

# Trophic ecology of zooplankton at a frontal transition zone: fatty acid signatures at the subtropical convergence, Southern Ocean

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Received June 1, 2010; accepted in principle September 1, 2010; accepted for publication September 7, 2010

Corresponding editor: Mark J. Gibbons

Regional variations in fatty acid composition were assessed in zooplankton communities situated immediately north and south of the subtropical convergence (STC), where warm (maximum 21°C) nutrient-poor waters of the southwest Indian Ocean converge with cool (minimum 11°C) nutrient-rich subantarctic waters of the Southern Ocean. I hypothesized that food web structures differ from north to south based on average spatial differences in primary productivity, and that zooplankton in the more productive region show enhanced herbivorous feeding. Several taxonomic groups including euphausiids showed differences in their fatty acid signatures from north to south, indicating the existence of measurable within- and among-species differences in trophic relationships between the two communities, presumably stemming from variations in food quality and availability. The indices 22:6 $\omega$ 3/20:5 $\omega$ 3, 18:1 $\omega$ 9/18:1 $\omega$ 7 and  $\Sigma\omega$ 3/ $\Sigma\omega$ 6 indicated that carnivory was the dominant feeding mode in the north, whereas herbivory was more prevalent in the south, a pattern that was not detected in the same region using stable isotope ratios. Such conflicting results substantiate the importance of utilizing more than one method to investigate feeding relationships in aquatic systems. Fatty acid profiles of the amphipod *Themisto gaudichaudii* revealed some distinct herbivory markers, indicating more complex feeding habits by this important pelagic species than previously reported. My findings shed new light on the regional variations in zooplankton diet and food web dynamics in a poorly understood but critically important frontal zone bordering the Southern Ocean.

**KEYWORDS:** plankton studies; food web; diet; regional variations; euphausiids

## INTRODUCTION

Lipid profiles provide a large amount of information on the development, reproduction, condition and feeding ecology of organisms, and pelagic zooplankton have been extensively studied in this regard (Lee *et al.*, 2006; Mayzaud *et al.*, 2007). Fundamental discoveries

involving lipid storage dynamics and fatty acid composition of oceanic zooplankton have been made over the past several decades, particularly in Arctic and Antarctic regions (Phleger *et al.*, 1998; Falk-Petersen *et al.*, 1999; Petursdottir *et al.*, 2008), although species like the euphausiid *Euphausia superba* have received

significantly more attention than most others. Cripps *et al.* (Cripps *et al.*, 1999) described regionally distinct sub-groups of *E. superba* based on fatty acid profiles, and these authors concluded that krill tissue lipids from different zones reflected dissimilar feeding histories (i.e. the food environments of the krill, measured as dominant algal species and copepod condition, were spatially diverse). Numerous additional studies have indicated that fatty acid profiles of zooplankton vary according to seasonal or geographical variations in food availability (e.g. Falk-Petersen *et al.*, 2000; Stevens *et al.*, 2004; Schmidt *et al.*, 2006). Because zooplankton represent the consumer base of the marine food web, leading to upper level consumers including fish, birds and mammals, it is important that we gain a better understanding of the spatial and temporal variations in food sources and how they affect the feeding dynamics and general condition of planktonic communities.

Of particular interest in food web studies are the polyunsaturated fatty acids (PUFAs), as for the most part these must first be synthesized by primary producers and then consumed and incorporated into the tissues of grazers and secondary consumers (Dalsgaard *et al.*, 2003). In particular, the PUFAs 20:4 $\omega$ 6, 20:5 $\omega$ 3 and 22:6 $\omega$ 3 cannot be synthesized by most heterotrophs, but are essential components of membranes, and as such are termed essential fatty acids (EFAs). The source and fate of these EFAs are of significant interest in aquatic food web studies and can provide fundamental information about plankton condition and trophodynamics. Furthermore, the relative quantities of marker fatty acids in consumers, including 22:6 $\omega$ 3, 20:5 $\omega$ 3, 18:1 $\omega$ 9, and long chain monounsaturated fatty acids (MUFAs), are often used as indicators of specific food sources (Falk-Petersen *et al.*, 2000; Dalsgaard *et al.*, 2003). Ratios such as 18:1 $\omega$ 9/18:1 $\omega$ 7, 22:6 $\omega$ 3/20:5 $\omega$ 3 and 16 carbon/18 carbon fatty acids ( $\Sigma$ C16/ $\Sigma$ C18) are also useful when determining the relative contributions of animal prey or different phytoplankton groups (e.g. dinoflagellate versus diatom food) to consumer diets (Dalsgaard *et al.*, 2003). In this manner, fatty acid profiles can inform us about the predominant food and feeding plasticity of herbivorous and omnivorous zooplankton. Alterations in fatty acids occur from one trophic level to the next, but valuable dietary information can be retained despite these metabolic modifications (Dalsgaard *et al.*, 2003).

Despite a growing body of lipid-related data pertaining to plankton, there remains a lack of available information on zooplankton trophodynamics in some of the key oceanographic regions of the world's oceans, including the subtropical convergence (STC). This is an ecologically significant transitional zone, as it is here that the

warm nutrient-poor waters of the southwest Indian Ocean converge with cool nutrient-rich subantarctic waters of the Southern Ocean. Seasonal changes may be less important than regional differences in food quality and availability in this frontal zone, stemming from less-pronounced seasonal oscillations in the light regime compared with those in polar environments (Llido *et al.*, 2005). Intense phytoplankton blooms during a short summer, followed by long periods of limited production, are not predominant features of the STC (Lutjeharms *et al.*, 1985) as is typical at high latitudes (Lee *et al.*, 2006). One would not expect zooplankton at the STC to accumulate large stores of lipid during summer to provide them sufficient energy to reproduce and/or survive through to the following year, as is common in many polar zooplankton (Lee *et al.*, 2006). It follows that trophic dynamics of zooplankton communities at the STC, as described using fatty acid compositional data, are likely very different from those documented in frontal zones of the high Arctic or Antarctic. Hydrological and physical processes contribute to unique primary production dynamics and plankton community structure north and south of the STC (Lutjeharms *et al.*, 1985; Barange *et al.*, 1998; Pakhomov *et al.*, 1999), and distinct regional differences in the nitrogen sources for phytoplankton and zooplankton are apparent from stable isotope ratios (Richoux and Froneman, 2009).

Although the STC contributes substantially to total annual carbon production in the Southern Ocean (Llido *et al.*, 2005), open ocean sections of the convergence zone remain poorly understood with respect to planktonic food web ecology. To determine regional variations in fatty acid composition of zooplankton communities inhabiting the STC, samples from a variety of zooplankton groups were collected north and south of the 14°C surface isotherm chosen to demarcate the mid-point of the transition zone. The focus was primarily on euphausiids, although copepods, decapods, mysids, amphipods and a variety of gelatinous forms were collected to evaluate spatial differences in community trophic dynamics. Based on pronounced changes in temperature, salinity and nitrogen sources (Froneman *et al.*, 2007; Richoux and Froneman, 2009), I hypothesized that food web structure of the zooplankton differ from north to south. Furthermore, based on previous *in situ* measurements of periodically enhanced chlorophyll biomass immediately to the north of the STC (Lutjeharms *et al.*, 1985), I hypothesized that zooplankton in the north have fatty acid profiles indicative of increased herbivory relative to the south. However, ocean colour data collected during the months prior to this study (monthly averaged SeaWiFS data accessed through NASA's Giovanni product) suggested an alternative hypothesis that

zooplankton in the south experienced a food environment enriched in phytoplankton biomass over a medium term (3 months) period. Should the alternative hypothesis hold true, zooplankton in the south would show increased levels of herbivory relative to the north.

