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Identification of the food sources of sympatric ghost shrimp (*Trypaea australiensis*) and soldier crab (*Mictyris longicarpus*) populations using a lipid biomarker, dual stable isotope approach

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Abstract Fatty acids (FAs) profiles and stable isotope signatures of the ghost shrimp, *Trypaea australiensis* and the soldier crab, *Mictyris longicarpus* were determined at an unvegetated sandbank of Southport (Gold Coast, Queensland, Australia), in November 2005 and February 2006. Additionally, the FAs composition of the faeces and feeding pellets of *M. longicarpus* and the surface sediment at the study site were also analysed. *Trypaea australiensis* was found to selectively feed principally on benthic diatoms, as revealed by the high contribution of the marker lipid (20:5 (n-3)) to tissue total FAs and the δ^{13}C and δ^{15}N isotopic signatures of shrimp tissues. Although the diet of *T. australiensis* did not change between the two sampling periods, the shrimps appeared to reduce their feeding activity in summer, presumably in relation to a restricted metabolism, as revealed by a decrease in the contribution of the microalgal markers in their tissues. The FAs composition of the tissues of the soldier crab indicated that bacteria and diatoms constituted the base of its diet (contributions of branched 15:0 and 17:0, 18:1 (n-7) and 20:5 (n-3)). However, the isotopic signatures of the crabs suggested that meiofauna may represent an intermediate link between the crab and these micro-organisms.

Key words: food source, lipid biomarker, *Mictyris longicarpus*, stable isotope, *Trypaea australiensis*.

INTRODUCTION

Benthic macrofauna influence sediment properties and microbial processes through their feeding, bioturbation, burrow construction and burrow irrigation activities (Welsh 2003). These activities influence sediment organic matter loads, the vertical distribution and mineralization rates of organic particles within the sediment, and exchanges of oxygen and dissolved nutrients between the sediment and water column (Berg *et al.* 2001; Welsh 2003). The relative degree of these effects is to a large extent dependent upon the behaviour of the individual species and in particular their feeding mode (Sandnes *et al.* 2000; Welsh 2003). In the intertidal zones of the eastern coast of Australia, the ghost shrimp *Trypaea australiensis* (Thalassinidea: Callianassidae; commonly known as the 'marine yabby') and the soldier crab, *Mictyris longicarpus* (Brachyura: Mictyridae) are conspicuous and abundant large bioturbators which differ considerably in their feeding mode (Webb & Eyre 2004a,b).

Thalassinidean shrimps are common members of the infaunal communities of intertidal and subtidal marine sediments. These shrimps are intense bioturbators and construct complex burrow galleries, which can penetrate to more than 1 m deep, and have multiple openings at the sediment surface (Atkinson & Taylor 2005). *Trypaea australiensis* has been found to depths of at least 50 cm (Stapleton *et al.* 2001) and population densities of 50 to more than 100 individuals per square metre have often been reported (e.g. Contessa & Bird 2004). This species obtains food particles through subsurface deposit feeding (Boon *et al.*

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1997), but is also potentially able to collect food from its burrow walls and by resuspending sediment (Stapleton *et al.* 2001). *Trypaea australiensis* is known to selectively ingest fine particles (<63 μm in diameter; Stapleton *et al.* 2001). Although the feeding behaviour of this species is well known, its diet has not been clearly identified. To date, only Boon *et al.* (1997) have attempted to elucidate the diet of *T. australiensis* using natural abundance ^{13}C and ^{15}N isotope analyses in a system dominated by seagrasses and macroalgae as potential food sources and thus microalgae were not considered as a potential dietary source. Consequently, although yabbies in unvegetated sediments are suspected to feed on benthic microalgae (see references in Webb & Eyre 2004a), to our knowledge this has still not been directly demonstrated.

Similarly to *T. australiensis*, the behaviour of the soldier crab *M. longicarpus* has been widely studied, especially in relation to its feeding activity. Like other Myctiridae, *M. longicarpus* resides in non-permanent burrows during periods of tidal inundation and emerges to feed at low tide (Dittmann 1998). After emerging, hundreds to thousands of individuals aggregate to form dense 'armies' which roam across the sediment surface, giving them their common name of the 'soldier crabs' (Cameron 1966; Dittmann 1998). During feeding, the crabs scoop sediment into their buccal cavity where it is sorted and food particles selected. Unselected particles and sediment are discarded forming feeding (or pseudofaecal) pellets (Cameron 1966; Quinn 1986), which are visible on the sediment surface, and can sometimes cover the entire exposed intertidal area (Dittmann 1993). During tidal inundation, soldier crabs also appear to be able to feed in a similar manner on subsurface sediments while buried within their temporary burrows (Quinn 1986; Dittmann 1993). Although sometimes referred to as a 'grazer' (Webb & Eyre 2004b), implying that their diet consists largely of algal cells, this is not supported by studies on food ingestion by *M. longicarpus*. These indicate that this crab is an indiscriminate feeder, with a diet composed of detrital material, bacteria, diatoms and meiofauna (Cameron 1966; Quinn 1986; Dittmann 1993). However, these studies have relied on observations of the gut contents or the impact of the crabs on target potential food sources in the sediment and therefore at best, results are only indicative of the types of foods ingested and not those assimilated by the crabs. For example, in a previous study, it was proposed that observed changes in the richness and diversity of sediment meiofauna populations were probably a result of the intense sediment disturbance caused by the soldier crab *M. platycheles* leading to the death of some individuals rather than predation (Warwick *et al.* 1990). Thus, as with *T. australiensis*, there is a need to directly identify not only the food sources ingested by *M. longicarpus*, but those which are actually assimilated by the animals.

Because of the widespread distributions and often high population densities of *T. australiensis* and *M. longicarpus*, investigations on their major food sources are critical to understand the biogeochemistry and trophic structure of the sediments they inhabit. More specifically, these species may have a key role in the energy flow from the sediment to higher trophic levels, and as such significantly contribute to carbon removal from the sediment, as they constitute important prey items for higher predators, such as fish (e.g. Contessa & Bird 2004) and birds (e.g. Zharikov & Skilleter 2004a,b).

The aim of this study was to identify the food sources of coexisting and potentially competing *T. australiensis* and *M. longicarpus* populations on an unvegetated sandflat in south-eastern Queensland (Australia), employing both fatty acid biomarker and natural abundance stable isotope techniques. Recent studies have demonstrated that this dual approach provides a powerful tool to identify organic matter sources and trophic interactions in coastal marine ecosystems (Kharlamenko *et al.* 2001; Cook *et al.* 2004; Alfaro *et al.* 2006).

METHODS

Study site

All samples were collected in mid November 2005 and early February 2006 from a large (~1.0×0.4 km) unvegetated intertidal sandbank of the Broadwater, Southport (Gold Coast), about 50 km south of Moreton Bay (Queensland, Australia; Fig. 1). This site was chosen because it (i) supported large populations of both *M. longicarpus* and *T. australiensis*, which were by far the dominating macrofauna species present (N. Spilmont & D. T. Welsh, pers. obs. 2005); and (ii) was situated about 300 m offshore and separated from the beach by a subtidal channel at low tide, and therefore was rarely disturbed by anthropogenic activities.

