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Using biochemical and isotopic tracers to characterize organic matter sources and their Incorporation into Estuarine food webs (Rufiji Delta, Tanzania)

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Abstract (Max 200 words)

Fatty acid biomarkers and stable isotope signatures were used to identify the sources of particulate and sedimentary organic matter and its input into the food web through the dominant consumer within the mangrove dominated Rufiji estuary, Tanzania. Specific fatty acids were used to identify the preferred basal sources of dominant fauna (i.e., filter feeder bivalves, snails, crabs, shrimps and three fish species), and their presence in the water and sediment samples in the estuary. Both fatty acid and stable isotope results revealed that food web in the Rufiji estuary depended on a variety of carbon sources (mangroves, allochthonous terrestrial inputs, macroalgae, and phytoplankton), contributing to a different degree into the diets of primary consumers and members of near-shore fish, but none of them were obligatory for the survival of these species. The $\delta^{15}\text{N}$ values of major primary producers and consumers/predators revealed a trend for $\delta^{15}\text{N}$ enrichment with increasing trophic level. The ratio of docosahexaenoic acid to eicosapentaenoic acid (DHA:EPA) decreased from pelagic to benthic feeding fish. This indicated that fish with different feeding modes derived their fatty acids from different primary sources of nutrition, and suggested that the DHA:EPA ratio may be a useful indicator of feeding mode.

Key words: Fatty acid, stable isotopes, organic matter, food web, Rufiji estuary.

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1. Introduction

Estuaries have the potential to play an important role in the global carbon cycle through the exchange and modification of materials transported from land to coastal waters [1,2]. The organic matter (OM) pool in these systems comprises a spectrum of dissolved, colloidal and particulate material originating from a variety of sources. Foremost among these are terrestrial river-borne compounds, including those produced in the river itself or originating from the surrounding watershed, deriving from atmospheric fall out, industrial and municipal discharges, and marine-derived OM from adjacent coastal waters. The relative importance of these sources varies between and within estuaries [3–6]. Moreover, the composition and reactivity of OM delivered from different sources may also vary significantly, and consequently influence a number of key environmental processes such as the supply of food to benthic and pelagic organisms, water chemistry, sediment cohesiveness and stability, and the distribution of contaminants [4]. Generally terrigenous OM derived from terrestrial vegetation is more refractory and has a longer retention time in an estuary than planktonic OM [7]. However, in cases where terrigenous discharges are influenced by wastewater and other anthropogenic sources, OM tends to be more labile and enriched in increasing bacterial population associated with high organic loading [8].

Characterization of the source inputs, chemical nature, and OM reactivity in estuaries has been achieved through application of different approaches involving biological markers. For example, considerable attention has been focused on the utilization of stable isotopes as a tool for inferring their sources and following pathways of OM cycling in aquatic systems [9–13]. The stable isotope (C,N) signals have been coupled with organic C/N elemental ratios to provide additional resolution and understanding of ongoing biogeochemical processes. The use of these tracers relies on the existence of gross differences between respective OM pools and the general enrichment of marine derived organic material in ^{13}C , ^{15}N and elemental nitrogen relative to terrigenous OM [10,14]. The use of this approach is based on the assumptions that OM isotopic and C/N ratios are conservative, and that their distributions in natural systems reflect only physical mixing of material from compositionally distinct end-member sources. However, isotope-based source assignments for OM are frequently confounded by potential modification of end-member signatures by biogeochemical processes, which are known to alter the isotopic and elemental composition of OM pools in estuaries [5,10,15].

Fatty acids also can be used reliably to distinguish OM sources within complex aquatic ecosystems. Because of their biological specificity, these fatty acids have been used to trace the origin and trajectory of OM in the ecosystems by distinguishing: autochthonous primary producers (e.g., dinoflagellates, diatoms); OM of primary (e.g., algal) and secondary (e.g., zooplankton, bacteria) trophic levels, and relative importance of terrigenous sources along the estuarine continuum [10,16,17]. However, these biomarkers have limitations too. For example, some fatty acid markers may be metabolized and transformed once consumed by the animal, and only their relative rather than absolute amounts can be measured.

While both the above approaches (fatty acids and stable isotopes) have limitations, the combination of these techniques has significantly improved the source determination of OM in aquatic systems [10,18]. In this study, we used fatty acids and stable isotopes (^{13}C and ^{15}N) to investigate the origin of particulate and sedimentary OM, and its fate into the food web in a mangrove estuary. This information is then used to gain insights into the trophic position and feeding relationships of three most common and important species of teleost representing pelagic, benthic-pelagic, and benthic feeders in the Rufiji estuary in Tanzania. To the best of our knowledge, the study provides the first overview on characteristics of the OM pools, and their incorporation into the food webs within Rufiji estuary.

2. Methods and materials

2.1. Study site

The study sites are located in Rufiji estuary, 100 km south of Dar es Salaam city, in the Rufiji River delta (Fig. 1). This river has a catchment area of 177400 km² and is over 640 km long [19]. About 30 km from the coast, the lower Rufiji River branches out into a series of channels forming an impressive delta, covering approximately 1,200 km² with 530 km² covered by mangrove forests [20]. The four northern tributaries presently carry the bulk of the discharge into Rufiji River. The southern part of the delta, which is sheltered, receives little freshwater, and the water in front of this part of the delta are clear and saline and feature seagrass meadows and scattered patch reefs. Tidal influence in the delta is considerable [21].

In the upstream part of Rufiji catchment area, some small towns are located. The downstream part is free of industries, but in the delta, various pesticides are used in mangrove sections that have partially been cleared for rice cultivation [22]. The estuary of the Rufiji delta is an integral part of the largely undisturbed saline swamps, tidal marshes, woodland and mangrove forests. It is by far the largest delta in eastern Africa, and contains the largest continuous block of mangrove forest on the eastern seaboard of the African continent.

The mangroves (*Heritiera littoralis*, *Avicennia marina*, and *Rhizophora mucronata*) provide the dominant habitat in the estuary, but tidal marshes, seagrass meadows, and mud flats are also present. Several omnivorous crustaceans of commercial importance spend part of their life cycle in mangroves feeding on the detritus, live benthic microalgae, occasional animal material and fine inorganic particles [22].

The present study was carried out in three locations (Muhoro – station 1, Msomeni-station 2, and Kalale – station 3), representing the south, middle and northern parts of the Rufiji delta (Fig. 1). These locations were chosen because they are representative of the entire estuary, and include a variety of adjacent habitats (i.e., mangrove stands, mud flats, and seagrass meadows) with sufficient diversity and densities of consumer organisms.

2.2. Sample collection

All flora, fauna, sediment and water samples were collected from three locations at the Rufiji estuary in June and July 2014 as part of a reconnaissance survey. Enough material was collected for three replicates. The main primary producers, including mangrove leaf tissue (*A. marina*, *H. littoralis*, and *R. mucronata*), and thalli of most abundant algae (*Ulva* sp. and *Padina* sp.) were collected from 3 stations. Seagrass blades (*T. hemprichii*), were only found in sufficient quantity at station 1 (Muhoro area). A range of animals, including polychaetes, gastropods (*Terebralia* sp.), clams (*Crassostrea cucullata*), crabs (*Uca* sp.), were collected from all three locations; and Panaeid shrimps (*Panaeus monodon*) were collected from Stations 1 and 3. The animals under investigation are the dominant species at respective stations, within the Rufiji delta (21). Ten to twenty individuals of gastropods, bivalves, crabs and shrimps were dissected to remove muscle tissues, used for preparing the dry tissue pool. Dissected tissues were frozen (-20 °C) and stored for analysis.

Teleost (*Trichiurus lepturus* and *Arius thalassinus*) were collected from Stations 2 and 3, while *Hilsa kelee* were collected from Stations 1 and 3, where they were found in sufficient quantity. Six individuals of each species of fish were randomly selected from the total catch of each sample, and were euthanized in an ice slurry; Muscle tissues (skin removed) were collected from both sides of each fish, for biochemical analyses within 24 h of sample collection. All the samples were kept frozen (-20 °C) until analysis, conducted within three months of sample collection.

The sediment samples were obtained by scraping the top 1-2 cm off the surface sediment at each three locations. Four liters of water were collected from each sampling site, and were transported back to the laboratory, where they were filtered through pre-combusted (4 h at 450°C) glass fibre filters (Whatman GF/F, 0.7µm). Three filter papers were used for each replicate and were put into cold storage at -20 °C until further extraction, that were conducted within three months of sampling.