## METHOD

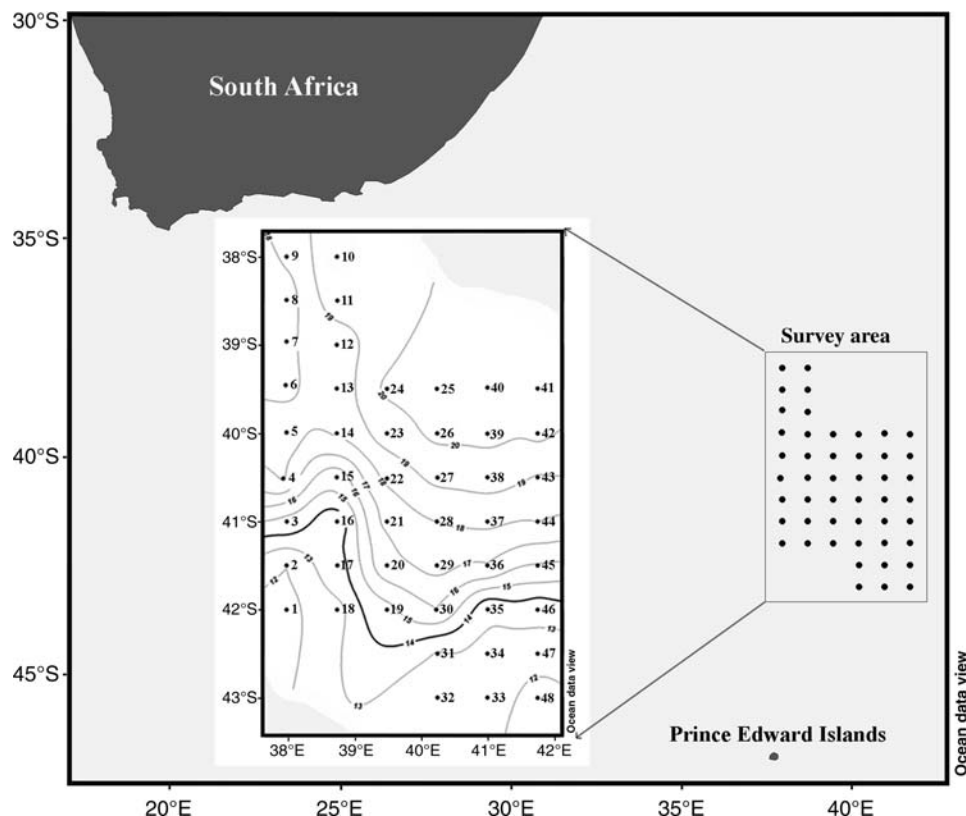
### Sample collection

Collections of biological and physical (salinity, temperature, chlorophyll *a*, nutrients) data took place from the 17th to 27th of April 2007 during Voyage 135 of *S.A. Agulhas* from Cape Town, South Africa. Six transects across the STC were completed between 38 and 43°S, and 38 and 42°E, for a total of 48 stations sampled (Fig. 1). A Seabird SBE 9/11 recorded salinity and temperature profiles to 1500 m depth, and concentrations of nitrate, nitrite, silicate and phosphate were measured, using standard colorimetric analyses, in water samples collected from 10 depths between 25 and 1500 m (Table I). Total chlorophyll *a* (Chl *a*) concentrations were determined from Niskin bottle-collected water sampled

between 25 and 150 m. Chl *a* procedures were performed on route using 90% acetone and a standard fluorometric procedure (Parsons *et al.*, 1984; Turner 10AU fluorometer). References are made to longer term ocean colour data, accessed through NASA's Giovanni product, that represent monthly averages of 9-km resolution (SeaWiFS.R2009), and long-term sea-surface temperature data derived from monthly averages (11 micron day MODIS-Aqua.R2009, available at <http://gdata1.sci.gsfc.nasa.gov/giovanni>). Zooplankton were collected from 200 m (night) or 300 m (day) to surface using a Bongo net (200- $\mu$ m mesh) fitted with flow and depth metres. Animals were coarsely sorted and immediately frozen at  $-25^{\circ}\text{C}$  for the duration of the voyage. Surface water aliquots (2–4.4 L) for the determination of particulate organic matter (POM) fatty acid profiles were pre-screened through a 200  $\mu$ m Nitex mesh, filtered onto pre-ignited GF/F Whatman glass fibre filters (47 mm filters; <5 bar vacuum) and also frozen.

### Sample treatment

In the laboratory, frozen plankton samples were further sorted and measured on ice under a



**Fig. 1.** Survey area in the STC region during Voyage 135 of *S.A. Agulhas*, April 2007. Enlargement shows the 6 transects and 48 biological sample stations, in collection order, superimposed on sea surface temperature isotherms. The thickened  $14^{\circ}\text{C}$  isotherm represents the surface division of the STC between northern and southern water masses.

Table I: Water samples at the subtropical convergence

	North	South
Salinity range (psu)	(34.1–35.7)	(33.9–34.4)
Temperature range (°C)	(14.3–20.7)	(11.2–14.2)
Chlorophyll <i>a</i> (mg m <sup>-2</sup> ; integrated 0–150 m)	18.9 ± 6.7 (12.0–40.0)	26.0 ± 7.5 (13.0–38.6)
Chlorophyll <i>a</i> (mg m <sup>-3</sup> ; surface)	0.19 ± 0.08 (0.03–0.42)	0.31 ± 0.10 (0.12–0.42)
Nitrite (μM L <sup>-1</sup> )	0.43 ± 0.15	0.53 ± 0.11
Nitrate (μM L <sup>-1</sup> )	2.64 ± 1.31	13.19 ± 4.13
Silicate (μM L <sup>-1</sup> )	5.68 ± 2.20	5.44 ± 3.09
Phosphate (μM L <sup>-1</sup> )	0.69 ± 0.23	1.28 ± 0.32
Fatty acids (%TFA), <i>N</i>	North, 7	South, 4
14:0	5.0 ± 1.6	6.0 ± 0.7
15:0	1.5 ± 0.5	1.4 ± 0.2
i-15:0	0.6 ± 0.4	1.0 ± 0.5
ai-15:0	1.0 ± 0.7	2.1 ± 0.9
16:0	25.4 ± 6.2	23.6 ± 4.4
ai-16:0	1.0 ± 1.3	1.2 ± 1.1
17:0	1.0 ± 0.3	0.8 ± 0.2
18:0	15.5 ± 7.6	10.5 ± 2.5
20:0	3.8 ± 5.0	5.1 ± 3.8
ΣSFA	61.5 ± 9.4	57.9 ± 3.7
16:1ω7	1.4 ± 0.8	2.3 ± 0.3
16:1ω5	1.3 ± 1.1	1.7 ± 0.4
17:1	0.8 ± 0.6	1.1 ± 0.3
18:1ω9	3.8 ± 2.3	4.3 ± 1.3
18:1ω7	1.2 ± 1.0	2.1 ± 1.4
20:1ω9	0.5 ± 0.3	0.8 ± 0.3
ΣMUFA	9.7 ± 4.3	13.9 ± 2.0
18:2ω6	3.2 ± 0.8	3.0 ± 1.9
20:2ω6	0.6 ± 0.5	1.4 ± 1.2
16:3ω4	1.9 ± 2.4	1.4 ± 0.2
18:4ω3	1.2 ± 0.8	1.3 ± 0.4
20:4ω6	0.8 ± 0.6	0.7 ± 0.1
20:5ω3	5.6 ± 3.1	4.3 ± 1.2
22:5ω6	2.1 ± 1.1	1.4 ± 0.5
22:6ω3	12.4 ± 4.8	14.1 ± 4.4
ΣPUFA	28.8 ± 7.9	28.3 ± 4.0
ΣEFA	18.8 ± 7.5	19.1 ± 5.3
ΣBAFA	9.9 ± 3.2	10.8 ± 3.4
ΣPUFA/ΣSFA	0.5 ± 0.2	0.5 ± 0.1
22:6ω3/20:5ω3	2.6 ± 1.0	3.3 ± 0.8
Σ(22:1+20:1)	0.7 ± 0.6	1.1 ± 0.6
ω3/ω6	2.9 ± 0.7	3.2 ± 1.3
18:1ω9/18:1ω7	2.9 ± 3.1	1.3 ± 0.1
ΣC16/ΣC18	1.4 ± 0.3	1.5 ± 0.1
TFA (μg mg <sup>-1</sup> DM)	1.9 ± 0.9	2.2 ± 0.6

Environmental parameters and particulate fatty acids >0.05%TFA (mean ± SD, ranges in brackets). Nutrient values represent depth-averaged concentrations between 25 and 150 m from the first transect of the research cruise (nutrient concentration trends along the other five transects were similar to that of transect 1 so data are not included here).

dissecting microscope. The smallest animals (e.g. copepods) were pooled, sometimes by the hundreds, to generate sufficient material for an adequate signal, whereas larger animals were processed as individuals. Euphausiids, amphipods and tunicates were identified to species using taxonomic keys (Baker *et al.*, 1990; Esnal, 1999; Esnal and Daponte, 1999; Gibbons *et al.*, 1999; Vinogradov, 1999),

whereas most other taxa were placed into broad taxonomic groupings. It is possible that individuals within a species may have varied somewhat in reproductive maturity, although the within-species body size ranges were relatively small (Table II), and reproductive females were not included. Small zooplankters were vacuum filtered onto GF/C filters, and all filters and animals were visually inspected for contaminant material, placed in pre-ignited foil envelopes and lyophilized for 24 h. Animals were homogenized using a mortar and pestle, and dried and weighed animal subsamples and filters [up to 100 mg dry mass (DM)] were placed in 2 mL chloroform under nitrogen in lipid-cleaned vials and stored at -20°C. The total lipids were extracted from each sample using a modified Folch procedure (Folch *et al.*, 1957) within 1 year of collection. Whole lipids were extracted in 2:1 (v/v) chloroform/methanol, and washed with 0.5 mL of chloroform-extracted water. The lower lipid layers were removed and combined following several chloroform washes. A known quantity of internal standard (19:0) was added to each extract for quantifying fatty acid methyl esters (FAMEs). FAMEs were prepared by heating the extracts suspended in hexane and 14% boron trifluoride-methanol at 80°C for 1.5 h (method adapted from Budge and Parrish, 1998).

