

Trophodynamics of three decapod crustaceans in a temperate estuary using stable isotope and fatty acid analyses

Emily S. Antonio*, Nicole B. Richoux

Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa

ABSTRACT: Temporal variations in stable isotope ratios and fatty acid composition were examined in 3 crustacean species and their potential food sources in a temperate estuary. We hypothesized that the shrimp *Palaemon peringueyi*, the mud prawn *Upogebia africana* and the sand prawn *Callichirus kraussi* have disparate diets due to interspecific differences in metabolism and feeding mode and that diets of the crustaceans all change temporally due to variations in available food. The species with the most variable diet was expected to have the greatest niche differentiation. We sampled the 3 crustaceans and their food sources on occasions spanning 4 seasons. The diet of *P. peringueyi* changed from a dominance of benthic microalgae in spring and winter to increased contributions of particulate organic matter (POM) in summer, with preferential sequestration of 22:6 ω 3. *U. africana* showed a stable dependence on estuarine POM as the main source of its energy, while *C. kraussi* relied more on marine POM, except in winter, when benthic microalgae was an important energy source. The 2 infaunal prawns showed elevated proportions of 16:1 ω 7 and 20:5 ω 3 throughout most of the year, probably originating from diatoms. *P. peringueyi* showed a different preferred diet than the 2 prawns, resulting in an isotopic niche segregation from the infaunal prawns, but the fatty acid niche of the shrimp overlapped with the prawns. Stable isotope values revealed the temporal variations in the origins and proportions of different food sources contributing to the diet of each species, while fatty acid data provided information on finer-scale trophic interactions. Temporal variations in estuarine consumer diets should be considered in estuarine food web studies whenever possible.

KEY WORDS: Benthos · Diet · Niche · Temporal variation · Shrimps · Prawns · South Africa

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INTRODUCTION

An estuary is a meeting place of terrestrial drainage systems with the coast, and it represents habitats that promote high biological production as a result of the synergy of terrestrial and marine-origin nutrients. The seaward flow of freshwater and the intrusion of marine water into the river function to transport organic matter into and out of the estuary, and these natural fluctuations create a highly variable environment for the resident organisms (Boyn-ton et al. 1982, Kasai et al. 2010). Consumers have

evolved different feeding mechanisms (e.g. filter feeding, epiphyte grazing, detritus feeding, carnivory and omnivory) to take advantage of the variety and temporally transient nature of the food sources available in estuaries (Garm et al. 2003, Yokoyama et al. 2005, Antonio et al. 2010). Terrestrial vegetation, littoral plants, benthic microalgae and freshwater or marine phytoplankton are some of the potential producers in estuarine food webs (Kang et al. 2003, Guest et al. 2004, Richoux & Froneman 2008). Determining the diets of estuarine animals is difficult due to the complexity inherent in these detritus-

driven systems, as energy sources vary substantially through space and time (Galois et al. 1996, Antonio et al. 2012, Gonçalves et al. 2012). Estuaries are important rearing grounds and permanent habitats for many fish and invertebrates, and the benthos constitute major consumers in these biologically rich ecosystems (Napolitano et al. 1997, Kang et al. 2003, Antonio et al. 2011). By examining the diets of estuarine benthic consumers through time, we can form an understanding of how organisms respond to variable sources of organic matter in an estuary, and we can better inform coastal managers interested in sustainable management of marine and estuarine resources.

Prawns and shrimps are ecologically important decapods throughout the world (Guary et al. 1975, Ziebis et al. 1996, Pillay et al. 2007). They tolerate wide ranges of water salinities, temperatures and habitat types ranging from oceanic to temporarily closed or permanently open estuaries (Froneman 2001, Antonio et al. 2011). Burrowing prawns such as upogebiids and callianassids are benthic ecosystem engineers inhabiting sheltered intertidal zones (Dworschak 1987, Nickell & Atkinson 1995, Pillay et al. 2007). Epibenthic swimming shrimps such as *Palaemon* spp. are commonly associated with seagrass or other macrophyte beds in estuaries (Guerao & Ribera 1996, Froneman 2001). Prawns and shrimp are significant food sources for economically important fish and they are harvested by humans as bait or for direct consumption (Teshima et al. 1992, Volkman et al. 1998, Hodgson et al. 2000). Despite their ecological and economic importance, limited information exists on the feeding niches and trophic interactions among these common estuarine crustaceans (Garm et al. 2003, Richoux & Froneman 2007, Antonio et al. 2011). Upogebiid prawns are typically labeled as suspension feeders (Schaefer 1970, Harris et al. 1991), callianassid prawns as deposit feeders (Ziebis et al. 1996) and *Palaemon* shrimp as omnivorous detrital feeders (Richoux & Froneman 2008). Food actually assimilated by these animals is ambiguous since organic matter available in an estuary is composed of diverse materials that vary in proportion both spatially and temporally. In general, we know relatively little about the basic food sources of these invertebrates and how the quality of their food changes over time.

Researchers studying estuarine systems have incorporated a variety of methods to assess consumer diets; most recently, these include stable isotope (SI) and fatty acid (FA) analyses. The 2 tracer methods provide time-integrated information about food as-

similated into the tissues of consumers, rather than snapshot descriptions of recognisable food recently ingested (from gut content analysis) or direct observations of feeding activities (Budge et al. 2008, Kelly & Scheibling 2012). Predictable changes in the ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) isotopes from prey to predator allow us to determine the most likely sources of carbon and the trophic positions of consumers within food webs (Peterson & Fry 1987, Vander Zanden & Rasmussen 1999). As long as the food sources are isotopically distinct, the conservative $\delta^{13}\text{C}$ value of a potential food source is a useful tracer in estuarine studies (Richoux & Froneman 2007, Antonio et al. 2012), while the carbon to nitrogen ratio (C:N) is an indicator of the origin of particulate organic matter (POM) (Kasai et al. 2010). FA analysis is based on the concept that primary producers establish particular FA patterns that may be transferred conservatively to consumers, thus indicating the quality and quantity of dietary sources assimilated by the animals within a period of time (Napolitano et al. 1997, Dalsgaard et al. 2003). Such dietary indicators are obscured as the trophic level increases (Budge et al. 2002) and as the origins of the FAs diversify (Galois et al. 1996, Richoux & Froneman 2008). Combining information derived from FA and SI analyses has proven to be particularly effective in identifying major sources of organic matter contributing to the diets of benthic invertebrates (Budge et al. 2008, Gonçalves et al. 2012). Relative changes in SI and FA values have been useful for assessing spatial and temporal variability in diets within species (Cripps & Atkinson 2000) and for assessing diets among co-occurring species (Guest et al. 2004, Antonio et al. 2012). SI data can also be used to infer characteristics of population or community structure and statistically compare isotopic niche space (range of isotopic values) and niche areas (standard ellipse areas in bi-plot space), and these can provide insights into resource partitioning and specialization (Jackson et al. 2011, Layman et al. 2012, Ryan et al. 2013).