2.3. Laboratory analyses

2.3.1. Lipid extraction and analysis

For lipid extraction, 10 g wet weight of flora, 5 g dry weight (dw) of sediments, 3 g dry weight (dw) of filtrates, and between 300 and 500 mg dw of animal tissues, including three replicates for each type of sample at each location were made, following the modified extraction protocol in marine particles and sediment samples [23–25]. Dry weight samples were obtained by freeze-drying and homogenization of samples in batches. Sample was extracted with a mixture of CH₂Cl₂ and CH₃OH (9:1 v/v ratio) in an automated solvent extractor (Dionex ASE 300; 3 cycles at 1500 psi and 100 °C). Recovery standard (50 mg/l *n*-hexatriacontane - d₅₀) was added prior to extraction. The extracts were reduced in volume on a Büchi Syncore SPE to ca. 0.5 ml, and later they were blown dried under a gentle stream of N₂. The total lipid extract (TLE) was subsequently separated into two fractions using 6 ml glass columns packed with 500 mg of Supelco Superclean LC-NH₂ [26]. The columns were eluted with CHCl₃/isopropanol (15 ml of 2:1 v/v, neutral fraction) and 2 % acetic acid in diethyl ether (15 ml; acid fraction). The acid fraction was derivatized with 1 ml of bromotrifluoride (BF₃) in methanol and heated (100 °C for

2 hours) to convert the fatty acids into their corresponding fatty acid methyl esters (FAMES); the samples were further extracted with NaCl and hexane, and separation was achieved by centrifugation (10 min at 3000 rpm). FAMES were removed from the upper hexane phase. 10 ppm of an internal standard (deuterated eicosanoic acid methyl ester) was added to each sample before analysis. To ensure that contamination was not introduced during extraction and/separation of lipid fractions, blanks were prepared following the same protocol.

The analysis of FAMES was performed using an Agilent 6890N GC interfaced to an Agilent 5973 MSD mass spectrometer operating at 70 eV, and scanning from m/z 40–600 (at 2.62 scans/sec), and the run-time was 68.42 min. The extracts were injected in splitless mode (10 μ l; inlet pressure of 10 psi with a flow rate 54.3 ml/min) and separated on a HP-5 MS capillary column (5% diphenyl dimethyl polysiloxane; 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness). The samples were run at constant flow (1.3 ml/min) with helium as carrier gas. The interface temperature was set at 300 °C; the source temperature was set at 230 °C and the MS quadrupole was maintained at 150 °C, respectively. The samples were injected at 35 °C and the oven was programmed to 130 °C at 20 °C/min and then to 320 °C at 6 °C/min, where it was held isothermally for 15 mins. The FAMES were identified by comparing their characteristic mass spectra, ion fragments (base peak and molecular ion), retention times, and elution order published in literature [27,28], NIST online library, and Archives of Mass Spectrometry (http://lipidlibrary.aocs.org/ms/arch_xyz/index1.htm). Quantification was based on specific fragments targeted in the total ion chromatograms generated from the lipid extracts analyzed in full scan-mode. Recovery of deuterated hexatriacontane added prior to extraction ranged from 80 to 90%. Internal standard (deuterated eicosanoic acid methyl ester) was used for quantification based on their response factors. Detection limit in the different standards ranged from 0.1 to 1 ng/g. Reproducibility of internal standards was in the range of ± 3.5 –7% for different compounds.

Fatty acids were designated as X:Y ω Z, where X is the number of carbon atoms, Y is the number of double bonds and Z is the position of the last double bond from the terminal methyl group. The fatty acid biomarkers (specific fatty acids and ratios of fatty acids) of the major potential food sources found in the Rufiji estuary were identified by comparing with the published literature (Table 1).

2.3.2. Stable isotope analysis

For stable isotope analyses, two replicate from all samples (primary producers, animals, sediment and POM) were oven-dried for 24 h at 60 °C and finely crushed (<200 µm). The filters (containing suspended OM) and sediment were acidified by HCl fumes in order to remove carbonates prior to drying and grinding [29].

Analyses were carried out on a fully automated Thermo scientific 20/20 isotope analyzer. Samples were combusted and the resulting gases were separated by gas chromatography, and then analyzed by continuous flow-mass spectrometry. Data are reported in δ notation relative to V-PDB standard and atmospheric nitrogen for carbon and nitrogen, respectively where:

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

where $X = {}^{13}\text{C}$ or ${}^{15}\text{N}$, and $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. Data quality control throughout the analysis was ensured by running a reference standard after every 10 runs. Analytical precision was $\pm 0.1\text{‰}$ and $\pm 0.3\text{‰}$ for ${}^{13}\text{C}$ and ${}^{15}\text{N}$, respectively.

2.3.3. Data analysis

Statistical analyses (ANOVA) were conducted with Statistica 6.0 software. All data were checked for and met the normality and homogeneity variance requirements for parametric analyses. Non-metric multidimensional scaling (NMDS) was also run using PRIMERv6 software to explore similarities and differences in the fatty acid profiles of fish, invertebrates and organic matter sources.

3. Results

3.1 Fatty acids in primary producers

Preliminary analyses for primary producers have indicated no significant differences between locations (ANOVA; $p > 0.05$). Therefore, their samples were combined for all three locations (stations 1, 2, 3). Palmitic acid (16:0) was the most abundant compound in the FA profiles of plant samples, except for LCFAs which were more abundant in mangrove leaves (Table 2). In the leaves of three mangroves species, LCFAs (long-chain fatty acids, with a carbon number greater than 24), indicator of vascular plants, contributed between 34.8 and 42.4% of the

total fatty acids (Table 2). The contribution of PUFA (polyunsaturated fatty acids) 18:3 ω 3, 18:3 ω 6 to mangrove leaves ranged between 3.8 to 11.8% and 2.3 to 7.1%, respectively. In seagrass samples, α -linolenic acid (18:3 ω 3) accounted for 27.8 % of the total fatty acids, followed by PUFA 18:2 ω 6 and 18:3 ω 6, which represented 24.2 and 16.4 %, respectively, of the total fatty acids. The dominant fatty acids in green and brown macro algae, *Ulva* sp. and *Padina* sp., in addition to 16:0 and 18:0, were 18:3 ω 6 and 18:2 ω 6, comprising 28.9 % and 12.7%, respectively, of the total fatty acids for *Ulva* sp; 11.2% and 3.8% for *Padina* sp. (Table 2). Among MUFA (monounsaturated fatty acids), oleic acid (18:1 ω 9) had the highest concentration (22.6% of the total) in brown algae, whereas in green macro algae they only represented 5.2 % of the total fatty acids.

3.2. Fatty acids in estuarine particulate (POM) and sedimentary organic matter (SOM)

Fatty acid profiles of estuarine POM and SOM are given in Table 3. Ubiquitous 16:0 and 18:0 contributed 29.4, 34.7, and 36.8% of total fatty acids in POM for stations 1-3, respectively, and about 28.5, 39.7 and 34.2 % of the total fatty acids in SOM for stations 1-3, respectively. Other fatty acids present in relatively high amount in POM were PUFA 18:3 ω 6 (3.5, 10.9 and 9.6 % for stations 1-3, respectively), 18:2 ω 6 (1.7, 10.1, and 14.7% total fatty acids for stations 1-3, respectively). 22:6 ω 3, indicative of dinoflagellates, and 20:5 ω 3, indicative of diatoms, significantly contributed (19.3% and 4.6, respectively) to the total POM fatty acids for station 1. The contribution of the odd-branched fatty acids (15:0 and 17:0, iso and anteiso) and the cis-vaccenic acid (18:1 ω 7), both indicative of bacterial population, was about 11, 15.6, and 20.9 % of total fatty acids in POM from stations 1-3, respectively, and 23.9, 38.8, and 26.9 % of total fatty acids in sediments from stations 1-3, respectively (Table 3). The LCFAs, indicators of terrestrial inputs, were detected in both POM and surface sediment of each station, but then maximum contribution occurred in surface sediments at stations 2-3 (8.5 and 11% of total fatty acids, respectively). Results from one-way ANOVA for fatty acid biomarkers for major food sources across locations indicate that there is a significant difference ($p < 0.05$) in diatoms biomarkers which appeared to be more abundant in sediment samples at station 1 compared to stations 2 and 3. Dinoflagellates and brown algal derived biomarkers were significantly high in POM samples at station 1 compared to stations 2 and 3.

