Temporal and spatial variability in stable isotope ratios of SPM link to local hydrography and longer term SPM averages suggest heavy dependence of mussels on nearshore production

Jaclyn M. Hill*, Christopher D. McQuaid* and Sven Kaehler*

*Coastal Research Group, Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa

Abstract

Temporal changes in hydrography affect suspended particulate matter (SPM) composition and distribution in coastal systems, potentially influencing the diets of suspension feeders. Temporal variation in SPM and in the diet of the mussel *Perna perna*, were investigated using stable isotope analysis. The δ^{13}C and δ^{15} N ratios of SPM, mussels and macroalgae were determined monthly, with SPM samples collected along a 10 km onshore–offshore transect, over 14 months at Kenton-on-Sea, on the south coast of South Africa. Clear nearshore (0 km) to offshore (10 km) carbon depletion gradients were seen in SPM during all months and extended for 50 km offshore on one occasion. Carbon enrichment of coastal SPM in winter (June–August 2004 and May 2005) indicated temporal changes in the nearshore detrital pool, presumably reflecting changes in macroalgal detritus, linked to local changes in coastal hydrography and algal seasonality. Nitrogen patterns were less clear, with SPM enrichment seen between July and October 2004 from 0 to 10 km. Nearshore SPM demonstrated cyclical patterns in carbon over 24-h periods that correlated closely with tidal cycles and mussel carbon signatures, sampled monthly, demonstrated fluctuations that could not be correlated to seasonal or monthly changes in SPM. Macroalgae showed extreme variability in isotopic signatures, with no discernable patterns. IsoSource mixing models indicated over 50% reliance of mussel tissue on nearshore carbon, highlighting the importance of nearshore SPM in mussel diet. Overall, carbon variation in SPM at both large and small temporal scales can be related to hydrographic processes, but is masked in mussels by long-term isotope integration.

Introduction

Coastal ecosystems constitute complex and dynamic environments in terms of both nutrient regimes and hydrography (Ostrom et al. 1997). Benthic and intertidal communities are profoundly influenced by their environments through the physical processes that drive food and nutrient delivery (Blanchette et al. 2006). Consequently changes in these processes can affect intertidal community structure at a variety of spatial and temporal scales (Roughgarden et al. 1988; Bustamante et al. 1995; Nielsen and Navarrete 2004). Intertidal mussel beds are generally thought to obtain their organic carbon through the ingestion of pelagic phytoplankton (Widdows et al. 1979; Asmus and Asmus 1991; Dame and Prins 1998), although several studies have shown that inshore and estuarine production, including macroalgal carbon from detrital food webs, may be of equal importance (e.g. Stuart et al. 1982; Dunton and Schell 1987; Duggins et al. 1989; Bustamante and Branch 1996).

In many cases, the biological application of stable isotope analysis has successfully linked primary producers to higher trophic levels in marine and estuarine environments (DeNiro and Epstein 1978; Deegan and Garritt 1997; Kaehler et al. 2000; Vizzini et al. 2002), but only recently has temporal variation in the isotopic ratios of marine producers and consumers become a focus of trophic investigations (Goering et al. 1990; Reira and Richard 1997; Kang et al. 1999). Frazer et al. (1997) discovered that winter δ^{13}C ratios of Antarctic larval krill were enriched relative to summer, while Megens et al. (2001) reported temporal enrichment of carbon values of particulate organic matter (POM) in the North Sea that was related to season. Rolff (2000) described inter-annual variation in both δ^{13}C and δ^{15}N in the plankton of temperate fresh and marine waters and Kreeger and Newell (2001) described monthly changes in hydrography and climate that can affect SPM composition and distribution in coastal systems. This suggests that a more detailed understanding of intertidal food webs may require attention to temporal variation in the composition of food sources. Investigations into high frequency temporal variations at lower trophic levels may then provide a more complete picture of the range of variability in primary producers and its effects further up the food chain, while elucidating the temporal coupling of isotopic variation at different trophic levels.

A previous study of isotope ratios of SPM and mussel diets in coastal waters around southern Africa showed distinct δ^{13}C depletions from nearshore to offshore environments that occurred during both austral summer and winter (Hill et al. 2006). That study, however, lacked an assessment of temporal variation in both SPM and the trophic relationships between mussels and their food sources. Establishing trophic links between producers and consumers in dynamic marine environments is a complex undertaking, as the turnover of consumer tissues tends to be slower than that of their diet (O'Reilly et al. 2002). Consumer isotope ratios are time-integrated (reflecting average diet over a period of time) and may not reflect short-term dietary variations in δ^{13}C or δ^{15}N. Consequently, consumer isotope signatures are believed to experience far less temporal variation than primary producers.

This study investigates temporal variation in the δ^{13}C and δ^{15}N ratios of macroalgae, suspended particulate matter (SPM) and intertidal mussels. This was done to evaluate (1) the temporal variability of nearshore/offshore gradients in δ^{13}C on a monthly timescale, (2) the short-term temporal variability of nearshore SPM, as determined over 24-h periods, (3) longer term (seasonal) temporal variability of mussel diet and its relation to distinct sources of production, and (4) the relative importance of nearshore detritus versus pelagic (i.e. phytoplankton) and estuarine primary production in the diet of the intertidal mussel *Perna perna*.

Methods

Site

Kenton-on-Sea (Fig. 1) is located on the south coast of South Africa, possessing rocky shores comprised of aeolian dune rock, with three permanently open estuaries in close proximity. Both the Kariega Estuary which enters the sea directly at Kenton and the Kowie River, approximately 25 km east along the coastline, have heavily impounded catchment areas with extremely limited outflow to the sea, while the Great Fish River, approximately 55 km east along the coastline, has a strong flow regime, delivering considerable volumes of freshwater to the coastal environment (Allanson and Read 1995).

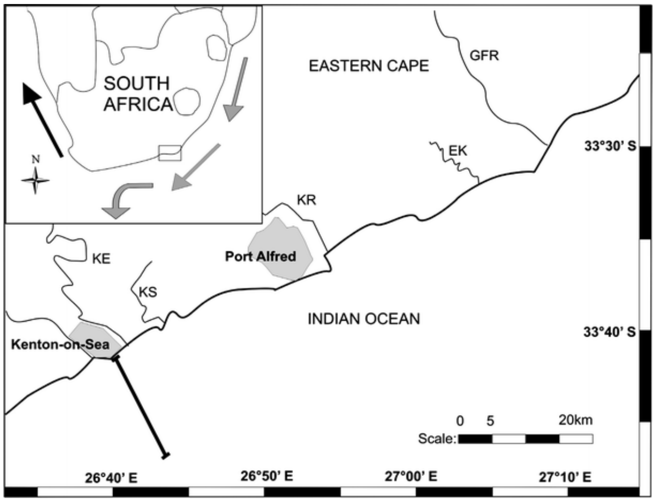

Fig. 1 Map of study area, including three permanently open estuaries in close proximity to Kenton-on-Sea (*KE* Kariega Estuary, *KR* Kowie River and *GFR* Great Fish River). The Kasouga Estuary (*KS*) and the East Kleinemonde (*EK*) are temporarily open-closed estuaries. The sampling transect runs along the perpendicular line offshore at Kenton-on-Sea. *Grey* and *black arrows* represent the Agulhas and Benguela Currents, respectively

Sample collection

In order to determine SPM isotope ratios inshore and offshore, two individual samples were obtained from 5-l surface water samples on a monthly basis, along a transect perpendicular to the coast at distances of 0, 0.25, 0.5, 1, 2, 4 and 10 km from the shoreline at Kenton-on-Sea (Fig. 1). The sampling period lasted from April 2004 to May 2005. Each month, five intertidal mussels (*Perna perna*) and duplicate samples of three of the most abundant macroalgae species (*Gelidium pristoides*, *Hypnea spicifera* and *Ulva* sp.) were collected from the rocky intertidal zone at Kenton-on-Sea.

