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Assessment of storage lipid accumulation patterns in eucalanoid copepods from the eastern tropical Pacific Ocean

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ABSTRACT

Members of the copepod family Eucalanidae are widely distributed throughout the world's oceans and have been noted for their accumulation of storage lipids in high- and low-latitude environments. However, little is known about the lipid composition of eucalanoid copepods in low-latitude environments. The purpose of this study was to examine fatty acid and alcohol profiles in the storage lipids (wax esters and triacylglycerols) of *Eucalanus inermis, Rhincalanus rostrifrons, R. nasutus, Pareucalanus attenuatus,* and *Subeucalanus subtenuis,* collected primarily in the eastern tropical north Pacific near the Tehuantepec Bowl and Costa Rica Dome regions, noted for its oxygen minimum zone, during fall 2007 and winter 2008/2009. Adult copepods and particulate material were collected in the upper 50 m and from 200 to 300 m in the upper oxycline. Lipid profiles of particulate matter were generated to help ascertain information on ecological strategies of these species and on differential accumulation of dietary and modified fatty acids in the wax ester and triacylglycerol storage lipid components of these copepods in relation to their vertical distributions around the oxygen minimum zone. Additional data on phospholipid fatty acid and sterol/fatty alcohol fractions were also generated to obtain a comprehensive lipid data set for each sample. *Rhincalanus* spp. accumulated relatively large amounts of storage lipids (31–80% of dry mass (DM)), while *E. inermis* had moderate amounts (2–9% DM), and *P. attenuatus* and *S. subtenuis* had low quantities of storage lipid (0–1% DM). *E. inermis* and *S. subtenuis* primarily accumulated triacylglycerols (> 90% of storage lipids), while *P. attenuatus* and *Rhincalanus* spp. primarily accumulated wax esters (> 84% of storage lipids). Based on previously generated molecular phylogenies of the Eucalanidae family, these results appear to support genetic predisposition as a major factor explaining why a given species accumulates primarily triacylglycerols or wax esters, and also potentially dictating major fatty acid and alcohol accumulation patterns within the more highly modified wax ester fraction. Comparisons of fatty acid profiles between triacylglycerol and wax ester components in copepods with that in available prey suggested that copepod triacylglycerols were more reflective of dietary fatty acids, while wax esters contained a higher proportion of modified or *de novo* synthesized forms. Sterols and phospholipid fatty acids were similar between species, confirming high levels of regulation within these components. Similarities between triacylglycerol fatty acid profiles of *E. inermis* collected in surface waters and at > 200 m depth indicate little to no feeding during their ontogenetic migration to deeper, low-oxygen waters.

1. Introduction

The two major types of storage lipids found in copepods are wax esters (WEs) and triacylglycerols (TAGs). Generally, copepods residing at high latitudes or below 500 m depth accumulate larger amounts of storage lipids and, of those storage lipids, a greater proportion are WEs (Lee and Hirota, 1973; Lee et al., 1971a). Additionally, copepods that undergo diapause tend to accumulate WEs, which provide energy stores during this period of diminished physical activity and potentially aid in buoyancy (Lee et al., 2006; Pond, 2012). WEs are proposed to be a better long-term storage lipid than TAGs, as they usually are mobilized only after TAG depletion during starvation (Håkanson, 1984; Lee and Barnes, 1975; Lee et al., 1974; Sargent et al., 1977). Two different lipases are likely responsible for mobilization of WEs and TAGs, with TAGs being able to be quickly hydrolyzed for immediate energy needs (Lee et al., 2006). WE catabolism is thought to occur via a hormone-sensitive lipase, perhaps associated with specific life events (preparation for reproduction, diapause, etc.) (Sargent and

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Henderson, 1986). Storage lipids, particularly WEs, appear to be formed from a combination of direct incorporation of dietary fatty acids, incorporation of modified dietary fatty acids, and *de novo* biosynthesis and esterification of fatty acids and alcohols (Graeve et al., 2005; Graeve et al., 1994a; Kattner and Hagen, 1995; Sargent and Falk-Petersen, 1988). As high-latitude copepods generally have the highest total lipid content, as well as the largest proportion of storage lipid, the vast majority of copepod lipid studies have examined high latitude or temperate species. Such information on lipid content can be particularly useful to investigate food sources, diet, and trophic position of the studied copepods (e.g., Brett et al., 2006; Escribano and Pérez, 2010; Falk-Petersen et al., 2002; Graeve et al., 1994b; Pond et al., 1995). To our knowledge, there are only two published papers on copepod lipid profiles in equatorial systems (latitudes lower than 20°) (Cass et al., 2011; Schukat et al., 2014), and only a few papers have examined copepod lipids in detail for latitudes lower than 40° (Escribano and Pérez, 2010; Håkanson, 1984; Lavaniegos and López-Cortés, 1997; Lee and Hirota, 1973; Lee et al., 1971a; Saito and Kotani, 2000; Schnack-Schiel et al., 2008; Sommer et al., 2002).

Members of the copepod family Eucalanidae (genera: *Rhincalanus, Eucalanus, Subeucalanus*, and *Pareucalanus*) occur throughout the world's oceans (Bradford-Grieve et al., 1999; Goetze, 2003; Grice, 1962; Lang, 1965). These species, including those inhabiting latitudes below 40°, often have visible storage lipid sacs (Lee et al., 2006; Lee and Hirota, 1973), with total lipid content of 5–69% of dry weight, of which storage lipids usually comprise $>40\%$ of total lipids (Cass et al., 2011; Flint et al., 1991; Lee, 1974; Lee and Hirota, 1973; Lee et al., 1971a; Morris and Hopkins, 1983; Ohman, 1997; Schnack-Schiel et al., 2008). It is not known why these copepods accumulate such large amounts of lipids, although some eucalanoid species in highly seasonal environments or upwelling systems have been found to undergo diapause or seasonal dormancy (Kasyi, 2006; Ohman et al., 1998; Schnack-Schiel et al., 2008; Schukat et al., 2013). The amount of WEs and TAGs accumulated are variable, and seem to depend on the genus. *Rhincalanus* spp. consistently show predominantly WE accumulation (e.g., Graeve et al., 1994a; Kattner et al., 1994; Ohman, 1988; Sommer et al., 2002), while *Eucalanus* spp. tend towards TAG accumulation (Lee, 1974; Lee and Hirota, 1973; Ohman, 1988; Saito and Kotani, 2000). To our knowledge, no storage lipid patterns have been recorded for *Subeucalanus* or *Pareucalanus* spp., although some of the unknown *Eucalanus* spp. identified in Lee and Hirota (1973) may be members of these genera, as taxonomic revision of the *Eucalanus* genus into *Eucalanus, Subeucalanus,* and *Pareucalanus* genera occurred after that paper was published (Geletin, 1976).

Members of all four genera of Eucalanidae occur in the eastern tropical north Pacific (ETNP), a geographical area within 0–20°N and 80–130°W (Chen, 1986; Saltzman and Wishner, 1997b; Sameoto, 1986; Vinogradov et al., 1991). This region is characterized by a severe oxygen minimum zone (OMZ), with dissolved oxygen concentrations $<4.5\ \mu M$ (Brinton, 1979; Levin et al., 1991; Saltzman and Wishner, 1997a; Sameoto, 1986; Vinogradov et al., 1991). In this region, the core of the OMZ (the area of lowest oxygen levels) can occur anywhere between approximately 300 and 1000 m, with an overall thickness of the OMZ between 200 m and over 1000 m (Fiedler and Talley, 2006). 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 (approximately 40–50 m depth) (Herman, 1989). The ETNP also supports many higher trophic level organisms, including abundant tuna and cetacean populations (summarized in Ballance et al., 2006).

Copepods, including the abundant Eucalanidae family, have varied and distinct vertical distributions in the ETNP, likely related to the oxygen environment (Chen, 1986; Saltzman and Wishner, 1997b; Sameoto, 1986; Vinogradov et al., 1991). *Eucalanus inermis*, a species endemic to the ETNP, is found throughout the water column, but has higher abundances at depths associated with the surface chlorophyll maximum and the upper and lower edges of the OMZ core (Wishner et al., 2013). *E. inermis* likely does not undergo diel vertical migrations (Saltzman and Wishner, 1997b; Sameoto, 1986), but performs ontogenetic migrations to deeper waters as part of their life cycle (Wishner et al., 2013). *Subeucalanus subtenuis*, one of the most abundant copepods in this region (Longhurst, 1985), and *Pareucalanus attenuatus* are usually concentrated in the shallow euphotic zone where oxygen concentrations are highest. *Rhincalanus rostrifrons* and *R. nasutus*, on the other hand, can be found throughout the water column, but are concentrated above and below the OMZ core, from about 200–800 m depth, depending on structure of the OMZ. *R. nasutus* has been documented to display small-scale diel vertical migration (about 30–40 m) (Sameoto, 1986). These differences in vertical structure around the oxygen environment suggest that a variety of ecological strategies occur within this family, which could include distinct life history patterns or different feeding strategies that are reflected within storage lipid profiles.

Given the diversity, high abundance, relatively large size (2–6 mm), variable ecological niches, and storage lipid capacity of eucalanoid copepods of the ETNP, this group represents a unique opportunity to explore lipid composition and its variability in tropical copepods. The purpose of this study was two-fold. First, this work aimed to fill a substantial research gap in copepod lipid composition by providing a comprehensive data set of all lipid fractions in five species of tropical copepods (*E. inermis, S. subtenuis, P. attenuatus, R. nasutus*, and *R. rostrifrons*). Second, storage lipids (TAGs and WEs) were compared between and within species to better understand the controls on storage lipid accumulation patterns, as well as determine general information about ecological strategies of these five species in the ETNP OMZ region. As part of this second aspect of the study, fatty acid profiles of particulate matter at depths of high eucalanoid copepod abundance were analyzed to provide an indication of available food in different regions of the water column.

2. Methods

2.1. Study area

Samples for this project 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*. The cruise transect ran between two major stations: the Tehuantepec Bowl region off of southern Mexico (13°N, 105°W) and the Costa Rica Dome (9°N, 90°W) (Fig. 1). Bottom depths were >3000 m at all stations. Overall, primary productivity and zooplankton biomass vary greatly throughout the region, with the Costa Rica Dome (a wind-driven upwelling area) having 4–6 times higher primary productivity and approximately 2–4 times greater total zooplankton and micronekton biomass compared the Tehuantepec Bowl (a non-upwelling region) (Olson and Daly, 2013; Sameoto, 1986). The strongest upwelling at the Costa Rica Dome normally occurs in the late fall and early winter (Pennington et al., 2006). During our cruises, the OMZ occurred at a much shallower depth and was more vertically expansive at the Tehuantepec Bowl site as compared to the Costa Rica Dome (Fig. 2). At both stations, fluorescence usually peaked in the upper 50 m, with additional peaks between 70 and 100 m depth at the Tehuantepec Bowl site (Fig. 2).

