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Eucalanoid copepod metabolic rates in the oxygen minimum zone of the eastern tropical north Pacific: Effects of oxygen and temperature

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ABSTRACT

The eastern tropical north Pacific Ocean (ETNP) contains one of the world's most severe oxygen minimum zones (OMZs), where oxygen concentrations are less than 2 μmol kg^{-1}. OMZs cause habitat compression, whereby species intolerant of low oxygen are restricted to near-surface oxygenated waters. Copepods belonging to the family Eucalanidae are dominant zooplankters in this region and inhabit a variety of vertical habitats within the OMZ. The purpose of this study was to compare the metabolic responses of three species of eucalanoid copepods, *Eucalanus inermis, Rhincalanus rostrifrons*, and *Subeucalanus subtenuis*, to changes in temperature and environmental oxygen concentrations. Oxygen consumption and urea, ammonium, and phosphate excretion rates were measured via end-point experiments at three temperatures (10, 17, and 23 °C) and two oxygen concentrations (100% and 15% air saturation). *S. subtenuis*, which occurred primarily in the upper 50 m of the water column at our study site, inhabiting well-oxygenated to upper oxycline conditions, had the highest metabolic rates per unit weight, while *E. inermis*, which was found throughout the water column to about 600 m depth in low oxygen waters, typically had the lowest metabolic rates. Rates for *R. rostrifrons* (found primarily between 200 and 300 m depth) were intermediate between the other two species and more variable. Metabolic ratios suggested that *R. rostrifrons* relied more heavily on lipids to fuel metabolism than the other two species. *S. subtenuis* was the only species that demonstrated a decrease in oxygen consumption rates (at intermediate 17 °C temperature treatment) when environmental oxygen concentrations were lowered. The percentage of total measured nitrogen excreted as urea (% urea-N), as well as overall urea excretion rates, responded in a complex manner to changes in temperature and oxygen concentration. *R. rostrifrons* and *E. inermis* excreted a significantly higher % of urea-N in low oxygen treatments at 10 °C. At 17 °C, the opposite trend was observed as *E. inermis* and *S. subtenuis* excreted a higher % of urea-N in the high oxygen treatment. This unique relationship has not been documented previously for crustacean zooplankton, and warrants additional research into regulation of metabolic pathways to better understand nitrogen cycling in marine systems. This study also compared metabolic data for *E. inermis* individuals captured near the surface versus those that were resident in the deeper OMZ. Deeper-dwelling individuals had significantly higher nitrogen excretion rates and O:N ratios, suggesting an increased reliance on lipids for energy while residing in the food-poor waters of the OMZ.

1. Introduction

Recent studies indicate that the oceans are decreasing in oxygen in response to climate change, primarily through surface heating and increased stratification (Emerson et al., 2004; Keeling and Garcia, 2002; Keeling et al., 2010). In addition, regions of the ocean classified as oxygen minimum zones (OMZs), which are layers of oxygen deficient waters at intermediate depths, appear to be expanding and are characterized by having a greater than average decrease in oxygen concentrations (Bograd et al., 2008; Gilly et al., 2013; Stramma et al., 2008, 2010). These OMZs ($O_2 < 20$ μM) occupy about 7% of total ocean volume (Paulmier and Ruiz-Pino, 2009) and are typically maintained as a result of poor ventilation, sluggish circulation, oxygen-poor sources waters, and decomposition of sinking particles (Keeling et al., 2010; Wyrtki, 1962). The extent of low oxygen or hypoxic waters in coastal regions (usually defined as $O_2 < 2$ mg l^{-1} or < 60 μM) also has increased in the last three decades due to natural and human activities (Helly and Levin, 2004; Rabalais et al., 2009). Little is

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known, however, about the effects of OMZs and hypoxic coastal regions on carbon and nitrogen cycles, marine biota, and the efficiency of the biological pump.

Metabolic rates of marine organisms, in particular, will be sensitive to changing ocean conditions. Increasing water temperatures and decreasing O_2 and pH levels will exceed physiological tolerances of many marine organisms and eventually limit suitable habitats (Prince and Goodyear, 2006). Metabolic rates of marine zooplankton are known to be influenced by a number of different factors, including temperature (e.g., Childress, 1977; Hirche, 1987; Ikeda et al., 2001), body mass (Conover and Gustavson, 1999; Ikeda et al., 2001), salinity (Barber and Blake, 1985), season (Conover, 1959; Conover and Gustavson, 1999; Torres et al., 1994), depth of occurrence (Childress, 1975; Seibel and Drazen, 2007; Torres et al., 1994), life strategy (Company and Sardà, 1998), feeding activity or feeding history (Bohrer and Lampert, 1988; Ikeda, 1971, 1977; Mayzaud, 1976), swimming activity (Childress, 1968; Swadling et al., 2005; Torres and Childress, 1983), and *in situ* oxygen concentrations (Childress, 1975, 1977; Cowles et al., 1991; Donnelly and Torres, 1988). Other metabolic parameters, such as ammonia, urea, and phosphate excretion rates also may be influenced by many of the same factors, including temperature (e.g., Aarset and Aunaas, 1990; Ikeda et al., 2001; Quarmby, 1985), salinity (Barber and Blake, 1985), body mass (Conover and Gustavson, 1999; Ikeda et al., 2001) and feeding history (Ikeda, 1977; Mayzaud, 1976; Miller and Roman, 2008; Saba et al., 2009). The metabolic ratios of O:N, N:P and O:P (which compare the molar ratios of oxygen consumed and ammonium and phosphate excreted) are useful as indicators of metabolic substrate catabolized during respiration (primarily lipids, proteins, and/or carbohydrates), and have been documented to vary with season (Gaudy et al., 2003; Hatcher, 1991; Snow and Williams, 1971), timing in reproductive cycle (Barber and Blake, 1985), dry weight (Ikeda et al., 2001), feeding history (Hatcher, 1991; Ikeda, 1977; Mayzaud and Conover, 1988; Quetin et al., 1980), and temperature (Aarset and Aunaas, 1990). Unlike respiration rates, however, excretion rates and metabolic ratios have rarely been examined in relation to variable *in situ* oxygen concentrations.

The lethal and sublethal effects of coastal hypoxic oxygen concentrations are well documented for many benthic organisms (Vaquer-Sunyer and Duarte, 2008), and some work has examined deleterious effects of low oxygen levels on pelagic crustaceans (Ekau et al., 2010). Crustacean studies on effects of low oxygen have largely concentrated on changes in oxygen consumption rates, egg production, growth, development, activity rates and survival (Auel and Verheye, 2007; Svetlichny and Hubareva, 2002; Svetlichny et al., 2000). Few studies, if any, have examined the effects of low oxygen conditions on ammonia, urea or phosphate excretion rates or on metabolic substrate use. One study on white shrimp (*Penaeus setiferus*) found that protein catabolism dominated at low oxygen, whereas substrate use switched to a combination of lipid and protein catabolism at higher oxygen levels (Rosas et al., 1999). Thus, low *in situ* oxygen concentrations have the potential to influence other metabolic parameters besides respiration rates, and changes in metabolic pathways could influence the composition of excreted by-products and, therefore, impact elemental cycling.

The eastern tropical north Pacific (ETNP) is the largest low oxygen oceanic biome (Paulmier and Ruiz-Pino, 2009). The ETNP is characterized by a strong, shallow pycnocline and a pronounced oxycline (Fiedler and Talley, 2006), where chlorophyll, primary production, and copepod maxima occur (Herman, 1989). Oxygen concentrations $< 50\ \mu M$ occur as shallow as 40 m and can reach values as low as $0.5\ \mu M$ in the OMZ core (Brinton, 1979; Levin et al., 1991; Saltzman and Wishner, 1997a; Vinogradov et al., 1991). Studies examining the vertical distribution of organisms within the ETNP have found that all taxa, from zooplankton to micronekton to benthic fauna, seem to have distinct layers of peak abundance often related to oxygen concentrations (e.g., Brinton, 1979; Sameoto, 1986; Wishner et al., 1995, 2013).

Members of the copepod family Eucalanidae are among the dominant zooplankton in this region and adult females have a broad depth distribution (Chen, 1986; Longhurst, 1985; Saltzman and Wishner, 1997b; Sameoto, 1986). The most abundant members of the group, *Subeucalanus subtenuis* (formerly *Eucalanus subtenuis* (Geletin, 1976)) and *Eucalanus inermis*, are consistently in the top 10–12 most abundant copepod species in this region. Recorded abundances are extremely variable, but these two species can each comprise 2% to more than 50% of the total copepod population in the region, and occur in densities of tens to several hundred individuals per cubic meter. *S. subtenuis* females are found in highest abundance in the shallow euphotic zone, while *E. inermis* adult females have a vertical range which spans much of the upper 1000 m, with peaks in abundance near the chlorophyll maximum, the upper oxycline and the lower oxycline. This species is also present in small numbers throughout the core of the OMZ, where oxygen levels are nearly zero. It is thought that the presence of these females at depths below 200 m represent an ontogenetic migration (Wishner et al., 2013). Neither of these species has been reported to have diel vertical migration patterns in the ETNP. *Rhincalanus rostrifrons* is less abundant than *E. inermis* and *S. subtenuis*. *R. rostrifrons* often occurs in densities of 5–10 individuals per cubic meter and has maximum abundances in the upper oxycline. The occurrence of diel vertical migration does not seem to be consistent for this species, but one study did report migration to deeper depths at night (modal depth change from 97 to 193 m) (Longhurst, 1985). Such differences in vertical distributions allow us to examine the metabolic response of closely related species, or even different life history stages, to low *in situ* oxygen concentrations in the open ocean. Members of this family occurring in other low-oxygen regions have a variety of responses to the presence of the OMZ, including avoidance, dormancy, reduced metabolic rates, and the presence of anaerobic pathways (e.g., Flint et al., 1991; Ohman et al., 1998; Teuber et al., 2013b; Wishner et al., 2008).