Chlorophyll-*a* (chl-*a*) samples (3 × 250 ml) were collected at each station along the same transect from September 2004 to May 2005 to investigate changes in primary producer standing stocks.
The majority of estuarine SPM is derived from angiosperms and freshwater macrophytes, and may result in isotope ratios that are quite different from marine SPM. In order to determine estuarine SPM isotope ratios, 5-l estuarine surface water samples (2×) were collected from the Kariega Estuary mouth on five separate outgoing (April, July, November 2004, February, April 2005) and incoming tides (May, August, October 2004, January, March 2005).

SPM samples (2×) were also collected once, along a 50 km transect with samples from 0 to 7.5 km collected in June 2003 and then from 3.5 to 50 km offshore in August 2003 (total of 14 stations) to investigate isotopic gradients in offshore water.

On two occasions (winter June 21–22 and summer November 18–19, 2005) nearshore (0 km) SPM samples were collected at Kenton-on-Sea at hourly intervals for 24 h to investigate short-term variation in δ^{13}C and δ^{15} N ratios.

Sample preparation

Water samples were filtered through pre-combusted (500°C, 6 h) Whatman® GF/F filters (0.45 µm pore size), using a vacuum pump (≤4 cm Hg) and oven dried at 60°C for 24 h. Zooplankton and other large particle contaminants were manually removed under a dissecting microscope (16× magnification). Mussel adductor muscle tissue was dissected from fresh samples, rinsed in distilled water (dH_2O) and oven dried (60°C, 48 h). All macroalgae were rinsed in dH_2O, and visible epiphytes removed before drying (60°C, 48 h). Size fractionated chl-*a* was determined through serial filtration of 250 ml samples, using a vacuum pump (≤4 cm Hg), through 20.0 (Nitex), 2.0 and 0.20 µm (plastic Nucleopore™) filters and extracted in 90% acetone for 24 h in the dark. Chl-*a* concentrations (mg chl-*a* m^{-3}) were determined fluorometrically (Turner designs 10AU fluorometer) before and after acidification (Holm-Hansen and Reimann *1978*).

Isotope analysis

δ^{13}C and δ^{15}N ratios of all samples were determined using a continuous flow Isotope Ratio Mass Spectrometer (IRMS), after sample combustion in on-line Carlo-Erba preparation units at the University of Cape Town, South Africa and the Environmental Engineering Research Centre, Queens University Belfast, Northern Ireland. Beet sugar and Merck gelatine were used as standards, calibrated against International Atomic Energy reference materials. Results are expressed in standard delta notation, $\delta X = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \times 1000$, where X is the element in question and R is the ratio of the heavy over the light isotope. Precision of replicate determinations for both carbon and nitrogen was ±0.05‰.

Data analysis

Carbon and nitrogen contour graphs were created using Ocean Data View 3 (Schlitzer 2005; available at http:\\www.awi-bremerhaven.de/GEO/ODV). Monthly positional patterns of the Agulhas Current were investigated via sea surface temperatures (SST) through MODIS (Moderate Resolution Imaging Spectroradiometer) real time weighted daily images (weighted average product is a weighted average of day−1 (weight = 1), day (weight = 3), and day+1 (*w* = 1) with 1.1 km resolution (M. Roberts, personal comunication)) and PATHFINDER climatology. This was done to determine the distance offshore at which the current was found (measured in UTHSCSA Image Tool v3 (2002)), with the Agulhas Front designated as water with an SST of 22°C. Monthly climatological chl-*a* concentrations were also explored through MODIS real time weighted daily images and the ocean colour Sea-viewing Wide Field-of-view Sensor (SeaWiFS) to compare temporal changes in primary production using chl-*a* as an indicator of phytoplankton biomass. Correlation analyses were performed in Statistica v7 (StatSoft Inc. 2004) between $\delta^{13}C$ ratios at 0, 0.25, 0.5, 1, 2, 4 or 10 km and wind direction, wind speed, rainfall, water temperature or the distance offshore of the Agulhas Current to investigate their effects on the variability in isotopic ratios of SPM.

Two one-way ANOVA were performed using Statistica v7 (StatSoft Inc. 2004) on $\delta^{13}C$ and $\delta\ ^{15}N$ ratios of mussels to assess temporal (monthly) variability in isotope ratios. The IsoSource model described by Phillips and Gregg (2003) was applied as a two isotope system ($\delta^{13}C$ and $\delta^{15}N$) with three sources (average nearshore (0 km), average offshore (10–50 km) and average estuarine SPM) to determine the percentage contribution of organic carbon and nitrogen to mussel diet of nearshore SPM during each month, using an average fractionation of 1‰ for $\delta^{13}C$ (as reported by DeNiro and Epstein 1978, Fry and Sherr 1984, and Peterson and Howarth 1987). Although a mean $\delta^{15}N$ fractionation value between trophic levels of 3–4‰ is accepted as the norm (DeNiro and Epstein 1981; Fry and Sherr 1984; Post 2002), this is an average value over a wide variety of species and some of the literature suggests that bivalves may have smaller fractionation values of approximately 1.7‰ (Fry 1988; Raikow and Hamilton 2001; Moore and Suthers 2005). Consequently a trophic correction of 1.7‰ for nitrogen was used in the IsoSource model. Isotopic ratios for estuarine SPM were used from low tide values only, as this is the only time (except in extreme floods) that the Kariega may outflow to the sea.

The IsoSource model was also applied as a two isotope system ($\delta^{13}C$ and $\delta^{15}N$) with three sources (average of all macroalgal species over 14 months, average estuarine SPM and monthly offshore SPM (10 km) as a proxy for phytoplankton) to determine the percentage contribution of organic carbon to monthly nearshore SPM by macroalgae, estuarine and phytoplankton sources. IsoSource models reported a range of source proportions that satisfy the isotopic mass balance in the mixing model (Phillips and Gregg 2003), however as some of our source proportions were reported to make contributions across a wide range of percentages (e.g. contributions of nearshore SPM to mussel diet in July 2004 could be between 53 and 100%) we have presented the generated mean with standard deviations.

Results

Distinct spatial patterns existed in $\delta^{13}C$, with consistent nearshore to offshore depletions seen during all months (Fig. 2), with an average carbon ratio of −15.83 ± 1.48‰ nearshore (0 km) and −19.88 ± 1.30‰ offshore (10 km). The extended offshore transect reaching 50 km from the coast of Kenton-on-Sea, also showed clear, strong carbon depletion from directly inshore (0 km; −14.26 ± 0.75‰) to 50 km from the coast (−18.53 ± 0.61‰; Fig. 3). Initial $\delta^{13}C$ values decreased rapidly with distance, leveling out around 20 km and remaining within 2.0‰ of the offshore average (−19.88‰). The $\delta^{13}C$ contour plots of monthly data revealed projections of nearshore SPM with enriched $\delta^{13}C$ values extending several kilometres into the offshore environment between the months of June–August 2004 and again in May 2005. During May and September 2004 nearshore SPM enrichment remained close inshore and highly depleted offshore water incursions (approx. −20.00‰) reached as far inshore as 3 km from the beach. In May 2005, SPM ratios showed both projections of nearshore water and incursions of offshore water (Fig. 2). In contrast to carbon signatures, inshore to offshore gradients were not observed in SPM $\delta^{15}N$, although temporal changes occurred throughout the 14-month period

(Fig. 2). Nitrogen ratios along the entire transect from July to October 2004 were higher (8.0–9.0‰) than during the remaining months which stayed within the range previously reported for oceanic POM (5.0–7.0‰; Miyake and Wada 1967; Minagawa and Wada 1984).