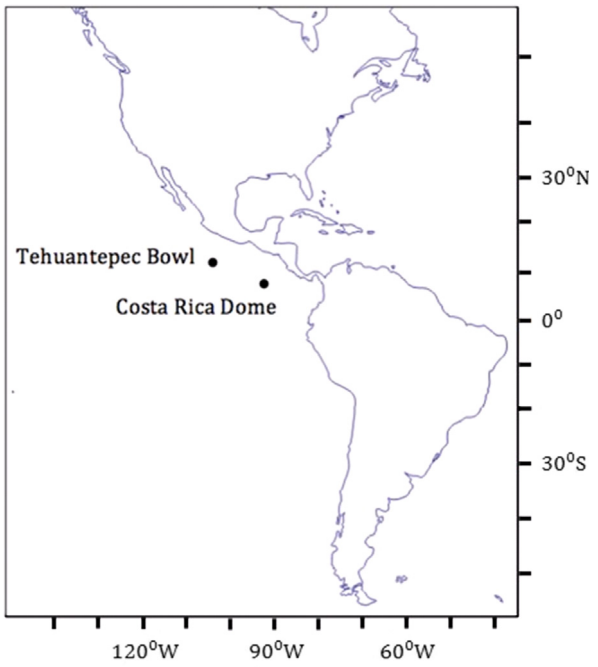

Fig. 1. Map of eastern tropical north Pacific sampling sites. Solid circles represent approximate locations of the two primary sampling sites: the Tehuantepec Bowl (13°N, 105°W) and the Costa Rica Dome (9°N, 90°W).

2.2. Copepod collection and measurement

Copepods included in lipid samples were collected at both the Tehuantepec Bowl and Costa Rica Dome using bongo tows, Tucker trawls, and MOCNESS (Multiple Opening/Closing Net and Environmental Sampling System) (Wiebe et al., 1976) tows in the upper 300 m of the water column. Copepods were collected from their respective depths of maximum abundance as determined by the MOCNESS (data courtesy of K. Wishner). Adult female *Subeucalanus subtenuis* and *Pareucalanus attenuatus* were targeted in the upper 50 m, while *Rhincalanus rostrifrons* and *R. nasustus* were primarily collected in the 200–300 m range (for further information on *Rhincalanus* spp. collection, please see Cass et al. (2011)). *Eucalanus inermis* adult males were collected from the upper 50 m and adult females were collected from both the upper 50 m and 200–300 m depths (designated as shallow and deep individuals, respectively). Due to variations in abundance and spatial distribution between years, adult female *R. nasutus* were only collected in 2007 and adult female *P. attenuatus* were only collected in 2008. During both years, the Costa Rica Dome tows contained a wider diversity of the target species. Therefore, copepods used for these analyses were primarily collected at the Costa Rica Dome station. Exceptions to this include the *S. subtenuis* sample from 2007 and the *E. inermis* male, *P. attenuatus*, and *S. subtenuis* samples from 2008, where copepods collected at both stations were pooled to obtain the number of individuals needed for the lipid sample. *E. inermis* females from the upper 50 m in 2007 were all collected at the Tehuantepec Bowl.

Immediately after capture, copepods were sorted and individuals of each species were separated into small vessels containing 0.2 μm filtered seawater at *in situ* temperature and held for approximately 3–12 h to allow them to empty their guts. All individuals were frozen in cryovials at −80 °C on board the ship and in land-based laboratory facilities. After both cruises, samples were shipped in dry ice between the port and the University of South Florida to ensure appropriately low storage temperatures were maintained.

Prior to lipid extraction, individuals were thawed and quickly measured for total and prosome length (in mm). Length (l), width (w) and height (h) dimensions were also recorded for visible lipid sacs and the volume of each lipid sac was estimated using the equation for an ellipsoid ($V=(4/3)*\pi*(h/2)*(w/2)*(l/2)$). Copepods were then grouped into batches of 30–85 individuals and refrozen at −80 °C until lipid extraction occurred. Wet masses (WMs) (in mg) were estimated using length–weight equations derived from measurements on additional individuals collected on both cruises (Cass, 2011). Dry masses (DMs) were estimated by conversion from WMs based on the average percent water of each species at each location (Cass, 2011). Conversions of lipid sac volume to mass were made based on the density (reviewed in Sargent, 1976) and relative amounts of triacylglycerol or wax ester determined to be accumulated by each species. Data based on individual lipid sac measurements are reported in medians and quartile ranges, as few of these data sets were normally distributed.

2.3. Particulate matter collection

Particulate matter (PM) samples were collected during both cruises at the Costa Rica Dome site. During 2007, water was collected using Niskin bottles on a CTD rosette at the chlorophyll maximum (35 m), and at depths where relatively high abundances of *Eucalanus* and *Rhincalanus* were observed (260 and 325 m). At each collection depth, 3–10 l were pre-filtered through a 200 μm mesh screen to remove large copepods and subsequently filtered through a precombusted GF/F filter. In 2008, PM for lipid analyses was collected using a McLane WTS-LV *in situ* filtration system at 28, 264, and 540 m depth. These depths corresponded to the chlorophyll maximum, upper oxycline, and lower oxycline, respectively. The two deepest depths also coincided with layers of abundant *Eucalanus*. Approximately 2000 L of water was filtered at each depth, using a 53 μm mesh screen prefilter. Lipid samples were analyzed from a subsample of the total lipid extract obtained from particles collected on a double layer GF/F filter array.

2.4. Lipid extraction and analysis

Lipids were extracted by homogenizing copepods or filters in 2:1 dichloromethane (DCM):methanol (MeOH) using a tissue grinder. Liquid was then transferred to a capped centrifuge tube containing a few ml of salt water, and shaken. The DCM layer was removed, more DCM was added to the centrifuge tube, and then the process was repeated several times. A total lipid extract was obtained by drying the extracted DCM with anhydrous sodium sulfate and evaporating the sample using a rotary evaporator setup. The 2008 McLane pump samples were Soxhlet-extracted using 9:1 v/v DCM:MeOH for 8 h. Extracted lipids were then partitioned into DCM and dried using anhydrous sodium sulfate. Lipid samples processed in this study were a 5% split of total lipids extracted for each filter from the McLane system.

Separation of lipid classes was attained with silica columns using 5% deactivated silica gel (Merck silica gel 60, 70–230 mesh; Cass et al., 2011; Wakeham and Volkman, 1991). Five of the resulting fractions were utilized – WEs, TAGs, free fatty alcohols and sterols, free fatty acids (FFAs), and phospholipids (PLs) (listed in order of elution). For this study, the "sterol" fraction contained both steroid alcohols and steroid ketones, which were grouped together. The WE, TAG, FFA, and PL fractions were saponified by heating the sample to 100 °C for two hours with 0.5 N KOH in MeOH. Neutral fractions (fatty alcohols and sterols) were extracted first with hexane. The remaining solution was then acidified ($pH < 2$) and hexane used again to recover the acidic fraction (fatty acids). The fatty acids were converted to fatty acid methyl esters (FAMEs) by addition of diazomethane. The neutral WE fraction and the free fatty alcohol and sterol fraction were

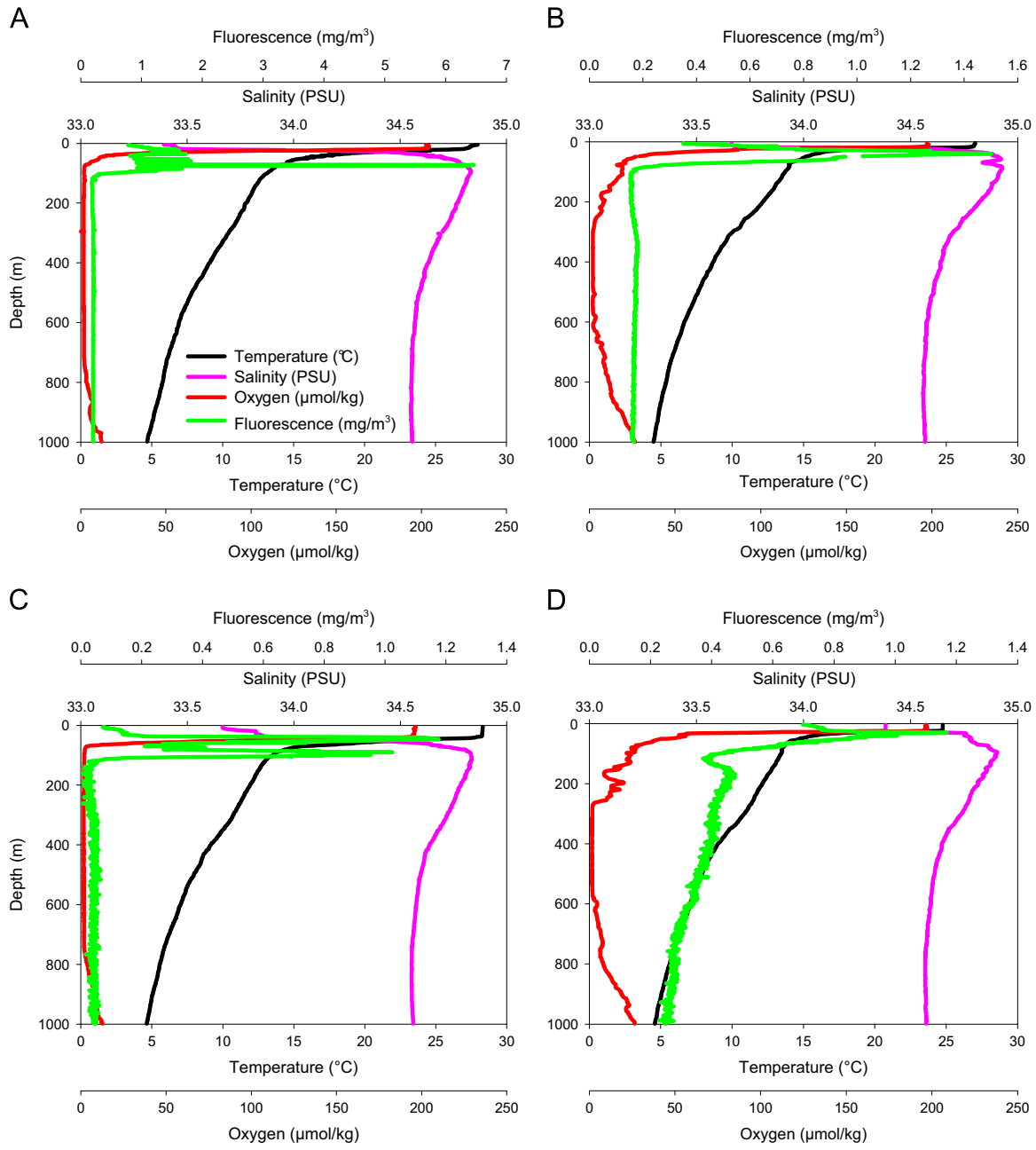

Fig. 2. Water column salinity (pink), oxygen (red), temperature (black), and fluorescence (green) profiles in the upper 1000 m at two stations during the eastern tropical north Pacific cruises. 2007 profiles are depicted for the (A) Tehuantepec Bowl and (B) Costa Rica Dome. 2008–2009 cruise profiles are shown for the (C) Tehuantepec Bowl and (D) Costa Rica Dome. Data were collected with a Sea-Bird *3plus* temperature sensor, Sea-Bird 4C conductivity sensor, Sea-Bird *9plus* digital quartz pressure sensor, Sea-Bird 43 oxygen sensor, C-Point chlorophyll fluorescence sensor (2007), and a Wet Labs ECO-AFL/FL fluorometer (2008/2009).