Distributions of microbial (Podlaska et al., 2012), microzooplankton (Olson and Daly, 2013), and zooplankton communities (Wishner et al., 2013) were previously reported for our study. In addition, the results of Maas et al. (2012) indicated that metabolic suppression occurred in thecosomatous pteropods, as an effect of low temperature and hypoxia. The goal of this study was to assess respiration and excretion rates of the eucalanoid copepods *S. subtenuis*, *R. rostrifrons* and *E. inermis* in order to investigate the metabolic responses of these three closely related species to low oxygen concentrations in the ETNP OMZ system. To our knowledge, no metabolic rates have been previously measured for *R. rostrifrons*, and only oxygen consumption rates for *S. subtenuis* from the Atlantic Ocean have been recently reported (Teuber et al., 2013a). Herein, we present results on oxygen consumption, and ammonium, phosphate, and urea excretion rates, as well as O:N, N:P, and O:P metabolic ratios. Measurements were obtained at high (100% saturation) and low (15–20% saturation) oxygen concentrations at representative temperatures for this study site, in order to compare baseline metabolic rates between these three species and assess their strategies to cope with a low oxygen environment.

2. Methods

2.1. Study area

Sample collection for this work occurred during two cruises to the eastern tropical north Pacific (ETNP) from 18 October to 17 November

2007 aboard the R/V *Seward Johnson* and 8 December 2008–6 January 2009 aboard the R/V *Knorr*. Primary sampling locations included the Costa Rica Dome (CRD; 9°N, 90°W) and the Tehuantepec Bowl (TB; 13°N, 105°W) (Fig. 1). At both locations, during both sampling periods, the OMZ was extensive, with the $<20\ \mu mol\ kg^{-1}$ O_2 layer occupying approximately 800–1000 m of vertical space within the water column (Table 1). The OMZ of the TB was more vertically expansive than the CRD in both years, with oxygen values $<20\ \mu mol\ kg^{-1}$ occurring as shallow as 39 m depth. During the 2008–2009 cruise, upwelling was apparent at the CRD, with surface temperatures a few degrees cooler and surface salinities slightly higher than at the TB during 2008–2009 and both sites during 2007. The mixed layer was relatively shallow during both cruises, between 20 and 45 m. Geometric mean chlorophyll *a* concentrations in the upper 15 m of the water column ranged from 0.26 to 0.31 mg chl m^{-3}. The depth of the chlorophyll maximum was usually below the mixed layer depth (20–51 m), with maxima concentrations ranging from 0.36 to 0.48 mg chl m^{-3}. Microzooplankton were dominated by heterotrophic dinoflagellates and aloricate spirotrich ciliates, and their grazing activity accounted for 33–108% of the surface primary production (Olson and Daly, 2013). The copepods studied here were generalist feeders, ingesting phytoplankton, microzooplankton and detrital particles (M. B. Olson and K. L. Daly, unpublished data).

2.2. Copepod collection

Adult female copepods were collected using bongo tows, Tucker trawls, and MOCNESS (Multiple Opening/Closing Net and Environmental Sampling System) tows to capture individuals for experiments (Fig. 2). *S. subtenuis* were collected from the upper 50 m and *R. rostrifrons* in the 200–300 m range. *E. inermis* were collected from both depths and analyzed separately to assess potential intra-population metabolic variability. Immediately after capture, adult female copepods were sorted and individuals of each species were separated into small containers containing 0.2 μm filtered seawater at *in situ* temperature. Copepods were kept at *in situ* temperatures for approximately 3–12 h prior to experimentation to allow them to empty their guts and recover from possible capture stress. Extended acclimation periods were avoided in order to reduce potential effects of starvation on rate measurements (e.g., Mayzaud, 1976). After the initial sorting, handling was gentle and minimal to decrease the effects of stress during experimentation (Ikeda and Skjoldal, 1980).

2.3. Shipboard and laboratory techniques

End point metabolic experiments were performed using 60 ml BOD bottles, in order to simultaneously measure oxygen consumption, and ammonium, phosphate, and urea excretion rates of copepods. BOD bottles were filled with filtered seawater containing antibiotics (25 mg l^{-1} each of streptomycin and ampicillin). Undersaturated oxygen conditions were obtained by bubbling nitrogen or a low-oxygen gas mixture into the filtered seawater. Typically, 2–15 copepods were then gently added to each BOD bottle, depending on the size of species and temperature of the experiment. This number of copepods was optimal to achieve a measurable drawdown of oxygen and production of excretory products, while trying to avoid crowding effects. Eucalanoid copepod densities in thin layers within the upper 300 m of the water column were estimated to be up to 4 individuals per 60 ml in the ETNP based on data recorded by the Shadowed Image Particle Profiling Evaluation Recorder (Remsen et al., 2004) deployed vertically at each station where experimental copepods were collected (A. Remsen, personal communication). These observed densities were similar to or slightly lower than densities

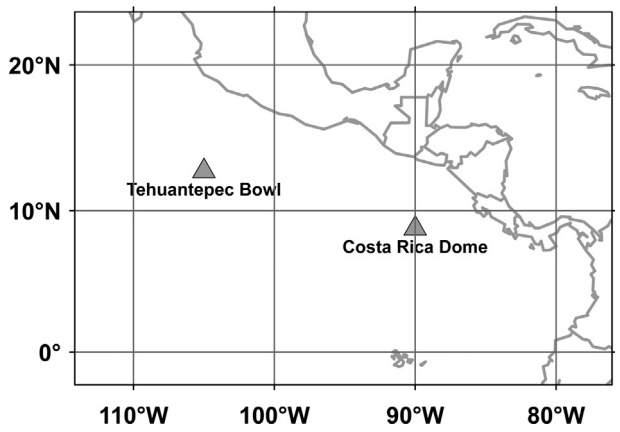

Fig. 1. Map of eastern tropical north Pacific sampling sites. Gray triangles represent approximate locations of the two primary sampling sites: the Tehuantepec Bowl (13°N, 105°W) and the Costa Rica Dome (9°N, 90°W).

Table 1
Environmental conditions at the Costa Rica Dome (CRD) and Tehuantepec Bowl (TB) during 2007 and 2008–2009 sampling periods. Data were collected with a Sea-Bird *3plus* temperature sensor, Sea-Bird *9plus* digital quartz pressure sensor, Sea-Bird 4C conductivity sensor, and Sea-Bird 43 oxygen sensor. Oxygen and salinity sensor data were verified by analysis of water samples. Surface data represent the average of the upper 10 m of the water column. Chlorophyll *a* concentrations in the upper 15 m and chlorophyll maxima are the geometric means of discrete samples collected from several CTD/Niskin water bottle casts at each station. Chlorophyll maxima depths are the range of depths where maxima occurred.

	2007		2008–2009	
	CRD	TB	CRD	TB
Surface temperature (°C)	27.0	27.7	24.8	28.3
Surface salinity (PSU)	33.7	33.4	34.4	33.7
Surface dissolved O_2 ($\mu mol\ kg^{-1}$)	197.5	203.8	196.5	196.1
Depths of $<20\ \mu mol\ kg^{-1}$ O_2 (m)	73–916	39–1068	139–909	64–1064
Depths of $<2\ \mu mol\ kg^{-1}$ O_2 (m)	329–429	135–716	272–574	98–749
Mixed layer depth (m)	20	20	25	45
Chlorophyll *a* (mg m^{-3})	0.28	0.26	0.31	0.24
Chlorophyll *a* maxima (mg m^{-3})	0.48	0.36	0.47	0.39
Chlorophyll maxima depths (m)	28–35	20–29	21–33	5–51

used in incubations during this study. Experiment duration was typically 12–24 h. Bottles were kept in the dark and in water baths to maintain the desired temperature. Subsamples for oxygen, phosphate, ammonium, and urea concentrations were taken immediately before and after each experiment.

