Hierarchical effects of biogeography and upwelling shape the dietary signatures of benthic filter feeders

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ABSTRACT: Benthic filter feeders have key functional roles in coastal ecosystems as an intermediate trophic level in food webs and as bioengineers. The food available to such species depends on the local composition of the water column, reflecting factors operating across multiple spatial scales. Here, we examined how upwelling and biogeography influence their dietary signatures. The diet regimes of 2 mussels and 1 barnacle were investigated using fatty acid (FA) and stable isotope (SI) analyses across 13 sites spanning 3000 km across 3 biogeographic regions corresponding to the west, south and east coasts of South Africa. SI and FA signatures showed similar patterns for all taxa. $\delta^{15}N$ signatures of filter feeders increased from the oligotrophic east to the eutrophic west coast, with no difference between upwelling and non-upwelling sites, while $\delta^{13}C$ signatures significantly decreased at upwelling sites. The lower $\delta^{13}C$ signature associated with upwelling indicates different food sources from non-upwelling sites. FA signatures changed among coasts, with west coast samples differing from the south and east coasts. Upwelling affected the FA signature of filter feeders only on the west coast, where upwelling events are stronger. These specimens were enriched in polyunsaturated FA, a marker of high food quality. Hence, a powerful effect of upwelling was detectable in filter feeder diets, but depended on upwelling intensity and frequency, and was nested within the overriding effects of biogeography.

KEY WORDS: Trophic ecology · Fatty acids · Stable isotopes · Spatial scale · Intertidal ecology · *Mytilus galloprovincialis* · *Perna perna* · *Chthamalus dentatus*

INTRODUCTION

The main factors driving large spatial scale (i.e. 1000s of km) patterns in primary production are temperature, solar radiation and nutrient availability (Field et al. 1998, Cramer et al. 1999, Elser et al. 2007). Temperature affects metabolic rates and thus acts on growth, reproduction and productivity of primary producers (Grime 1977, Xiong et al. 2000); solar radiation affects rates of photosynthesis (Bondeau et al. 1999); and macro- and micronutrients frequently limit primary production (Howarth 1988). These factors vary at biogeographic scales and collectively interact to control the type of primary producers and the level of primary production. This, in turn, controls the quantity and quality of the food available for primary consumers (McGuire et al. 1997). In aquatic systems, primary consumers of planktonic organisms, such as benthic filter feeders, play an important role as an intermediate trophic pathway between autotrophic and small heterotrophic organisms and higher predators (Smith et al. 2009). Many are also critical ecosystem engineers within these systems, providing habitat for associated organisms and enhancing local diversity (Levinton 1995, Crooks 2002, Gutiérrez et al. 2003). For these reasons, modi-

fying the base of the food web can affect the physiology and distribution of higher consumers, and ultimately the functioning of the entire ecosystem (Connell 1985, Dodson et al. 2000, Menge 2000).

In marine systems, nutrient supply is strongly affected by the prevailing hydrographic regime (Field et al. 1998, Rohde 1999). For instance, oceanographic currents play a fundamental role in coastal areas by mixing coastal and offshore production, potentially leading to changes in food availability for benthic populations (Hill et al. 2006, Smith et al. 2009). Bustamante et al. (1995) and Hill et al. (2006) showed changes of intertidal chlorophyll a (chl a) concentration and stable isotope signatures of suspended particulate organic matter (SPM) respectively among the 3 coasts of South Africa (i.e. east, south and west). The origin of this pattern was suggested to be linked to the influence of the warm oligotrophic Agulhas Current on the south and east coasts and the eutrophic cold water Benguela Current on the west coast.

At smaller spatial scales (i.e. mesoscales of 10s to 100s of km), localized oceanographic effects, such as upwelling or local currents, are amongst the main drivers of variability in marine primary production (Basterretxea & Arístegui 2000, Demarcq 2009). By bringing nutrient-rich water into the euphotic zone, upwelling enhances primary production locally; it influences the species composition of primary producers in the water column and thus affects the quantity and/or quality of food available for benthic populations (Figueiras et al. 2002, Blanchette et al. 2006). The advection of nutrient-rich waters allows the proliferation of diatoms, which can reach extremely high concentrations (Kiørboe et al. 1998). After an upwelling event, diatoms are usually replaced by dinoflagellates as they become limited by the availability of silicon, the main constituent of their frustules (Humborg et al. 2000, Tilstone et al. 2000). Such changes in food availability can have profound consequences for benthic populations, influencing recruitment, survival, growth and reproduction (Wing et al. 1995, Sanford & Menge 2001).

At local (i.e. from 1 to a few km) and small (i.e. from cm to a few m) spatial scales, other factors contribute to local variability in food availability. Common factors include local hydrodynamics or tidal cycles, wave exposure and the presence of kelp beds (Fréchette & Bourget 1985, Carter 1988, Kingsford et al. 1991, Dubois et al. 2007). However, these factors are often very chaotic and difficult to predict, being specific to the area investigated and highly variable in time. Because feeding involves particle capture at extremely small scales (mm), the diets of filter feeders potentially reflect factors operating across multiple spatial scales, making it difficult to predict how they will respond to long-term environmental changes.

We investigated the balance among such factors by examining how the dietary regime of intertidal filter feeders varies at different spatial scales. Using 2 complementary techniques (fatty acid and stable isotope analyses), we aimed to establish (1) the effects of biogeography (i.e. large scale), (2) whether the diets of benthic filter feeders are influenced by upwelling events (i.e. mesoscale), and (3) the degree to which local scale processes influence filter feeder diets.

MATERIALS AND METHODS

Study area and sample collection

The South African coast (34.4–29.1° S, 17.9–31.3° E) can be divided into 3 main bioregions corresponding to the 3 coasts: west, south and east (Emanuel et al. 1992). Each coast exhibits localized upwelling events, which differ strongly in duration, intensity and frequency among the 3 coasts. The west coast is characterized (1) by the northwards-flowing Benguela Current, which brings cold eutrophic waters, and (2) by strong, wind-driven upwelling events, occurring seasonally in the south of this coast, becoming more frequent northwards, with persistent upwelling around the region 60 km north of Groenrivier (site 1 in Fig. 1; Andrews & Hutchings 1980, Carr & Kearns 2003). On the south and east coasts, the Agulhas Current carries oligotrophic warm water flowing south-westwards along the coast from the Mozambique Channel (Probyn et al. 1994, Lutjeharms et al. 2000). The south coast experiences wind-driven upwelling, with a semi-permanent upwelling cell close to Port Alfred (site 9 in Fig. 1). On this coast, upwelling events occur frequently over the year, but they are less intense than on the west coast (Schumann et al. 1982, Walker 1986). The east coast experiences some upwelling events, but these are rare and weak (Lutjeharms 2006), and were not considered in the present study. Overall, there is a gradient from the east through the south to the west coast of decreasing water temperatures, increasing nutrient levels and increasing prevalence of upwelling (Shannon et al. 1983, Probyn et al. 1994, Lutjeharms 2006).

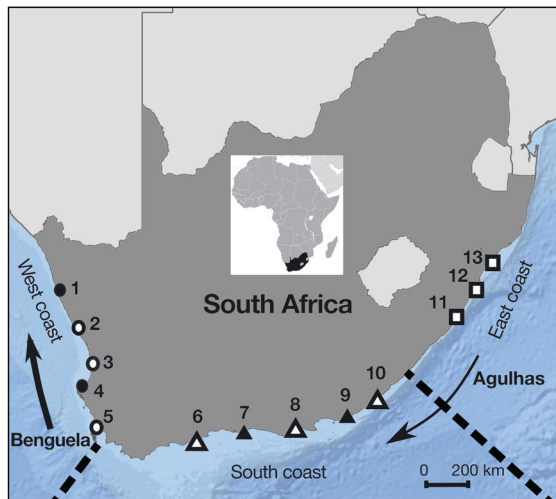We sampled 13 sites (Fig. 1) separated by 100s of km with 2 locations separated by 1 to 3 km within

Fig. 1. South Africa, showing the sampling sites on 3 coasts (34.4–29.1°S, 17.9–31.3°E): west (circles), south (triangles) and east (squares). Sites were in either upwelling (black) or non-upwelling (white) areas. Site 1 (Groenrivier), site 2 (Doring Bay), site 3 (Lambert's Bay), site 4 (Cape Columbine), site 5 (Bloubergstrand), site 6 (Jongensfontein), site 7 (Brenton on Sea), site 8 (St. Francis Bay), site 9 (Port Alfred), site 10 (Kidd's Beach), site 11 (Mbotyi), site 12 (Pennington) and site 13 (Ballito). All sites in white were used to test the effects of biogeography

each site, in May 2012. Locations were nested in site. In order to test the effects of biogeography, 3 non-upwelling sites were chosen on each of the 3 coasts. These sites were 2, 3 and 5 on the west coast; 6, 8 and 10 on the south coast; and 11, 12 and 13 on the east coast. To test the effects of upwelling, 2 sites characterized by upwelling (sites 1, 4, 7, 9) and 2 without upwelling (sites 2, 5, 8, 10) were sampled along each of the south and the west coasts. The sites of upwelling and non-upwelling were chosen based on the work of Cole & McQuaid (2010) and Xavier et al. (2007) who identified upwelling based on chl *a* and temperature data, respectively.

We investigated the diet of 3 filter feeders: 2 species of mussels, viz. the introduced *Mytilus galloprovincialis* and the indigenous *Perna perna*, and 1 species of barnacle, *Chthamalus dentatus*. Unlike the barnacle *C. dentatus*, neither species of mussel occurs around the whole coast of South Africa (Griffiths et al. 2009), and for this reason, *P. perna* was collected on the south and east coasts and *M. galloprovincialis* on the west coast. *C. dentatus* was sampled on all 3 coasts. At each location, randomly selected replicates of mussels and barnacles were collected. Three replicates of each taxon were used for the fatty acid (FA) analyses and 5 for the stable isotope (SI) analyses. Each barnacle replicate was

represented by a pool of animals (8–10 individuals) due to their small size, while for mussels, the adductor muscle of a single individual was used due to the low turnover rate of this tissue (approximately 9 mo for SI and 1 mo for FA; Pirini et al. 2007, Hill & McQuaid 2009), which renders it representative of a time-integrated diet. All samples were dissected, rinsed with Milli Q water to remove sediment, pieces of shell and other material and placed in 2 ml cryotubes. Mussel and barnacle samples were transported in a liquid nitrogen container until returned to the laboratory, where they were stored in a −80°C freezer until processing.