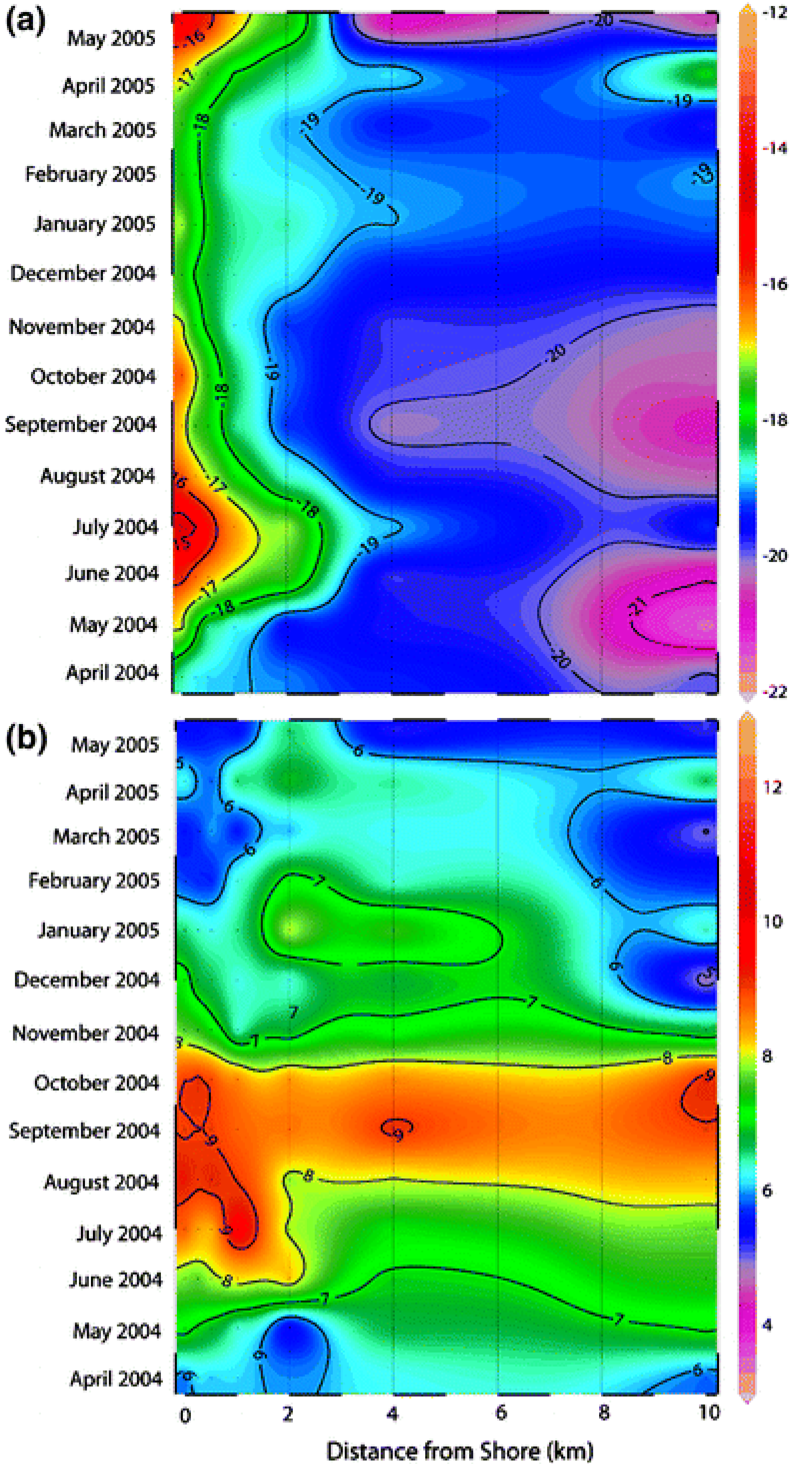

Fig. 2 Contour graphs of δ^{13}C (a) and δ^{15}N (b) signatures from monthly SPM transects at Kenton-on-Sea

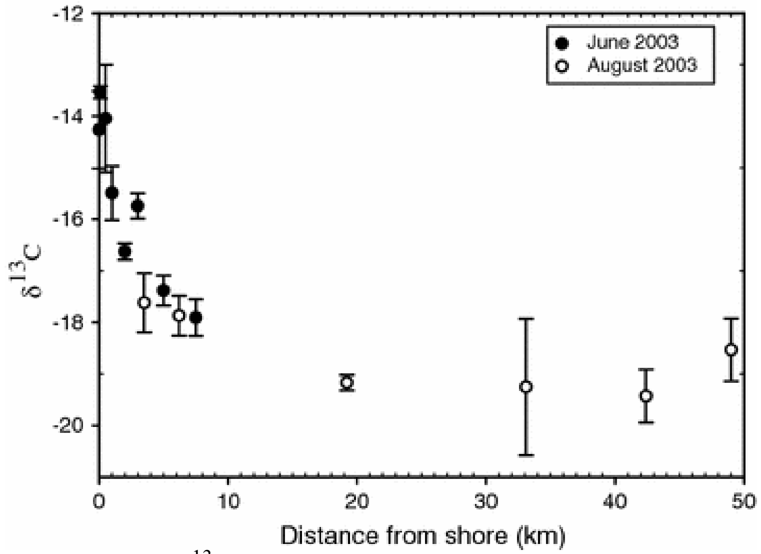

Fig. 3 Nearshore to offshore gradient in $\delta^{13}C$ from 0 to 50 km offshore. Values are means ± SD

Correlation analyses showed no significant relationships between SPM carbon ratios at any distance offshore when related to wind direction, wind speed, rainfall, water temperature or the distance of the Agulhas Current (Fig. 5a) on any sampling occasion ($P > 0.05$ in all analyses). As this involved a large number of correlation analyses, none of which were significant, the data are not presented.

Mussel adductor tissue showed significant temporal differences (homogenous groupings, Newman–Keuls $P < 0.05$) in $\delta^{13}C$ ratios over the 14-month period, although the differences were very small (within 1.0‰) and could not be related to season (Fig. 4). No significant differences were seen in $\delta^{15}N$ ratios ($P > 0.05$). In 11 out of 14 months $\delta^{13}C$ ratios of mussel adductor tissue were similar or identical to carbon values of nearshore SPM, while three out of 14 months showed mussel $\delta^{13}C$ ratios that fell between those for nearshore and offshore SPM (Fig. 5b). However, no consistent 1‰ fractionation was seen between nearshore or offshore SPM and mussel ratios. The two isotope system, three source model, using average $\delta^{13}C$ and $\delta^{15}N$ ratios of nearshore, offshore and estuarine SPM ratios for all months, showed mussels demonstrating over 50% dependence on nearshore SPM for incorporated carbon and nitrogen in 12 out of 14 months (Fig. 5c).