Animals and sediment sampling

Trypaea australiensis individuals were collected manually using a 'yabby pump' and stored on ice until being frozen alive in the laboratory (within 1–2 h). Three individuals were sampled for fatty acid and three for stable isotope analyses respectively, on each sampling date. Muscular tissues from the thorax (carefully excluding the digestive tract) and the abdomen of individual animals were pooled and utilized for fatty acid and stable isotope analyses.

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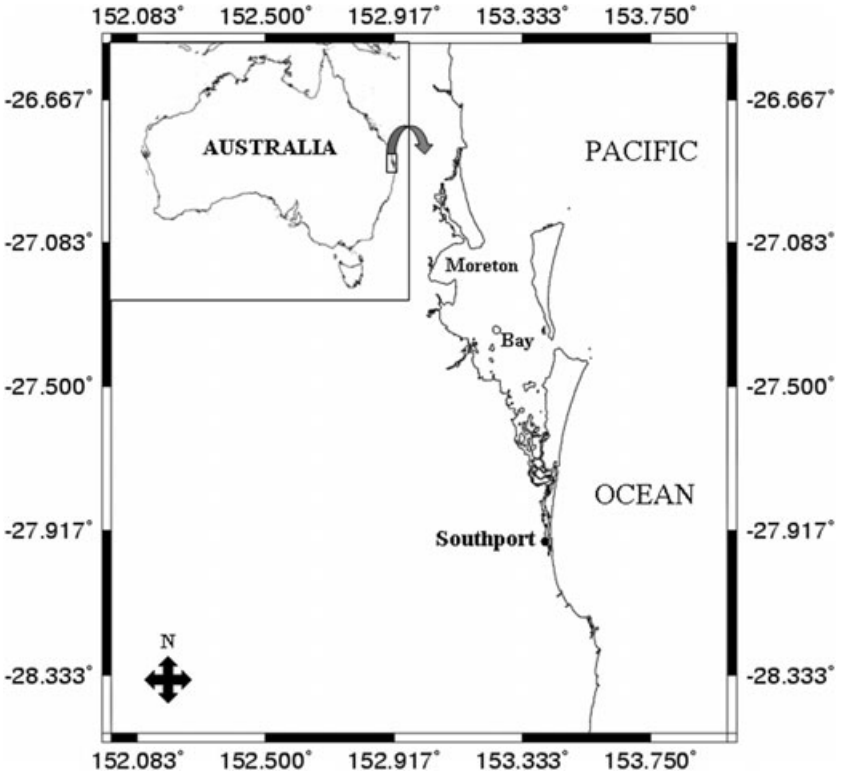

Fig. 1. Location of the study site (Southport) along the coast of southern Queensland (Australia).

Approximately 30 adult soldier crabs (large, blue coloured individuals; Dittmann 1998) were randomly collected while they were actively feeding at the sediment surface and placed in a clean aquarium for transport to the laboratory. Crabs were kept in the aquarium for approximately 3 h before being removed and frozen. The faecal pellets deposited in the aquarium were collected using clean forceps, pooled and frozen for fatty acid analysis. Crabs for both fatty acid and isotopic analyses were thawed, their carapaces removed and tissue from the cephalothorax (excluding the gut) collected ($n = 5$ in November; $n = 3$ in February).

Undisturbed surface sediments (first cm) were collected using 1.3 cm^2 plastic cores ($n = 5$ and 3 in November and February, respectively) and approximately 5 g of fresh sorted sediments; 'feeding pellets' from *M. longicarpus* were collected from the sediment surface using a clean spatula, which was rinsed with distilled water between replicate samples ($n = 5$ and 3 in November and February, respectively). Both types of sediment sample were transferred to plastic tubes, put on ice and frozen once back in the laboratory.

Additional samples of unsorted sediments ($n = 6$ at both sampling dates) were collected for the determination of the Chl*a* content, a common index for microphytobenthos biomass (e.g. Seuront & Spilmont 2002). Surface sediment was scraped with a clean spatula, placed in 8 mL acetone and stored in a cool box in darkness. Pigments were extracted for 24 h and Chl*a* contents were calculated as described by Seuront and Spilmont (2002), taking into account the dry weight of each sediment sample measured after 48 h at 60°C (results expressed in μg of Chl*a* per gram of dried sediment; μg g^{-1}).

Fatty acids analysis

Lipid extractions were conducted following a slightly modified version of the method of Bligh and Dyer (1959), as previously described by Meziane and Tsuchiya (2000). Briefly, lipids were extracted twice by homogenization of dried tissue/sediment samples for 2 min followed by 20 min of ultrasonication in 20 mL of distilled water : methanol : chloroform (1:2:1, v:v:v). Addition of 10 mL of a distilled water- : chloroform mixture (1:1, v:v) formed an aqueous-organic 2-layer system. The lipids migrated into the lower organic phase and separation was enhanced by centrifugation. The organic phase was collected, concentrated by rotary evaporation and dried under nitrogen. Extracts were saponified (2 h at 100°C) under reflux with 3 mL of 2 mol L^{-1} sodium hydroxide dissolved in a 2:1 (v:v) distilled water : methanol

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Table 1. Biomarkers (fatty acids and ratios of fatty acids) used to identify potential food sources

Biomarkers	Source	References
Branched 15:0 + 17:0, 18:1 (n-7)	Bacteria	Rajendran *et al.* 1993; Carrie *et al.* 1998; Meziane & Tsuchiya 2000
20:5 (n-3)	Diatoms	Canuel *et al.* 1995; Meziane & Tsuchiya 2000
22:6 (n-3)/20:5 (n-3) <1	Diatoms	Budge & Parrish 1998
16:1 (n-7)/16:0	Diatoms	Copeman & Parrish 2003
Long chain fatty acids, 18:2 (n-6), 18:3 (n-3)	Vascular plants	Budge & Parrish 1998; Meziane & Tsuchiya 2002; Dalsgaard *et al.* 2003; Meziane *et al.* 2007
18:2 (n-6), 18:3 (n-3)	Macrophytes	Kharlamenko *et al.* 1995; Kharlamenko *et al.* 2001

mixture. After acidification with ultra pure concentrated HCl, lipids were recovered by successive addition of 2 × 2 mL of chloroform. The pooled chloroform extracts were evaporated to dryness under a nitrogen stream and the fatty acids were converted to methyl esters under reflux for 10 min with 1 mL of 14% BF_3-methanol. After evaporation to dryness under a nitrogen stream, extracts were redissolved in chloroform : methanol (2:1, v:v).

Methyl ester fatty acids (FAMEs) were separated by high performance thin-layer chromatography using Merk plates coated with silica gel (Meziane & Tsuchiya 2002). The FAME containing bands were scraped-off and extracted from the powdered silica gel in a mixture of chloroform : methanol (2:1, v:v) and stored at –20°C until analysed by gas chromatography.

Methyl ester fatty acids were analysed on a Varian 2200 gas chromatograph (GC). The GC was equipped with a flame ionization detector (FID) and a Supelco Omegawax 320, fused-silica capillary column (30 m × 0.32 mm internal diameter, 0.25 μm film thickness). Nitrogen was used as the carrier gas. The injector temperature was set at 240°C and the detector temperature was held at 260°C. The initial column temperature was 60°C, which was held for 1 min, then increased to a final temperature of 240°C at a rate of 3°C min^{-1}; this final temperature was maintained for a further 10 min. The resulting peaks were identified by comparing their retention times with those of authentic FAME standards (Supelco Inc.). For some samples, lipid identifications were confirmed by GC-mass spectrometry (MS) (Varian Saturn 2200). Helium was used as the carrier gas for the MS; the column and the temperature programme for the GC were the same as for FID analysis.

