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Fatty acid profiles reveal temporal and spatial differentiation in diets within and among syntopic rocky shore suspension-feeders

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ABSTRACT: Regional and temporal variations in the diets of rocky shore suspension-feeders (the volcano barnacle *Tetraclita serrata*, the brown mussel *Perna perna* and the reef-building polychaete *Gunnarea gaimardi*) were assessed using fatty acid profiling. Specimens were collected up-current and down-current of a river mouth in 2 coastal regions ~50 km apart along southeastern South Africa during March and July of 2009. One of the rivers represents a marine-dominated system, and the other a freshwater-dominated system. Our aims were to assess any dietary differences among the 3 suspension-feeders, spatial changes in diet within each species (at regional and local scales—50 and 15 km, respectively), and temporal changes in diet within each species. Fatty acid profiles clearly distinguished the species, with barnacles characterised by dinoflagellate and zooplankton-associated fatty acids; polychaetes, by diatom-associated fatty acids; and mussels, by a combination of mixed phytoplankton and mollusc-specific fatty acids (non-methylene interrupted). These interspecific differences probably arose in part from the contrasting feeding mechanisms employed. The distinctions in diet contribute to ecological partitioning of the suspended food within a highly competitive habitat. Regional- and local-scale intraspecific differences in diets were minimal to absent, but temporal distinctions in intraspecific diets were dominant features in the data set, confirming that the trophic environment for suspension-feeders can change markedly throughout a year.

KEY WORDS: *Perna perna* · *Tetraclita serrata* · *Gunnarea gaimardi* · River discharge · Suspended particulate matter · Niche partitioning · South Africa

INTRODUCTION

Sessile suspension-feeders are the dominant features of wave-exposed rocky shores (Sink et al. 2005). Filtration by such consumers allows the transfer of energy from the water column to the benthos, therefore creating an important trophic connection between adjacent habitats (Dolmer 2000). Coastal suspension-feeders derive their food from a variety of particulate sources including phytoplankton, bacteria, zooplankton and detritus (the latter comprised of a plethora of potential sources including macroalgae, microalgae, higher plants and animal material; Duggins et al. 1989, Ward & Shumway 2004). Terrestrially derived organic matter can represent an important additional source of nutrition for nearshore coastal communities, particularly those in the vicinity of estuaries (Grange & Allanson 1995, Tallis 2009). However, higher plants generally represent low-quality food to suspension-feeders unless the material is substantially decomposed (McLeod & Wing 2009), and researchers have shown that terrestrial carbon has little impact in some rocky shore regions (Hill et al. 2008). As plankton communities and primary productivity in coastal and offshore waters are dynamic (Brown 1992, Barlow et al. 2010), and rocky shores may receive variable quantities and types of particulates (Bustamante & Branch 1996, Tallis 2009),

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the diets of these ecologically important consumers likely change through space and time.

Fatty acids are useful for identifying the presence of various organic matter sources in an aquatic environment and estimating source contributions to animal diets (Graeve et al. 1997, Peters et al. 2006). As such, fatty acids can reveal seasonal and spatial changes in the diets of consumers. Fatty acids have been successfully used to describe food web interactions in a variety of ecological studies involving microalgae or macroalgae (Shin et al. 2008), zooplankton (Peters et al. 2006), freshwater and marine fish (Tocher 2003) and seabirds (Richoux et al. 2010). Some studies have focussed specifically on estuarine and/or marine suspension-feeding invertebrates (Zhukova 2000, Freites et al. 2002, Alfaro et al. 2006). Researchers that have examined the contributions of different food to estuarine consumers have noted that minor to substantial proportions of terrestrial plants support secondary production of bacteria, zooplankton and benthic invertebrates (Alfaro et al. 2006, Richoux & Froneman 2008). Fewer researchers have assessed spatial or temporal patterns in the natural food quality or availability for rocky shore suspension-feeders using fatty acid profiles. Ventrella et al. (2008) examined the diet of the subtidal mussel *Mytilus galloprovincialis* at 3 sites (each separated by >15 km) in the Adriatic Sea using fatty acids, and during 2 periods of a single year (April and October). In that study, the collection time substantially influenced mussel fatty acid compositions rather than site, with ω3 polyunsaturated fatty acid (PUFA) levels generally greater in April and saturated fatty acids (SFA) more prominent in October (Ventrella et al. 2008). Results like these are typically explained by variations in the seston composition (Ventrella et al. 2008) or temperature (Hall et al. 2002). In a South African study, Allan et al. (2010) examined fatty acid and stable isotope signatures in the mussel *Perna perna* at several locations along the southeast coast, and found strong evidence of the effects of localised upwelling on mussel diets. In addition to spatial and temporal variations in food sources, animal food preferences or selective abilities can influence their fatty acid profiles (Kharlamenko et al. 2001). Even within the same species, body size had a large effect on fatty acid composition in barnacles in the Sea of Japan, whereas separate species of barnacles had similar profiles, thus indicating similar diets (Zhukova 2000). Further studies are needed on additional organisms and regions to increase our understanding of spatial and temporal changes in the diets of rocky shore suspension-feeders, and the interspecific differences between syntopic organisms occupying the same feeding guild.

The aim of our research was to assess the influences of location and time on the fatty acid profiles of select rocky shore suspension-feeders in the vicinity of 2 contrasting estuaries. The consumer profiles, in turn, were used to infer the contributions of potential sources to consumer diets. The general approach was to collect 3 invertebrate species from 2 coastal regions adjacent to estuary mouths, on 2 separate occasions within a single year. Collections were made at 2 sites up-current and down-current of each estuary mouth to evaluate diet quality and to assess spatial variations at a subregional scale. The following hypotheses were tested: (1) all 3 species fall within the same trophic guild (suspension-feeding sessile benthos) and have access to the same food sources, but they exhibit disparate fatty acid profiles owing to interspecific differences in feeding mechanisms or selective abilities, (2) fatty acids in the invertebrates vary regionally due to differences in freshwater inputs (which influence the detrital pool), (3) given that the coastal region of the study area is dominated by a major southern-flowing current, consumers down-current (south) of an estuary mouth demonstrate a greater dependence on terrestrially derived food sources than those up-current (north) and (4) fatty acid signatures in the invertebrates change temporally as result of variations in available food sources.

MATERIALS AND METHODS

Study regions

The Kariega and Great Fish Rivers drain into the Indian Ocean from the warm-temperate southeast coast of South Africa (Fig. 1). Both river systems remain permanently open to the ocean. The Kariega Estuary is a freshwater-deprived and well-mixed marine-dominated system with abundant seagrasses and salt marshes along the banks, and a catchment of ~680 km^2 (Taylor 1988). The total chlorophyll concentrations in the Kariega Estuary are typically low (<0.5 mg l^{-1}; Richoux & Froneman 2007), generally reflecting the slow phytoplankton growth rates conferred by limited macronutrient availability resulting from the reduced freshwater inflow (Grange & Allanson 1995). Suspended particulate material (SPM; ranging from 39 to 76 mg l^{-1}) in the estuary comprises macrophyte fragments, phytoplankton, inorganic particles, bacteria and other microorganisms (Grange &

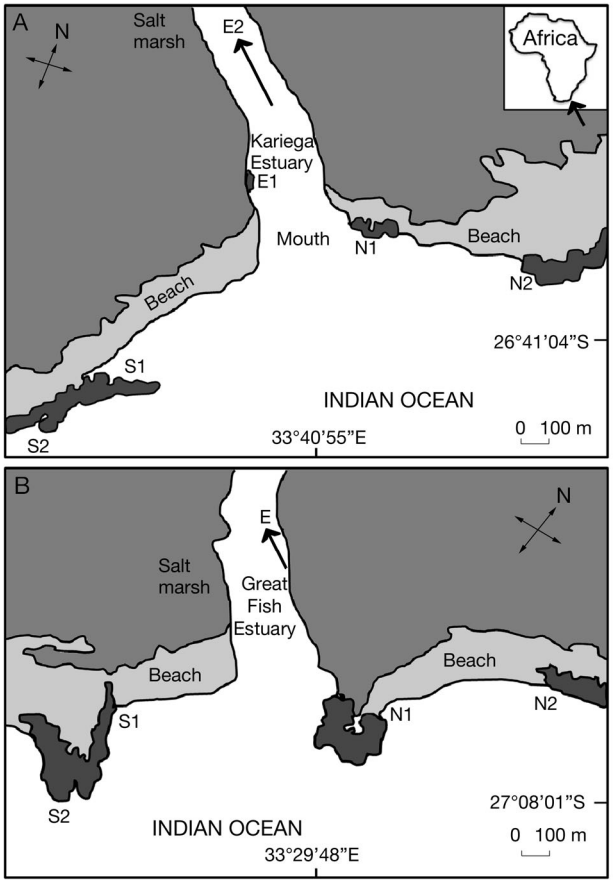

Fig. 1. Sampling locations in the (A) Kariega and (B) Great Fish coastal regions along the southeast coast of South Africa. Dark grey patches indicate rocky shore habitat. E: estuarine sites. Arrows next to E and E2 indicate that these sites are located upstream, 1 and 15 km, respectively; N: northern sites; S: southern sites

Allanson 1995). The Great Fish River drains a larger catchment of ~30 400 km^2 and regularly receives freshwater; it is therefore classified as a river-dominated meso-eutrophic system with significant terrestrial inputs (Grange et al. 2000, Jennings 2005). A continuous supply of nutrients can result in elevated phytoplankton biomass within the Great Fish system (Grange et al. 2000), although high turbidity from allochthonous detrital inputs generally limits phytoplankton production (Jennings 2005). Periodic pulses of enhanced phytoplankton biomass can accumulate along with detrital matter through hydrodynamic trapping (Grange & Allanson 1995), and these pulses may represent important transfers of organic material to nearshore regions. Average concentrations of SPM (126 to 509 mg l^{-1}), particulate organic matter (POM; 29 to 76 mg l^{-1}) and chlorophyll *a* (chl *a*; 0.4 to 21.8 µg l^{-1}) are all generally higher in the Great Fish system than those in the Kariega system (Grange et al. 2000, Vorwerk et al. 2001). As an indication of the freshwater fluxes through each region, daily average flow rates (m^3 s^{-1}) in March 2009 were 0.0008 (Kariega) and 14.48 (Great Fish), and in July 2009 were 0.005 (Kariega) and 5.11 (Great Fish; Department of Water Affairs and Forestry database, www.dwaf.gov.za/hydrology).