Diet analyses

Isotopic analyses

In the laboratory, samples for SI analyses were dried at 60°C for 48 h. The samples were ground into a fine powder in a ball mill, and 1 mg subsamples were placed in tin foil capsules. Samples were analysed for SI ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) using a continuous flow Isotopic Ratio Mass Spectrometer (Europa Scientific 20-20 IRMS linked to ANCA SL Prep Unit, IsoEnvironmental Laboratory, Rhodes University, Grahamstown, South Africa). Results are expressed in standard unit notation:

$$\delta X = ([R \text{ sample}/R \text{ standard}] - 1) \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N, R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Beet sugar, ammonium sulphate and casein were used as standards, calibrated against multiple International Atomic Energy reference standards. Measurement precision for both carbon and nitrogen was ±0.05‰.

FA analyses

Total lipids were extracted and trans-esterified using a modified Indarti 1-step procedure (Indarti et al. 2005). All samples were taken from the −80°C freezer and immediately transferred to a freeze-drier (VirTis BenchTop K) for 24 h and subsequently transferred back into a −80°C freezer for 1 or 2 d until FA extraction. Extreme care was taken to avoid potential degradation of the samples by keeping them at cold temperatures as much as possible (in a −80°C freezer when stored and on ice when working in the laboratory) and by reducing exposure to oxygen (tubes

were closed under nitrogen flow before storing). Samples were then homogenized into 4 ml of a fresh solution of a mixture of methanol, concentrated sulphuric acid and chloroform containing 0.01% of an anti-oxidant, butylated hydroxytoluene (1.7/0.3/2.0 v/v/v) and closed under nitrogen. The extraction and transesterification reactions occurred at 100°C for 30 min. The FA methyl esters (FAMEs) formed were then stored at −80°C until gas chromatography (GC) analysis. FAME composition of each sample was determined by GC (Agilent Technologies 7890A, at the National Research Foundation Fatty Acid Facility at Rhodes University) equipped with a ZB-Waxplus 320 capillary column, with helium as the carrier gas at a flow rate of 1.664 ml min^{-1}. The injector was at a temperature of 250°C, and splitless injection was used for all samples. The flame ionization detector (FID) was set at 260°C, and the oven was initially set at 70°C. After 1 min, the oven temperature was increased by 40°C min^{-1} until 170°C and then raised to 250°C at a rate of 2.5°C min^{-1} and held for 4.5 min. Peaks were integrated using GC ChemStation software (Agilent Technologies, version B.04.02), identified by comparison with retention times of external known standards (37-component FAME mix Supelco, marine polyunsaturated FA (PUFA) no. 1 Supelco, menhaden oil PUFA no. 3, bacterial acid methyl ester mix Supelco), as well as by mass spectrometry analyses (Agilent Technologies 7000 GC/MS Triple Quad; Agilent Mass Hunter, version B.05.00) using the NIST library. Each FA was measured as a proportion of the total FA (TFA) composition (% by weight of TFA), and peak areas were corrected according to the FID response to FA chain length (Ackman 2002). FAs are reported using a shorthand notation of A:Bn-x, where A indicates the number of carbon atoms, B is the number of double bonds, and x indicates the position of the first double bond relative to the terminal methyl group (Budge et al. 2006).

Data analyses

SIs

To examine spatial differences in δ^{13}C and δ^{15}N among coasts and between upwelling and non-upwelling areas, 2 mixed analysis of variance (ANOVA) designs were used. To test the effects of biogeography, the design was composed of the factors: coast (3 levels, fixed), site (3 levels, random and nested in coast), location (2 levels, random and nested in site) and taxon (2 levels, fixed and crossed with coast, site

and location). This design was performed using 3 non-upwelling sites on each of the 3 coasts. A second design used to test the effects of upwelling had the following factors: upwelling (2 levels, fixed), site (2 levels, random and nested in upwelling), location (2 levels, random and nested in site) and taxon (2 levels, fixed and crossed with upwelling, site and location). A preliminary run of the second design included the factor coast, which was crossed with upwelling and taxon. This preliminary analysis indicated that biogeography was an overriding factor that masked other effects and prevented us from separating the effects of upwelling from those of biogeography. Consequently, the second analysis used to test the effects of upwelling was applied on the west and south coasts separately, with 2 upwelling sites and 2 non-upwelling sites on each. In the event of significant results, Tukey HSD post hoc tests were performed. The violation of homogeneity of variances was considered to be acceptable because ANOVA is relatively robust to heterogeneous variances for large designs such as the one in this study (Underwood 1997). The values are expressed as mean ± standard deviation. Analyses were performed using STATISTICA v12 (StatSoft).

FAs

We compared the FA composition of specimens among bioregions and between upwelling and non-upwelling conditions with the same experimental designs as for the SI data analyses, using a multivariate permutation analysis (PERMANOVA, Anderson 2001)—a program that allows the analysis of datasets with multiple variables. A test of homogeneity of dispersions was conducted for each analysis (PERMDISP; Anderson et al. 2008). In the case of a significant (p < 0.05) PERMDISP result, the dispersions were homogenized using standard transformations (square root). Each term in the PERMANOVA analyses was tested using >9999 permutations as the relevant permutable units (Anderson & Braak 2003). In the event of significant results, PERMANOVA pairwise tests were performed. Similarity percentage (SIMPER) analyses were used to assess the FA(s) responsible for the differences among groups of samples. Non-metric multidimensional scaling (MDS) was used to visualize differences among species; relationships between samples were considered well represented when stress was <0.2. All analyses were based on Bray-Curtis dissimilarities calculated from percentage data after transformation. Although the

filter feeders chosen for this comparison had a few FAs in common, the presence of non-methylene-interrupted FAs (NMI FAs), FAs synthesized de novo by mussels but not by barnacles (Barnathan 2009), and of other FAs that characterize only 1 taxon or the other, prevented a direct comparison between the 2 taxa. The FA compositions of barnacles and mussels were therefore analysed separately in each of the analyses. NMI FAs can be synthesized by mussels, and neither species of mussel occurs on all 3 coasts. Consequently, in the comparison between mussel species from the 3 biogeographic regions, we ran the analysis without NMI FAs, as differences could be driven by species-specific effects as well as by environmental conditions, confounding the effects of biogeography and species. NMI FAs were omitted only for testing biogeographic effects, while for the upwelling analyses, they were retained because this effect was evaluated separately for each coast, and thus the same mussel species was under investigation (i.e. *P. perna* on the south coast and *M. galloprovincialis* on the west coast). Only FAs forming >1% of TFAs were considered in the analyses. All analyses were conducted using the PERMANOVA+ add-on package of PRIMER v6 (Clarke & Gorley 2006, Anderson et al. 2008).

RESULTS

Differences among coasts

Filter feeders clearly revealed different SI and FA signatures depending on the coasts considered (Tables 1 & 2). West coast samples were grouped significantly apart from samples from the south and east coasts (Tukey HSD and PERMANOVA pairwise test, $p < 0.001$), which showed more variability in δ^{13}C, δ^{15}N and FA signatures (Figs. 2 & 3). These patterns were observed for both mussels and barnacles (Tukey HSD and PERMANOVA pairwise test, $p < 0.05$).

Variations in SI were also observed within each coast, with δ^{15}N of mussels decreasing from 8.7 ± 0.3 to 7.5 ± 0.3‰ from site 13 to 11 on the east coast, increasing from 6.9 ± 0.3 to 8.7 ± 0.2‰ from site 10 to 6 on the south coast and slightly decreasing from 8.5 ± 0.3 to 8.0 ± 0.3‰ from site 5 to 2 on the west coast. The δ^{13}C signatures of mussels varied widely among the 3 coasts, but with a clear overall trend of increasing values from east to west. Globally, samples from the west coast were more enriched in carbon δ^{13}C than those from the other 2 coasts (values ranged from −17 to −15.5‰ for the east coast,

Table 1. ANOVA of stable isotope analyses performed on 2 groups of filter feeders (barnacles *Chthamalus dentatus* and mussels *Mytilus galloprovincialis* and *Perna perna*) on the 3 South African coasts (n = 5 per location, hence n = 10 per site). Co: coast, Ta: taxon, Si: site, Lo: location; **$p < 0.01$, ***$p < 0.001$

Biogeography	δ^{13}C				δ^{15}N			
	df	MS	*F*	p	df	MS	*F*	p
Co	2	103.46	789.67	<0.001***	2	14.60	132.45	<0.001***
Ta	1	44.49	339.60	<0.001***	1	63.27	574.10	<0.001***
Co × Ta	2	3.95	30.18	<0.001***	2	21.48	194.96	<0.001***
Si (Co)	6	2.06	15.74	<0.001***	6	11.41	103.58	<0.001***
Si (Co) × Ta	6	0.46	3.53	0.005**	6	0.70	6.40	<0.001***
Lo (Si (Co))	9	0.61	4.62	<0.001***	9	0.25	2.27	0.221
Lo (Si (Co)) × Ta	9	0.36	2.76	0.003**	9	0.09	0.78	0.631
Error	109	0.13			109	0.11		

Table 2. PERMANOVA results on the fatty acid composition of mussels *Mytilus galloprovincialis* and *Perna perna* and barnacles *Chthamalus dentatus* in the 3 biogeographic provinces (n = 3 per location, hence n = 6 per site). Co: coast, Si: site, Lo: location; *$p < 0.05$, ***$p < 0.001$