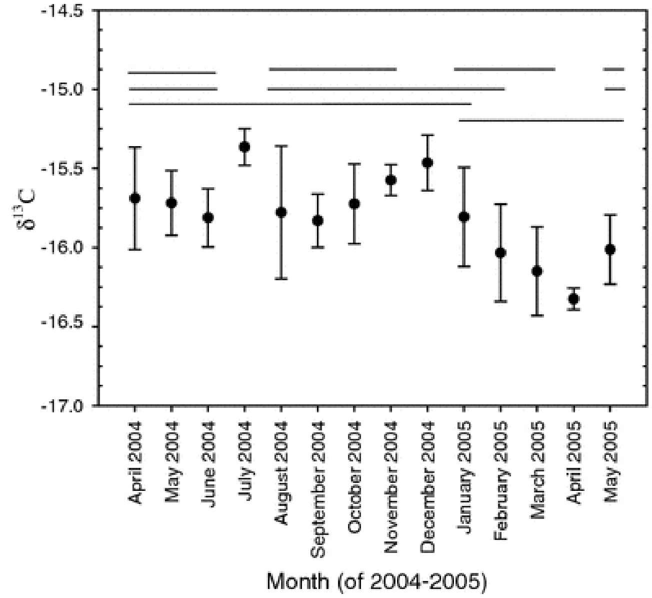

Fig. 4 Monthly $\delta^{13}C$ signatures of adductor tissue of *Perna perna* ($n = 5$) collected from the rocky intertidal zone at Kenton-on-Sea from April 2004 to May 2005. Values are means ± SD. *Lines* above graph indicate homogenous groups (Newman–Keuls, $P < 0.05$)

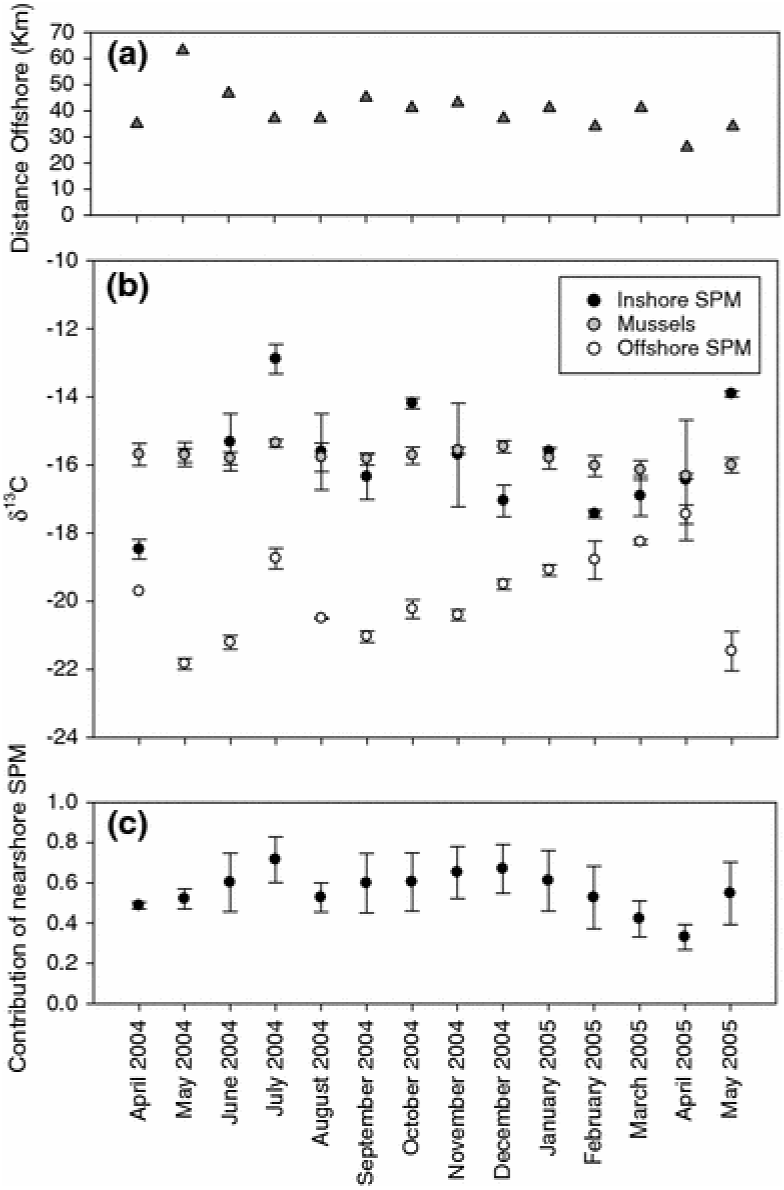

Fig. 5 a Position of the Agulhas Current offshore at Kenton-on-Sea for all months (km); b $\delta^{13}C$ relationships among mussels, nearshore and offshore SPM for 2002–2004; c percentage $\delta^{13}C$ and $\delta^{15}N$ contribution of average nearshore SPM for all months to mussel diet determined using IsoSource (Phillips and Gregg 2003). Models calculated for each month using an increment of 1% and mass-based tolerance of 0.08‰

Macroalgal ratios lacked clear temporal trends in either $\delta^{13}C$ or $\delta^{15}N$, with inconsistent enrichments and depletions in consecutive months and a wide range in both carbon and nitrogen ratios. Both *Hypnea spicifera* and *Gelidium pristoides,* for example, demonstrated large-scale temporal variations, ranging between −10.23 to −18.37 and −12.64 to −17.40‰ for $\delta^{13}C$, respectively, and between 3.29 to 7.43 and 5.84 to 8.19‰ for $\delta^{15}N$, respectively. Average $\delta^{13}C$ values for *H. spicifera*, *G. pristoides* and *Ulva* sp. were −14.93 ± 2.36, −14.68 ± 1.48, and −14.95 ± 1.23‰, respectively. Average $\delta^{15}N$ values were 5.89 ± 1.02, 6.85 ± 0.74, and 6.26 ± 1.05‰, respectively. Such variation in macroalgal isotopic ratios complicated links between mussel diet and nearshore SPM, but the application of IsoSource modeling showed that macroalgae accounted for over 40% of nearshore SPM in nine out of 14 months (April, August, December 2004 and February, April 2005 between 0 and 38%). Estuarine (<10%) and phytoplankton (<20%) made minimal contributions to nearshore SPM in most months, however contributions increased for estuarine SPM (90–100%) in August and December 2004, and for phytoplankton (60–100%) in April 2004, February and April 2005.

Chl-*a* in all three size fractions showed large inshore variability with February and March 2005 showing high concentrations (mg chl-*a* m^{-3}) in the 20.0 and 2.0 µm size classes that decreased with distance offshore, however overall there were no consistent temporal trends. Monthly SeaWiFS satellite photos of chl-*a* concentrations (mg chl-*a* m^{-3}) for each sampling occasion showed seasonal peaks in primary production during austral spring and autumn around Kenton-on-Sea and also allowed a daily overview of basic coastal hydrography. Satellite photos showed nearshore primary production around Kenton-on-Sea being drawn offshore and south-west along the coastline on four occasions (June–August 2004 and May 2005; available at www.rsmarinesa.org.za, reproduced Fig. 6). These occasions corresponded to the months that demonstrated projections of enriched nearshore SPM away from the coast.