Gas chromatographic (GC) analyses of FAMEs were performed with a Hewlett Packard 5890A GC equipped with a bonded and cross-linked 78% cyanopropyl methylpolysiloxane-fused silica capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness; Quadrex Corporation) with helium as the carrier gas. One microlitre sample aliquots were manually injected at 250°C with the oven set at 100°C for the first 3 min. The oven temperature was increased to 150°C at a rate of 5°C min<sup>-1</sup>, held for 1 min, and raised to 220°C at 3.5°C min<sup>-1</sup> (flame ionization detector was set at 260°C). Peaks were integrated using 32 Karat 5.0 software (Beckman Coulter Inc.) and identified using mass spectral (MS) data derived from a subset of the samples and by comparing retention times with those of external standards (37 component FAMEs and marine PUFA no. 1, Supelco). Each fatty acid was reported qualitatively as a proportion of the total identified fatty acids (%TFA), and quantitatively as μg mg<sup>-1</sup> DM. The fatty acid 22:4ω6 could not be definitively identified using standards or MS techniques (unfortunately an MS was not always available when needed); therefore, it must be considered a tentative identification. However, I chose not to exclude 22:4ω6 from the analysis, as it was clearly an important distinguishing characteristic for copepods. Follow-up studies will be needed to confirm the identity of this component.

Bacterial fatty acids (BAFAs) include odd-numbered carbon chains and iso- (*i*-) and anteiso- (*ai*-) branches

Table II: Zooplankton at the subtropical convergence

Species or taxonomic category	n	Size (mm)	TFA ( $\mu\text{g mg}^{-1}$ DM)		PUFA (%TFA)		
			North	South	North	South	
Euphausiacea	<i>Euphausia similis</i>	5, 8	17–22	48.5 $\pm$ 13.4	50.2 $\pm$ 21.0	57.0 $\pm$ 1.2	58.1 $\pm$ 1.4
	<i>Euphausia spinifera</i>	2, 2	24–25	91.8 $\pm$ 55.0	54.8 $\pm$ 12.7	44.3 $\pm$ 1.9	53.1 $\pm$ 14.2
	<i>Euphausia longirostris</i>	0, 6	19–23	–	41.2 $\pm$ 20.1	–	55.6 $\pm$ 5.1
	<i>Euphausia recurva</i>	5, 0	12–17	84.4 $\pm$ 46.8	–	57.6 $\pm$ 4.9	–
	<i>Nematoscelis megalops</i>	5, 7	17–22	94.6 $\pm$ 34.9	62.7 $\pm$ 17.6	60.3 $\pm$ 3.9	57.2 $\pm$ 6.6
	<i>Stylocheiron abbreviatum</i>	1, 1	20–23	44.3	61.6	49.0	36.4
	<i>Thysanoessa longicauda</i>	2, 3	10–15	90.5 $\pm$ 55.2	84.4 $\pm$ 16.1	50.8 $\pm$ 8.8	65.3 $\pm$ 2.9
Amphipoda	<i>Phronima sedentaria</i>	3, 2	18–22	36.0 $\pm$ 19.0	24.2 $\pm$ 7.2	62.6 $\pm$ 5.6	70.2 $\pm$ 0.8
	<i>Themisto gaudichaudii</i>	2, 6	15–26	73.9 $\pm$ 17.2	48.6 $\pm$ 17.0	79.9 $\pm$ 11.0	66.5 $\pm$ 12.3
	<i>Vibilia armata</i>	5, 1	4–9	77.2 $\pm$ 14.3	125.1	69.4 $\pm$ 2.6	70.2
Copepoda	Mixed copepods	17, 5	1–6	105.6 $\pm$ 73.5	97.0 $\pm$ 37.7	52.6 $\pm$ 10.4	35.9 $\pm$ 10.6
Decapoda	Penaeidean shrimps	7, 0	33–45	67.8 $\pm$ 38.2	–	69.0 $\pm$ 5.7	–
Mysidacea	<i>Siriella thompsoni</i>	1, 2	10–11	307.9	104.7 $\pm$ 29.3	56.0	65.2 $\pm$ 1.8
Pyrosomatida	<i>Pyrosoma atlanticum</i>	5, 3	150–280	31.7 $\pm$ 8.3	24.1 $\pm$ 7.6	63.7 $\pm$ 5.5	50.1 $\pm$ 3.8
Salpida	<i>Salpa thompsoni</i>	3, 4	32–40	181.0 $\pm$ 124.2	59.6 $\pm$ 22.4	65.2 $\pm$ 5.5	62.0 $\pm$ 10.1
Chaetognatha	Chaetognaths	1, 3	16–20	58.0	77.7 $\pm$ 15.3	70.1	61.6 $\pm$ 5.0
Ostracoda	Halocyprididae	2, 0	1–4	231.4 $\pm$ 48.7	–	42.5 $\pm$ 5.0	–
Pteropoda	Pteropods	9, 2	4–10	23.3 $\pm$ 16.5	111.4 $\pm$ 101.9	61.8 $\pm$ 9.7	60.6 $\pm$ 0.7
Cnidaria	Jellyfish	3, 0	8–19	68.1 $\pm$ 41.0	–	52.4 $\pm$ 10.2	–
Osteichthyes	Myctophid larvae	3, 1	25–55	44.1 $\pm$ 8.1	201.7	55.8 $\pm$ 3.8	67.7

Body length range (mm), total fatty acids (TFA,  $\mu\text{g mg}^{-1}$  DM  $\pm$  SD) and polyunsaturated fatty acids (PUFAs, %TFA  $\pm$  SD). n = sample size (north, south), –, no data, see Supplementary tables for detailed fatty acid profiles.

(Budge and Parrish, 1998), and EFAs include 20:4 $\omega$ 6, 20:5 $\omega$ 3 and 22:6 $\omega$ 3 (Dalsgaard *et al.*, 2003). The ratio  $\Sigma\omega$ 3/ $\Sigma\omega$ 6 (sum of  $\omega$ 3 PUFA relative to sum of  $\omega$ 6 PUFA) represents an herbivory index (Sargent and Falk-Petersen, 1981), and the ratios 18:1 $\omega$ 9/18:1 $\omega$ 7 and 22:6 $\omega$ 3/20:5 $\omega$ 3 are carnivory indices (Cripps and Atkinson, 2000; Dalsgaard *et al.*, 2003), although in environments where dinoflagellates and diatoms are the dominant primary producers this latter ratio is often used as an indicator of relative dominance of dinoflagellates over diatoms (Parrish *et al.*, 2000). The ratio  $\Sigma$ PUFA/ $\Sigma$ SFA (saturated fatty acids) can be a useful freshness (Derieux *et al.*, 1998) or diatom (Claustre *et al.*, 1989) index for suspended particulates, or a potential carnivory index in some consumers (Cripps and Atkinson, 2000), and the diatom or herbivory marker  $\Sigma(16:1\omega$ 7+16:1 $\omega$ 5)/16:0 (Graeve *et al.*, 1994) was also considered.