Our aim was to evaluate how 3 crustaceans with divergent feeding modes and lifestyles respond to temporal variability of organic matter in an estuary. We tested 3 hypotheses: (1) organism diets change temporally as a result of variations in the available food sources, (2) diets of the different species are distinct owing to differences in their feeding behaviours, and (3) the hyperbenthic swimming shrimp exhibits greater niche differentiation relative to the infaunal prawns owing to its mobility and hence potentially increased access to a greater variety of

food sources. To address these hypotheses, we measured time-integrated tracers (SI ratios and FA profiles) in the 3 consumers over a 9 mo period.

MATERIALS AND METHODS

Study site and sample collection

The Kowie River is a temperate permanently open system on the southeast coast of South Africa that empties into the Indian Ocean (Fig. 1). It is one of the longest tidal rivers in the country, and marine water can extend 21 km from the river mouth (Heineken & Grindley 1982). Sampling trips were conducted during neap tides in 2011 (May, June, August, October, November) and 2012 (January). The target species were the shrimp *Palaemon peringueyi* (Stebbing, 1915), the mud prawn *Upogebia africana* (Ortmann, 1894) and the sand prawn *Callichirus kraussi* (Stebbing, 1900). The mud prawns and shrimp were collected from a mud flat at the lower estuary ~3 km from the river mouth, while the sand prawns were collected from a sand flat ~1.3 km closer to the mouth (Fig. 1). A prawn bait pump or tin can was used to extract the sand and mud prawns, while *P. peringueyi* shrimp were captured using a hand net (50 cm² mouth diameter, 1 mm mesh size) pulled along the shore through seagrass patches. Animals were kept alive in buckets of fresh estuarine water. We collected all potential food sources of the 3 crustacean species: local estuarine POM, benthic microalgae (BMA), surface sediments, marsh plants, seagrass, detritus and marine POM. Potential food sources were collected at the same places and times as the animal collections. About 6 l of surface water were collected from each site to derive POM tracer data and chlorophyll *a* (chl *a*) concentrations. Triplicate samples of the top 5 cm of sediments were scraped from about 50 cm² of the benthos and spread flat onto trays (30 × 20 cm²) to later extract the BMA. Three cores (3.2 cm² diameter) of surface sediments (top 10 mm) were taken from each site to later derive chl *a* data. Coastal marine water was collected ~3 km south of the river mouth to derive tracer data of the marine POM entering the Kowie Estuary.

Sample processing and laboratory analyses

In the laboratory, animals were kept alive overnight in aerated and filtered estuarine water to allow them to clear their guts. The animals were sexed,

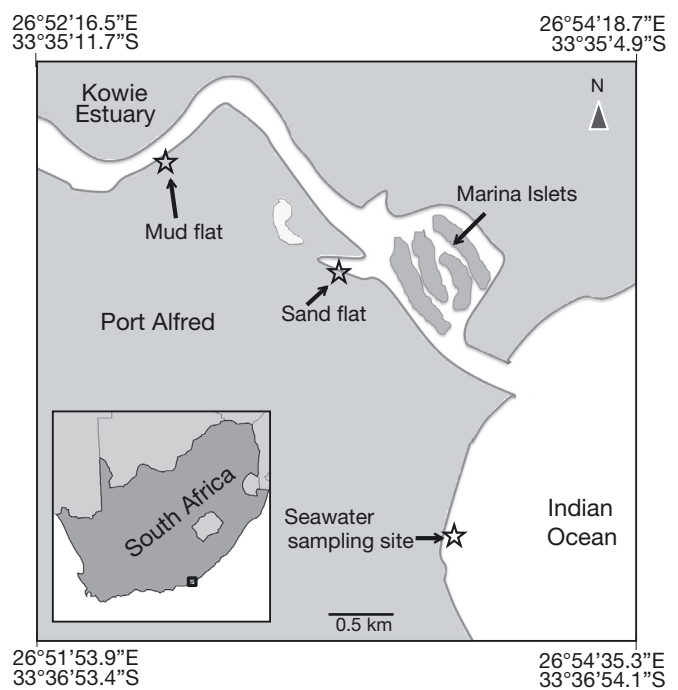


Fig. 1. Location of sampling sites (stars) along the Kowie Estuary and coastal area of Port Alfred, southeast coast of South Africa

placed in individual foil envelopes and frozen at -80°C . Water samples were vacuum-filtered onto ashed and pre-weighed glass fiber filters (GF/F Whatman, 47 mm) until they clogged. Filtered samples intended for SI analysis were treated with a few drops of 1.2 M HCl to remove carbonates, rinsed with distilled water, placed in individual foil envelopes and stored at -80°C . Filtered samples intended for FA analysis were frozen immediately at -80°C . Surface sediments for extracting BMA were covered with nylon mesh (63 μm) and a 5 mm layer of pre-treated sand (acid-washed and combusted at 450°C for 5 h) and then exposed to artificial light. BMA that migrated into the wetted pre-treated sand were separated according to Antonio et al. (2012). Microalgal biomass was estimated from 250 ml aliquots of water and ~300 mg subsamples of sediment immersed separately in 8 ml of 90% acetone for 24 h at -20°C . Total chl *a* concentrations were measured using a Turner Designs 10AU fluorometer.

Animals, filters and sediment samples were lyophilized at -60°C for 24 h (VirTis BenchTop 2K) and weighed. Headless and unshelled animals were individually homogenized using a mortar and pestle. Animal samples for SI analysis were soaked in 1:2 methanol-chloroform to remove lipids, lyophilized at -60°C overnight, and re-homogenized. The $\delta^{13}\text{C}$ and

$\delta^{15}\text{N}$ values of the animals and filtered samples were determined using a mass spectrometer with a Europa Scientific 20-20 IRMS linked to an ANCA SL prep unit. Casein was used as a protein standard, nitrogen was expressed (with analytical precision within $\pm 0.2\%$) relative to atmospheric nitrogen, and carbon was expressed relative to Vienna Pee-Dee Belemnite. Isotope ratios are expressed in the δ unit notation in the following equation: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where X represents ^{13}C or ^{15}N and R is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio, respectively.

