Ecological characteristics of eucalanoid copepods of the eastern tropical North Pacific Ocean: Adaptations for life within a low oxygen system

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ABSTRACT

The eastern tropical North Pacific Ocean (ETNP) is home to one of the largest and most severe mid-water oxygen minimum zones (OMZs). Members of the copepod family Eucalanidae are abundant in this region and display varied vertical distributions throughout the OMZ. This research assessed the diversity of ecological strategies used by these copepods to cope with the presence of the OMZ based on their biochemical, physiological, and behavioral characteristics. Five species of copepods (*Eucalanus inermis*, *Pareucalanus attenuatus*, *Rhincalanus nasutus*, *Rhincalanus rostrifrons*, and *Subeucalanus subtenuis*) were collected at the Costa Rica Dome (9°N, 90°W) and Tehuantepec Bowl (13°N, 105°W) during cruises in fall of 2007 and winter of 2008–2009. Adult females of all species were collected and analyzed for water, ash, carbon, nitrogen, hydrogen, phosphorus, protein and lipid content, lactate dehydrogenase (LDH) activity, and survivorship when exposed to prolonged low-oxygen conditions. Four distinct ecological strategies were observed for the five species based on genus. *E. inermis*, found throughout the OMZ and surface waters, had very high water content (94% of wet weight (WW)), leading to low organic content per unit WW. However, when corrected to ash-free dry weight (AFDW), protein and storage lipid contents of this species had intermediate (46 and 8% of AFDW, respectively) values compared to other species. *E. inermis* demonstrated high survivorship under low-oxygen conditions (>90%), and had detectable levels of LDH, indicating an ability to rely on anaerobic pathways. *Rhincalanus* spp., found primarily in the lower-oxygen subsurface waters, also had detectable LDH activity and high survivorship under low-oxygen conditions (>85%), but had much higher storage lipid levels (>37% of AFDW), very low protein levels (28–33% of AFDW), and low water content (87% of WW). *S. subtenuis* and *P. attenuatus* are both distributed primarily in surface waters, but showed distinct ecological strategies. *S. subtenuis* protein levels were very high (67% AFDW), storage lipid levels low (0%), and water content low (87% of WW). They also had very low survivorship under low-oxygen conditions (22%) and no detectable LDH activity. *P. attenuatus* also did not have detectable LDH activity or large lipid stores. *P. attenuatus*, however, had much lower protein content (30% AFDW) and higher water content (89% of WW), indicating a distinct ecological strategy. Comparisons of ETNP individuals with conspecifics or congeners from higher-oxygen environments suggest that low protein levels (indicative of lower overall activity) seen in some groups may be an adaptation for survival in lower-oxygen regions.

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1. Introduction

The oceans are decreasing in oxygen in response to global warming, primarily through surface heating and increased stratification (Emerson et al., 2004; Keeling and Garcia, 2002). Additionally, regions of the ocean having oxygen minimum zones (OMZs), which are characterized by oxygen deficient waters at intermediate depths, appear to be expanding (Stramma et al., 2008, 2010). The suboxic regions in open water OMZs are typically maintained as a result of poor ventilation, sluggish circulation, oxygen-poor source waters, and decomposition of sinking particles (Wyrtki, 1962). Most tropical and subtropical regions in the Atlantic and western Pacific oceans have moderate OMZs, with minimum oxygen levels of 60 to 80 μM (Paulmier and Ruiz-Pino, 2009). One of the largest and most severe open water OMZs is located in the eastern tropical North Pacific (ETNP) (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 μM occur as shallow as 40 m and often reach <4.5 μM in the OMZ core (the region of lowest oxygen concentrations) (Brinton, 1979; Levin et al., 1991; Saltzman and Wishner, 1997b; Vinogradov et al., 1991). Some oxygen levels have been reported below 0.5 μM (Chen, 1986; Levin et al., 1991; Sameoto, 1986). The vertical oxygen gradients in OMZs structure biological assemblages and

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biogeochemical processes. As a result, habitats of organisms intolerant to low oxygen may be compressed into the shallow, near-surface oxygenated waters (Prince and Goodyear, 2006).

Members of the family Eucalanidae are dominant copepods in the ETNP, and include all four genera (*Rhincalanus*, *Eucalanus*, *Subeucalanus* and *Pareucalanus*) (Chen, 1986; Longhurst, 1985; Saltzman and Wishner, 1997a; Sameoto, 1986). Like many other zooplankters in the region, these copepods display a variety of vertical distributions that are likely related to the oxygen environment (Chen, 1986; Saltzman and Wishner, 1997a; Sameoto, 1986; Vinogradov et al., 1991). *Eucalanus inermis* (Giesbrecht, 1892), endemic to the ETNP, is found throughout the upper 1000 m, often with maximum concentrations in the chlorophyll maximum and the upper and lower edges of the OMZ core. *Subeucalanus subtenuis* (Giesbrecht, 1888), *Subeucalanus subcrassus* (Giesbrecht, 1888), *Subeucalanus pileatus* (Giesbrecht, 1888), and *Pareucalanus attenuatus* (Dana, 1849) are usually concentrated in the shallow euphotic zone. *Rhincalanus rostrifrons* (Dana, 1849) [sometimes referred to as *Rhincalanus cornutus rostrifrons* (Lang, 1965)] and *Rhincalanus nasutus* (Giesbrecht, 1888), on the other hand, are often absent from the surface mixed layer, and instead are concentrated above and below the OMZ core. This suggests that a variety of ecological strategies occur within this family in the ETNP region.

Even though eucalanoid copepods are abundant in the ETNP, little is known about their ecology. As severe OMZ regions appear to be expanding (Stramma et al., 2008, 2010), understanding the strategies employed by zooplankton in current OMZ systems may help us to predict the effects of decreasing oxygen on marine ecosystems in other regions of the ocean. Eucalanidae is a relatively small family of copepods (24 described species) that are distributed throughout a majority of the world's oceans and occur in coastal and open water systems (Bradford-Grieve et al., 1999; Goetze, 2003; Grice, 1962; Lang, 1965). The abundance of eucalanoid copepods in the ETNP system, coupled with their broad distribution in other regions, make them a useful group for comparative studies within and between ecosystems. These copepods also have been the focus of several recent genetic studies, which aimed to further describe separate lineages within species (Goetze, 2003, 2005, 2006, 2010; Goetze and Ohman, 2010). Consequently, their phylogeny is fairly well described, which provides a basis for understanding differences in ecological characteristics between species and populations within the family.

The primary objective of this study was to assess whether eucalanoid copepods employed different ecological strategies in the ETNP OMZ system based on their biochemical, physiological and behavioral characteristics. Based on published vertical distributions in this region (Chen, 1986; Saltzman and Wishner, 1997a; Sameoto, 1986; Vinogradov et al., 1991), it was hypothesized that *P. attenuatus* and *S. subtenuis* would have similar characteristics and strategies, and *R. nasutus* and *R. rostrifrons* would be similar to each other, whereas *E. inermis* would have different strategies relative to the other two groups. The results of our study were compared with findings for eucalanoid copepods in other regions of the world to assess whether the observed characteristics were adaptations to the OMZ system, or simply general features of a species or genus.

Additional attention was paid to *E. inermis*, which is one of the most abundant and widely vertically distributed copepods in this region (Chen, 1986; Longhurst, 1985; Saltzman and Wishner, 1997a; Wishner et al., 2013). Adult females occur in near-surface waters, but a large resident population also occurs in the upper and lower oxyclines and in the OMZ. Their occurrence at low oxygen depths may be due to an ontogenetic migration, possibly related to their reproductive cycle (Wishner et al., 2013). However, this hypothesis has not been well substantiated in the literature. We hoped to learn more about how individuals taking part in this migration may differ from conspecifics in shallower depths to better understand the role of such a migration within a tropical system. Also, differences in vertical

distribution have been documented between adult males and females, with adult males often concentrated at shallower depths than females (Longhurst, 1985; K. Wishner, personal communication). Therefore, the ecological strategies of both males and females were examined.

2. Methods

2.1. Collection site and methods

Copepods were collected during two cruises to the eastern tropical North Pacific (ETNP) during 18 October–17 November 2007 aboard the R/V *Seward Johnson* and 8 December 2008–6 January 2009 aboard the R/V *Knorr*. Sampling occurred primarily at the Costa Rica Dome (9°N, 90°W) and to a lesser extent the Tehuantepec Bowl (13°N, 105°W) (Fig. 1). Copepods were collected using bongo tows, Tucker trawls, and MOCNESS (Multiple Opening/Closing Net and Environmental Sampling System) (Wiebe et al., 1976) tows at depths of high abundances. Adult female *S. subtenuis* and *P. attenuatus* were collected from the upper 50 m, while *R. rostrifrons* and *R. nasutus* were collected in the 200–300 m range. *E. inermis* adult females were collected at both shallow and deep depths and adult males collected in the upper 50 m. In addition, adult female *R. cornutus* were obtained during a cruise to the Gulf of Mexico (GOM) at a station offshore of Florida (27°N, 86°W) during June 25–29 2007 aboard the R/V *Suncoaster*. Adult female *R. nasutus* individuals also were collected from the Guaymas Basin, Gulf of California (GOC) (approximate location 27°N, 111°W) during June 4–12 2007 aboard the R/V *New Horizon*. These *R. cornutus* and *R. nasutus* were both collected using a Tucker trawl.