3.3. Fatty acids in animal samples

The fatty acids corresponding to potential food sources identified in various animal tissues are presented in Table 4. Independent of the location, ubiquitous fatty acid (16:0 and 18:0) contribution was relatively high, accounting for 20.4 – 23.7% of the total fatty acids in the muscle tissues of polychaeta. The relative contribution of other predominant polyunsaturated fatty acids, PUFA (20:4 ω 6 and 20:5 ω 3) and bacterial fatty acids (BAFA) represented 19.4 – 27.1%, 5.3 – 14.2% and 15.1 – 29.6%, of the total fatty acids in polychaeta, respectively. Diatoms markers (20:5 ω 3) contribution to the tissue of Polychaeta was significantly higher ($p < 0.05$) in station 1 than in stations 2 and 3 (Table 4). The most abundant fatty acids in the tissues of *Terebralia* sp. was saturated fatty acids (SAFAs) (16:0 and 18:0) and BAFAs accounting for 22.1 – 29 % and 31.3 – 41.0 %, respectively, of the total fatty acids. Terrestrial inputs of long-chain fatty acids (LCFAs) accounted for 8.9 and 12 % of the total fatty acids in these gastropods. In addition to ubiquitous fatty acid varieties (16:0 and 18:0), the filter feeders (*C. cucullata*), had highest contribution (20.1%) of dinoflagellate markers (22:6 ω 3), followed by diatoms (20:5 ω 3, 9.2%), and LCFAs (6.9%), arachidonic acid (20:4 ω 6, 7.7 %), and brown algal markers (18:1 ω 9, 6.6 %). For crabs (*Uca* sp.), SAFA (16:0 and 18:0) contributed between 32.3 to 39.9% of total fatty acids, followed by LCFAs (14.9 – 19.5 %), and BAFAs (18.5 – 18.9%) at stations 2 and 3. Diatoms marker contributed significantly ($p < 0.05$, 15.5%) to the muscle tissues of *Uca* sp. at station 1 (Table 4). The fatty acid profiles of Panaeid shrimp (*P. monodon*) contained high amounts of SAFAs (16:0 and 18:0) (22 – 23.7%), BAFAs (11.9 – 27.2%) and terrestrial (LCFAs) inputs (11 - 16.9%). Diatoms (20:5 ω 3), brown algae (18:1 ω 9), and dinoflagellates (22:6 ω 3), contributed significantly ($p < 0.05$, 15.7, 12.2, and 4.5%, respectively) to the tissues of shrimps collected from station 1.

Fatty acid compositions in teleost (*H. kelee*, *T. lepturus* and *A. thalassinus*) are presented in supplementary data, Table S1, and the major contributing fatty acids markers are given in figure 2. The analysis of teleost samples revealed the presence of biomarkers from variety of food sources, indicative of their higher trophic level. Regardless of the species and locations, SAFA (16:0 and 18:0) contributions were the highest (29.2 – 32.3%, of the total fatty acids). The pelagic feeders, *H. kelee*, contained relatively high amounts of brown algae (18:1 ω 9, 15.6%) at station 1,

and dinoflagellates markers (22:6 ω 3) at both stations 1 and 3 (24.5% and 13.7%, respectively (Fig. 2). The LCFA inputs were relatively low (2.4 - 6.7%). For benthic-pelagic and benthic feeders fish, *T. lepturus* and *A. thalassinus*, the contribution of BAFAs, diatoms (20:5 ω 3), and LCFAs were the highest (17.5 – 24.0%, 11.4 – 14.1%, and 9.9 – 13.6%, respectively), followed by arachidonic acid (20:4 ω 6, Fig. 2). The contribution of dinoflagellates (22:6 ω 3) and brown algae (18:1 ω 9) was very low (Fig. 2).

A two-way ANOVA on food-derived biomarker input among animals and between stations revealed significant differences among species ($p < 0.05$), but not between the stations ($p > 0.05$), for almost all biomarkers, except the dinoflagellates (22:6 ω 3) and brown algae (18:1 ω 9), which significantly contributed to the tissues of *H. kelee* in station 1 than in station 3. Additionally, the non-metric multidimensional scaling (NMDS) plot (Fig. 3) indicated that three fish species in station 2 and 3 were trophically connected with crabs (*Uca* sp.), polychaetes, penaeid shrimps as they were likely to feed on/linked with sediment (SOM) and particulate (POM) organic matter. *H. kelee* in station 1 presented a FA profile close to POM and green algae (*Uva* sp.) and it seemed that this population was different from the population collected in station 2 and 3 (distance in between). Furthermore, crabs (*Uca* sp.) was possibly the important group that connected *T. lepturus* and *A. thalassinus* to the primary producers.

3.5 Stable isotope composition

Stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) data for primary producers, consumers, estuarine POM, and sediments are presented in Table 5. Distinctive groupings and separations of various sources of organic matter across different trophic level provided by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are shown in Figure 4. The $\delta^{13}\text{C}$ values for primary producers ranged from -28.5‰ for mangrove leaves to -13.01‰ green macro algae thalli, while those for $\delta^{15}\text{N}$ ranged from 5.6‰ (mangrove leaves) to 7.63‰ (brown algae thalli). The estuarine POM and sediment distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranged from 27.7 to 29.6‰ and 2.77 to 7.0‰, respectively. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of consumers (5 species of macrozoobenthic and 3 species of teleost) also varied greatly; $\delta^{13}\text{C}$ ranged from -21.8‰ (polychaetes) to -13.9 ‰ (*A. thalassinus*), and for $\delta^{15}\text{N}$ from 9.5‰ (polychaetes) to 16.43‰ (*A. thalassinus*), indicating a general enrichment with increasing trophic level (Fig. 4, Table 5). The

baseline value for $\delta^{15}\text{N}$, which was derived from the mean $\delta^{15}\text{N}$ values of primary producers (mangrove leaves, seagrass blades, and brown and green algae thalli) was 6.5. Taking into account a trophic fraction of 3.4 ‰ per trophic level (Vander Zanden and Rasmussen 2001), the food web spanned *ca.* 3 trophic levels between primary producers and fish species (Fig. 5). Except for the shrimp (*P. monodon*), which occupied the same trophic level (3-4) as the predatory fish species, other invertebrates were generally located between trophic level 1 and 3 (Fig. 5).

4. Discussion

4.1 Fatty acids in primary producers.

The FA profiles of major primary producers in the Rufiji estuary confirmed that specific biomarkers could be traced with confidence to the higher trophic levels. Consistent with this, LCFAs were found in high concentrations in mangrove leaves as reported by other researchers [30–32]. These saturated FAs were not found in significant quantity in other major food sources of primary producers (e.g., seagrass and macroalgae), thus providing a good tracer for mangrove derived organic matter input. Considerable amount of 18:3 ω 3, markers for seagrass [32–34] was found in the samples of seagrass blades cleaned of epiphytes. Although this FA was found in mangrove leaves and thalli of brown macroalgae, it occurred in much lower concentrations. Thus, seagrass biomarkers results are analyzed carefully to apportion source inputs, taking this fact into consideration. Green macroalgae (*Ulva* sp.) was the most abundant algal species in stations 2 and 3, contributing to the OM input for organisms in the estuary. Samples of *Ulva* sp. contained high proportion of 18:2 ω 6 and 18:3 ω 6, which have been used as markers for green algae [30,31,35]. Contrary to previous reports [30,31] these FAs were also found in considerable amount in seagrass blades. Brown algae (*Padina* sp.) contained high proportion of oleic acid (18:1 ω 9), which has been reported as a diagnostic biomarker for brown algae [32,36]. Although this fatty acid was also found in other primary producers, its proportions was much low, confirming the reliability of this biomarker as a diagnostic source indicator in this study.

4.2. Fatty acids in surface water and sediment

POM and sediment samples collected from three stations contained a wide range of fatty acids, indicating the contribution from a variety of OM sources. Station 1, which was statistically different from stations 2 and 3 in terms of dinoflagellates contributions to POM and diatoms to sediments, indicated that OM was predominantly derived from a mixture of autochthonous sources, including fresh and detrital phytoplankton, zooplankton and bacteria. This was supported by high concentrations of compounds indicative of a mixed plankton community such as 14:0, 16:0, 16:1 ω 7, and PUFA C₁₈, C₂₀, and C₂₂ fatty acids. The abundance of these more ubiquitous compounds, in addition to other compounds specific to phytoplankton, indicated that OM in lower reaches of the Rufiji estuary (station 1) was mostly composed of a planktonic/microbial source. For examples, the presence of 16:1 ω 7 and 20:5 ω 3 have been attributed as contributions of diatoms [37,38]. These fatty acids contributed 10.2 and 15.1% of total fatty acids in POM and sediment, respectively, in station 1. In addition, 22:6 ω 3, commonly attributed to dinoflagellate sources of phytoplankton [37,39] were also present in significant amount in POM samples from station 1. LCFAs, derived predominantly from vascular plant sources (terrestrial inputs) were present in station 1. However, the reduced contribution of these fatty acids in both POM and sediment samples from station 1 indicate that, quantitatively, the mangrove biomass and terrigenous inputs were not a significant contributor to the OM pool within this part of the estuary. The small contribution of OM derived from terrigenous sources was not surprising given the fact that the southern part of the Rufiji estuary, where station 1 is located, is sheltered and receives little freshwater [40], thereby reducing the export of mangrove-derived detritus and terrestrial inputs to the adjacent habitats.