Homogenized animal subsamples intended for FA analysis were weighed (20 to 70 mg) and put into test tubes. Total lipids were extracted and their FAs were methylated using a one-step method modified from Indarti et al. (2005). Briefly, filtered and animal samples were covered with 2 ml 0.01% butylated hydroxytoluene in chloroform; anhydrous sulphuric acid/methanol solution (1.3:0.7 v/v) was added to each sample and the mixture was placed at 100°C for 30 min. Following the addition of MilliQ filtered water, samples were vortexed and centrifuged at 3000 rpm. The top aqueous layer was discarded, while the lower fatty acid methyl ester (FAME)-containing layer was dried with sodium sulphate. Aliquots (1 μl) of the FAME suspensions were injected at 260°C into an Agilent 7890A/7000 gas chromatograph/mass spectrometer (GC/MS) equipped with a Zebtron ZB-WAXplus capillary column with helium as the carrier gas. FAME peaks were created using a flame ionization or MS detector and visualized using Chemstation (B04.02) or Masshunter (B.05.00) software; peak identities were confirmed using external standards (marine polyunsaturated FA [PUFA] no. 1 and no. 3, Bacterial and Supelco 37 component FAMES mix; Sigma) and a NIST 08 MS library. FAs were reported as percentages of the total fatty acids (% TFA, mean \pm SD) and grouped as saturated (SFA), monounsaturated (MUFA), PUFA, essential (EFA), bacterial (BFA) or higher plant (HPFA) FAs. The ratio of 16:1 ω 7/16:0 (>1.0) and the relative prevalence of 20:5 ω 3 were considered as diatom indicators (Ackman et al. 1968, Graeve et al. 1994), while the abundance of 18:4 ω 3 and ratios of 18:1 ω 9/18:1 ω 7 and 22:6 ω 3/20:5 ω 3 were evaluated as potential dinoflagellate or carnivory indices in consumers (Cripps & Atkinson 2000, Parrish et al. 2000). However, estuarine POM tends to incorporate a mixture of detritus arising from diverse sources including phytoplankton (Goñi et al. 2009, Antonio et al. 2010); therefore, we employed a diatom index (16:1 ω 7+20:5 ω 3)/(18:1 ω 9+18:4 ω 3+22:6 ω 3) as a potential indicator of relative dominance of diatoms over dinoflagellates.

Data analyses

Exploratory Stable Isotope Analysis in R (SIAR; Parnell et al. 2010) models for each consumer were performed using estuarine POM, BMA, surface sediment, marsh plants, seagrass, detritus and marine POM as potential food sources. Sources that had $<5\%$ contribution to animal diets were subsequently removed, leaving estuarine POM and BMA from muddy or sandy locations and marine POM in the final SIAR models for all 3 species. Normality and equality of variance in the SI data were confirmed using the Shapiro-Wilk test. One-way ANOVA with Tukey's post hoc tests were used to test the effect of time on the isotopic ratios of each food source and consumer. Fractionation values of 1.0% for $\delta^{13}\text{C}$ (DeNiro & Epstein 1978) and 3.0% for $\delta^{15}\text{N}$ (DeNiro & Epstein 1981) were incorporated into the models. Although other prey-predator fractionations were found (e.g. McCutchan et al. 2003), conventional $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionation values may still be applied for benthic consumers that lack species-specific fractionation values (Yokoyama et al. 2005). The trophic level of each consumer was calculated following Vander Zanden & Rasmussen (1999): $\text{Trophic position}_{\text{consumer}} = 1 + [(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{food}}) (\Delta\delta^{15}\text{N})^{-1}]$, where $\delta^{15}\text{N}_{\text{consumer}}$ is the $\delta^{15}\text{N}$ value for a given consumer, $\delta^{15}\text{N}_{\text{food}}$ represents the average $\delta^{15}\text{N}$ of the basal food source, and $\Delta\delta^{15}\text{N}$ is the trophic fractionation of 3% between adjacent trophic levels (DeNiro & Epstein 1981).

For the FA data, non-metric multidimensional scaling (NMDS) of the untransformed %TFA profiles (all 45 FAs detected in each species were included in each model) was used to examine the temporal changes in animal diets. Analyses of similarities (ANOSIM) were performed on each Euclidean similarity matrix to determine resemblance in the FA profiles of consumers among sampling periods, and whether diets differed among the species. The test statistic R and significance values were reported. Similarity percentages (SIMPER) and principal components analysis (PCA) were used to evaluate which FAs were most influential on any differences between species. The Stable Isotope Bayesian Ellipses in R (SIBER) routine in SIAR was used to analyse resource partitioning (isotopic niche space) among the 3 decapod species using their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Jackson et al. 2011) and the x and y coordinates of the NMDS analysis of FA profiles of each consumer. The areas of the convex hulls and ellipses represent the calculated isotopic and FA niche widths, parallel to the isotopic niche described by

Jackson et al. (2011) and Layman et al. (2012). By incorporating all sampled individuals ($n > 30$ for each species), this approach allowed us to quantify the feeding niche occupied by each population (Semmens et al. 2009), measure the degree of potential niche overlap (Jackson et al. 2011), and compare information derived from the 2 methods (SI and FA analyses). Analyses were conducted using PAST 2.00 (Hammer et al. 2001), Primer v 6 (Plymouth Routines in Multivariate Ecological Research) or R (Parnell et al. 2010).

RESULTS

Stable isotopes of food sources and consumers

Significant temporal variations were observed in the suspended and sedimented microalgal biomass (chl *a*) at the 2 sites of the Kowie Estuary (Fig. 2). POM microalgal biomass in the mud flat was relatively high from June to October (winter to spring), and in January (late summer) both the sediment and POM reached their peak microalgal biomass (Fig. 2A). At the sandy site, both sediment and POM showed high microalgal biomass only in October relative to the rest of the year. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of potential food sources changed significantly (Fig. 2B & Fig. 3, Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m504p193_supp.pdf) over time at the mud and sand flats. The $\delta^{13}\text{C}$ values among food sources were particularly distinct, BMA being highest, estuarine POM being lowest, and marine POM showing intermediate values. The mean C:N values of estuarine (muddy and sandy) POM and marine POM were high (>8) during June to August and low (<8) in other months (Fig. 2C). Diatom indices of muddy and sandy sediments were greater compared with the particulates suspended above them, except in October, when POM diatom indices were similar to those of the sediments (Fig. 2D).