Experiments were run at 10 and 23 °C during the 2007 cruise and 10 and 17 °C during the 2008–2009 cruise. The experimental temperatures were representative of different depths: 10 °C for the base of the upper oxycline, 17 °C for chlorophyll maximum depths, and 23 °C for near-surface waters (see Fig. 2). The high oxygen treatment was representative of surface waters above the pycnocline and the low oxygen treatment was representative of conditions in the upper oxycline near the chlorophyll maximum, which is the region of maximum abundance for many species of copepods in the ETNP (Herman, 1989; Longhurst, 1985; Saltzman and Wishner, 1997b). Due to the relatively low abundances of *R. rostrifrons* present during our study, *R. rostrifrons* were only incubated at 10 and 17 °C, while *E. inermis* and *S. subtenuis* were incubated at all three temperatures. During both years, experiments were run at high (100% air saturation; 201–325 μM initial oxygen concentration, 66–296 μM ending oxygen concentration) and low (15–20% air saturation; 36–78 μM initial oxygen concentration, 7–69 μM ending oxygen concentration) oxygen treatments (see Table 2 for replicate numbers). A few experiments were run

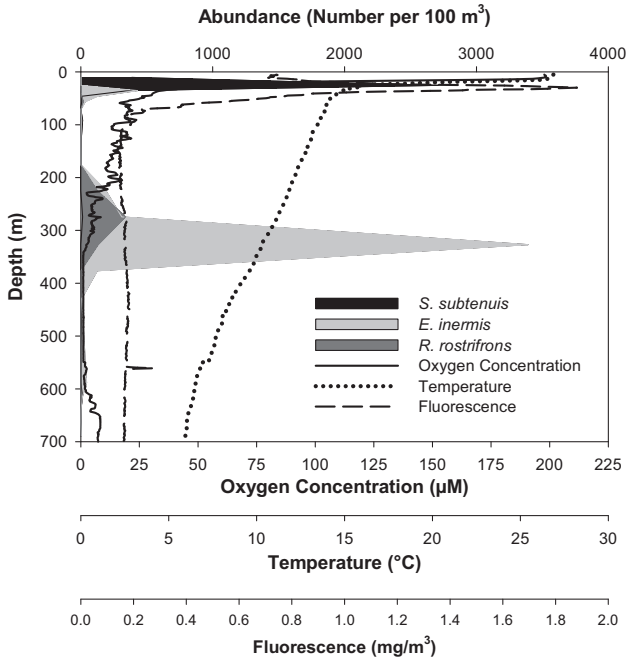

Fig. 2. The vertical distributions of adult female eucalanoid copepods in relation to temperature, fluorescence, and oxygen concentrations during 2007 at the Costa Rica Dome site (9°N, 90°W). Abundance data for *Subeucalanus subtenuis*, *Eucalanus inermis* and *Rhincalanus rostrifrons* are from day tows (MOCNESS data courtesy of K. Wishner and D. Outram).

Table 2
Number of replicate experiments for all rate measurements at high (100% saturation) and low O_2 (15% saturation).

Species	2007				2008–2009			
	10 °C		23 °C		10 °C		17 °C	
	High	Low	High	Low	High	Low	High	Low
E. inermis								
Shallow	8	8	1	4	5	5	4	5
Deep	1	1	–	–	9	8	–	–
R. rostrifrons	3	3	–	–	2	1	1	1
S. subtenuis	6	6	5	4	6	4	6	5

at lower oxygen concentrations (5% saturation; 8–17 μM initial oxygen concentration, 4–16 μM ending oxygen concentration) at 10 °C, which represented the lowest oxygen levels able to be used accurately for end point oxygen experiments. Although oxygen consumption rates could be measured at 5% saturation, more experiments were not completed at this oxygen concentration because copepod excretion rates were unmeasurable. Additionally, higher mortality of *S. subtenuis* at <20 μM led to many fewer usable runs for that species. Due to these factors, collected organisms were used preferentially for the 100% and 15% oxygen saturation treatments. Control bottles without any copepods also were run under the same conditions. Only experimental runs where all copepods survived the entire incubation were used to assess rate measurements.

Oxygen samples were analyzed aboard the ship using a Clark-type oxygen electrode (Strathkelvin instruments). Pre- and post-experiment, a 1.5 ml gas tight syringe was used to collect water from the BOD bottles and inject it into a small chamber surrounding the electrode to obtain a reading. Electrodes were calibrated daily to high and low points using air saturated and nitrogen bubbled water, respectively. Samples for ammonium, phosphate, and urea were frozen at −20 °C until laboratory analyses could be performed. Ammonium and phosphate concentrations were determined using a Technicon Autoanalyzer II (Gordon et al., 2000), while urea concentrations were measured by the manual spectrophotometric method using diacetylmonoxime without deproteinization (Rahmatullah and Boyd, 1980; Whitledge et al., 1981).

At the end of each experiment, copepods were frozen at −80 °C in cryovials containing a small amount of filtered seawater. In the lab, individuals from each BOD bottle were thawed, briefly submerged in DI water to remove adhering salts, blotted dry, placed in an aluminum capsule, and weighed on a Mettler Toledo UMX2 microbalance to obtain wet weight (WW). Samples were then dried in an oven at 60 °C for several days and weighed again to measure dry weight (DW). Wet and dry weights of copepods were used to determine mass-specific rates. Additionally, to account for the impact of body size on respiration rate (Ikeda et al., 2001), median oxygen consumption rates for each species at each treatment were also scaled to an organism of 1 mg WW (based on equations from Teuber et al., 2013a). A scaling factor of −0.25 was used, which is a good estimate when empirical scaling data are not available (Schmidt-Nielsen, 1984) and is similar to previously reported scaling factors for copepods (e.g., Pavlova, 1994; Thuesen et al., 1998).

2.4. Data analysis

Metabolic ratios were calculated using changes in oxygen, phosphate, urea, and ammonium concentrations. N:P and O:N ratios were calculated twice, once using ammonium excretion values and a second time using total measured nitrogen excretion (urea and ammonium nitrogen excretion combined). Theoretical ratios are derived assuming only ammonium excretion (Mayzaud and Conover, 1988), although total measured nitrogen values may be more accurate for comparison purposes when urea excretion is a major component.

Q_{10} values were calculated for 10 and 23 °C using values from 2007 and 10 and 17 °C using values from 2008 to 2009. When no significant difference was seen between oxygen treatment levels, values were combined. When differences existed, only the high oxygen data points were used. Only *E. inermis* individuals collected from the upper 50 m were used for Q_{10} comparisons.

Prior to statistical analyses, data were tested for normality, and then appropriate parametric or non-parametric tests were used (Zar, 1984). Comparisons between species and treatment groups were made using ANOVAs and parametric and non-parametric pair-wise comparison tests in SigmaPlot 11.0. *T*-tests assuming unequal variances and Mann–Whitney rank sum tests also were used for some comparisons. Significance was assessed at $\alpha=0.05$. Due to the non-normality of many of the rate data sets, central values here will be reported as medians and quartile ranges, rather than averages and standard deviations or errors. Unless otherwise noted, statistics were performed on rates per unit WW and all reported significant differences are based on wet mass-specific rate measurements. Owing to space limitations, tables report rate measurements for each species at the different temperatures tested. Unless significant differences existed for other treatment variables (year, collection depth, oxygen level), those results are combined under the appropriate temperature treatments. All rates are reported in mass-specific units.

3. Results

3.1. Vertical distribution of copepods in relation to environmental parameters

The exact depths of the surface mixed layer, upper oxycline, and OMZ varied between sites and years (Table 1 and summarized in Wishner et al., 2013). However, in general, adult female *S. subtenuis*

Table 3
Oxygen consumption rates for three eucalanoid copepod species at three temperatures. Values are medians (25th quartile–75th quartile) and number of replicates. Rates per mg wet weight (WW) and dry weight (DW) are shown at all temperatures. "Size-scaled" average rates per mg WW are also given after scaling to an organism of 1 mg WW. Outside of temperature and species, more specific data breakdowns are only given when significant differences existed between the treatment groups. Notations for Tables 3–8 are as follows: "2007" and "2008" denote the 2007 and the 2008–2009 cruises."High O_2" and "Low O_2" refer to rates obtained at 100% and 15% air saturation oxygen treatments, respectively. For *E. inermis*, "Deep" and "Shallow" refer to collection depths of 200–300 m and ca. 50 m, respectively.

Species	O_2 consumption (nmol (mg WW)$^{-1}$ h^{-1})	O_2 consumption (nmol (mg DW)$^{-1}$ h^{-1})	Size-scaled O_2 consumption (nmol (mg WW)$^{-1}$ h^{-1})
E. inermis			
10 °C	**1.71 (1.28–2.27) *45***	**27.8 (21.3–35.8) *45***	**2.61**
2007	1.15 (0.49–1.43) *18*	19.7 (8.1–25.7) *18*	
Shallow	1.00 (0.36–1.38) *16*	18.0 (6.1–24.7) *16*	
2008	1.95 (1.68–2.42) *27*	32.0 (27.3–37.2) *27*	
Shallow	1.88 (1.64–2.27) *10*	30.0 (25.6–37.3) *10*	
17 °C	**2.38 (2.30–2.60) *9***	**43.9 (37.1–45.7) *9***	**3.63**
23 °C	**5.55 (5.31–7.09) *5***	**90.5 (89.8–140.9) *5***	**8.46**
R. rostrifrons			
10 °C	**6.06 (3.75–6.59) *9***	**40.3 (24.8–51.3) *9***	**5.52**
17 °C	**7.01 (6.18–7.85) *2***	**55.8 (48.8–62.8) *2***	**6.39**
S. subtenuis			
10 °C	**7.58 (5.65–10.22) *22***	**56.8 (43.0–77.2) *22***	**7.46**
17 °C	**11.49 (7.66–12.31) *11***	**85.2 (55.8–91.2) *11***	**11.31**
High O_2	12.31 (12.14–12.56) *6*	91.2 (90.6–92.9) *6*	
Low O_2	7.54 (7.35–7.78) *5*	53.2 (50.7–58.4) *5*	
23 °C	**16.01 (15.73–17.82) *9***	**144.9 (121.9–166.5) *9***	**15.76**

were most abundant mid-thermocline, in the vicinity of the chlorophyll maximum (CRD site: 25 m; T=16 °C; 60 μM O_2) as illustrated in Fig. 2. *R. rostrifrons* females were largely observed slightly above the base of the upper oxycline, just above the core of the OMZ at 275 m (T=11 °C; 6 μM O_2). *E. inermis* had a peak maximum abundance at 325 m, also near the base of the upper oxycline (T=10 °C; 2 μM O_2), and extending through the OMZ in low concentrations to the top of the lower oxycline. In addition, adult females of this species had a secondary peak in the thermocline near the chlorophyll maximum at 35 m (T=15 °C; 35 μM O_2).