The fatty acids profiles were examined with respect to the literature to identify biomarkers of potential food sources (Table 1).

Stable isotope analyses

Only animal muscular tissues (the most appropriate tissue for isotopic analysis of decapod crustaceans; Yokoyama *et al.* 2005) without carbonate were analysed. Frozen tissue samples were thawed, dried at 60°C for a minimum of 24 h and then ground to a fine powder using a mortar and pestle. The powder was then weighed into tin capsules, and oxidized at high temperature (EA3000 Eurovector Elemental Analyser) with analysis of the resultant CO_2 and N_2 in a continuous flow-isotope ratio mass spectrometer (Isoprime, GV Instruements). Ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were expressed as the relative difference (‰) between the sample and conventional standards (ANU sucrose for carbon and atmospheric N_2 for nitrogen) as:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000\ (‰) \qquad (1)$$

where $X = {}^{13}C$ or ^{15}N and $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$

Data analysis

Fatty acids data were analysed using the program PRIMER version 5 (Clarke & Warwick 2001). A cluster analysis was conducted based on the Bray-Curtis similarity coefficient (with no transformation applied to the data). Similarities between sampling events were investigated using the SIMPER function and statistical differences were determined using ANOSIM.

Non-parametric statistical tests (Sokal & Rohlf 1995) were used to assess the results from the fatty acid profiles and the stable isotope analysis because of the small size of the datasets, in which case they are more robust and powerful than parametric tests (e.g. Maxwell & Delaney 2004). Comparisons between fatty acid profiles and stable isotope analysis were conducted using the Kruskal-Wallis test (KW test hereafter) and the Mann–Whitney *U*-test (test between two samples, MW test hereafter). After the KW test, subsequent non-parametric multiple comparison procedures based on the Tukey test (Sokal & Rohlf 1995) were used to identify distinct groups of measurements.

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RESULTS

Fatty acids

The fatty acids compositions (%) of all the samples analysed in mid November 2005 and early February 2006 are presented in Table 2.

There was a high degree of within-group similarity for the fatty acids (FA) profiles for each of the sampling events (78–94% similarity, SIMPER analysis). A two-way crossed analysis of similarities showed that there was a significant difference in the FA profiles from different sample types (e.g. soldier crabs tissues, feeding pellets, etc; ANOSIM, $R = 0.808$, $P = 0.001$) and between the two different sampling periods (ANOSIM, $R = 0.744$, $P = 0.001$).

The cluster analysis (Fig. 2) clearly showed that *T. australiensis* tissue samples were the most dissimilar from all other groups (cluster I, 26–49% average dissimilarity, SIMPER analysis). The second cluster (cluster II) constituted soldier crab tissue samples and was separated from the third cluster (cluster III: surface sediments, and soldier crab feeding pellets and faeces samples) by an average dissimilarity of 21–34%. Within each cluster, samples also appeared to be separated by sampling date (November/February).

In all samples, the most abundant fatty acid was palmetic acid (16:0), which represented from 18.3% to 34.0% of total fatty acids (Table 2). The polyunsaturated 20:5 (n-3) made a similar contribution in *T. australiensis* tissue samples in November (MW test, $P > 0.05$). Palmitoleic acid (16:1 (n-7)) was the next most abundant fatty acid in all samples except the faeces of *M. longicarpus,* where palmitoleic acid concentrations were depleted and the saturated fatty acid 18:0 was the second most common fatty acid. Saturated fatty acids represented more than 50% of total FAMEs in all samples, except *T. australiensis* tissues in November, and maximally represented more than 60% of total fatty acids in the faeces of *M. longicarpus*.

Contributions of long chain fatty acids (number of carbons >24) which are characteristic of terrestrial vegetation were below detection limits in all samples in both February and November. Similarly, the polyunsaturated marker lipids for marine macrophytes, 18:2 (n-6) and 18:3 (n-3) were present at only low (0.2–2.3%) and relatively similar concentrations in the sediment during both sampling seasons.

The bacterial markers, 18:1 (n-7) and odd-branched FAs were detected in all samples with the exception of branched FAs in the faeces of *M. longicarpus*. The relative contribution of 18:1 (n-7) was not significantly different in surface sediments and the discarded, sorted sediments, which formed the feeding pellets of *M. longicarpus* on both sampling dates (MW test, $P > 0.05$). In November, the proportion of this bacterial marker was higher in animal tissues than in either surface sediment or *M. longicarpus* feeding pellets. In contrast, the reverse situation was observed in February (KW test and subsequent non-parametric multiple comparisons test $P < 0.05$). The low concentrations of monounsaturated 18:1 (n-7) in *M. longicarpus* faeces was also responsible for a large part of the dissimilarity between the tissues of *M. longicarpus* and its faeces (12% and 17% in November and February, respectively, SIMPER analysis). Regarding the other bacterial markers (odd-branched 15:0 and 17:0), their contribution was lower in *M. longicarpus* tissues and feeding pellets than in surface sediment samples on both sampling dates. The polyunsaturated fatty acid 20:5 (n-3), known as a marker of diatoms, was also present in all samples, with a particularly high contribution recorded in the tissues of *T. australiensis* in February. Its contribution to total FAs in surface sediment samples did not vary significantly between the two sampling dates (MW test, $P > 0.05$) and was higher than in the sorted sediments composing *M. longicarpus* feeding pellets during both periods (MW test, $P > 0.05$). The values of the ratio 16:1 (n-7)/16:0, which is also an indicator of microalgae, were similar in all the samples between the two sampling periods, except in *T. australiensis* tissues where it was significantly higher in November (MW test, $P > 0.05$). As previously mentioned, the concentration of 20:5 (n-3) was particularly high in *T. australiensis* tissues in November, whereas it made a lower but still important contribution in February being the third most abundant FA. This fatty acid was also the major contributor to the dissimilarity between *T. australiensis* tissue FA profiles and those of all other samples (SIMPER analysis) and was also largely responsible for the high contribution of polyunsaturated fatty acids (PUFAs) in the tissues of the yabbies collected in November. The values of the ratio 22:6 (n-3)/20:5 (n-3) were lower than 1 for all the samples at both sampling dates.

The polyunsaturated 20:5 (n-3) made a similar contribution to *M. longicarpus* tissues on both sampling dates and was responsible for 15% of the dissimilarity found between the soldier crab tissues and their faeces in November (SIMPER analysis).