Three indigenous species, the grey volcano barnacle *Tetraclita serrata* Darwin, the brown mussel *Perna perna* (Linnaeus, 1758) and the polychaete *Gunnarea gaimardi* (Quatrefages, 1848), were selected on the basis of their common sessile suspension-feeding mode and their healthy abundances along the coastline. *T. serrata* can probably capture a range of particles from zooplankton (upper range unknown) and large (20 to 200 µm) to small (2 to 20 µm) phytoplankton (Zhukova 2000, Riisgård & Larsen 2010). *P. perna* can filter small (2 to 100 µm) particles that may contain substantial amounts of free-living bacteria and microflagellates (Berry & Schleyer 1983), as well as larger particles up to 2 mm (Wong & Levinton 2006). Sabellid polychaetes like *G. gaimardi* build extensive banks of tubes made of sand grains, and they can filter a variety of particles ranging from phytoplankton to smaller micro- and macroalgal detritus, flagellates and bacteria (Davies et al. 1989).

Collections

Specimen collections were completed in 2009 during early austral autumn (March) and mid-winter (July). Five sites were selected at each of 2 coastal regions adjacent to the mouths of the Kariega Estuary (Fig. 1A) and the Great Fish Estuary (Fig. 1B). Of the 5 sites in each region, 2 were north of the mouth, 2 were south and one was located within the estuary mouth (Fig. 1). Sites within a region were 0.5 to 1.5 km distant from each other, and the 2 regions were ~50 km apart. Because the Kariega is a marine-dominated system, we collected water samples from the upper reaches of the estuary (~15 km upstream from the mouth) to obtain a true estuarine signature. The estuary mouth site (Site E) in the Kariega region was therefore divided into 2 sub-sites: Site E1 (estuary mouth) and Site E2 (upper reaches). Physical and chemical data (temperature, salinity, chl *a*, SPM concentrations) were collected from all sites to provide some basic information about the environment at the time of sampling; however, we expect that some of these factors can vary extensively through time, so our data represent mere snapshots. Water samples of

500 ml were collected in triplicate at all sites to determine chl *a* (µg l^{-1}) and SPM/POM (mg l^{-1}) concentrations. An additional 5 l of water was collected at each site for determination of SPM fatty acid profiles (each sample provided information on SPM at one instance in time and therefore is not representative of the long-term trophic environment of the suspension-feeders). Water was stored on ice in a cooler box during transport to the laboratory.

For the invertebrate collections, a single mussel (*Perna perna*), 10 barnacles (*Tetraclita serrata*) and 5 polychaetes (*Gunnarea gaimardi*) each constituted 1 fatty acid sample, multiplied by replicates of 3 per site and time. The level of replication was limited by the accessibility of the sites during each low tide cycle and processing times in the laboratory. Mussels could not be collected at the most northern site (Site N2) in the Great Fish region during March, owing to a rapid tidal shift on that occasion, and most species did not occur at the estuary mouth sites.

Sample preparation

All water samples were filtered gently (<5 cm Hg vacuum) onto pre-combusted (450°C) and pre-weighed filters (GF/F Whatman, 47 mm). Filters were visually inspected after filtration to remove large debris. Filters intended for fatty acid analysis were frozen at −80°C. Filters for determination of SPM dry mass (DM) were oven-dried at 50°C for 24 h and weighed (mg l^{-1}), after which the filters were ashed for 12 h at 450°C and re-weighed to determine POM DM (mg l^{-1}) concentrations. Chl *a* determinations followed Parsons et al. (1984); each filter was topped with 8 ml of 90% acetone and stored at −20°C overnight. Fluorometric readings were recorded (Turner Designs 10AU) before and after the addition of 2 drops of 1 M hydrochloric acid, and the readings were converted to chl *a* concentrations per unit volume (µg l^{-1}).

Collected animals were kept alive and aerated in ambient seawater for 1 d to allow their guts to clear prior to processing. Dissections were performed (on ice) where possible (e.g. polychaete dissections proved to be extremely problematic) to further ensure that recently digested food and reproductive tissues were not included in the analyses and enable ecologically valid interspecific comparisons: whole barnacles minus their guts and seminal vesicles, mussel adductor muscles and mantle margins, and whole polychaetes were retained and processed. Samples were frozen at −80°C, and all filters and tissues intended for fatty acid analyses were lyophilised (VirTis BenchTop K) for 24 h. Animal tissues were homogenised using a mortar and pestle, and subsamples (20 to 100 mg) were weighed into 15 ml glass tubes. Filter and animal samples were covered with 2 ml 0.01% butylated hydroxytoluene in chloroform, flushed with nitrogen gas and stored at −20°C.

Lipid extractions were done using a method modified from Folch et al. (1957), where samples were immersed in 2:1 chloroform/methanol (*v*/*v*) and stored overnight at −20°C. A solution of 0.9% potassium chloride was added to create 2 phases, and the top (aqueous) layers were discarded after several rinses. The final extract was dried with anhydrous sodium sulfate, concentrated to dryness under nitrogen atmosphere and stored in anhydrous dichloromethane. Known quantities of an internal standard (23:0; Supelco) were added to each sample for later quantification of fatty acid methyl esters (FAMEs; see Allan et al. 2010 for FAME synthesis protocol). Gas chromatogram (GC) analysis was completed on a Hewlett Packard 5890A Series II (splitless injections) fitted with a Zebron-Waxplus 320 capillary column (GC parameters as per Allan et al. 2010). FAME peaks were visualised using Clarity 2.6 software, identified using external standards (marine PUFA No. 1, 37 component FAMEs mix; Supelco) and confirmed using mass spectrometry on an Agilent 7000A GC/MS-QQQ coupled with a NIST 08 MS library (column and methods as per the GC runs). Each fatty acid was measured quantitatively as micrograms per milligram DM, and proportionally as a percentage of the total identified fatty acids (%TFA), and data are reported as mean ± SD.

Data analyses

Similarities in the untransformed qualitative fatty acid data (%TFA) were explored using non-metric multidimensional scaling (n-MDS). ANOSIM (analysis of similarity) procedures were performed on each Euclidean similarity matrix to determine whether species differed statistically (from each other, between regions, among sites, or between sample times) based on their entire fatty acid profiles (up to 39 fatty acids in total). The ANOSIM test statistic R, based on the ratio of the between-group to within-group similarity ranking, was reported as a value between −1 and +1 (1 indicates complete dissimilarity, 0 indicates complete similarity, with negative values rarely occurring) together with a corresponding

significance value. For factors with >2 levels (3 species, 2 or 3 site types [north of mouth: 2 sites, south of mouth: 2 sites, and in some cases estuary mouth]), R and significance values were calculated using pairwise comparisons as part of the ANOSIM procedure. SIMPER (similarity percentage) was used to assess which fatty acids were primarily responsible for any differences between groups. SIMPER results were compared with the loading results following principal components analysis (PCA) using the same data as outlined above, and since loadings are visually useful as projections onto PCA plots, we used the location of the PCA loadings as guideline estimates to superimpose the SIMPER results onto the n-MDS plots (as a visual aid only).

Differences of fatty acid groupings or marker ratios among species and between sample dates were determined on pooled data (regions and sites pooled based on the outcomes of the similarity analyses; raw or log-transformed data used depending on outcomes of residual analyses) using general linear models (GLM) and post hoc Tukey tests. Differences in quantitative SPM between March and July were assessed using *t*-tests. Analyses were conducted using Primer (v5), PAST 2.00 (Hammer et al. 2001), or Statistica 10.

RESULTS

Interspecific comparisons (Hypothesis 1)

We detected 39 fatty acids, each of which occurred in at least 1 of the species (Table 1), with 32 of these fatty acids detected in greater than trace amounts. Fatty acid profiles differed substantially among the species collected in each region and between months, with the polychaetes generally the most distinct from the mussels and barnacles (Fig. 2; Table A1 in the Appendix). The mussels and barnacles were similar to one another during March, but highly distinct from each other in both regions during July (Fig. 2; Table A1). Several fatty acids were influential in the interspecific differences: 20:5ω3, 20:1ω9, 14:0, 22:5ω3 and 22:4ω6 all contributed to the distinct profiles of the polychaetes. The fatty acids 20:2ω9, 22:2 (6,11) and 20:4ω6 were influential in separating the mussels from the other species, and 22:6ω3, 18:1ω9, 18:1ω7, 18:0 and 16:0 (the latter particularly in March) contributed substantially to the distinct profiles of the barnacles (Fig. 2).

The qualitative data (%TFA) indicated some interesting interspecific differences in some of the fatty acid groupings and ratio markers (Fig. 3, Table 1). PUFA, SFA and essential fatty acid (EFA) levels were generally consistent among the 3 species, whereas the macrophyte fatty acids were more prevalent in the mussels (Fig. 3). The diatom-associated fatty acids were most dominant in the polychaetes, and the dinoflagellate-associated fatty acids were much greater in the mussels and barnacles (Fig. 3). Quantitative data (μg mg^{-1} DM) showed some different patterns, with mussels having significantly decreased quantities of all sub-categories compared with the other 2 species (Fig. 4). The macrophyte-related fatty acids were not as dominant in the mussels (Fig. 4). Bacterial-associated fatty acids were found at similar levels in all 3 species (Table 1), although quantitatively there was a clear decrease of this component in the mussels (Fig. 4).

Regional and site comparisons (Hypotheses 2 and 3)

Potential regional (Kariega vs. Great Fish) and site differences were assessed using the fatty acid profiles of each species. Significant regional differences were detected in the mussels (Fig. 5A) and barnacles (Fig. 5C) during March, but there was no regional effect on the polychaetes (Fig. 5E, Table A2 in the Appendix). SIMPER (and PCA loadings) indicated that where there were regional differences, the animals from the Kariega region showed increased EFAs relative to those in the Great Fish region (Fig. 5A,C). There were no regional effects on any species during July (Fig. 5B,D,F, Table A2). To detect any effects of site within each region on the fatty acid profiles of each species, a 'site' category (north of mouth, south of mouth and in some cases estuary mouth) was included as a factor in the n-MDS and ANOSIM. Site effects occurred infrequently: north vs. south distinctions were apparent only in the barnacles during March (Tables A2 & A3 in the Appendix) and in the Kariega mussels (Table A3). Specific fatty acids contributing to any site differences were not readily apparent, as the site effects were statistically and ecologically weak (global R terms for 'site' in Tables A1, A2 & A3, all <0.3).