converted to trimethylsilyl-ethers (TMS-ethers) using BSTFA (N, O-bis(trimethylsilyl)trifluoro-acetamide) and pyridine.

Samples were analyzed on a GC (Agilent 6890 gas chromatograph with an FID detector) or GC/MS (Agilent 6890 gas chromatograph coupled to an Agilent 5793 mass spectrometer). FAME fractions were run on a Restek RTX-WAX column, while TMS ethers were analyzed using a J&W DB-XLB column. Internal standards of methylnonadecanoate for FAMEs and 5-α(H)-cholestane for the TMS ethers were added to each sample prior to injection on the GC. Identification of compounds was accomplished using mass spectra and retention times. Total lipid mass and percent mass of each lipid class or compound were calculated by summing identified lipid compounds in all or relevant fractions.

Detailed fatty acid, sterol and alcohol profiles have been reported previously for *Rhincalanus* spp. analyzed as part of this study (Cass et al., 2011). However, their results are reported again as part of this paper in order to create a full comparison among the different eucalanoid genera present within the ETNP.

2.5. Statistics

Cluster analyses were performed using PRIMER 6 to examine relative similarity between samples. Resemblance matrices for fatty acid, alcohol or sterol profiles of samples were generated using Euclidian distance calculations performed on data seta sets

where each lipid component was represented as % mass of total fatty acids, alcohols or sterols within the lipid fraction of interest.

3. Results

3.1. Copepod storage Lipids

Storage lipid (WE and TAG) accumulation patterns varied among different genera (Table 1). *Eucalanus inermis* and *Subeucalanus subtenuis* accumulated primarily TAGs ($\geq$90% of storage lipid [TAG+WE], 13–75% of total lipid). In contrast, the two *Rhincalanus* species stored almost exclusively WEs ($>$90% of storage lipids, 86–97% of total lipids). *Pareucalanus attenuatus* similarly tended towards WE accumulation (84% of storage lipid, 41% of total lipid), but also biosynthesized a considerable proportion of TAG (16% of storage lipid, 8% of total lipid).

Overall, lipid sacs were smallest in *S. subtenuis* and *P. attenuatus* (median sizes of 0–2 $\times 10^{-3}$ mm^3) and largest in *Rhincalanus* spp. (55–64 $\times 10^{-3}$ mm^3), with *E. inermis* occupying an intermediate range (7–30 $\times 10^{-3}$ mm^3) (Table 1). Similar trends held when volumes were converted to mass and corrected for the weights of each individual, with average storage lipid levels estimated at 0 – 1% of DM for *S. subtenuis* and *P. attenuatus*, 2–9% DM for *E. inermis*, 31%DM for *R. nasutus* and 54–80%DM for *R. rostrifrons*.

Rhincalanus spp. had only small amounts of non-storage lipid, with sterols (0.6–4.7%), FFAs (1.5–2.6%), PLs (0.4–1.8%) and free fatty alcohols (0.1–1.0%) each comprising $<$5% of the total lipid (Table 1). *E. inermis, P. attenuatus,* and *S. subtenuis* had lower total storage lipids, so other non-storage fractions comprised a larger proportion of total lipids. Storage lipids in *E. inermis, P. attenuatus,* and *S. subtenuis* were still the most abundant form of lipids, although lower proportionally than for the other species (13–76%), followed by FFAs (11–54%), sterols (5–49%), PLs (2–30%) and free fatty

Table 1
Lipid classes (mass%) and storage lipid content of copepods. Lipid classes: WE=wax ester; TAG=triacylglycerol; FFAlc=free fatty alcohol; FFA=free fatty acid; PL=phospholipid. Lipid sac volumes (median (25th percentile, 75th percentile), units of "x10^{-3} mm^3") for individuals included in each pooled sample are given. Estimates of storage lipid mass based on sac volume are given as percentage of individual wet mass (WM) and dry mass (DM). Number of individuals pooled for each sample is given as "*N*." All copepods were adult females except where noted.

	Eucalanus inermis						*Pareucalanus attenuatus*	*Rhincalanus rostrifrons*[a]		*Rhincalanus nasutus*[a]	*Subeucalanus subtenuis*	
	Shallow female		Deep female		Male							
	2007	2008	2007	2008	2007	2008	2008	2007	2008	2007	2007	2008
WE	2.1	0.1	0.5	0.4	1.1	0.2	40.9	85.6	96.5	91.6	2.6	1.0
TAG	36.1	66.8	12.6	72.7	75.2	57.7	7.8	5.2	0.5	2.6	22.3	44.3
FFAlc	0.2	0.2	0.6	0.2	0.1	0.4	0.7	1.0	0.1	0.1	1.0	0.4
Sterol	19.7	8.2	48.8	6.2	7.5	5.6	8.9	4.7	0.6	2.4	12.2	9.6
FFA	32.0	18.2	28.0	12.8	11.8	26.4	36.8	2.6	1.8	1.5	53.6	15.0
PL	10.0	6.5	9.5	8.1	4.3	9.7	5.0	0.9	0.4	1.8	8.4	29.7
Sac Vol	30	7	7	18	7	10	2	64	56	55	0	0
	(24,34)	(2,26)	(3,26)	(5,34)	(0,14)	(1,19)	(1,9)	(48,98)	(47,72)	(41,99)	(0,0)	(0,1)
%WM	0.6	0.1	0.1	0.3	0.3	0.3	0.1	9.4	7.5(6.3,9.4)	4.1(2.9,6.4)	0.0	0.0
	(0.5,0.8)	(0.0,0.4)	(0.0,0.3)	(0.1,0.5)	(0.0,0.5)	(0.0,0.7)	(0.0,0.2)	(6.4,12.1)			(0.0,0.0)	(0.0,0.1)
%DM	9.2	1.6	1.7	5.3	4.4	5.3	0.7(0.3,1.8)	80.1	53.8	31.0	0.0	0.0
	(7.5,13.6)	(0.5,6.7)	(0.7,5.1)	(1.0,8.3)	(0.2,8.2)	(0.4,11.6)		(54.6,103.5)	(45.6,67.9)	(21.9,47.8)	(0.0,0.3)	(0.0,0.7)
N	37	36	38	34	41	41	30	46	30	33	80	85

[a] Lipid class proportions as previously reported in Cass et al. (2011).

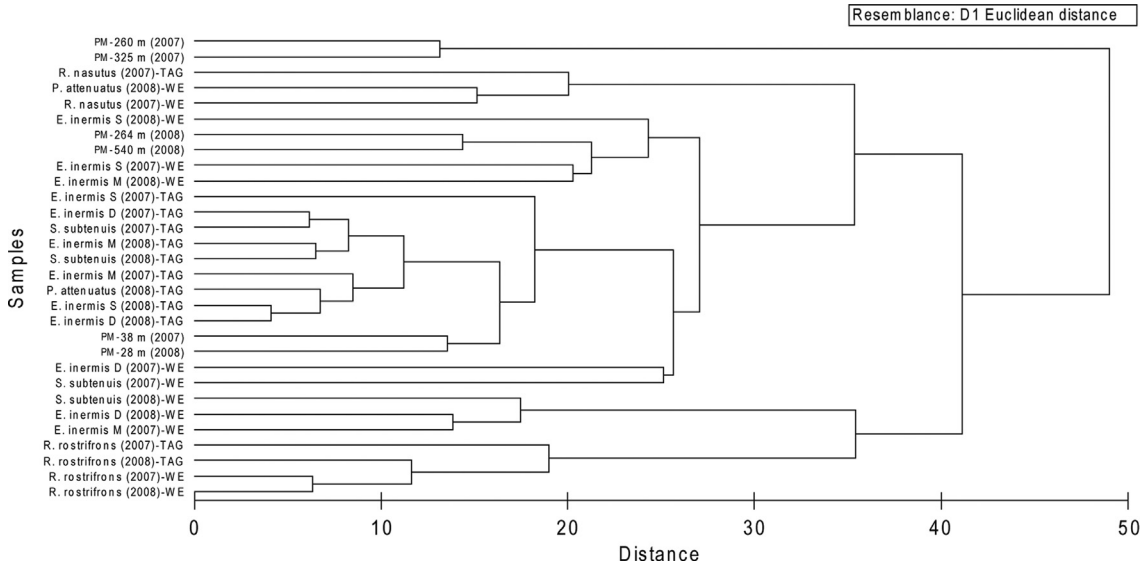

Fig. 3. Cluster analysis comparing total fatty acid profiles for particulate material and storage lipid fatty acid profiles for copepods. Species (*E. inermis, P. attenuatus, R. nasutus, R. rostrifrons, S. subtenuis*) or depth of particulate material (PM) samples and year are noted for each sample. Storage lipid type is denoted as wax ester (WE) or triacylglycerol (TAG). For *Eucalanus inermis*, samples include "M" (adult males), "S" (adult females collected from the upper 50 m of the water column) and "D" (adult females collected between 200 and 300 m).

alcohols (< 1.0%). The amount of each type of lipid class was highly variable within species and years, and no consistent patterns were observed interannually. Generally, samples with lower percentages of storage lipid had higher percentages of FFAs, likely due to autolysis of PL, TAG, and WE fatty acids following the death of animals or during the freeze–thaw cycle during sample processing (Ohman, 1996). However, it is unlikely that such degradation would have preferentially mobilized specific fatty acids within each lipid class and, therefore, the reported fatty acid relative abundances within lipid classes should be representative of initial conditions prior to any degradation (Sasaki and Capuzzo, 1984).

