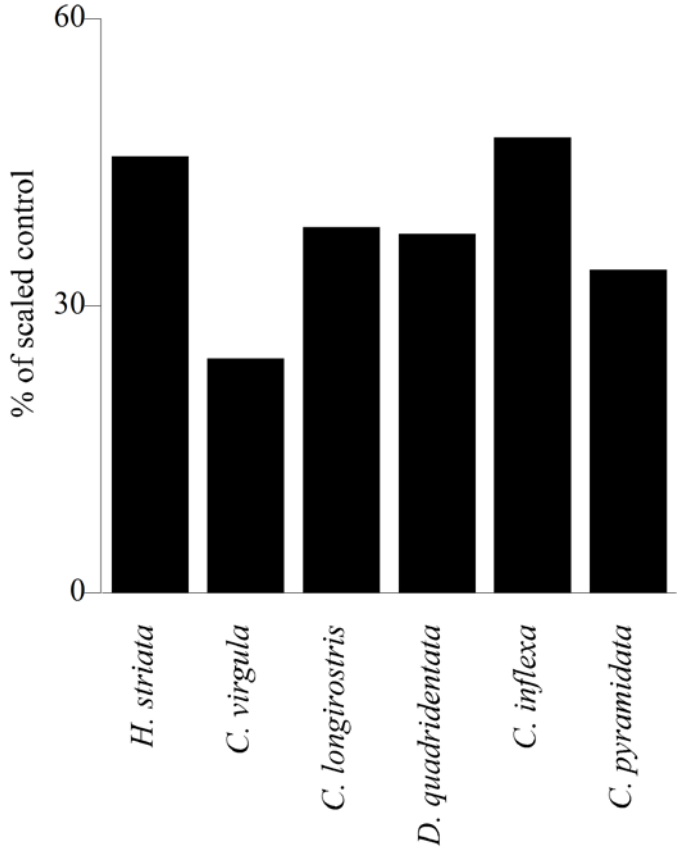
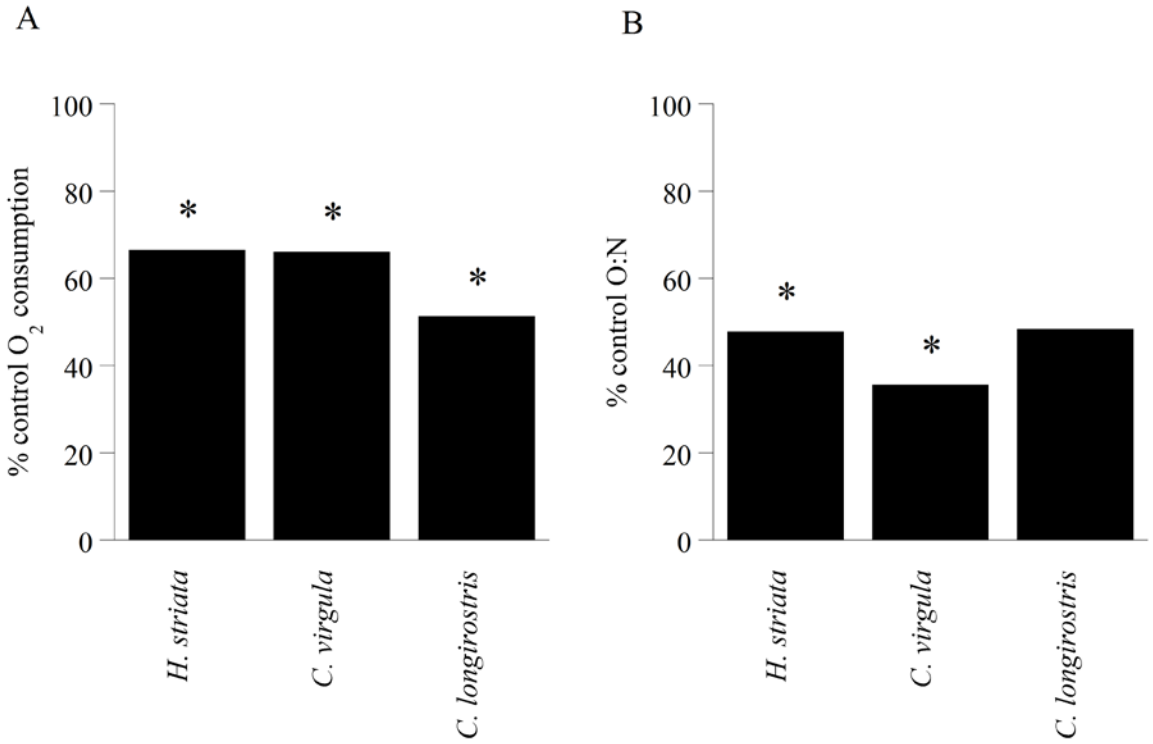

60
30
0
% of scaled control
H. striata
C. virgula
C. longirostris
D. quadridentata
C. inflexa
C. pyramidata

Fig. 5:


A
B
100
80
60
40
20
0
% control O_2 consumption
% control O:N
*
H. striata
C. virgula
C. longirostris