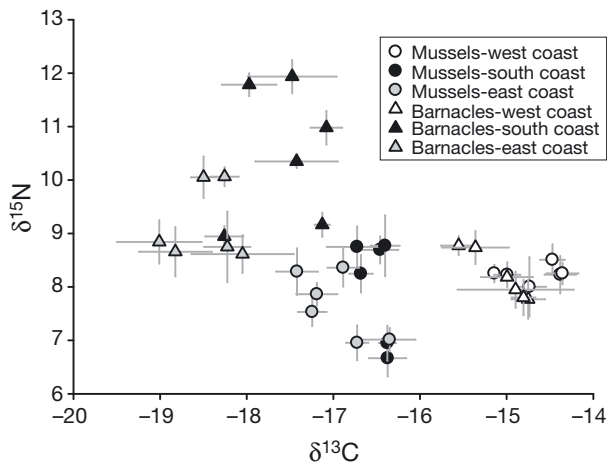| Biogeography	Mussels				Barnacles			
	df	MS	*F*	p	df	MS	*F*	p
Co	2	331.95	46.48	0.021*	2	1831.5	3.41	0.039*
Si (Co)	6	71.41	37.52	<0.001***	6	536.84	12.21	<0.001***
Lo (Si (Co))	9	19.01	1.31	0.200	9	43.95	0.97	0.496
Residual	36	14.58			36	44.94		

Fig. 2. Stable isotope signatures (mean ± SD; n = 5) of mussels *Mytilus galloprovincialis* and *Perna perna* (circles) and barnacles *Chthamalus dentatus* (triangles), collected at locations in the 3 South African biogeographic provinces: east (grey), south (black) and west (white) coasts. Each symbol represents the average value of replicates from 1 location (n = 5)

−16.5 to −15.5‰ for the south coast and −14.7 to −14.5‰ for the west coast). Barnacles showed the same pattern in terms of both nitrogen and carbon, but with generally higher average values than the mussels ($\delta^{15}N$ values = 9.3 ± 0.5, 10.5 ± 0.5, 8.3 ± 0.4‰; $\delta^{13}C$ values = −18.6 ± 0.3, −17.5 ± 0.3, −15.2 ± 0.5‰, respectively, for the west, south and east coasts, Fig. 2). Significant differences among sites within the same coast were found for both types of filter feeder for $\delta^{13}C$ and $\delta^{15}N$ (Table 1), although neither taxon showed a clear pattern.

Twenty-seven FAs contributing >1% of the TFAs were found for mussels and barnacles (Tables 3 & 4). Mussels generally showed high levels of PUFAs (40–54%) on all coasts, followed by saturated FAs (SFAs, 30–38%) and monounsaturated FAs (MUFAs, 14–24%). Overall, n−3 FAs (mainly 22:6n-3, 20:5n-3), 20:4n-6 and 16:0 were the predominant FAs accounting for 50 to 60% of TFAs (Table 3). For the barnacle *Chthamalus dentatus*, the proportion of SFAs was relatively high on the south and east coasts, with 16:0 and 18:0 (~20–60%), while the west coast was characterized by a high proportion of PUFAs at all sites (40–50% of TFAs). As expected, 20:5n-3 and 22:6n-3 formed a high proportion of the TFAs on all coasts (25–40%, Table 4). PERMANOVA revealed significant differences in the FA signatures of both filter feeders among coasts, with samples from the west coast being different from specimens from the other 2 coasts (PERMANOVA pairwise test; Fig. 3). For mussels, the differences were mainly due to 20:5n-3 and

dinoflagellate trophic markers (TMs; i.e. 18:4n-3 and 22:6n-3), which were more abundant on the west coast than the other coasts; while 18:2n-6, 18:3n-3, 20:4n-6, 22:2n-6, 22:4n-6 and 22:5n-6 were predominant on the south and west coasts (SIMPER). For barnacles, diatom TMs (i.e. 16:1n-7 and 20:5n-3) and dinoflagellate TMs typified the west coast, whereas bacterial FAs (bacterial acid methyl esters, BAMEs), 16:0, 18:0 and 20:4n-6 were more abundant on the south and east coasts (SIMPER). These FAs contributed to 55% of the dissimilarities among mussels on the 3 coasts (SIMPER). The kelp TM 20:4n-6 was more abundant in mussels than barnacles and had higher proportional values on the south and east coasts than on the west coast (Tables 3 & 4; for mussels: 4.33 ± 0.52, 6.96 ± 0.77, 7.08 ± 1.13, and for barnacles: 0.13 ± 0.08, 2.43 ± 0.5, 2.63 ± 0.32, for the west, south and east coast, respectively). PERMANOVA pairwise tests did not highlight any dissimilarities between samples from the south and east coasts for either taxon. These results were confirmed by the MDS (Fig. 3).

No FA or $\delta^{15}N$ differences were recorded between locations within the same sites; however, variability
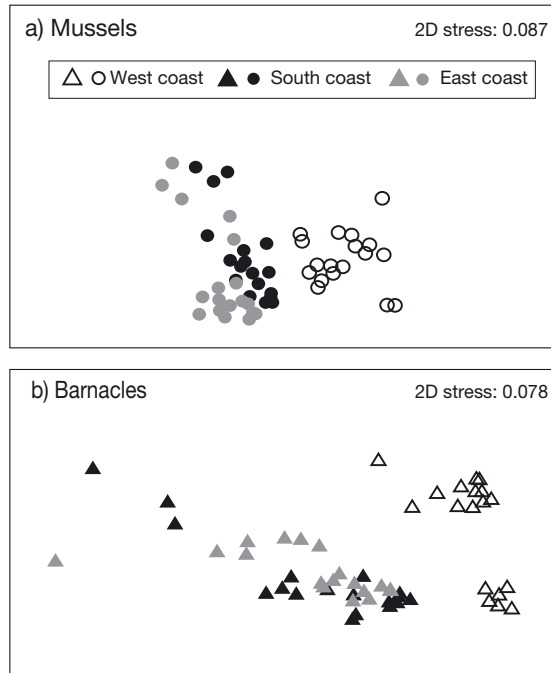

Fig. 3. Non-metric multidimensional scaling analyses of total fatty acid (TFA) composition of (a) mussels *Mytilus galloprovincialis* and *Perna perna* (circles) and (b) barnacles *Chthamalus dentatus* (triangles) in the 3 biogeographic provinces: west, east and south coast. Each symbol represents a single replicate (n = 3 per location; hence, n = 6 per site)

Table 3. Total fatty acid (TFA) composition (% of TFA) of mussels *Mytilus galloprovincialis* and *Perna perna* collected at 9 sites across 3 coasts of South Africa. The values are percentages expressed as mean ± SD (n = 3 per location; hence, n = 6 per site). Only FAs contributing >1% to TFA are displayed. BAME: bacterial FA, PUFA: polyunsaturated FA, MUFA: monounsaturated FA, SFA: saturated FA. Non-methylene-interrupted FAs were omitted from the biogeography effect analyses

| Fatty acid | West coast | | | South coast | | | East coast | | |
	Site 2 Doring Bay	Site 3 Lambert's Bay	Site 5 Blouberg-strand	Site 6 Jongens-fontein	Site 8 St. Francis Bay	Site 10 Kidd's Beach	Site 11 Mbotyi	Site 12 Pennington	Site 13 Ballito
14:0	1.30 ± 0.19	1.00 ± 0.10	1.72 ± 0.17	2.25 ± 0.22	2.44 ± 0.30	3.15 ± 0.33	2.65 ± 0.27	3.88 ± 0.53	3.71 ± 0.83
16:0	23.56 ± 1.37	20.16 ± 1.16	20.25 ± 1.47	20.37 ± 2.06	19.07 ± 2.20	21.06 ± 2.43	15.72 ± 0.63	18.09 ± 2.01	24.69 ± 5.09
16:1n-7	4.48 ± 0.58	2.83 ± 0.68	4.69 ± 0.78	2.93 ± 0.24	3.64 ± 0.34	4.64 ± 0.20	4.95 ± 0.51	6.83 ± 0.96	6.23 ± 1.06
18:0	6.52 ± 0.81	5.70 ± 0.31	5.42 ± 0.85	7.66 ± 0.94	7.21 ± 1.65	9.00 ± 1.72	7.56 ± 0.60	7.04 ± 0.92	10.18 ± 1.31
18:1n-9	1.52 ± 0.20	1.25 ± 0.29	1.72 ± 0.24	1.60 ± 0.11	1.70 ± 0.22	2.36 ± 0.30	1.87 ± 0.07	2.51 ± 0.39	3.31 ± 0.33
18:1n-7	2.13 ± 0.22	2.49 ± 0.29	2.96 ± 0.14	2.42 ± 0.23	2.93 ± 0.40	3.06 ± 0.26	3.04 ± 0.43	3.31 ± 0.59	2.64 ± 0.38
18:2n-6	1.83 ± 0.08	1.91 ± 0.13	1.19 ± 0.05	2.90 ± 0.30	2.37 ± 0.37	2.00 ± 0.46	3.91 ± 0.51	4.07 ± 0.49	2.22 ± 0.85
18:3n-3	0.26 ± 0.09	0.48 ± 0.09	0.60 ± 0.09	0.96 ± 0.18	0.95 ± 0.27	0.55 ± 0.29	1.49 ± 0.15	1.21 ± 0.17	0.35 ± 0.30
18:4n-3	1.33 ± 0.31	3.76 ± 0.24	1.30 ± 0.35	0.70 ± 0.13	0.96 ± 0.40	0.84 ± 0.20	3.15 ± 0.16	1.08 ± 0.17	3.06 ± 0.16
20:1n-11	1.43 ± 0.10	1.54 ± 0.18	1.67 ± 0.12	1.77 ± 0.20	1.76 ± 0.15	1.75 ± 0.13	3.01 ± 0.43	2.68 ± 1.42	2.48 ± 0.60
20:1n-9	5.44 ± 0.64	5.06 ± 0.29	4.33 ± 0.28	4.57 ± 0.73	4.84 ± 0.70	6.13 ± 0.80	3.23 ± 0.11	4.12 ± 0.64	7.88 ± 1.53
20:1n-7	0.92 ± 0.25	1.00 ± 0.09	1.45 ± 0.13	0.80 ± 0.19	1.65 ± 0.28	1.98 ± 0.34	0.93 ± 0.11	1.90 ± 0.82	1.14 ± 0.29
20:4n-6	4.36 ± 0.49	3.74 ± 0.46	3.54 ± 0.62	5.95 ± 0.58	8.53 ± 0.91	5.05 ± 0.82	9.14 ± 0.55	6.12 ± 1.28	4.25 ± 1.57
20:5n-3	11.48 ± 1.16	13.55 ± 0.90	18.09 ± 2.17	9.74 ± 1.02	8.45 ± 2.23	9.45 ± 1.49	9.74 ± 1.18	9.13 ± 1.55	7.54 ± 1.26
22:2n-6	1.14 ± 0.26	1.28 ± 0.24	0.80 ± 0.14	2.37 ± 0.34	2.60 ± 0.34	2.35 ± 0.37	2.29 ± 0.28	2.39 ± 0.58	1.14 ± 0.61
22:4n-6	1.59 ± 0.08	1.42 ± 0.06	1.46 ± 0.22	1.55 ± 0.30	2.02 ± 0.27	2.38 ± 0.24	2.95 ± 0.44	2.14 ± 0.44	1.21 ± 0.55
22:5n-6	0.65 ± 0.08	2.29 ± 0.11	1.28 ± 0.08	2.45 ± 0.11	1.72 ± 0.14	1.31 ± 0.12	1.85 ± 0.25	1.81 ± 0.24	1.05 ± 0.41
22:5n-3	1.49 ± 0.10	1.88 ± 0.16	2.91 ± 0.11	2.31 ± 0.21	2.87 ± 0.40	2.71 ± 0.43	2.79 ± 0.60	1.31 ± 0.07	1.25 ± 0.35
22:6n-3	23.45 ± 1.48	24.65 ± 1.52	19.44 ± 0.83	21.70 ± 2.11	20.02 ± 1.49	16.11 ± 1.84	16.65 ± 1.16	16.35 ± 0.93	9.65 ± 2.74
BAME	5.12 ± 0.33	4.01 ± 0.26	5.18 ± 1.28	5.01 ± 0.52	4.26 ± 0.83	4.12 ± 0.89	3.08 ± 0.26	4.03 ± 0.40	6.01 ± 1.15
SFA	36.50 ± 2.22	30.87 ± 1.00	32.57 ± 3.49	35.29 ± 3.55	32.98 ± 4.81	37.33 ± 4.99	29.01 ± 1.22	33.04 ± 3.00	44.59 ± 7.22
MUFA	15.92 ± 1.14	14.17 ± 1.35	16.82 ± 0.65	14.09 ± 1.19	16.52 ± 1.08	19.92 ± 1.20	17.03 ± 0.58	21.35 ± 1.20	23.69 ± 1.85
PUFA	47.58 ± 2.47	54.96 ± 1.75	50.61 ± 3.02	50.63 ± 4.30	50.49 ± 5.53	42.75 ± 5.87	53.96 ± 1.48	45.61 ± 3.81	31.72 ± 8.22