Euclidean distance matrices revealed that WE and TAG fatty acid profiles within single samples were usually distinct from each other (Fig. 3). *E. inermis, S. subtenuis*, and *P. attenuatus* samples had distances > 24 units between the fatty acids of the two lipid classes. *R. nasutus* had a slightly higher similarity (distance of 23 units). *R. rostrifrons* showed the most consistency (distances of 12–19 units) between the lipid fractions. Given these differences, WE and TAG profiles will be discussed separately.

Cluster analyses indicated that TAG lipid profiles fell into three different groups of samples having distances of < 20 units within the groups: *R. nasutus, R. rostrifrons,* and a final group containing all *E. inermis, P. attenuatus*, and *S. subtenuis* samples (Fig. 3). *R. nasutus* was characterized by high 18:1($n-9$) (25%), 16:1($n-7$) (17%), 18:0 (11%), 16:0 (10%), and 20:5($n-3$) (9%) (Table 2). *R. rostrifrons* TAGs were primarily composed of 16:0 (34–35%) and 14:0 (22–34%), with smaller amounts of 18:0 (3–9%), 22:6($n-3$) (4–5%), 20:5($n-3$) (3–5%) and 18:1($n-9$) (2–5%). *E. inermis, P. attenuatus*, and *S. subtenuis* all showed profiles dominated by 16:0 (12–25%), 14:0 (7–19%), 16:1($n-7$) (11–15%), 20:5($n-3$) (10–18%), 18:1($n-9$) (4–14%), and 22:6($n-3$) (2–13%).

The fatty acid profiles of the copepod WE fractions were more diverse. *R. nasutus* and *P. attenuatus* formed one group with a distance of 15 units between samples (Fig. 3). Their WEs primarily were composed of 16:1($n-7$) and 18:1($n-9$) (47–65% combined), with smaller amounts of 20:5($n-3$) (6–9%), 18:2($n-6$) (3–5%) and 22:6($n-3$) (2–6%) (Table 3). The two *R. rostrifrons* samples formed a second group, with a distance of 6 units between samples. This cluster was characterized by high levels of 14:0 and 16:0 (79–82% combined) with some 18:0 (3–5%) and 16:1($n-7$) (2%). The remaining clusters included *E. inermis* and *S. subtenuis*, which accumulated WEs as < 10% of storage lipids. The third group consisted of the *S. subtenuis* and *E. inermis* deep samples from 2008 and the *E. inermis* male sample from 2007 (distances of 13–18 units between samples). The WEs of these samples were primarily 16:0 (49–56%), 18:0 (12–21%), 14:0 (9%), and had lower 18:1($n-9$) (0–4%). The remaining *E. inermis* and *S. subtenuis* samples were loosely related in a group with distances of 20–40 units between samples. Samples in this group had high levels of 16:0 (17–31%) and varying amounts of 18:0 (3–23%), 18:1($n-9$) (0–25%), 22:6($n-3$) (0–25%), and 20:5($n-3$) (0–22%).

3.2. Comparison of particulate matter lipids and copepod storage lipids

Cluster analysis revealed close coupling (< 15 units distance) between three pairs of particulate matter (PM) total fatty acid

Table 2
Triacylglycerol (TAG) profiles for copepods. Values are in percent of total mass. All copepods were adult females except where noted.

	Eucalanus inermis						*Pareucalanus attenuatus*	*Rhincalanus rostrifrons*[a]		*Rhincalanus nasutus*[a]	*Subeucalanus subtenuis*	
	Shallow female		Deep female		Male							
	2007	2008	2007	2008	2007	2008	2008	2007	2008	2007	2007	2008
12:0	n.d.[b]	0.2	0.1	0.1	n.d.	0.4	0.2	0.1	0.2	0.3	n.d.	0.1
14:0	18.7	10.4	13.8	8.9	6.9	12.5	9.4	22.3	34.0	3.2	13.9	15.0
14:1($n-5$)	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.2
15+17 Branched	0.9	2.2	1.7	2.4	1.5	1.5	1.1	0.7	1.3	0.4	0.7	1.7
15:0	0.5	1.4	1.5	1.6	1.0	1.0	1.3	0.8	1.1	0.2	0.9	1.5
16:0	12.4	19.6	22.2	17.9	13.8	24.6	16.4	35.2	33.6	10.4	18.9	22.3
16:1($n-5$)	0.2	n.d.	0.4	n.d.	0.4	n.d.	n.d	0.3	0.3	0.2	0.6	n.d.
16:1($n-7$)	13.6	11.5	14.9	10.8	11.7	14.0	11.9	2.7	2.5	16.6	13.0	11.7
16:2($n-6$)	1.5	1.1	1.7	1.3	1.6	1.6	1.4	0.8	0.7	1.0	1.5	1.4
16:3($n-4$)	1.3	0.5	0.6	0.8	0.6	1.1	0.4	1.4	0.1	1.2	0.8	1.3
16:4($n-1$)	2.1	0.7	1.3	1.0	0.7	1.6	1.4	2.0	2.9	1.2	1.7	1.9
Phytanic Acid	3.1	1.5	1.7	1.5	2.2	1.3	0.7	2.7	1.2	7.0	1.7	0.4
17:0	n.d.	1.5	0.6	1.5	n.d.	0.4	2.0	n.d.	0.4	n.d.	0.6	1.3
17:1	0.2	0.9	0.7	1.2	0.8	0.5	1.5	0.2	0.8	0.3	0.4	1.1
18:0	1.2	2.5	2.2	2.2	2.3	3.6	3.8	9.0	3.0	10.6	1.6	1.5
18:1($n-7$)	2.4	0.4	2.8	0.1	2.5	0.1	0.2	1.2	0.4	1.9	3.0	0.3
18:1($n-9$)	4.3	9.8	5.3	8.5	10.6	11.2	13.9	4.5	2.1	24.7	5.5	9.5
18:2($n-6$)	3.7	2.2	2.9	2.8	3.1	2.3	2.1	1.2	0.4	1.9	3.5	2.5
18:3($n-6$)	0.3	0.9	n.d.	1.1	1.4	0.7	0.6	0.6	n.d.	n.d.	0.5	0.6
18:3($n-3$)	0.4	1.2	1.6	1.5	2.0	1.1	1.0	0.1	n.d.	0.3	0.9	0.9
18:4($n-3$)	1.1	1.6	1.8	2.6	4.7	1.2	1.4	0.7	1.5	0.7	1.3	2.5
20:0	0.3	0.7	0.6	0.8	1.0	0.6	0.4	0.9	0.4	0.3	0.3	0.3
20:1($n-11$)	0.3	0.5	0.4	0.5	0.5	n.d.	1.1	0.2	0.3	1.0	0.4	0.3
20:3($n-6$)	0.7	0.5	0.4	0.5	0.4	n.d.	0.7	0.3	0.5	0.5	0.8	n.d.
20:4($n-6$)	5.5	1.3	0.8	1.2	1.3	1.2	1.3	1.4	0.6	1.9	3.3	1.6
20:4($n-3$)	0.5	0.5	1.2	0.6	0.5	0.7	0.7	0.3	0.7	0.6	1.1	0.6
20:5($n-3$)	17.7	10.5	10.1	13.2	10.6	10.7	10.2	5.1	3.3	8.8	13.8	9.6
22:4($n-6$)	0.6	0.2	n.d.	0.1	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	0.2	n.d.
22:5($n-6$)	0.2	0.6	n.d.	0.5	0.5	n.d.	0.8	n.d.	n.d.	n.d.	0.3	0.4
22:5($n-3$)	1.1	0.9	1.0	1.1	0.9	0.5	0.9	n.d.	1.0	n.d.	1.4	0.6
22:6($n-3$)	2.3	10.0	6.2	10.0	13.3	3.3	8.1	3.5	5.3	4.0	5.7	6.8
24:1	0.4	0.4	n.d.	0.5	0.6	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	0.3
Other:	2.5	3.6	1.8	3.0	2.4	1.9	4.5	1.7	1.5	0.8	1.7	1.8

[a] Converted from molar percentages previously reported in Cass et al. (2011).
[b] n.d. = not detected.

Table 3
Wax ester (WE) fatty acid profiles for copepods. Values are in percent of total mass. All copepods were adult females except where noted.