Stable isotopes

The isotopic compositions of animal tissues are shown in Table 3. These $\delta^{13}C$ and $\delta^{15}N$ signatures did not vary significantly between the two sampling periods for either *T. australiensis* or *M. longicarpus* (MW test, $P > 0.05$). *Trypaea australiensis* tissues were isotopically depleted for carbon and enriched for nitrogen. In comparison, soldier crab tissues were isotopically more enriched in carbon and slightly more depleted for nitrogen than those of *T. australiensis*. In order to

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Table 2. Fatty acid composition (% of total FAs ± SD) of the tissues of *Trypaea australiensis* and *Mictyris longicarpus*, the faeces and feeding pellet of *M. longicarpus*, and the surface sediment collected in November 2005 and February 2006

	T. australiensis				*M. longicarpus*				Surface sediment	
	Tissues		Tissues		Faeces		Feeding pellets			
Fatty acids	November (n = 3)	February (n = 3)	November (n = 5)	February (n = 3)	November (n = 1)	February (n = 1)	November (n = 5)	February (n = 3)	November (n = 5)	February (n = 3)
14:0	8.7 ± 0.6	14.1 ± 1.3	3.4 ± 0.3	3.5 ± 1.5	5.5	4.8	2.6 ± 0.3	6.1 ± 2.1	3.6 ± 1.1	5.0 ± 0.8
15:0	0.8 ± 0.1	1.5 ± 0.1	3.3 ± 0.6	3.8 ± 2.0	1.6	2.4	1.3 ± 0.2	2.0 ± 0.5	1.9 ± 0.7	1.1 ± 0.9
16:0	18.3 ± 1.5	28.9 ± 1.7	33.0 ± 2.0	34.0 ± 8.3	32.7	28.9	29.4 ± 2.3	31.8 ± 4.0	29.4 ± 2.5	32.2 ± 2.5
17:0	1.1 ± 0.1	1.5 ± 0.1	1.8 ± 0.2	2.2 ± 0.5	1.0	1.2	0.9 ± 0.1	0.7 ± 0.8	0.8 ± 0.2	0.6 ± 0.5
18:0	3.0 ± 0.6	4.3 ± 0.2	10.2 ± 2.8	7.7 ± 6.8	25.1	22.2	20.1 ± 7.0	14.3 ± 8.6	14.5 ± 5.6	13.6 ± 4.0
20:0	–	0.6 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	1.5	1.0	0.8 ± 0.2	0.7 ± 0.6	0.7 ± 0.5	0.7 ± 0.6
22:0	–	–	–	0.1 ± 0.2	–	1.6	–	–	–	–
15:0 iso	0.4 ± 0.0	0.8 ± 0.0	–	0.4 ± 0.4	–	–	–	0.6 ± 1.1	0.4 ± 0.4	0.5 ± 0.4
15:0 anteiso	–	–	–	0.1 ± 0.2	–	–	–	–	0.1 ± 0.2	1.5 ± 0.0
16:0 iso	–	–	1.0 ± 0.4	0.4 ± 0.8	–	–	1.9 ± 0.6	0.5 ± 0.8	1.4 ± 0.9	0.8 ± 0.7
17:0 iso	1.0 ± 0.0	1.2 ± 0.2	0.2 ± 0.4	0.8 ± 0.5	–	–	–	–	0.5 ± 0.5	–
18:0 iso	–	–	–	–	–	–	1.3 ± 0.4	–	1.1 ± 0.7	–
15:1	–	–	0.6 ± 0.6	–	–	–	1.6 ± 0.5	1.0 ± 0.9	1.1 ± 0.8	0.8 ± 0.7
16:1	0.6 ± 0.0	0.7 ± 0.1	–	0.4 ± 0.0	–	–	0.1 ± 0.3	–	0.6 ± 0.4	–
16:1 (n-7)	11.8 ± 0.2	14.7 ± 0.4	13.6 ± 1.4	18.1 ± 3.6	6.6	6.6	12.7 ± 4.5	13.7 ± 0.3	17.5 ± 4.2	18.5 ± 1.4
17:1	1.6 ± 0.5	2.3 ± 0.6	7.0 ± 1.7	6.2 ± 0.8	1.2	–	0.8 ± 0.1	0.7 ± 0.7	1.1 ± 0.4	0.6 ± 0.5
18:1 (n-9)	5.7 ± 0.2	5.4 ± 0.4	4.3 ± 0.4	3.6 ± 2.1	4.9	8.3	8.9 ± 1.7	6.6 ± 2.4	5.7 ± 2.2	5.3 ± 0.3
18:1 (n-7)	3.8 ± 0.4	4.2 ± 0.4	3.9 ± 0.6	2.8 ± 0.2	2.6	5.7	2.7 ± 0.1	8.9 ± 2.2	2.7 ± 0.5	7.6 ± 2.4
20:1	–	2.8 ± 4.4	0.6 ± 0.4	0.3 ± 0.3	1.1	–	0.7 ± 0.4	3.4 ± 0.8	0.9 ± 0.7	1.8 ± 1.6
20:2	–	–	0.1 ± 0.3	–	–	–	0.3 ± 0.6	–	–	–
18:2 (n-6)	2.3 ± 0.2	1.9 ± 0.2	1.9 ± 0.4	1.6 ± 0.7	0.8	1.3	1.1 ± 0.4	0.3 ± 0.5	0.7 ± 0.5	0.6 ± 0.5
22:2	0.6 ± 0.1	–	–	–	–	–	–	–	–	–
18:3 (n-6)	0.6 ± 0.0	0.2 ± 0.3	0.3 ± 0.4	0.1 ± 0.2	–	–	0.6 ± 0.5	0.3 ± 0.5	0.4 ± 0.5	–
18:3 (n-3)	2.5 ± 0.6	1.8 ± 0.1	1.0 ± 0.2	0.6 ± 0.2	0.8	–	1.2 ± 1.3	0.7 ± 0.6	1.0 ± 0.2	0.6 ± 0.5
20:3 (n-3)	–	–	–	–	–	2.7	–	–	0.2 ± 0.6	–
20:3 (n-6)	2.5 ± 0.6	–	–	0.3 ± 0.3	–	–	–	–	–	–
18:4 (n-3)	4.0 ± 0.6	2.1 ± 0.1	0.5 ± 0.4	0.3 ± 0.3	–	–	–	1.0 ± 0.9	0.1 ± 0.3	–
20:4 (n-6)	–	1.2 ± 0.2	5.5 ± 1.5	5.0 ± 3.0	–	4.2	1.2 ± 0.4	2.0 ± 0.9	2.0 ± 0.6	1.8 ± 0.3
20:5 (n-3)	18.7 ± 1.3	6.8 ± 2.1	4.3 ± 1.3	3.9 ± 1.5	3.3	3.3	1.6 ± 0.6	2.7 ± 2.5	3.2 ± 1.7	4.5 ± 0.9
22:5 (n-3)	0.5 ± 0.0	–	1.1 ± 0.3	1.6 ± 1.0	9.7	1.2	7.1 ± 1.3	–	7.1 ± 2.2	–
22:6 (n-3)	10.7 ± 1.5	2.9 ± 2.6	1.8 ± 0.6	0.6 ± 0.2	1.4	1.7	–	2.0 ± 0.9	0.3 ± 0.6	2.1 ± 0.6
Not identified	0.8 ± 0.3	0.2 ± 0.4	–	0.6 ± 0.6		2.8	0.9 ± 0.6	–	0.9 ± 0.6	–
Total %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Σ Saturated FAs (SAFAs)	31.9 ± 2.9	50.9 ± 2.6	52.4 ± 3.1	52.0 ± 4.9	67.5	62.1	55.2 ± 6.7	55.6 ± 6.5	51.0 ± 5.9	53.0 ± 6.4
Σ Branched FAs (BFAs)	1.5 ± 0.1	2.0 ± 0.2	1.2 ± 0.5	1.8 ± 0.1	0.0	0.0	3.2 ± 0.9	1.1 ± 1.0	3.4 ± 1.5	2.8 ± 0.5
Σ Monounsaturated FAs (MUFAs)	23.4 ± 0.8	27.2 ± 0.8	29.9 ± 1.6	31.4 ± 3.3	16.4	20.7	27.5 ± 4.6	34.4 ± 6.1	29.6 ± 4.2	34.5 ± 5.2
Σ Polyunsaturated FAs (PUFAs)	42.4 ± 3.5	19.7 ± 2.4	16.5 ± 3.9	14.2 ± 6.5	16.1	14.5	13.0 ± 2.7	8.9 ± 3.5	15.1 ± 2.6	9.6 ± 0.2
Σ Long chain FAs (LCFAs)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ branched 15:0 + 17:0	1.5 ± 0.1	2.0 ± 0.2	0.2 ± 0.4	1.3 ± 0.8	0.0	0	0.0	0.6 ± 1.1	1.0 ± 0.3	2.0 ± 0.5
22:6 (n-3)/20:5 (n-3)	0.6 ± 0.1	0.4 ± 0.3	0.4 ± 0.0	0.2 ± 0.1	0.4	0.5		0.5 ± 0.2	0.1 ± 0.1	0.5 ± 0.1
16:1 (n-7)/16:0	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.2	0.2	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.6 ± 0.1