3.2. Comparison of rates among species

At both 10 and 17 °C, *E. inermis* had significantly lower oxygen consumption (Table 3) and ammonium, urea and total measured nitrogen excretion rates (rates per mg wet weight; Tables 4 and 5) than *S. subtenuis*. *E. inermis* also had significantly lower oxygen consumption and ammonium and phosphate (Table 5) excretion rates at 23 °C than the other two species. In addition, *S. subtenuis* had significantly higher %-urea N values (Table 4) and phosphate excretion rates (Table 5) compared to that of *E. inermis* at 10 °C, and *S. subtenuis* had an order of magnitude higher urea and percentage of nitrogen excreted as urea (% urea-N) than *E. inermis* rates at 23 °C, but, due to a low number of replicates, statistical significance could not be tested.

Metabolic rates for *R. rostrifrons* were not consistent in how they related to rates of the other two species. At both 10 and 17 °C, *R. rostrifrons* had oxygen consumption rates which were significantly higher than rates of *E. inermis*, but were not statistically different from those of *S. subtenuis* (Table 3). At 10 °C, *R. rostrifrons* phosphate excretion was similar to that of *S. subtenuis* (Table 5). In terms of excretion rates of nitrogenous waste products, both *R. rostrifrons* and *E. inermis* had lower ammonium excretion rates at 10 as well as 17 °C and lower urea excretion at 10 °C (Table 4). *R. rostrifrons* was not significantly different from either species with regards to rates of urea excretion at 17 °C (Table 4). Total measured nitrogen excretion rates for *R. rostrifrons* were similar to that of *S. subtenuis* under 10 °C conditions, and not statistically different from rates of *E. inermis* at 17 °C (Table 5). *S. subtenuis* consistently had higher wet weight-specific metabolic rates at all temperature and oxygen level treatments compared to the other species.

E. inermis was the largest of the three species, with an average wet weight (WW) of 5.41 mg (Table 6). *R. rostrifrons* and *S. subtenuis* were substantially smaller, with average WWs of 0.69 and 0.94 mg, respectively. When oxygen consumption rates were corrected for body size, weight-corrected trends corroborated our mass-specific rate measurements (Table 3), and indicated that body size could not explain the lower oxygen consumption rates of *E. inermis* compared to the other species. *E. inermis* also had significantly higher water content, averaging around 94% of WW, as compared with the other two species, which both had average values around 87%. Data for average nitrogen, protein and phosphorus content of individuals of the three species showed that, in terms of percentage of WW, *S. subtenuis* had the highest percentage of these three components, while *E. inermis* uniformly had the lowest. Estimates of storage lipid mass indicated that such lipids comprised a much higher percentage of body mass in *R. rostrifrons* than the other two species, with *S. subtenuis* having the overall lowest percentage of storage lipids per unit mass. In general, when corrected for average body nitrogen and phosphorus values, the daily turnover rates of nitrogen and phosphorus did not significantly vary between the three species (Tables 5 and 6). The one exception is at 10 °C, where *E. inermis* had significantly lower phosphorus turnover rates than the other two species.

Metabolic ratios examined (O:N, N:P, O:P) were highly variable and, thus, did not generally show any significant differences among species (Table 7). However, *E. inermis* did have a significantly higher N:P ratio at 10 °C as compared to *R. rostrifrons* and *S. subtenuis*.

3.3. Temperature

E. inermis and *S. subtenuis* had significantly higher oxygen consumption and ammonium and phosphate excretion rates, as well as % N and % P turnover rates in the 23 °C treatment compared to the 10 °C treatment (Tables 3–5). Additionally, total N excretion rates for *S. subtenuis* were significantly higher at 23 °C compared to rates at 10 °C. Rates measured at the intermediate temperature (17 °C) did not show any consistent statistical trends as compared to the 10 and 23 °C treatments.

Unlike many of the other metabolic parameters, urea excretion rates were not influenced by temperature. While the median value % urea-N increased with decreasing temperature, the overall trend was not statistically significant owing to the considerable variation in rates.

S. subtenuis had a significantly higher N:P ratio at 17 °C compared to ratios at 10 °C. However, O:N, N:P, and O:P ratios were generally not influenced significantly by temperature for any of the species examined.

3.4. Oxygen level

Environmental oxygen concentrations between 15 and 100% saturation did not appear to strongly influence metabolic rates, except for those of *S. subtenuis*. At 17 °C, *S. subtenuis* had significantly lower respiration rates (about a factor of 2/3) in the low oxygen treatment compared to that in the 100% air saturation experiment (Table 3). At 10 °C, *E. inermis* showed significantly higher phosphate excretion and % P turnover rates during the low oxygen experiments (Table 5).

Table 4
Ammonium and urea excretion rates and percent urea of total measured nitrogen excretion for eucalanoid copepods. Notations as described in Table 3. Excretion rates are in nmol urea or ammonium (NH_4^+). NS denotes data that were not significantly different from other treatments.

Species	NH_4^+ excretion (nmol (mg WW)$^{-1}$ h^{-1})	NH_4^+ excretion (nmol (mg DW)$^{-1}$ h^{-1})	Urea excretion (nmol (mg WW)$^{-1}$ h^{-1})	Urea excretion (nmol (mg DW)$^{-1}$ h^{-1})	% Urea N (nmol Urea-N^{-1} (nmol Total N)$^{-1}$)
E. inermis					
10 °C	**0.23 (0.16–0.34) *42***	**3.9 (2.7–5.6) *42***	**0.03 (0.00–0.06) *39***	**0.4 (0.1–1.1) *39***	**14.8 (2.1–39.0) *38***
High O_2	NS	NS	0.00 (0.00–0.02) *20*	0.1 (0.0–0.2) *20*	2.4 (0.0–12.9) *20*
Low O_2	NS	NS	0.06 (0.03–0.08) *19*	0.9 (0.4–1.3) *19*	34.7 (20.6–40.6) *18*
2007	0.34 (0.26–0.49) *16*	5.7 (4.8–8.2) *16*	NS	NS	NS
2008	0.19 (0.14–0.31) *26*	3.1 (2.2–4.9) *26*	NS	NS	NS
17 °C	**0.40 (0.32–0.60) *9***	**6.6 (5.1–9.4) *9***	**0.01 (0.00–0.06) *9***	**0.1 (0.0–0.1) *9***	**1.9 (0.0–17.5) *9***
High O_2	NS	NS	0.06 (0.04–0.09) *4*	1.0 (0.7–1.5) *4*	19.9 (13.6–30.1) *4*
Low O_2	NS	NS	0.00 (0.00–0.00) *5*	0.0 (0.0–0.0) *5*	0.0 (0.0–0.0) *5*
23 °C	**0.77 (0.60–1.00) *4***	**14.4 (10.2–19.2) *4***	**0.01 *1***	**0.2 *1***	**2.4 *1***
R. rostrifrons					
10 °C	**0.21 (0.00–0.43) *9***	**1.3 (0.0–3.5) *9***	**0.19 (0.01–0.72) *5***	**1.9 (0.1–4.1) *5***	**77.5 (5.6–100.0) *5***
High O_2	NS	NS	0.01 (0.00–0.01) *2*	0.1 (0.0–0.1) *2*	2.8 (1.4–4.2) *2*
Low O_2	NS	NS	0.72 (0.46–1.96) *3*	4.1 (3.0–12.3) *3*	100.0 (88.8–100.0) 3
17 °C	**0.29 (0.23–0.35) *2***	**2.3 (1.9–28) *2***	**0.08 (0.04–0.12) *2***	**0.7 (0.3–1.0) *2***	**22.3 (11.1–33.4) *2***
S. subtenuis					
10 °C	**0.94 (0.66–1.22) *22***	**8.0 (4.4–10.5) *22***	**0.29 (0.19–0.45) *19***	**2.0 (1.3–3.7) *19***	**43.9 (34.2–62.6) *19***
17 °C	**0.99 (0.77–1.30) *11***	**7.8 (5.1–9.7) *11***	**0.27 (0.11–0.53) *11***	**2.0 (0.7–3.9) *11***	**39.6 (14.9–50.0) *11***
High O_2	NS	NS	0.40 (0.29–0.57) *6*	3.0 (2.1–4.2) *6*	42.4 (39.7–52.7) *6*
Low O_2	NS	NS	0.08 (0.00–0.14) *5*	0.6 (0.0–0.8) *5*	13.0 (0.0–22.0) *5*
23 °C	**3.16 (2.37–3.40) *7***	**26.1 (20.3–30.8) *7***	**0.30 (0.28–0.30) *3***	**2.7 (2.5–2.8) *3***	**14.4 (13.0–15.4) *3***

Table 5
Total measured nitrogen and phosphate excretion rates and daily percent body nitrogen and phosphorus turnover for eucalanoid copepods. Notations as described in Table 3.