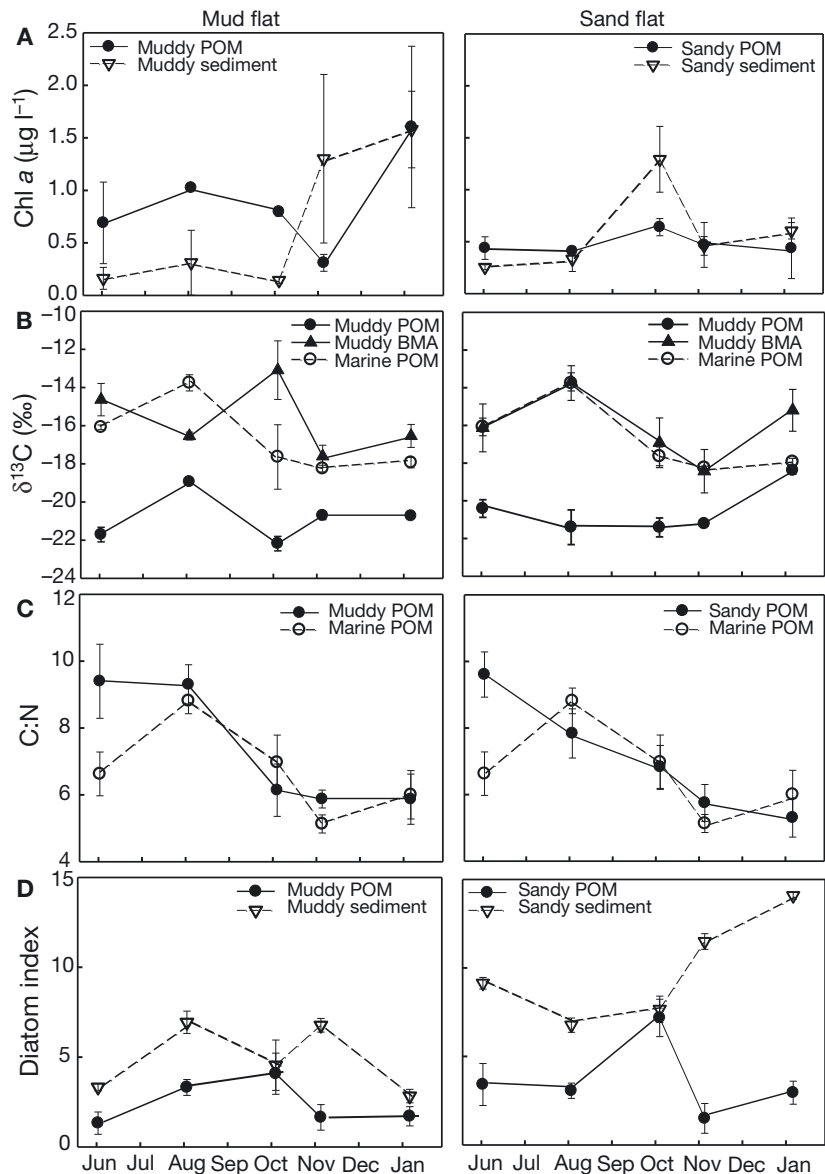


Fig. 2. Temporal fluctuations in (A) chlorophyll *a* concentration ($\mu\text{g l}^{-1}$), (B) $\delta^{13}\text{C}$ (‰), (C) C:N ratio and (D) fatty acid diatom index (16:1 ω 7+20:5 ω 3)/(18:1 ω 9+18:4 ω 3+22:6 ω 3) of the particulate organic matter (POM) and sediment in the 2 sites of Kowie Estuary, South Africa. Error bars are standard deviations

Among the 3 consumers, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the shrimp *Palaemon peringueyi* changed the most throughout the year (Fig. 3, Tables S1 & S2). The relatively high $\delta^{13}\text{C}$ values of *P. peringueyi* (-13 to -14 ‰) in May to June were similar to the high $\delta^{13}\text{C}$ values of BMA (-13 to -15 ‰), while the lower $\delta^{13}\text{C}$ values (-16 to -19 ‰) in the shrimp from August to January were intermediate among the food sources (Fig. 3). The relatively high $\delta^{15}\text{N}$ values of the shrimp were similar to those of muddy BMA throughout the year. Similarly, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Upogebia*

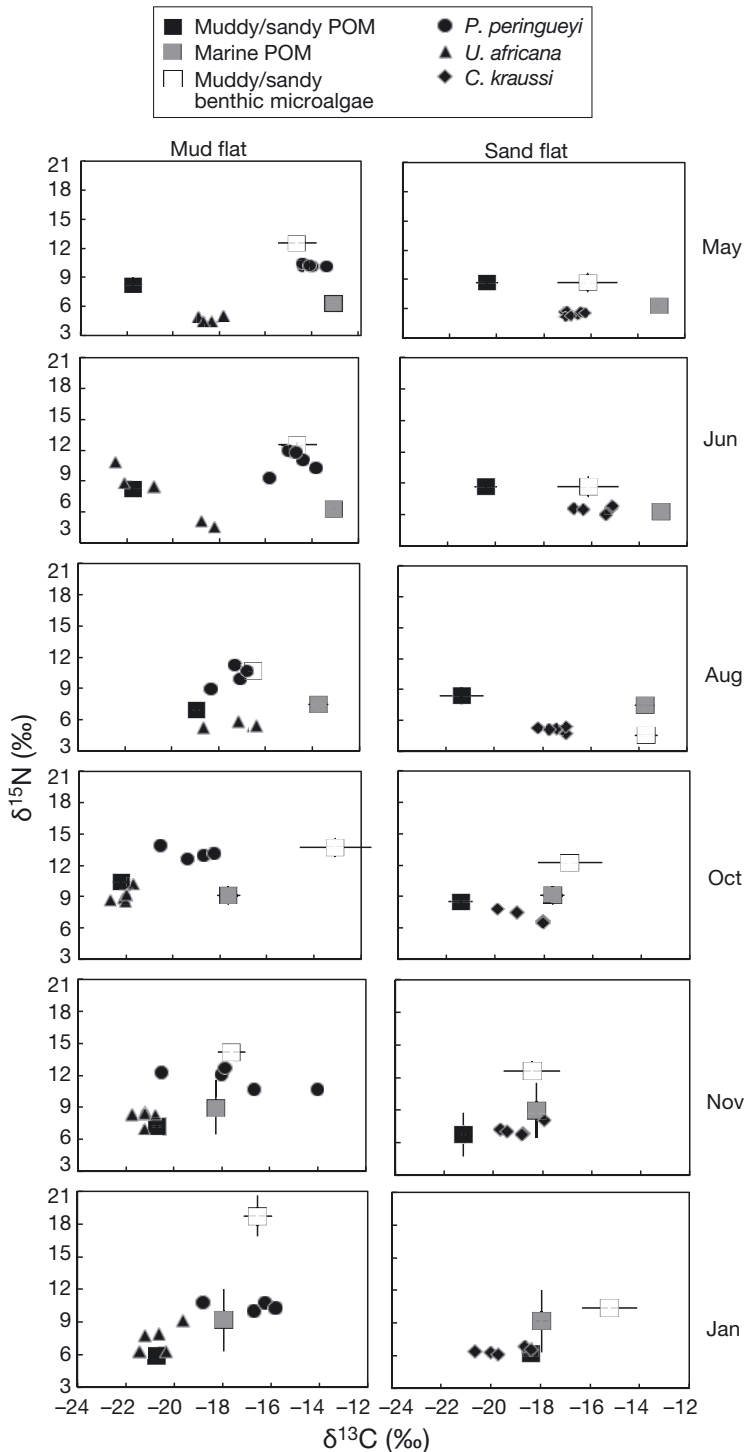


Fig. 3. $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) values of food sources (muddy/sandy particulate organic matter [POM], marine POM and muddy/sandy benthic microalgae) and consumers (*Palaemon peringueyi*, *Upogebia africana* and *Callichirus kraussi*) collected from the muddy and sandy sites of the Kowie Estuary, South Africa, from May 2011 to January 2012. Fractionation factors of 1 and 3‰ have been subtracted from each consumer's carbon and nitrogen signatures, respectively. Error bars are standard deviations

africana varied significantly throughout the year (Fig. 3, Tables S1 & S2). The seasonal shifts in the $\delta^{13}\text{C}$ values of the mud prawn (–16 to –20‰) were similar to those of the mud flat POM (–19 to –22‰). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the sand prawn *Callichirus kraussi* varied significantly throughout the year (Fig. 3, Tables S1 & S2), with high $\delta^{13}\text{C}$ values from May to August (–14 to –17‰) and relatively low values from October to January (–17 to –19‰), following the trends in the marine POM. In general, the 3 consumers showed higher $\delta^{13}\text{C}$ values in autumn to winter and lower values in spring to summer, while $\delta^{15}\text{N}$ were higher in spring relative to the rest of the year. The high $\delta^{13}\text{C}$ values of BMA were reflected in all 3 species in winter, while the low $\delta^{13}\text{C}$ values of POM were reflected in the animals from October to January. The shrimp showed the highest trophic level (2.5 annual mean) compared with the 2 prawns, which were similar to each other (annual means of 1.1 and 1.3; Table S1).