'–': absence or traces. BFA, branched fatty acids; FA, fatty acids; LCFA, long chain fatty acids typical markers of vascular plants; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids.

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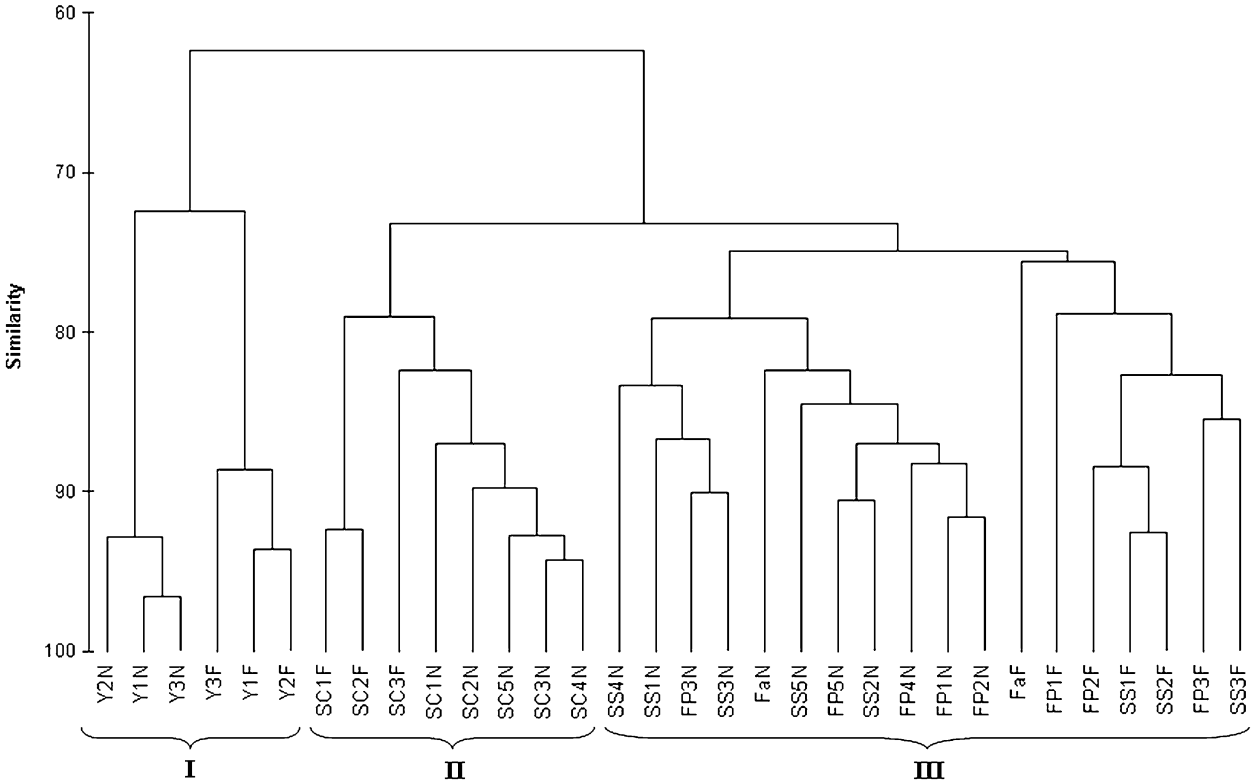

Fig. 2. Hierarchical classification (Bray-Curtis similarity coefficient) of samples (Y: tissues from the yabby *Trypaea australiensis*; SC: tissues from the soldier crab *Mictyris longicarpus*; FP: feeding pellets from *M. longicarpus*; Fa: faeces from *M. longicarpus*; SS: surface sediment) collected in November 2005 (N) and February 2006 (F).

Table 3. δ13C and δ15N (mean ± SD, ‰) of the tissues of *Trypaea australiensis* and *Mictyris longicarpus* individuals collected in November 2005 and February 2006

Sample	δ^{13}C	δ^{15}N	*n*
T. australiensis November	–22.1 ± 0.4	7.8 ± 0.5	3
T. australiensis February	–21.0 ± 1.6	8.7 ± 0.8	2
M. longicarpus November	–14.1 ± 0.7	6.3 ± 0.9	3
M. longicarpus February	–14.2 ± 0.6	5.9 ± 1.0	3

identify the potential food sources of each species, trophic enrichments of 1.0‰ for δ^{13}C and 3.4‰ for δ^{15}N were used (Vander Zanden & Rasmussen 2001; Post 2002). Using this assumption, the theoretical average isotopic signatures of the food sources would be –22.7 ± 1.0‰ (δ^{13}C) and 4.8 ± 0.7‰ (δ^{15}N) for *T. australiensis*, and –15.2 ± 0.6‰ (δ^{13}C) and 2.7 ± 0.9‰ (δ^{15}N) for *M. longicarpus*.

DISCUSSION

The relative contributions to the FA profiles, in particular in surface sediments, of typical markers of vascular plants (LCFAs) were nil in all the samples, indicating that the study site was not under any terrestrial influence. Moreover, although fairly extensive seagrass beds are present in the subtidal channels surrounding the studied sandbank (McClennan & Sumpton 2005), the low contribution of their most abundant fatty acids to the fatty acid profiles of the surface sediments indicate that seagrass detritus did not contribute to the sedimentary pools of organic matter of the sandbank.

The surface sediment of the study site exhibited similar fatty acids profiles at both sampling dates (79% similarity), which indicated that the food sources available to the studied invertebrates were similar in November and February. The contributions of marker fatty acids, branched 15:0 and 17:0 and 18:1 (n-7), and 20:5 (n-3) (Table 1) indicated that bacteria and diatoms respectively were the principle food web basis. In the case of diatoms, which usually dominate intertidal microphytobenthos populations (MacIntyre *et al.* 1996), this was corroborated by the absence of change in the Chlorophyll *a* content of the surface sediment (1.34 ± 0.27 μg g^{-1} and 1.28 ± 0.25 μg g^{-1} in November and February, respectively; MW test, $P > 0.05$).