Table 4. Total fatty acid (TFA) composition of barnacles *Chthamalus dentatus* on the west, south and east coasts of South Africa. The values are percentages expressed as mean ± SD (n = 3 per location; hence, n = 6 per site). Only FAs contributing >1% to TFA are displayed. BAME: bacterial FA, PUFA: polyunsaturated FA, MUFA: monounsaturated FA, SFA: saturated FA. Non-methylene-interrupted FAs were omitted from the biogeography effect analyses

| Fatty acid | West coast | | | South coast | | | East coast | | |
	Site 2 Doring Bay	Site 3 Lambert's Bay	Site 5 Blouberg-strand	Site 6 Jongens-fontein	Site 8 St. Francis Bay	Site 10 Kidd's Beach	Site 11 Mbotyi	Site 12 Pennington	Site 13 Ballito
14:0	4.21 ± 0.74	3.33 ± 0.21	5.66 ± 0.49	2.42 ± 0.46	2.73 ± 0.30	4.09 ± 0.31	3.57 ± 0.41	4.13 ± 0.46	3.00 ± 0.27
16:0	28.01 ± 4.18	19.03 ± 1.11	18.68 ± 1.25	24.36 ± 2.84	33.42 ± 4.30	22.64 ± 0.37	23.03 ± 1.71	38.89 ± 1.93	26.30 ± 1.36
16:1n-7	5.56 ± 0.57	5.73 ± 0.20	5.91 ± 0.60	2.35 ± 0.34	1.87 ± 0.28	4.90 ± 0.29	4.49 ± 0.21	2.85 ± 0.88	3.50 ± 0.24
16:4n-1	0.17 ± 0.21	0.03 ± 0.03	2.09 ± 0.22	0.06 ± 0.05	0.00 ± 0.00	0.24 ± 0.03	0.48 ± 0.19	0.08 ± 0.04	0.17 ± 0.06
18:0	2.89 ± 0.44	2.94 ± 0.21	5.62 ± 0.35	14.76 ± 2.09	22.31 ± 3.01	8.51 ± 0.32	11.36 ± 1.33	19.48 ± 3.11	14.45 ± 1.26
18:1n-9	4.93 ± 0.32	4.75 ± 0.66	8.18 ± 0.32	5.98 ± 0.23	3.82 ± 0.93	5.85 ± 0.13	6.01 ± 0.41	3.12 ± 0.87	5.18 ± 0.29
18:1n-7	4.68 ± 0.51	3.77 ± 0.31	5.22 ± 0.61	3.10 ± 0.11	2.53 ± 0.67	2.81 ± 0.16	2.95 ± 0.23	2.83 ± 0.91	2.76 ± 0.38
18:2n-6	1.08 ± 0.12	1.50 ± 0.08	0.91 ± 0.04	1.05 ± 0.18	1.35 ± 0.16	0.88 ± 0.02	1.07 ± 0.19	0.76 ± 0.28	1.20 ± 0.13
18:3n-3	0.38 ± 0.19	0.29 ± 0.01	0.40 ± 0.04	0.44 ± 0.12	0.36 ± 0.13	0.56 ± 0.03	0.83 ± 0.26	0.41 ± 0.16	0.78 ± 0.11
18:4n-3	4.23 ± 1.16	7.68 ± 0.37	1.68 ± 0.15	0.45 ± 0.16	2.35 ± 0.19	0.94 ± 0.09	1.13 ± 0.30	1.05 ± 0.47	2.74 ± 0.11
20:1n-11	1.36 ± 0.07	1.32 ± 0.29	1.99 ± 0.31	0.48 ± 0.24	0.80 ± 0.33	0.81 ± 0.14	1.09 ± 0.13	0.67 ± 0.13	0.49 ± 0.14
20:1n-9	0.94 ± 0.12	0.80 ± 0.02	0.99 ± 0.07	0.60 ± 0.09	0.56 ± 0.13	0.48 ± 0.02	0.43 ± 0.03	0.49 ± 0.10	0.59 ± 0.21
20:4n-6	0.13 ± 0.04	0.10 ± 0.05	0.16 ± 0.15	2.88 ± 0.58	1.56 ± 0.69	2.87 ± 0.23	2.91 ± 0.47	1.62 ± 0.27	3.37 ± 0.22
20:5n-3	12.97 ± 1.77	14.69 ± 0.51	24.10 ± 1.18	12.17 ± 2.26	8.16 ± 2.73	19.17 ± 0.35	14.88 ± 2.20	5.98 ± 1.59	11.62 ± 0.79
22:0	0.32 ± 0.07	0.17 ± 0.05	0.21 ± 0.06	4.95 ± 0.22	2.58 ± 0.21	0.86 ± 0.03	3.15 ± 0.23	1.79 ± 0.40	1.61 ± 0.20
22:1n-11	0.20 ± 0.07	0.34 ± 0.03	0.44 ± 0.01	0.37 ± 0.03	0.31 ± 0.05	0.33 ± 0.06	0.83 ± 0.21	0.52 ± 0.22	1.19 ± 0.03
22:5n-3	0.98 ± 0.17	0.04 ± 0.05	0.40 ± 0.04	0.73 ± 0.32	0.28 ± 0.18	1.63 ± 0.06	1.32 ± 0.20	0.40 ± 0.19	0.77 ± 0.07
22:6n-3	23.29 ± 2.67	29.53 ± 0.70	15.12 ± 1.04	17.87 ± 3.14	8.49 ± 2.94	19.03 ± 1.07	15.65 ± 2.43	8.10 ± 1.14	15.03 ± 1.65
24:1n-9	0.15 ± 0.08	0.16 ± 0.04	0.22 ± 0.14	0.44 ± 0.10	0.25 ± 0.16	0.66 ± 0.19	0.88 ± 0.14	0.73 ± 0.12	0.92 ± 0.09
BAME	3.53 ± 0.61	3.80 ± 0.12	2.00 ± 0.13	4.55 ± 0.43	6.28 ± 0.62	2.73 ± 0.12	3.92 ± 0.81	6.11 ± 0.18	4.34 ± 0.37
SFA	38.96 ± 5.23	29.28 ± 1.12	32.18 ± 1.65	51.04 ± 5.60	67.31 ± 8.31	38.83 ± 0.64	45.03 ± 3.91	70.40 ± 4.88	49.71 ± 2.38
MUFA	17.82 ± 0.99	16.87 ± 0.94	22.96 ± 0.83	13.31 ± 0.38	10.14 ± 1.80	15.86 ± 0.35	16.68 ± 1.11	11.21 ± 2.37	14.63 ± 0.69
PUFA	43.23 ± 4.69	53.85 ± 1.40	44.86 ± 2.02	35.65 ± 6.01	22.54 ± 6.76	45.32 ± 1.17	38.28 ± 5.33	18.39 ± 2.90	35.67 ± 2.44

at the scale of the location was found for $\delta^{13}C$ at a few sites. Tukey HSD tests highlighted dissimilarities between locations at sites 8 and 12 for mussels and site 10 for barnacles (p < 0.01), but these did not appear to affect the results for the other factors.