Immediately after capture, adult 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 to allow them to empty their guts. Once their guts were emptied, copepods collected in the ETNP were divided up for various analyses. Most individuals were frozen at −80 °C for later body content, enzyme, and weight analyses. Some individuals of *S. subtenuis*, *R. rostrifrons*, and *E. inermis* were used for metabolic end point incubation experiments (see Cass and Daly, 2014). All *Rhincalanus* spp. collected during the GOM and GOC cruises were frozen at sea, either in liquid nitrogen (GOM) or a −80 °C freezer (GOC).

2.2. Body composition

Subsets of individuals from each species were used for carbon/ hydrogen/nitrogen (CHN) content analyses. Copepods were thawed,
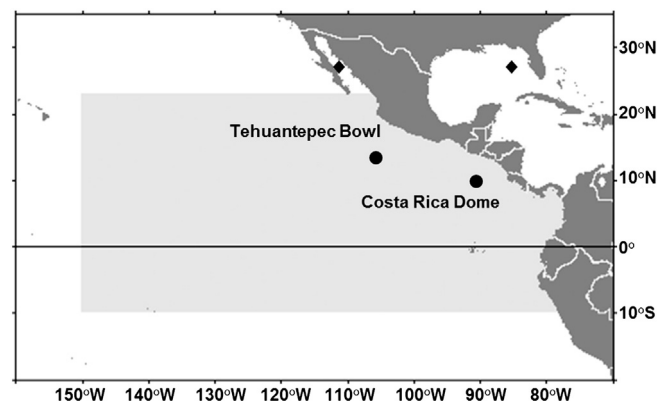

Fig. 1. Map of sampling sites. Black dots show the two main sampling regions, the Tehuantepec Bowl and Costa Rica Dome, in the eastern tropical North Pacific. Black diamonds show the Gulf of California and Gulf of Mexico sites. Gray area denotes the extent of the eastern tropical Pacific Ocean as designated by Fiedler and Lavín (2006). Map modified from SWFSC NOAA website (http://swfsc.noaa.gov/).

measured for body size, and then grouped into batches of 5–15 individuals onto pre-combusted (2 h at 450 °C) and pre-weighed GF/F filters. Copepods on filters were briefly rinsed with DI water using hand-pump filtration and then dried at 60 °C for several days prior to weighing on a Mettler XP2U Ultramicrobalance. After weighing, filters were placed into pre-combusted 20 ml glass scintillation vials. Copepods and filter blanks were analyzed for carbon (C), hydrogen (H), and nitrogen (N) content at the University of California, Santa Barbara Marine Science Institute Analytic Laboratory. Blank filters assessed contamination during handling and sample preparation.

Protein content of copepods was determined on individuals using the Lowry assay (Lowry et al., 1951). Phosphorus (P) content was assessed on individuals using the method outlined in Anderson and Hessen (1991). For these analyses, individual copepod were thawed, measured, and then placed in a small amount of reagent in a clean scintillation vial. Copepod integument was disrupted through agitation with a sonication probe. For a subset of individuals, estimations of storage lipid mass were made through measurements of storage lipid sacs. Height (h), length (l), and width (w) were measured and volume was estimated using the equation for an ellipsoid (V = $(4/3) * \pi * (h/2) * (w/2) * (l/2)$). Conversions to mass were made based on the density (reviewed in Sargent, 1976) and relative amounts of triacylglycerol or wax ester known to be accumulated by each species (Cass et al., 2011, 2014). Total wet mass of individuals was determined through established length to weight conversions for these species in this sampling region (Cass, 2011).

Water and ash content was determined using batches of 2–10 copepods for each species. Copepods were thawed, measured, dipped briefly in DI water, blotted dry, and sealed into a small aluminum capsule. WW was measured using the XP2U Mettler ultramicrobalance. Capsules were reopened and dried at 60 °C for several days prior to resealing and reweighing for DW. Capsules were then combusted (500 °C for 6 h) and reweighed to obtain ash weight.

2.3. Enzyme analyses

Lactate dehydrogenase (LDH) facilitates the final step in the glycolysis pathway, which can be used under anaerobic conditions to generate ATP (adenosine triphosphate). Measurement of LDH activity has been used to assess anaerobic potential of marine organisms (e.g., Teuber et al., 2013; Vetter and Lynn, 1997). Levels of LDH were measured on batches of 3–35 individuals using the methods described in Childress and Somero (1979). Copepod batches were homogenized using a glass tissue grinder in a 50 mM imadazole/HCl buffer solution (pH = 7.2 at 20 °C). Buffer solution was added in ratios of 10:1 or 15:1 μL of buffer to mg of wet mass, giving a final homogenate weight to volume dilution of 1:11 or 1:16, respectively. Activity levels were assessed for all copepods at 10 °C using a thermostated Cary 1000 UV/Visible spectrophotometer with data analysis software. Twenty microliters of homogenate were added to 1 mL of lactate dehydrogenase stock solution (80 mM Imadazole buffer, 0.15 mM NADH, 5.0 mM sodium pyruvate) in a 1.5 mL cuvette. Activity levels corresponded to rates of change in absorbance at 340 nm measured over a 60 second interval due to the oxidation of NADH substrate. Samples were run in triplicate, with the average value reported. Activity level is expressed in units (μmol substrate converted to product per min) per g wet mass or g protein.

2.4. Survivorship studies

Metabolic end-point experiments were run on various species of eucalanoid copepods (methods described in Cass and Daly, 2014). Experiments were run at approximately 5, 15, and 100% air oxygen saturation. Only experiments where all individuals survived were used for rate measurements. Here, survivorship during all metabolic experiments at 10 °C (the only temperature where all three oxygen saturation

levels were explored) is investigated. Experiments at 5 and 15% saturation levels were approximately 12–15 h in duration, while 100% saturation experiments were 22–36 h.

2.5. Statistical analyses

Statistical analyses were run in SigmaPlot 11.0. One and two way ANOVAs and t-tests were used to compare the central trend of data. When raw data did not fit the assumptions of equal variance or normality, ranked data were substituted or a comparable non-parametric test was utilized. Significance was assessed at α = 0.05. Unless otherwise noted, values listed in the text are means and standard deviations.

3. Results

3.1. Environmental parameters and copepod distributions

Temperature, oxygen, and fluorescence profiles for the Tehuantepec Bowl and Costa Rica Dome stations during the 2007 and 2008/2009 ETNP cruises are depicted in Fig. 2. Temperature profiles were generally similar between stations and years. However, surface temperatures were slightly cooler and salinities slightly higher at the Costa Rica Dome site compared to those at the Tehuantepec Bowl, consistent with upwelling. In addition, surface nitrate concentrations (2007: 4–5 μM; 2008/9: 8–12 μM) at the Cost Rica Dome site also indicated upwelling, in contrast to surface nitrate concentrations at the Tehuantepec Bowl, which were below the limit of detection (K. Daly, unpublished data). Fluorescence profiles often showed two major peaks in the Tehuantepec Bowl region, usually one near the base of the mixed layer, about the depth of the nutricline (20–30 m), and one near the base of the thermocline. Only one peak was observed at the Costa Rica Dome. In both locations, oxygen rapidly declined between the base of the surface mixed layer and about 50–60 m. In the Costa Rica Dome, however, oxygen intrusions were present between 100 and 300 m, resulting in somewhat higher oxygen concentrations at these mid-water depths than at the Tehuantepec Bowl site.

Eucalanoid copepod vertical distributions at the Costa Rica Dome during 2007 and 2008 were largely similar to those reported previously (Fig. 3; K. Wishner, personal communication). Overall, the minimum oxygen concentrations encountered were highest for adult female *Pareucalanus* spp. (the majority of which were likely *P. attenuatus* (e.g., Grice, 1962; Saltzman and Wishner, 1997a; Sameoto, 1986)) and *S. subtenuis*, and lowest for *E. inermis* adult females. *Rhincalanus* spp. adult females and *E. inermis* adult males experienced minimum oxygen concentrations between those of the other two groups. For example, in 2008, *S. subtenuis* and *Pareucalanus* spp. adult females had peak abundances in the upper 40–60 m (>40 μM O_2) (Fig. 3). Daytime abundances of adult female *E. inermis* were highest between 200 and 300 m (2–20 μM O_2) and 20–100 m (25–200 μM O_2), and 500–600 m (1–2 μM O_2). Adult males were found between 20 and 100 m (25–200 μM O_2). Adult female *R. rostrifrons* were found primarily at 50–150 m (15–45 μM O_2) during the day, with smaller numbers present at 200–250 m (10–15 μM O_2) and 0–20 m (>200 μM O_2). *R. nasutus* adult females were found in low numbers at 50–100 m (25–45 μM O_2).