Cluster analysis (Fig. 2) showed that the FA profiles obtained from the tissues of *T. australiensis* (Cluster I) were highly distinct from those of the surface sediment

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(Cluster III). In contrast, those obtained from the tissues of *M. longicarpus* (Cluster II) were more similar to the FA profiles of the surface sediment, suggesting that the soldier crab probably fed on most of the food sources available within the surface sediment of the study site, whereas the yabbies were more selective.

Food sources of *Trypaea australiensis*

The dominance of the diatom-specific marker, 20:5 (n-3) to the FA profiles of the shrimps clearly indicates that diatoms were the predominant food source for *T. australiensis*. In contrast, in November the polyunsaturated 22:6 (n-3) also made a high contribution to the FA composition of *T. australiensis* tissues. This FA has been interpreted as being an indicator of the presence of dinoflagellates in the diet of marine invertebrates in some studies (Bachok *et al.* 2003; Alfaro *et al.* 2006). However, the ratio of 22:6 (n-3)/20:5 (n-3) is thought to be a much more suitable index to discriminate between dietary contributions of diatoms and dinoflagellates in marine environments (Budge & Parrish 1998). Diatoms produce 22:6 (n-3) at much lower levels than 20:5 (n-3) in contrast to dinoflagellates (Budge & Parrish 1998). Ratios between 22:6 (n-3) and 20:5 (n-3) smaller and greater than 1 are thus typical of diatoms and dinoflagellates, respectively (Budge & Parrish 1998). In the present study, this ratio was 0.6 in November and 0.4 in February, indicating that diatoms were always the primary source for *T. australiensis*.

The natural abundance isotopic signatures obtained from the tissues of *T. australiensis* did not vary significantly between the two sampling dates, suggesting that the diet of the shrimp was similar. Although the isotopic signature of diatoms was not measured at the study site, the estimated signature of the potential food source of *T. australiensis* of -22.7 ± 1.0‰ for $\delta^{13}C$ and 4.8 ± 0.7‰ for $\delta^{15}N$ matches with those previously obtained for microphytobenthos collected at nearby sites in Moreton Bay (Melville & Connolly 2003; Guest *et al.* 2004). Furthermore, the $\delta^{15}N$ signature of the shrimps indicates that this food source was directly ingested by the shrimps without any trophic intermediate. These results together with the cluster analysis (Fig. 2) suggest that during feeding *T. australiensis* actively selected diatoms from among the potential food sources that were available within the surface sediments.

However, the observed difference in the contribution of the diatom specific marker lipid 20:5 (n-3) to the FAs profiles of *T. australiensis* tissues, as well as the difference in the value of the ratio of 16:1 (n-7)/16:0 between November and February cannot be explained by changes in diatom availability at the sediment surface, as FAs profiles were similar in both seasons, as were Chl*a* contents. Because this difference in the FAs profiles of *T. australiensis* does not appear to be linked to changes in the abundance of diatoms which represented their main food source, an alternative mechanism may be involved. Paterson and Thorne (1995) have demonstrated experimentally that *T. australiensis* reduced its metabolic rate under anoxic conditions, which for instance explained the absence of reproduction in the species during summer (McPhee & Skilleter 2002). At our site enhanced microbial respiration in the sediment associated with high summer temperatures during February might have resulted in the establishment of anoxic conditions within the shrimp burrows, especially during periods of emersion when the shrimps are unable to irrigate their burrows. The consequent reduction in metabolic rates may have caused the shrimps to reduce their feeding activity, which in turn could explain the lower contributions of the diatom lipid biomarkers to the tissues of *T. australiensis* in February compared with November, despite the similar availability of this food source during both periods.

Our conclusion that *T. australiensis* fed primarily on benthic diatoms contrasts strongly with the results of Boon *et al.* (1997). Based on natural abundance ^{13}C and ^{15}N signatures, they considered mangrove leaves and seagrasses as being the main component of the yabbies diet in Western Port (Victoria, Australia), where these plants were dominant. At our study site sediment lipid profiles demonstrated that mangrove detritus was absent or present at negligible levels as the LCFAs typical of mangrove and terrestrial vegetation were never detected in sediment or animal tissue samples. Seagrass was present close to the studied sandbank and the seagrass markers, polyunsaturated fatty acids 18:2 (n-6) and 18:3 (n-3), were present at low levels in the sediment and *T. australiensis* tissues. There was, however, little evidence from either FA or isotopic analyses that the shrimps preferentially selected seagrass biomass from the sediment, or that *T. australiensis* accumulated seagrass debris in its burrow chamber and used it as a nutritional source, as previously described for other thallassinidean shrimps (*Corallianassa longiventris* and *Pestrella tyrrhena*: Abed-Navandi & Dworschak 2005; Abed-Navandi *et al.* 2005). These shrimps may thus have a plastic feeding strategy making them able to adapt to the local availability of potential food sources and this may go a long way to explaining the widespread distribution of *T. australiensis* over a range of ecosystem types along the Australian coastline. However, complementary experiments, for example, employing isotopically labelled food choice assays would be useful to test the dietary preference of *T. australiensis* in systems where the potential organic matter sources are numerous (microalgae, mangrove leaves and detritus, seagrasses) and their availability variable through the year (e.g.

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seasonal cycle of the abundance and composition of the litter in mangroves).

Food sources of *Mictyris longicarpus*

On both sampling dates, the sediments which composed the feeding pellets of *M. longicarpus* contained relatively higher quantities of saturated fatty acids (SAFAs) and lower contributions of PUFAs than the surface sediments at the study site. This indicated that the quality of the sediment organic matter pool was modified by the soldier crabs during their feeding activity. These modifications suggest that *M. longicarpus* actively selected organic matter types from the ingested sediment, as previously shown for other intertidal crab species (Meziane *et al.* 2002).

The significant decrease observed in the proportion of bacterial markers, branched 15:0 and 17:0 FAs between the surface sediment and the feeding pellets is consistent with previous evidence that *M. longicarpus* actively ingested bacteria (Quinn 1986). In addition, although tissue levels of these bacterial marker fatty acids were not high, the absence of the marker FAs in the crab's faeces during both seasons suggests that bacteria were well assimilated. Therefore, the relatively low tissue levels of branched 15:0 and 17:0 may be more indicative of their rapid turnover in the crab tissues rather than poor assimilation. However, it is not possible to definitively conclude that the crabs actively selected bacteria from the food sources available in the sediments, as ingestion of bacteria may simply result from the bacteria preferentially colonizing organic particles which were then selected by the crabs.