	Eucalanus inermis						*Pareucalanus attenuatus*	*Rhincalanus rostrifrons*[a]		*Rhincalanus nasutus*[a]	*Subeucalanus subtenuis*	
	Shallow females		Deep females		Male							
	2007	2008	2007	2008	2007	2008	2008	2007	2008	2007	2007	2008
12:0	0.7	6.2	n.d.[b]	3.6	n.d.	3.7	0.1	0.1	0.1	0.1	0.3	3.7
14:0	6.1	8.2	3.6	9.0	9.3	4.7	1.2	39.1	44.4	2.0	5.3	8.8
14:1($n-5$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.3	0.3	0.4	n.d.	n.d.
15+17 Branched	1.5	4.6	1.2	n.d.	n.d.	n.d.	0.7	1.0	1.4	0.1	6.1	n.d.
15:0	1.2	11.1	2.1	6.5	3.4	5.7	0.2	1.2	1.4	n.d.	0.1	3.1
16:0	26.6	30.9	17.0	55.9	53.6	27.4	1.1	40.3	37.9	0.8	23.0	49.2
16:1($n-5$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.2	0.4	0.5	n.d.
16:1($n-7$)	3.6	n.d.	6.7	n.d.	n.d.	3.2	21.7	2.3	2.1	33.0	4.4	7.1
16:2($n-6$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.3	0.5	n.d.	3.5	n.d.	n.d.
16:3($n-4$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.5	n.d.	2.7	n.d.	n.d.
16:4($n-1$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	0.7	0.4	1.7	n.d.	n.d.
Phytanic Acid	n.d.	n.d.	0.6	n.d.	n.d.	n.d.	2.0	2.2	2.7	4.0	n.d.	n.d.
17:0	1.5	6.3	1.7	n.d.	4.5	4.7	n.d.	n.d.	n.d.	n.d.	2.5	6.5
17:1	n.d.	n.d.	n.d.	n.d.	n.d.	2.2	2.1	0.1	0.2	0.3	n.d.	n.d.
18:0	22.8	3.3	5.9	15.6	21.1	16.6	0.5	5.4	3.2	0.6	17.9	12.4
18:1($n-7$)	4.7	n.d.	3.2	n.d.	8.2	n.d.	4.4	0.6	0.5	1.4	1.9	n.d.
18:1($n-9$)	16.8	17.6	9.9	3.8	n.d.	25.0	25.7	1.5	1.2	32.0	n.d.	n.d.
18:2($n-6$)	n.d.	n.d.	n.d.	5.6	n.d.	n.d.	4.9	0.2	0.2	2.6	2.4	n.d.
18:3($n-6$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.6	0.2	n.d.	0.6	8.3	n.d.
18:3($n-3$)	n.d.	n.d.	0.8	n.d.	n.d.	n.d.	1.3	n.d.	n.d.	0.3	1.5	n.d.
18:4($n-3$)	n.d.	n.d.	1.2	n.d.	n.d.	n.d.	1.4	0.2	0.2	0.8	n.d.	n.d.
20:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.5	0.4	n.d.	0.9	n.d.
20:1($n-11$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.1	n.d.	n.d.	0.7	n.d.	n.d.
20:3($n-6$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.0	n.d.	n.d.	0.5	n.d.	n.d.
20:4($n-6$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.6	n.d.	n.d.	1.8	n.d.	n.d.
20:4($n-3$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.1	0.4	n.d.	0.5	n.d.	n.d.
20:5($n-3$)	14.5	10.1	21.6	n.d.	n.d.	n.d.	8.9	1.0	0.7	6.4	7.7	9.3
22:4($n-6$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	n.d.	n.d.	n.d.	n.d.	n.d.
22:5($n-6$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.6	n.d.	n.d.	n.d.	n.d.	n.d.
22:5($n-3$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	n.d.	n.d.	n.d.	n.d.	n.d.
22:6($n-3$)	n.d.	n.d.	24.6	n.d.	n.d.	n.d.	6.1	1.0	1.7	2.0	17.3	n.d.
24:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Other:	0.0	1.8	0.0	0.0	0.0	6.8	2.3	0.6	1.0	0.6	0.0	0.0

[a] Converted from molar percentages previously reported in Cass et al. (2011).
[b] n.d.=not detected.

profiles: the two chlorophyll maxima samples (38 and 28 m in 2007 and 2008, respectively), the two deepest samples in 2007 (260 and 325 m) and the two deepest samples in 2008 (264 and 540 m) (Fig. 3). Major fatty acids for the chlorophyll maxima group included 16:0 (23–30%), 14:0 (13%), 16:1($n-7$) (9–11%), 22:6($n-3$) (7–13%), 18:0 (4–8%), 18:1($n-9$) (4–5%) and 20:5($n-3$) (4%) (Table 4). Deep samples from 2007 had primarily 18:0 (42–53%) and 16:0 (27–33%) fatty acids with smaller amounts of 18:1($n-9$) (5–7%), 16:1($n-7$) (2–3%), 15 and 17 carbon branched fatty acids (2%), 22:6($n-3$) (2%) and 18:1($n-7$) (2%). The 2008 deep samples had a wider range of moderately abundant fatty acids, including 16:0 (24–25%), 18:0 (10–19%), 18:1($n-9$) (6–14%), 16:1($n-7$) (5–8%), 22:1 (5–10%), 22:6($n-3$) (6–7%), 15 and 17 carbon branched (4%), 18:1($n-7$) (3–5%), 20:5($n-3$) (3–4%) and 14:0 (2–5%).

Copepod storage lipids were compared to lipid profiles from the bulk PM samples to ascertain the degree of modification in each storage lipid component as compared to available food. When the total fatty acid profiles for the PM were compared to the TAG fatty acid profiles of the copepods, the two surface samples (28 and 38 m) clustered closely with the *E. inermis*/*P. attenuatus*/*S. subtenuis* group with distances of fewer than 20 units (Fig. 3). WE profiles for *E. inermis* and *S. subtenuis* were less similar to these surface samples (distance values of 2–22 units higher than TAGs when WE and TAGs are compared within a given copepod sample). Species that accumulated primarily WEs (*Rhincalanus* spp. and *P. attenuatus*) had even larger differences between TAG and WE similarities to PM samples. For PM at all depths, TAG fractions were 6–27 units more similar to the PM samples than WE fractions within the same copepod sample.

3.3. Copepod phospholipids

The phospholipids fractions were very similar among the copepods (Table 5), consistent with their role as membrane lipids and relatively independent of the animals' storage lipid composition. Major fatty acids included 22:6($n-3$) (23–46%), 16:0 (18–25%), 18:0 (5–24%), 20:5($n-3$) (5–12%) and 18:1($n-9$) (4–11%). Cluster analyses indicated that phospholipid fractions among copepods were relatively similar (<25 units of distance among samples), but still divided into two groups that had distances among samples of <15 units. *R. rostrifrons* formed its own group, which was slightly higher in 18:0 (18–24% versus 5–11%) and lower in 22:6 ($n-3$) (23–27% versus 25–46%) than the other species.

3.4. Copepod fatty alcohols and sterol fractions

Pareucalanus and the *Rhincalanus* species were the only copepods to accumulate WEs as the primary storage lipid (Table 1) and, thus, the only copepods with significant amounts of fatty alcohols. The other copepod species that did not accumulate WEs as the primary storage lipid had very small amounts of fatty alcohols. Although their fatty alcohol composition is reported here (Table 6), they will not be considered in further discussion due to their very

Table 4
Total fatty acid profiles for particulate samples. Values are in percent of total mass.

Fatty acids	2007			2008		
	38 m	260 m	325 m	28 m	264 m	540 m
12:0	0.4	0.2	0.3	0.1	0.4	0.5
14:0	12.8	0.8	1.5	13.1	5.3	2.0
14:1($n-5$)	1.3	0.2	0.2	0.2	0.2	n.d[a]
15+17 Branched	1.8	1.6	1.9	1.4	3.5	3.9
15:0	1.9	0.7	1.2	1.0	1.5	1.7
16:0	23.2	27.4	33.3	30.6	25.3	23.8
16:1($n-5$)	0.5	0.1	0.2	0.5	1.5	1.3
16:1($n-7$)	10.5	2.3	2.7	8.5	8.0	5.3
16:2($n-6$)	4.7	n.d.	n.d.	0.7	0.4	n.d.
17:0	1.1	1.0	1.2	0.3	0.2	n.d.
17:1	0.4	0.4	0.4	0.7	2.1	3.2
18:0	8.0	53.4	42.0	4.0	9.6	18.8
18:1($n-7$)	0.9	1.9	2.2	4.5	4.0	2.5
18:1($n-9$)	4.1	4.8	7.4	4.9	13.5	5.9
18:2($n-6$)	1.8	0.9	1.1	1.6	1.3	1.6
18:3($n-3$)	1.8	0.1	0.1	0.8	0.6	n.d.
18:4($n-3$)	7.8	n.d.	0.1	2.1	0.8	0.2
20:0	3.1	0.6	0.7	2.9	1.0	1.0
20:1($n-11$)	0.0	0.0	0.2	0.2	1.7	2.0
20:4($n-6$)	n.d.	n.d.	n.d.	0.4	0.6	0.3
20:5($n-3$)	4.0	0.4	0.6	4.0	4.1	3.0
22:0	0.2	0.5	0.2	0.9	1.0	2.7
22:1	1.6	0.3	0.6	0.5	4.8	10.4
22:6($n-3$)	6.7	1.6	1.6	12.9	6.1	7.3
24:0	n.d.	n.d.	n.d.	n.d.	n.d.	1.1
Even C Branched	1.2	0.5	0.5	0.2	1.8	1.9
Other:	0.8	0.4	0.2	2.2	0.7	0.5

[a] n.d.=not detected.

low overall alcohol content and erratic accumulation patterns. Within the *Pareucalanus* and *Rhincalanus* genera, each species showed very different fatty alcohol accumulation patterns, with distances of >55 units between species (Table 6). *R. rostrifrons* accumulated primarily 18:1 (71%), 16:1 (24–25%) and 16:0 (4–5%) fatty alcohols. *R. nasutus* profiles only contained 16:0 (60%), 14:0 (31%) and 18:0 (8%) alcohols, while *P. attenuatus* had a more general accumulation pattern, with 18:0 (29%), 18:1 (27%), 14:0 (18%), 16:1 (15%) and 16:0 (11%) being almost equally abundant.

Sterol profiles among the copepods were highly similar, with cholest-5-en-3β-ol (75–96%) and cholesta-5,22E-dien-3β-ol (3–25%) as the only sterols regularly observed at $>1\%$ of total sterols (Table 7). Cluster analyses indicated that although sterols in all copepods were generally similar (distances of <30 units between all samples), three different groups of copepod samples emerged with distances of <10 units within groups. One group was comprised of *R. rostrifrons* (cholest-5-en-3β-ol content: 75–76%), another included *S. subtenuis* (cholest-5-en-3β-ol content: 94–96%), and the remaining group included *E. inermis*, *R. nasutus*, and *P. attenuatus* (cholest-5-en-3β-ol content: 83–89%).

4. Discussion

4.1. Comparison of ETNP copepods with congeners

Storage lipid fatty acid profiles generated from *Eucalanus inermis*, *Subeucalanus subtenuis*, *Pareucalanus attenuatus* and *Rhincalanus* spp. from the ETNP are largely similar to previous findings from high and low-latitude congeners. Of the group, members of the *Rhincalanus* genus have been the most comprehensively studied globally. Based on the cluster analyses, *R. rostrifrons* and *R. nasutus* FA profiles were distinct from one another, with *R. rostrifrons* primarily accumulating 14:0 and 16:0 fatty acids and *R. nasutus* having storage lipids dominated by 16:1($n-7$) and 18:1($n-9$) fatty acids. *R. nasutus* from all other ocean regions also show the same trends, with 16:1($n-7$) and 18:1($n-9$) fatty acids as major contributors to WEs or total lipids (Cass et al., 2011; Lavaniegos and López-Cortés, 1997; Lee et al., 1971a; Schnack-Schiel et al., 2008; Schukat et al., 2014; Sommer et al., 2002). Furthermore, *R. gigas* from the Southern Ocean have a dominance of 16:1($n-7$) and 18:1($n-9$) fatty acids (Graeve et al., 1994a; Kattner et al., 1994; Kattner and Hagen, 1995), and a specific pathway for WE fatty acid and alcohol biosynthesis has been proposed (Kattner and Hagen, 1995), which *R. nasutus* likely shares (Cass et al., 2011). Although *R. rostrifrons* lipid profiles have not been generated for other ocean regions, congener *R. cornutus* from the Gulf of Mexico also has a dominance of 14:0 and 16:0 fatty acids in storage lipids (Cass et al., 2011).