### Statistical analysis

For many species or taxonomic groups, sufficient replicates were not collected at each station in the transect grid. As a result, similar samples collected from each region (north, south and, occasionally, the middle transition area of the STC represented by the 14°C isocline) were used as replicates from that region. Qualitative data were arcsine square root transformed to improve normality and then subjected to principal component analysis (PCA) and/or non-metric multidimensional scaling (n-MDS) if transformations did not completely normalize

the data. Quantitative data were highly variable and were not subjected to statistical analysis. All fatty acids occurring at levels >0.05%TFA were included in each multivariate analysis of the different taxonomic groups, which helped to minimize potential exaggerated effects of any minor fatty acid variables. Thirty-five fatty acids were included in the northern and southern food web multivariate analyses: 14:0, i-15:0, ai-15:0, 15:0, 15:1, ai-16:0, 16:0, 16:1 $\omega$ 7, 16:1 $\omega$ 5, i-17:0, ai-17:0, 17:0, 16:3 $\omega$ 4, 17:1, 16:4 $\omega$ 3, 18:0, 18:1 $\omega$ 9, 18:1 $\omega$ 7, 18:1 $\omega$ 5, 18:2 $\omega$ 6, 18:2 $\omega$ 4, 18:3 $\omega$ 6, 18:3 $\omega$ 3, 18:4 $\omega$ 3, 20:0, 20:1 $\omega$ 9, 20:2 $\omega$ 6, 20:4 $\omega$ 6, 20:4 $\omega$ 3, 20:5 $\omega$ 3, 22:4 $\omega$ 6, 22:1 $\omega$ 9, 22:5 $\omega$ 6, 22:5 $\omega$ 3, 22:6 $\omega$ 3. Although n-MDS was the more appropriate analysis for the whole food webs, PCA graphics were presented in the results because of their close similarities with the n-MDS results, and the most influential fatty acid variables described by both PCA loadings and SIMPER were nearly identical, whereas PCA loadings were easier to represent visually.

## RESULTS

### Physical data

Comparison of water samples collected throughout the study region indicated enhanced nitrate and Chl *a* concentrations from surface to 150 m depth in the southern region (Table I; transition line dividing the north from the south is represented by the 14°C temperature

isocline in Fig. 1), although the highest Chl *a* readings ( $0.42 \text{ mg m}^{-3}$  in surface samples) were well below those indicative of bloom events ( $0.7 \text{ mg m}^{-3}$ ; Llado *et al.*, 2005). SeaWiFS data confirmed that Chl *a* concentrations in the region of interest were generally below  $0.5 \text{ mg m}^{-3}$  throughout April 2007, and that a wide and diffuse band of low Chl *a* values between  $0.2$  and  $0.5 \text{ mg m}^{-3}$  spanned the transect area. In contrast, SeaWiFS Chl *a* concentrations indicated a thinner (between  $41$  and  $44^\circ\text{S}$ ) band of enhanced productivity between January and March 2007, with averages up to  $1 \text{ mg m}^{-3}$  in the STC region [potentially concentrated in the southern region of the STC, located according to monthly averages of sea-surface temperature (MODIS-Aqua, NASA Giovanni)]. Prior to January 2007, average STC conditions varied from month to month, with enhanced ( $0.7$ – $2 \text{ mg m}^{-3}$ ) Chl *a* concentrations situated north of the STC in December, throughout the STC region in November, and south of the STC in October of 2006. Nutrient analysis of water samples collected between  $150$  and  $1500 \text{ m}$  indicated no distinctions from north to south (see Fig. 2 in Richoux and Froneman, 2009).

### Fatty acids in particulate organic matter

Water particulates were dominated by SFA (up to 70%TFA, largely owing to 16:0 and 18:0), followed by PUFA (up to 37%TFA resulting from high levels of 22:6 $\omega$ 3 and 20:5 $\omega$ 3) and very low levels of MUFA (Table I). Quantitative concentrations of fatty acids in particulate samples were extremely low, with TFA totals no greater than  $3 \mu\text{g mg}^{-1} \text{ DM}$  (Table I), further confirming that algal blooms were not occurring at the time (e.g. during bloom periods in Trinity Bay, Newfoundland, mean TFA content of suspended particulates reached  $41 \mu\text{g mg}^{-1} \text{ DM}$ ; Budge and Parrish, 1998). BAFAs were relatively abundant (up to 14%TFA in the particulates), indicating the importance of the microbial food web in the region, and low  $\Sigma\text{PUFA}/\Sigma\text{SFA}$  (freshness index; Derieux *et al.*, 1998) confirmed the dominance of detrital material. PCA revealed no north to south geographical trends in the fatty acids of particulates.

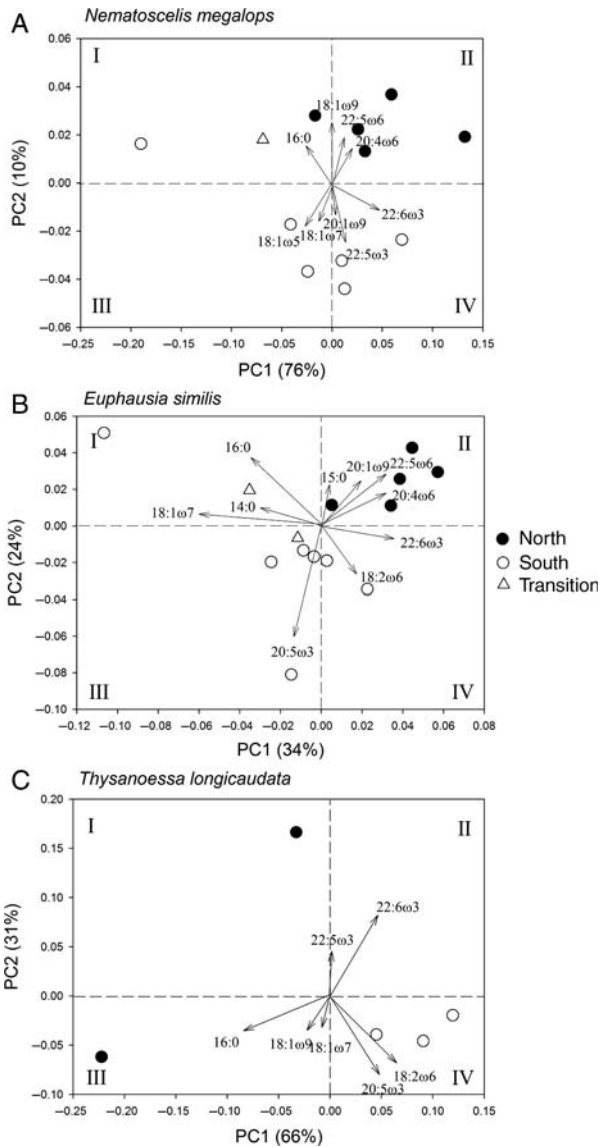
### Fatty acids in euphausiids

PUFA proportions in the seven euphausiid species analysed were extremely high (up to 68%TFA), primarily owing to high levels of the EFAs 22:6 $\omega$ 3 and 20:5 $\omega$ 3 (Table II and Supplementary data, Tables S1 and S2). The next most abundant were the SFAs, due to consistently high levels of 16:0 in all species. MUFAs were the

least dominant overall, the most abundant of those being 18:1 $\omega$ 9 and 18:1 $\omega$ 7. In some species, TFA ( $\mu\text{g mg}^{-1} \text{ DM}$ ) appeared enhanced in northern specimens (e.g. *Nematoscelis megalops* and *Euphausia spinifera*) and in others, TFA were greater in southern specimens (e.g. *Stylocheiron abbreviatum* and *Euphausia similis*; Table II), although variability among replicates obscured any significant regional differences. ANOVA models performed to compare individual and summary fatty acid categories within the different euphausiid species, using both quantitative and qualitative data, resulted in few significant north/south differences. Variability among replicate samples within each region was generally high, as indicated by large coefficients of variation, therefore multivariate analyses were chosen as superior tools for further statistical explorations.