Upwelling effects

Significant upwelling effects were found on the SI signatures of mussels and barnacles on both coasts and on the FA signatures of both taxa in the west coast samples only (ANOVA and PERMANOVA, both p < 0.01) (Tables 5 & 6, as well as Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m543p037_supp.pdf). Mussels and barnacles from upwelling sites on the west coast had depleted $\delta^{13}C$ signatures compared to non-upwelling sites (Fig. 4). This effect was stronger at site 1 than site 4. The 2 non-upwelling sites on the west coast (2 and 5) had similar $\delta^{13}C$ values for mussels, while they were significantly different from each other for barnacles, with site 2 being depleted in $\delta^{13}C$ compared to site 5 (Tukey HSD, p > 0.05). On the south coast,

depletion of $\delta^{13}C$ ratios at upwelling sites was only significant for mussels, and the effect was weaker than on the west coast, while only barnacles from upwelling site 9 showed depletion of $\delta^{13}C$. For barnacles, the other site of upwelling, site 7, was not different from the 2 non-upwelling sites (Tukey HSD, p < 0.01; Fig. 4). Mussels from the non-upwelling site 8 revealed enrichment of $\delta^{13}C$ compared to the other non-upwelling site 10 (−16.37 ± 0.1‰ and −15.55 ± 0.2‰, respectively, for sites 10 and 8), although they were both enriched in $\delta^{13}C$ compared to their corresponding upwelling sites (Tukey HSD, p < 0.05).

$\delta^{15}N$ was not affected by upwelling on either coast (ANOVA, p > 0.05). On the west coast there was variability among sites, with site 4 being $\delta^{15}N$ enriched compared to the other 3 sites for both taxa (Tukey HSD, p < 0.01; Fig. 4). $\delta^{15}N$ also differed strongly among sites on the south coast (ANOVA, p < 0.01). As for the biogeography effect, $\delta^{15}N$ increased from east to west, i.e. from site 10 to site 7 on the south coast, with no differences between either upwelling or non-upwelling sites (Fig. 4). This effect was observed for both taxa, with the minor variation that sites 10 and 9

Table 5. Total fatty acid (TFA) composition of barnacles *Chthamalus dentatus* in upwelling and non-upwelling conditions. The values are percentage expressed as mean ± SD (n = 3 per location; hence, n = 6 per site). Sites 1, 4, 7 and 9 are upwelling sites, and sites 2, 5, 8 and 10 are non-upwelling sites. Only FAs contributing >1% to TFA are displayed. BAME: bacterial FA, PUFA: polyunsaturated FA, MUFA: monounsaturated FA, SFA: saturated FA, NMI: non-methylene-interrupted FA

| Fatty acid | West coast | | | | South coast | | | |
	Site 1 Groenrivier	Site 2 Doring Bay	Site 4 Pasternoster	Site 5 Bloubergstrand	Site 7 Brenton on Sea	Site 8 St. Francis Bay	Site 9 Port Alfred	Site 10 Kidd's Beach
14:0	1.43 ± 0.24	4.33 ± 0.53	2.37 ± 0.16	5.94 ± 0.37	3.05 ± 0.26	2.75 ± 0.20	4.43 ± 0.51	4.09 ± 0.22
16:0	23.33 ± 1.92	28.84 ± 3.05	16.18 ± 0.38	19.59 ± 0.94	24.93 ± 1.65	33.62 ± 2.89	23.83 ± 2.61	19.64 ± 0.26
16:1n-7	3.98 ± 0.24	5.72 ± 0.41	3.54 ± 0.16	6.20 ± 0.45	2.39 ± 0.14	1.89 ± 0.21	4.40 ± 0.54	4.90 ± 0.20
16:4n-1	0.00 ± 0.00	0.18 ± 0.15	0.83 ± 0.14	2.19 ± 0.17	0.09 ± 0.04	0.00 ± 0.00	0.63 ± 0.28	0.24 ± 0.02
18:0	3.35 ± 0.38	2.97 ± 0.32	3.44 ± 0.08	3.80 ± 0.26	12.10 ± 1.41	22.44 ± 2.03	9.20 ± 1.33	8.51 ± 0.24
18:1n-9	6.89 ± 0.19	5.07 ± 0.23	8.86 ± 0.49	8.58 ± 0.24	5.05 ± 0.12	3.86 ± 0.68	5.06 ± 0.38	5.85 ± 0.09
18:1n-7	5.17 ± 0.34	4.81 ± 0.38	5.37 ± 0.40	5.47 ± 0.45	2.47 ± 0.09	2.57 ± 0.49	2.42 ± 0.38	2.82 ± 0.12
18:2n-6	3.92 ± 0.39	1.11 ± 0.09	2.40 ± 0.10	0.95 ± 0.03	1.24 ± 0.07	0.60 ± 0.11	0.75 ± 0.11	0.88 ± 0.02
18:3n-3	2.95 ± 0.37	0.39 ± 0.14	1.87 ± 0.10	0.42 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:4n-3	7.14 ± 1.27	4.53 ± 0.78	4.99 ± 0.48	1.77 ± 0.12	0.91 ± 0.24	0.30 ± 0.13	1.27 ± 0.24	0.93 ± 0.06
20:1n-11	1.27 ± 0.06	1.40 ± 0.06	2.99 ± 0.21	2.09 ± 0.23	0.52 ± 0.14	0.80 ± 0.23	0.63 ± 0.07	0.81 ± 0.10
20:1n-9	0.73 ± 0.03	0.97 ± 0.09	1.27 ± 0.02	1.03 ± 0.05	0.74 ± 0.13	0.57 ± 0.09	0.50 ± 0.12	0.48 ± 0.02
20:1n-7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.81 ± 0.23	0.35 ± 0.10	0.07 ± 0.07	0.17 ± 0.11
20:2n-6	0.96 ± 0.03	0.39 ± 0.03	1.00 ± 0.03	0.58 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:4n-6	0.69 ± 0.04	0.14 ± 0.03	1.18 ± 0.06	0.17 ± 0.11	1.56 ± 0.07	1.59 ± 0.50	1.61 ± 0.25	2.87 ± 0.16
20:5n-3	16.02 ± 1.10	11.29 ± 1.29	21.96 ± 0.38	25.27 ± 0.85	12.18 ± 1.01	6.27 ± 1.98	15.62 ± 2.64	19.36 ± 0.28
22:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.69 ± 0.19	2.60 ± 0.14	1.24 ± 0.31	0.86 ± 0.02
22:5n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.09	0.28 ± 0.13	1.11 ± 0.26	1.63 ± 0.04
22:6n-3	20.03 ± 0.81	23.97 ± 1.94	19.66 ± 0.42	13.77 ± 0.76	21.22 ± 2.48	8.61 ± 2.14	17.42 ± 2.16	19.03 ± 0.76
BAME	2.14 ± 0.20	3.90 ± 0.51	2.11 ± 0.06	2.19 ± 0.10	8.65 ± 0.45	10.90 ± 0.91	9.82 ± 1.74	6.92 ± 0.29
SFA	30.25 ± 2.69	40.04 ± 3.85	24.09 ± 0.42	31.52 ± 1.24	50.42 ± 3.44	72.31 ± 6.08	48.51 ± 5.89	40.02 ± 0.57
MUFA	18.04 ± 0.43	17.98 ± 0.73	22.02 ± 0.30	23.37 ± 0.58	11.98 ± 0.50	10.04 ± 1.34	13.08 ± 1.17	15.03 ± 0.29
PUFA	51.71 ± 3.12	41.98 ± 3.33	53.89 ± 0.43	45.11 ± 1.42	37.60 ± 3.82	17.65 ± 4.83	38.41 ± 5.02	44.95 ± 0.85

Table 6. Total fatty acid (TFA) composition of mussels *Mytilus galloprovincialis* and *Perna perna* in upwelling and non-upwelling conditions. The values are percentages expressed as mean ± SD (n = 3 per location; hence, n = 6 per site). Sites 1, 4, 7 and 9 are upwelling sites, and sites 2, 5, 8 and 10 are non-upwelling sites. Only FAs contributing >1% to TFA are displayed. BAME: bacterial FA, PUFA: polyunsaturated FA, MUFA: monounsaturated FA, SFA: saturated FA, NMI: non-methylene-interrupted FA