No previous work exists on *Subeucalanus* spp., but information on the storage or total lipid fatty acid profiles of congeners of *E. inermis* and *P. attenuatus* have been published. *E. hyalinus* from the Benguela upwelling region in the tropical Atlantic showed high abundances of 16:0 (24–35%), 20:5($n-3$) (12–26%), 22:6($n-3$) (6–11%), 16:1($n-7$) (7–9%), 18:1($n-9$) (6–9%) and 18:0 (4–8%) fatty acids in an analysis of their total lipids (Schukat et al., 2014). As TAGs and PLs made approximately equal contributions to these individuals, these results are largely consistent with our results for *E. inermis*, although the abundant 14:0 fatty acid (7–19%) from the *E. inermis* TAG fraction is not as well represented in *E. hyalinus* (3–5%). TAG profiles from *E. bungii* collected near 50°N shows a largely similar pattern to *E. inermis*, with 16:0 (22%), 16:1 (20%), 20:5 (17%), and 18:1 (12%) fatty acids all being major components (Lee, 1974). However, *E. inermis* from our study had substantially higher values for 14:0 (7–19 v. 6%) and 22:6($n-3$) (2–13 v. 1%) than *E. bungii*. It is likely that these differences occurred due to diet, as 14:0 and 22:6(n–3) fatty acids were readily available (7–13% each of totally fatty acids) in shallow-water PM during our study (Table 4). As the fatty acid 22:6(n–3) is often used as a biomarker for dinoflagellates (e.g., Parrish et al., 2000), this particle signature might be reflective of the dominance of dinoflagellates in the micrograzer community in the ETNP (Olson and Daly, 2013). Further, its presence in *E. inermis* is consistent with observed ingestion of dinoflagellates during grazing experiments (Olson and Daly, unpublished data). Total lipid fatty acids for *P. sewelli* from the Gulf of California showed relatively high levels of 16:1 and 18:1 fatty acids (about 12% each) (Lavaniegos and López-Cortés, 1997), suggesting similar accumulation patterns to those observed in our *P. attenuatus* sample.

4.2. Implications of triacylglycerol fatty acid profiles

The similarities between the TAG profiles of the shallow-dwelling eucalanoid copepods (*P. attenuatus S. subtenuis*, and some *E. inermis*) are not surprising given their overlapping vertical ranges (K. Wishner, pers. comm.). As storage lipid composition often reflects dietary preferences (Lee et al., 2006), these similarities suggest similar feeding preferences for these three species. Their resemblances to the overall PM collected near the chlorophyll maximum (Fig. 3) also are indicative of a varied diet that is likely generally reflective of the available prey spectrum. This is consistent with feeding experiments conducted during the same cruises using *E. inermis* females, which documented ingestion of a wide variety of available prey items, including copepod nauplii, diatoms, heterotrophic dinoflagellates, and ciliates (Olson and Daly, unpublished data). Stable isotope data also support general particle feeding by *E. inermis* and *S. subtenuis* in the ETNP (Williams, 2013). However, the close resemblance between TAG fatty acid profiles for *E. inermis* collected shallower than 50 m with those collected deeper than 200 m in the water column was unexpected. There are adequate food sources potentially available

Table 5
Phospholipid fatty acid profiles for copepods. Values are in percent of total mass. All copepods were adult females except where noted.

	Eucalanus inermis						*Pareucalanus attenuatus*	*Rhincalanus rostrifrons*[a]		*Rhincalanus nasutus*[a]	*Subeucalanus subtenuis*	
	Shallow female		Deep female		Male							
	2007	2008	2007	2008	2007	2008	2008	2007	2008	2007	2007	2008
12:0	0.1	0.1	0.1	0.6	n.d[b]	0.3	0.4	0.3	1.0	0.2	0.1	0.1
14:0	2.4	1.0	1.1	1.8	1.0	2.3	2.5	2.4	3.9	1.0	1.7	2.9
14:1($n-5$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15+17 Branched	0.3	1.0	0.8	0.7	0.8	0.8	0.9	0.8	0.3	0.2	0.8	0.8
15:0	0.2	0.4	0.4	0.5	0.5	0.4	0.6	0.5	0.6	0.2	0.4	0.5
16:0	21.9	22.7	24.8	21.1	21.8	23.1	23.0	18.6	25.5	18.1	19.4	20.6
16:1($n-5$)	0.3	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	n.d.	0.1	0.1
16:1($n-7$)	3.0	1.5	1.9	1.4	1.4	1.8	1.7	1.9	1.2	1.7	1.6	1.5
16:2($n-6$)	0.1	0.1	0.1	0.1	n.d.	0.1	0.1	n.d.	n.d.	n.d.	0.1	n.d.
16:3($n-4$)	0.1	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	n.d.	0.1
16:4(n-1)	0.1	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	0.1	n.d.
Phytanic Acid	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	n.d.	0.2	n.d.	n.d.
17:0	1.5	3.0	2.1	2.6	2.3	1.9	3.8	1.0	1.6	0.7	2.1	2.7
17:1	0.1	0.6	0.3	0.6	n.d.	0.3	0.5	n.d.	n.d.	n.d.	0.2	0.5
18:0	8.5	5.1	4.9	5.4	9.5	11.1	10.3	23.6	17.5	11.2	7.2	5.9
18:1($n-7$)	3.7	0.4	4.8	0.3	2.5	0.4	0.5	1.6	1.6	2.0	2.3	3.1
18:1($n-9$)	4.3	6.0	4.1	6.7	4.5	5.0	4.9	9.0	5.2	10.6	2.3	3.9
18:2($n-6$)	1.4	0.7	1.0	0.9	n.d.	0.6	0.5	0.8	0.6	0.7	0.9	1.1
18:3($n-6$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18:3($n-3$)	0.1	0.2	0.3	0.2	n.d.	0.2	0.2	0.2	n.d.	n.d.	0.3	0.4
18:4($n-3$)	0.1	0.1	n.d.	0.2	n.d.	0.2	n.d.	n.d.	n.d.	n.d.	0.3	0.3
20:0	0.1	0.1	n.d.	0.2	n.d.	0.2	0.2	0.4	0.4	n.d.	n.d.	n.d.
20:1($n-11$)	0.3	0.2	0.1	0.2	n.d.	0.5	0.2	0.4	0.5	0.5	0.1	0.4
20:3($n-6$)	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20:4($n-6$)	5.5	1.4	1.5	1.6	1.5	1.3	0.9	3.5	2.1	2.5	2.1	2.9
20:4($n-3$)	n.d.	n.d.	0.3	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	n.d.	0.3
20:5($n-3$)	12.2	8.0	10.2	8.2	10.2	9.5	5.5	6.9	5.5	9.8	8.5	9.8
22:4($n-6$)	1.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.	n.d.	n.d.	n.d.
22:5($n-6$)	2.1	2.6	1.8	2.0	2.0	1.8	2.1	2.2	n.d.	3.3	1.5	1.9
22:5($n-3$)	3.8	1.0	1.2	1.2	n.d.	0.8	0.6	n.d.	n.d.	n.d.	1.3	0.7
22:6($n-3$)	25.1	37.5	37.6	32.7	41.5	32.6	36.1	23.3	26.6	36.9	46.1	38.0
24:1	n.d.	5.4	n.d.	9.3	n.d.	3.2	3.0	1.7	4.1	n.d.	n.d.	1.0
Other:	0.4	0.8	0.5	1.3	0.5	1.0	1.5	0.3	1.6	0.3	0.5	0.6

[a] Converted from molar percentages previously reported in Cass et al. (2011).
[b] n.d.=not detected.

Table 6
Wax ester fatty alcohol profiles for copepods. Values are in percent of total mass. All copepods were adult females except where noted.

	Eucalanus inermis						*Pareucalanus attenuatus*	*Rhincalanus rostrifrons*[a]		*Rhincalanus nasutus*[a]	*Subeucalanus subtenuis*	
	Shallow Female		Deep female		Male							
	2007	2008	2007	2008	2007	2008	2008	2007	2008	2007	2007	2008
12:0	43.0	1.6	n.d[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14:0	24.6	7.8	n.d.	44.4	n.d.	n.d.	18.1	0.1	n.d.	31.0	20.8	n.d.
15:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.9	n.d.	n.d.
16:0	n.d.	31.8	n.d.	12.8	94.7	0.8	11.3	3.6	4.6	60.3	58.0	19.6
16:1	n.d.	n.d.	n.d.	n.d.	2.4	n.d.	15.2	24.9	24.5	n.d.	n.d.	n.d.
18:0	32.4	15.5	100.0	11.7	2.9	100.0	28.8	0.4	n.d.	7.7	21.2	23.9
18:1	n.d.	43.3	n.d.	31.1	n.d.	n.d.	26.6	71.1	71.0	n.d.	n.d.	56.5

[a] Converted from molar percentages previously reported in Cass et al. (2011).
[b] n.d.=not detected.

in deeper water in this region. Increased POC concentrations were observed at our sampling locations at depths near the lower OMZ edge (S. G. Wakeham, unpublished data) and similar increases have been observed previously in this region at depths near the lower oxycline (Wishner et al., 1995). It is currently thought that high abundances of *E. inermis* below the surface layer (deeper than 200 m) represent an ontogenetic migration, although the occurrence of such a migration may vary temporally and spatially (Wishner et al., 2013). However, adult *E. inermis* females are found simultaneously in both deep and shallow regions (Wishner et al., 2013), making uncertain the role of the migration or cues associated with its start and/or termination. Also, a range of life stages of *E. inermis* are found in the shallow waters (Wishner et al., 2013), indicating non-synchronous reproduction by adults. Given the major differences between shallow and deeper-water PM (Table 4), it seems unlikely that *E. inermis* actively feeds at depth, as their TAG profiles would then reflect a different feeding history than their shallow-water conspecifics. The high similarities in TAG fatty acids between individuals collected at the two depths support a common feeding history (Fig. 3). Comparisons between stable isotope values for deep and shallow dwelling *E. inermis* individuals show no statistical difference (Williams, 2013),

Table 7
Sterol profiles for copepods. Values are in percent of total mass. Sterol notation is described in Table 8. All copepods were adult females except where noted.