Due to the shift in the drainage pattern of the Rufiji River in the 1970s [40], Stations 2 and 3, in the middle and north, of the estuarine delta, receive enhanced freshwater outflow (40). Accordingly, high contribution of LCFAs, 18:3 ω 6, and linoleic acid (18:2 ω 6) in both POM and sediment samples from stations 2 and 3 was expected. High concentrations of LCFAs particularly to the surface sediments in stations 2 and 3 suggested contribution of nearby mangrove biomass and terrestrial sources, perhaps due to the surrounding land use activities, such as rice cultivation, fishing, and the harvest of firewood, charcoal, and mangroves poles [21]. LCFAs contribution to these sites was comparable to those measured elsewhere in tidal flats receiving OM derived from mangrove forest/terrestrial plants [30,31,41,42] The high concentration of the PUFA (18:3 ω 6)

recorded at these stations indicated the contribution of *Ulva* sp. to the OM pool. The other most abundant fatty acid in these stations was linoleic acid (18:2 ω 6), which when found in large proportions is attributed to agricultural products [43]. Although, this fatty acid was particularly abundant in *Ulva* sp., consistently with the conjoint use of 18:3 ω 6 and 18:2 ω 6 as biomarkers of green macroalgae [30], which could mean high contribution of *Ulva* sp., to the OM pool, its relatively small contribution to OM pool at station 1 indicate that its input at stations 2 and 3 was most likely from the surrounding rice fields. Consistent with this, linoleic acid constituted > 38% of total FAs in rice [44]. Moreover, the green macroalgae has been described as a good indicator of nitrogen enrichment in an estuarine environment [45]. Although nutrients have not been measured in this study, the proliferation of green algae observed in these parts of the estuary is perhaps due to excess nitrogen, which is delivered to the estuary from agricultural activities in the catchment. Notably, fertilizers are intensively used in the rice fields around Rufiji delta.

Because they are usually synthesized by bacteria, the odd branched FAs and 18:1 ω 7 have been used as markers for the presence of microorganisms in the environment [30,46]. In the Rufiji estuary, the contribution of these FAs in POM was small (generally < 10%) of the total FAs concentration and this was most likely due to resuspension of bacterial communities associated with organic detritus on surface sediment [47]. However, in surface sediments, particularly in stations 2 and 3, these FAs constitute a significant fraction (23.9 to 31.8%) of the total FAs. This large quantity of bacterial derived FAs implied a rich microbial community on surface sediments. In this study, bacterial FAs strongly correlated with 18:2 ω 6 ($r = 0.75$, $p = 0.005$) and 18:3 ω 6 ($r = 0.96$, $p = 0.001$), markers of green algae. Assuming that the linoleic acid indicates agricultural inputs at stations 2 and 3, which might also supply enough nutrients for algal growth, the large presence of bacteria at these stations was most likely a response to a substantial input of organic matter from the surrounding watershed. Bacterial populations have been reported to respond positively to the increase of OM input which is used for metabolic activities [46,48].

Presence of 20:5 ω 3, a marker for diatoms and 22:6 ω 3 for dinoflagellates, made up a small fraction (generally < 3%) of the total FAs pool associated with POM at stations 2 and 3. The presence of these compounds was most likely due to growth of these organisms in the water column. 22:6 ω 3 was very negligible (< 0.5%) in the sediments samples at station 2, and not detected in station 3, which agreed with the more pelagic nature of dinoflagellates, and the low

concentration of this biomarker found in water samples. It is likely that dead dinoflagellate cells that sink to the bottom are quickly decomposed, and their biomarker signature is permanently lost from the sediment record.

4.3. Chemical proxies and dietary intake

The fatty acid composition of consumers and predators is helpful to evaluate the relative importance of various OM sources in their diets [32,35,49]. The FA profiles of polychaetes were dominated by bacterial markers (BAFAs), EPA (20:5 ω 3) and 20:4 ω 6. EPA, biomarkers of diatoms, and arachidonic acid (20:4 ω 6) have been reported to be major food sources for benthic organisms such as annelids and mollusks [50–52]. LCFAs were more abundant in polychaete tissues in stations 2 and 3 than in station 1. This could be associated with the availability and abundance of these FA in the sediments of stations 2 and 3 than in station 1. The main food source for the gastropod (*Terebralia* sp.) was bacteria, macroalgae and mangroves, judging by the relative contribution of their respective markers. The dominance of BAFAs and macroalgae sources in the diet of these organisms has also been reported in other sub-tropical estuaries [30,31]. However, contrary to Meziane and Tsuchiya [31], LCFAs – indicative of mangrove-derived OM – contributed substantially to the diet of gastropods in this study. It was not clear whether the LCFA contribution to gastropods was related to the availability of mangrove-derived OM in areas where gastropods were collected or preference of this food source by gastropods. Slim et al. [53] highlighted the dominant role of the snail *Terebralia palustris* in removing leaf litter in the Gazi Bay mangrove forest. On the other hand, the work of Tenore [54,55] documented the role of the detritus derived from vascular plants in supporting the bacterial biomass for deposit-feeders. Since *Terebralia* sp. collected from Rufiji estuary had high contribution of bacterial FAs, it is likely that mangrove-derived OM was ingested to assimilate the attached bacteria which are rich in nitrogen.

The filter feeding species studied (*C. cucullata*), had a high dinoflagellate (22:6 ω 3) contribution, followed by diatoms (20:5 ω 3), and brown algal markers (18:1 ω 9). The biomarkers for dinoflagellates and diatoms have been reported to be major food sources for filter feeders, and essential for their growth and survival [38,41,56]. In this study, the higher contribution of dinoflagellates compared to diatoms – as shown by eicosapentaenoic acid/docosahexaenoic acid

(EPA/DHA) ratio of < 1 – is likely to be related to the fact that dinoflagellates tend to be pelagic organisms, directly available to filter feeding bivalves from the water column. On the other hand, benthic diatoms which were mostly found on surface sediments must have been suspended into the water column to become available to filter feeding bivalves. Mangrove-derived LCFAs also contributed to the tissues of filter feeding bivalves in more or less abundance as FAs derived from diatoms and brown algae, suggesting that mangrove-derived OM was also consumed by bivalves. Alfaro et al. [32] found that mangrove-derived OM contributed to the diet of bivalves, at least when found in proximal areas. However, the bivalves collected in this study are on the tidal flat, *ca* 4 km away from mangrove forests near station 1. Previous studies have challenged the hypothesis that mangroves provide a major source of nutrients to primary consumers [31,57–59]. According to Meziane and Tsuchiya [31], the export of mangrove-derived organic matter was restricted to the nearest part of the intertidal flat; and bacterial, diatom and green macroalgae were the main food source of primary consumers in the area. In the present study, the presence of significant amounts of LCFAs in surface sediments, POM and in the muscle samples of consumers such as bivalves suggest that OM derived from mangrove forests were exported to the intertidal flat and contributed to the food source in primary consumers, supporting the hypothesis that mangroves contribute enough source of nutrients to consumers.

Bacteria and mangrove/terrestrial plants are the main food sources of crabs (*Uca* sp.), according to the relative contribution of BAFAs and LCFAs in the samples. Crabs are among few organisms able to directly ingest mangrove leaves accelerating the degradation time of mangrove litter and enhancing nutrient availability to microbial communities [60,61]. Thus crabs represent also a key crucial link between basal organic matter sources and high trophic level consumers. This result concurs with data reported from other estuaries [30,31,62]. The preference for BAFAs has been reported in an experimental study, which described high assimilation efficiency (about 98%) of microheterotrophs by *Uca vocans* [63]. Diatoms (20:5 ω 3) and brown algae (18:1 ω 9) contributed significantly to the diet of crabs at station 1 only. These FAs also significantly contributed to surface sediments and POM at this station; their utilization by crabs is most likely connected to the increase in the biomass of diatoms and brown algae within this area. Thus, their availability may certainly induce a better use of these microorganisms by primary consumers. Ingestion of algae has also been reported for other populations of *Uca vocans* [30,31,63]. According to Kharlmenko et al. [35], presence of dinoflagellates (22:6 ω 3) marker in the tissues

of macrozoobenthic species may reflect a carnivorous diet. However, very low contribution of dinoflagellates (22:6 ω 3) in the muscle tissue of crabs collected from all three stations indicates that meiofauna contributed meagerly to the diet of these crab species.

Panaeid shrimps are known to feed extensively on various kinds of food sources including microheterotrophic protozoans, meiofauna, detritus, benthic diatoms and algae [32,64–66]. In this study, the fatty acid profile of shrimp (*P. monodon*) showed a wide range of organic compounds, among which BAFAs, diatoms (20:5 ω 3), LCFAs were dominant at both stations. The contribution of dinoflagellates (22:6 ω 3) and brown algae (18:1 ω 9) markers was also high, in shrimps collected from station 1, reflecting the filter feeding behavior of these invertebrates [32,56,67,68].

PUFAs dominated the FA composition in all fish species (Fig. 2). The most common biomarkers found within *H. kelee* individuals were dinoflagellates (22:6 ω 3), brown algae (18:1 ω 9), which were readily available in the water column for direct consumption, or indirectly through filter-feeding preys which these fish consume. Diatom markers (20:5 ω 3) were also present, but in a relatively small quantity compared to dinoflagellates. The ratios between EPA and DHA, which could be used as a trophic marker for the feeding mode of estuarine fish (52), were higher (2.7 – 3.9) in *H. kelee* compared to other fish species. The high DHA: EPA ratio indicated the pelagic feeding mode of these species, which was consistent with previous studies reporting the importance of both pelagic flora and fauna in the diet of these fishes [68,69]. The contribution from bacterial markers (BAFAs) and LCFAs were high only in *H. kelee* collected from station 3, reflecting the opportunistic feeding habit on the most available food sources by this species.