3.2. Body composition—species differences

Body composition was variable among adult females of *E. inermis*, *S. subtenuis*, *P. attenuatus*, *R. nasutus* and *R. rostrifrons* in the ETNP (Table 1). Percent water content (% of WW) was significantly higher in *E. inermis* (93.9%) than *R. rostrifrons*, *R. nasutus*, and *S. subtenuis* (mean values of 86.6–87.2% of WW). *P. attenuatus* had intermediate water content (89.3%) and high variability. Ash content followed the same trends, with *E. inermis* having the highest values (41.8% of DW), while *S. subtenuis*, *R. nasutus* and *R. rostrifrons* showed significantly
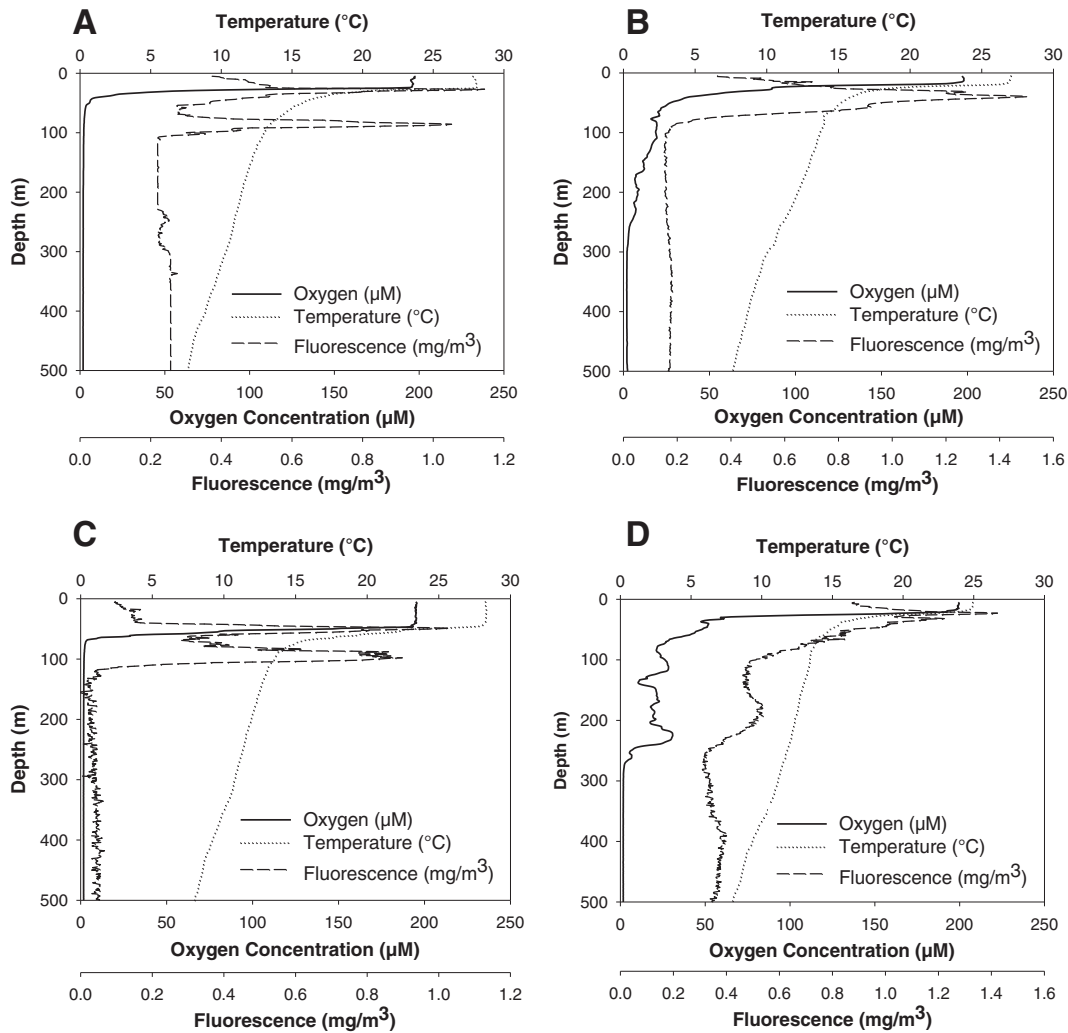
Fig. 2. Water column oxygen, temperature, and fluorescence profiles in the upper 500 m at two stations during the eastern tropical North Pacific cruises. 2007 profiles are depicted for the (A) Tehuantepec Bowl and (B) Costa Rica Dome. 2008–2009 cruise profiles are shown for the (C) Tehuantepec Bowl and (D) Costa Rica Dome. Data were collected with a Sea-Bird *3plus* temperature sensor, Sea-Bird *9plus* digital quartz pressure sensor, Sea-Bird 43 oxygen sensor and a C-Point chlorophyll fluorescence sensor.

lower values (13.9–18.0% of DW). *P. attenuatus* again had intermediate ash content (22.7% of DW).

C content was lowest for *E. inermis* based on % WW and DW, and highest for *R. rostrifrons* and *R. nasutus* (Table 1). *E. inermis* C content was significantly lower than *R. nasutus*, *R. rostrifrons* and *S. subtenuis*, based on WW and DW. However, when converted to % of ash-free dry weight (AFDW), *E. inermis* was more similar to *P. attenuatus* and *S. subtenuis*, and all three were lower than *Rhincalanus* spp.

N content (% WW) was significantly different between *S. subtenuis* and *E. inermis*, which showed the highest and lowest values, respectively (Table 1). When converted to % of DW, *P. attenuatus* and *S. subtenuis* grouped together with high values, while *E. inermis* and *Rhincalanus* spp. were lower and similar. When examining % of AFDW, *Rhincalanus* spp. showed the lowest values, while *S. subtenuis* and *P. attenuatus* had about a factor of two higher. *E. inermis* fell between the two.

H content per unit WW was significantly lower in *E. inermis* than *S. subtenuis* and *Rhincalanus* spp. (Table 1). Per unit DW, three distinct groups were formed, which were statistically distinct from one another. *E. inermis* had the lowest H content, *P. attenuatus* and *S. subtenuis* showed intermediate levels, and *Rhincalanus* spp. had the highest values, which were twice as high as *E. inermis*. Conversion to AFDW grouped *E. inermis* with *S. subtenuis* and *P. attenuatus*, which were all lower than *Rhincalanus* spp.

P content was highest in *S. subtenuis* and lowest in *E. inermis* based on WW, a significant difference (Table 1). Per unit DW, *E. inermis* was intermediate between two statistically distinct groups: *S. subtenuis*/ *P. attenuatus* and *Rhincalanus* spp. These trends continued when converted to % of AFDW.

Protein content per unit WW was lowest in *E. inermis* and highest in *S. subtenuis*. *E. inermis* showed significantly lower protein levels than *S. subtenuis* and *Rhincalanus* spp. (Table 1). *S. subtenuis* also had significantly higher protein contents than *P. attenuatus*. On a per unit DW basis, *S. subtenuis* was significantly higher than all other species. Comparisons using AFDW indicated that *P. attenuatus* and *Rhincalanus* spp. had the lowest protein contents, while *S. subtenuis* had levels about twice as high. *E. inermis* had intermediate protein levels.

C:N ratios were lowest in *S. subtenuis*, and highest in *Rhincalanus* spp. (C:N of about 4 versus 10) (Table 1). There were significant differences observed between *S. subtenuis* and *R. rostrifrons*, *R. nasutus* and *E. inermis*. C:P and N:P ratios (estimated from average C, N and P values for each species) showed two distinct groups. For both ratios, *Rhincalanus* spp. had higher values than the other three species.

Storage lipid mass (estimated through measurement of lipid sac dimensions) was lowest in *S. subtenuis* and highest in *Rhincalanus* spp., particularly *R. rostrifrons* (as percent WW, DW and AFDW) (Table 1). Statistically, *Rhincalanus* spp. grouped together, and had significantly
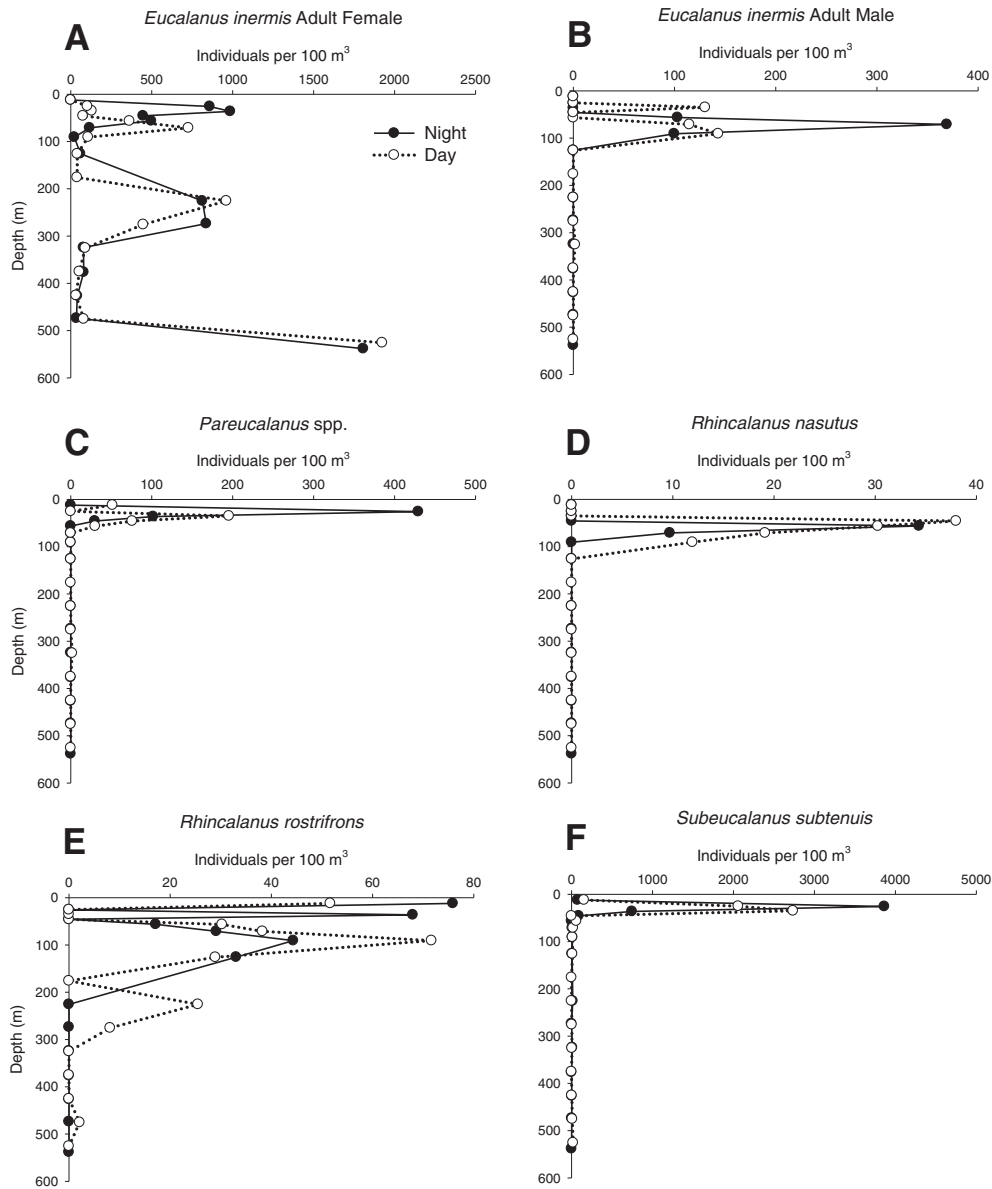
Fig. 3. Vertical profiles for copepods from the Costa Rica Dome in 2008/2009. Number of individuals per 100 m³ are given for mean MOCNESS net tow depths in the upper 550 m. Day (open circles, dashed lines) and night (filled circles, solid lines) abundances are reported separately. Abundance data is depicted for *Eucalanus inermis* (A) adult females and (B) adult males, and adult females of (C) *Pareucalanus* spp., (D) *Rhincalanus nasutus*, (E) *R. rostrifrons*, and (F) *Subeucalanus subtenuis*. Unpublished data provided by K. Wishner. Further information on collection procedures can be found in Wishner et al. (2013).