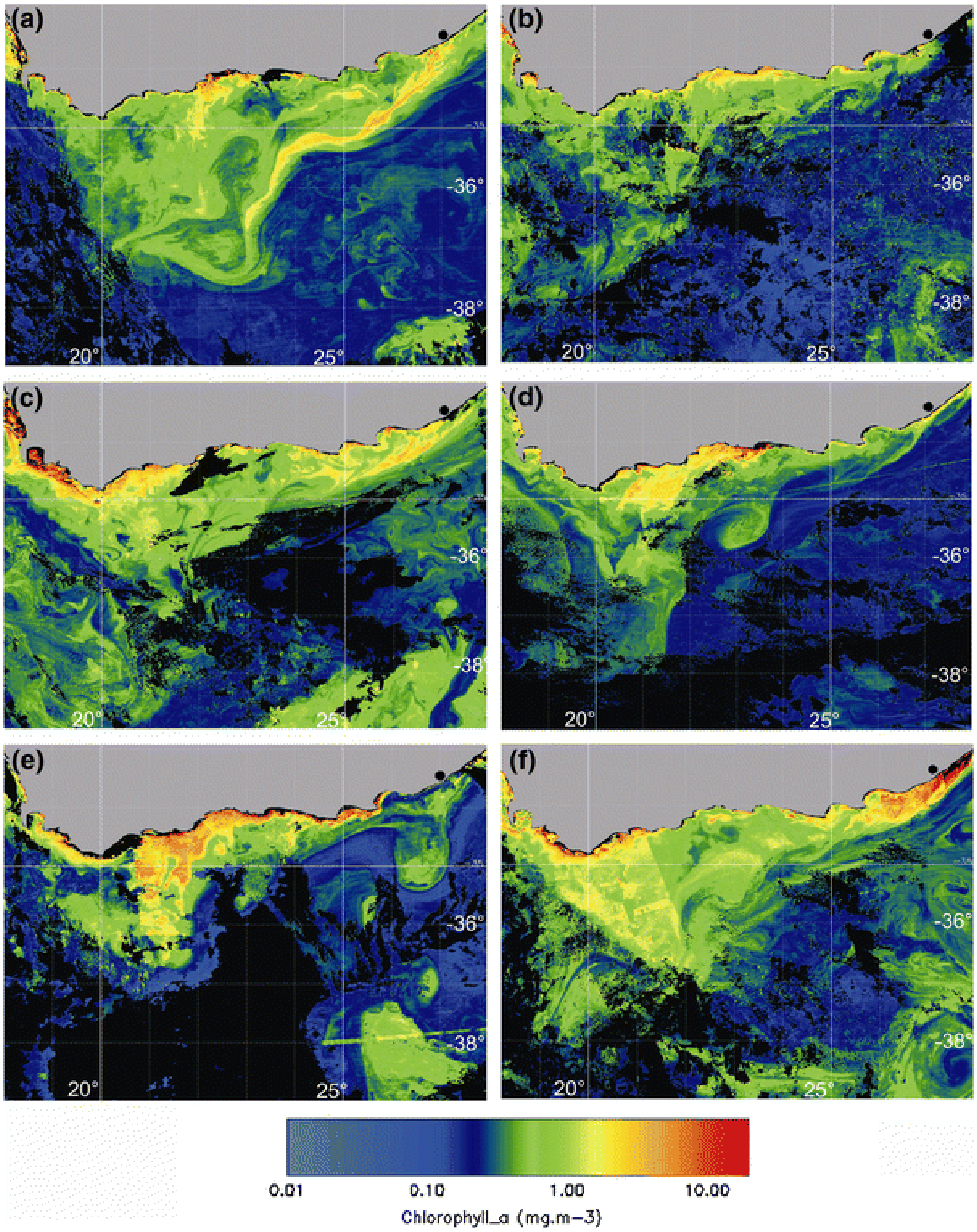

Fig. 6 SeaWiFS satellite photo (http://www.rsmarinesa.org.za/) of chlorophyll-*a* concentrations (mg chl-*a* m^{-3}) for the south coast of South Africa in June 2004 (a), July 2004 (b), August 2004 (c), May 2005 (d), May 2004 (e), September 2004 (f). *Black dot* indicates Kenton-on-Sea

Estuarine $\delta^{13}C$ values from the Kariega mouth for incoming and outgoing tides were −18.37 ± 0.14 and −15.80 ± 0.50‰, respectively, while $\delta^{15}N$ values were 6.85 ± 0.77 and 6.27 ± 0.54‰, respectively, with incoming tides being isotopically more representative of marine water.

$\delta^{13}C$ ratios of SPM showed cyclical enrichment patterns over a 24-h period. Inshore carbon ratios at high tides were enriched to a maximum of −14.84 ± 0.36‰ and at low tides depleted to a minimum of −17.39 ± 1.49‰, while variation in the SPM ratios of $\delta^{15}N$ was less clear, lacking any overall pattern (Fig. 7).

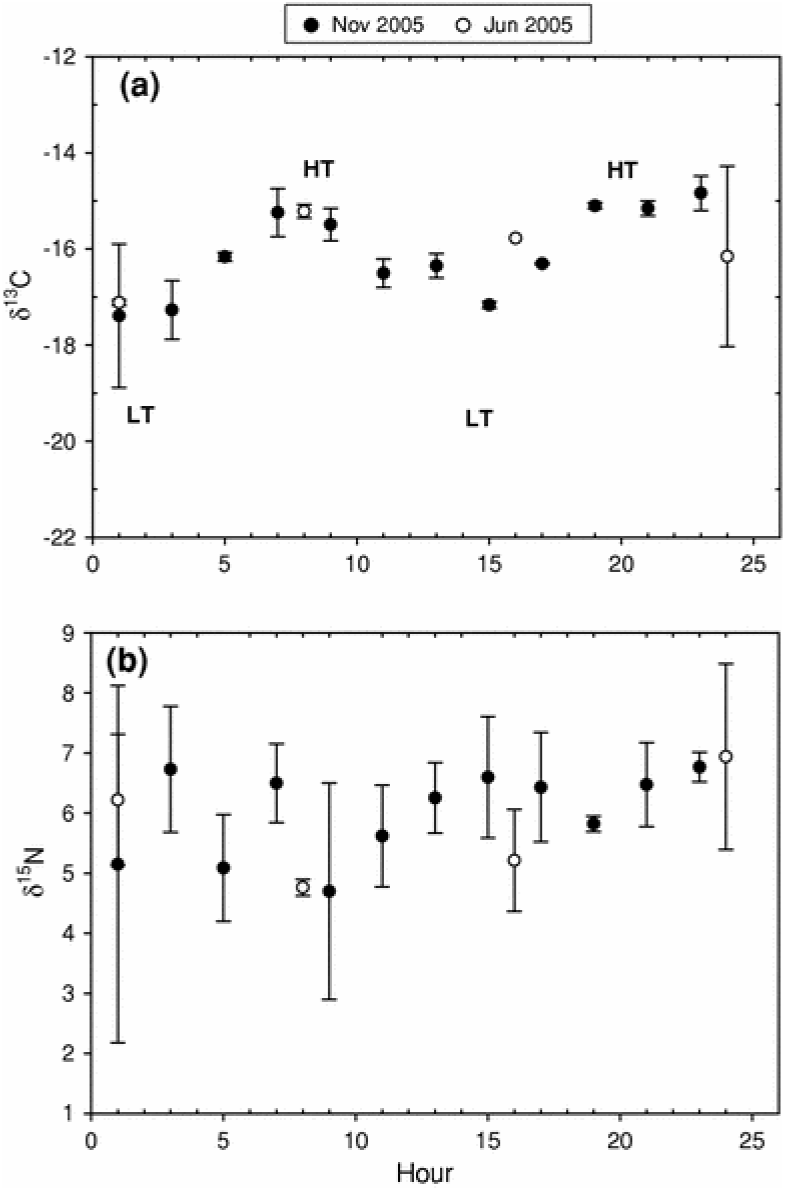

Fig. 7 $\delta^{13}C$ (a) and $\delta^{15}N$ (b) ratios of nearshore SPM over 24 h. Values are means ± SD. *LT* and *HT* indicate low tide and high tide, respectively