Fatty acid	West coast				South coast			
	Site 1 Groenrivier	Site 2 Doring Bay	Site 4 Pasternoster	Site 5 Bloubergstrand	Site 7 Brenton on Sea	Site 8 St. Francis Bay	Site 9 Port Alfred	Site 10 Kidd's Beach
14:0	0.72 ± 0.08	1.14 ± 0.13	0.77 ± 0.08	1.49 ± 0.13	4.09 ± 0.29	2.08 ± 0.17	2.33 ± 0.08	2.65 ± 0.19
16:0	19.85 ± 0.79	24.09 ± 0.96	18.76 ± 0.24	20.87 ± 0.52	30.18 ± 2.81	17.45 ± 1.22	15.52 ± 1.10	18.64 ± 1.47
16:1n-7	1.98 ± 0.24	3.03 ± 0.37	2.47 ± 0.35	4.11 ± 0.54	3.07 ± 0.21	3.11 ± 0.21	4.02 ± 0.20	3.90 ± 0.11
18:0	5.23 ± 0.30	6.54 ± 0.47	5.30 ± 0.28	5.42 ± 0.29	12.08 ± 1.12	8.57 ± 0.94	8.91 ± 0.37	10.10 ± 0.98
18:1n-9	1.34 ± 0.16	1.32 ± 0.11	1.91 ± 0.25	1.48 ± 0.18	1.98 ± 0.20	1.47 ± 0.13	1.45 ± 0.12	1.99 ± 0.18
18:1n-7	2.26 ± 0.16	1.85 ± 0.13	2.29 ± 0.06	2.56 ± 0.13	2.07 ± 0.10	2.51 ± 0.27	2.92 ± 0.23	2.57 ± 0.15
18:2n-6	1.50 ± 0.19	0.72 ± 0.05	1.63 ± 0.11	1.07 ± 0.02	1.33 ± 0.29	2.03 ± 0.23	2.30 ± 0.16	1.68 ± 0.28
18:3n-3	0.85 ± 0.15	0.23 ± 0.06	0.96 ± 0.13	0.55 ± 0.04	0.40 ± 0.19	0.73 ± 0.17	0.98 ± 0.21	0.47 ± 0.17
18:4n-3	1.08 ± 0.17	1.16 ± 0.19	1.54 ± 0.20	1.15 ± 0.21	0.34 ± 0.24	0.83 ± 0.25	0.69 ± 0.13	0.25 ± 0.12
20:1n-11	1.14 ± 0.11	1.24 ± 0.05	1.10 ± 0.06	1.46 ± 0.06	1.57 ± 0.13	1.50 ± 0.09	1.46 ± 0.07	1.48 ± 0.08
20:1n-9	4.61 ± 0.17	4.72 ± 0.36	5.19 ± 0.27	3.67 ± 0.12	6.70 ± 0.67	4.12 ± 0.39	3.58 ± 0.24	5.15 ± 0.46
20:1n-7	0.61 ± 0.09	0.80 ± 0.14	0.98 ± 0.10	1.20 ± 0.10	1.29 ± 0.16	1.41 ± 0.17	1.37 ± 0.11	1.66 ± 0.19
20:2 NMI1	5.95 ± 0.51	4.73 ± 0.31	6.09 ± 0.40	4.34 ± 0.39	2.55 ± 0.34	4.68 ± 0.32	4.07 ± 0.26	3.87 ± 0.30
20:2 NMI2	1.42 ± 0.24	1.26 ± 0.18	1.25 ± 0.14	1.10 ± 0.20	1.00 ± 0.27	0.99 ± 0.38	0.49 ± 0.11	0.84 ± 0.18
20:4n-6	4.78 ± 0.12	3.20 ± 0.27	5.40 ± 0.72	3.47 ± 0.41	2.31 ± 0.48	5.55 ± 0.56	5.27 ± 0.22	5.44 ± 0.52
20:5n-3	10.05 ± 0.65	8.26 ± 0.74	13.98 ± 1.07	17.33 ± 1.03	2.94 ± 1.03	7.28 ± 1.42	8.09 ± 0.68	5.09 ± 0.90
22:2n-6	0.84 ± 0.16	0.98 ± 0.15	1.39 ± 0.15	0.71 ± 0.08	1.18 ± 0.16	2.21 ± 0.18	2.17 ± 0.15	2.14 ± 0.22
22:2 NMI1	4.93 ± 0.54	3.99 ± 0.33	4.62 ± 0.38	5.21 ± 0.64	2.94 ± 0.49	6.42 ± 0.52	7.21 ± 0.28	6.58 ± 0.38
22:2 NMI2	0.91 ± 0.23	1.72 ± 0.16	1.00 ± 0.07	0.29 ± 0.13	2.01 ± 0.55	0.98 ± 0.28	1.99 ± 0.59	3.26 ± 0.88
22:3 NMI	1.85 ± 0.13	1.37 ± 0.08	1.71 ± 0.05	1.43 ± 0.07	0.44 ± 0.29	1.46 ± 0.15	1.53 ± 0.07	1.33 ± 0.11
22:4n-6	0.71 ± 0.04	0.51 ± 0.04	0.73 ± 0.11	0.41 ± 0.14	0.31 ± 0.15	1.73 ± 0.16	1.51 ± 0.15	2.09 ± 0.13
22:5n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.64 ± 0.17	1.06 ± 0.09	1.31 ± 0.08	1.11 ± 0.07
22:5n-3	1.97 ± 0.04	1.30 ± 0.07	1.68 ± 0.06	2.08 ± 0.13	1.06 ± 0.28	2.46 ± 0.26	3.21 ± 0.35	2.28 ± 0.26
22:6n-3	20.40 ± 0.72	20.43 ± 1.05	15.18 ± 0.54	14.31 ± 0.30	9.71 ± 2.17	14.58 ± 0.99	13.88 ± 0.88	10.21 ± 1.12
BAME	5.00 ± 0.35	5.42 ± 0.20	4.07 ± 0.10	4.29 ± 0.32	7.81 ± 0.85	4.78 ± 0.46	3.75 ± 0.31	5.25 ± 0.53
SFA	30.81 ± 0.61	37.19 ± 1.46	28.90 ± 0.47	32.07 ± 0.95	54.16 ± 4.91	32.90 ± 2.68	30.50 ± 1.73	36.63 ± 2.94
MUFA	11.93 ± 0.56	12.95 ± 0.61	13.93 ± 0.31	14.49 ± 0.79	16.67 ± 0.81	14.11 ± 0.63	14.81 ± 0.74	16.74 ± 0.66
PUFA	57.26 ± 0.79	49.86 ± 1.47	57.17 ± 0.43	53.44 ± 1.01	29.17 ± 5.42	52.99 ± 3.04	54.69 ± 2.23	46.63 ± 3.43

did not differ from each other in the case of barnacles (Tukey HSD, p < 0.01).

FA composition also responded to the effect of upwelling on the west coast (Fig. 5; PERMANOVA, p < 0.001), with specimens of both mussels and barnacles from upwelling areas being enriched in PUFAs such as 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3 or dinoflagellate TMs, and NMI FAs for mussels, while those from non-upwelling areas had higher values of 14:0, 16:0 or 16:1n-7 and 18n-9 (Table 6; SIMPER). Differences among sites on the west coast were also recorded for both taxa, with the 2 non-upwelling sites being different from each other for both taxa, as were the upwelling sites in the case of barnacles (PERMANOVA pairwise test, p < 0.05). For both taxa, site 2 had higher proportions of 16:0, 22:4n-3 and dinoflagellate TMs from site 5, which was typified by 16:4n-1, 22:5n-3 and diatom TMs (SIMPER). The differences between upwelling sites for barnacles were mainly due to 16:0, 18:2n-6, 18:3n-3 and dinoflagel-

late TMs, which characterized site 1, while 14:0, 16:4n-1, 20:1n-11 and 20:5n-3 were higher at site 4 (SIMPER). Another important source of variation was identified by a non-metric MDS plot analysis, which separated specimens from the northerly sites 1 and 2 from the other 2 sites (Fig. 5b). The FAs responsible for this variation were mainly 16:0, 16:1n-7, 18:2n-6 and dinoflagellate TMs, which were more abundant at sites 1 and 2, while 16:4n-1, 18:1n-9, 18:3n-4 20:1n-11 and 20:5n-3 characterized sites 4 and 5 (SIMPER).

In contrast, no effect of upwelling was detected on the FA composition of the south coast samples for either taxon (PERMANOVA, p > 0.05; Fig. 5c,d). Differences among sites were recorded for mussels, with the 2 upwelling sites, 7 and 9, being different from each other (PERMANOVA pairwise test, p < 0.01). Site 7 had a high proportion of SFAs (as BAMEs, 14:0, 16:0 and 18:0), while site 9 was characterized by PUFAs, for instance 20:2NMI1, 20:5n-3, 22:6n-3 or 22:3NMI (SIMPER). The 2 non-upwelling sites, 8 and
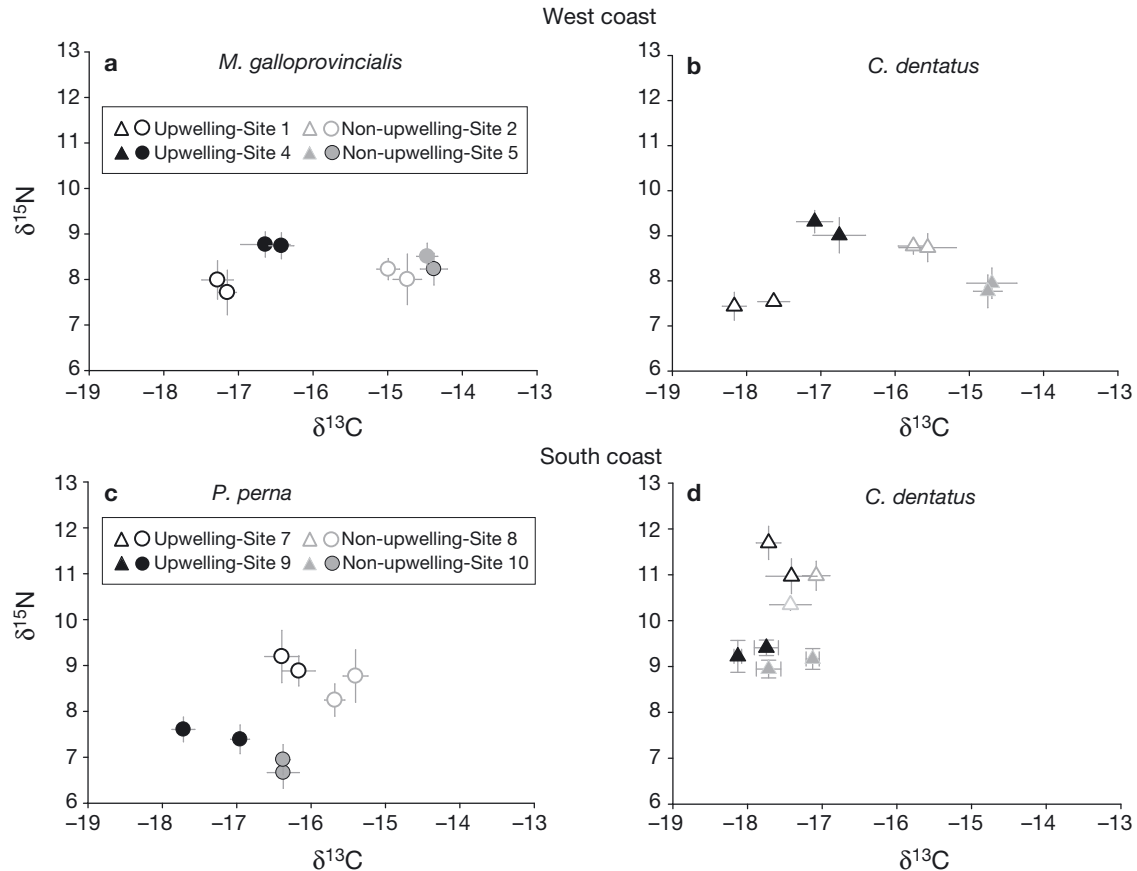
West coast

South coast

Fig. 4. Stable isotope (SI: δ¹³C and δ¹⁵N) signatures of intertidal mussels *Mytilus galloprovincialis* and *Perna perna* (circles) and barnacles *Chthamalus dentatus* (triangles) at locations within upwelling (black) and non-upwelling (grey) sites on the (a,b) west and (c,d) south coasts (mean ± SD; n = 5)

10, did not show dissimilarities between each other for either taxon, as well as the 2 upwelling sites for barnacles (PERMANOVA pairwise test, p > 0.05). These results were confirmed by the MDS for each taxon (Fig. 5).