higher storage lipid mass than the other three species. *E. inermis* also had significantly more storage lipid than *S. subtenuis*.

3.3. Body composition—Eucalanus inermis

Comparisons between male and female *E. inermis*, as well as between shallow and deep-dwelling *E. inermis* females showed only a few differences in body composition (Table 2). Males had significantly higher C, N, protein content, and C:N ratios than females. Interannual variability was observed in P content for all *E. inermis*, with 2008 yielding significantly lower concentrations of P. Shallow-dwelling females collected in 2007 had significantly higher water content and lower C and N than other *E. inermis* female groups. 2008 shallow females showed significantly lower protein content than other female groups.

For CHN content analyses, shallow *E. inermis* females collected in 2007 were grouped based on collection site and analyzed separately. Collection sites included the Tehuantepec Bowl (13°N, 105°W), Costa Rica Dome (9°N, 90°W), and an intermediate station between the other two sites (10°41.41′N, 96°56.60′W). One-way ANOVAs comparing CHN content at these stations found significant differences for all three parameters, particularly for comparisons based on WW.

3.4. Body composition—Rhincalanus spp.

The body composition of *R. nasutus* females was similar among individuals collected from the ETNP and the GOC. Due to a similarity in % water, trends observed were similar in terms of WW and DW (Table 3). *R. nasutus* individuals from the ETNP had significantly higher N and protein content than females from the GOC. On the other hand, individuals from the GOC had significantly higher storage lipid mass.

Comparisons of the closely related *R. rostrifrons* from the ETNP and *R. cornutus* from the GOM showed more distinct differences. Percent water was significantly higher in *R. rostrifrons* (86.7 versus 80.6% WW), leading to major differences in body components in terms of WW, but fewer differences in terms of DW. Per unit WW, *R. cornutus*

Table 1

Body composition for eastern tropical North Pacific adult female copepods. Copepod prosome lengths (PL) are given in mm. Parameters are listed in terms of percent wet weight (WW), dry weight (DW), and ash-free dry weight (AFDW) when applicable. Unless otherwise noted, percentages or weights are reported as the mean ± standard deviation (number of samples[a]). C:N, C:P, and N:P denote molar ratios involving carbon (C), nitrogen (N), and phosphorus (P) content.

	E. inermis	P. attenuatus	R. rostrifrons	R. nasutus	S. subtenuis
PL (mm)	5.2 ± 0.3 (413)	4.1 ± 0.3 (55)	2.8 ± 0.1 (157)	3.5 ± 0.2 (68)	2.9 ± 0.1 (375)
WW (mg)	5.36 ± 0.92 (140)	1.45 ± 0.37 (8)	0.68 ± 0.06 (34)	1.25 ± 0.22 (4)	0.92 ± 0.11 (102)
DW (mg)	0.34 ± 0.07 (140)	0.18 ± 0.08 (6)	0.10 ± 0.02 (25)	0.23 ± 0.01 (3)	0.12 ± 0.02 (64)
Water (% WW)	93.9 ± 0.5 (105)	89.3 ± 2.4 (3)	86.7 ± 2.3 (20)	86.6 (1)	87.2 ± 1.4 (52)
Ash (% DW)	41.8 ± 4.1 (105)	22.7 ± 8.1 (2)	13.9 ± 7.6 (20)	16.2 (1)	18.0 ± 3.8 (52)
Carbon					
% WW	1.48 ± 0.28 (34)	3.29 ± 0.26 (3)	7.82 ± 1.61 (5)	8.95 ± 0.44 (2)	5.44 ± 0.55 (12)
% DW	25.2 ± 3.0 (35)	38.4 ± 0.7 (3)	52.2 ± 3.6 (5)	55.2 ± 0.3 (2)	38.8 ± 2.6 (12)
% AFDW	44.5 ± 5.3 (35)	51.8 ± 0.8 (3)	61.6 ± 5.8 (5)	67.7 ± 0.1 (2)	49.3 ± 3.5 (12)
Nitrogen					
% WW	0.32 ± 0.04 (34)	0.80 ± 0.07 (3)	0.81 ± 0.07 (5)	0.88 ± 0.03 (2)	1.34 ± 0.13 (12)
% DW	5.5 ± 0.5 (35)	9.3 ± 0.2 (3)	5.5 ± 0.8 (5)	5.4 ± 0.4 (2)	9.5 ± 0.5 (12)
% AFDW	9.7 ± 0.9 (35)	12.6 ± 0.2 (3)	6.5 ± 1.2 (5)	6.7 ± 0.6 (2)	12.1 ± 0.7 (12)
Hydrogen					
% WW	0.23 ± 0.05 (34)	0.46 ± 0.09 (3)	1.23 ± 0.33 (5)	1.19 ± 0.09 (2)	0.72 ± 0.13 (12)
% DW	4.0 ± 0.9 (35)	5.4 ± 1.1 (3)	8.2 ± 1.3 (5)	7.3 ± 0.3 (2)	5.1 ± 0.8 (12)
% AFDW	6.8 ± 1.2 (35)	7.3 ± 1.6 (3)	9.6 ± 1.0 (5)	9.0 ± 0.3 (2)	6.5 ± 1.0 (12)
Phosphorus					
% WW	0.027 ± 0.007 (59)	0.085 ± 0.035 (3)	0.048 ± 0.013 (18)	0.049 ± 0.014 (9)	0.125 ± 0.019 (33)
% DW	0.45 ± 0.13 (59)	1.06 ± 0.43 (3)	0.36 ± 0.10 (18)	0.37 ± 0.10 (9)	0.98 ± 0.15 (33)
% AFDW	0.78 ± 0.22 (59)	1.38 ± 0.56 (3)	0.42 ± 0.12 (18)	0.44 ± 0.12 (9)	1.19 ± 0.18 (33)
Protein					
% WW	1.63 ± 0.27 (51)	2.47 ± 1.01 (6)	3.23 ± 0.57 (14)	3.93 ± 0.55 (9)	7.04 ± 1.48 (32)
% DW	26.9 ± 4.2 (51)	23.0 ± 9.5 (6)	24.3 ± 4.3 (14)	27.9 ± 3.9 (9)	54.9 ± 11.6 (32)
% AFDW	46.2 ± 7.2 (51)	29.8 ± 12.2 (6)	28.3 ± 5.0 (14)	33.3 ± 4.7 (9)	66.9 ± 14.1 (32)
Lipid sac mass[b]					
% WW	0.28 (0.05–0.54) 145	0.08 (0.03–0.20) 30	7.91 (6.27–11.12) 76	4.16 (2.94–6.41) 33	0.00 (0.00–0.06) 165
% DW	4.5 (0.9–.8.5) 145	0.7 (0. 3–1.8) 30	63.8 (50.5–92.4) 76	31.0 (21.9–47.8) 33	0.0 (0.0–0.4) 165
% AFDW	7.7 (1.5–14.6) 145	0.9 (0.4–2.4) 30	74.1 (58.6–107.4) 76	37.0 (26.2–57.1) 33	0.0 (0.0–0.5) 165
C:N[b]	5.4 (4.9–5.7) 35	4.8 (4.8–4.9) 3	12.1 (9.1–13.1) 5	11.9 (11.2–12.6) 2	4.8 (4.7–4.9) 12
C:P[c]	141.6	100.0	418.5	472.0	112.4
N:P[c]	26.4	20.8	37.4	39.9	23.7

[a] "Sample" refers to an independent measurement taken for a given parameter. Each sample may contain between 1 and 35 individuals. See Sections 2.2 and 2.3 for details about sample preparation for each parameter.

[b] Due to non-normality or use of ratios, these values are listed as median (25th–75th quartile) *number of samples*.

[c] These ratios are only estimates which were calculated using the average values for C, N, and P for each species.

showed significantly higher C, H, N, P, protein, and lipid sac mass. However, per unit DW, significant differences were only observed for H and protein content, for which *R. cornutus* still had higher values than *R. rostrifrons*.