	Eucalanus inermis						*Pareucalanus attenuatus*	*Rhincalanus rostrifrons*[a]		*Rhincalanus nasutus*[a]	*Subeucalanus subtenuis*	
	Shallow female		Deep female		Male							
	2007	2008	2007	2008	2007	2008	2008	2007	2008	2007	2007	2008
$C_{27}\Delta^{5,22}$	17.5	14.5	12.3	15.7	12.6	13.5	13.6	23.3	24.6	9.5	2.8	6.1
$C_{27}\Delta^{22}$	n.d[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.6	n.d.
$C_{27}\Delta^{5}$	82.5	85.1	86.8	83.9	87.4	86.5	86.4	75.7	75.4	89.2	95.6	93.9
$C_{27}\Delta^{0}$	n.d.	n.d.	0.9	n.d.	n.d.	n.d.	n.d.	1.0	n.d.	1.3	n.d.	n.d.
$C_{29}\Delta^{5}$	n.d.	0.5	n.d.	0.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Converted from molar percentages previously reported in Cass et al. (2011).
[b] n.d.=not detected.

Table 8
Shorthand, full, and common names of sterol compounds.

Sterol shorthand	Full compound name	Common compound name
$C_{27}\Delta^{5,22}$	Cholesta-5,22E-dien-3β-ol	22-Dehydrocholesterol
$C_{27}\Delta^{22}$	5α(H)-cholest-22E-dien-3β-ol	22-Dehydrocholestanol
$C_{27}\Delta^{5}$	Cholest-5-en-3β-ol	Cholesterol
$C_{27}\Delta^{0}$	5α(H)-cholestan-3β-ol	Cholestanol
$C_{29}\Delta^{5}$	24-Ethylcholest-5-en-3β-ol	Sitosterol

providing further support for a common food source for individuals found at all depths. Overall, this suggests feeding near the surface waters prior to descent for an ontogenetic migration, or, the presence of a non-synchronous diel vertical migration of a subset of the population between the surface and deeper depths with feeding occurring primarily in surface waters. *E. inermis* individuals have high water content and low weight-specific metabolic rates compared to many other crustacean plankton (Cass, 2011; Flint et al., 1991), allowing them the ability to reside in areas with lower environmental oxygen concentrations. Seeking refuge in such an area may provide these copepods with protection from predators that cannot cope with the OMZ.

4.3. Wax ester versus triacylglycerol accumulation

Generally consistent with previous work on congeners, *Rhincalanus* spp. accumulated high amounts of storage lipids (>31% dry mass (DM)) which were dominated by WEs while *E. inermis* had moderate amounts of storage lipids (2–9% DM) with TAGs as the dominant type (Table 1) (Cass et al., 2011; Flint et al., 1991; Lee, 1974; Lee and Hirota, 1973; Lee et al., 1971a; Morris and Hopkins, 1983; Ohman, 1988, 1997; Saito and Kotani, 2000; Schnack-Schiel et al., 2008; Schukat et al., 2014). *P. attenuatus* and *S. subtenuis* (whose storage lipid types had not been previously reported) showed the lowest amounts of storage lipid accumulation (0−1% DM), but divergent patterns of storage lipid type (WE for *P. attenuatus*, TAG for *S. subtenuis*). The results from *P. attenuatus* are inconsistent with general trends, which show that lipid-poor zooplankton (particularly those in tropical regions) have storage lipids dominated by TAGs (Lee and Hirota, 1973; Lee et al., 1971a). Although there is the possibility that our limited temporal sampling caught this species at a time when it had recently expended its normally more expansive lipid stores, several factors make this seem unlikely. Previous reports of the vertical distribution of *P. attenuatus* in this region have found this species to be primarily concentrated in the upper 100 m of the water column (Chen, 1986; Saltzman and Wishner, 1997b; Sameoto, 1986). When the upper 1000 m of the water column was surveyed during our cruise in 2008/2009, only 3% of adult female *Pareucalanus* spp. were found at depths below 100 m at the Costa Rica Dome, and no individuals were found below 100 m at the Tehuantepec Bowl site (unpublished data courtesy of K. F. Wishner). As *P. attenuatus* is often the only species in the *Pareucalanus* genus to be found in our sampling area of the ETNP (e.g., Grice, 1962; Saltzman and Wishner, 1997b; Sameoto, 1986), these data are likely to reflect the distribution of *P. attenuatus* as a single species. This general distribution does not indicate support for a life history which includes a dormant period at depth requiring large lipid stores. Additionally, multiple life stages have been found simultaneously during previous cruises (Chen, 1986) as well as our own work (unpublished data, K. F. Wishner), suggesting that reproduction is ongoing and females present are likely in a variety of states with regards to their reproductive status. Thus, there does not appear to be a reason to expect normally large lipid stores based on their life history or to conclude that our snapshot included only females that had recently expended lipids in egg production (Jónasdóttir et al., 2008; Richardson et al., 1999). Therefore, we further explore possible explanations as to why a tropical, shallow-dwelling copepod with low lipid stores would preferentially accumulate WEs.

Accumulating WEs as the primary storage lipid in copepods has long been thought to occur primarily in deep sea and high latitude herbivorous organisms (Lee and Hirota, 1973; Lee et al., 1971a), as prey are often patchy spatially and temporally. Copepods undergoing diapause also generally accumulate WEs (Lee et al., 2006), as those individuals require energy stores during periods of little to no feeding. However, numerous exceptions to these rules have been reported. Several marine and freshwater copepod species which have dormant stages accumulate primarily TAGs (summarized in Williams and Biesiot, 2004). Additionally, some species of temperate and high latitude copepods (e.g., *Temora longicornis, Acartia clausi, Centropages hamatus, Calanus propinquus, C. simillimus*, and *Euchirella rostromagna*) have been found to accumulate mainly TAGs (Hagen et al., 1993; Hagen et al., 1995; Kattner et al., 1981; Ward et al., 1996). This usually is explained through differing life history strategies, suggesting that these copepods continue feeding to some degree throughout the winter (usually omnivorous or carnivorous feeding) and, therefore, do not store WEs (Graeve et al., 1994a; Hagen et al., 1993). Omnivorous or carnivorous copepods, however, have been found to accumulate WEs and do not show evidence of seasonal dormancy (Albers et al., 1996; Hagen et al., 1995; Kattner et al., 2003). These species often accumulate shorter chain fatty acids and alcohols, which leads to lower energy WE formation more typical of non-diapausing copepods (Kattner et al., 2003).

Given most previous conclusions for WE versus TAG accumulation, there should not be any reason *a priori* for copepods residing in the upper 300 m of the ETNP (*Pareucalanus* or *Rhincalanus* spp.)

to accumulate WEs. WE accumulation does not fit the geographical trends, and, although *R. nasutus* from other regions are thought to undergo seasonal dormancy (Schnack-Schiel et al., 2008; Schukat et al., 2013), there has been no evidence of diapausing copepods in this area. Chlorophyll levels at the Costa Rica Dome site are relatively high (0.25–1.0 mg/m^3 throughout the year in the sampling region) when compared to the surrounding oligotrophic environment (<0.1 mg/m^3 on average), and primary productivity is estimated to be five-fold higher (Pennington et al., 2006). This does not support inadequate or seasonal food supply as an underlying cause for WE accumulation. The likeliest explanation has to do with genetic predisposition or taxonomic influences, as previously noted by several authors (Hagen et al., 1995; Lee et al., 1972; Williams and Biesiot, 2004). However, as information about lipids has largely been limited to high latitude areas, it has been difficult to reach firm conclusions about taxonomic influences, as often only a few species were examined within a given group due to low diversity at higher latitudes. *Rhincalanus* has been the only genus in Eucalanidae to consistently show preferential WE accumulation (Graeve et al., 1994a; Kattner et al., 1994; Kattner and Hagen, 1995; Lee and Hirota, 1973; Lee et al., 1971a; Ohman, 1988; Schnack-Schiel et al., 2008; Schukat et al., 2014; Sommer et al., 2002). E*ucalanus* spp. identified to the species level from tropical, subtropical, and temperate environments have shown primarily TAG accumulation (Lee, 1974; Lee and Hirota, 1973; Ohman, 1988, 1997; Ohman et al., 1998; Saito and Kotani, 2000; Schukat et al., 2014). No information on lipid classes for *Subeucalanus* and *Pareucalanus* spp. has been previously reported for comparison. Data from this study suggest that WE accumulation is a dominant strategy in the *Rhincalanus* spp., with *P. attenuatus* also showing WE preference. *E. inermis* and *S. subtenuis* are more similar and primarily store TAG, as suggested by previous *Eucalanus* findings. These results are consistent with recent genetic analyses of 16 S rRNA and ITS2 gene loci, in which *Pareucalanus* and *Rhincalanus* form one monophyletic group and *Subeucalanus* and *Eucalanus* form another group (Goetze, 2003). The match between phylogeny and primary storage lipid type is consistent with genetic predisposition playing a strong role in dictating storage lipid accumulation strategies for this family.

In addition to the unexpected result of WE accumulation in *P. attenuatus*, the difference between lipid profiles in the TAG and WE fractions of this species is particularly noteworthy (Fig. 4). Of the copepods that primarily accumulated WEs, *P. attenuatus* had the greatest difference between their TAG and WE fatty acid profiles, as these fractions were part of two distinct clusters (Fig. 3). Generally, the other WE-dominated species had WE and TAG profiles that were more similar to each other than to any of the other samples, likely due to the large amount of *de novo* synthesized fatty acids incorporated into both components of their storage lipids (Cass et al., 2011). In the literature, differences between the lipid profiles of WEs and TAGs within the same samples are largely unexplored. Many studies examining lipid composition determined the relative amount of each storage lipid class and then total fatty acid and alcohol profiles, making it difficult to illuminate differences between storage lipids and examine the functional implications. Previous reports on both TAG and WE lipid profiles for copepods are rare (Albers et al., 1996; Lee, 1974; Lee et al., 2006; Lee et al., 1971a), and to our knowledge have not included information on fatty acids available in local PM. Thus, this study provides a unique venue to further explore the sources of fatty acids in storage lipid components.