No location effect was recorded at sites used for the upwelling comparison for either SI or FA analyses, with the exception of δ¹³C at site 8 for mussels and site 10 for barnacles (Tukey HSD, p < 0.01), and for the FA signatures of barnacles at sites 7 and 8 (PERMANOVA pairwise test, p < 0.05 for both sites). Again, this minor effect of location did not influence the results of the other factors.

DISCUSSION

SI and FA signatures of mussels and barnacles showed very distinct patterns in response to both biogeography and upwelling. The upwelling results with samples of the south and west coast together showed the main driver of variability to be bioregion.

This indicates that biogeography is the main factor generating variability in filter feeder diets along the South African coast, and we should consider the effects of upwelling to be effectively nested within those of biogeography.

Effect of biogeography

Mussels and barnacles showed similar SI patterns among bioregions, but these differed between δ¹⁵N and δ¹³C. For both taxa, δ¹⁵N decreased from northeast to southwest on the east coast, increased from east to west along the south coast and remained roughly constant along the west coast. δ¹³C increased continuously from east to west along the east and south coasts, while the west coast samples were enriched compared to the other 2 coasts. This accords with a previous study which found a gradient of δ¹³C and δ¹⁵N signatures of the SPM and intertidal benthic organisms along the South African coast (Hill et al. 2006). The strong spatial pattern in SI signatures of
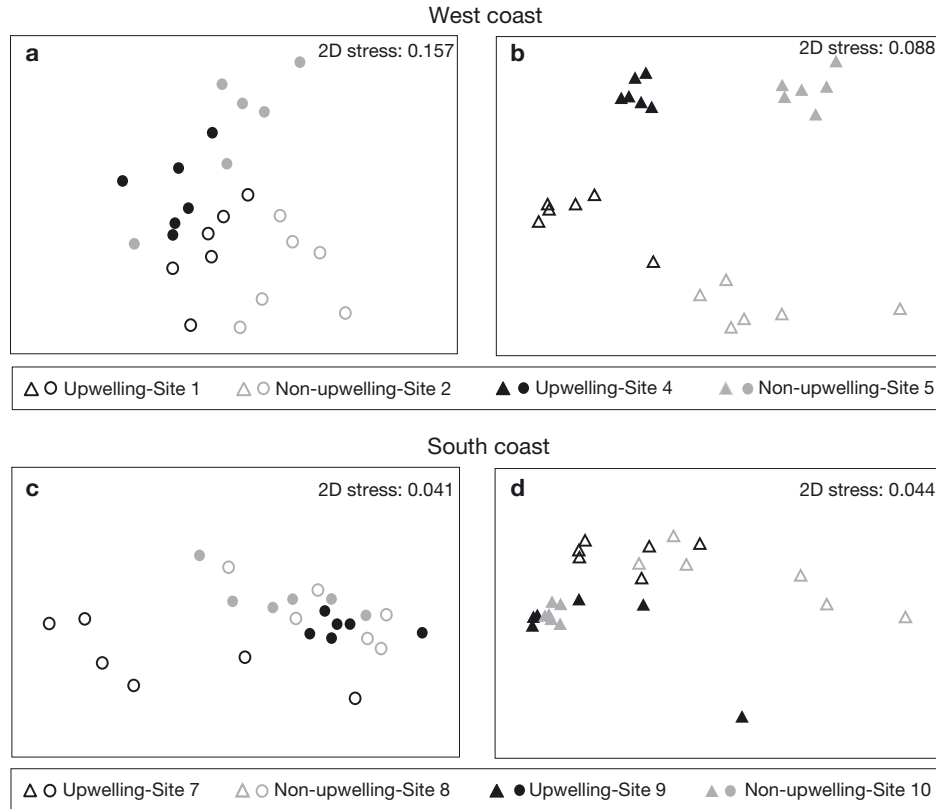
Fig. 5. Non-metric multidimensional scaling analyses of total fatty acid (TFA) composition of mussels *Mytilus galloprovincialis* and *Perna perna* (circles) and barnacles *Chthamalus dentatus* (triangles) on the (a,b) west and (c,d) south coasts at upwelling (black) and non-upwelling (grey) sites. Each symbol represents a single replicate (n = 3 per location; hence, n = 6 per site)

benthic primary consumers between the south and east coasts versus those on the west coast reflect the effects of the Agulhas and the Benguela Currents, respectively. The Benguela Current is a highly productive eutrophic system along the west coast that promotes high primary production dominated by phytoplankton (Andrews & Hutchings 1980, Brown et al. 1991, Pitcher et al. 1992), whereas the south and east coasts are oligotrophic, strongly influenced by the nutrient-depleted Agulhas Current (Schleyer 1981, Probyn et al. 1994, Machu et al. 2005). Oligotrophic systems are generally depleted in $\delta^{15}N$ compared to more productive environments due to the high levels of recycling that occur in nutrient depauperate waters (Miyake & Wada 1967). Consequently, we suggest that the differences in $\delta^{15}N$ signatures measured in the taxa of this study result from the dependence of primary producers on recycled nitrogen in oligotrophic waters (Hill et al. 2006). The concomitant increase of $\delta^{13}C$ is probably related to the offshore deflection of the Agulhas Current passing from the east to the south coast as it follows the con-

tinental shelf break, which diverges from the coast towards the south-west. Coastal water is usually $\delta^{13}C$ enriched compared to offshore water (Hill et al. 2006), and the continental shelf is narrow on the east coast (Goschen & Schumann 1990) so that onshore water is well mixed with offshore water, while this mixing becomes weaker as the Agulhas Current is deflected offshore along the south coast (Roberts 2005). Similar SI results on filter feeder signatures of South Africa were found by Allan et al. (2010) on the easterly side of the south coast and by Bustamante & Branch (1996) on the west coast.

FA signatures of filter feeders on the west coast were enriched in n-3 PUFAs compared to the other 2 coasts, which were not different from each other for either taxon and were enriched in n-6 PUFAs. High levels of n-3 PUFAs are indicative of microalgae, while macroalgae are more n-6 PUFA enriched (Dalsgaard et al. 2003), indicating stronger contributions by microalgae on the west coast and of macroalgae on the east and south coasts. In addition, mussel samples from the west coast were typi-

fied by dinoflagellate TMs (i.e. 18:4n-3 and 22:6n-3), and barnacles had high values of both dinoflagellate and diatom TMs (i.e. diatom TMs: 16:1n-7 and 20:5n-3). Specimens from the other 2 coasts, on the other hand, were not characterized by any specific TM. This suggests that filter feeder diets were more dependent on phytoplankton on the west coast and on macroalgal detritus on the south and east coasts. This pattern is probably due to the promotion of frequent phytoplankton blooms on the west coast by the eutrophic Benguela upwelling ecosystem on the west coast (Shannon et al. 1983, Mitchell-Innes et al. 2000), so that specimens from the west coast are exposed to a considerable phytoplankton input, compared to benthic filter feeders on the other 2 coasts. Another aspect to consider is that different macroalgal taxa dominate these coasts. The west coast is characterized by extensive forests of the kelps *Ecklonia maxima* and *Laminaria pallida* (Velimirov 1980, Bustamante et al. 1995), while the south and east coasts are typified by rhodophytes and coralline algae (Griffiths & Branch 1997, Bolton et al. 2004). These macroalgae become partially available for filter feeders through degradation and could thus be identifiable in their FA signatures, for instance, using the FA 20:4n-6, which is characteristic of kelp (Hanson et al. 2010). Kelp detritus has been shown to be central to the west coast ecosystem (Bustamante & Branch 1996) and in other systems worldwide (Dunton & Schell 1987, Miller & Page 2012), but surprisingly our results showed that kelp TMs were found in higher proportions on the south and east coasts than on the west coast. The differences in the kelp TMs between the west and the south and east coasts are not unexpected since these biogeographical regions are characterized by different hydrographic regimes. The high abundances of phytoplankton on the west coast perhaps override the presence of other food sources in the water, potentially masking the importance of kelp to benthic filter feeders. In contrast, the oligotrophic Agulhas system inputs smaller amounts of nutrients in the water, obliging filter feeders on the south and east coasts to rely heavily on macroalgal detritus. In addition, it is also difficult to highlight the importance of kelps because only 1 FA is strongly associated with them, in contrast to other organisms such as diatoms or dinoflagellates that are characterized by multiple FAs (Parrish et al. 2000, Hanson et al. 2010). Nevertheless, a few other studies showed that a kelp food source was not important for marine organisms (Porri et al. 2011, McLeod et al. 2013), suggesting that the role of kelp in natural systems

may be different depending on the organism and ecosystem under study.

While it has been shown that the SI signatures of co-existing *Perna perna* and *Mytilus galloprovincialis* do not differ (Hill et al. 2006), the differences observed among coasts in mussel FA signatures could also reflect differences between the 2 species of mussels sampled. A few studies have evaluated the FA signatures of co-occurring mussels and have indicated minor differences between their FA values, which were probably related to species selectivity or differences in their metabolism (Beninger & Stephan 1985, Ezgeta-Bali et al. 2014). Although we recognize the confounding effect of having different mussel species for the west and south coasts, it is important to highlight that the barnacle *Chthamalus dentatus*, which occurs along the entire South African coast, showed the same geographical pattern as mussels.

Effect of upwelling

Upwelling had a profound effect on SI signatures within both coasts, and on FA signatures within the west coast. Carbon signatures were significantly depleted at upwelling sites for all species on both the west and the south coasts, with values lying between those of filter feeders from non-upwelling sites (−15.5 to −18‰, this study) and offshore phytoplankton (−20 to −22‰; Hill et al. 2006). δ^{13}C is an indicator of food source, indicating that the food source of filter feeders at upwelling sites was different from non-upwelling sites. Previous SI studies showed that intertidal organisms feed predominantly on macroalgal detritus (France 1995, Bustamante & Branch 1996, Hill et al. 2006), and others have highlighted that the δ^{13}C of SPM displays significant depletion when moving from the near to the offshore environments (Hill et al. 2008, Mallela & Harrod 2008). Therefore, we suggest that the depletion of δ^{13}C in specimens from upwelling sites reflects a diet mainly based on SPM brought onshore during periods of downwelling and relaxation conditions (Roughan et al. 2005), or when upwelled water is retained within the centres where it is generated (Nielsen & Navarrete 2004, Wieters 2005).