3.5. Enzyme activity

Lactate dehydrogenase (LDH) activity was detected in all *E. inermis* adult males and females, as well as in adult female *R. rostrifrons* and *R. nasutus* (Table 4). LDH activity levels in *S. subtenuis* and *P. attenuatus* were below detection limits for this assay. In *E. inermis*, shallow dwelling males showed the highest activity levels, while deep dwelling females had the lowest, a statistically significant difference. Results were similar using both units per g WW and per g protein. Statistical comparisons were not able to be made among species, owing to small sample sizes. LDH activity per gram WW appeared to be similar among adult females of *E. inermis*, *R. rostrifrons* and *R. nasutus*. However, in units of activity per gram protein, *E. inermis* showed 2–3 times higher enzyme activity, on average, than *Rhincalanus* spp.

3.6. Survivorship studies

Survivorship was high (96–100%) for *S. subtenuis*, *E. inermis* and *R. rostrifrons* in the 15% (36–78 μM) and 100% (201–325 μM) oxygen saturation level metabolic experiments at 10 °C (Fig. 4). In contrast, at 5% oxygen saturation (≤20 μM O$_2$), only *E. inermis*, *R. rostrifrons* and *R. nasutus* still had high survivorship (96, 93, and 87%, respectively), while *S. subtenuis* had only 22% survivorship after 12–15 h.

4. Discussion

Results of this study indicated that adult females of *E. inermis*, *P. attenuatus*, *S. subtenuis*, *R. rostrifrons* and *R. nasutus* could be separated into four groups based on similar ecological attributes within genera. Our observed vertical distributions of these copepod species were similar to those of previous studies (Chen, 1986; Longhurst, 1985; Saltzman and Wishner, 1997a; Sameoto, 1986).

4.1. Eucalanus inermis

E. inermis, an endemic species from the eastern tropical Pacific, is found throughout the upper 1000 m of the water column, with peaks in abundance in OMZ regions near the chlorophyll maximum and above and below the OMZ core (Chen, 1986; Saltzman and Wishner, 1997a; Sameoto, 1986; Vinogradov et al., 1991). *E. inermis* body composition was characterized by high water and low organic matter content per unit WW. This low level of body tissue for its size allowed *E. inermis* to have a low metabolic rate (medians of 1.71 and 2.38 nmol (mg WW)$^{-1}$ h^{-1} at 10 and 17 °C, respectively) compared to *S. subtenuis* (median rates of 7.58 and 11.49 nmol (mg WW)$^{-1}$ h^{-1} at 10 and 17 °C, respectively) and *R. rostrifrons* (median rates of 6.06 and 7.01 nmol (mg WW)$^{-1}$ h^{-1} at 10 and 17 °C, respectively) (Cass and Daly, 2014). *E. inermis* also had moderate LDH activity, indicating an ability to utilize the glycolytic pathway for energy production under hypoxic conditions (Hochachka and Somero, 2002). LDH activity, coupled with low metabolic rates, likely allows *E. inermis* to have a high tolerance for low oxygen environments.

Table 2
Body composition for eastern tropical North Pacific *Eucalanus inermis* copepods. Copepod prosome lengths (PL) are given in mm. Parameters are listed in terms of percent dry weight (DW) or wet weight (WW). Samples are separated by sex, collection depth, and year. Unless otherwise noted, percentages or weights are reported as the mean ± standard deviation (number of samples). C:N, C:P, and N:P denote molar ratios involving carbon (C), nitrogen (N), and phosphorus (P) content.

	Eucalanus inermis					
	Female				Male	
	Shallow		Deep			
	2007	2008	2007	2008	2007	2008
PL (mm)	4.9 ± 0.4 (109)	5.3 ± 0.3 (111)	5.4 ± 0.2 (88)	5.3 ± 0.3 (105)	4.0 ± 0.2 (68)	4.1 ± 0.2 (69)
WW (mg)	5.05 ± 1.16 (46)	5.22 ± 0.87 (34)	5.67 ± 0.41 (8)	5.80 ± 0.43 (52)	2.26 ± 0.29 (12)	2.55 ± 0.20 (8)
DW (mg)	0.28 ± 0.05 (46)	0.35 ± 0.07 (34)	0.38 ± 0.04 (8)	0.38 ± 0.04 (52)	0.16 ± 0.01 (6)	0.16 ± 0.04 (3)
Water (% WW)	94.3 ± 0.4 (31)	93.8 ± 0.4 (26)	93.6 ± 0.5 (4)	93.6 ± 0.4 (44)	94.2 ± 0.3 (3)	ND
Ash (% DW)	43.7 ± 4.1 (31)	42.5 ± 3.7 (24)	35.4 ± 4.8 (4)	40.4 ± 3.2 (41)	38.9 ± 6.4 (3)	ND
Carbon						
% WW	1.35 ± 0.28 (15)	1.58 ± 0.33 (8)	1.66 ± 0.15 (3)	1.57 ± 0.16 (8)	1.72 ± 0.12 (3)	1.82 ± 0.14 (3)
% DW	23.5 ± 3.0 (15)	25.1 ± 2.8 (8)	27.8 ± 1.4 (4)	27.1 ± 1.4 (8)	30.8 ± 2.8 (3)	31.7 ± 6.7 (3)
Nitrogen						
% WW	0.30 ± 0.04 (15)	0.34 ± 0.04 (8)	0.33 ± 0.04 (3)	0.34 ± 0.02 (8)	0.33 ± 0.03 (3)	0.36 ± 0.01 (3)
% DW	5.2 ± 0.5 (15)	5.5 ± 0.3 (8)	5.6 ± 0.3 (4)	5.9 ± 0.4 (8)	6.0 ± 0.3 (3)	6.2 ± 1.0 (3)
Hydrogen						
% WW	0.23 ± 0.07 (15)	0.23 ± 0.03 (8)	0.19 ± 0.01 (3)	0.24 ± 0.03 (8)	0.25 ± 0.03 (3)	0.26 ± 0.01 (3)
% DW	3.9 ± 1.0 (15)	3.7 ± 0.3 (8)	4.9 ± 1.5 (4)	4.1 ± 0.5 (8)	4.5 ± 0.6 (3)	4.5 ± 1.0 (3)
Phosphorus						
% WW	0.033 ± 0.007 (16)	0.022 ± 0.007 (13)	0.028 ± 0.004 (15)	0.025 ± 0.007 (15)	0.030 ± 0.009 (13)	0.020 ± 0.004 (13)
% DW	0.56 ± 0.12 (16)	0.37 ± 0.11 (13)	0.48 ± 0.07 (15)	0.39 ± 0.10 (15)	0.51 ± 0.16 (13)	0.35 ± 0.08 (13)
Protein						
% WW	1.72 ± 0.17 (14)	1.33 ± 0.28 (15)	1.76 ± 0.14 (10)	1.79 ± 0.12 (12)	1.65 ± 0.42 (14)	1.81 ± 0.41 (14)
% DW	29.6 ± 2.9 (14)	22.8 ± 4.8 (15)	27.7 ± 2.1 (10)	28.1 ± 1.8 (12)	28.4 ± 7.2 (14)	31.1 ± 7.0 (14)
Lipid sac mass[a]						
% WW	0.57 (0.46–0.84) 37	0.09 (0.03–0.38) 36	0.11 (0.04–0.32) 38	0.34 (0.06–0.53) 34	0.26 (0.01–0.48) 41	0.31 (0.03–0.67) 41
% DW	9.2 (7.5–13.6) 37	1.6 (0.5–6.7) 36	1.7 (0.7–5.1) 38	5.3 (1.0–8.3) 34	4.4 (0.2–8.2) 41	5.3 (0.4–11.6) 41
C:N[a]	5.1 (4.9–5.5) 15	5.3 (5.1–5.5) 8	5.7 (5.7–5.8) 4	5.3 (5.0–5.7) 8	6.1 (5.8–6.2) 3	6.2 (5.8–6.2) 3
C:P[b]	105.7	185.5	153.2	162.2	148.0	235.1
N:P[b]	20.2	34.3	26.1	30.1	24.4	39.9

[a] Due to non-normality or use of ratios, these values are listed as median (25th–75th quartile) *number of samples*.
[b] These ratios are only estimates which were calculated using the average values for carbon, nitrogen and phosphorus for each species.

Table 3
Body composition for *Rhincalanus cornutus* from the Gulf of Mexico and *R. nasutus* from the Gulf of California. Copepod prosome lengths (PL) are given in mm. Parameters are listed in terms of percent dry weight (DW) or wet weight (WW). Unless otherwise noted, percentages or weights are reported as the mean ± standard deviation (number of samples).