Species	Total N excretion (nmol (mg WW)$^{-1}$ h^{-1})	Total N excretion (nmol (mg DW)$^{-1}$ h^{-1})	% N turnover (nmol Total N (nmol Body N)$^{-1}$ day^{-1})	PO_4^{3-} excretion (nmol (mg WW)$^{-1}$ h^{-1})	PO_4^{3-} excretion (nmol (mg DW)$^{-1}$ h^{-1})	%P turnover (nmol PO_4^{3-} - P (nmol Body P)$^{-1}$ day^{-1})
E. inermis						
10 °C	**0.34 (0.21–0.43) *38***	**5.4 (3.4–7.2) *38***	**3.6 (2.2–4.6) *38***	**0.006 (0.001–0.011) *36***	**0.09 (0.01–0.19) *36***	**1.4 (0.2–2.8) *36***
High O_2	NS	NS	NS	0.001 (0.000–0.007) *16*	0.02 (0.00–0.11) *16*	0.3 (0.1–1.8) *16*
Low O_2	NS	NS	NS	0.009 (0.002–0.015) *20*	0.15 (0.04–0.24) *20*	2.5 (0.7–4.5) *20*
Shallow	0.37 (0.31–0.45) *21*	5.9 (5.2–8.1) *21*	4.0 (3.5–5.0) *21*	NS	NS	NS
Deep	0.20 (0.15–0.38) *20*	3.2 (2.7–5.8) *17*	2.0 (1.6–3.7) *17*	NS	NS	NS
2007	0.43 (0.34–0.53) *14*	7.9 (5.6–8.5) *14*	4.5 (3.6–5.6) *14*	NS	NS	NS
Shallow	0.41 (0.34–0.51) *13*	7.7 (5.6–8.4) *13*	4.4 (3.3–5.2) *13*	NS	NS	NS
Deep	2.05 *1*	31.1 *1*	20.6 *1*	NS	NS	NS
2008	0.28 (0.18–0.37) *24*	4.6 (2.9–5.8) *24*	3.2 (1.8–3.7) *24*	NS	NS	NS
Shallow	0.33 (0.31–0.39) *8*	5.2 (4.8–6.1) *8*	3.8 (3.5–4.4) *8*	NS	NS	NS
Deep	0.20 (0.16–0.37) *16*	3.2 (2.6–5.6) *16*	2.0 (1.6–3.7) *16*	NS	NS	NS
17 °C	**0.51 (0.25–0.66) *9***	**8.6 (5.4–11.4) *9***	**5.9 (2.9–7.5) *9***	**0.053 (0.027–0.082) *3***	**0.93 (0.47–1.23) *3***	**18.0 (9.0–27.7) *3***
23 °C	**0.90 *1***	**17.8 *1***	**9.6 *1***	**0.041 (0.036–0.052) *4***	**00.77 (0.61–1.00) *4***	**9.3 (8.1–11.8) *4***
R. rostrifrons						
10 °C	**0.54 (0.45–1.87) *5***	**3.8 (3.7–10.7) *5***	**2.1 (1.8–7.3) *5***	**0.06 (0.03–0.09) *8***	**0.4 (0.2–0.8) *8***	**8.7 (4.6–13.3) *8***
17 °C	**0.46 (0.32–0.60) *2***	**3.7 (2.5–4.8) *2***	**1.8 (1.2–2.3) *2***	**0.48 *1***	**33.8 *1***	**68.8 *1***
S. subtenuis						
10 °C	**1.35 (1.08–2.25) *19***	**10.0 (8.2–18.9) *19***	**3.3 (2.8–6.0) *19***	**0.08 (0.02–0.12) *16***	**0.6 (0.2–1.0) *16***	**4.2 (0.4–7.3) *16***
17 °C	**1.23 (1.06–2.49) *11***	**9.1 (7.7–18.6) *11***	**3.0 (2.6–6.1) *11***	**0.17 (0.12–0.23) *10***	**1.3 (0.8–1.6) *10***	**9.5 (7.0–13.0) *10***
23 °C	**4.13 (3.96–4.31) *3***	**37.5 (36.4–38.3) *3***	**11.0 (10.5–11.5) *3***	**0.39 (0.25–0.42) *7***	**3.5 (2.0–3.9) *7***	**24.1 (15.8–26.4) 7**

The few experiments carried out at approximately 5% saturation at 10 °C showed, on average, lower oxygen consumption rates (0.83, 1.04, 1.06 nmol O_2 (mg WW)$^{-1}$ h^{-1}) for *E. inermis* at 10 °C versus rates measured at 15–20% and 100% saturation (median of 1.71 nmol O_2 (mg WW)$^{-1}$ h^{-1} with 25th to 75th quartile ranges of 1.28 to 2.27). However, the lower two of these three data points were collected in 2007, when averages for *E. inermis* rates at 15 and 100% saturation were much lower (1.15 nmol O_2 (mg WW)$^{-1}$ h^{-1} with 25th to 75th quartile ranges of 0.49–1.43) than rates measured in 2008. *R. rostrifrons*, whose sole oxygen consumption measurement at 5% saturation was 1.62 nmol O_2 (mg WW)$^{-1}$ h^{-1}, showed a substantially lower rate than those obtained at the higher oxygen concentrations (median value of 6.06 O_2 nmol (mg WW)$^{-1}$ h^{-1} with 25th to 75th quartile ranges of 3.75 to 6.59). Due to high mortality of *S. subtenuis* in 5% oxygen treatments (78% of experimental organisms), no measurements were successfully obtained for this species.

Table 6
Adult female copepod weight and body composition for eucalanoid species. Weights are in mg per individual. Percent water, carbon, nitrogen, protein, phosphorus and storage lipid are in terms of wet weight. Carbon, hydrogen, and nitrogen content were analyzed at the University of California, Santa Barbara Marine Science Institute Analytic Laboratory. Protein content was determined with the Lowry et al. (1951) assay and phosphorus content followed the methods of Anderson and Hessen (1991). Storage lipid was estimated based on the measured volume of the visible storage lipid sac. Data are reported in the form of "mean ± standard deviation (number of replicates)" or "median (25th quartile–75th quartile) *replicates*".

Species	Wet weight (mg)	Dry weight (mg)	Water (%)	Carbon (%)[1]	Nitrogen (%)[1]	Protein (%)[1]	Phosphorus (%)[1]	Storage lipid (%)[2]
E. inermis	5.41 ± 0.79 (100)	0.33 ± 0.06 (100)	93.9 ± 0.5 (100)	1.48 ± 0.28 (34)	0.32 ± 0.04 (34)	1.63 ± 0.27 (51)	0.027 ± 0.007 (59)	0.28 (0.06–0.54) *145*
R. rostrifrons	0.69 ± 0.05 (20)	0.09 ± 0.02 (20)	86.7 ± 2.3 (20)	7.82 ± 1.61 (5)	0.81 ± 0.07 (5)	3.23 ± 0.57 (14)	0.048 ± 0.013 (18)	7.91 (6.27–11.12) *76*
S. subtenuis	0.94 ± 0.11 (52)	0.12 ± 0.02 (52)	87.2 ± 1.4 (52)	5.44 ± 0.55 (12)	1.34 ± 0.13 (12)	7.04 ± 1.48 (32)	0.125 ± 0.019 (33)	0.00 (0.00–0.06) *165*

[1] Body composition from copepods collected at the same time as the metabolic experimental animals (Cass, 2011).
[2] Storage lipid sac volume estimates from copepods collected at the same time as the metabolic experimental animals (Cass et al., 2014).

Table 7
Metabolic ratios for eucalanoid copepods. Notations as described in Table 3. NS denotes data that were not significantly different from other treatments.

Species	O:N		N:P		O:P
	NH_4^+–N only	Total N	NH_4^+–N only	Total N	
E. inermis					
10 °C	**13.6 (8.6–23.5) *42***	**11.1 (7.3–20.2) *38***	**46.9 (15.4–98.4) *27***	**64.3 (27.6–107.3) *26***	**433.3 (198.7–1870.1) *29***
Shallow	11.5 (7.8–15.1) *23*	8.2 (5.1–11.1) *21*	NS	NS	NS
Deep	22.1 (12.5–41.3) *19*	19.5 (12.2–23.6) *17*	NS	NS	NS
2007	8.2 (2.6–12.1) *16*	5.6 (1.7–7.8) *14*	NS	NS	NS
2008	21.6 (14.2–33.1) *26*	14.2 (10.7–22.8) *24*	NS	NS	NS
17 °C	**11.5 (7.9–18.3) *9***	**9.0 (7.8–14.0) *9***	**6.1 (5.8–351.4) *3***	**13.0 (6.6–354.9) *3***	**116.9 (59.8–18692.2) *3***
23 °C	**16.0 (13.4–19.1) *4***	**15.8 *1***	**17.0 (16.8–17.9) *4***	**20.9 *1***	**297.9 (226.0–362.8) *4***
R. rostrifrons					
10 °C	**30.7 (22.3–42.7) *5***	**22.3 (9.6–29.0) *5***	**8.2 (3.2–10.2) *5***	**4.7 (2.7–13.6) *4***	**177.6 (108.6–291.3) *8***
17 °C	**51.6 (47.0–56.2) *2***	**42.2 (32.8–51.5) *2***	**0.4 *1***	**0.4 *1***	**22.2 *1***
S. subtenuis					
10 °C	**22.6 (9.1–27.0) *20***	**12.7 (4.3–16.6) *19***	**11.5 (8.3–13.5) *11***	**16.8 (11.3–26.6) *11***	**206.7 (93.1–305.0) *12***
17 °C	**16.9 (15.2–23.3) *11***	**11.5 (9.1–19.1) *11***	**4.5 (4.0–7.3) *9***	**8.4 (4.5–9.3) *9***	**107.1 (56.3–120.1) *8***
23 °C	**11.3 (9.6–13.8) *7***	**8.0 (6.2–8.7) *3***	**8.5 (7.5–9.4) *7***	**9.6 (9.6–10.1) *3***	**90.0 (78.3–127.8) *7***