Temporal comparisons (Hypothesis 4)

Temporal differences in the fatty acid profiles of the 3 species were highly dominant features in all of the analyses performed and they overshadowed

Table 1. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*, suspended particulate material (SPM). Qualitative (%TFA, percentage of total identified fatty acids) and quantitative (µg mg^{-1} dry mass) fatty acid or fatty acid group data (mean ± SD). Regions and sites have been pooled (following ANOSIM, which for the most part revealed minimal to no ecologically relevant regional or site distinctions within each species). Fatty acids found in trace amounts but not included in the table: ai-15:0, 18:2ω3, 22:1ω11, 20:3ω6, 19:0, 20:4ω3 and 22:1ω9. NMID: non-methylene interrupted diene; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: saturated fatty acids; EFA: essential fatty acids, including 20:4ω6, 20:5ω3, 22:6ω3, 18:3ω3 and 18:2ω6; bacterial: all odd numbered and branched (i- or ai-) fatty acids; macrophytes: 18:2ω6 + 18:3ω3; sum ω3: sum of all ω3 fatty acids; sum ω6: sum of all ω6 fatty acids; ω3/ω6: ratio of ω3 to ω6 fatty acids; P/S: PUFA to SFA ratio; diatom index: (16:1ω7 + 20:5ω3)/(18:2ω6 + 18:2ω3 + 18:3ω3 + 18:4ω3 + 18:4ω3 + 22:6ω3); total FA: total fatty acids

	Perna perna (mussels)		*Tetraclita serrata* (barnacles)		*Gunnarea gaimardi* (polychaetes)		SPM	
	Mar	Jul	Mar	Jul	Mar	Jul	Mar	Jul
Fatty acid (%TFA)								
14:0		3.6 ± 1.4	5.2 ± 2.3	2.4 ± 0.9	8.0 ± 2.1	6.1 ± 1.3	3.5 ± 1.6	3.4 ± 1.1
i-15:0	0.2 ± 0.2	0.2 ± 0.1	0.7 ± 0.5	0.2 ± 0.1	1.6 ± 0.5	1.6 ± 0.4	0.7 ± 0.3	0.6 ± 0.3
15:0	1.1 ± 0.4	0.9 ± 0.3	0.9 ± 0.4	0.5 ± 0.2	1.3 ± 0.5	1.2 ± 0.2	2.5 ± 1.1	3.1 ± 1.3
i-16:0	0.4 ± 0.2	0.3 ± 0.1	1.7 ± 0.6	2.0 ± 0.4	0.9 ± 0.3	0.7 ± 0.2	0.9 ± 0.6	0.4 ± 0.5
16:0	20.1 ± 3.2	17.9 ± 4.3	22.7 ± 3.0	17.0 ± 2.3	16.4 ± 2.0	15.3 ± 1.7	24.4 ± 2.8	23.2 ± 3.4
i-17:0	1.0 ± 0.3	1.0 ± 0.3	0.5 ± 0.2	0.3 ± 0.1	1.1 ± 0.2	0.9 ± 0.5	0.3 ± 0.3	0.4 ± 0.3
17:0	1.8 ± 0.5	1.8 ± 0.4	1.6 ± 0.3	1.4 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	2.6 ± 1.0	2.4 ± 1.0
i-18:0	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.0 ± 0.1	0.1 ± 0.2	0.8 ± 0.9	1.9 ± 0.9
18:0	5.9 ± 1.2	5.1 ± 0.6	9.2 ± 1.5	8.2 ± 0.8	6.8 ± 0.8	5.6 ± 0.8	8.7 ± 1.8	7.9 ± 1.2
20:0	0.5 ± 0.6	1.3 ± 0.6	0.7 ± 0.6	0.8 ± 0.2	0.4 ± 0.3	0.2 ± 0.3	0.7 ± 1.2	0.5 ± 0.4
22:0	0.5 ± 0.3	0.6 ± 0.3	0.9 ± 0.3	1.0 ± 0.5	0.2 ± 0.3	0.3 ± 0.3	2.8 ± 1.4	3.8 ± 0.9
24:0	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	2.5 ± 2.0	1.7 ± 0.4
16:1ω7	6.1 ± 3.2	4.5 ± 2.7	4.5 ± 1.0	2.0 ± 0.4	3.0 ± 0.7	3.2 ± 0.6	3.6 ± 1.5	4.3 ± 0.9
16:1ω5	1.0 ± 0.3	1.1 ± 0.4	0.1 ± 0.2	0.3 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.2 ± 0.6	0.0 ± 0.1
18:1ω9	1.7 ± 0.5	2.5 ± 1.1	5.6 ± 0.5	5.4 ± 0.9	1.9 ± 0.8	1.8 ± 0.3	10.3 ± 9.6	15.9 ± 2.2
18:1ω7	2.7 ± 0.9	2.3 ± 1.0	5.6 ± 1.5	5.8 ± 0.8	5.4 ± 0.7	4.6 ± 0.4	12.7 ± 9.3	5.9 ± 3.2
20:1ω9	1.1 ± 1.0	2.4 ± 0.6	0.0 ± 0.1	2.0 ± 0.7	5.6 ± 0.6	4.9 ± 0.7	0.0 ± 0.1	0.0 ± 0.0
20:1ω7	2.6 ± 0.9	1.2 ± 0.7	1.5 ± 0.5	1.1 ± 0.6	1.6 ± 0.3	1.9 ± 0.3	0.9 ± 2.9	8.5 ± 2.2
18:2ω6	2.2 ± 0.5	2.0 ± 0.3	1.2 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	2.7 ± 3.0	1.0 ± 0.5
20:2ω9	3.9 ± 2.0	4.5 ± 1.6	0.4 ± 0.6	0.6 ± 0.5	0.6 ± 0.3	0.6 ± 0.3	6.3 ± 2.6	3.4 ± 2.1
20:2ω6	0.6 ± 0.4	0.3 ± 0.1	0.9 ± 0.6	0.6 ± 0.3	0.9 ± 0.2	0.7 ± 0.2	4.9 ± 4.3	0.2 ± 0.4
22:2 9,17 NMID	1.5 ± 0.7	1.6 ± 0.6	0.7 ± 0.8	0.0 ± 0.0	2.0 ± 0.5	1.9 ± 0.3	0.1 ± 0.2	0.2 ± 0.5
22:2 6,11 NMID	4.1 ± 1.5	4.5 ± 1.3	0.0 ± 0.1	0.0 ± 0.1	3.5 ± 0.8	3.5 ± 0.6	0.6 ± 0.8	0.0 ± 0.0
18:3ω3	1.4 ± 0.9	1.3 ± 0.4	0.9 ± 0.3	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.6	1.2 ± 0.6
18:4ω3	1.4 ± 0.7	1.5 ± 0.6	1.3 ± 0.6	0.9 ± 0.5	0.2 ± 0.2	0.3 ± 0.2	1.0 ± 1.3	1.1 ± 0.6
20:4ω6	5.0 ± 1.1	5.0 ± 1.3	1.7 ± 0.5	2.1 ± 0.4	3.7 ± 0.6	4.2 ± 1.0	0.2 ± 0.3	0.7 ± 0.5
22:4ω6	1.6 ± 0.5	1.8 ± 0.5	0.6 ± 0.7	0.2 ± 0.3	3.8 ± 1.0	4.1 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
20:5ω3	10.4 ± 2.8	9.4 ± 2.1	12.2 ± 2.8	17.9 ± 2.0	18.8 ± 1.6	21.5 ± 1.4	1.1 ± 0.5	3.3 ± 1.5
21:5ω3	1.4 ± 0.5	1.6 ± 0.4	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.1	0.7 ± 0.3	0.7 ± 1.0	0.1 ± 0.2
22:5ω6	1.1 ± 0.4	1.3 ± 0.6	0.5 ± 0.5	0.4 ± 0.1	0.6 ± 0.3	0.7 ± 0.6	0.1 ± 0.3	0.1 ± 0.3
22:5ω3	2.1 ± 0.6	2.2 ± 0.6	0.8 ± 0.2	0.7 ± 0.2	3.9 ± 0.9	4.2 ± 0.6	0.0 ± 0.2	0.1 ± 0.2
22:6ω3	11.0 ± 2.8	14.3 ± 3.3	13.4 ± 3.7	21.5 ± 2.7	2.5 ± 0.5	4.0 ± 0.7	0.7 ± 1.0	1.4 ± 0.9
Fatty acid group (%TFA)								
Sum PUFA	48.2 ± 6.8	51.9 ± 7.9	36.0 ± 6.3	47.6 ± 3.6	43.3 ± 5.0	48.8 ± 3.5	20.0 ± 6.3	13.6 ± 2.3
Sum MUFA	15.4 ± 3.6	14.5 ± 3.0	18.1 ± 2.4	17.2 ± 1.6	18.5 ± 1.6	17.8 ± 1.3	29.0 ± 3.7	36.4 ± 4.4
Sum SFA	36.4 ± 5.4	33.6 ± 5.7	45.9 ± 4.8	35.2 ± 3.4	38.2 ± 4.7	33.4 ± 3.3	50.9 ± 6.5	50.0 ± 3.6
Sum EFA	30.0 ± 3.5	32.1 ± 4.8	29.4 ± 6.6	43.1 ± 4.1	26.7 ± 2.3	31.6 ± 1.8	5.4 ± 2.7	7.7 ± 2.0
Bacterial	6.5 ± 1.4	6.5 ± 0.9	7.2 ± 1.6	5.6 ± 0.8	6.8 ± 1.3	6.7 ± 0.9	9.1 ± 2.0	9.5 ± 2.3
Macrophytes	3.6 ± 1.3	3.3 ± 0.6	2.1 ± 0.4	1.6 ± 0.3	1.7 ± 0.3	1.9 ± 0.3	3.5 ± 3.1	2.3 ± 1.0
Sum ω3	28.0 ± 4.5	30.6 ± 4.7	30.0 ± 6.7	42.7 ± 3.9	26.8 ± 2.6	31.6 ± 2.0	5.1 ± 2.1	8.0 ± 2.2
Sum ω6	14.8 ± 3.3	15.2 ± 3.4	5.0 ± 1.0	4.3 ± 0.7	13.8 ± 2.4	14.7 ± 2.7	8.5 ± 5.0	2.0 ± 1.0
ω3/ω6	2.0 ± 0.6	2.1 ± 0.5	6.3 ± 2.2	10.2 ± 2.0	2.0 ± 0.3	2.2 ± 0.4	0.9 ± 0.9	5.1 ± 3.2
P/S	1.4 ± 0.4	1.6 ± 0.4	0.8 ± 0.2	1.4 ± 0.2	1.2 ± 0.3	1.5 ± 0.3	0.4 ± 0.2	0.3 ± 0.1
22:1 + 20:1	3.9 ± 1.2	4.0 ± 0.8	2.2 ± 0.9	3.7 ± 1.2	7.5 ± 0.9	7.4 ± 1.3	1.7 ± 3.3	9.6 ± 2.2
18:1ω9 + 18:1ω7	4.4 ± 1.2	4.7 ± 1.3	11.2 ± 1.8	11.2 ± 1.3	7.3 ± 1.3	6.4 ± 0.6	22.9 ± 2.2	21.8 ± 3.4
Diatom index	1.1 ± 0.4	0.8 ± 0.3	1.0 ± 0.1	0.8 ± 0.1	5.0 ± 0.6	4.1 ± 0.7	1.6 ± 2.1	1.5 ± 0.6
22:6ω3/20:5ω3	1.1 ± 0.4	1.6 ± 0.5	1.1 ± 0.2	1.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.6 ± 0.9	0.5 ± 0.2