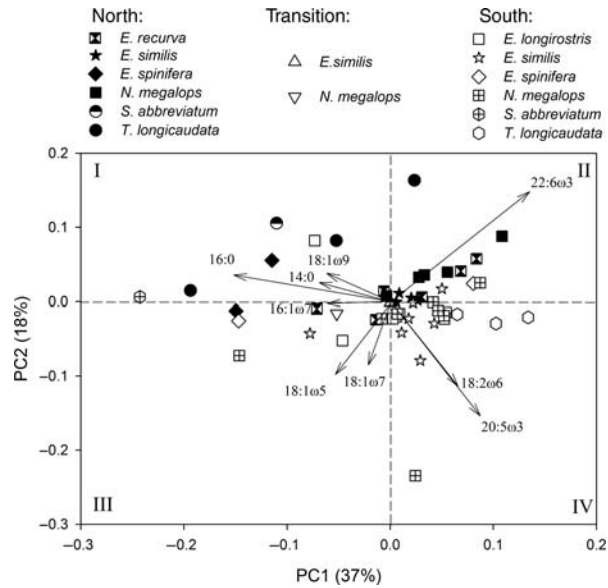
PCA were applied to the three euphausiid species having sufficient sample sizes to warrant investigations of potential within-species regional variations (*N. megalops*, *E. similis* and *Thysanoessa longicaudata*; Fig. 2). The PCA results for *N. megalops* and *E. similis* were remarkably similar, with northern samples clustering in Quadrant II, most of the southern samples clustering in Quadrants III and IV and a few transitional zone samples located somewhere in between the two (Fig. 2A and B). The PCA results of *T. longicaudata* were slightly different, with southern samples clustering closely into Quadrant IV and the two northern samples located distantly in Quadrants I and III (Fig. 2C). Some influential fatty acids (i.e. PCA loadings) were consistent among the three species, such as 20:4 $\omega$ 6 and 22:5 $\omega$ 6 in northern samples, and 18:2 $\omega$ 6, 18:1 $\omega$ 7 and 20:5 $\omega$ 3 in southern samples. Few samples of the remaining species were collected, although 20:5 $\omega$ 3 and 18:1 $\omega$ 7 were associated with southern *Euphausia spinifera*, and 16:0 and 22:6 $\omega$ 3 were associated with northern specimens (Supplementary data, Tables S1 and S2).

All euphausiid samples (representing seven species) were combined into an additional PCA (Fig. 3), and two ecologically relevant gradients became readily apparent. PC1 separated species according to their diet, with Quadrants II and IV containing the species showing greater degrees of herbivory (*N. megalops* and *E. similis*), Quadrants I and III containing species showing more carnivory (*S. abbreviatum*, *E. spinifera* and *Euphausia longirostris*), and the more varied and hence omnivorous species (*Euphausia recurva* and *T. longicaudata*) scattered between the two extremes. This dietary spectrum was indicated by the predominance of algal markers 22:6 $\omega$ 3, 20:5 $\omega$ 3 and 18:2 $\omega$ 6 towards Quadrants II and IV, and the importance of 14:0 and the animal prey markers 18:1 $\omega$ 9 and 16:0 (Stevens *et al.*, 2004) in Quadrants I and III (Fig. 3). PC2 distinguished the euphausiids on



**Fig. 2.** Euphausiids. PCA scores of arcsine square root-transformed qualitative fatty acid data (%TFA) for (A) *Nematoscelis megalops*, (B) *Euphausia similis* and (C) *Thysanoessa longicaudata*. Fatty acids >0.05%TFA were included in the analyses (22 fatty acids in total; see Supplementary data, Table S1 for list). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Roman numerals are quadrant labels. Arrows represent the net direction and relative strength of the dominant fatty acid loadings (those greater than 0.2), and the cross-section of the dashed lines represent the origin.

the basis of their location north or south of the STC, with the majority of the northern specimens clustered into Quadrants I and II, and the southern specimens clustered into Quadrants III and IV (Fig. 3). In general, 22:6 $\omega$ 3, 16:0, 18:1 $\omega$ 9 and 14:0 were important fatty acid loadings in northern specimens, and 20:5 $\omega$ 3, 18:1 $\omega$ 7, 18:1 $\omega$ 5 and 18:2 $\omega$ 6 were influential in the



**Fig. 3.** Euphausiids. PCA scores of arcsine square root-transformed qualitative fatty acid data (%TFA), all species included. See Fig. 2 legend for other details.

southern specimens, with within-species diet flexibility complicating the interpretation. The herbivory index  $\Sigma\omega 3/\Sigma\omega 6$  was higher in euphausiids collected from the south (mean ratio ranged from 7.4 to 13.9 in the north and 9.4 to 23.0 in the south), and the carnivory index 22:6 $\omega$ 3/20:5 $\omega$ 3 was greater in northern euphausiids (mean ratio ranged from 2.0 to 2.6 in the north and 1.6 to 2.0 in the south), but  $\Sigma$ PUFA/ $\Sigma$ SFA showed no trends (mean ratio ranged from 1.2 to 2.6 in the north and 1.1 to 3.2 in the south) (Supplementary data, Table S1). Other traditional markers for diatoms (16:1 $\omega$ 7) and dinoflagellates (18:4 $\omega$ 3) were found in negligible amounts in the zooplankton.

### Fatty acids in other zooplankton

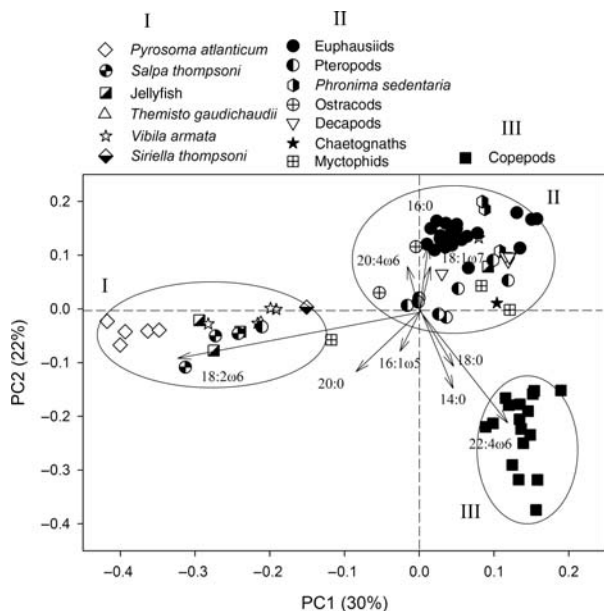
Copepod samples were represented by pooled samples that included some large (primarily *Calanus simillimus* and *Pleuromamma abdominalis*) and small specimens (predominantly *Clausocalanus breviceps* and *Oncaea confifera*; Daly, 2008). Multivariate analysis did not reveal specialized differences between large and small copepod fractions, so all samples were collectively grouped under the coarse taxonomic heading of copepods. As a group, mixed copepods contained elevated levels of two fatty acids: 22:4 $\omega$ 6 (tentative identification) and 14:0 (Supplementary data, Tables S3 and S4). PUFA and EFA quantities were high, similar to the euphausiids, followed by SFAs and MUFAs. The copepod PCA

scores were highly variable and showed no north/south distinctions (PCA data not shown).

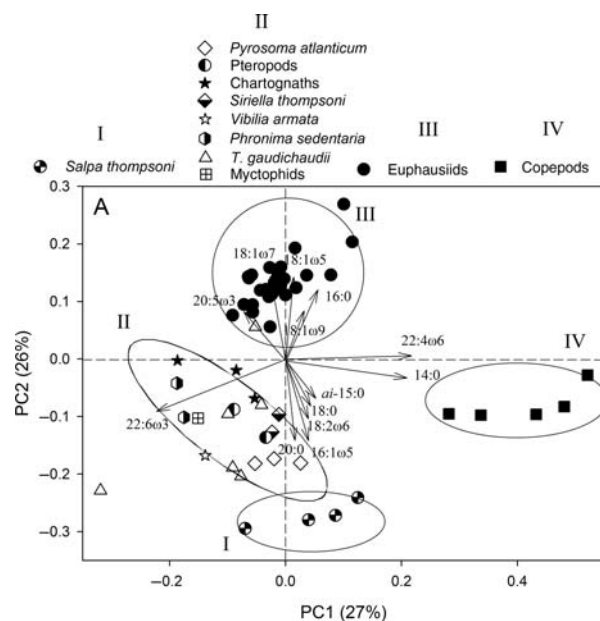
PUFA levels were high in all zooplankton groups and species (Table II). A few specimens contained very high TEFA concentrations of up to 200 or 300  $\mu\text{g mg}^{-1}$  DM (equivalent to 20–30% DM; e.g. some of the salps, mysids, pteropods, ostracods, copepods and myctophids; Table II). PCA indicated distinct north/south trends only in the pyrosomes and the amphipods *Vibilia armata* owing to enhanced quantities of 18:2 $\omega$ 6 in the northern specimens (Supplementary data, Tables S3–S6). In the remaining groups, among-sample variability obscured potential geographical trends, or the sample size was too small to observe patterns. However, in the omnivorous taxonomic groups (mysids *Siriella thompsoni*, hyperiid amphipods *Themisto gaudichaudii*, copepods, chaetognaths and myctophids), the marker ratio 22:6 $\omega$ 3/20:5 $\omega$ 3 was greater in specimens north of the STC, suggesting increased carnivory in the north (exceptions were *V. armata* and *Phronima sedentaria*). Furthermore, the herbivory index  $\Sigma\omega$ 3/ $\Sigma\omega$ 6 was enhanced in southern specimens of *Pyrosoma atlanticum*, *T. gaudichaudii*, *V. armata*, pteropods, chaetognaths, *Phronima sedentaria* and myctophids.