Outputs from SIAR models indicated a temporally variable diet composition in *P. peringueyi* (Fig. 4A, Table S3 in the Supplement). BMA contributed largely (up to 65%) to the shrimp diets throughout the study, whereas contributions from POM of estuarine and marine origin were more variable. *U. africana* showed a consistent preference for estuarine POM (muddy site) throughout the year (contributions of up to 88%; Fig. 4B, Table S3), whereas *C. kraussi* showed an annual mixed diet (Fig. 4C, Table S3), with generally high contributions from marine and estuarine POM (sandy site). SIBER analysis revealed a relatively large isotopic niche width (areas of convex hull and standard ellipse) in the shrimp *P. peringueyi*, and no overlap with the 2 prawns (Fig. 5A). The sand prawn *C. kraussi* showed the smallest isotopic feeding niche and substantial convex hull overlap with the mud prawn *U. africana*, but there was minimal overlap between their ellipses (Fig. 5A).

Fatty acid profiles of food sources and consumers

Forty FAs were detected (annual mean proportions >1%) in estuarine POM and BMA, and 38 were detected in marine POM (Table S4 in the Supplement). Major FAs detected in POM and BMA samples included SFAs 14:0, 15:0,

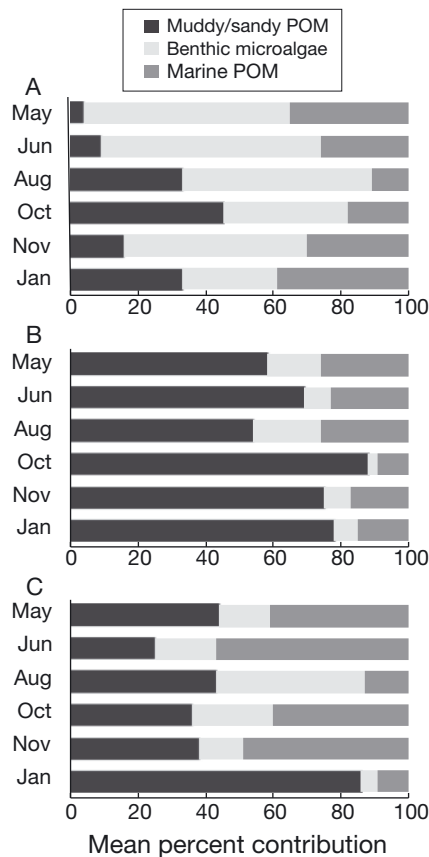


Fig. 4. Mean contributions (%) of food sources to the diets of (A) *Palaemon peringueyi*, (B) *Upogebia africana* and (C) *Callichirus kraussi*, as estimated by Stable Isotope Analysis in R (SIAR). Proportion distribution and standard deviations are shown in Table S3 in the Supplement (at www.int-res.com/articles/suppl/m504p193_supp.pdf). POM: particulate organic matter

16:0, 18:0, 22:0 and 24:0; MUFAs 16:1 ω 7, 18:1 ω 7 and 18:1 ω 9; and PUFAs 20:5 ω 3 and 22:3 ω 6, and these varied through time. The SFAs, particularly 16:0, were consistently high in all of the food sources, although proportions shifted temporally. The proportions of PUFA and EFA in POM were highest in June in the sandy POM, but for muddy and marine POM the highest proportions occurred in January. BMA showed consistently higher PUFA and EFA levels than POM throughout the year. Proportions of BFAs and HPFAs were generally <10% in the food sources. Although a higher diatom index was observed in the sediments during winter and summer, the index increased during spring in the water column.

Forty-one FAs were detected (mean annual concentrations >1%) in at least 2 of the consumers (Table S5 in the Supplement). FA profiles of consumers were similar between genders within a species and thus were pooled, and juveniles were not

included in the data set. The PUFA 20:5 ω 3 (23–25% annual mean) and SFA 16:0 (16–18% annual mean) were the most abundant constituent FAs in the 3 crustaceans. Major FAs in the 3 species included PUFAs 18:4 ω 3, 20:4 ω 6, 20:5 ω 3 and 22:6 ω 3; SFAs 16:0, 18:0 and 14:0; and MUFAs 16:1 ω 7, 18:1 ω 7 and 18:1 ω 9. Elevated proportions of 20:5 ω 3 and 22:6 ω 3 were common to the 3 consumers in June and August, producing high PUFA and EFA levels in winter. In contrast, SFA and MUFA were greatest in spring (October) in the 3 species. The BFAs and HPFAs occurred in proportions of <10% throughout the year. The sand prawn *C. kraussi* showed the highest values of the diatom index among the 3 decapod species.

NMDS and ANOSIM of FA profiles revealed large temporal shifts in the diets of the 3 decapods (Fig. 5, Table S6 in the Supplement). Interspecific differences were greatest during May and January, with *P. peringueyi* and *C. kraussi* showing the most disparate profiles overall. Apart from some overlapping (mainly during June and November between the mud prawns and either the sand prawns or the shrimp), the FA profiles of the 3 species were remarkably distinct from each other throughout much of the year. In general, the shrimp showed smaller temporal and among-individual variability in their FA profiles compared with the mud and sand prawns. SIMPER analysis (>50% cumulative percent contribution) and PCA revealed the FAs that most influenced the differences among the 3 species (superimposed in Fig. 6). The PUFA 22:6 ω 3 was consistently influential in isolating *P. peringueyi* from the 2 prawns, while FAs 20:5 ω 3 and 16:1 ω 7 were important distinguishers for the prawns. SIBER analysis of pooled FA data indicated some potential overlap in the feeding niches of the 3 species, with *P. peringueyi* showing the smallest niche width (convex hull and ellipse) compared with the 2 prawns (Fig. 5B). The FA feeding niches of the 3 decapod species showed more overlapping relative to the isotopic feeding niches (Fig. 5).