Table 1 (continued)

	Perna perna (mussels)		*Tetraclita serrata* (barnacles)		*Gunnarea gaimardi* (polychaetes)		SPM	
	Mar	Jul	Mar	Jul	Mar	Jul	Mar	Jul
Fatty acid group (µg mg^{-1} dry mass)								
Total FA	21.7 ± 9.3	11.6 ± 2.9	56.7 ± 20.4	29.4 ± 7.4	54.5 ± 21.1	27.3 ± 7.2	6.6 ± 5.9	2.6 ± 0.9
Sum PUFA	10.2 ± 4.1	5.9 ± 1.5	20.3 ± 8.0	13.9 ± 3.5	23.3 ± 8.7	13.2 ± 3.2	1.4 ± 1.6	0.4 ± 0.1
Sum MUFA	3.4 ± 1.7	1.7 ± 0.6	10.2 ± 3.6	5.1 ± 1.3	10.2 ± 4.2	4.9 ± 1.3	2.0 ± 1.9	1.0 ± 0.4
Sum SFA	8.1 ± 4.0	3.9 ± 1.3	26.2 ± 9.8	10.4 ± 2.9	21.0 ± 9.0	9.2 ± 2.9	3.2 ± 2.7	1.3 ± 0.4
Sum EFA	6.4 ± 2.6	3.7 ± 1.1	16.7 ± 7.1	12.6 ± 3.1	14.4 ± 5.3	8.6 ± 2.3	0.3 ± 0.2	0.2 ± 0.1
Bacterial	1.4 ± 0.7	0.7 ± 0.2	4.0 ± 1.6	1.7 ± 0.5	3.7 ± 1.7	1.8 ± 0.6	0.6 ± 0.6	0.3 ± 0.1
Macrophytes	0.8 ± 0.5	0.4 ± 0.1	1.2 ± 0.6	0.5 ± 0.2	0.9 ± 0.4	0.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.0
Sum ω3	5.9 ± 2.4	3.6 ± 1.1	17.0 ± 7.1	12.5 ± 3.2	14.5 ± 5.4	8.6 ± 2.3	0.4 ± 0.5	0.2 ± 0.1
Sum ω6	3.2 ± 1.5	1.6 ± 0.4	2.8 ± 1.2	1.3 ± 0.4	7.3 ± 2.8	3.9 ± 0.9	0.6 ± 0.7	0.1 ± 0.0
22:1 + 20:1	0.8 ± 0.3	0.5 ± 0.1	1.2 ± 0.6	1.1 ± 0.5	4.0 ± 1.6	2.0 ± 0.6	0.1 ± 0.2	0.3 ± 0.1
18:1ω9 + 18:1ω7	1.0 ± 0.5	0.6 ± 0.2	6.3 ± 2.3	3.3 ± 0.9	4.1 ± 1.8	1.7 ± 0.5	1.6 ± 1.6	0.6 ± 0.3

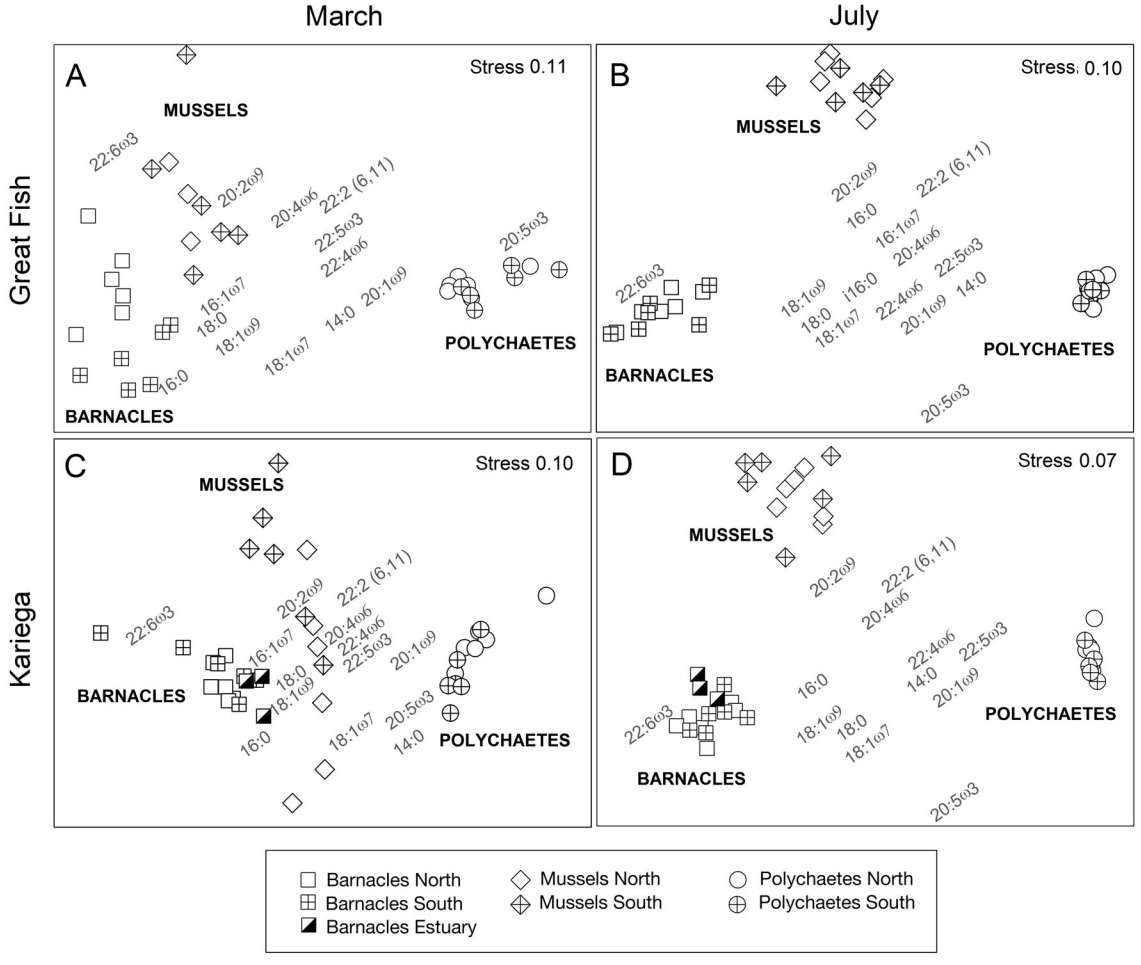

Fig. 2. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*. n-MDS output of consumers in the Great Fish (A,B) and Kariega (C,D) regions during March (A,C) and July (B,D) 2009. Fatty acids influential in separating the species (derived from SIMPER and PCA) are superimposed; refer to Table A1 in the Appendix for ANOSIM output

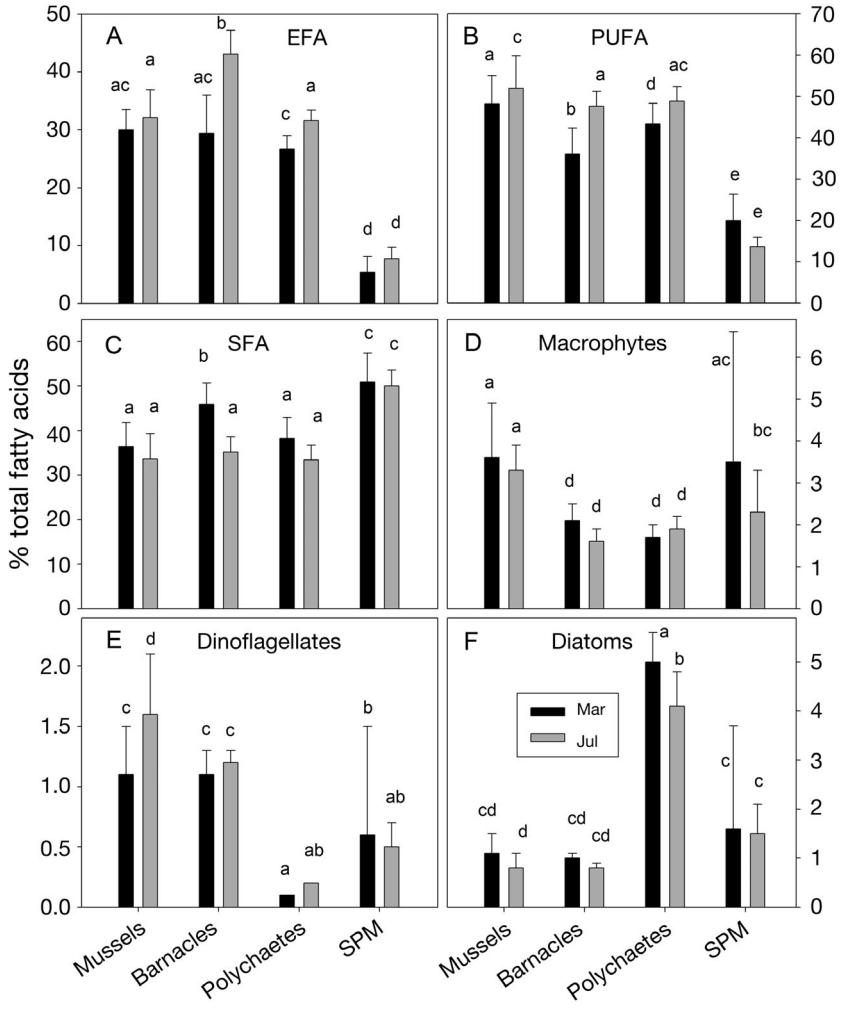

Fig. 3. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*, suspended particulate material (SPM). Comparisons of qualitative data (% total fatty acids) for (A) EFA (essential fatty acids), (B) PUFA (polyunsaturated fatty acids), (C) SFA (saturated fatty acids), (D) macrophyte (18:3ω3 + 18:2ω6), (E) dinoflagellate (22:6ω3/20:5ω3) and (F) diatom (16:1ω7 + 20:5ω3)/(18:2ω6 + 18:2ω3 + 18:3ω3 + 18:4ω3 + 18:4ω3 + 22:6ω3) indices in March and July (Kariega and Great Fish regions pooled). Lower-case letters: significant differences as per the general linear model output (factors 'species' and 'month'; n = 166 for each model, significance at $p < 0.05$)