Discussion and conclusions

Temporal variability of nearshore/offshore gradients

The temporal consistency of the observed nearshore to offshore depletions (Fig. 2) establishes the existence of a consistent $\delta^{13}C$ gradient at kilometer scales when moving from nearshore to offshore water. Offshore (10 km) $\delta^{13}C$ (average −19.88‰) appears to be typical of Agulhas Current water (Hill et al. *2006*; Kaehler, unpublished) and the depletion gradient extends a great distance from the coast; as far as 20 km offshore, and remaining within 2.0‰ of the offshore average up to 50 km out to sea (Fig. 3). These data support the findings of Hill et al. (*2006*) that showed biannual 0–10 km carbon depletions along the coastline of southern Africa and confirm that such trends are consistent in time as well as ubiquitous around the coast. This implies a shift from a nearshore carbon supply to one more representative of an offshore pelagic carbon source in surface water at increasing distances during different parts of the year. It was not possible to link δ^{13}C values directly to distinct sources of production but the nearshore (0 km) δ^{13}C ratios fell within a range of values previously reported for intertidal macroalgae (Bustamante and Branch *1996*), likely representing inshore primary production (Hill et al. *2006*). Although nearshore SPM carbon ratios fell within the range of macroalgal values found at Kenton-on-Sea in this study, the two could not be directly linked. This is presumably because SPM samples represent a mixture of phytoplankton plus detritus from different algal species that have ratios modified by degradation and that occur in unknown proportions. However IsoSource modeling showed that macroalgae accounted for over 40% of nearshore SPM at nine out of 14 sites relative to offshore phytoplankton and estuarine inputs.

Temporal patterns in carbon enrichment were seen at Kenton-on-Sea during late austral autumn and winter with protrusions of δ^{13}C-enriched nearshore SPM extending several km offshore in both June–August 2004 and May 2005. Although nearshore values ranged between −12.88 ± 0.44 and −16.44 ± 0.74‰ (Fig. 2) and the δ^{13}C of outgoing water from the Kariega mouth averaged −15.80 ± 0.50‰, according to the mixing model, in all but 2 months, estuarine sources made negligible (<10%) contributions to nearshore SPM. In addition, the highly enriched nature of nearshore δ^{13}C values and the absence of significant correlations with water temperature, rainfall, wind speed or wind direction suggest that these seasonal increases in nearshore carbon values are unrelated to estuarine inputs or pelagic primary production. Local macroalgal communities show strong seasonality in both species biomass, and their biochemical composition (McQuaid *1985a*, *b*) and changes in nearshore SPM ratios presumably reflect changes in the pool of macroalgal detritus. Other contributions to SPM including detritus from intertidal macroalgae not addressed in this study and production from benthic and subtidal habitats may also influence nearshore SPM signatures and should be considered in future research. Monthly (MODIS) satellite photos of SST allowed us to determine the position of the inshore edge of the Agulhas Current (the Agulhas Front) and its distance offshore at Kenton-on-Sea on each sampling occasion (Fig. 5a), but there was no correlation between the position of the Agulhas Front and SPM values at any distance. Brown (*1992*) demonstrated a cyclical pattern of primary productivity in this part of the southeastern Indian Ocean, using chl-*a* concentrations as an estimate of phytoplankton biomass. She showed increased phytoplankton production in spring and autumn and decreased production in summer and winter. These seasonal peaks in primary production were not seen from our chl-*a* measurements in any size class, which is probably due to the instantaneous nature of spot measurements, they were, however, obvious from SeaWiFS satellite images (averaged over whole days) for the 14-month sampling period.

SeaWiFS imagery provides estimates of temporal and spatial variation in chlorophyll-*a* which helps to visualize local hydrography which may then be related to this study's isotopic ratios of SPM, providing a broad overview of SPM variability, although they cover different temporal scales. The four months that exhibited projections of carbon enriched nearshore water away from the shoreline also showed nearshore primary production being drawn offshore and south-west along the coast in SeaWiFS satellite images (Fig. 6), suggesting similar patterns of water movement. Although these projections may not be directly related to the position of the Agulhas Front, they are likely to be related to hydrographic features inshore of the current. During three months there were incursions of offshore water as close as 3 km from the shore (May, September 2004 and May 2005; Fig. 2) and corresponding SeaWiFS satellite images show strong mixing between nearshore and offshore waters in May and September 2004 with offshore water coming particularly close inshore in May 2005 (Fig. 6). This is consistent with the patterns seen in nearshore and offshore projections (Fig. 2), indicating strong ties between coastal SPM δ^{13}C ratios and oceanic hydrography.

Interpretations of SPM δ^{15}N ratios were more complicated. It is possible that the highly enriched ratios seen between July and October 2004 (Fig. 2) are due to inputs from the two temporary open-closed estuaries in proximity to Kenton-on-Sea (Kasouga River and East Kleinemonde), both of which experienced large breaching events between July and October 2004 (W. Froneman, personal communication). However, the enriched ratios extend along the entire sampling transect, suggesting that coastal nutrients were being pulled offshore. This conflicts with the carbon data showing pulses of offshore water coming close inshore during the months in question. Therefore the more likely possibility is that a supply of enriched offshore nitrogen was being forced into the coastal environment, the source of which is currently unknown. Further investigations into isotopic variation in inshore and offshore nitrogen are clearly needed.

Short-term temporal variability of nearshore SPM

Although there was consistent nearshore to offshore carbon depletion in SPM during all months, the slopes of the curves varied implying temporal changes in nearshore SPM which may result from changes in carbon sources, carbon abundance or mixing. The investigation of nearshore (0 km) SPM variation over 24-h periods showed a clear cyclical pattern of δ^{13}C enrichment and depletion that was tightly correlated with the daily tidal cycle. SPM δ^{13}C was most depleted at low tides and gradually increased to be most enriched at high tides (Fig. 7). The range of variability at the tidal scale was much smaller than at the seasonal scale, remaining within 2.0‰. This enrichment/depletion sequence is the opposite of that expected if offshore or estuarine water directly influenced the intertidal zone at high tide. During high tide, when riverine water is forced up the estuary by incoming seawater, nearshore SPM ratios did not reflect high tide estuarine ratios (−18.37 ± 0.14‰), but were more enriched with an average of −15.20 ± 0.25‰, conversely, at low tide when estuarine water (average −15.80 ± 0.50‰) empties into the sea, nearshore SPM values were depleted (average −17.59 ± 1.54‰), suggesting that terrigenous or estuarine inputs are not a major contributor to nearshore SPM. These results are not surprising as both the Kariega and the Kowie Rivers are highly saline, impounded estuaries with minimal output to the sea. Although the strong flow regime of the Great Fish River contributes large freshwater outputs to the coastal zone which often extend south-west down the coastline (Vorwerk 2007), δ^{13}C ratios of SPM from the Great Fish River are extremely depleted (approx. −28.0; Froneman 2002). Such depleted carbon ratios clearly did not influence nearshore water at Kenton-on-Sea. Instead the influence of the tidal cycle on isotopic ratios is believed to reflect the effects of detrital resuspension, which may significantly alter food composition (Fréchette et al. 1989). It is important to recognize that differences in both temporal and spatial scales between our local measurements (short and long term) and the remote sensing data are sources of uncertainty. Nevertheless, it is clear that the large variation in isotope ratios at both small (hourly) and large (inter-annual and inter-monthly) temporal scales appears to be closely linked to coastal and oceanic hydrography.