The primary differences between TAG and WE fractions are mostly believed to be due to TAGs being more reflective of recent feeding, while WEs represent longer-term diet (Lee et al., 2006). Additionally, there is evidence that WEs contain a larger amount of modified or *de novo* fatty acids, while TAGs contain fatty acids

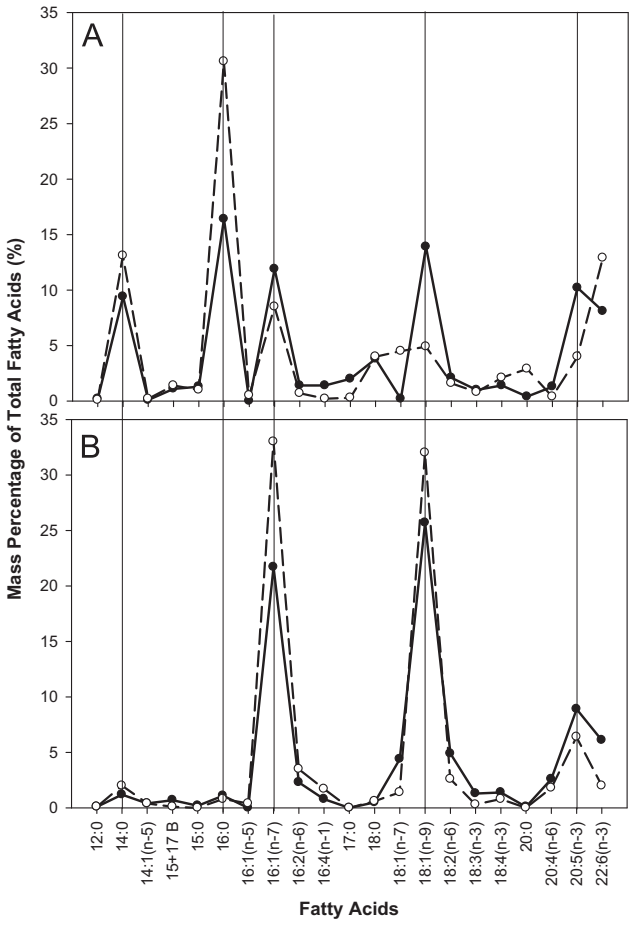

Fig. 4. A comparison of (A) triacylglycerol (TAG) and (B) wax ester (WE) fatty acid profiles of *Pareucalanus attenuatus*. The proportion of each fatty acid is noted by a circle and lines are used to connect circles solely to emphasize trends. *P. attenuatus* samples are depicted by the black circles and solid lines, while open circles and dashed lines in (A) represent total lipids from particulate matter collected in 2008 at 28 m depth and (B) 2007 *Rhincalanus nasutus* WEs.

incorporated more directly from the diet (Albers et al., 1996; Lee et al., 2006; Sargent et al., 1981). Although we cannot assess present versus long-term diet in this study, our results do provide further support that TAGs more directly reflect recent diet in copepods, while WE contain a greater proportion of modified fatty acids. Differences between TAG and WE fractions are most clearly illustrated by *P. attenuatus* lipid composition (Figs. 3 and 4). TAGs consisted almost entirely of recent dietary fatty acids (as indicated by a close coupling with shallow PM, Fig. 4(A)), while many of the fatty acids of WEs were likely modified from the diet or synthesized *de novo*. Given the high similarity between *P. attenuatus* and *R. nasutus* WE fatty acid profiles (Fig. 4(B)), it is likely that *P. attenutatus* utilizes a synthesis pathway similar to the one employed by *R. nasutus* and *R. gigas* (Cass et al., 2011). The presence of a set synthesis pathway for *P. attenuatus* (as well as other *Pareucalanus* spp.) is also supported by their lipid profile similarity to congener *P. sewelli* (Lavaniegos and López-Cortés, 1997). Due to the lack of substantial WE accumulation in *E. inermis* and *S. subtenuis*, it is possible that they do not have an active fatty acid or alcohol synthesis pathway, explaining the erratic accumulation patterns observed in their wax ester fractions (Tables 3 and 6). Polyunsaturated fatty acid (PUFA) content of *Pareucalanus* and *Rhincalanus* spp. also support TAGs reflecting recent feeding, as the percentages of 20:5($n-3$) and 22:6($n-3$) were consistently higher in TAG lipids than WEs. As PUFA can only be attained from

food sources (Brett and Müller-Navarra, 1997), this suggests that a higher proportion of unmodified dietary fatty acids occur in TAGs. Similarly, for all copepod species, TAG profiles were more similar than WE profiles to available PM, suggesting a larger dietary component within the TAG fraction.

4.4. Non-storage lipid fractions

Sterol profiles were relatively similar between these five different copepod species. Their profiles were dominated by cholest-5-en-3β-ol and cholesta-5,22E-dien-3β-ol, suggesting that sterol composition is highly regulated. This likely is due to cholest-5-en-3β-ol having many important functions in cellular membranes, including stabilizing membrane structure, affecting membrane permeability and altering the activity of membrane proteins (Crockett, 1998). Such specific sterol compositions are probably attained through preferential retention of dietary cholest-5-en-3β-ol and other dietary phytosterols (e.g., 24-methylcholesta-5,22E-dien-3β-ol and 24-methylenecholesterol) that can be easily dealkylated to cholest-5-en-3β-ol, and subsequent conversion of assimilated sterols to needed forms (Goad, 1978; Harvey et al., 1989; Teshima, 1971). One major difference between the sterol profiles reported in this study versus previous work is the absence of cholesta-5,24-dien-3β-ol (desmosterol), which is often the second most abundant sterol found in zooplankton (Harvey et al., 1987; Mühlebach et al., 1999; Serrazanetti et al., 1992; Serrazanetti et al., 1994). Cholesta-5,24-dien-3β-ol is usually thought to occur because it is an intermediate in the conversion of dietary phytosterols to cholest-5-en-3β-ol (Goad, 1978). These studies have all occurred at temperate or polar latitudes, where seasonal phytoplankton blooms contribute to available food. Microplankton counts at our study site indicated that heterotrophic organisms were major components of available prey (Olson and Daly, 2013). Therefore, heterotrophic prey were likely to be common in the diet, resulting in lower amounts of phytosterols for conversion. It is also possible that these copepods have a more efficient or rapid conversion of ingested phytosterols to cholest-5-en-3β-ol, such that cholesta-5,24-dien-3β-ol was not able to accumulate in the body.

Like sterols, phospholipid profiles are generally highly regulated, as fatty acid composition is an important factor in membrane function. The copepods in this study illustrated this point well, with phospholipid FA profiles having <25 units of distance among all copepod samples. The major FAs found in these copepods (22:6($n-3$), 16:0, 18:0, 20:5($n-3$) and 18:1($n-9$)) have also been observed in other eucalanoid and general copepod phospholipid profiles (e.g., Albers et al., 1996; Lee, 1974; Lee et al., 1971a; Scott et al., 2002). Minor divergences in the amounts of dominant fatty acids may results from variable environmental conditions, including differences in habitat temperature (Pruitt, 1990).

Fatty alcohol profiles appeared to be largely species-specific, with *R. rostrifrons, R. nasutus* and *P. attenuatus* each having their own distinctive alcohol signature. Fatty alcohols of WEs are believed to be synthesized *de novo* (Sargent et al., 1981), indicating that such differences might be expected. Fatty alcohol composition has not been previously determined for *P. attenuatus*. Observed profiles for *R. nasutus* are nearly identical to previous findings (Lee et al., 1971a; Schnack-Schiel et al., 2008; Schukat et al., 2014; Sommer et al., 2002), and also similar to those seen in *R. gigas* (Graeve et al., 1994a; Kattner et al., 1994; Kattner and Hagen, 1995). In addition, *R. cornutus* individuals from the Gulf of Mexico share a fatty alcohol signature with *R. rostrifrons* reported in this study (Cass et al., 2011). This strongly suggests a genetic component in fatty alcohol accumulation patterns for those copepods that store primarily WE.

Free fatty acid (FFA) classes (2–54% total lipid) in this study were in some cases higher, on average, than expected. FFAs are generally a minor component in zooplankton, often not even reported separately. These lipids may comprise <3–4% of the total lipids (Lee et al., 1971b; Ohman, 1996; Sargent and Falk-Petersen, 1988; Schnack-Schiel et al., 2008), although there have been reports of FFAs comprising >30% of total lipids (Falk-Petersen et al., 1982). Some of the increase in FFAs may have been due to a higher FFA pool within the copepod body itself for use in anabolic and catabolic pathways (Falk-Petersen et al., 1982). FFAs were particularly high in species which spent substantial amounts of their time near the surface (*E. inermis, P. attenuatus, S. subtenuis*), where they were exposed to warmer temperatures (15–28 °C; Olson and Daly, 2013) and, consequently, metabolic rates were likely quite high (e.g., Ikeda et al., 2001). In addition, some portion of the high FFA levels may be attributed to the length measuring step where copepods were briefly thawed, and it is likely that lipases became active and broke down other lipid components (particularly phospholipids) into FFAs (Ohman, 1996; Sasaki and Capuzzo, 1984). However, such post-death degradation should not have preferentially mobilized particular fatty acids within phospholipid or other lipid fractions and, therefore, the reported fatty acid relative abundances within each lipid class should be representative of initial conditions prior to any degradation (Sasaki and Capuzzo, 1984).

5. Conclusions

Storage lipid fatty acid profiles generated for *Rhincalanus* spp., *P. attenuatus*, and *E. inermis* were consistent with previous findings of storage or total lipid fatty acids for congeners in low as well as high-latitude environments. Storage lipid type and total amount varied greatly between these species, with *Rhincalanus* spp. accumulating very large amounts of storage lipids that were primarily WEs, *E. inermis* accumulating moderate amounts of TAG-dominated storage lipids, and *P. attenuatus* and *S. subtenuis* accumulating primarily WEs and TAGs, respectively, but in low quantities. The patterns of WE versus TAG accumulation for storage lipids are best explained by genetic predisposition, as the split between the two groups follows current molecular phylogenies for the Eucalanidae family, and other commonly utilized life history-related factors do not adequately explain the observed differences. A genetic component is also likely involved in dictating the types of fatty acids and alcohols synthesized by the WE-dominated species for incorporation into their storage lipids.

Comparison of fatty acids observed between TAG and WE fractions for the same species support that TAGs contain more fatty acids directly incorporated from recent feeding, while WEs contain a greater proportion of modified or newly synthesized fatty acids. The similar fatty acid profiles for *P. attenuatus*, *S. subtenuis* and *E. inermis* TAGs suggest a similar feeding strategy is utilized by all three species which incorporates a broad range of prey. Additionally, high levels of similarity between TAG profiles of *E. inermis* adult females gathered in shallow and deep water indicate that this species likely feeds primarily at the surface and may not actively feed during its migration to the deeper waters of the ETNP.

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