### Food web

Multivariate analyses of food webs north (Fig. 4) and south (Fig. 5) of the STC revealed some interesting



**Fig. 4.** Food web north of the STC. PCA scores of arcsine square root-transformed qualitative fatty acid (%TFA; 35 FA included). Ellipses indicate the three distinct groups (I, II and III), as indicated by ANOSIM following n-MDS analysis. See Fig. 2 legend for other details.



**Fig. 5.** Food web south of the STC. PCA scores of arcsine square root transformed qualitative fatty acid data (%TFA; 35 FA included). Ellipses indicate the four distinct groups (I, II, III and IV), as indicated by ANOSIM following n-MDS analysis. See Fig. 2 legend for other details.

spatial differences in plankton trophodynamics. The northern food web formed three main groupings, as indicated by ANOSIM, with Group I containing parasitoid amphipods and the more herbivorous particle feeders, Group II containing omnivores and carnivores, and Group III composed solely of the omnivorous copepods (Fig. 4). In contrast, the southern food web formed four groupings, with Group I consisting of salps, Group II of particle feeders, parasitoids, plus two carnivores (myctophids and chaetognaths represented by a mixture of *Eukrohnia hamata*, *Sagitta gazellae* and *S. zetesios*; Sterley, 2008), Group III solely of euphausiids, and Group IV representing the copepods (Fig. 5).

Although the salps *Salpa thompsoni* appeared to show different trophic relationships in the two regions, with southern specimens forming a group distant from all other zooplankton including other gelatinous small particle feeders (Fig. 5), qualitative fatty acid profiles of the salps were nearly identical in the north and south (Supplementary data, Table S5; quantitative data were highly variable in the north and obscured any trends). In actuality, it was *Pyrosoma atlanticum*, *T. gaudichaudii*, *V. armata* and the pteropods that differed from north to south, mainly from enhanced quantities of 18:2 $\omega$ 6 (a marker for non-diatom algae; Dalsgaard et al., 2003) in northern specimens. In the south, however, *Salpa thompsoni* was the only species to maintain high levels of 18:2 $\omega$ 6, thus causing its distinction from the other



particle feeders (Fig. 5). The remaining herbivorous gelatinous plankton, represented mainly by pyrosomes, clustered into Group II with other herbivores, omnivores and even carnivores such as chaetognaths and *Phronima sedentaria*.

An interesting result from the multivariate analyses of the food webs was the close association of the small ostracods and pteropods with the large omnivores and carnivores within northern Group II (Fig. 4). The pteropods were dominated by the thecosomate species *Limacina retroversa*, but included larger specimens of *Clio* and *Cavolinia* spp. (Daly, 2008). The ostracods were represented by a variety of halocyprids.

## DISCUSSION

### Euphausiids

Euphausiids have been classified as herbivores, omnivores or carnivores, and are generally more carnivorous than copepods (Mauchline *et al.*, 1989). Diets are diverse and can include an array of items including phytoplankton, protozoans, detritus and small zooplankton such as copepods and fish eggs (Atkinson and Snyder, 1997; Gibbons *et al.*, 1999; Schmidt *et al.*, 2006). Euphausiids show complex feeding plasticity; typical herbivores can resort to carnivory when their preferred food is not available (Atkinson and Snyder, 1997) and carnivores can periodically resort to herbivory (Mayzaud *et al.*, 2007). As such, feeding behaviour is strongly influenced by the nature of the food environment (Gibbons *et al.*, 1999; Schmidt *et al.*, 2006). Unfortunately, the knowledge of large- or small-scale variations in feeding plasticity of euphausiids is not well developed.

Multivariate analysis of euphausiid fatty acid signatures at the STC revealed ecologically important gradients on PC1, with species in the right-hand quadrants having diets reflecting enhanced phytoplankton feeding and those towards the left having characteristics reflecting carnivory (Fig. 3). The scenario became more complicated when PC2 was considered, revealing within-species variations from north to south. It appears that herbivores had greater access to heterotrophic dinoflagellate food (as shown by 22:6 $\omega$ 3) in the north, and diatom (20:5 $\omega$ 3, 18:1 $\omega$ 7) and chlorophyte or cyanobacterial food (18:2 $\omega$ 6) south of the STC. There is a possibility that regional differences in fatty acid profiles may have partially developed from differential adaptive responses of zooplankton to divergent temperature regimes (Mayzaud *et al.*, 2000; Hall *et al.*, 2002). In this context, increased 20:5 $\omega$ 3 in the southern specimens

could also represent a physiological response to maintain membrane fluidity at the lower temperatures (Hall *et al.*, 2002), but this is unlikely a dominant process in the euphausiids inhabiting the study region as the minimum temperatures are relatively high ( $\sim 11^{\circ}\text{C}$ ).

The north to south separation of the more carnivorous species in Quadrants I and III could indicate differences in the dominant animal prey consumed (e.g. 18:1 $\omega$ 7 and 18:1 $\omega$ 5 were more dominant in southern chaetognaths compared with northern chaetognaths). The carnivory index 22:6 $\omega$ 3/20:5 $\omega$ 3 was higher in the north in all euphausiid species collected from both regions, and combined with the increase in the animal marker 18:1 $\omega$ 9 and the low prevalence of classic diatom markers such as 16:1 $\omega$ 7 in northern specimens, it appears very likely that euphausiids were generally more carnivorous in the north. Interestingly, an increase in carnivory within the northern euphausiids was not detected using stable isotope ratio data, as the significantly enriched  $\delta^{15}\text{N}$  signatures in northern specimens were accounted for by concurrent increases in the  $\delta^{15}\text{N}$  values for particulates and primary consumers (Richoux and Froneman, 2009). Such discrepancies among methods are not uncommon, as independent molecular techniques can integrate dietary information at different time scales. To seek an explanation for the differences in trophic behaviour among and within species indicated by lipid composition, attention must return to potential spatial and temporal variations in the food environment.

Longitudinal transect water collections and satellite images used to quantify Chl *a* concentrations have revealed periodically enhanced primary productivity immediately to the north and/or south of different sectors along the STC (Lutjeharms *et al.*, 1985; Froneman *et al.*, 1997; Llido *et al.*, 2005; SeaWiFS, NASA Giovanni). These events occur year-round, although frequency of occurrence increases somewhat during spring and summer (Llido *et al.*, 2005). Measurable differences in phytoplankton species composition and diversity also periodically correspond with the distinct water masses meeting at the STC (Boden *et al.*, 1988; Froneman *et al.*, 1997). At the location and time (April 2007) of the present study, size-fractionated Chl *a* measurements indicated the dominance of picophytoplankton ( $<2\ \mu\text{m}$ ; up to 66% total Chl *a*), followed by nanophytoplankton (2–20  $\mu\text{m}$ ) size classes, both north and south of the STC, with microphytoplankton ( $>20\ \mu\text{m}$ ) representing the lowest proportion ( $<10\%$ ) of the total Chl *a* biomass throughout the region (Froneman *et al.*, 2007; Daly, 2008). Average total Chl *a* and nitrate concentrations (integrated and averaged, respectively, to 150 m depth) were slightly greater

south of the STC, but regional differences in surface water particulate fatty acids were not detected (Table 1), thus indicating no measurable differences in particulate food quantity or quality from north to south. High levels of both SFA and BAFA indicated the predominance of detritus and bacteria, respectively, in the particulate component of the water relative to live algal cells (Derieux *et al.*, 1998; Mayzaud *et al.*, 2007). However, algal markers 22:6 $\omega$ 3, 20:5 $\omega$ 3, 18:2 $\omega$ 6 and 18:1 $\omega$ 7, and the animal marker 18:1 $\omega$ 9, were all present in the particulate component of the water and played important roles in the planktonic food web. The zooplankton community was dominated by copepods, although no regional changes in copepod abundance or biomass were detected (Daly, 2008). The larger zooplankton fraction was dominated by chaetognaths and euphausiids (up to 87% of total abundance), and euphausiids and amphipods (up to 72% of total biomass), although regional trends were also absent in April 2007 (Sterley, 2008). Unlike high latitude regions, seasonal trends in primary production are not intense at the STC (Llido *et al.*, 2005), although more frequent blooms in the north or south could create important average differences in the food quality and quantity available to consumers in the region. Because lipid compositions reflect a time-integrated assimilation history by consumers (Dalsgaard *et al.*, 2003), fatty acid profiles of the STC zooplankton represent time-averaged consumer diets, whereas Chl *a* and zooplankton abundance/biomass indicate only snap shots of the recent food environment.