Urea excretion rates were affected by an interaction between starting oxygen concentration and temperature, although not in a consistent manner. For example, both *R. rostrifrons* and *E. inermis* had higher excretion rates in lower oxygen concentrations at low temperature (10 °C), conditions similar to those that they experience in the upper oxycline (Table 4). In contrast, *S. subtenuis* and *E. inermis* had a 5–6 fold decrease in rates when exposed to lower oxygen concentrations at intermediate temperatures (17 °C). However, given the high variability in urea excretion rates, none of these trends were statistically significant. An identical trend existed with % urea-N. *R. rostrifrons* and *E. inermis* excreted a significantly higher % of urea-N in low oxygen treatments at the low temperature (10 °C) (Fig. 3). At 17 °C, *E. inermis* and *S. subtenuis* also excreted a significantly (or nearly significantly) higher % of urea-N in the high oxygen treatment (*p*-values of 0.032 and 0.063, respectively).

3.5. Year

Interannual variability was assessed by comparing metabolic rates observed at 10 °C between the October–November 2007 and December–January 2008–2009 sampling periods. *E. inermis* was the only species in which interannual variability was observed in any of the measured rates. In 2007, *E. inermis* had significantly higher ammonium, total measured nitrogen excretion, and %N turnover rates but significantly lower oxygen consumption rates (Tables 3–5). However, this variability primarily reflects differences observed between individuals collected in the upper 50 m versus those collected in the deeper (200–300 m) range, as many more of the deeper-dwelling *E. inermis* were used in experiments in 2008–2009 (see Section 3.6. *E. inermis* Depth of Collection). When only *E. inermis* collected in the upper 50 m (which were well-represented in both sampling years) were compared, oxygen consumption rates were the only parameters that showed significant differences. When rates at 10 °C were compared, shallow-dwelling *E. inermis* collected in 2007 had oxygen consumption rates that were approximately half of those collected in 2008–2009 (median values of 1.00 and 1.88 nmol (mg WW)$^{-1}$ h^{-1}, respectively; $p=0.003$). *E. inermis* from the upper 50 m did not show significant differences between years when total N excretion, % N turnover, and ammonium excretion rates were considered.

3.6. *E. inermis* depth of collection

Significant differences in total N excretion rates, %N turnover rates, and O:N metabolic ratios were observed between *E. inermis* collected at < 50 m depth ("shallow") versus those collected at 200–300 m depth ("deep"). Deep individuals had significantly lower total N excretion and % N turnover rates and higher O:N ratios than shallow-dwelling individuals. Additionally, deep *E. inermis* collected in 2008–2009 had about half the ammonium excretion rate of shallow individuals collected in the same year (median: 0.16 v. 0.31 nmol (mg WW)$^{-1}$ h^{-1}). While this difference itself is not statistically significant ($p=0.058$), it likely contributed to the interannual variability observed for this parameter.

3.7. Q_{10} ratios

Q_{10} ratios indicate the sensitivity of certain rates to temperature changes (Hochachka and Somero, 2002). Q_{10} values were generally in the 1.5–2.5 range, except for phosphate excretion

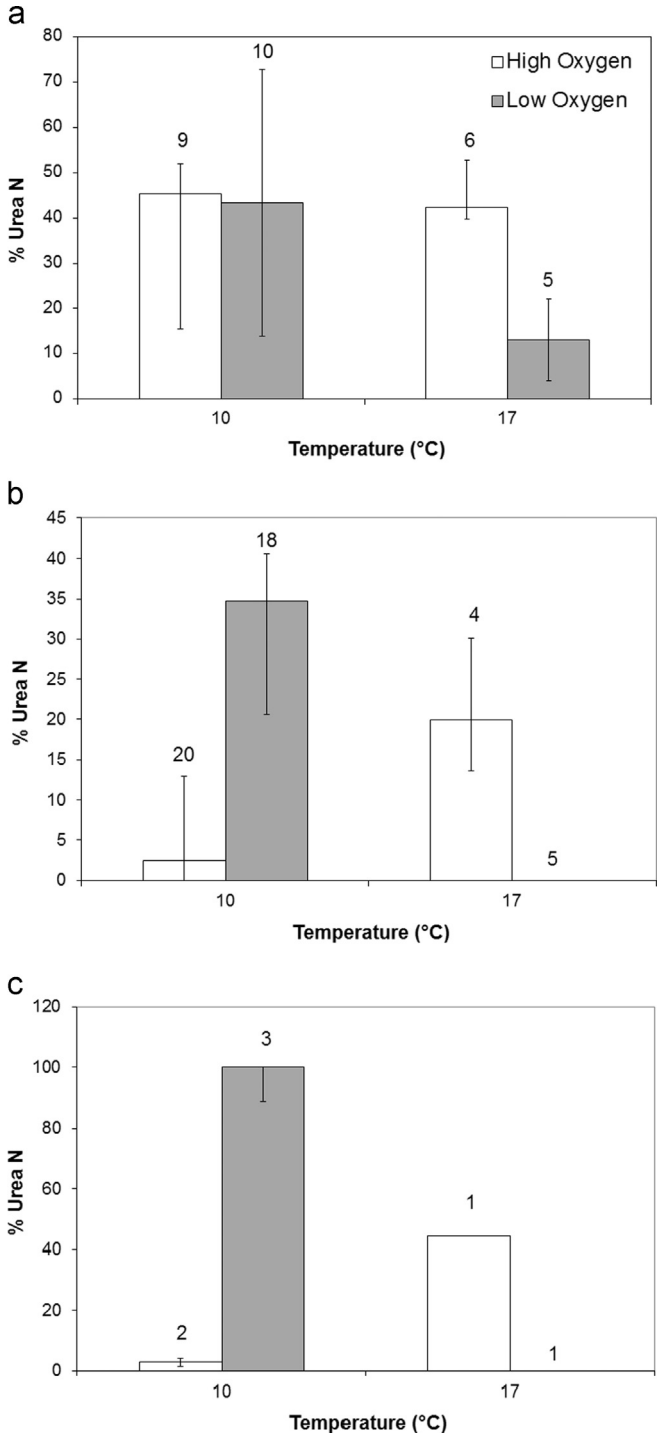

Fig. 3. Percent urea-nitrogen at high (white bars) and low (gray bars) oxygen treatments for (a) *Subeucalanus subtenuis*, (b) *Eucalanus inermis*, and (c) *Rhincalanus rostrifrons*. Columns mark the median value and error bars denote 25th–75th quartiles. Values above error bars indicate the number of replicates.

rates, which were approximately 3–4 (Table 8). Values generally were similar between 2007 and 2008–2009, with the exception of oxygen consumption and phosphate excretion rates for *E. inermis*. Oxygen consumption rates for this species had a higher Q_{10} in 2007 that 2008–2009 (4.10 v. 1.26), while phosphate excretion rates had a lower Q_{10} (2.94 v. 3.99). No values are reported for *R. rostrifrons*, as no significant temperature differences existed. Urea excretion rates also were not examined, as there was no significant temperature variation within any species.

Table 8
Q_{10} ratios for *Eucalanus inermis* and *Subeucalanus subtenuis*. Metabolic rates from 2007 (10 v. 23 °C) and 2008–2009 (10 v. 17 °C).

	E. inermis		*S. subtenuis*	
	10 v. 17 °C	10 v. 23 °C	10 v. 17 °C	10 v. 23 °C
O_2 consumption	1.26	4.10	1.69	2.12
NH_4^+ excretion	2.20	2.03	1.43	2.04
Total N excretion	1.54	1.75	1.35	1.21
PO_4^{3-} excretion	3.99	2.94	3.20	3.49

4. Discussion

4.1. Metabolic parameter variation among species

E. inermis had significantly lower wet weight-specific oxygen consumption and ammonium, urea, and phosphate excretion rates than *S. subtenuis* in all temperature treatments, but particularly at 10 °C. This was not surprising, as *E. inermis* has been reported to be a "jelly-bodied" copepod, having metabolic rates and a body composition per wet mass that are more similar to gelatinous plankton than calanoid copepods (Flint et al., 1991). Our body composition results support this finding as well (Table 6). *E. inermis* had a percent water content of about 94% WW, while the other two species contained about 87% water (more typical for crustacean plankton).