DISCUSSION

Temporal variation in available food sources reflected in consumers

We utilized SI and FA tracers in food sources and consumers to determine whether temporal shifts in consumer diets result from changes in the quality or quantity of organic matter in the water column or

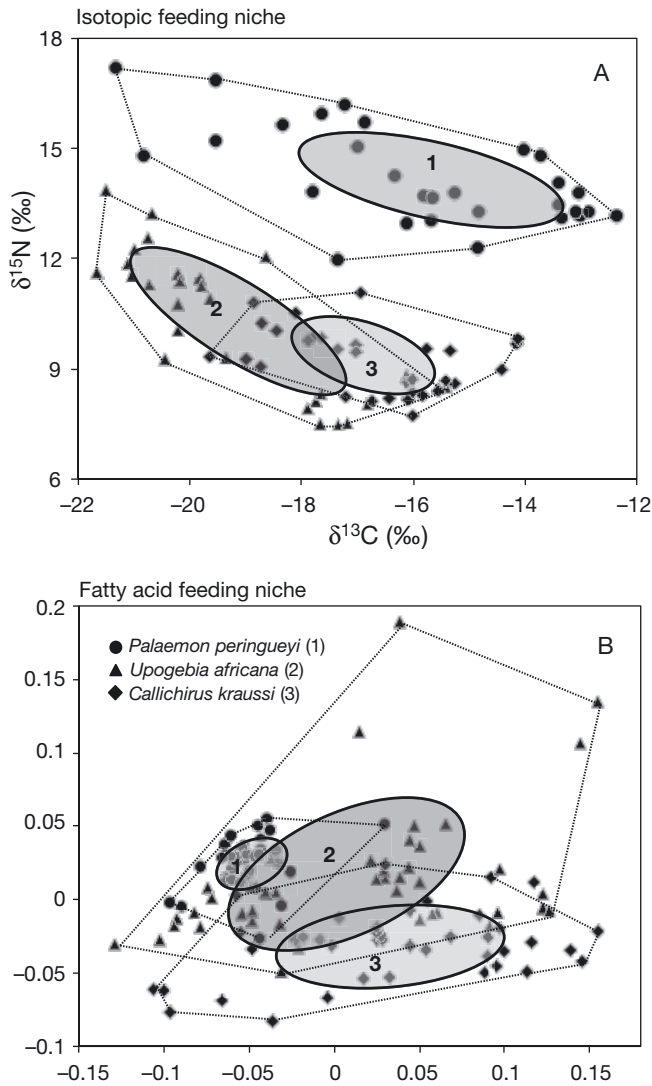


Fig. 5. SIBER output using (A) pooled $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ biplot and (B) non-metric multidimensional scaling (NMS) x–y ordinates of fatty acid profiles of *Palaemon peringueyi* (1), *Upogebia africana* (2) and *Callichirus kraussi* (3). The area of the convex hulls (dotted polygons) and ellipses (shaded) represent the calculated (A) isotopic and (B) fatty acid feeding niche widths of each species

sediments. Estuaries are typically characterized by minimum productivity levels in winter and maximum productivity in summer (Boynton et al. 1982, Galois et al. 1996), so we expected some basic differences in food quality based on seasonality. Kowie Estuary was slightly unusual as it showed high phytoplankton and BMA biomass in spring, particularly near the river mouth (sandy site), and this pelagic production may have been transported upstream towards the mud flats occupied by mud prawns and shrimp, where microalgal biomass dramatically increased in summer (Fig. 2). Similar results were observed in the

Yura Estuary, Japan, where high phytoplankton biomass produced at the river mouth in early spring was transported upstream by estuarine circulation (Kasai et al. 2010). Advection and strong tidal currents in summer can bring marine phytoplankton from the coast into the estuary and result in high phytoplankton standing stocks inside an estuary (Goñi et al. 2009, Antonio et al. 2012). Estuarine POM with high microalgal biomass and low C:N ratios <8.0 (near the Redfield value of 6.78) indicate live phytoplankton of marine origin, whereas elevated C:N ratios >8.0 indicate increased terrestrial and freshwater contributions (Galois et al. 1996, Kasai et al. 2010, Antonio et al. 2012). High C:N values and high proportions of HPFA and BFA in POM were observed in winter relative to spring and summer at both sites of the Kowie Estuary, evidence of higher contributions of terrestrial allochthonous matter to the suspended food during this period. Thus, the Kowie Estuary was generally freshwater dominated in winter, and marine dominated from spring to summer.

Tracer data indicated that diatoms were an important food source in the Kowie Estuary. The FA diatom index value in the sediments was greater than that in POM throughout the year, implying the consistent presence of benthic diatoms (Fig. 2D, Table S4). Elevated diatom indices in POM and sediments denoted the importance of diatoms in other estuarine food webs (Ackman et al. 1968, Budge et al. 2008, Richoux & Froneman 2008). In our study, BMA was an important alternative carbon source for decapod consumers in August (late winter), as reflected in the SIAR model output of BMA contributions (20–56%). This nutritionally rich food source likely becomes suspended in the water column through water movements; hence, more active hydrodynamic forces increase its contributions to the diets of filter feeders in particular. Other researchers (Kang et al. 2003) have highlighted the importance of BMA as an energy subsidy for some estuarine and shallow coastal food webs during periods of low phytoplankton production. During warmer months in the Kowie Estuary, the abundant microalgal biomasses (denoted by high chl *a*, high diatom indices, and high EFAs in the POM and BMA) all likely contributed to a high quantity and quality of food for the estuarine benthos. Napolitano et al. (1997) observed that high proportions of 14:0, 16:1 ω 7, 16:4 ω 1 and 20:5 ω 3 reflected diatoms as the major component throughout a phytoplankton bloom in Bahia Blanca Estuary, Argentina. Similarly, temporal variations in the availability and quality of the organic matter in the Kowie Estuary appeared to strongly affect the benthic consumer diets.