The relative contribution of the FA marker of diatoms (polyunsaturated 20:5 (n-3)) in the soldier crabs feeding pellets was considerably lower than that in the surface sediment on both sampling dates. This indicates that *M. longicarpus* actively sorted, selected and ingested diatoms after the initial uptake of surface sediment. Although direct counting was not performed here, the proportion of 20:5 (n-3) in the tissues of the soldier crab indicates that diatoms were assimilated. This result is congruent with previous gut content analysis (Cameron 1966) and observed differences in the Chl*a* concentrations between 'grazed' sediment and enclosures from which soldier crabs were excluded for 8 weeks (Webb & Eyre 2004b). However, the similar proportions of 20:5 (n-3) found in the faeces and in the crab tissues, suggest that the ingested diatoms were not completely utilized by the crabs. The high proportion of the monounsaturated 18:1 (n-7) (found in high concentrations in most micro-organisms in general; e.g. Kharlamenko *et al.* 1995) in the tissues of *M. longicarpus* supports our conclusion that bacteria and diatoms were dominant components in the diet of soldier crabs.

As for *T. australiensis*, the isotopic signatures of the tissues of *M. longicarpus* indicated that its diet did not change between the two sampling periods. However, the $\delta^{13}C$ and $\delta^{15}N$ values obtained for the crab were distinct from those of the shrimps, which as discussed above appeared to feed selectively on microphytobenthos and particularly diatoms. These discrepancies could be ascribed to the presence of bacteria in the crab's diet, to the occurrence of an intermediate between the base (constituted by diatoms and bacteria) and *M. longicarpus*, and/or by a contribution of seagrass to their diet. This indicates that consumption of primary consumers such as meiofauna could be partly responsible for the isotopic signatures obtained for the crabs, with the meiofauna representing an intermediate link between the crabs and the microorganisms (i.e. diatoms and bacteria). This predation on meiofauna is supported by the results of the impacts of soldier crab foraging on benthic meiofauna populations (Dittmann 1993), even if such indirect assessments of predation are subject to artefacts such as the intense sediment disturbance caused by the crabs leading to the death of some meiofauna (Warwick *et al.* 1990). The enriched isotope ratio of the crab tissues implies that a seagrass contribution to the crabs' food web was very likely. However, because the markers of the seagrass exhibited a relatively low contributions to the FA profiles in all the samples, this would imply that either the bacteria, on which the crabs feed actively, are obtaining their carbon from seagrass organic matter or that the seagrass degraded detritus present in the sediment and ingested by the crabs was qualitatively different from a fresh seagrass material in terms of fatty acids composition (i.e. less polyunsaturated FAs), as a result of differential microbial degradation. These mechanisms either separately or in tandem would explain the concomitant, but opposite results of enriched carbon isotope ratio (i.e. major contribution of seagrasses in the crab diet) with the low contribution of the seagrass markers in the crab tissues (i.e. minor contribution of these plants to the crab diet). Moreover, the selection of degraded seagrass detritus and its associated bacteria during sediment sorting would also at least partially explain the decrease observed in the contribution of bacterial marker FAs to the FA profiles of the surface sediment and the sorted sediments which comprise the feeding pellets.

Overall, the results on lipid biomarkers and natural abundance isotopes indicate that *M. longicarpus* is an indiscriminate feeder exploiting several sources of organic matter in the surface sediments. These sources include microphytobenthos (primarily diatoms at our study site), bacteria, probably meiofauna and also at least some organic detritus, as cluster analysis revealed a high degree of similarity between the fatty acids profiles of the crab tissues and the surface sediments

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on which they feed. This conclusion is consistent with the proposed feeding mechanism of the crabs, where sediment is scooped into the buccal cavity and mixed with water, allowing lighter food particles to be separated and harvested by specific gravity, with heavier sediment particles being ejected as pseudofaecal feeding pellets (Cameron 1966; Quinn 1986).

CONCLUSION

The present study indicated that, as previously described, the base of the diet of *M. longicarpus* was mainly composed of minute organisms, that is, bacteria and diatoms, and that meiofauna could partly represent a trophic intermediate between diatoms and the soldier crab. At our study site, *T. australiensis* fed primarily on microphytobenthos; consequently, there was a potential competition between the two organisms for the same food source (diatoms). However, because of their feeding behaviour, this competition would be limited in space, as *M. longicarpus* explores large areas during feeding, whereas *T. australiensis* collects sediment from a restricted area surrounding its burrow.

In terms of energy flow through the ecosystem, the autochthonous primary production can be exported to adjacent areas by predators such as fish and birds which feed on both crustaceans via one or two intermediates whether predators feed on yabbies or soldier crabs (if we consider that meiofauna was an intermediate between diatoms and *M. longicarpus*). Thus, in both cases, the transfers of matter and energy are rapid and make the functioning of this system very singular.

Overall, these results show how the combined fatty acid and stable isotope approach can be helpful in situations such as the one faced here, that is, attempting to resolve the nature of food assimilated by invertebrates potentially feeding on several important food sources. These procedures, combined together, are promising in terms of resolving the nature of the sources in freshwater, estuarine and coastal food webs that have hitherto proved difficult to understand.

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REFERENCES

Abed-Navandi D. & Dworschak P. C. (2005) Food sources of tropical thalassinidean shrimps: a stable-isotope study. *Mar. Ecol. Prog. Ser.* **291,** 159–68.

Abed-Navandi D., Koller H. & Dworschak P. C. (2005) Nutritional ecology of thalassinidean shrimps constructing burrows with debris chambers: the distribution and use of macronutrients and micronutrients. *Mar. Biol. Res.* **1,** 202–15.

Alfaro A. C., Thomas F., Sergent L. & Duxbury M. (2006) Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuar. Coast. Shelf Sci.* **70,** 271–86.

Atkinson R. J. A. & Taylor A. C. (2005) Aspects of the physiology, biology and ecology of Thallassinidean shrimps in relation to their burrow environment. *Oceanogr. Mar. Biol. Ann. Rev.* **43,** 173–210.

Bachok Z., Mfilinge P. L. & Tsuchiya M. (2003) The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. *J. Exp. Mar. Biol. Ecol.* **292,** 187–97.

Berg P., Rysgaard S., Funch P. & Sejr M. K. (2001) Effects of bioturbation on solids and solutes in marine sediments. *Aquat. Microb. Ecol.* **26,** 81–94.

Bligh E. G. & Dyer W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37,** 911–17.

Boon P. I., Bird F. L. & Bunn S. E. (1997) Diet of the intertidal callianassid shrimps *Biffarus arenosus* and *Trypaea australiensis* (Decapoda: Thalassinidea) in Western Port (southern Australia), determined with multiple stable-isotope analyses. *Mar. Freshwater Res.* **48,** 503–11.

Budge S. M. & Parrish C. C. (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. *Org. Geochem.* **29,** 1547–59.

Cameron A. M. (1966) Some aspects of the behaviour of the soldier crab, *Mictyris longicarpus*. *Pac. Sci.* **20,** 224–34.

Canuel E. A., Cloern J. E., Ringelberg D. B., Guckert J. B. & Rau G. H. (1995) Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. *Limnol. Oceanogr.* **40,** 67–81.

Carrie R. H., Mitchell L. & Black K. D. (1998) Fatty acids in surface sediment at the Hebridean shelf edge, west of Scotland. *Org. Geochem.* **29,** 1583–93.

Clarke K. R. & Warwick R. M. (2001) *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 2nd edn. PRIMER-E, Plymouth.

Contessa L. & Bird F. L. (2004) The impact of bait-pumping on populations of the ghost shrimp *Trypaea australiensis* Dana (Decapoda: Callianassidae) and the sediment environment. *J. Exp. Mar. Biol. Ecol.* **304,** 75–97.