The three euphausiid species showing highest degrees of carnivory overall (*Stylocheiron abbreviatum*, *Euphausia spinifera* and *Euphausia longirostris*) were distinguished by increased levels of the animal markers 16:0 and 18:1 $\omega$ 9, but also elevated 14:0 content that may have originated in part from copepod prey. A carnivorous trophic position for these species was further supported by nitrogen stable isotope data measured on specimens collected at the same time and locations (average trophic level for all three species ranged from 3.7 to 3.8 throughout the study region; Richoux and Froneman, 2009). *Euphausia spinifera* collected north of the Agulhas Front, where it divides the Indian Ocean from the Southern Ocean near 63°E, showed differences in feeding mode compared with specimens from the south (Perissinotto *et al.*, 2001; Mayzaud *et al.*, 2007). Animals were more omnivorous in the north, as indicated by increased proportions of 22:6 $\omega$ 3 and  $\Sigma(20:1 + 22:1)$  MUFAs, but decreased proportions of C16 PUFAs and 18:5 $\omega$ 3 compared with southern specimens (Mayzaud *et al.*, 2007). The north/south differences in *E. spinifera* were attributed to differences in both metabolic

processes and trophic environments (Mayzaud *et al.*, 2007). *Euphausia longirostris* at the Prince Edward Islands had a large metazoan gut content (53% of total items counted) relative to phytoplankton (30% of total items counted; Gurney *et al.*, 2001) and was considered omnivorous in that region, and *S. abbreviatum* is generally known as a non-migrating and active predator throughout its distribution (Kinsey and Hopkins, 1994 and references therein).

The euphausiids *Thysanoessa longicaudata* and *Euphausia recurva* showed high variability in their fatty acid profiles and occupied various positions along PC1 of the PCA (Fig. 3), thus were classified as omnivores. *Thysanoessa longicaudata* was more carnivorous in the north relative to the south STC. *Thysanoessa* spp. in polar oceans have similarly been categorized as carnivores feeding primarily on *Calanus* copepods (Falk-Petersen *et al.*, 2000), or omnivores that feed on both animal and phytoplankton material (Falk-Petersen *et al.*, 1999). *Euphausia recurva* is also reported as an omnivore throughout the literature (e.g. Pillar *et al.*, 1991), with a feeding basket capable of collecting small particles mainly in the 10–20  $\mu$ m range (Suh and Choi, 1998).

Of the two euphausiid species showing increased levels of herbivory in general (*Nematoscelis megalops* and *Euphausia similis*), *N. megalops* was the most unexpected, as stable nitrogen isotopes placed this species in an intermediate trophic position rather than an herbivorous one relative to the other euphausiids (trophic position 3.6 in the STC region; Richoux and Froneman, 2009). Furthermore, although it is also known for its dietary plasticity, *N. megalops* was predominantly carnivorous in the Prince Edward Islands region (Gurney *et al.*, 2001), in the vicinity of the Agulhas Front (Mayzaud *et al.*, 2007) and in the northern Benguela (Barange *et al.*, 1991). Its large eyes and lack of a feeding basket for filtering small particles (Mauchline *et al.*, 1989) also suggest a carnivorous lifestyle for this species. Interestingly, despite enhanced levels of algal PUFAs in *N. megalops*, it contained similar or greater amounts (quantitative and qualitative) of the animal marker 18:1 $\omega$ 9 compared with the other euphausiids at the STC (but low 16:0), so obviously the trophodynamics of this species are highly complex. *Nematoscelis megalops*, particularly in the north, contained the greatest quantities of TFA among all the euphausiids at the STC, perhaps indicating its enhanced ability to derive the highest quality diet from the environment in any food availability scenario. *Euphausia similis* at the Prince Edward Islands showed size-selection behaviour for small phytoplankton cells from 10 to 20  $\mu$ m (Perissinotto, 1992), a size class that was abundant at the STC during April 2007.

## Other zooplankton

Although some high latitude copepods are mainly herbivorous suspension-feeders (Kattner and Hagen, 1995), most copepods show flexibility in their diets and show preference for heterotrophic ciliates and flagellates over algae (Nejstgaard *et al.*, 1997; Mayzaud *et al.*, 2007). Conclusions about copepod diet at the STC are generally limited here, as samples represented size-fractionated pools that were later combined. However, the fatty acid profiles of these copepod samples represent a valuable average prey signature in this pelagic food web. In general, markers for both herbivory (22:6 $\omega$ 3 and 20:5 $\omega$ 3) and carnivory (18:1 $\omega$ 9) were important in the copepod samples, with no indication of increased carnivory in the north. In both the northern and southern food webs, copepods separated distinctly from all other zooplankton (Figs 4 and 5), primarily due to the influence of 22:4 $\omega$ 6 and 14:0. Consumers of copepods were obviously modifying these fatty acids, as they were not accumulated in sizable quantities in any other zooplankton. This scenario is not uncommon, as some fatty acids are synthesized or highly modified by consumers (Cook *et al.*, 2000). However, 22:4 $\omega$ 6 represents a potential qualitative marker for copepod consumption at the STC, since small amounts were recorded in typical consumers of copepods (euphausiids, mysids, chaetognaths and myctophids), whereas it was absent in the particle feeders and measured only in traces in the remaining omnivores (*Phronima sedentaria*, decapods, *Themisto gaudichaudii* and *Vibilia armata*). This fatty acid is not particularly common in the copepod literature, although it represented up to 3%TFA in copepods off the coast of Iceland (Petursdottir *et al.*, 2008).

The long-chain monoenes often associated with copepods (20:1 and 22:1; Dalsgaard *et al.*, 2003) were not particularly abundant in STC specimens and did not play a key role in the food web. The large pool of literature on copepod compositional fatty acid data was derived primarily from high latitude calanoid copepods (Falk-Petersen *et al.*, 1999; Stevens *et al.*, 2004; Petursdottir *et al.*, 2008), and the trends revealed may not be valid in regions having alternative environmental regimes and species assemblages. For example, omnivorous calanoid (Ward *et al.*, 1996; Peters *et al.*, 2006) and non-calanoid (Hazzard and Kleppel, 2003) copepods can be dominated by other components such as 18:1 $\omega$ 9, 16:0 and 16:1 $\omega$ 7, while containing very low levels of 20:1 and 22:1 fatty acids or alcohols. Synthesis of large quantities of these long-chain monoenes during brief and pronounced phytoplankton blooms allows copepods to survive prolonged periods of food shortage,

as this anabolic process prevents the negative feedback inhibition of further fatty acid synthesis (Falk-Petersen *et al.*, 1999). The low quantities of long-chain monoenes within STC copepods provide compelling evidence that these animals are not herbivores experiencing episodic food shortages during the year.

The colonial pyrosome *Pyrosoma atlanticum* filters particulates from the water column (Esnafl, 1999), with high retention efficiency for particles >10  $\mu$ m (Perissinotto *et al.*, 2007), whereas the hyperiid amphipod *Vibilia armata* is best known as a symbiotic ectoparasite of gelatinous animals (Vinogradov, 1999). Regional distinctions in the food environment of these species were indicated by increased levels of 18:2 $\omega$ 6, a biomarker for chlorophytes or cyanobacteria (Volkman *et al.*, 1998; Gugger *et al.*, 2002), in the northern specimens. As in the euphausiids, these regional differences in fatty acid composition were probably reflecting differences in the recent food environment from north to south, although 18:2 $\omega$ 6 did not differ regionally in the particulate samples during April. *Pyrosoma atlanticum* may be selectively retaining 18:2 $\omega$ 6 after ingestion, and *V. armata* is presumably assimilating this marker through its close parasitic association with the gelatinous forms. Stable nitrogen isotopes reflected omnivorous feeding in both species throughout the STC region (trophic positions ranged from 2.6 to 3.0; Richoux and Froneman, 2009), thus providing further confirmation of a large heterotrophic and/or detrital component in the particulates.