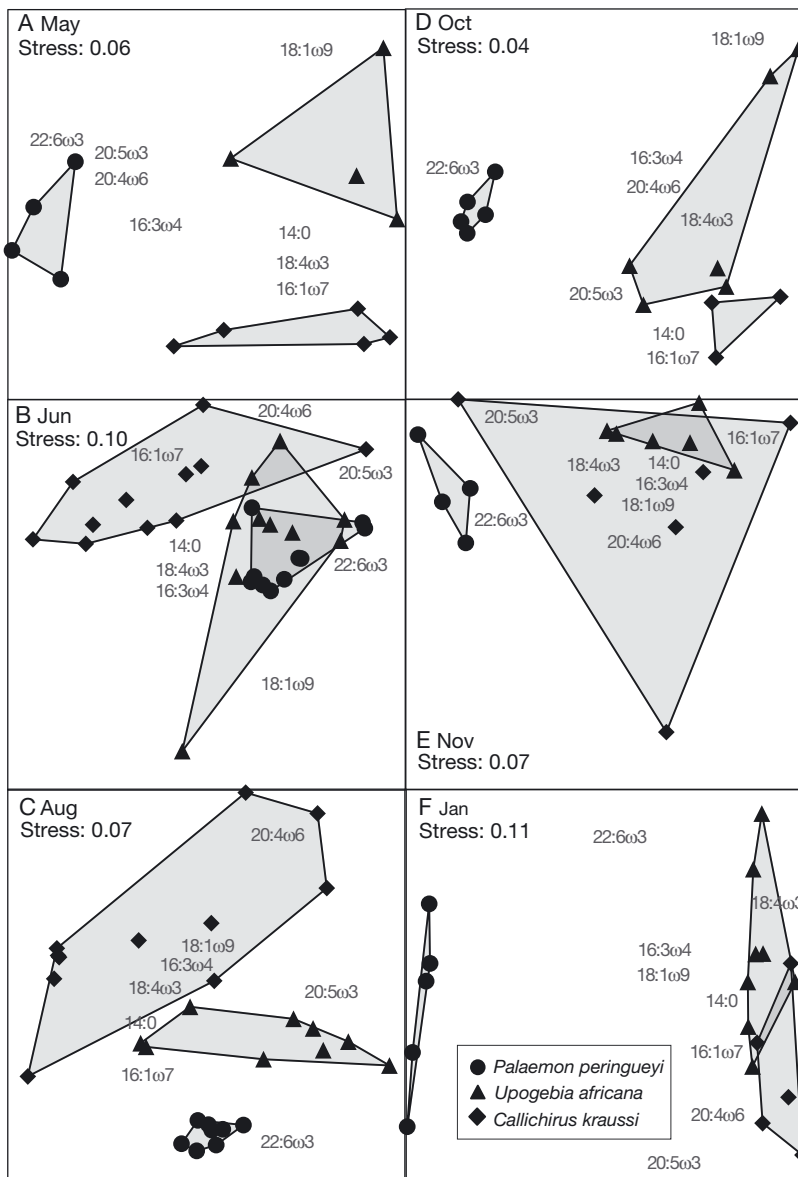


Fig. 6. Non-metric multidimensional scaling output using fatty acid profiles of *Palaemon peringueyi*, *Upogebia africana* and *Callichirus kraussi* during 6 sampling months (A–F). Polygons represent the fatty acid feeding niche of each species per sampling month. Fatty acids influential in separating the 3 species (derived from SIMPER and PCA) are superimposed in the plots. Refer to Table S6 in the Supplement (at www.int-res.com/articles/suppl/m504p193_supp.pdf) for ANOSIM output

The SI and FA data from the 3 decapods both imparted detailed information about the temporal variability in diets. For example, BMA were increasingly utilized by the 3 consumers during winter to supplement their nutrition during periods of potentially lower food quality and availability in the POM. Similarly, consistently high levels of PUFA and EFA in all 3 species reflected their utilization of nutrition-

ally rich food sources throughout the year, so their nutritional flexibility allowed them to maintain their condition despite fluctuations in their food supply. Such temporal fluctuations typically result from shifts in primary production; for example, increased phytoplankton abundance in certain seasons (e.g. spring and summer) can be reflected in the FA profiles of consumers (Parrish et al. 2005). Seasonal changes in the phytoplankton community composition can also affect the FA profiles of POM and, hence, the organisms that derive their nutrition from it (Napolitano et al. 1997). The PUFAs 20:5 ω 3 and 22:6 ω 3 are particularly essential for survival and growth of molluscs, prawns, fish and other heterotrophs (reviewed by Volkman et al. 1998), and these components must be obtained primarily from feeding (Teshima et al. 1992, González-Baró & Pollero 1998). In general, high levels of PUFA correspond with good nutritional condition in the field (high quantity and quality food) and in laboratory experiments (Pazos et al. 2003, Narváez et al. 2008). It was therefore surprising that the peak proportions of PUFAs in the Kowie Estuary decapods occurred during winter, when the food sources were generally more deficient in PUFAs. Some researchers have reported selective retention of certain FAs by consumers during periods of low food quality (González-Baró & Pollero 1998, Piveteau et al. 1999). Changes in temperature can also cause increases in PUFA levels in consumers such as *Penaeus japonicus* (Guary et al. 1975), although seasonal shifts in temperature are not large in the Kowie system (Heineken & Grindley 1982). It is plausible that the Kowie Estuary decapods preferentially retained PUFAs in their muscle tissues during periods of relative food shortage. Animals may also selectively retain or mobilize certain lipid components for reproductive purposes, thus affecting their FA compositions. The decapods that we studied typically spawn in June–August (winter peak) and November–March (summer peak) in South African estuaries (Hill 1967, Forbes 1973), a

schedule potentially influenced by the winter and summer increase in their PUFA levels (Table S5). For example, the EFAs 20:4 ω 6, 20:5 ω 3 and 22:6 ω 3 were significantly higher in June and August relative to the rest of the year in the 3 decapods, suggesting that reproduction could potentially mask diet changes in these consumers.

Interspecific differences in feeding behaviour

The SI and FA values of the 3 decapod species revealed substantial interspecific differences in their diets. Of the 3 consumers, *Palaemon peringueyi* had diets generally most dominated by BMA, except in spring to summer, when increased assimilation of POM was observed. *P. peringueyi* in a neighboring South African estuary showed high $\delta^{13}\text{C}$ (–14 to –16‰), which suggested substantial dietary contributions of epibionts and BMA associated with seagrasses and marsh plants (Richoux & Froneman 2007), whereas low proportions of higher plant FAs in their tissues indicated that macrophytes were not an important food source (Richoux & Froneman 2008). Instead, *P. peringueyi* shrimp living in estuaries may have selectively utilized the epibionts attached to the macrophytes and detritus as their source of energy (Froneman 2001, Richoux & Froneman 2007). There is evidence of selective detritus feeding by a related species (*P. adspersus*) that uses its second maxillae as bimodal sensors to mechanically and chemically assess detrital food before consuming or rejecting it (Garm et al. 2003). *P. peringueyi* may also possess such an ability to select specific food items (such as nutritious BMA) from detritus associated with macrophyte beds. Our study confirmed the importance of BMA as a key food source for *P. peringueyi*, and also suggested POM as a significant alternative food source during warmer months. Moreover, this species showed a higher trophic position than the 2 prawns (2.5 annual mean), implying carnivory. Guerao & Ribera (1996) similarly revealed predation on various benthic invertebrates by *P. serratus*. Our analysis of the shrimp's FA composition showed the prominence of 22:6 ω 3 as the most influential FA, which significantly distinguished the shrimp from the 2 other species, implying an enhanced ability of shrimp to preferentially assimilate 22:6 ω 3 from their diet. The EFA 22:6 ω 3 is typically abundant in phytoplankton (Parrish et al. 2000, Dalsgaard et al. 2003), and crustaceans generally do not synthesize this compound, so it must be acquired from their diet (Teshima et al. 1992). In the Kowie

Estuary, although proportions of 22:6 ω 3 were low in BMA and POM, the shrimp maintained elevated proportions of 22:6 ω 3 and generally high proportions of PUFAs and EFAs throughout the year (>45%), indicating a generally good quality diet.