Other authors have also highlighted the importance of vertical sedimentation of SPM for demersal ecosystems following upwelling events (Corbisier et al. 2014). In our case, this effect was stronger at sites with continuous or very frequent upwelling events on both coasts (i.e. sites 1 and 9). These upwelling

effects were confirmed for both taxa, with the exception of barnacles at 1 site on the south coast. In this case, barnacle $\delta^{13}C$ was depleted only at site 9, while site 7 was not different from its paired non-upwelling site. The discrepancy can be related to dissimilarity in the frequency and intensity of upwelling at the 2 sites, which are well documented (Velimirov 1980, Newell et al. 1982, Schumann et al. 1982, Lutjeharms et al. 2000). Site 7, which did not show any upwelling effect, experiences only sporadic and ephemeral upwelling, while site 1 (Groenrivier) and site 9 (Port Alfred) are centres of either continuous or frequent upwelling, and site 4 (Cape Columbine) exhibits seasonal events which occur mostly during summer. Thus, we found that the impact of upwelling on the SI signatures of filter feeders was dependent on the intensity and frequency of the upwelling, with sites known to exhibit frequent and powerful upwelling events showing the strongest effects. The fact that the influence of upwelling is more pronounced where upwelling is continuous or intense has previously been shown (Navarrete et al. 2005, Xavier et al. 2007), and reinforces the interpretation of SI as integrated signatures, with a gradient from non-upwelling to weak to strong upwelling sites characterized by more depleted $\delta^{13}C$. Differences were also recorded between non-upwelling sites. On the west coast, barnacles at site 2 were $\delta^{13}C$ depleted relative to site 5, although both were still $\delta^{13}C$ enriched compared to the 2 upwelling sites. A similar effect was observed between non-upwelling sites on the south coast for both taxa. Such differences may be driven by site-specific hydrodynamics. For example, the tidal cycle can influence benthic food availability. Fréchette & Bourget (1985) showed a depletion of SPM concentration over an immersed mussel bed passing from high to low tide. While tidal effects will be similar among sites, this indicates how local currents or fronts could influence food supply to the shore (Iverson et al. 1979).

In contrast to the results for $\delta^{13}C$, upwelling had no significant effect on the $\delta^{15}N$ signatures of either taxon or coast. As $\delta^{15}N$ is usually an indicator of trophic level (Peterson & Fry 1987), this indicates that the trophic level of these filter feeders was not influenced by upwelling. Our results are in contrast to the outcome of another study which evaluated the effect of upwelling on filter feeders on the Chilean coast (Reddin et al. 2015). Those authors found $\delta^{15}N$ to be an important discriminator between upwelling and non-upwelling sites. They suggested that the difference was due to the use of 'new', isotopically heavier nitrogen by primary producers in upwelling centres compared to the recycled-depleted nitrogen used by specimens at non-upwelling sites. The contrasting results between their study and our work may be related to differences in the hydrodynamics of the regions (Wieters et al. 2009). Another study on the Banc d'Arguin gulf (Mauritania) found that the effect of upwelling differed between the north and south part of the gulf, with benthic invertebrates and fishes living in the northwestern part being dependent on the nearby upwelling phytoplankton production, while this food source did not support the intertidal benthic community in the southeastern part (Carlier et al. 2015). The various results obtained from these studies and the present work suggest that the effect of upwelling may not be ubiquitous. Further investigation is needed in order to assess whether the influence of upwelling may be generalized across ecosystems.

Variability in $\delta^{15}N$ signatures was observed among sites, particularly on the south coast. On this coast, there was an enrichment of $\delta^{15}N$ from the more eastern to the more western sites, presumably reflecting the proximity of the Agulhas Current to the south coast as previously described.

FA composition of mussels and barnacles also showed differences depending on the occurrence and intensity of upwelling; however, this effect was only observed on the west coast for both taxa. This again may be related to the low intensity of upwelling on the south coast (Lutjeharms et al. 2000). Samples from upwelling sites on the west coast showed higher percentages of PUFAs such as 18:2n-6, 20:4n-6, 20:5n-3, dinoflagellate TMs and NMI FAs for mussels, compared to non-upwelling sites, which had high values of SFAs and MUFAs. High levels of PUFAs are usually associated with highly productive systems and are an indication of good food quality (Brett & Müller-Navarra 1997, Müller-Navarra et al. 2000, Dalsgaard et al. 2003). Hence, our results suggest that filter feeders experienced better food quality at upwelling sites compared to non-upwelling sites. In addition, the presence of dinoflagellate TMs in the upwelling samples may represent a proxy for upwelling events, as dinoflagellates are among the first groups of phytoplankton to occur during a bloom (Tilstone et al. 2000), indicating that filter feeders at upwelling areas were directly influenced by the effects of upwelling. The high levels of NMI FAs in the mussels of upwelling sites support this interpretation, as NMI FAs are synthesized de novo by bivalves by elongation and Δ5 desaturation of 18:1n-9 and 16:1n-7, which are both abundant in phytoplankton

and in heterotrophic organisms (Zhukova 1991, Dalsgaard et al. 2003, Peters et al. 2006).

Another important aspect that emerged from the FA analyses on the west coast was the dissimilar pattern between sites 1–2 and 4–5, indicating the degree of potential variability within the same biogeographic region. Sites 1 and 2 were characterized by dinoflagellate TMs compared to the more southerly sites 4 and 5, which were not typified by any specific TM. Other studies have shown intra-region variability for the fauna associated with mussel beds (Cole & McQuaid 2010), mussel distribution (Smith et al. 2009) and intertidal filter-feeder recruitment (Connolly et al. 2001), which can be driven by numerous processes including differences in upwelling intensity, larvae retention or temperature over the spatial range of the region. In our case, the stronger intensity of upwelling in the northern part of the west coast (Andrews & Hutchings 1980, Carr & Kearns 2003), compared to the seasonal upwelling in the southern part of the west coast, could have been the primary factor responsible for the pattern observed.

PERMANOVA highlighted other dissimilarities between sites within the same condition of either upwelling or non-upwelling. At both upwelling sites on the west coast, *C. dentatus* was characterized by dinoflagellate TMs (site 1) or by diatom TMs (site 4). Similarly, the 2 non-upwelling sites showed marked differences between each other for both taxa, with site 2 being typified by dinoflagellate TMs and site 5 by diatom TMs. The fact that even the 2 non-upwelling sites of the west coast presented phyto-plankton TMs, and that all samples from the west coast were characterized by high proportions of PUFAs compared to the other 2 coasts, highlights the importance of the Benguela upwelling system in regulating trophodynamics on the west coast. Although differences were observed among upwelling and non-upwelling sites, this system supports generally high levels of primary production (Bustamante et al. 1995, Cury et al. 2000), an effect that is probably detectable throughout the west coast.

Variability between locations

$\delta^{13}C$ and FA composition of primary consumers also differed at smaller scales with occasional differences observed between locations at the same sites. Such scales of variability can be attributed to a wide range of processes. For example, McQuaid & Mostert (2010) experimentally manipulated water flow at centimetre scales around mussel beds, which altered growth rates, while Kon et al. (2007) observed that the diet of bivalves in an Australian mangrove forest changed according to the microhabitat (tidal creek, mangrove forest, mangrove forest gap) inhabited. Schaal et al. (2011) also suggested that processes happening at the microhabitat level, such as bacterial degradation of macroalgae, can change the food available for intertidal consumers that are living only centimetres apart.

Differences among taxa

SI and FA signatures differed between mussels and barnacles. All samples were collected from shores with similar wave action, and both taxa are non-selective filter feeders; thus, these differences in FA and SI signatures must be driven by other factors, such as body size (Rubenstein & Koehl 1977), differences in filtration mechanisms related to the morphology of the feeding apparatus and food acquisition (Nixon et al. 1971, Rubenstein & Koehl 1977, Zardi et al. 2008), or different metabolic pathways (Napolitano 1999). Nevertheless, little knowledge is available on the FA metabolic pathways of filter feeders. Specifically, there is little information on how and which FAs are assimilated by different filter feeder species, how they are stored or used by the organisms, and whether they are apportioned differently to the consumer's tissues and, if so, in which proportions. It is also important to note that, while *C. dentatus* was sampled as a whole organism, only the adductor muscle was used for *P. perna*. Animal tissues exhibit different levels of metabolic activity (Dahlhoff et al. 2002), and organs might metabolise FAs differently, depending on their function (Ezgeta-Balí et al. 2014). In addition, mussels in the present study were much larger than the barnacles (mean 50 mm and 10 mm, respectively), suggesting they might feed on particles of different size. What is remarkable in the present study is that, despite strong interspecific differences, all taxa showed the same pattern of higher proportions of PUFAs on the west coast compared to the other coasts, and higher proportions of phytoplankton TMs (a proxy for upwelling) at upwelling sites compared to non-upwelling centres on the west coast. The analogous patterns shown by filter feeders along the South African coast confirm that the main factors influencing trophic signatures were biogeography and upwelling, with the effects of the latter nested in the former.

CONCLUSIONS

Our results highlight the importance of the linkages between oceanographic processes acting at different spatial scales on the diets of benthic primary consumers. Biogeography had a paramount influence on filter feeder diets and, within each bioregion, upwelling profoundly affected them, with strength of these effects depending on the frequency and intensity of the upwelling. Given a scenario of climate change, ocean currents and upwelling are likely to respond to alterations in temperature, precipitation and wind intensities (Timmermann et al. 1999). Bakun (1990) and Narayan et al. (2010) showed that the intensity and frequency of upwelling is increasing because of rising greenhouse gas concentrations in the atmosphere which cause an increase in the land–sea pressure gradient and alongshore wind stress on the ocean surface. This will influence nutrient supply to the upper mixed layer and thus primary production, with cascading effects on the population dynamics of consumers (Harley et al. 2006). Although these changes are likely to influence biological productivity, the effects are likely to be coast-specific and at this stage they are not yet predictable (Harley et al. 2006).

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