	R. cornutus	*R. nasutus*
PL (mm)	2.7 ± 0.3 (69)	3.4 ± 0.2 (82)
WW (mg)	0.80 ± 0.12 (10)	1.11 ± 0.13 (11)
DW (mg)	0.12 ± 0.03 (3)	0.15 ± 0.04 (14)
Water (% WW)	80.6 ± 0.6 (3)	85.8 ± 2.5 (11)
Ash (% DW)	ND	15.3 ± 3.8 (11)
Carbon		
% WW	10.53 ± 0.48 (3)	7.39 ± 0.91 (3)
% DW	54.2 ± 2.4 (3)	52.5 ± 2.5 (3)
Nitrogen		
% WW	1.14 ± 0.02 (3)	0.74 ± 0.03 (3)
% DW	5.9 ± 0.1 (3)	5.3 ± 0.3 (3)
Hydrogen		
% WW	2.02 ± 0.06 (3)	1.44 ± 0.21 (3)
% DW	10.4 ± 0.4 (3)	10.2 ± 0.7 (3)
Phosphorus		
% WW	0.078 ± 0.027 (7)	0.043 ± 0.011 (14)
% DW	0.40 ± 0.20 (7)	0.32 ± 0.08 (14)
Protein		
% WW	6.90 ± 0.64 (5)	3.30 ± 0.70 (16)
% DW	35.5 ± 3.3 (5)	23.4 ± 5.0 (16)
Lipid sac mass[a]		
% WW	13.3 (8.8–17.5) 24	10.3 (8.3–14.8) 28
% DW	68.7 (45.4–90.1) 24	72.8 (58.5–104.5) 28
C:N[a]	9.2 (9.0–9.4) 3	10.2 (9.6–10.4) 3
C:P[b]	135.0	171.9
N:P[b]	14.6	17.2

[a] Due to non-normality or use of ratios, these values are listed as median (25th–75th quartile) *number of samples*.
[b] These ratios are only estimates which were calculated using the average values for carbon, nitrogen and phosphorus for each species.

Although adult female *E. inermis* body contents were low based on WW relative to other eucalanoid species, comparing components on the basis of %AFDW puts *E. inermis* within the range of the other species. One interesting trend that emerged with AFDW comparisons was that *E. inermis* actually had intermediate protein levels (Table 1). Thus, *E. inermis* had more muscle mass per unit AFDW than *Rhincalanus* spp. or *P. attenuatus*, although not as much as *S. subtenuis*, suggesting an intermediate activity level. As *E. inermis* has a wide vertical range, muscle (i.e., protein) might be needed for making long migrations (Donnelly et al., 1993). Stable isotopes as well as fatty acid profiles of storage lipids suggested that adult *E. inermis* largely fed near the surface (Cass et al., 2014; Williams, 2013), regardless of their collection depth, suggesting stored energy is likely needed to fuel their metabolism during their ontogenetic migration to deeper depths (Wishner et al.,

Table 4
Lactate dehydrogenase (LDH) activity for eastern tropical North Pacific copepods. LDH activity was measured in units (μmol substrate utilized per min) per g wet weight (WW) or protein. Activity levels are reported as the mean ± standard deviation (number of samples). BD denotes levels of LDH that were below detection. All copepod homogenates were 1:11 dilutions (mg wet weight to μL buffer volume) except *P. attenuatus*, which was a 1:16 dilution.

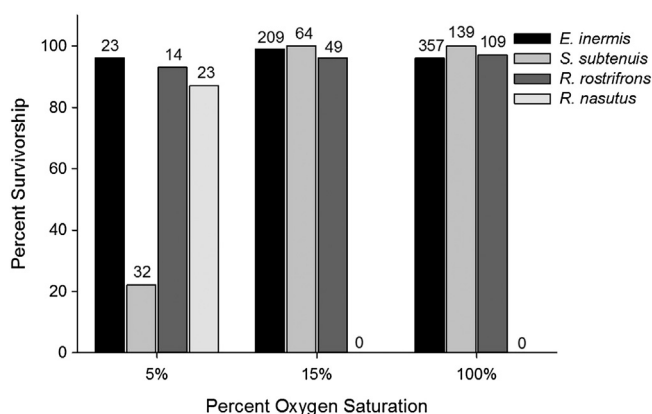| Species	LDH activity (units per g WW)	LDH activity (units per g protein)
E. inermis	2.29 ± 1.31 (8)	139.0 ± 79.4 (8)
Female	1.73 ± 1.00 (6)	108.9 ± 66.6 (6)
Shallow	2.34 ± 1.02 (3)	154.3 ± 66.9 (3)
Deep	1.12 ± 0.38 (3)	63.5 ± 21.3 (3)
Male	3.96 ± 0.29 (2)	229.1 ± 16.9 (2)
P. attenuatus	BD (1)	BD (1)
R. rostrifrons	1.59 (1)	49.3 (1)
R. nasutus	1.43 (1)	36.4 (1)
S. subtenuis	BD (2)	BD (2)

Fig. 4. Percent survivorship of eucalanoid copepods during metabolic incubations. Bars represent the percentage of individuals surviving at the end of an incubation experiment at 10 °C and either 5, 15, or 100% oxygen saturation. Numbers above bars indicate total number of individuals used in experiments. Results are shown for *Eucalanus inermis*, *Subeucalanus subtenuis*, *Rhincalanus rostrifrons*, and *R. nasutus* (respectively, bars from left to right). Note that data for *R. nasutus* are only available for 5% saturation.

2013). *E. inermis* stored primarily triacylglycerols (Cass et al., 2014), and their average storage lipid sac mass was higher per unit WW and DW than those of *S. subtenuis* and *P. attenuatus*, but not nearly as high as *Rhincalanus* spp. These moderate lipid stores would supply them with energy during times of reduced feeding at depth. Relatively low metabolic demand and ability to use alternative metabolic pathways is another strategy that would allow *E. inermis* females to reside in low oxygen waters for periods of time.

Few differences were seen among the three groups of adult *E. inermis* examined in this study. It appeared that males had higher C, N and protein levels than females, possibly indicating greater muscle mass and organic matter. These males might have been more active than females, as they occurred almost exclusively in the upper 100 m of the water column (Fig. 3). Coupled with their smaller size (Table 2), increased mobility would aid in avoiding potential visual predation in the euphotic zone.

LDH levels also appeared to be variable, with males having the highest activities. Similar variations between sexes have been found in other species, including the deep-sea copepod *Gaussia princeps* (Thuesen et al., 1998). As males had about half the mass of females, these activity differences could be due to a difference in size, which has been found to affect enzyme activity rates (Thuesen et al., 1998). It was also possible that males, having higher protein levels, might have higher metabolic rates, which require supplementation of anaerobic pathways in relatively low oxygen conditions encountered below the surface mixed layer. Unfortunately, no metabolic data were available for males to further explore this explanation. Additionally, assays were primarily carried out to establish presence or absence of LDH, so sample numbers were low. Further samples should be run to ascertain firm differences among the groups.

This study also hoped to illuminate differences between deeper and shallower dwelling adult females to learn more about their proposed ontogenetic migration (Wishner et al., 2013). In more temperate and seasonal environments, *E. elongatus* and *E. californicus* were seen to undergo ontogenetic migration and have a dormant period at depth (Kasyi, 2006; Ohman et al., 1998). While dormancy was not expected within the ETNP system, it was unclear whether the deeper *E. inermis* females might represent, for instance, a particular stage in the reproductive cycle. However, no consistent differences were observed during our fall and winter collection periods between all shallow and deeper-dwelling females. Additionally, substantial variation was seen in the shallow females in terms of C, H, and N content based on collection site. This suggested that differences observed in this study were likely the result of natural variability captured during sampling. Additional

comparisons using oxygen consumption rates indicated similar metabolic rates between the two groups, although metabolic ratios suggested increased lipid utilization by the deeper individuals (Cass and Daly, 2014). This was consistent with lipid and stable isotope data, which suggested that all adult *E. inermis* shared a food source, likely near the surface layer (Cass et al., 2014; Williams, 2013). These findings suggest that feeding at depth was rare, and thus lipid stores could be used as an energy supplement when individuals resided there. Overall, data from this study were inconclusive to ascertain if deep and shallow females represented different cohorts or reproductive stages. Further work comparing egg and gonad development in shallow and deep individuals would help to determine if there is vertical separation based on timing in the reproductive cycle.

4.2. Subeucalanus subtenuis

S. subtenuis employed a different strategy than *E. inermis*. *S. subtenuis* primarily occurs in the upper 100 m of the water column, in more oxygenated waters (Chen, 1986; Saltzman and Wishner, 1997a; Sameoto, 1986). Their vertical distribution appeared to be limited due to oxygen levels. *S. subtenuis* did not have detectable levels of LDH, indicating little anaerobic ability to help cope with oxygen-limited conditions. Survivorship studies showed that only 22% of adult females were able to survive 12–15 h at <20 μM oxygen, while survivorship was 100% in more highly oxygenated water (Fig. 4). Thus, vertical distributions would be limited, as oxygen levels of <20 μM occurred as shallow as 50 m in the Tehuantepec Bowl and somewhat deeper in the Costa Rica Dome (Fig. 2). Indeed, *S. subtenuis* abundance decreased to almost zero at depths where oxygen levels were <20 μM during our cruises (Fig. 3).

Of the five species, *S. subtenuis* had the highest protein content. This suggested that they were the most active and, therefore, required a greater muscle mass. The higher activity also was supported by metabolic data (Cass and Daly, 2014), which showed that, across a large temperature range, *S. subtenuis* had the highest metabolic rate (median rates of 7.58, 11.49 and 16.01 nmol $(mg\ WW)^{-1}\ h^{-1}$ at 10, 17 and 23 °C, respectively) compared to *R. rostrifrons* (median rates of 6.06 and 7.01 nmol $(mg\ WW)^{-1}\ h^{-1}$ at 10 and 17 °C, respectively) and *E. inermis* (median rates of 1.71, 2.38 and 5.55 nmol $(mg\ WW)^{-1}\ h^{-1}$ at 10, 17 and 23 °C, respectively). Observations of live *S. subtenuis* also indicated that they were more active than other eucalanoid species observed in this study. As *S. subtenuis* was found within a relatively narrow depth range, vertical migration probably did not occur to any great extent. Instead, activity was within the surface layer, and possibly included predator avoidance and prey capture. Their storage lipid fatty acid profiles were similar to *E. inermis*, suggesting a similar feeding strategy (Cass et al., 2014). However, they did not accumulate as much storage lipid as *E. inermis*, although these two species shared triacylglycerols as the major type of storage lipid (Cass et al., 2014). This suggested one of several possibilities. First, their metabolism may be sufficiently high that little excess lipid was available for storage. Another scenario is that storage lipids were not necessary for this surface-dwelling, active copepod, and therefore they did not prioritize the accumulation of storage lipids. As this species may not experience food limitation or undergo diapause, storage lipids would not be necessary.