any regional or site effects. The profiles of each species were distinct between March and July in both the Kariega and Great Fish regions (Fig. 6, Table A3). Temporal changes were most distinct and highly statistically significant when considering the quantitative data (Table 1, Fig. 4), with fatty acid concentrations consistently greater in all species during March. This overwhelming quantitative dominance of fatty acids in March was not reflected in the proportional data (Table 1, Fig. 3), and in many cases the suspension-feeder tissues were qualitatively enhanced during July (Fig. 6).

Water characteristics

The fatty acid profiles of the SPM were assessed to provide only snapshot indications of the dietary environment for the suspension-feeders at the times of their collection (i.e. the data do not shed light on feeding history). Like the consumers, quantitative concentrations (not qualitative levels) of fatty acids were measurably higher in the SPM collected in March compared with July (Fig. 4). Ordination analysis using n-MDS and ANOSIM revealed significant regional (global R = 0.34, significance <0.001) and temporal (global R = 0.66, significance <0.05) effects on the SPM fatty acid profiles (Fig. 7), although the SIMPER (and PCA loadings) results did not reveal ecologically meaningful influential fatty acid results relative to those of the consumers.

The water physico-chemical data were also snapshots and they indicated few remarkable distinctions among sites or between regions. SPM concentrations (mean ± SD; mg l^{-1}) were typically variable: Great Fish coastal region in March = 96 ± 81 and July = 32 ± 8, Kariega coastal region in March = 32 ± 6 and July = 25 ± 5. POM concentrations (mean ± SD; mg l^{-1}) were fairly comparable between regions and dates: Great Fish coastal region in March = 8.1 ± 3.9 and July = 7.8 ± 5.5, Kariega coastal region in March = 5.6 ± 0.8 and July = 4.9 ± 1.2. Chl *a* concentrations were fairly low at all sites in both March and July (ranged from 0.1 to 0.7 µg l^{-1}). Changes in mean water temperatures between regions and months were minimal (Kariega in March = 18.5°C and July = 17.7°C, Great Fish in March = 15.0°C and July = 17.3°C). Salinity readings along the coast were between 35 and 37 and did not indicate a measurable influence of freshwater outflow from either estuary (apart from a single salinity reading of 27 just south of the Great Fish Estuary during March). Hypersaline conditions were apparent in the upper reaches of the Kariega Estuary (Site E2), which is typical for this system during dry periods.

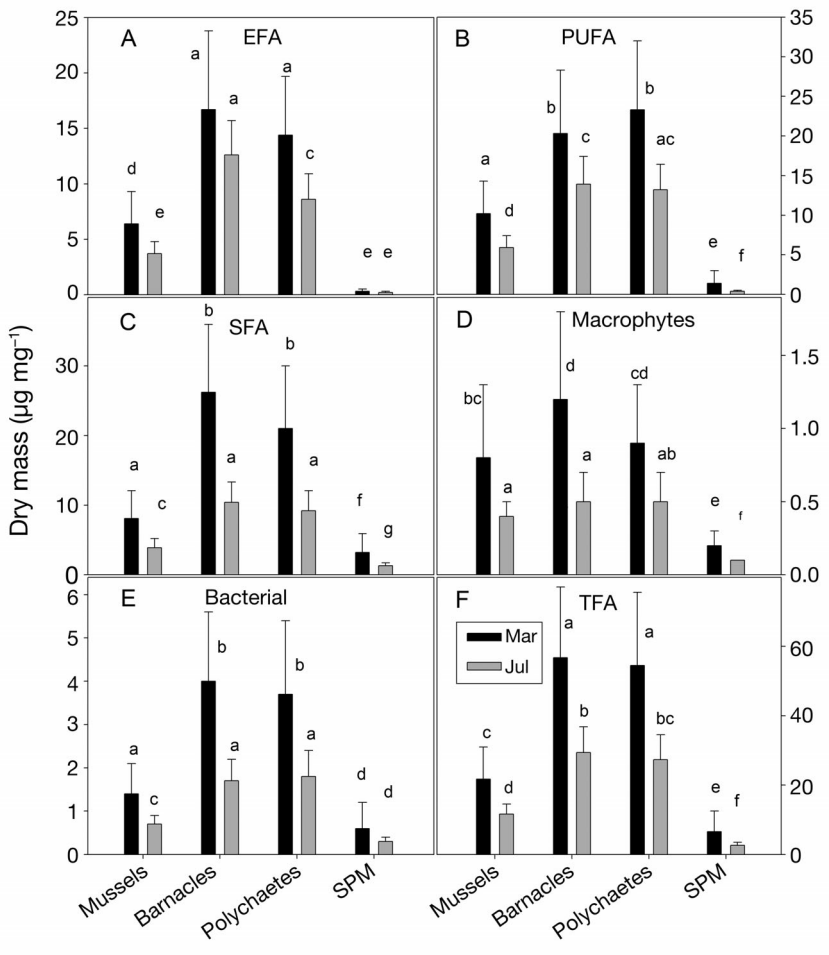

Fig. 4. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*, suspended particulate material (SPM). Comparisons of quantitative data (µg mg^{-1} dry mass) for (A) EFA (essential fatty acids), (B) PUFA (polyunsaturated fatty acids), (C) SFA (saturated fatty acids), (D) macrophyte (18:3ω3 + 18:2ω6), (E) bacterial (sum of odd chain and branched fatty acids) and (F) TFA (total fatty acids) in March and July (Kariega and Great Fish regions pooled). Lower-case letters for the consumers: significant differences as per the general linear model output (factors 'species' and 'month'; n = 166 for each model, significance at $p < 0.05$). SPM was analysed separately from the consumers using *t*-tests (n = 19 for each model, significance at $p < 0.05$)

DISCUSSION

Interspecific differences in diet (Hypothesis 1)

Three syntopic invertebrates (mussels *Perna perna*, barnacles *Tetraclita serrata* and reef-building polychaetes *Gunnarea gaimardi*) from 2 regions of the southeastern South African coastline had highly distinct fatty acid profiles. These 3 species occupy the same sessile suspension-feeding trophic guild and are exposed to similar physical and biological conditions over time in each region. There are, however, a number of factors that may have contributed to the observed species-specific fatty acid profiles, including disparate particle-capturing mechanisms, basic metabolic physiology and intertidal locations.

Particle-capturing mechanisms are distinct among the species studied, so the animals probably have very different selective abilities when exposed to a complex seston mixture and different responses to shifts in the composition of that mixture. As a result, the species have different diets despite their common locale. Such distinctions may contribute to the partitioning of the trophic environment and hence facilitate coexistence of these abundant organisms in a highly competitive environment (i.e. for space). *Tetraclita serrata* is an acorn barnacle that can filter-feed actively by generating feeding currents with its setose feeding legs (cirri), but it typically feeds passively by orienting the cirri into the flow of water (Hunt & Alexander 1991). Stomach content analysis of *T. squamosa* indicated an ability for this species to capture a large size range and type of particles (10 µm to 1 mm), with the contents dominated by flagellates, diatoms and zooplankton, and some minor contributions of filamentous algae, fine particulates and foraminiferans (Hunt & Alexander 1991). Direct observations of actively feeding barnacles have confirmed their ability to select 'preferred' particles (potentially through chemoreception) and reject unwanted material (Hunt & Alexander 1991), but information about the selective abilities of different species is scarce relative to groups such as bivalves.

Large-particle capturing characteristics were not readily apparent from the fatty acid profiles of *Tetraclita serrata* when considering some previously described markers for carnivory (e.g. prevalence of 22:1 + 20:1 or high PUFA/SFA and 18:1ω9/18:1ω7 ratios; Graeve et al. 1997, Cripps & Atkinson 2000). However, levels of 18:1ω9 and 18:1ω7 were higher in the barnacles (11.2%TFA) compared with the mussels (4.5%) and polychaetes (6.9%; Table 1). Controlled feeding experiments have indicated significantly higher levels of 18:1ω7 in rotifers relative to their microalgal diets (Shin et al. 2008); thus, it is possible that the C18 monounsaturates in the barnacle