Our *E. inermis* oxygen consumption rates were similar to those reported by Flint et al. (1991) and Dagg et al. (1980). Dagg et al. (1980) also measured nitrogen excretion rates of *E. inermis* from the Peru Current; however, they were substantially higher than those obtained by our study (27% versus 6–10% body N daily). This was mostly due to differences in the body nitrogen contents of *E. inermis*, as levels in our study were 2–2.5 times higher than Dagg et al. (1980) reported. In addition, recently reported weight-specific oxygen consumption rates for *S. subtenuis* near an Atlantic Ocean OMZ during March–June 2010 were at least twice as high as the rates reported in our study when similar temperatures were compared (Teuber et al., 2013a). This could represent a significant difference between *S. subtenuis* in these two locations, with ETNP *S. subtenuis* adapted to have lower metabolic rates to cope with the shallower and more severe OMZ. In the ETNP, their depth range overlaps with the upper oxycline, and they may be exposed to oxygen concentrations $<50\ \mu M$ near the chlorophyll maximum, especially at the TB site. The observed difference could also be an artifact of body size (Ikeda et al., 2001), sampling season (Conover, 1959; Conover and Gustavson, 1999; Torres et al., 1994) or feeding history (Bohrer and Lampert, 1988; Ikeda, 1971, 1977; Mayzaud, 1976).

The metabolic ratios of all three copepod species indicated that metabolism was predominantly supported by protein catabolism (Mayzaud and Conover, 1988). The median O:N ratio of *R. rostrifrons*, however, was higher than those for the other species while the N:P ratio was lower, suggesting more reliance on lipid catabolism relative to the other species. This result was not surprising, as storage lipid content was usually more than 10 times greater in *R. rostrifrons* when compared to *E. inermis* and *S. subtenuis* (Table 6).

Given that adult female *R. rostrifrons* reside almost exclusively at depth (Fig. 2), where oxygen levels are much lower than the "low" oxygen treatments, it is noteworthy that its observed oxygen consumption rate was similar to that of an active surface species (*S. subtenuis*). One possible explanation for this was the observed variability in behavior and activity rates detected between species. These experiments were conducted in the dark with no food present; therefore, the rate measurements are likely representative of routine metabolic rates, which include minimal

activity. During sorting, *S. subtenuis* individuals were by far the most active, while *R. rostrifrons* females were the least active (C. Cass, personal observations). Thus, while both species had similar rates when there was minimal activity and no feeding, *R. rostrifrons* likely continued this low activity lifestyle *in situ*, giving this species an advantage in oxygen-limited environments. This observation also was supported by its low body protein content relative to that in *S. subtenuis* (Table 6), indicating less muscle mass per weight. In contrast, *E. inermis* had an intermediate level of swimming activity and appeared to have adapted to a low-oxygen environment by having the lowest amount of actively metabolizing tissue for its size. These combined factors would allow adult *E. inermis* to migrate from surface waters as well as survive in the OMZ.

Percent urea-N excreted was variable among species and between treatment groups, yielding few significant differences. Overall levels for *E. inermis* ranged from 0 to 63% across the three temperatures, with median values of 2–15%. This was consistent with the results reported by Dagg et al. (1980), who found average urea excretion rates of 8–25% of total N excretion for *E. inermis*. Overall, rates for the three species were comparable with previously reported values (5–60%) for other copepod species in various temperate to tropical regions (Eppley et al., 1973; Miller and Roman, 2008; Mitamura and Saijo, 1980; Saba et al., 2009; Smith and Whitledge, 1977; Steinberg et al., 2002).

4.2. Temperature

Temperature was a major factor influencing almost all of the measured rates (oxygen consumption, ammonium excretion, phosphate excretion, total N excretion, %N and %P turnover). The highest temperature (23 °C) had the highest metabolic rates, which was expected based on previous studies (e.g., Barber and Blake, 1985; Donnelly and Torres, 1988; Ikeda et al., 2001). Q_{10} ratios were primarily in the 1.5–2.5 range, similar to previously determined values for crustacean metabolic rates (e.g., Aarset and Aunaas, 1990; Irwin et al., 2007; Mauchline, 1998). Phosphate excretion rates for both *E. inermis* and *S. subtenuis* had Q_{10} ratios in the 3–4 range, suggesting higher sensitivity (Childress, 1977) of phosphate excretion to temperature changes. Higher Q_{10} values for phosphate excretion have been observed before (Alcaraz et al., 2013), although a specific mechanism has not been proposed for the observed differences. However, such variation in Q_{10} ratios could have impacts on the larger ecosystem by contributing towards differential rates of nitrogen and phosphorus cycling if these species are forced into habitats of greater temperature. For instance, if OMZs continue to expand vertically (Gilly et al., 2013; Stramma et al., 2010), *S. subtenuis* could be further pushed into the high-temperature surface waters to avoid the encroaching low-oxygen waters.

In contrast, urea excretion rates and % urea-N did not vary significantly with temperature for any species. One other study using prawns showed that these rates may either increase or decrease with temperature (Quarmby, 1985), depending on the sex and size of the prawn. Thus, there does not appear to a standard response in urea production in crustaceans to changes in temperature. Additionally, metabolic ratios also did not appear to be influenced by temperature, indicating that substrate usage was not temperature dependent. This was consistent with most previous work comparing O:N, N:P and O:P ratios and temperature (Ikeda, 1985; Ikeda et al., 2001).

4.3. Oxygen level

At the concentrations tested, oxygen saturation level did not appear to influence many metabolic parameters, except for those of *S. subtenuis*. At higher temperatures, *S. subtenuis* had lower oxygen consumption rates in the low oxygen than high oxygen treatment (although this was only significant at 17 °C). This suggested that when oxygen consumption rates were reduced at lower temperatures, *S. subtenuis* did not consume enough oxygen to be affected by external oxygen conditions of 15–20% saturation. However, as temperature increased and routine metabolic rates increased, oxygen started to become limiting. This was interesting, as the *in situ* condition of 17 °C and 15–20% air saturation occurred near the chlorophyll/fluorescence maximum in this region (Fig. 2), which was the approximate depth of maximum abundance for *S. subtenuis*. Thus, this species appeared to be functioning at a depth where they may be slightly stressed metabolically, but likely achieving optimal food resources. Additionally, based on their high mortality during exposure to even lower hypoxic conditions (78% mortality at 10 °C over 12–15 h of exposure to ≤ 20 μM O_2 or 5% saturation), it seems unlikely that *S. subtenuis* would be able to function at depths much below the chlorophyll maximum in the OMZ. Indeed the vertical depth distribution of *S. subtenuis* (Fig. 2) indicates that this species was restricted to near surface waters in this region.

Neither *E. inermis* nor *R. rostrifrons* showed differences in oxygen consumption rates between the low and high oxygen treatments at any temperature, suggesting that oxygen was not limiting for them at 15–20% air saturation. However, preliminary results at 10 °C and 5% saturation indicated a possible decrease for both of these species in oxygen consumption rate between 15% and 5% air saturation, which warrants further experimentation to confirm. *E. inermis* and *R. rostrifrons* would experience such oxygen levels in the oxyclines and OMZ core, which includes depths of maximum abundance for these species. Several coping mechanisms have already been identified for these species to reduce aerobic demand if such suppression exists. Both species can utilize anaerobic pathways to supplement aerobic metabolism (Cass, 2011). Additionally, stable isotope and lipid biomarker data suggest that *E. inermis* does not feed while within the OMZ during their ontogenetic migration, which would reduce necessary activity when in low-oxygen waters (Cass et al., 2014; Williams, 2013).

In addition to oxygen consumption rates, urea excretion rates and % urea-N were the only other parameters that showed significant differences with oxygen level (Fig. 3). Here, lower temperatures (10 °C) and decreased environmental oxygen led to an increase in % urea-N for *E. inermis* and *R. rostrifrons*, while at higher temperatures (17 °C), *E. inermis* and *S. subtenuis* showed the opposite trend. This was particularly interesting given that, within high oxygen treatments, urea excretion rates seem to be insensitive to temperature.

The formation of urea as an excretory product in crustaceans happens through two different pathways (Claybrook, 1983). In the first pathway, the enzyme arginase catalyzes the reaction of the amino acid arginine to ornithine and urea. A second pathway involves the formation of uric acid and subsequently urea from breakdown of purines. Once urea is formed, urease can catalyze the reaction for full breakdown into ammonia. Oxygen is only directly involved in one step of the purine catabolism pathway. Changing temperature or oxygen concentrations could affect the urea output by signaling to the organism to change the substrates that are being catabolized or via up- or down-regulation of the activity or amount of some of the enzymes involved in these pathways, such as arginase or urease. As marine copepods do not thermoregulate (Willmer et al., 2005), cellular temperature largely mirrors environmental temperature and thus temperature changes should cause variation in the rates of enzyme reactions (Weiner, 2006). While activity of these enzymes is largely unstudied in crustaceans, it has been found that urease is inhibited in denitrifying soil bacteria under high oxygen conditions (Ruan et al., 2009). Additionally, work on rat liver cells has found up-regulation of gene expression in regions coding for arginase I and other enzymes when cellular oxygen levels are increased (Miralles et al., 2000). This suggests that up- or down-regulation of

activity or expression of relevant enzymes in response to different oxygen environments is possible in crustaceans.