4.3. Rhincalanus rostrifrons and R. nasutus

R. rostrifrons and *R. nasutus* together comprised the third group. *R. rostrifrons* is much more abundant than *R. nasutus* in the ETNP, however, they both often show similar vertical distributions (Chen, 1986; Saltzman and Wishner, 1997a; Sameoto, 1986). Many reports specifically placed these species at the edge of the OMZ core, but not within it, and have suggested that these species are limited by oxygen concentrations below 0.5 μM O₂ (Saltzman and Wishner, 1997a; Sameoto, 1986; Vinogradov et al., 1991). This is largely consistent

with our study, during which *Rhincalanus* spp. abundances were highest directly above the OMZ core, in the upper oxycline (Fig. 3). Like *E. inermis*, LDH activity was observed in *Rhincalanus* spp. All LDH values reported for *E. inermis* and *Rhincalanus* spp. are within ranges found for other copepod species (e.g., Geiger et al., 2001; Thuesen et al., 1998). However, based on activity per g protein, LDH activity levels in *Rhincalanus* spp. were only about one-half to one-third the levels found in *E. inermis*. This may point towards a lower anaerobic capacity, explaining their absence from the lowest oxygen regions. However, within taxa, trends have been found with size (Thuesen et al., 1998). The WW of *E. inermis* was approximately 5–10 times higher than WWs of *Rhincalanus* spp., indicating a large size gap. Differences in LDH activity might be due to such a relationship, and not necessarily represent differential adaptation to OMZs. Even though the metabolic rate of *R. rostrifrons* was similar to that of *S. subtenuis* at 10 °C, its tolerance for low oxygen was superior. Survivorship at <20 μM was more than 85% for *Rhincalanus* spp., compared to only 22% for *S. subtenuis*. Their swimming activity level also was much lower generally than *S. subtenuis*, and often little to no motion was observed in *Rhincalanus* spp. during sorting. Protein levels indicated that *S. subtenuis* had approximately twice as much muscle mass as *Rhincalanus* spp., providing further support for observations of large differences in activity levels.

One of the most distinctive features of *Rhincalanus* spp. was the large storage lipid sac that occupied a major portion of its body cavity. The percent of wet mass of storage lipids was more than 10 times that of *E. inermis*, *P. attenuatus* or *S. subtenuis*. *Rhincalanus* spp. also had higher C and H content (the major components of lipids), as well as C:N ratios near 10, due to high lipid and low protein levels. The need for such large amounts of storage lipids in this environment was not clear, and may be due to a genetic predisposition (Cass et al., 2014). *Rhincalanus* spp. globally are known for their extremely high total and storage lipid levels, with total lipid levels between 16 and 69% of dry weight (Kattner et al., 1994; Lee and Hirota, 1973; Morris and Hopkins, 1983; Schnack-Schiel et al., 2008; Sommer et al., 2002). Storage lipids, often present in a large oil sac (Lee et al., 2006), commonly comprised 61–92% of total body lipid in adults (Cass et al., 2011; Graeve et al., 1994; Kattner and Hagen, 1995; Kattner et al., 1994; Lee and Hirota, 1973; Lee et al., 1971; Schnack-Schiel et al., 2008; Sommer et al., 2002).

Additionally, the composition of storage lipids for this group was primarily wax esters, not triacylglycerols as found in *E. inermis* and *S. subtenuis* (Cass et al., 2014). Predominance of one type of storage lipid over another is thought to occur due to a variety of factors. For instance, accumulating wax esters as the primary storage lipid in copepods has long been thought to occur primarily in deep sea (>600 m) and high latitude herbivorous organisms (Lee and Hirota, 1973; Lee et al., 1971), as well as those undergoing diapause (Lee et al., 2006), as those individuals require surplus energy stores for periods of little to no feeding. *Rhincalanus* spp. in the ETNP, however, did not meet those criteria, as there was no evidence of, or necessity for, diapause, and they occurred primarily above 400 m. This is only one of many exceptions to the rules that have been noted in the literature (e.g., Hagen et al., 1993, 1995; Kattner et al., 1981; Ward et al., 1996; Williams and Biesiot, 2004). In the case of the Eucalanidae family, there is likely a large genetic component controlling storage lipid accumulation patterns (Cass et al., 2014). Genetic analyses of 16S rRNA and ITS2 gene loci indicated that *Pareucalanus* and *Rhincalanus* form their own monophyletic group and *Subeucalanus* and *Eucalanus* form another, consistent with the observation of was ester versus triacylglycerol dominance (Cass et al., 2014; Goetze, 2003).

4.4. Pareucalanus attenuatus

P. attenuatus comprised a final group. This was surprising, as the vertical distribution of this copepod was similar to that of *S. subtenuis*. *P. attenuatus* has been found primarily in the upper 100–150 m, with peak abundances from about 30 to 100 m (Chen, 1986; Saltzman and Wishner, 1997a; Sameoto, 1986). During our cruises, adult females were concentrated in the upper 60 m of the water column, largely overlapping with *S. subtenuis* (Fig. 3). *P. attenuatus* also shared several other characteristics with *S. subtenuis*. Both species had high levels of P, and C:P and N:P ratios were similar to each other and to those in *E. inermis*. Also, both *P. attenuatus* and *S. subtenuis* had no detectable LDH activity, indicating little use of anaerobic pathways. These results suggested that *P. attenuatus* also resided in near-surface waters due to an inability to tolerate prolonged exposure to low oxygen. Furthermore, storage lipid mass, C and H contents, and C:N ratios were similar between *P. attenuatus* and *S. subtenuis*. However, *P. attenuatus* primarily accumulated wax ester storage lipids, while *S. subtenuis* accumulated mostly triacylglycerols (Cass et al., 2014). As mentioned above, this difference might be due to genetic factors. Triacylglycerol fatty acid profiles indicated that *S. subtenuis* and *P. attenuatus* (along with *E. inermis*) likely shared a common feeding strategy (Cass et al., 2014).

Another major difference between *S. subtenuis* and *P. attenuatus* was protein content. *P. attenuatus* had protein levels and presumably muscle mass that were similar to those in *Rhincalanus* spp., the least active copepod group. They also had slightly higher water and ash content than *Rhincalanus* spp. and *S. subtenuis*, indicating that less tissue material was present in the body. Thus, this species appeared to occupy an intermediate position between the other three groups in terms of an ecological strategy. Like *S. subtenuis*, *P. attenuatus* remained in near-surface waters to minimize exposure to extremely low oxygen levels. Thus, substantial lipid storage was not necessary, as food levels are relatively consistent throughout the year. Reduced activity levels and lower respiring tissue per WW are strategies that would help this species tolerate lower oxygen levels observed in the upper 50 to 100 m. Unfortunately, *P. attenuatus* was only present in low abundances in our sampling area, limiting the number of measurements. Additional studies of this species would be useful in further elucidating the extent of differences between *P. attenuatus* and the other eucalanoid species of this region.

4.5. Comparison with other eucalanoid copepods

While it was apparent that these four groups of copepods utilized different strategies within the ETNP region, it was difficult to ascertain if such findings were specific to this region or based on more general differences among taxa. In a review on adaptations to low oxygen zones, Childress and Seibel (1998) pointed out that many features of organisms inhabiting low oxygen regions of the water column are adaptive, but not necessarily adaptations specifically for life in such areas. In other words, some organisms are able to exploit low oxygen zones because of previously evolved traits, and not due to adaptations that have evolved specifically in response to oxygen limitation. To determine if patterns observed here were similar to other systems, ETNP copepod traits were compared with traits in conspecifics and congeners from other collection sites within other relatively low-latitude systems (approximately 35°N to 35°S).

Rhincalanus spp. in the ETNP OMZ were characterized by low protein content, high lipid content, and detectable LDH activity, suggesting that these organisms save available energy by decreasing swimming activity (potentially by reducing feeding and relying on storage lipids) and have the ability to use anaerobic pathways in the absence of sufficient oxygen. High amounts of storage lipids appear to characterize all low-latitude members of this group (*R. nasutus*, *R. rostrifrons*, and *R. cornutus*), as reported measurements of storage or total lipids exceed 30% of DW (Flint et al., 1991; Lee et al., 1971; Morris and Hopkins, 1983; Ohman et al., 1998; Schukat et al., 2014). Although useful for a low-energy lifestyle, it does not appear that high lipid content is an adaptation specifically for OMZ *Rhincalanus* spp.