The main food sources for *T. lepturus* and *A. thalassinus*, regardless of the location, were BAFAs, diatoms (20:5 ω 3), and mangrove/terrestrial-derived inputs (LCFAs). The LCFAs and BAFAs were also present in high concentration in surface sediments collected from stations 2 and 3. Hence, their dominance in the diet of these species reflects the food preference related to OM input in areas where these animals were collected. Notably, arachidonic acid (20:4 ω 6) contribution was also high for these species, indicating a food chain based on available benthic food sources [50,51]. Benthic organisms such as annelids, molluscs and crabs showed an elevated content of FA (20:4 ω 6) in their tissue (Table 4), indicating their important contribution to the diet of these fish. The DHA:EPA ratio in *T. lepturus* and *A. thalassinus* was very low (0.12 to 0.23,

and 0.02 to 0.08, respectively) compared to those in *H. kelee*. These two fatty acids are integral parts of cell membranes, and are therefore, retained in the tissues of each organism. This fact makes it more difficult to use DHA and EPA as trophic markers, since each organism incorporate these PUFAs into their membranes and not solely use them for nutritional purposes [70,71]. Thus, the contents of these fatty acids can vary from one species to another depending on their physiology and metabolism. However, the decreasing DHA:EPA ratio from pelagic (*H. kelee*) to benthic (*A. thalassinus*) feeding fish is a trend that was also observed in the DHA:EPA ratio in invertebrates. Although the pelagic fauna has not been investigated in this study, previous study by Ulimboka and Shilla (2013, unpublished data) on pelagic fauna (calanoids and cyclopoid copepods, and fish larvae) collected from the same locations as in this study, indicated high DHA:EPA ratios (3.3 to 4.1) compared to the benthic fauna sampled (0.0 to 0.3). The high DHA:EPA ratio (2.2) for invertebrates in this study was only recorded for filter-feeding bivalves (*C. cucullata*), which reflected its pelagic feeding mode. Based on these facts it is likely that not only the physiology of fish, with regard to maintaining membrane fluidity, but also the dietary composition influenced the DHA:EPA ratio in respective fish species. Thus, the DHA:EPA ratio could be used as a trophic marker for tracing the feeding characteristics in these fishes. However, this particular trend needs to be further tested by incorporating a wide spatial and temporal coverage for both fish species and their prey in future studies.

Since isotope compositions of muscle tissue reflect animal feeding history [72], the stable isotope ratios of these primary consumers further provide useful information regarding to their food sources and diet. In the present study, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures for all primary producers cluster away from all consumers studied, although values are within range of other previous studies [32,48,73]. Consumers such as polychaetes worms, bivalves, crabs appeared between mangrove leaves and algae, although slightly closer to mangrove leaves (Fig. 3). The isotopic variation between primary producers and consumers may be the result of long-term dietary differences among individual species, and the fact that the consumers probably feed on two or more food sources within their surroundings. Further studies, incorporating a wider temporal range, may need to be conducted to clearly identify the long-term dietary differences. As expected, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of shrimps and fish species confirm the wide variety of carbon sources and correctly place these invertebrates within the higher trophic levels of the local food web.

Unlike FAs profile which revealed significant differences ($p < 0.05$) on food-derived biomarkers input among animals and between stations, the isotopic values for primary producers and consumers did not differ significantly among sites ($p > 0.05$). Based on this, the analysis of presumed food sources in this study relied much on the FAs profile as discussed above; and isotopic variation, particularly those of $\delta^{15}\text{N}$, were used to describe the trophic structure and estuarine food web as discussed in the following subsection.

4.4 Trophic structure and estuarine food webs

The $\delta^{15}\text{N}$ values of fish within Rufiji estuary do not vary greatly among these species of differing feeding modes. Assuming a fractionation of 3.4‰ per trophic level [74,75], the $\delta^{15}\text{N}$ of each fish collected in the Rufiji estuary spanned *ca* 1.1 trophic levels, with all values lying within the range of one trophic level. Therefore, all studied fish species occupied a similar trophic level in the Rufiji estuary. The $\delta^{15}\text{N}$ values for fish in this study ranged from 11.7 to 16.4‰, which is slightly higher than those recorded for closely related species in other tropical estuary [76,77]. These differences in $\delta^{15}\text{N}$ values are likely to reflect a difference in the source values of $\delta^{15}\text{N}$ within different estuaries and habitats. Previous studies have shown that nitrate-enriched wastewater input into estuaries causes enriched $\delta^{15}\text{N}$ values in estuarine plants [78,79]. The catchment in the Rufiji estuary is used for agriculture, mainly rice cultivation and nutrient loading from cleared areas under cultivation is relatively high [22]. These nutrient sources could cause shifts in the stable isotopic composition of primary producers and consumers in the food web [79].

Both fatty acid and stable isotope results revealed that the food web in Rufiji estuary depended on a variety of carbon sources, contributing to different degrees, to the diets of primary consumers. The major primary producers (mangroves, macroalgae, seagrass and phytoplankton) are all potential food sources to the three abundant members of the near-shore fish species as shown by fatty acid profiles and stable isotope values. The non-metric multidimensional scaling (NMDS) plot (Fig. 3), as described in results subsection 3.3, further provide an evidence that variety of potential food sources support the food web in Rufiji estuary. Terrestrial/mangrove-derived material appeared to be more important at stations 2 and 3, which receive large amount of

freshwater inflow. Unlike in other studies [31,32,59–61], mangrove-derived material seemed to contribute to the food of primary consumers in adjacent habitats (mud flats). This suggests that terrestrial OM is exported further into the open coastal waters in Rufiji estuary. Furthermore, the contribution of mangrove litter to the microbial food web appeared to be important to a variety of primary consumers, which are then consumed by secondary consumers and predators higher up the food chain.

As expected, the brown algae and phytoplankton (benthic diatoms and dinoflagellates) contributed significantly to the estuarine food web at station 1. In contrast, seagrass although it was abundant at this site, contributed minimally to the food source. Terrestrial/mangrove derived sources were present at this station, but their contribution to the food web was less important than autochthonous (estuarine and marine related OM). The small contribution of OM derived from terrestrial sources could be related to freshwater flow, the primary mechanism for delivery of terrestrial OM, which is usually very little in this part of the estuary compared to other stations (Fig. 1, [40]). While this study did not look at the seasonal variations in carbon and nutrient sources, it is likely that the importance of these carbon sources may differ. For example, during wet seasons, increased rainfall and river flow could potentially increase the delivery of terrigenous carbon sources, making them available to the food web. Further research on abundance and transport of various sources of nutrients throughout the estuary, and over different seasons, which may provide further insight on the food web dynamics of this economically and ecologically important estuary, is of paramount importance.

5. Synthesis and concluding remarks

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopes provided a generally good distinction between trophic levels and carbon sources. However, the addition of fatty acid profile analyses proved to be a powerful and sensitive in describing the origin and pathways of material transfer between primary producers and abundant members of the fish fauna in Rufiji estuary. Based on fatty acids results obtained from this study, it appears that the food web at Rufiji estuary depends on a variety of carbon sources. The major primary producers (mangroves, macroalgae, seagrass and phytoplankton) all contributed, to a different degrees, to the diets of primary consumers. The prevalence of different organic matter supporting the food web is an indication of presence of stable structures of

estuarine food webs. This is because there are various trophic resources base in the food webs. On this lieu, the multiple trophic connectivity of consumers at higher trophic levels to the primary producers is viable. However, through anthropogenic disturbances such as pollution and high rate of removal (via fishing) of species like *A. thalassinus* that demonstrated high omnivore index and trophically connected directly to the primary producers and consumers at different trophic levels, can threaten the estuarine food web structure and ultimately the ecosystem health. This knowledge is of crucial importance in our understanding of the ecological balance and potential responses of these ecosystems to human impacts.

Like many estuaries throughout the world. Rufiji estuary is characterized by both high species diversity and habitat complexity. However, specific comparison with other estuaries, for example, Matapouri estuary, New Zealand [32], Okukubi estuary, Japan [31], and Bahamas Islands [59], shows that mangrove-derived materials in the Rufiji estuary have much wider effect on the food web since much of its organic matter appear to be exported to the adjacent habitats and contribute to the diet of primary consumers. Ongoing research on the transport of various sources of nutrients throughout the estuary, and over different seasons, may provide further insight on the processes/factors which modulate the spatial and temporal variability of food sources in this rich and diverse tropical estuarine system.

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Table captions

Table 1: Fatty acids used as biomarkers for different food sources.

Table 2: Relative fatty acid composition of potential food sources at Rufiji estuary, Tanzania (% of the total fatty acids; standard deviations are shown in parentheses; none detected are shown with a dash).

Table 3: Relative fatty acid composition in suspended organic matter (POM) and surface sediment from three locations at Rufiji estuary, Tanzania (% of the total fatty acids; standard deviations are shown in parentheses; none detected are shown with a dash).