Multivariate analyses revealed intriguing differences in food web relationships north and south of the STC (Figs 4 and 5). Overall, the unusual grouping of mixed feeders in southern Group II (Fig. 5) suggested a larger incidence of herbivory in the diets of several typical omnivores and carnivores in this region. Enhanced proportions of the fatty acids 18:2 $\omega$ 6 and 20:0 distinguished particle-feeding and parasitic zooplankton (pyrosomes, salps, *T. gaudichaudii*, *V. armata* and *S. thompsoni*), represented by Group I in the north. In the south, pronounced decreases in 18:2 $\omega$ 6 in all particle-feeding (or parasitoid) taxa apart from the salps caused their increased association with the other zooplankton groups, excepting the euphausiids and copepods. Trophic levels between 2 and 3, calculated using stable nitrogen isotope ratios, confirmed that the particle-feeding Group I animals (apart from the jellyfish) represented mid-level consumers (Richoux and Froneman, 2009). Chaetognaths are often categorized as gelatinous zooplankton, and at the STC they contained increased levels of 18:1 fatty acids typical of most carnivores, but low levels of the fatty acid 18:2 $\omega$ 6 characteristic of the gelatinous small particle feeders. Myctophids dominate the mesopelagic ichthyofauna of

the Southern Ocean and are important opportunistic predators of zooplankton including copepods, amphipods, ostracods, euphausiids, chaetognaths, fish eggs and fish larvae (Stowasser *et al.*, 2009). Fatty acid profiles (Figs 4 and 5) suggested recent feeding on amphipods *T. gaudichaudii* and mysids by some myctophid specimens throughout the STC, and a few specimens in the north may have recently fed on pteropods, chaetognaths and euphausiids.

The close association of the jellyfish, typical carnivores, with the small particle feeders in the north probably resulted from similar biochemical compositions among the water-loaded and membrane-heavy gelatinous forms (Bailey *et al.*, 1995). The inclusion of the small pteropods in northern Group II was somewhat unexpected, as thecosomate pteropods consume microplankton collected in a mucous trap (van der Spoel and Dadon, 1999 and references therein). However, when the food environment is dominated by heterotrophic protozoans, as presumably occurred in the northern STC prior to the April sampling event, the pteropod diet signatures could thus reflect heterotrophic feeding. As one could make the same argument for salps and pyrosomes, fatty acid profiles obviously represent a complex combination of both diet- and species-specific metabolic processes, and using multiple techniques to approach the elucidation of these food webs provides the most informative results. The halocyprid ostracods, although small, can actively feed on organisms much larger than themselves (Angel, 1999), hence explaining their association with Group II in the north.

The fatty acid 18:2 $\omega$ 6, a biomarker for chlorophytes or cyanobacteria, occurred at concentrations >3%TFA in particulates collected from the STC. The small mysids *Siriella thompsoni* and amphipods *Themisto gaudichaudii* and *V. armata* throughout the STC region were similar to the gelatinous small particle feeders in containing high levels of 18:2 $\omega$ 6. Certainly, the hyperiid amphipods are known to feed on gelatinous animals (Phleger *et al.*, 1998), although low levels of 20:0 differentiated them from the gelatinous forms. *Themisto gaudichaudii* is extremely common and abundant throughout the Southern Ocean (Pakhomov *et al.*, 1999), and it serves important roles in the planktonic food web as both a consumer and a food source for higher predators such as myctophids (Stowasser *et al.*, 2009). However, in Antarctic regions, it is typically classified as an obligate carnivore using data derived from both biomarker and traditional gut content techniques, with gut pigments assumed to arise from secondary feeding (Pakhomov and Perissinotto, 1996; Nelson *et al.*, 2001). Both stable isotope ratios (Richoux and Froneman, 2009) and fatty acid profiles (present study) contradict these previous

studies and clearly indicate an omnivorous existence for *T. gaudichaudii* in the STC region (certainly less carnivorous than the euphausiids).

Salps *Salpa thompsoni* were interesting in that their diet was virtually undistinguishable north and south of the STC using fatty acid compositional data. Similar to the pteropods, salps feed while they are swimming by filtering suspended particles (1 mm to <1  $\mu$ m) using a mucous net (Esnal and Daponte, 1999), thus they could effectively clear the pico- and nanophytoplankton at the STC. However, pteropod fatty acid profiles reflected a different food environment history from north to south. This distinction between the two mucous feeders may have resulted from more rapid feeding and growth rates in the salps, and hence higher turnover rates of dietary fatty acids. *Salpa thompsoni* gut contents reflect the natural composition of the phytoplankton in the environment, thus confirming that they are not selective feeders, but they can show extremely high feeding rates (Tanimura *et al.*, 2008).

Although the use of proportional data increases the validity of comparing fatty acid compositions among different taxonomic groups, some of the food web associations depicted in Figs 4 and 5 may have been influenced by variations in lipid class composition among groups. For example, gelatinous forms are often lipid poor (e.g. 6% DM in *Pyrosoma atlanticum*) and dominated by membrane polar lipids (Perissinotto *et al.*, 2007), whereas crustaceans can be relatively lipid rich [e.g. krill tend to have substantial lipid contents of up to 48% DM (Hagen *et al.*, 2001)] with potentially large proportions of neutral storage lipids (Falk-Petersen *et al.*, 1999). Tropical and sub-tropical zooplankton are generally lipid poor compared with polar zooplankton (Lee *et al.*, 2006), although some differences could potentially occur among the various zooplankton groups collected at the STC. TFA content in an organism is a function of both total lipid content and lipid class composition, but it can be used as a coarse proxy to show variations in total lipid content (Richoux *et al.*, 2005). Mean TFA content in the euphausiid species at the STC generally fell between 4 and 9% DM, with most of the remaining zooplankton groups showing similar mean TFA content, although the copepods, mysids and ostracods showed larger mean TFA contents that were highly variable (>10% DM; Supplementary data, Tables S2, S4 and S6). Some food web studies focus strictly on the fatty acids in neutral storage lipids, as these tend to reflect recent changes in diet (Lee *et al.*, 2006), although dietary PUFAs can also be deposited relatively unchanged into polar fractions of consumers (Dalsgaard *et al.*, 2003). The analyses of TFA in this study of the STC, which include fatty acids occurring within both

neutral and polar lipid fractions of the zooplankton, represent longer term signatures and a more holistic approach to viewing the STC food web. Within-group and within-species comparisons (e.g. euphausiids) support the contention that there are distinct and measurable distinctions in the food web north and south of the STC over medium temporal scales (weeks to months).

## Summary

Whereas seasonality is critical in structuring plankton communities in polar regions (e.g. Falk-Petersen *et al.*, 2000), regionality is a prevalent feature at the STC. North/south differences in zooplankton food web structure and fatty acid profiles of euphausiids, pyrosomes and a hyperiid amphipod species indicated differences in food quality during the recent past in the two regions of the STC. Sampling was done during a period of low phytoplankton productivity at the STC, therefore the signatures of the small animals having rapid turnover of major lipid components (e.g. over 2–3 weeks; Graeve *et al.*, 1994) reflected this lack of distinction in productivity from north to south. Larger animals, however, are subject to slower biochemical turnover rates and thus their tissue biochemistry reflected previous disparate food conditions occurring in the north and south STC regions at somewhat longer time scales. Of particular significance, the prevalence of carnivory in the northern STC and of herbivory in the south was not detected using stable isotope ratios, thus emphasizing the need to utilize several methods to deduce feeding habits in aquatic systems. Molecular methods such as stable isotope and fatty acid signatures probably reflect disparate time scales and are differentially sensitive to dietary inputs. As an additional result of interest, fatty acid profiles indicated some herbivory by *Themisto gaudichaudii*, a species known as a strict carnivore based on previous data on gut contents, stable isotope ratios, and feeding appendage structures; thus, feeding dynamics in *T. gaudichaudii* are probably more complicated than previously thought. This study sheds light on regional differences in pelagic food webs at an important transition zone in the Southern Ocean.

## SUPPLEMENTARY DATA

Supplementary data can be found online at <http://plankt.oxfordjournals.org>.

## ACKNOWLEDGMENT

I acknowledge the officers and crew of the *S.A. Agulhas* and scientific personnel from Rhodes University and the University of Cape Town (UCT) for their assistance during sampling. Nutrient data were provided courtesy of the Oceanography team from UCT. The ocean colour and long-term temperature data were acquired using the GES-DISC Interactive Online Visualization and aNalysis Infrastructure (Giovanni) as part of the NASA's Goddard Earth Sciences (GES) Data and Information Services Center (DISC). Collection and analysis comply with the current laws in South Africa.

## FUNDING

Funding was provided through grants to P. W. Froneman, Southern Ocean Group, from the Department of Environmental Affairs and Tourism (via the South African National Antarctic Program) and administered by the National Research Foundation. Additional funds and facilities from Rhodes University and the University of Cape Town are appreciated.

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