Upogebia africana depended mostly on estuarine POM as the main source of carbon (>80% in some cases; Fig. 4B) relative to the other 2 species. The $\delta^{13}\text{C}$ values of *U. africana* and estuarine POM (both ranging from –15 to –19‰) have been documented in another temperate South African system (the Kariiega Estuary) and indicated a POM-based diet (Richoux & Froneman 2007). The elevated proportions of 16:1 ω 7 and 20:5 ω 3 in estuarine POM was reflected in the mud prawns, revealing the assimilation of diatoms by *U. africana*, a finding consistent with data on mud prawns collected from the Kariiega Estuary (Richoux & Froneman 2008). Upogebiids filter feed on phytoplankton and fine particles deposited and resuspended in and around their burrows (Dworschak 1987, Nickell & Atkinson 1995) using a setal filtering basket. Schaefer (1970) investigated the foregut of *U. africana* and found that setae in the foregut were efficient at extracting liquid from the fine mud particles. Gut contents of *U. africana* have confirmed the presence of plant fragments, whole and fragmented diatoms, and bacteria all embedded in a matrix of very fine organic material (Hill 1967, Harris et al. 1991). Thus, the existence of a sophisticated filtration mechanism in *U. africana* strongly supports the data derived from gut contents, SI and FA analyses, which all consistently suggest the ability of this consumer to effectively filter diatoms from estuarine POM.

The SIAR models suggested that the sand prawn *Callichirus kraussi* assimilated marine-origin carbon in general, and utilized BMA during low pelagic production in winter (Fig. 4C). The assimilation of marine POM by the sand prawns was expected since they were located closer to the ocean compared with the mud prawns. FA profiles of the sand prawns showed high proportions of the diatom-associated FAs 16:1 ω 7 and 20:5 ω 3 throughout the year, so like the mud prawns and the shrimp, the sand prawns were able to extract nutrient-rich diatoms from their environment, albeit from different origins (i.e. mainly from either marine or estuarine POM) that changed in dominance over time. SI and FA data from *C. kraussi* also showed that these decapods could utilize benthic diatoms in the sediments when pelagic food was in low supply. Such plasticity in their nutritional ecology certainly would contribute towards the successful establishment of large numbers of sand

prawns in a temporally variable habitat characteristic of most estuaries. Our sand prawn results contrast somewhat with other studies that described callianassids as deposit feeders, removing organic material from sand grains within their burrows or on the sediment surfaces (Dworschak 1987, Ziebis et al. 1996). However, there have been some reports of callianassid prawns showing suspension-feeding behaviour described as a sifting process of detritus and micro-organisms from the sand with their maxillipeds (Forbes 1973). Gut contents data derived from *C. kraussi* have revealed the presence of plant fragments, diatoms, protozoans and bacteria (Harris et al. 1991), a collection similar to that found in mud prawn guts. Our SI and FA results clearly indicated a suspension-feeding life style for *C. kraussi*, information that was not possible to determine using traditional gut content analysis.

Niche differentiation among consumers

Interspecific differences in diet among sympatric consumers should maximize the utilization of available resources and decrease competitive interactions. Such niche partitioning can be assessed using SIBER (Jackson et al. 2011) to measure the isotopic niches occupied by different species, and we have adopted the isotopic model to assess FA niches as an additional measure of trophic niche. SI analysis showed the general preferences of *P. peringueyi* on BMA, *U. africana* on estuarine POM, and *C. kraussi* on marine POM. Thus, the more unique isotopic niche of the shrimp was due to its selective consumption of nutritious BMA from its environment or carnivory on BMA-eating organisms, while the isotopic niche overlap between the mud and sand prawns was due to their assimilation of similar dietary components from the POM. As the sand prawns were located closer to the ocean than the mud prawns, the sand prawns assimilated more of the marine-origin carbon than that of river origin. The standard ellipse area overlap between the 2 prawns (from -17.5 to -18.5‰ $\delta^{13}\text{C}$ and from 9 to 10‰ $\delta^{15}\text{N}$) that represented the isotopic niche overlap between the 2 populations may signify the transition niche space between estuarine- and marine-feeding consumers. The 2 prawns occupied similar trophic positions (~ 1 annual mean) since they are primary consumers of POM, also contributing to some overlapping in isotopic niche space. *P. peringueyi*, having a higher trophic position than the 2 prawns (2.5 annual mean) and largely consuming epibionts and detritus attached

to macrophytes (Froneman 2001, Richoux & Froneman 2007, 2008, this study), could be regarded as an omnivore, thus having a wider isotopic niche width that is probably related to its flexible diet and high mobility. The hyperbenthic shrimp could readily extract quality food among the detritus of the macrophyte bed and suspended in the water column, giving this species a nutritional edge over the infaunal prawns.

To our knowledge, ours is the first study to use SIBER metrics on FA profiles of consumers to investigate aspects of trophic niche. Interestingly, the FA niches of the 3 decapods (Fig. 5B) showed more overlap than the isotopic feeding niches. *U. africana* occupied the widest FA niche width, which overlapped with the other 2 species, while *P. peringueyi* showed the smallest FA niche width, implying minimum inter-population niche variation (Semmens et al. 2009). These results contrast somewhat with the SI analysis and suggest that *U. africana* assimilates a broad range of FA components from the filtered estuarine POM compared with *P. peringueyi*, which has the ability to preferentially assimilate highly nutritious FAs from its variable diet, highlighting the role of *P. peringueyi* in sequestering high quality organic material. However, since the SIBER metrics were performed on consumers pooled from all sampling times, such contrasting results compared with SI output could reflect a higher stability in lipid components in the shrimp over time, perhaps a result of their increased trophic flexibility compared with the infaunal prawns. The overlapping FA standard ellipse areas in the 2 prawns (both having high levels of 16:1 ω 7 and 20:5 ω 3) was consistent with their common suspension-feeding behaviour from burrows. In general, more niche segregation among the decapods was observed using the SI ellipse areas, illustrating the variable origin of the dietary components of the consumers, while more overlap in their FA niches points to increased similarity in the FA components of the available food. For example, even though the shrimp assimilated more BMA, which was isotopically different from the POM primarily assimilated by the 2 prawns, the FA components of these food sources were relatively similar (at least during different times in the year) and hence created similar bulk FA profiles in the pooled consumers. This is an important distinction of the 2 tracer methods, because consumers in the same system may consume prey with more isotopic variation but with less FA variation. Further research is needed to better understand the implications of SIBER output from FA profiles in relation to trophic niches.

Many studies using SI and/or FA analyses to assess consumer diets have been completed in true marine environments (Budge et al. 2002, Parrish et al. 2005), and the knowledge derived from these studies are difficult to translate into an estuarine context owing to the major differences in the physical and biological factors involved. Relatively few researchers have incorporated both SI and FA tracer techniques (Budge et al. 2008, Gonçalves et al. 2012) to study feeding relationships among consumers. Our use of a combination of tracer methods revealed interesting temporal changes in both consumers and food sources in a complex estuarine environment, and the limitations of one technique were balanced by the strengths of the other. The 3 decapod species in a temperate estuary exhibited distinct diets due to their different feeding behaviours and their different locations within the estuary, and their diets shifted over time due to temporal changes in their food sources. In contrast to our expectations, the hyperbenthic shrimp showed the most variable diet over time compared with the infaunal prawns. Our data indicated some trophic niche partitioning among the 3 decapod species, which probably contributes to their successful co-habitation. The temporal changes revealed through SI and FA data from the food sources in relation to the consumers reiterates the importance of incorporating a temporal aspect and the use of more than one diet tracer when studying estuarine food webs.

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