Temporal variability in mussel diet

Numerous investigations of bivalve molluscs have shown that these suspension feeders respond to changing food conditions through preferential selection and ingestion of SPM (e.g. Newell et al. 1989; Ward et al. 1998; Milke and Ward 2003). Subsequently, Hill et al. (2006) speculated that mussels with δ^{13}C ratios reflecting those of nearshore water either feed non-selectively or else preferentially select/assimilate macroalgal detritus, while if mussels demonstrated δ^{13}C ratios similar to offshore SPM it could be explained through preferential selection of phytoplankton. During this study, carbon ratios of mussel tissue were more enriched than or similar to the values of nearshore SPM in 11 out of 14 months, while for three months mussel δ^{13}C ratios fell between those of nearshore and offshore SPM (Fig. 5b). The species composition of phytoplankton communities in surface waters can differ significantly between nearshore and offshore (Kaneta et al. 1985, Caldeira et al. 2001) suggesting a third alternative, that mussels may feed non-preferentially or passively on a diet of nearshore phytoplankton dominated by coastal diatom species. Barlow et al. (2005) reported that diatoms generally dominated inshore and small flagellates offshore in the southern Benguela region and Kukert and Riebesell (1998) reported distinct isotopic enrichment of diatom dominated SPM relative to flagellate-dominated samples, but we have no data from Kenton-on-Sea to test this possibility.

δ^{13}C ratios of mussel tissues demonstrated a pattern of temporal variability that could not be clearly correlated to season. With the exception of July 2004, carbon ratios seemed to be more depleted in spring (September/October) and autumn (April/May) when phytoplankton blooms were visible in the MODIS satellite images, but the results of post hoc tests were not clear cut (Fig. 4). Moreover, mussel and SPM ratios did not show consistent 1‰ carbon fractionation (DeNiro and Epstein *1978*; Fry and Sherr *1984*; Peterson and Howarth *1987*). The ambiguity of this relationship illustrates the significance of integrated isotope assimilation in consumers, demonstrating that time-integrated tissue does not reflect short-term changes in δ^{13}C, with important implications for food web studies. Isotopic ratios of primary producers are subject to more variation than higher trophic levels as variability in coastal hydrography may drive changes in nutrient sources and/or the isotope composition of the macroalgae themselves at short temporal scales (O'Reilly et al. *2002*). Consequently, temporal integration must be considered when comparing samples which average isotopic ratios over different time periods (O'Reilly et al. *2002*; Phillips and Eldridge *2006*). The situation is further complicated by seasonal variation in metabolic rates (in both producers and consumers) that can result in temporal changes in fractionation unrelated to diet (Lorrain et al. *2002*). Indirect measurements of mussel tissue turnover via whole body protein turnover rates in the mussel *Mytilus edulis* have suggested 100% turnover requires 244–350 days (Hawkins *1985*), so that integrated tissue samples are unlikely to reflect the instantaneous nature of monthly SPM measurements. Furthermore SPM measurements are only snapshots in time and are not necessarily indicative of monthly means, so that it makes little sense to try to relate instantaneous SPM measurements with time-integrated mussel tissue. The use of longer term isotopic averages of producers (as used in this study's IsoSource modeling) therefore should provide better insight into trophic dynamics of consumers and the elucidation of rocky shore food webs.

Relative importance of nearshore, pelagic and estuarine SPM in mussel diet

Clearly temporal variation occurs at different scales in consumers and SPM. Accordingly, the application of IsoSource modeling (Phillips and Gregg *2003*) in this study used SPM values averaged over all months to investigate the percentage contribution of nearshore organic carbon relative to offshore and estuarine sources to the δ^{13}C and δ^{15}N ratios of each mussel sample, allowing us to assess the overall importance of nearshore SPM to mussel diet (Fig. 5c). In all but two months, mussels at Kenton-on-Sea showed more than 50% dependence on nearshore SPM for their organic carbon and nitrogen, demonstrating stronger ties to nearshore than offshore or estuarine carbon sources, indicating a high degree of dependence on nearshore primary production.

Sandy beaches often possess a well-developed surf zone, with the combined intertidal/surf zone acting as a semi-autochthonous ecosystem driven largely by primary production by the surf zone diatom *Anaulus birostratus* (Brown and McLachlan *1990*). Such systems support at least three (interstitial, microbial and macroscopic) food chains and act as a self-contained ecosystem that provides an interface between oceanic and coastal communities. We propose that in the same fashion, the rocky intertidal zone represents a moderately self-sustaining ecosystem that typically exports materials to adjacent ecosystems, and is only poorly dependent upon offshore inputs. However, external hydrographic processes may influence the balance between imported and exported primary production in rocky shores. Although much work has been done on ecosystem regulation through top-down or bottom-up controls (Menge *1992*, *2000*; Hunter and Price *1992*; Seitz and Lipcius *2001*; Posey et al. *2006*), current research has begun to recognize the importance of oceanographic processes in ecosystem dynamics (Menge et al. *1997*, *1999*, *2003*; Blanchette et al. *2006*). There are strong δ^{13}C links between mussels and nearshore SPM and overall δ^{13}C patterns of SPM at both small (hourly) and large (inter-annual and inter-monthly) temporal scales. As these may be associated with coastal hydrography, we conclude that local hydrographic processes play a principle role in the ecosystem dynamics of intertidal rocky shores.

Acknowledgments Dr. S. Davis and Dr. R. Kalin at the EERC, Queens University Belfast provided invaluable assistance, guidance and instruction as well as the use of their facilities. Dr. M. Villet and Dr. F. Porri, Rhodes University, provided statistical help and direction. Isotope analysis was done both through the EERC, Queens University Belfast and the stable light isotope unit, University of Cape Town. All of this help is gratefully acknowledged.

References

Asmus R, Asmus H (1991) Mussel beds: limiting or promoting phytoplankton? J Exp Mar Biol Ecol 48:215–232

Allanson BR, Read GHL (1995) Further comment on the response of Eastern Cape Province estuaries in South Africa. S Afr J Aquat Sci 21:56–70

Barlow R, Sessions H, Balarin M, Weeks S, Whittle C, Hutchings L (2005) Seasonal variation in phytoplankton in the southern Benguela: pigment indices and ocean colour. Afr J Mar Sci 27:275–287

Blanchette C, Broitman B, Gaines S (2006) Intertidal community structure and oceanographic patterns around Santa Cruz Island, CA, USA. Mar Biol 149:689–701

Brown PC (1992) Spatial and seasonal variation in chlorophyll distribution in the upper 30 m of the photic zone in the Southern Benguela/Agulhas ecosystem. S Afr J Mar Sci 12:515–525

Brown AC, McLachlan A (1990) Ecology of sandy shores. Elsevier, Amsterdam

Bustamante RH, Branch G (1996) The dependence of intertidal consumers on kelp-derived organic matter on the west coast of South Africa. J Exp Mar Biol Ecol 196:1–28

Bustamante R, Branch G, Eekhout S, Robertson B, Zoutendyk P, Schleyer M, Dye A, Hanekom N, Keats D, Jurd M, McQuaid C (1995) Gradients of intertidal primary productivity around the coast of South Africa and their relationships with consumer biomass. Oecologia 102:189–201

Caldeira RMA, Russell P, Amorim A (2001) Evidence of an unproductive coastal front in Baía d'Abra, an embayment on the south east of Madeira Island, Portugal. Bull Mar Sci 69:1057–1072

Dame R, Prins T (1998) Bivalve carrying capacity in coastal ecosystems. Aquat Ecol 31:409–421

Deegan L, Garritt RH (1997) Evidence for spatial variability in estuarine food webs. Mar Ecol Prog Ser 147:31–47

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Dunton KH, Schell DM (1987) Dependence of consumers on macroalgal (*Laminaria solidungula*) carbon in an arctic kelp community: δ^{13}C evidence. Mar Biol 93:615–625