Reported protein content of *Rhincalanus* spp. has been more variable. *R. nasutus* has been studied in regions characterized by severe oxygen

depletion (concentrations <20 μM), including the ETNP, Gulf of California (GOC) (the present study) and California Current (Ohman et al., 1998), as well as in the North Pacific (Omori, 1969) and African coast (Flint et al., 1991), which have higher oxygen concentrations (minimum oxygen levels >40 μM; Paulmier and Ruiz-Pino, 2009). In the low-oxygen regions, protein contents have averaged 25–33% DW, while higher oxygen regions have nitrogen or protein content at least twice as high. For instance, Flint et al. (1991) reported *R. nasutus* off the coast of western Africa to have protein content of 11.3% of WW (although the true values are likely closer to 8% WW (twice as high as ETNP individuals), given that the general equation they used to estimate WW underestimates WWs in *R. nasutus* by approximately 30% (Cass, 2011)). Additionally, Flint et al. (1991) reports that *R. nasutus* from Pacific eastern boundary current regions (low-oxygen zones) have similar body compositions to their coastal African counterparts, although no specific data are reported for those individuals. Although no data exists for *R. rostrifrons* in higher-oxygen environments, several studies have reported on *R. cornutus* in the Gulf of Mexico (this study; Morris and Hopkins, 1983) and off of West Africa (Flint et al., 1991), all regions with higher oxygen levels. Historically, the morphologically similar *R. cornutus* and *R. rostrifrons* have been both classified under the name *R. cornutus* (Lang, 1965); however, recent genetic work supports separation into two closely-related species whose ranges are restricted to different ocean basins (Goetze, 2003, 2010). On a dry weight basis, *R. cornutus* (Table 3) and *R. rostrifrons* (Table 1) appear to have similar protein contents to each other and to previously reported values (Morris and Hopkins, 1983). However, *R. cornutus* has lower water content (Table 3; Morris and Hopkins, 1983) than *R. rostrifrons*, leading to protein per unit WW being approximately twice as high in *R. cornutus* (Flint et al., 1991). In sum, the relationship between oxygen environment and protein content is suggestive that low protein (and hence low activity) may be an adaptation within this genus specifically for survival in low-oxygen environments. However, too few comparisons exist at this time to draw firm conclusions on this relationship and additional data points are needed in both high and low-oxygen regions.

LDH activity has only been reported previously for *R. nasutus*, and the levels were reported as zero (Flint et al., 1991). It was unclear whether individuals for this assay were from the Atlantic or Pacific collection sites, allowing no speculation concerning the relationship between LDH activity and environmental oxygen conditions. However, another enzyme study examining *Calanus pacificus* determined that LDH activity was present, when Flint et al. reported no activity for that species (Thuesen et al., 1998). Thus, it is possible that the Flint et al. (1991) study had an insufficient amount of material or used a less sensitive assay, or that LDH accumulation is variable among locations or seasons, etc. Indeed, we combined many individuals in our assays to ensure that activity would be detected if present. Given the limited studies available, we cannot draw conclusions about the presence of LDH in *R. nasutus* as an adaptation specifically for OMZ habitats.

Features of *E. inermis* which were conducive for habitation in an OMZ included high water content (indicative of a lower weight-specific metabolism), moderate protein levels (which supported necessary but not excess activity), low oxygen consumption rates (Cass and Daly, 2014), and the presence of LDH to indicate utilization of anaerobic pathways when oxygen is limiting. LDH activity has been noted in *E. inermis* residing in other low-oxygen regions of the Pacific (Flint et al., 1991), but not reported for other congeners, allowing no comparisons with higher-oxygen regions. Protein contents of *E. inermis* in the ETNP OMZ (1.6% of WW, 27% of DW) are similar to or slightly higher than reported values for conspecifics in other low-oxygen regions (~1% of WW, Flint et al., 1991), as well as congener *E. californicus* in the California Current (20% of DW, Ohman et al., 1998) and *E. hyalinus* in the eastern Pacific Ocean (~0.9% of WW, Flint et al., 1991). These locations also include low-oxygen habitats (Paulmier and Ruiz-Pino, 2009). However, *E. hyalinus* found in the high-oxygen GOM region (Morris and Hopkins, 1983) did

have slightly higher protein content (2.7% of WW) than that reported for *E. inermis*, and three times higher protein content than *E. hyalinus* values reported by Flint et al. (1991) in low oxygen regions. This further supports the idea that high protein content might be associated with life in higher-oxygen environments for the eucalanoid group.

Previous reports of water content in low-latitude *Eucalanus* spp. from a variety of oxygen environments indicate that values of 92–94% water are normal for this genus (Morris and Hopkins, 1983; Ohman, 1997; Ohman et al., 1998). It is thought that the *Eucalanus* genus comprises a distinct physiological group, characterized by high water and low organic matter content, leading to less actively respiring tissue and allowing a lifestyle more similar to gelatinous plankton (Flint et al., 1991; Ohman et al., 1998). Such characteristics would allow them to exploit some of the benefits of the "lethargic" lifestyle employed by typical gelatinous organisms, including decreased metabolic rate and only slight negative buoyancy. This would be advantageous in low-oxygen conditions by decreasing oxygen demand by the organism, but does not necessarily seem to be an adaptation specifically for such an environment.

P. attenuatus in the ETNP had higher water content than *S. subtenuis* and *Rhincalanus* spp., suggesting that this species, like *E. inermis*, may exploit some benefits of a more jelly-bodied existence. They also show low protein levels, suggesting that a reduction in general activity may assist to further decrease metabolic oxygen demands. No other reports exist for *P. attenuatus* protein or water content, but congener *P. sewelli* from the high-oxygen GOM also shows higher water content when compared to co-occurring *R. cornutus* and *S. monachus* (Morris and Hopkins, 1983). This suggests that higher water content may be a general feature of this genus, although more data are needed to further explore this possibility. However, protein content of *P. sewelli* was much higher (43% of DW, Morris and Hopkins, 1983), more similar to the levels observed in *S. subtenuis* in the ETNP. It was unclear whether this indicates that *P. attenuatus* in general had low protein content, or *P. attenuatus* specifically in the ETNP had low protein content. Regardless, we can conclude that low protein content is not characteristic of the entire *Pareucalanus* genus. Low lipid contents might be conserved across species, as *P. sewelli* still showed low lipid levels despite their occurrence in deeper depths in the GOM.

S. subtenuis appeared to have few to no biochemical or physiological features to allow them to cope with low oxygen, as evidenced by their high mortality at oxygen concentrations below 20 μM. Their only adaptation for OMZ life appeared primarily to be behavioral, with more than 98% of adult individuals observed shallower than 60 m (K. Wishner, personal communication). Such a distribution, which largely eliminates them from the lowest oxygen waters, has been commonly reported for *S. subtenuis* in OMZ regions of the eastern Pacific Ocean (Chen, 1986; Escribano et al., 2009; Saltzman and Wishner, 1997a; Sameoto, 1986). However, this species is commonly concentrated in the epipelagic zone in low as well as high-oxygen environments (e.g., Hopkins et al., 1981; Madhupratap and Haridas, 1990; Padmavati et al., 1998; Teuber et al., 2013; Yin et al., 2006). Low abundances have also been documented in deeper, oxygen-poor waters (Wishner et al., 2008), but such concentrations (several hundred individuals per 1000 m³), are no higher than those observed during our cruises below 100 m for this species. While individuals in the ETNP may be concentrated more shallowly to avoid the OMZ, published distributions (which group the water column into 100–200 m bins) do not allow such detailed comparisons among locations to assess minor differences in vertical structure related to oxygen concentration in the epipelagic zone.

4.6. Conclusions

In conclusion, this study determined that *E. inermis*, *S. subtenuis*, *P. attenuatus*, *R. rostrifrons* and *R. nasutus* formed four groups based on genera that differed in biochemical, physiological, and ecological

characteristics in the ETNP. *R. rostrifrons* and *R. nasutus* were the only two species similar enough to classify together. *E. inermis* was able to tolerate exposure to low oxygen through adopting a physiological strategy more similar to gelatinous plankton than typical copepods. Adult females had high water content and low organic matter, resulting in a decreased metabolic demand. This species also had detectable levels of LDH, suggesting that it is metabolically poised to utilize anaerobic pathways to increase its tolerance for low oxygen conditions. *Rhincalanus* spp. were characterized by particularly high lipid content and low protein levels. LDH was present in moderate levels, leading to tolerance for low oxygen conditions when coupled with low general activity. *S. subtenuis* appeared to avoid the OMZ, as survivorship for this species was much lower than for *E. inermis* or *Rhincalanus* spp. at <20 μM O_2. *S. subtenuis* also had the highest protein and lowest lipid levels of the group. *P. attenuatus* showed a similar vertical distribution to *S. subtenuis*, but had a different lipid accumulation strategy and one of the lowest protein levels, indicating low muscle mass and activity. These factors appeared to separate the two species in this region.

It was difficult to tell whether some of the observed features of these species were adaptations to conditions in the ETNP OMZ system, or if they were simply characteristics of the species or genera that allowed them to exploit the OMZ habitat. *P. attenuatus*, *E. inermis* and *Rhincalanus* spp. in the ETNP generally had lower protein content than congeners or conspecifics residing in higher oxygen environments. Such differences in protein levels (i.e., muscle mass) might be adaptive for low oxygen environments, as lower activity levels (a potential behavioral adaptation) would lead to decreased metabolic demand. However, too few comparisons exist in the literature to draw firm conclusions. Metabolic studies thus far have not recorded large differences in metabolic rates between populations of species that reside in high versus low oxygen environments (Childress and Seibel, 1998). Nevertheless, these studies often measured routine or resting metabolic rates (RMR), in which activity was minimal (Withers, 1992). RMR can differ based on maximal activity rates, as an energetic lifestyle may lead to increased mitochondrial density or size of energetically active organs (Reinhold, 1999). However, active rates can be 5–20 times higher than the standard metabolic rate (measured or extrapolated as the rate for zero activity) (Hochachka and Somero, 2002), so it is still likely that major differences would be most pronounced during activity. An interesting topic for future research would be to explore how different protein levels impact in situ active metabolic rates, which would allow for firmer conclusions relating protein content to behavioral adaptations to low oxygen zones.

Role of the funding source

Acknowledgments

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