Table 4: Relative fatty acid composition in animal samples from three locations at Rufiji estuary, Tanzania (% of the total fatty acids; standard deviations are shown in parentheses; none detected are shown with a dash).

Table 5: Means ($\pm$ SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition of potential food sources and consumers from Rufiji Estuary. The range of values are shown in parentheses.

Table S1 (Supplementary data): Relative fatty acid composition in fish samples from three locations at Rufiji estuary, Tanzania (% of the total fatty acids; standard deviations are shown in parentheses; none detected are shown with a dash).

Figure captions

Figure 1: Map of the study site at Rufiji Estuary, Tanzania. Three sampling locations are indicated in Muhoro stream (station 1), Msomeni (station 2), and Kalale stream (station 3).

Figure 2: Biomarker composition (percent $\pm$ SD) for potential food sources in fish samples (*H. kelee*, *T. lepturus* and *A. thalassinus*) collected within three locations at Rufiji Estuary. Food sources include: diatom biomarkers (20:5 ω 3); Dfng: dinoflagellates (22:6 ω 3), BAFA (odd branched fatty acids, and 18:1 ω 7), B.algae: brown algae (18:1 ω 9); G.algae: green algae (18:3 ω 6); Arach. acid: Arachidonic acid (benthic food sources) (20:4 ω 6), and terrestrial plant (LCFAs).

Figure 3: Non-metric multidimensional scaling ordination (NMS) for the fatty acid profiles of fish invertebrates and organic matter sources. Numbers indicate sampling stations (station 1, 2 and 3), the points without numbers represented the pooled data from all

sampling stations. All data points in ordination space were presented in average to avoid crowding.

Figure 4: Mean ($\pm$ SD) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of potential food sources and consumers from Rufiji Estuary. The food sources include mangrove (Av: *Avicennia marina*; Her: *Heritiera littoralis*; and Rhz: *Rhizophora mucronata*); algae (Ulv: *Ulva* sp. and Pad: *Padina* sp.); seagrass (Thl: *Thalassia hemprichii*); sediment from three stations (SD1, SD2, and SD3); particulate matter (PM1, PM 2, and PM3). The consumers include Pol: polychaetes, gastropods (Ter: *Terebralia* sp); clams (Cr: *Crassostrea cucullata*); Crabs (Uc: *Uca* sp.); Panaeid shrimps (Pan: *Panaeus monodon*); Teleost (HK: *Hilsa kelee*, Tl: *Trichiurus lepturus* and At: *Arius thalassinus*).

Figure 5: Mean ($\pm$ SD) $\delta^{15}\text{N}$ of primary producers and consumers (invertebrates and fish species) in the Rufiji estuary. The primary producers include: Av: *Avicennia marina*; Her: *Heritiera littoralis*; Rhz: *Rhizophora mucronata*; algae, Ulv: *Ulva* sp.; and Pad: *Padina* sp.); seagrass, Thl: *Thalassia hemprichii*. The consumers include: Pol: polychaetes, gastropods (Ter: *Terebralia* sp), clams (Cr: *Crassostrea cucullata*), Crabs (Uc: *Uca* sp, Panaeid shrimps (Pan: *Panaeus monodon*), Teleost (Hk: *Hilsa kelee*, Tl: *Trichiurus lepturus* and At: *Arius thalassinus*).

List of Figures

Figure 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

List of Tables

Table 1. Fatty acids used as biomarkers for different food sources.

Source	Biomarkers	Reference
Diatoms	20:5 ω 3	Parrish et al. 2000
Dinoflagellates	22:6 ω 3	Parrish et al. 2000
Bacteria	15:O iso, 15:O anteiso, 17:O iso, 17:O anteiso, 18:1 ω 7	Carrie et al. 1998
Terrestrial plants (mangroves)	Long chain fatty acids (LCFAs; fatty acids with more than 24 carbons)	Wannigama et al. 1981
Seagrass	18:2 ω 6, 18:3 ω 3	Kharlamenko et al. 2001
Brown algae	18:1 ω 9	Johns et al. 1979
Green macroalgae	18:3 ω 6, 18:2 ω 6,	Kharlamenko et el. 1995

Table 2

Fatty acids	<i>A. marina</i>	<i>H. littoralis</i>	<i>R. mucronata</i>	<i>T. hemprichii</i>	<i>Ulva</i> sp.	<i>Padina</i> sp.
SFA						
16:0	28 ± 1.5	22.3 ± 2.0	17.4 ± 1.5	8.3 ± 1.0	34.7 ± 3.6	15.4 ± 1.2
18:0	9.4 ± 0.9	11.2 ± 1.0	13.8 ± 1.1	2.5 ± 0.5	4.9 ± 0.8	8.9 ± 0.8
Others	1.2 ± 0.5	0.7 ± 0.2	3.2 ± 0.4	7.8 ± 2.1	0.9 ± 0.2	3.2 ± 0.2
Sum (%)	38.6 ± 2.1	34.2 ± 1.6	35.3 ± 1.4	18.6 ± 1.2	40.5 ± 3.2	27.5 ± 1.7
BAFA						
15:0 anteiso	0.3 ± 0.4	-	-	-	-	-
17:0 iso	0.8 ± 0.2	-	0.3 ± 0.2	0.2 ± 0.1	2.3 ± 0.2	-
17:0 anteiso	-	1.8 ± 0.6	-	-	-	-
Sum (%)	1.1 ± 0.5	1.8 ± 0.5	0.3 ± 0.1	0.2 ± 0.3	2.3 ± 1.0	0 ± 0.0
MUFA						
17:01	0.3 ± 0.2	-	-	1.2 ± 0.2	-	0.8 ± 0.2
18:1ω9	4.7 ± 0.7	3.2 ± 0.7	11.1 ± 1.1	4.5 ± 0.9	5.2 ± 0.8	22.6 ± 2.4
18:1ω7	0.4 ± 0.2	-	-	1.3 ± 0.5	2.7 ± 0.2	3.4 ± 0.8
Others	0.7 ± 0.2	1.7 ± 0.3	3.3 ± 0.4	1.6 ± 0.5	2.5 ± 0.6	1.3 ± 0.7
Sum (%)	5.8 ± 0.8	4.9 ± 0.9	14.4 ± 1.2	7.4 ± 1.1	10.4 ± 1.1	27.3 ± 3.2
PUFA						
18:2ω6	6.7 ± 0.6	1.6 ± 0.3	0.7 ± 0.2	24.2 ± 2.2	12.7 ± 1.2	3.8 ± 0.4
18:3ω6	7.1 ± 0.6	2.3 ± 0.5	5.7 ± 0.5	16.4 ± 2.8	28.9 ± 2.1	11.2 ± 1.1
18:3ω4	-	0.4 ± 0.2	-	-	0.7 ± 0.2	-
18:3ω3	4.8 ± 0.8	11.8 ± 1.2	3.8 ± 0.6	27.8 ± 3.2	2.8 ± 0.8	7.6 ± 0.9
20:4ω6	-	0.3 ± 0.1	0.7 ± 0.3	-	0.6 ± 0.2	16.4 ± 1.2
20:5ω3	-	-	-	-	-	4.7 ± 1.0
22:6ω3	0.4 ± 0.1	-	-	-	-	-
Others	0.8 ± 0.6	0.2 ± 0.7	0.0 ± 0.0	1.3 ± 0.3	0.5 ± 0.1	0.6 ± 0.11
Sum (%)	19.8 ± 1.2	16.6 ± 0.9	10.9 ± 1.0	69.7 ± 4.6	46.2 ± 3.6	44.3 ± 4.1
LCFA						
Sum (%)	34.8 ± 2.4	42.5 ± 2.4	39.1 ± 1.8	3.5 ± 1.1	0.6 ± 0.1	0.9 ± 0.3
Total FAMES (µg/g dry wt)	482.4 ± 14.6	609.7 ± 12.6	658.3 ± 10.9	821.8 ± 25.8	233.7 ± 14.1	263.5 ± 10.2