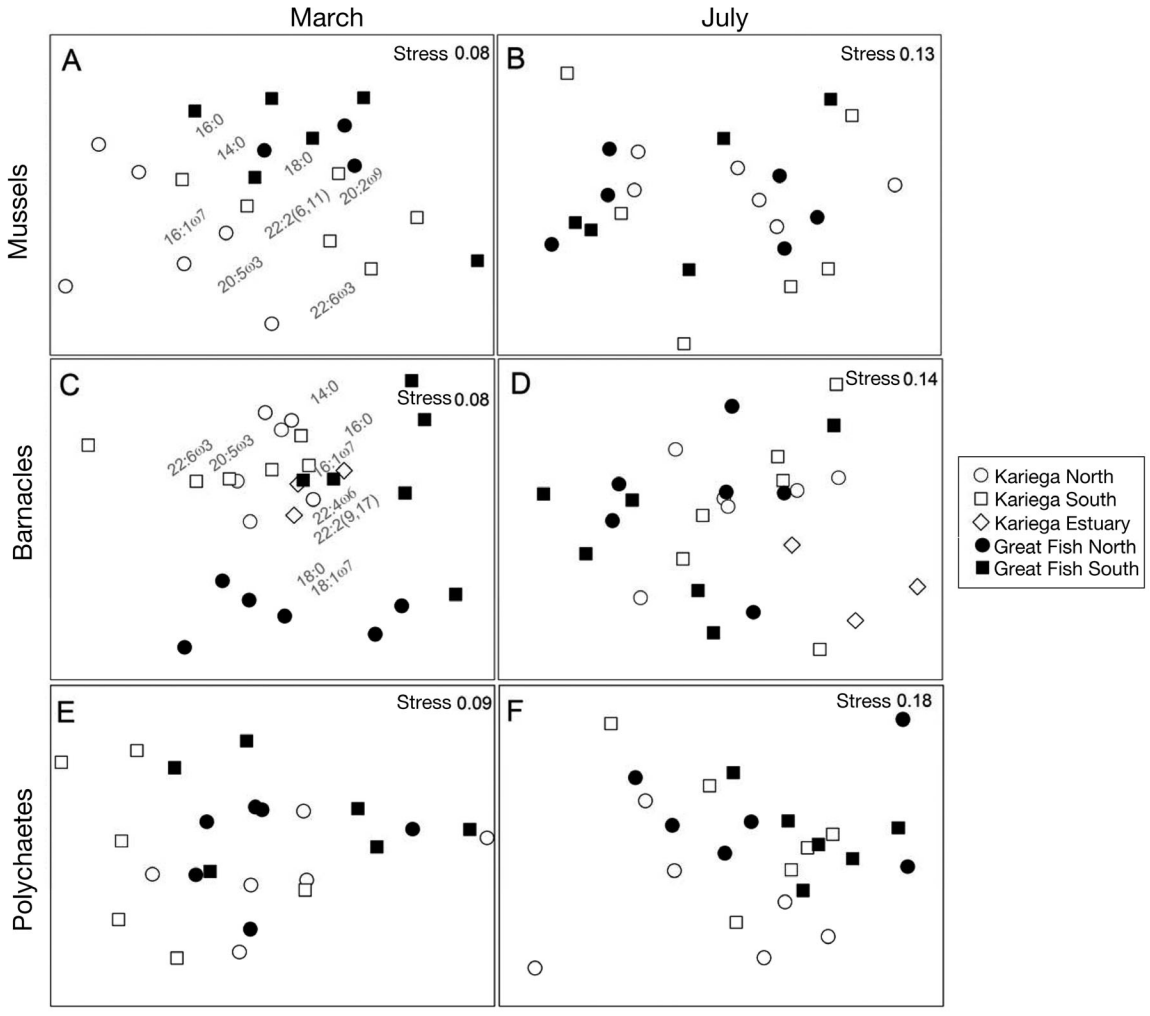

Fig. 5. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*. n-MDS output showing regional and site comparisons for each species during March and July 2009. Fatty acids influential in separating the species (derived from SIMPER and PCA) are superimposed; refer to Table A2 in the Appendix for ANOSIM output

diets originated from zooplankton that were preferentially accumulating these fatty acids (or possibly modifying 18:1ω7 from 16:1ω7; Shin et al. 2008). Furthermore, Sargent & Falk-Petersen (1988) have documented 18:1ω9 as a predominant feature in heterotrophic marine organisms; thus, this fatty acid has potential as a carnivory marker for suspension-feeders in our study, particularly if it is combined with 18:1ω7 (Table 1). Significant levels of carnivory were strongly reflected in the nitrogen isotopic signatures of *T. serrata*, which were the most enriched (10.7‰) compared with *Perna perna* (7.9‰) and *Gunnarea gaimardi* (9.2‰; Richoux et al. 2013). The fatty acid profiles of *T. serrata* also suggest diets that include significant contributions of diatoms and particularly dinoflagellates (20:5ω3 and 22:6ω3, respectively; potential markers described in Kharlamenko et al. 1995, 2001, Graeve et al. 1997). The main fatty acid that distinguished *T. serrata* from the other species was 22:6ω3 (especially in specimens collected in July; Table 1, Fig. 2). The prevalence of 22:6ω3 in the barnacles may indicate their preferential selection of dinoflagellates (a taxonomic group containing many heterotrophic forms), which may contribute further to the higher trophic level of barnacles relative to the other suspension-feeders. This important PUFA, 22:6ω3, can serve as an indicator of carnivory in invertebrates in some cases (Kharlamenko et al. 1995).

Suspension-feeding mussels feed in a very different manner from both acorn barnacles and sabellid polychaetes, as the bivalves are obligate active feeders that create currents using bands of lateral cilia running along their highly folded and filamented

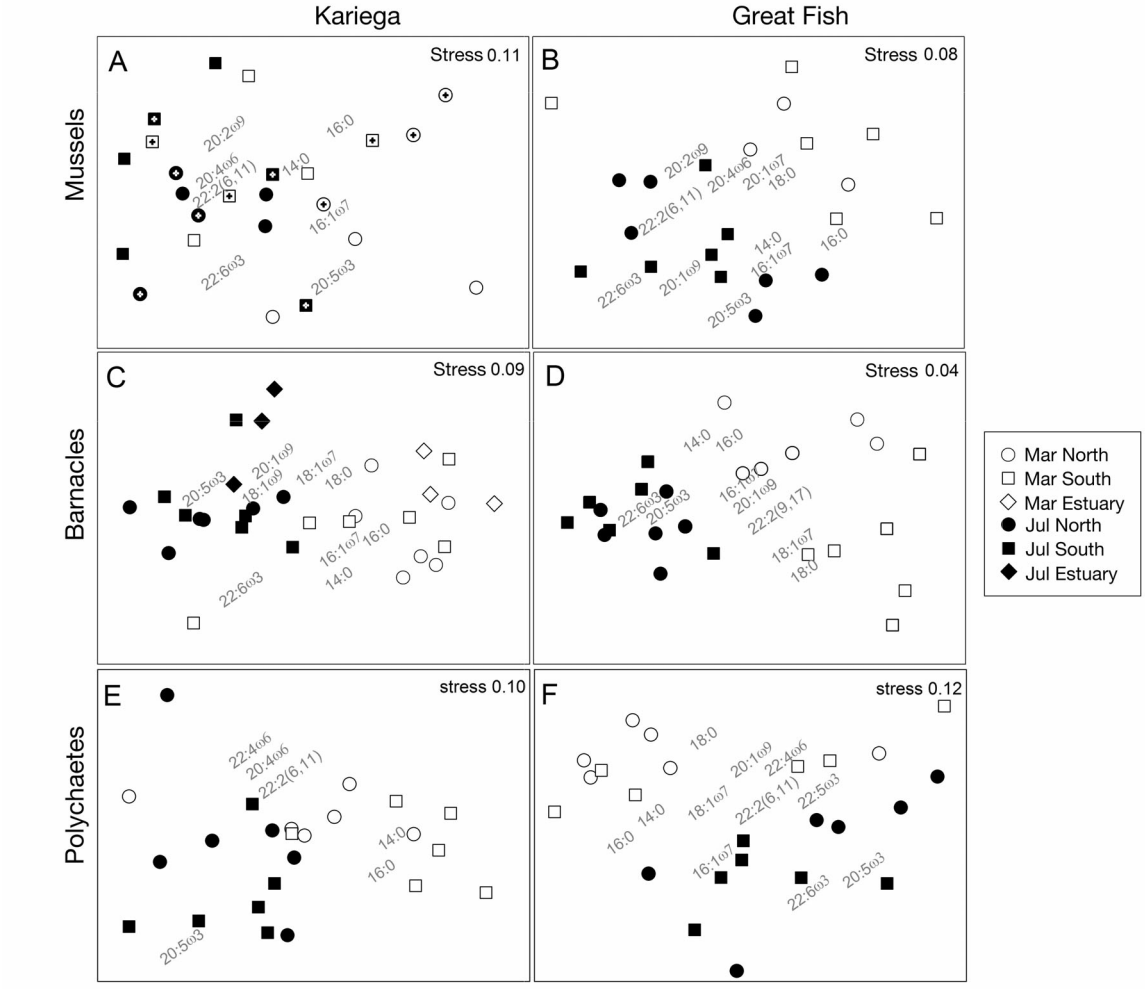

Fig. 6. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*. n-MDS output showing temporal and site comparisons in the Kariega and Great Fish regions in 2009. Fatty acids influential in separating the species (derived from SIMPER and PCA) are superimposed; refer to Table A3 in the Appendix for ANOSIM output

gills contained within a mantle cavity (Riisgård & Larsen 2010). Although the traditional assumption is that mussels attain 100% retention efficiency for particles between 4 and 7 µm, recent evidence indicates retention efficiencies vary both spatially and seasonally within a species, possibly in response to changes in seston composition (Strohmeier et al. 2012). Captured particles are sorted by the gill surface and labial palps, and mussels are thus capable of selecting specific phytoplankton cells from a complex suspension of similar-sized cells (Shumway et al. 1997). Researchers previously assumed that mussels derived their nutrition solely from phytoplankton and other small cells including bacteria and detritus, but evidence that mussels can be important consumers of actively moving zooplankton is accumulating (Wong & Levinton 2006, Shin et al. 2008). However, capture efficiency and selective preference are generally

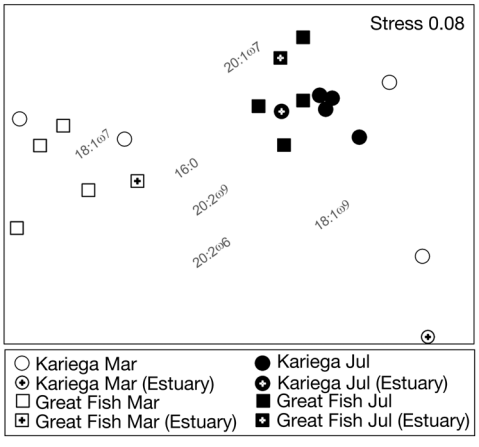

Fig. 7. Suspended particulate matter. n-MDS output showing temporal and site comparisons in the Kariega and Great Fish regions during 2009. Influential fatty acids (derived from SIMPER and PCA) are superimposed

greater for small and medium particles (i.e. phytoplankton size) and are reduced for larger particles (zooplankton size range of 200 µm to 2 mm; Strohmeier et al. 2012).