Frazer TK, Ross RM, Quetin LB, Montoya JP (1997) Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. J Exp Mar Biol Ecol 212:259–275

Fréchette M, Butman CA, Geyer WR (1989) The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnol Oceanogr 34:19–36

Froneman PW (2002) Food web structure in three contrasting estuaries determined using stable isotope δ^{13}C analysis. Afr J Aquat Sci 27:107–115

Fry B, Sherr EB (1984) $^{\delta 13}$C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib Mar Sci 27:13–17

Fry B (1988) Food web structure on Georges Bank from stable C, N and S isotopic compositions. Limnol Oceanogr 33:1182–1190

Goering J, Alexander V, Haubenstock N (1990) Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific bay. Estuar Coast Shelf Sci 30:239–260

Hawkins A (1985) Relationships between the synthesis and breakdown of protein, dietary absorption and turnovers of nitrogen and carbon in the blue mussel, *Mytilus edulis* L. Oecologia 66:42–49

Hill J, McQuaid C, Kaehler S (2006) Biogeographic and nearshore/offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of southern Africa. Mar Ecol Prog Ser 318:63–73

Holm-Hansen O, Reimann B (1978) Chlorophyll-*a* determination: improvements in methodology. Oikos 30:438–447

Hunter M, Price P (1992) Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology 73:724–732

Kaehler S, Pakhomov E, McQuaid C (2000) Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by δ^{13}C and δ^{15}N analysis. Mar Ecol Prog Ser 208:13–20

Kaneta PJ, Levandowskyl M, Esaias W (1985) Multivariate analysis of the phytoplankton community in the New York Bight. Mar Ecol Prog Ser 23:231–239

Kang CK, Sauriau P-G, Richard P, Blanchard GF (1999) Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. Mar Ecol Prog Ser 187:147–158

Kreeger DA, Newell RIE (2001) Seasonal utilization of different seston carbon sources by the ribbed mussel, *Geukenseia demissa* (Dillwyn) in a mid-Atlantic salt marsh. J Exp Mar Biol Ecol 260:71–91

Kukert H, Riebesell U (1998) Phytoplankton carbon isotope fractionation during a diatom spring bloom in a Norwegian fjord. Mar Ecol Prog Ser 173:127–137

Lorrain A, Paulet Y-M, Chauvaud L, Savoye N, Donval A, Saout C (2002) Differential $\delta^{13}C$ and $\delta^{15}N$ signatures among scallop tissues: implications for ecology and physiology. J Exp Mar Biol Ecol 275:47–61

McQuaid CD (1985a) Seasonal variation in the ash-free calorific value of nine intertidal algae. Bot Mar XXVIII:545–548

McQuaid CD (1985b) Seasonal variation in biomass and zonation of nine intertidal algae in relation to changes in radiation, sea temperature and tidal regime. Bot Mar XXVIII:539–544

Megens L, van der Plicht J, de Leeuw JW (2001) Temporal variations in ^{13}C and ^{14}C concentrations in particulate organic matter from the southern North Sea. Geochim Cosmochim Acta 65:2899–2911

Menge B (1992) Community regulation: under what conditions are bottom-up factors important on rocky shores? Ecology 73:755–765

Menge B (2000) Top-down and bottom-up community regulation in marine rocky intertidal habitats. J Exp Mar Biol Ecol 250:257–289

Menge B, Daley B, Lubchenco J, Sanford E, Dahlhoff E, Halpin P, Hudson G, Burnaford J (1999) Top-down and bottom-up regulation of New Zealand rocky intertidal communities. Ecol Monogr 69:297–330

Menge B, Daley B, Wheeler P, Strub P (1997) Rocky intertidal oceanography: an association between community structure and nearshore phytoplankton concentration. Limnol Oceanogr 42:57–66

Menge B, Lubchenco J, Bracken M, Chan F, Foley M, Freidenburg T, Gaines S, Hudson G, Krenz C, Leslie H, Menge D, Russell R, Webster M (2003) Coastal oceanography sets the pace of rocky intertidal community dynamics. PNAS 100:12229–12234

Milke L, Ward J (2003) Influence of diet on pre-ingestive particle processing in bivalves. II. Residence time in the pallial cavity and handling time on the labial palps. J Exp Mar Biol Ecol 293:151–172

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between ^{15}N and animal age. Geochim Cosmochim Acta 8:1135–1140

Miyake Y, Wada E (1967) The abundance ratio of $^{15}N/^{14}N$ in marine environments. Rec Oceanogr Work Jpn 9:32–53

Moore SK, Suthers IM (2005) Can the nitrogen and carbon stable isotopes of the pygmy mussel, *Xenostrobus securis*, indicate catchment disturbance for estuaries in northern New South Wales, Australia? Estuaries 28:714–725

Newell R, Shumway S, Cucci T, Selvin R (1989) The effects of natural seston particle size and type of feeding rates, feeding selectivity and food resource availability for the mussel *Mytilus edulis* Linneaus, 1758 at bottom culture sites in Maine. J Shellfish Res 8:187–196

Nielsen K, Navarrete S (2004) Mesoscale regulation comes from the bottom-up: intertidal interactions between consumers and upwelling. Ecol Lett 7:31–41

O'Reilly CM, Hecky RE, Cohen AS, Plisnier P-D (2002) Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. Limnol Oceanogr 47:306–309

Ostrom NE, Macko SA, Deibel D, Thompson RJ (1997) Seasonal variation in the stable carbon and nitrogen isotope biogeochemistry of a coastal cold ocean environment. Geochim Cosmochim Acta 61:2929–2942

Peterson B, Howarth R (1987) Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnol Oceanogr 32:1195–1213

Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261–269

Phillips D, Eldridge P (2006) Estimating the timing of diet shifts using stable isotopes. Oecologia 147:105–203

Posey M, Alphin T, Cahoon L (2006) Benthic community responses to nutrient enrichment and predator exclusion: influence of background nutrient concentrations and interactive effects. J Exp Mar Biol Ecol 330:105–118

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83:703–718

Raikow DF, Hamilton SK (2001) Bivalve diets in a midwestern U.S. stream: a stable isotope enrichment study. Limnol Oceanogr 46:514–522

Reira P, Richard P (1997) Temporal variation of $\delta^{13}C$ in particulate organic matter and oyster *Crassostrea gigas* in Marennes-Oleron Bay (France): effect of freshwater inflow. Mar Ecol Prog Ser 147:105–115

Rolff C (2000) Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of size-fractionated plankton at a coastal station in the northern Baltic proper. Mar Ecol Prog Ser 203:47–65

Roughgarden J, Gaines S, Possingham H (1988) Recruitment dynamics in complex life cycles. Science 241:1460–1466

Seitz R, Lipcius R (2001) Variation in top-down and bottom-up control of marine bivalves at differing spatial scales. ICES J Mar Sci 58:689–699

Stuart V, Field J, Newell R (1982) Evidence for absorption of kelp detritus by the ribbed mussel *Aulacomya ater* using a new ^{51}Cr-labelled microsphere technique. Mar Ecol Prog Ser 9:263–271

Vizzini S, Sara G, Michener RH, Mazzola A (2002) The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. Acta Oecol 23:277–285

Vorwerk PD (2007) A preliminary examination of selected biological links between four eastern Cape estuaries and the inshore marine environment. PhD Thesis, Rhodes University, p 268

Ward J, Levinton J, Shumway S, Cucci T (1998) Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Mar Biol 131:283–292

Widdows J, Fieth P, Worrall CM (1979) Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar Biol 50:195–207