Table 3

Fatty acids	POM			Sediment (SOM)		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
SFA						
16:0	16.7 ± 1.1	19.1 ± 0.2	22.9 ± 1.8	18.1 ± 1.3	21.9 ± 5.2	18.6 ± 2.1
18:0	12.7 ± 1.1	15.6 ± 0.9	13.9 ± 0.7	10.4 ± 1.1	17.8 ± 2.8	15.6 ± 2.4
Others (%)	7.9 ± 0.8	4.3 ± 0.9	10.0 ± 0.10	4.3 ± 0.11	0.0 ± 0.0	5.2 ± 0.13
Sum (%)	37.3 ± 1.8	39 ± 2.4	46.8 ± 2.1	32.8 ± 3.4	39.7 ± 4.5	39.4 ± 3.2
BFA						
15:0 anteiso	2.2 ± 0.6	4.2 ± 0.8	2.0 ± 0.4	6.8 ± 1.0	6.5 ± 0.9	-
17:0 iso	1.8 ± 0.9	3.5 ± 0.5	-	4.6 ± 0.4	4.7 ± 0.5	7.1 ± 1.4
17:0 anteiso	-	0.8 ± 0.2	4.1 ± 1.0	0.5 ± 0.4	3.3 ± 0.2	4.9 ± 1.2
Sum (%)	4 ± 0.8	8.5 ± 1.1	6.1 ± 1.2	11.9 ± 1.1	14.5 ± 2.2	12 ± 3.1
MUFA						
17:01	0.8 ± 0.3	0.6 ± 0.1	-	0.3 ± 0.3	0.3 ± 0.5	-
18:1ω9	4.9 ± 1.0	2.4 ± 0.7	1.8 ± 0.6	6.9 ± 0.7	0.2 ± 0.1	3.5 ± 0.7
18:1ω7	7.14 ± 1.1	14.8 ± 1.0	12.6 ± 1.5	12 ± 2.2	17.3 ± 2.1	14.9 ± 3.6
Others (%)	8.2 ± 0.3	3.1 ± 0.4	0.9 ± 0.5	0.6 ± 0.1	0.0 ± 0.0	0.8 ± 0.4
Sum (%)	21.04 ± 1.6	20.9 ± 2.3	15.3 ± 3.4	21.04 ± 3.4	17.8 ± 2.4	19.2 ± 2.7
PUFA						
18:2ω6	1.7 ± 0.3	10.1 ± 1.8	14.7 ± 2.3	3.8 ± 0.5	13.9 ± 3.2	12 ± 1.4
18:3ω6	3.5 ± 0.6	10.9 ± 0.9	9.6 ± 1.2	0.8 ± 0.4	2.1 ± 1.2	3.7 ± 0.6
18:3ω3	4.8 ± 0.9	0.6 ± 0.1	0.7 ± 0.2	7.8 ± 0.4	-	0.4 ± 0.2
20:4ω6	0.7 ± 0.2	-	0.4 ± 0.1	-	-	-
20:3ω3	0.2 ± 0.1	-	-	-	-	-
20:5ω3	4.6 ± 0.5	2.6 ± 0.4	0.9 ± 0.2	15.1 ± 3.3	3.2 ± 1.2	1.3 ± 0.6
22:6ω3	19.3 ± 1.2	1.5 ± 0.5	0.5 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	-
Others (%)	0.0 ± 0.0	0.8 ± 0.3	0.5 ± 0.4	1.1 ± 0.5	0.0 ± 0.0	1.0 ± 0.7
Sum (%)	34.8 ± 3.2	26.5 ± 2.2	27.3 ± 3.1	28.8 ± 3.2	19.5 ± 1.8	18.4 ± 4.2
LCFA						
Sum (%)	2.8 ± 0.9	5.1 ± 2.2	4.5 ± 0.9	5.9 ± 1.0	8.5 ± 0.9	11 ± 1.0
Total FAMES (µg/g dry wt)	159.5 ± 14.8	233.1 ± 8.2	187.4 ± 21.2	312.7 ± 12.1	334.9 ± 9.2	368.7 ± 34.4

Table 4.

Fatty acids	Polychaete		<i>Terebralia</i> spp		<i>C. cucullata</i>	<i>Uca</i> sp			<i>Panaeus monodon</i>	
SFA	Station 1	Station 2 &3	Station 1	Station 2 &3	All 3 stations	Station 1	Station 2	Station 3	Station 1	Station 3
16:00	11.8 ± 2.5	14.5 ± 3.8	18.4 ± 1.8	15.3 ± 2.56	22.1 ± 2.6	26.6 ± 7.0	29.6 ± 8.2	26.3 ± 6.5	17.6 ± 2.5	14.8 ± 08
17:00	1.4 ± 1.0	0.4 ± 0.2	-	1.5 ± 0.6	1.2 ± 0.8	2.8 ± 2.0	0.9 ± 0.1	1.1 ± 0.5	0.6 ± 0.1	0.6 ± 0.5
18:00	8.6 ± 1.2	9.2 ± 1.2	10.2 ± 2.1	6.8 ± 1.5	11.4 ± 2.6	5.7 ± 1.1	10.1 ± 1.4	7.4 ± 1.5	6.1 ± 0.8	7.2 ± 4.0
Others (%)	1.1 ± 1.0	1.2 ± 1.0	4.5 ± 1.2	2.5 ± 1.3	4.1 ± 1.4	5.3 ± 1.2	1.5 ± 0.6	2.4 ± 1.1	2.9 ± 1.8	2.3 ± 1.9
Sum (%)	22.9 ± 5.1	25.3 ± 3.4	33.4 ± 7.5	26.1 ± 4.2	38.8 ± 4.7	40.4 ± 6.5	42.1 ± 9.1	37.2 ± 10.4	27.2 ± 8.2	24.9 ± 6.8
BFA										
15:0 anteiso	2.2 ± 0.8	2.1 ± 0.8	18.78 ± 6.2	21.4 ± 10.7	0.5 ± 0.2	2.4 ± 0.8	7.0 ± 0.2	9.1 ± 1.1	3.4 ± 0.3	7.2 ± 1.3
17:0 iso	5.2 ± 0.8	9.9 ± 2.1	6.4 ± 0.7	4.8 ± 1.2	0.8 ± 0.2	4.0 ± 0.7	1.9 ± 1.4	3.8 ± 0.8	2.2 ± 0.9	4.4 ± 1.5
17:0 anteiso	1.1 ± 0.4	8.4 ± 0.9	2.2 ± 0.5	6.1 ± 1.1	1.3 ± 0.6	0.4 ± 0.2	4.3 ± 1.2	4.6 ± 1.6	2.7 ± 1.2	4.7 ± 2.2
Sum (%)	8.5 ± 2.8	20.4 ± 2.5	27.4 ± 4.2	32.3 ± 7.6	2.6 ± 1.2	6.8 ± 2.1	13.2 ± 6.1	17.5 ± 3.7	8.3 ± 3.4	16.3 ± 3.8
MUFA										
17:01	-	0.4 ± 0.2	-	0.9 ± 0.2	-	2.6 ± 0.7	0.5 ± 0.2	1.8 ± 0.7	-	0.4 ± 0.2
18:1ω9	1.9 ± 0.5	0.6 ± 0.5	-	0.4 ± 0.4	6.6 ± 0.45	8.1 ± 1.7	1.7 ± 1.0	1.6 ± 0.9	12.2 ± 2.9	2.8 ± 0.8
18:1ω7	6.6 ± 1.2	9.2 ± 1.0	3.9 ± 1.1	8.7 ± 0.8	1.3 ± 0.2	1.2 ± 0.9	5.3 ± 2.3	1.4 ± 1.5	3.6 ± 2.2	10.9 ± 3.3
Others (%)	4.6 ± 1.5	0.6 ± 0.1	2.0 ± 0.7	2.0 ± 0.8	1.7 ± 0.9	6.8 ± 0.1	0.7 ± 0.1	1.5 ± 2.0	3.8 ± 1.2	2.4 ± 0.4
Sum (%)	13.1 ± 1.4	10.8 ± 2.8	5.9 ± 1.2	12 ± 1.2	9.6 ± 2.7	18.7 ± 4.1	8.2 ± 1.1	6.3 ± 0.5	19.6 ± 4.6	16.5 ± 5.8
PUFA										
18:2ω6	2.8 ± 0.8	6.8 ± 1.8	2.3 ± 0.4	5.6 ± 1.4	0.5 ± 0.2	1.2 ± 0.8	3.8 ± 1.2	1.9 ± 0.8	0.7 ± 0.2	1.2 ± 0.8
18:3ω6	6.7 ± 0.9	1.4 ± 0.8	11.6 ± 0.8	8.5 ± 0.8	2.8 ± 2.3	-	1.3 ± 0.8	1.6 ± 0.4	0.6 ± 0.1	0.8 ± 0.5
18:3ω3	3.4 ± 0.4	0.5 ± 0.2	1.1 ± 0.6	-	-	0.9 ± 0.3	-	-	0.9 ± 0.3	-
20:4ω6	27.1 ± 3.6	19.4 ± 2.5	3.4 ± 0.9	1.5 ± 0.5	7.7 ± 1.3	8.2 ± 2.6	7.1 ± 0.4	6.6 ± 0.8	11.6 ± 5.6	12.5 ± 4.5
20:5ω3	14.2 ± 2.4	5.3 ± 0.8	2.5 ± 0.7	0.9 ± 0.2	9.2 ± 1.4	15.5 ± 1.7	6.7 ± 0.9	5.4 ± 1.2	15.7 ± 2.0	6.7 ± 1.5
22:6ω3	-	-	-	-	20.1 ± 4.1	0.4 ± 0.2	-	0.5 ± 0.2	4.5 ± 1.3	1.7 ± 1.5
Others (%)	6.5 ± 1.8	2.8 ± 1.5	3.5 ± 0.8	1.1 ± 0.1	1.8 ± 1.2	1.9 ± 0.7	2.7 ± 1.1	3.5 ± 1.2	0.0 ± 0.0	2.5 ± 1.2
Sum (%)	60.7 ± 6.7	36.2 ± 5.7	24.4 ± 4.2	17.6 ± 4.2	42.1 ± 8.2	28 ± 8.6	21.6 ± 5.3	19.5 ± 3.1	34 ± 7.8	25.4 ± 8.3
LCFA										
Sum (%)	2.8 ± 1.0	7.3 ± 0.2	8.9 ± 2.4	12 ± 2.6	6.9 ± 2.1	5.3 ± 2.1	14.9 ± 3.2	19.5 ± 6.1	11 ± 6.2	16.9 ± 1.7
Total FAMES (µg/g dry wt)	1223.3 ± 57.9	1411.7 ± 43.8	1804.9 ± 81.8	1786.4 ± 76.5	1503.8 ± 37.8	1393.5 ± 56.8	1402.5 ± 16.8	1413.6 ± 49.5	1398.7 ± 91.2	1402.4 ± 52.7