The feeding characteristics of mussels were reflected in both the fatty acid profiles of *Perna perna* (Table 1) and their isotopic signatures (relatively depleted N signatures; Hill & McQuaid 2008, Richoux et al. 2013). Moderate levels of 16:1ω7 and 20:5ω3 indicated a diet characterised by diatoms, but dinoflagellate-associated fatty acids (22:6ω3) were also well represented in mussel tissues (especially compared with the polychaetes; Table 1, Fig. 3). Mussels contained high levels of 20:4ω6 (Table 1, Fig. 2), a microalgal-produced EFA (Kharlamenko et al. 2001) that is preferentially accumulated by mussels when available in their food (Shin et al. 2008). Enhanced levels of 20:4ω6 have also been noted in local macrophytes (*Gelidium pristoides* and *G. enterobium*; Allan et al. 2010), which may represent additional sources of this EFA for the suspension-feeders. The higher prevalence of 20:2ω9 and the C22 non-methylene-interrupted dienes characteristic of bivalves (Barnathan 2009) also influenced the distinction of *P. perna* from the other 2 suspension-feeders (Table 1, Fig. 2). There was no indication (fatty acids or isotopes; Hill & McQuaid 2008, Allan et al. 2010, Richoux et al. 2013, present study) of significant consumption of zooplankton by *P. perna* along the South African coastline. Previous measures of fatty acid profiles of animals in the *Perna* genus (Narváez et al. 2008, Allan et al. 2010) support our findings to a degree in that the dominant fatty acids were largely consistent, but not necessarily at the same quantities.

Tube-dwelling sabellid polychaetes are ciliary downstream collectors (Riisgård & Larsen 2010). These reef-builders use ciliated tentacles to create a current and transfer particles from the upstream water current downstream to a second band of cilia that move the particles to the mouth (Riisgård & Larsen 2010). The lower limit of particles retained depends on the ciliary spacing, usually 1 to 2 µm (Riisgård & Larsen 2010), whereas the upper limit appears to depend on cilia length (about 20 µm in *Sabella penicillus*; Mayer 1994). Owing to the physical structure of the feeding crown, these tentaculate polychaetes generally retain small particles including detritus, bacteria and phytoplankton (Mayer 1994, Licciano et al. 2005), whereas larger particles trapped by the cilia are rejected in the mouth region (Nicol 1931). Isotopically, *Gunnarea gaimardi* occupies a trophic level between *Tetraclita serrata* and *Perna perna* (Hill & McQuaid 2008, Richoux et al. 2013), and fatty acid profiles indicated a large influence of diatoms (20:5ω3), particularly in comparison with dinoflagellates (diatom index; Table 1), in addition to consistently strong influences of 14:0 (potentially arising from diatoms or decaying macroalgae; Shin et al. 2008, Allan et al. 2010) and 20:1ω9 (Fig. 2). The latter fatty acid (20:1ω9) is an indicator of feeding on zooplankton in some environments (Sargent & Falk-Petersen 1988), although Shin et al. (2008) noted a decrease in 20:1ω9 in the mussel *P. viridis* feeding on rotifers over a 14 d period, so this fatty acid is an unlikely carnivory marker in our study (particularly since it was not prevalent in the barnacles). It appears that the polychaetes are also consuming some zooplankton, but smaller amounts or perhaps different species compared with the barnacles.

Of the 3 South African species, the polychaetes *Gunnarea gaimardi* and mussels *Perna perna* have the most similar feeding structures (both are active ciliary feeders), and one might expect that their profiles would be most similar. For example, 2 intertidal species (the oyster *Crassostrea gigas* and the mussel *Mytilus galloprovincialis*) in Jiaozhou Bay, China, had remarkably similar fatty acid profiles and carbon isotope signatures that reflected their phytoplanktonic diet and similar ciliary feeding organs (Xu & Yang 2007). However, in our comparisons, the tentacles of the polychaetes extend out of their protective calcareous tubes to enable feeding; thus, the delicate feeding structures are exposed to stronger currents than those experienced by the covered gills of the mussels. As such, the mussels may have more control over their particle capturing and processing activities compared with the polychaetes, and the selective abilities of mussels are indeed reported to be highly sophisticated (Ward & Shumway 2004).

Although we typically think of marine EFAs as arising from phytoplankton, macroalgae can supply a significant proportion of these essential nutrients to rocky shore feeders. For example, kelp is a large contributor to suspension-feeder diets in cold oceanic regions (Bustamante & Branch 1996, Tallis 2009), and intertidal macroalgal detritus was estimated to contribute largely to *Perna perna* diets along the south-east coast of South Africa (Hill et al. 2008). Detrital macroalgae may have contributed to the diets of *P. perna*, *Tetraclita serrata* and *Gunnarea gaimardi* in our study, as some of the EFAs typically allocated to phytoplankton (20:5ω3, 20:4ω6) are prevalent in at least 2 macroalgal species (Allan et al. 2010); however, the near complete absence of 22:6ω3 in any macroalgal species analysed (Allan et al. 2010) compared with the large levels found in consumers

(particularly the mussels and barnacles; Table 1) suggests a strong and direct reliance of the suspension-feeders on phytoplankton. We recommend that mixing model output from isotopic data on rocky shore consumers be interpreted with additional caution, especially when models indicate that macroalgae such as *Ulva* sp. are main contributors to suspension-feeder diets, whereas the lipids of this macroalgae are relatively depauperate of PUFAs (at least periodically; Allan et al. 2010) for it to represent a dominant food source in this habitat.

Besides food-capturing structures and mechanics, interspecific differences in basic metabolism may have contributed to the distinct profiles among the rocky shore suspension-feeders. For example, different species vary in their abilities to metabolise ω3 fatty acids (Tocher 2003), and, even within species, changes in body size can influence consumer diets (Zhukova 2000, Tallis 2009). In our study of South African suspension-feeders, we attempted to minimise any intraspecific body size effects by selecting similar-sized individuals. Small-scale differences in intertidal location could also contribute to differential fatty acid profiles among the species. Barnes & Powell (1953) noted that barnacles found lower on the shore grew faster than those located higher on the shore. The polychaetes in our study were located lowest on the intertidal slope, so they would have the greatest filtration time of the 3 species. The precise effects of intertidal location on fatty acid profiles are not known, but animals at lower intertidal locations should presumably have the advantage of assimilating more essential nutrients per day relative to animals at higher locations. The barnacles and mussels were collected from the same intertidal areas, so influences of small-scale location should have been minimal.

Spatial differences in diets (Hypotheses 2 and 3)

Regional differences in the fatty acid profiles of the suspension-feeders were generally not apparent, nor were up-current/down-current distinctions from each river mouth. Other researchers have noted significant spatial variability in recruitment, growth, biomass, and/or feeding rates of sessile suspension-feeders at a range of scales (e.g. 10s to 100s of kilometers) resulting from distinctions in nearshore productivity (Menge et al. 1997), temperature (Phillips 2005) and current speed or wave action (Emanuel et al. 1992). The lack of consistent spatial differences in our data indicate that the 2 regions were not disparate enough with respect to fatty acid compositions of the food sources, at least during 2009, to be reflected in the consumer diet profiles. In contrast, Allan et al. (2010) found a distinctive large-scale, down-current effect along the southeastern South African coastline during 2008, whereby the changing food conditions for *Perna perna* along a 600 km stretch of coastline (which envelopes the Kariega and Great Fish regions) were measurable as distinct fatty acid profiles (sites at ~50 km intervals). Furthermore, because we found measurable regional differences in the profiles of mussels and barnacles during March 2009, it seems that there is potential for regional differentiation in the trophic environment for the invertebrates at this scale, at least periodically. Researchers measuring stable isotopes in rocky shore consumers have described 4 biogeographic regions along the South African coastline arising from the prevailing broad-scale oceanographic and smaller scale hydrographic processes (Hill et al. 2008). The boundaries between these biogeographic regions are unclear, and the Kariega and Great Fish regions may easily fall within the same 'southeast coast' group, as deduced from isotopic signatures (Hill et al. 2008), for a prevailing duration of any given year. In addition to the broad oceanographic patterns that affect different regions of the South African coastline, small-scale factors such as local currents and wave action can affect nearshore productivity and hence the ecology of the suspension-feeders (Bustamante et al. 1995).

In addition to our question of a potential regional (~50 km scale) effect on the diets of rocky shore suspension-feeders, we assessed whether there was a measureable up-current/down-current effect on consumers (according to the prevalent direction of the Agulhas Current) arising from terrestrial output from each river mouth. We expected that terrestrial macrophyte-associated fatty acids (18:2ω6 and 18:3ω3) would be enhanced in consumers located down-current (south) of an estuary, particularly in the Great Fish region, as it has greater annual freshwater throughput than the Kariega Estuary (Grange et al. 2000). Such an effect of riverine subsidies on suspension-feeders was demonstrated in the Pacific Northwest, where isotopic signatures in mussels and barnacles located down-current of river mouths reflected the stimulation of the microbial loop by river-borne dissolved organic carbon inputs (Tallis 2009). In contrast, we rarely detected north versus south site effects (except for barnacles in the Great Fish region during March and mussels in the Kariega region during March; Figs. 5 & 6), and these trends

were not strong or consistent among species, nor did they result from any distinctions in terrestrial-associated fatty acids (Kariega mussels during March: north 2.9 ± 0.4 % and south 3.2 ± 0.4 %; Great Fish barnacles during March: north 2.1 ± 0.4 % and south 1.7 ± 0.4 %) or any other biologically relevant fatty acids. Relatively small levels of terrestrial-associated fatty acids (18:3ω3 and 18:2ω6) were detected in the other suspension-feeders, with the highest mean (3.6 ± 1.3 %, both regions inclusive) occurring in mussels during March (Table 1). The mussels may have derived these C18 PUFAs from detritus originating in higher plants (Richoux & Froneman 2008) or macroalgae (e.g. *Caulerpa filiformis* and *Codium extricatum*; Allan et al. 2010). The lack of sizable riverine inputs to marine consumers in the Kariega region was consistent with the findings of Hill et al. (2008), who noted only minor contributions (<10 %) of estuarine carbon to nearshore SPM in this same region during 2004/2005, and Vorwerk & Froneman (2009), who demonstrated measurable contributions of estuarine-derived material to zooplankton and subtidal suspension-feeders in both the Great Fish and Kariega regions, but showed little evidence for north/south distinctions in relation to distance from either river mouth. All of these studies have contributed information about spatial variations in consumer diets along the coast, but additional sites and sampling events are needed so that we may draw more robust conclusions about the causes of this variability.