Cook P. L. M., Revill A. T., Clementson L. A. & Volkman J. K. (2004) Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. III. Sources of organic matter. *Mar. Ecol. Prog. Ser.* **280,** 55–72.

Copeman L. A. & Parrish C. C. (2003) Marine lipids in a cold coastal ecosystem: Gilbert Bay, Mabrador. *Mar. Biol.* **143,** 1213–27.

Dalsgaard J., St John M., Kattner G., Muller-Navarra D. & Hagen W. (2003) Fatty acid trophic markers in the pelagic marine environment. *Adv. Mar. Biol.* **46,** 225–340.

doi:10.1111/j.1442-9993.2009.01994.x

Dittmann S. (1993) Impact of foraging soldiercrabs (Decapoda: Myctiridaea) on meiofauna in a tropical tidal flat. *Rev. Biol. Trop.* **41,** 627–37.

Dittmann S. (1998) Behaviour and population structure of soldier crabs *Mictyris longicarpus* (Latreille): observations from a tidal flat in tropical north Queensland, Australia. *Senckenb. Marit.* **28,** 177–84.

Guest M. A., Connolly R. M. & Loneragan N. R. (2004) Carbon movement and assimilation by invertebrates in estuarine habitats at a scale of metres. *Mar. Ecol. Prog. Ser.* **278,** 27–34.

Kharlamenko V. I., Zhukova N. V., Khotimchenko S. V., Svetashev V. I. & Kamanev G. M. (1995) Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). *Mar. Ecol. Prog. Ser.* **120,** 231–41.

Kharlamenko V. I., Kiyashko S. I., Imbs A. B. & Vyshkvartzev D. I. (2001) Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulphur isotope ratio and fatty acid analyses. *Mar. Ecol. Prog. Ser.* **220,** 103–17.

McClennan M. & Sumpton W. (2005) The distribution of seagrasses and the viability of seagrass transplanting in the Broadwater, Gold Coast, Queensland. *Proc. R. Soc. Queensl.* **112,** 31–8.

MacIntyre H. L., Geider R. J. & Miller D. C. (1996) Microphytobenthos: the ecological role of the 'secret garden' of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries* **19,** 186–201.

McPhee D. P. & Skilleter G. A. (2002) Aspects of the biology of the yabby *Trypaea australiensis* (Dana) (Decapoda: Thalassinidea) and the potential of burrow counts as an indirect measure of population density. *Hydrobiologia* **485,** 133–41.

Maxwell S. E. & Delaney H. D. (2004) *Designing Experiments and Analysing Data: A Model Comparison Perspective*, 2nd edn. Lawrence Erlbaum Associates, Mahwah.

Melville A. J. & Connolly R. M. (2003) Spatial analysis of stable isotope data to determine primary sources of nutrition for fish. *Oecologia* **136,** 499–507.

Meziane T. & Tsuchiya M. (2000) Fatty acids as tracers of organic matter in the sediment and web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. *Mar. Ecol. Prog. Ser.* **200,** 49–57.

Meziane T. & Tsuchiya M. (2002) Organic matter in a subtropical mangrove-estuary subjected to wastewater discharge: origin and utilisation by two macrozoobenthic species. *J. Sea Res.* **47,** 1–11.

Meziane T., Sanabe M. C. & Tsuchiya M. (2002) Role of fiddler crabs of a subtropical intertidal flat on the fate of sedimentary fatty acids. *J. Exp. Mar. Biol. Ecol.* **270,** 191–201.

Meziane T., Lee S. Y., Mfilinge P. L., Shin P. K. S., Lam M. H. W. & Tsuchiya M. (2007) Inter-specific and geographical variations in the fatty acid composition of mangrove leaves: implications for using fatty acids as a taxonomic tool and tracers of organic matter. *Mar. Biol.* **150,** 1103–13.

Paterson B. D. & Thorne M. J. (1995) Measurements of oxygen uptake, heart and gill bailer rates of the callianassid burrowing shrimp *Trypaea australiensis* Dana and its response to low oxygen tensions. *J. Exp. Mar. Biol. Ecol.* **194,** 39–52.

Post D. M. (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* **83,** 703–18.

Quinn R. H. (1986) Experimental studies of food ingestion and assimilation of the soldier crab, *Mictyris longicarpus* Latreille (Decapoda, Mictyridae). *J. Exp. Mar. Biol. Ecol.* **102,** 167–81.

Rajendran N., Suwa Y. & Urushigawa Y. (1993) Distribution of phospholipid ester-linked fatty acids biomarkers for bacteria in the sediment of Ise Bay. *Mar. Chem.* **43,** 39–56.

Sandnes J., Forbes T., Hansen R., Sandnes B. & Rygg B. (2000) Bioturbation and irrigation in natural sediments described by animal-community parameters. *Mar. Ecol. Prog. Ser.* **197,** 169–79.

Seuront L. & Spilmont N. (2002) Self-organized criticality in intertidal microphytobenthos patch patterns. *Physica A* **313,** 513–39.

Sokal R. R. & Rohlf F. J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman and Co Ltd, New York.

Stapleton K. L., Long M. & Bird F. L. (2001) Comparative feeding ecology of two spatially coexisting species of ghost shrimp, *Biffarus arenosus* and *Trypaea australiensis* (Decapoda: Callianassidae). *Ophelia* **55,** 141–50.

Vander Zanden M. J. & Rasmussen J. B. (2001) Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. *Limnol. Oceanogr.* **46,** 2061–6.

Warwick R. M., Clarke K. R. & Gee J. M. (1990) The effect of disturbance by soldier crabs *Mictyris longicarpus* H. Milne Edwards on meiobenthic community structure. *J. Exp. Mar. Biol. Ecol.* **135,** 19–33.

Webb A. P. & Eyre B. D. (2004a) Effect of natural populations of burrowing thalassinidean shrimp on sediment irrigation, benthic metabolism, nutrient fluxes and denitrification. *Mar. Ecol. Prog. Ser.* **268,** 205–20.

Webb A. P. & Eyre B. D. (2004b) The effect of natural populations of the burrowing and grazing soldier crab (*Mictyris longicarpus*) on sediment irrigation, benthic metabolism and nitrogen fluxes. *J. Exp. Mar. Biol. Ecol.* **309,** 1–19.

Welsh D. T. (2003) It's a dirty job but someone has to do it: the role of marine benthic macrofauna in organic matter turnover and nutrient recycling to the water column. *Chem. Ecol.* **19,** 321–42.

Yokoyama H., Tamaki A., Harada K., Shimoda K., Koyama K. & Ishihi Y. (2005) Variability of diet-tissue isotopic fractionation in estuarine macrobenthos. *Mar. Ecol. Prog. Ser.* **296,** 115–28.

Zharikov Y. & Skilleter G. A. (2004a) A relationship between prey density and territory size in non-breeding Eastern Curlews *Numenius madagascariensis*. *Ibis* **146,** 518–21.

Zharikov Y. & Skilleter G. A. (2004b) Why do eastern curlews *Numenius madagascariensis* feed on prey that lowers intake rate before migration? *J. Avian Biol.* **35,** 533–42.

doi:10.1111/j.1442-9993.2009.01994.x