Note: For statistically similar (p > 0.05) stations, FAs profiles are given as averages for stations where samples were collected from

Table 5

Species	13C	15N	Species	13C	15N
Primary producers			Consumers		
<i>Avicennia marina</i>	-27.6 ± 0.6 (-27.3 to -28.1)	6.0 ± 0.5 (5.6 to 6.6)	polychaetes	-21.8 ± 0.8 (-23.2 to -20.1)	9.5 ± 0.8 (8.7 to 10.4)
<i>Heritiera littoralis</i> ,	27.5 ± 0.3 (-26.4 to -28.8)	5.6 ± 0.4 (5.2 to 5.8)	<i>Terebralia</i> sp	-21.5 ± 0.7 (-22.6 to -20.7)	10.8 ± 0.6 (10.2 to 11.6)
<i>Rhizophora mucronata</i>	-28.5 ± 0.6 (-27.3 to -29.4)	5.8 ± 0.7 (5.4 to 6.1)	<i>Crassostrea cucullata</i>	-22.3 ± 1.6 (-23.3 to -21.7)	10.1 ± 0.5 (9.4 to 10.9)
<i>Thalasia hemprichii</i>	-14.8 ± 0.6 (-15.8 to -12.8)	6.1 ± 0.5 (5.9 to 6.8)	<i>Uca</i> sp	-21.6 ± 0.8 (-22.4 to -21.2)	10.7 ± 0.6 (10.3 to 11.8)
<i>Ulva</i> sp	-13.0 ± 0.4 (-13.7 to -11.9)	7.6 ± 0.2 (7.4 to 7.8)	<i>Panaeus monodon</i>	-17.3 ± 1.6 (-18.7 to -15.5)	13.2 ± 1.0 (12.7 to 13.6)
<i>Padina</i> sp	-13.2 ± 0.5 (-14.6 to -11.3)	7.6 ± 0.2 (7.3 to 7.8)	<i>Hilsa kelee</i>	-27.6 ± 0.6 (-21.2 to -15.3)	13.8 ± 0.8 (13.4 to 14.2)
POM			<i>Trichiurus lepturus</i>	-15.5 ± 0.7 (-16.4 to -14.8)	16.1 ± 1.0 (15.6 to 16.4)
station 1	-27.7 ± 0.5 (-28.2 to -27.4)	5.4 ± 1.2 (4.3 to 7.4)	<i>Arius thalassinus</i>	-13.9 ± 1.4 (-15.3 to -12.8)	16.4 ± 1.2 (15.9 to 16.9)
Station 2	-29.6 ± 0.4 (-30.7 to -28.1)	2.8 ± 2.6 (-0.92 to 4.8)			
Station 3	-28.7 ± 0.7 (-29.9 to -27.6)	4.1 ± 1.4 (2.7 to 5.2)			
Sediment					
Station 1	-28.3 ± 0.2 (-28.6 to -28.1)	7.0 ± 1.1 (5.7 to 8.5)			
Station 2	-29.4 ± 1.2 (-30.7 to -28.3)	6.0 ± 0.4 (5.7 to 6.3)			
Station 3	29.0 ± 1.1 (-30.2 to -27.1)	5.6 ± 1.4 (4.6 to 6.6)			

Table S1 (Supplementary data)

Fatty acids	<i>Hilsa kelee</i>		<i>Trichiurus lepturus</i>		<i>Arius thalassinus</i>	
	Station 1	Station 3	Station 2	Station 3	Station 2	Station 3
SFA						
14:0	1.1 ± 0.5	0.7 ± 0.3	2.2 ± 1.3	0.6 ± 0.1	-	1.4 ± 0.8
16:0	19.5 ± 4.7	23.2 ± 4.7	13.4 ± 3.9	17.4 ± 4.2	14.6 ± 8.3	15.7 ± 6.2
17:0	0.3 ± 1.2	-	0.4 ± 0.2	0.7 ± 0.4	0.8 ± 0.8	-
18:0	7.4 ± 1.1	8.8 ± 2.8	10.3 ± 6.1	7.6 ± 3.8	4.6 ± 1.9	6.1 ± 1.5
Others (%)	1.0 ± 0.2	1.5 ± 1.3	1.8 ± 1.0	2.5 ± 1.5	2.5 ± 1.6	0.8 ± 0.4
Sum (%)	29.3 ± 11.1	34.2 ± 9.2	28.1 ± 7.4	28.8 ± 12.7	22.5 ± 5.6	24 ± 11.3
BFA						
15:0 anteiso	1.0 ± 0.8	4.0 ± 0.2	4.3 ± 1.4	7.5 ± 3.1	5.9 ± 2.0	8.5 ± 4.1
17:0 iso	0.7 ± 0.2	2.6 ± 0.8	4.7 ± 0.8	1.9 ± 0.2	5.5 ± 6.1	2.9 ± 1.0
17:0 anteiso	-	0.8 ± 0.3	2.9 ± 0.9	3.8 ± 0.7	4.7 ± 2.7	3.7 ± 2.1
Sum (%)	1.7 ± 0.9	7.4 ± 2.1	11.9 ± 6.4	13.2 ± 5.6	16.1 ± 9.2	15.1 ± 3.9
MUFA						
16:1ω7	0.5 ± 0.4	1.3 ± 0.9	-	3.8 ± 1.4	0.6 ± 0.2	0.6 ± 0.9
18:1ω9	15.6 ± 4.8	3.8 ± 2.2	6.2 ± 0.8	1.9 ± 1.5	4.3 ± 3.6	1.1 ± 0.7
18:1ω7	0.7 ± 0.2	7.2 ± 2.8	5.8 ± 1.7	8.7 ± 2.6	7.2 ± 4.1	8.9 ± 3.6
Others (%)	2.8 ± 0.2	1.3 ± 0.7	4.1 ± 1.4	2.6 ± 0.5	3.2 ± 1.6	1.5 ± 0.7
Sum (%)	19.6 ± 3.7	13.6 ± 6.1	14.9 ± 3.6	16.1 ± 7.2	15.3 ± 2.2	11.1 ± 2.8
PUFA						
16:2ω4	5.9 ± 2.1	7.7 ± 2.0	3.2 ± 1.6	1.1 ± 0.8	3.2 ± 1.3	0.9 ± 1.3
18:2ω6	1.2 ± 0.9	3.2 ± 1.8	5.2 ± 1.9	2.1 ± 0.6	6.4 ± 4.0	8.8 ± 3.5
18:3ω6	0.9 ± 0.6	1.8 ± 0.6	0.7 ± 0.2	1.3 ± 1.0	1.7 ± 0.7	3.4 ± 0.5
18:3ω3	1.1 ± 0.7	-	0.3 ± 0.5	-	-	-
20:4ω6	2.6 ± 1.4	6.2 ± 2.9	8.4 ± 4.2	9.5 ± 7.3	11.4 ± 6.1	7.9 ± 3.7
20:5ω3	9.3 ± 2.5	3.5 ± 0.8	13.6 ± 3.5	11.4 ± 6.2	14.1 ± 0.5	13.8 ± 4.1
22:6ω3	24.7 ± 7.8	13.7 ± 4.7	3.1 ± 0.7	1.4 ± 0.7	0.4 ± 0.1	1.1 ± 0.9
Others (%)	1.3 ± 0.9	1.8 ± 0.6	0.7 ± 0.1	1.5 ± 0.8	0.0 ± 0.0	1.5 ± 0.7
Sum (%)	47 ± 11.6	37.9 ± 8.9	35.2 ± 9.3	28.3 ± 9.4	37.2 ± 11.4	37.4 ± 6.7
LCFA						
Sum (%)	2.4 ± 1.8	6.9 ± 2.1	9.9 ± 2.7	13.6 ± 2.9	8.9 ± 3.6	12.4 ± 7.5
Total FAMES						
(μg/g dry wt)	1856.8 ± 37.8	1966.3 ± 66.9	2414.6 ± 98.7	2343.9 ± 105.8	3014.7 ± 112.8	3238.4 ± 128.4