Temporal differences in diets (Hypothesis 4)

Fatty acid profiles of all 3 species were strongly influenced by the time of the year in which sampling took place. Environmental conditions are generally variable along the southeast coast of South Africa over time and space (Brown 1992, Jackson et al. 2012), so we did expect temporal differences in the diets of the suspension-feeders. Nearshore environmental factors (phytoplankton productivity, seston quality, temperature, current speeds) have important impacts on rocky intertidal populations (feeding, growth, recruitment) and communities (competitive interactions; Saurel et al. 2007). It is therefore not surprising that such factors could show effects at the level of the biochemical composition of animals, and several authors have documented increased abundances of PUFAs in suspension-feeders during periods of high primary productivity (Pazos et al. 1997, Freites et al. 2002, Narváez et al. 2008). Tallis (2009) also described an interesting seasonal shift in the diets and trophic levels of 4 rocky shore suspension-feeders that she ascribed to changes in riverine subsidies. In turn, direct effects of changing diet composition on consumer fatty acid profiles have been demonstrated in controlled feeding experiments (Shin et al. 2008). Temperature is an additional influential factor that can affect fatty acids of rocky shore suspension-feeders (Narváez et al. 2008), although temperatures along the South African coast do not reach the low values needed to trigger major storage of PUFAs (necessary for maintaining membrane fluidity) (Hall et al. 2002).

Lipid and fatty acid compositions of consumers may also shift through time, owing to changes in life cycle or reproductive status (Narváez et al. 2008). Although life cycle changes may affect the biochemistry of both gonad and non-gonad tissues in complex ways (Pazos et al. 1997), we have attempted to minimise the effects of reproduction by removing gonads of the consumers wherever possible. The reproductive cycles of *Perna perna* and *Tetraclita serrata* living in the southeastern region of South Africa have been documented, and spawning in the mussels can occur in different seasons (e.g. late summer, autumn, or spring; Hodgson 2010 and references therein). Similarly, spawning in *T. serrata* is variable and can extend throughout most of the year (i.e. between July and February, or even until May; Hodgson 2010 and references therein). There are no published data on the reproductive cycle of *Gunnarea gaimardi*. Fatty acids in the muscle tissues and gonads of *P. perna* have been traced over a full year in the Kariega region of South Africa (Ndhlovu 2013). Marked changes were noted in the fatty acid profiles of the mussel gonads, and these changes matched the build-up of reserves prior to spawning, whereas the profiles in the muscle tissues closely followed the changing trophic environment (Ndhlovu 2013). Equivalent detailed fatty acid data are not yet available for *T. serrata* or *G. gaimardi*, but fatty acid profiles in the whole bodies of *G. gaimardi* traced the patterns in *P. perna* muscle tissues over a 1 yr period, thus indicating that reproductive development did not mask the dietary signatures in the polychaetes (Ndhlovu 2013).

Analysis of temporal changes in the profiles for all 3 species indicated enhanced phytoplankton-associated fatty acids in the animal tissues during July, and qualitatively impoverished markers during March (Figs. 3 & 6). The quantitative data showed different patterns, with enhanced concentrations of

most fatty acids in all consumers during March compared with July (Table 1, Fig. 4). The quantitative results coincide with the typical patterns of productivity in the study region. The nearshore coast of southeastern South Africa, including both sampled regions, characteristically has enhanced primary productivity during spring and autumn, but low productivity during summer and winter (Brown 1992). As a result, the consumers collected during March (autumn) would have recently experienced increased phytoplankton productivity, hence the quantitatively enhanced fatty acids in the consumers (Table 1, Fig. 4). The consumers collected during July (winter) would have recently experienced a periodic low in primary productivity, which could explain the significant decreases in nearly all of the fatty acid categories in the SPM and the 3 consumers (Table 1, Fig. 4). In general, muscle tissue is a good reflector of long-term diet, whereas fast turnover tissues like digestive glands are superior for providing information on very short-term (i.e. scale of days) fluctuations in the trophic environment (Shin et al. 2008). The time frame for assimilation and tissue turnover after exposure to a new diet varies with species and a host of metabolic factors, but the fatty acid profiles of *Perna viridis* muscle tissue began to show changes after the animals had experienced a new diet for 7 d (Shin et al. 2008), and the oyster *Crassostrea gigas* showed significant shifts in fatty acid profiles in <3 wk on a new diet (Piveteau et al. 1999).

The opposite trends observed in the qualitative and quantitative data for *Perna perna*, *Tetraclita serrata* and *Gunnarea gaimardi* could have resulted from metabolic compensations. Several authors have documented selective retention of fatty acids by consumers during periods of decreased food quality (Piveteau et al. 1999, McLeod et al. 2013), so even when the occurrence of some components is low in the environment, the consumers showed enhanced levels in their tissues. When fatty acids are abundant in the environment, there may be no need for the consumers to selectively retain components. Selective mobilisation or retention of lipid components for reproductive purposes could also contribute to the metabolic changes in fatty acid profiles through time (Pazos et al. 1997). In essence, although fatty acids may be more abundant per unit DM during March, the consumers may be metabolically processing and storing fatty acids very differently from one season to another based on their basic energy and metabolic requirements versus reproductive allocation to gonads.

Conclusions

We demonstrated clear interspecific differences in fatty acid profiles of 3 syntopic rocky shore suspension-feeders. Considering the large variety and ever-changing composition of particles available to suspension-feeders (summarised in Ward & Shumway 2004), and the potentially large filtering capacities of highly abundant populations along the rocky shore (Sink et al. 2005), suspension-feeders occupy a fundamentally important ecological role in linking habitats (benthic to pelagic, offshore to nearshore) and recycling nutrients, and they can have profound top-down impacts on seston communities (Dolmer 2000). Distinctions in particle feeding dynamics among species within a community can thus have important implications on ecosystem processes by mediating recycling, deposition and secondary production (Ward & Shumway 2004). These distinctions also represent fundamental ways in which syntopic species partition their trophic environment to allow coexistence in a highly competitive environment (Tallis 2009). Regional- and local-scale distinctions in diet were not dominant features in our consumer data, but contributions of terrestrially derived matter to coastal consumers may change in the future with increased development of watersheds (Correll et al. 2001).

We have presented evidence on the main food sources for rocky shore suspension-feeders that in part contradicts previous research. To solve this controversy, it will be necessary to derive information about fatty-acid-specific stable isotope signatures in the tissues of each species. These data should allow us to determine the sources of EFAs (phytoplankton vs. macroalgal or macrophyte detritus) for the consumers. Controlled feeding experiments may help to shed light on the complexity of consumer nutrition in this highly variable environment.

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Appendix. Additional data

Table A1. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*. Species and site comparisons in each region: global R values (significance values from 2-way ANOSIM in parentheses) for the 3 species collected in the Great Fish and Kariega regions during March and July, and among the 3 site categories (north, south and estuary mouth), where applicable. NA: not applicable; *statistical difference

Factor	Great Fish		Kariega	
	Mar	Jul	Mar	Jul
Species	0.97 (<0.001)*	0.99 (<0.001)*	0.95 (<0.001)*	0.99 (<0.001)*
Barnacles vs. mussels	0.85 (<0.001)*	0.99 (<0.001)*	0.84 (<0.001)*	0.98 (<0.001)*
Barnacles vs. polychaetes	1.0 (<0.001)*	1.0 (<0.001)*	1.0 (<0.001)*	1.0 (<0.001)*
Mussels vs. polychaetes	0.98 (<0.001)*	1.0 (<0.001)*	0.98 (<0.001)*	1.0 (<0.001)*
Site category	0.16 (0.06)	0.02 (0.38)	0.18 (<0.05)*	0.06 (0.17)
North vs. south	NA	NA	0.18 (<0.05)*	NA
North vs. estuary	NA	NA	0.35 (0.07)	NA
South vs. estuary	NA	NA	−0.05 (0.51)	NA

Table A2. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*. Regional and site comparisons for each species: global R values (significance values from 2-way ANOSIM in parentheses) for the 3 species collected in the Great Fish and Kariega regions in March and July, and among the 3 site categories (north, south and estuary mouth), where applicable. NA: not applicable; *statistical difference

Factor	*Perna perna* (mussels)		*Tetraclita serrata* (barnacles)		*Gunnarea gaimardi* (polychaetes)	
	Mar	Jul	Mar	Jul	Mar	Jul
Region	0.32 (<0.05)*	−0.11 (0.82)	0.52 (<0.001)*	0.04 (0.30)	0.12 (0.07)	0.02 (0.38)
Site category	0.15 (0.09)	−0.04 (0.58)	0.25 (<0.01)*	0.07 (0.19)	0.08 (0.15)	0.09 (0.13)
North vs. south	NA	NA	0.27 (<0.01)*	NA	NA	NA
North vs. estuary	NA	NA	0.35 (0.07)	NA	NA	NA
South vs. estuary	NA	NA	−0.05 (0.51)	NA	NA	NA

Table A3. *Perna perna*, *Tetraclita serrata*, *Gunnarea gaimardi*. Temporal and site comparisons for each species: global R values (significance values from 2-way ANOSIM in parentheses) for the 3 species collected in the Great Fish and Kariega regions in March and July, and among the 2 or 3 site categories (north, south and estuary mouth), where applicable. *Statistical difference. Pairwise tests were not applicable for the 2 significant site effects, as these locations were represented by specimens from only north and south sites

Factor	*Perna perna* (mussels)		*Tetraclita serrata* (barnacles)		*Gunnarea gaimardi* (polychaetes)	
	Kariega	Great Fish	Kariega	Great Fish	Kariega	Great Fish
Month	0.35 (<0.01)*	0.37 (<0.01)*	0.74 (<0.01)*	0.90 (<0.01)*	0.48 (<0.01)*	0.47 (<0.01)*
Site category	0.18 (0.04)*	−0.14 (0.89)	0.09 (0.13)	0.27 (<0.05)*	0.1 (0.11)	0.07 (0.17)

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