Such differences in nitrogen excretion products with temperature and environmental oxygen may be important when looking at the contribution of zooplankton to the various nitrogen pools within a study region. As certain types of bacteria and plankton have different abilities to uptake and/or utilize the various forms of inorganic and organic N (Berg et al., 2003; Glibert and Terlizzi, 1999; Soloman et al., 2010), such distinctions are necessary for more accurate modeling and calculations. For instance, at the ETNP sites, the chlorophyll maximum (usually around 20–50 m depth) often occurred at oxygen levels of $\leq$30% air saturation (40–100 μM) and temperatures of 15–20 °C. Community nitrogen excretion rates for *E. inermis, S. subtenuis* and *R. rostrifrons* under conditions of 17 °C and 20% oxygen saturation can be estimated using 2007 MOCNESS abundance data from day tows at the Costa Rica Dome (data courtesy of K. Wishner and D. Outram). For the following calculations, the abundances of adult females of these species residing between 20 and 60 m depth (where conditions of 17 °C and 20% oxygen saturation are roughly accurate) were used. Total measured nitrogen excretion for adult females of these species averages 30–700 nmol N m^{-3} day^{-1} and, of that, urea-N accounts for about 9% (using estimates at 100% air saturation, urea-N would have accounted for 35%). Overall, their ammonium and urea excretion appears to be small compared to the standing nutrient concentrations. Ammonium concentrations ranged from 0.44 to 1.60 μM (2007) and from 0.01 to 1.61 μM (2008/2009) during our cruises (K. Daly, unpublished data). Hoch and Bronk (2007) reported urea values of ~0.07 μM N in the ETNP. Thus, these copepods excrete $<$1% of the standing stock each day. Reported rates of ammonium oxidation in the tropical Pacific Ocean are variable, with maximum rates of up to several hundred nmol NH_4^+-N l^{-1} day^{-1} (Beman et al., 2012; Lipschultz et al., 1990). Assuming a more typical rate of 50 nmol NH_4^+-N l^{-1} day^{-1}, eucalanoid ammonium excretion contributes $\leq$2% towards daily oxidation substrate. Even though substantial variations in excretion rates and products for these three species are unlikely to vary their total input by more than 1% on any of these parameters, these results give us a better starting point for assessing zooplankton community contributions to nutrient flux. By dry mass, adult females of *E. inermis*, *R. rostrifrons* and *S. subtenuis* represent only about 7% of the non-gelatinous zooplankton community in the upper 150 m (estimated with total biomass of 2131 mg m^{-2} from Wishner et al. (2013)), indicating that total zooplankton input is substantially higher than the eucalanoid contribution discussed here. Additionally, ammonium in this region is likely important for anaerobic ammonium oxidation pathways (anammox). In OMZ regions, anammox pathways are responsible for one quarter to one third of the loss of nitrogen (as dinitrogen gas) from those areas (Ward, 2013). Surveys of the distribution of ETNP anammox bacteria during our cruises indicated that such bacteria are not just restricted to the OMZ. Highest abundances were often found in near-surface waters, suggesting that ammonium excretion throughout the water column may provide a substrate to local anammox bacteria (Podlaska et al., 2012).

4.4. Year

The interannual variation observed for oxygen consumption rates in shallow-living *E. inermis* (higher rates observed during December–January 2008–2009 compared to October–November 2007) could be potentially due to two factors—the first of which was seasonal differences. Although tropical regions tend to be more stable temporally than high latitude systems, variation in wind-driven upwelling (Kessler, 2006), mean chlorophyll levels (Pennington et al., 2006) and zooplankton abundances (Fernández-Álamo and Färber-Lorda, 2006) can vary between October and January, providing mechanisms for such metabolic differences. Indeed, enhanced upwelling was observed during the 2008–2009 cruise at the CRD, as evidenced by cooler and more saline surface waters (Table 1). A second possibility was the influence of El Nino-Southern Ocean (ENSO) events. Both cruise years coincided with La Niña events (Multivariate ENSO Index (MEI) values were reported as −1.177 for October/November of 2007 and −0.752 for December 2008/January 2009 (NOAA Earth System Research Laboratory; http://www.esrl.noaa.gov/psd/enso/mei/)). La Niña events can lead to cooler surface temperatures due to a shoaling of the thermocline (Kang et al., 2008; Ryan et al., 2006; Saba et al., 2008), which could contribute to lower metabolic rates (e.g., Ikeda et al., 2001). However, such thermocline shoaling was not evident at our 2007 sampling sites as compared to 2008, which had approximately the same mixed layer depth (20–40 m) (Table 1).

4.5. Eucalanus inermis depth of collection

A comparison of day and night abundance profiles of adult females in this region indicates that a resident population of adult *E. inermis* likely exists at depth (Chen, 1986; Longhurst, 1985; Saltzman and Wishner, 1997b). It is thought that these deeper-dwelling individuals represent an ontogenetic migration (Wishner et al., 2013). The similarity between standard metabolic rates of *E. inermis* collected at different depths suggests that this migration does not represent a diapause state. Instead, the major difference observed between *E. inermis* collected at different depths was that lower nitrogen excretion rates occurred in deeper-dwelling individuals, leading to significantly decreased turnover of daily % body N. Such rates suggested decreased protein utilization and increased lipid catabolism in deeper, colder, low oxygen water. This finding was supported by significantly higher O:N ratios in deep individuals (median value of 19.5; shallow individuals had a median value of 8.2), suggesting that deeper living adult females may utilize a different metabolic strategy at depth. Comparison of storage lipid fatty acid composition between deep and shallow-dwelling individuals suggests that little feeding occurs by *E. inermis* outside of the euphotic zone (Cass et al., 2014). Thus, the observed differences between females at these two depths are best explained by reduced feeding during the migration, which is supplemented with lipid catabolism.

5. Conclusions

As expected, temperature and the species of copepod examined had the greatest overall effect on metabolic rates. In general, increases in temperature tended to increase metabolic rates, with Q_{10} values of around 2 for oxygen consumption and ammonium and nitrogen excretion rates and between 3 and 4 for phosphate excretion. Urea excretion rates were the sole exception, where no trend with temperature was observed. *S. subtenuis*, which were concentrated in the upper 100 m, had the highest metabolic rates, while *E. inermis*, which were found throughout the water column to about 600 m, typically had the lowest metabolic rates. *S. subtenuis* was an active species, having high protein content, indicative of large amounts of muscle tissue. This species also had decreased oxygen consumption rates at low oxygen levels and intermediate temperatures (17 °C), demonstrating potential oxygen limitation as shallow as 30 m in the ETNP. This suggests that the vertical distribution of *S. subtenuis* was likely constrained due to oxygen concentrations in the water column. In contrast, *E. inermis* had particularly high water and low organic content, resulting in a reduced amount of respiring tissue for its size, which contributed to its tolerance for low oxygen waters. *R. rostrifrons*, which was found predominantly in the upper oxycline, had an intermediate metabolic strategy between *S. subtenuis* and *E. inermis*. *R. rostrifrons* had typical water content for a copepod, but compensated for a limited

oxygen supply through decreased activity and, therefore, decreased metabolic demand. Large lipid reserves also contributed towards its metabolic needs. When excretion rates were examined as daily body N or P turnover rates, however, species differences largely disappeared, indicating that there was not necessarily a functional metabolic difference among these species.

As *S. subtenuis* and *R. rostrifrons* are circum-tropical and sub-tropical species, it would be interesting to see if the results from this study hold for individuals found in areas without such severe oxygen limitations. It has been suggested that some characteristics observed in organisms inhabiting oceanic low oxygen regions are not necessarily adaptations specifically for life at low oxygen, but rather general taxonomic features that allow them to exploit such a lifestyle (Childress and Seibel, 1998). A regional comparison would help to illuminate whether these copepods respond to low oxygen in this manner due to adaptation, or genetic pre-disposition.

One of the most interesting new findings of this study was the relationship between temperature, oxygen level, and nitrogen excretion in these species (Fig. 3). At 10 °C, low oxygen led to an increase in the amount of urea nitrogen produced relative to ammonium nitrogen in *E. inermis* and *R. rostrifrons*. The opposite trend was true at 17 °C for *E. inermis* and *S. subtenuis*. While the general pathways of urea and ammonia production in crustaceans are known, the mechanisms that regulate the relative amount of each produced are woefully understudied. Other studies have investigated relationships between urea, ammonium, and amino acid excretion with factors like food source, species, temperature, and life stage (Conover and Cota, 1985; Dagg et al., 1980; Miller and Roman, 2008; Mitamura and Saijo, 1980; Quarmby, 1985; Saba et al., 2009). Their findings illustrated that nitrogen excretion is complex and variable among individuals and species. Thus, our examination of three dominant copepod species only represents a small portion of the overall community picture. It is particularly important to understand the factors influencing nitrogen cycles in OMZ regions. It is thought that such regions contribute up to 50% of total nitrogen lost from the oceans to the atmosphere (Codispoti et al., 2001; Gruber and Sarmiento, 1997), primarily through denitrification and anaerobic ammonium oxidation (anammox) pathways. Our oceans are currently seeing decreases in oxygen concentrations, increases in temperatures, and expansion of OMZ systems (Bograd et al., 2008; Emerson et al., 2004; Gilly et al., 2013; Keeling and Garcia, 2002; Stramma et al., 2008, 2010). Thus, it is particularly important to understand the relationships between temperature, oxygen levels, and zooplankton excretory products as well as their impacts on OMZ food webs. Future work in this area should include more examination of enzyme levels to better understand how pathways themselves are affected, not just the end products.

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