



Isotopic studies in Pacific Panama mangrove estuaries reveal lack of effect of watershed deforestation on food webs



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ABSTRACT

Stable isotopic N, C, and S in food webs of 8 mangrove estuaries on the Pacific coast of Panama were measured to 1) determine whether the degree of deforestation of tropical forests on the contributing watersheds was detectable within the estuarine food web, and 2) define external sources of the food webs within the mangrove estuaries. Even though terrestrial rain forest cover on the contributing watersheds differed between 23 and 92%, the effect of deforestation was not detectable on stable isotopic values in food webs present at the mouth of the receiving estuaries. We used stable isotopic measures to identify producers or organic sources that supported the estuarine food web. N isotopic values of consumers spanned a broad range, from about 2.7 to 12.3‰. Mean $\delta^{15}\text{N}$ of primary producers and organic matter varied from 3.3 for macroalgae to 4.7‰ for suspended particulate matter and large particulate matter. The $\delta^{13}\text{C}$ consumer data varied between −26 and −9‰, but isotopic values of the major apparent producers or organic matter sampled could not account for this range variability. The structure of the food web was clarified when we added literature isotopic values of microphytobenthos and coralline algae, suggesting that these, or other producers with similar isotopic signature, may be part of the food webs.

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1. Introduction

Mangrove estuaries provide an important habitat for a high diversity of species (Nagelkerken et al., 2008). These estuaries are productive habitats that also support food webs, as they include substantial transfer of nutrients and organic matter among adjoining land-estuary-coastal ecosystems. Evidence for such couplings between contributing watersheds and estuaries has been inconsistent (Alongi, 2009). Some reports conclude that mangrove-derived materials subsidize coastal food webs (Dittmar and Lara, 2001a, b; Dittmar et al., 2001; Jennerjahn and Ittekkot, 2002), while others do not (Schwamborn et al., 2002; Bouillon et al., 2004; Guest et al., 2004; Connolly et al., 2005; Kon et al., 2007). Stable isotopic studies of mangrove food webs suggest that secondary producers may depend less on imports from land, and on primary production by mangrove trees than on other primary producers

(macroalgae, microphytobenthos, seagrasses, or detritus) (Hsieh et al., 2002; Kieckbusch et al., 2004). Allochthonous food sources (i.e. produced outside of the mangrove habitat) may also be used by consumers from the mangroves (e.g. Igulu et al., 2013). Generalizations are therefore difficult for a variety of reasons, including differences of primary producers and of consumers and in hydrodynamic conditions among different mangrove estuaries (Connolly et al., 2005; Nyunja et al., 2009; Vaslet et al., 2012).

One additional possible reason for contrasting results from one mangrove estuary to another may be that these tropical environments receive inputs from watersheds with rather different land covers. In many parts of the tropics, deforestation of watersheds has taken place (Valiela et al., 2013b). Deforestation creates significant differences in materials (water, sediment, nutrients) discharged to receiving waters (Valiela et al., 2013a,b), which raises the question about the possible effects on the food webs within the receiving mangrove estuaries.

The active transport and transformations of nutrients and suspended matter from the watershed to the estuaries pose the question whether food webs within the mangrove estuaries could be affected

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by watershed deforestation. This study focused on two questions. First, to assess the effect of watershed deforestation on food webs within the receiving mangrove estuaries. Second, to identify the sources of N, C, and S that support the food webs present within the mangrove estuaries. For both lines of study, we use stable isotopes of nitrogen, carbon, and sulfur. We use $\delta^{13}\text{C}$ as a proxy for food web sources, $\delta^{15}\text{N}$ as a proxy for trophic level structure within the food webs, and both of them as an indicator of the effect of deforestation. $\delta^{34}\text{S}$ was used as an additional indicator of origin of food sources.

2. Material and methods

2.1. Study site

The study was conducted in mangrove ecosystems that received inputs from watersheds with different percentage of deforestation. These coupled watershed-mangrove ecosystems were located at the Gulf of Chiriquí, in the Pacific coast of Panama (Fig. 1). These eight coupled watershed-estuary systems were selected as they offered a range of conversions from forest to pasture land covers, with forest cover ranging between 23 and 92% (Table 1). They are first order streams carrying mostly baseline freshwater discharge (with surface runoff after large rainfall events) down-gradient through mangrove estuaries. The mangrove forests in this region extend between watersheds and the sea and include mangrove forests, which largely include C3 photosynthetic plants. Watersheds are dominated by red mangrove, *Rhizophora mangle*, and the piñuelo mangrove, *Pelliciera rhizophorae*, within the saltier reaches of the estuaries. Other less-abundant species also found in salty reaches include the black mangrove *Avicennia germinans*, and the white mangrove *Laguncularia racemosa*, as well as a variety of other species (Valiela et al. submitted). More details about geological setting, precipitation, and other information were provided in Valiela et al. (2012, 2013a,b).

2.2. Sampling design

To capture the variation of the influence of seasonal and inter-annual contrasts in stable isotopic signatures, sampling was

Table 1

Selected properties of the watershed-estuary systems included in this study (Fig. 1).

Watershed-estuary	Area of watershed (Ha)	Land cover (% of area)		
		Forest	Pasture	Other
Pixvae	1429	73	23	4
De la Mona	1575	47	47	6
Manglarito	239	91	6	3
Limón	665	92	5	3
Luis	1007	73	18	9
Salmonete	195	29	52	19
Chamuscado	2229	66	28	6
Grande	9639	23	43	34

carried out at the end of wet and dry seasons during the years 2009, 2010, and 2011. All samples were collected at the mouth of the eight selected estuaries, in sites with salinity ranging between 30 and 35. The main primary producers and pools of organic matter were sampled: mangrove trees, macroalgae, large particulate organic matter (POM), suspended particulate matter (SPM) and sediment.

2.2.1. Producers and organic matter

Primary producers sampled included mangrove trees (*R. mangle*, *P. rhizophorae*, and *A. germinans*) and macroalgae. Samples of 3–5 leaves of each mangrove species were combined to make one composite sample from each sampling site, and seasons of the different years. Obtained values of the three major mangrove species were pooled together. Samples of several species of brown and filamentous green macroalgae were collected at low tide from the surface of mangrove prop roots. Green and brown macroalgae data were also pooled together. Mangrove leaves and macroalgae samples were rinsed with double-distilled water, dried at 60 °C, ground to a fine powder, and stored at room temperature in glass vessels until analysis.

SPM and POM were sampled using different methods. To sample SPM, we sampled water from the well-mixed water columns within the mangrove estuaries. Water samples were collected in 20 L carboys and kept cool during transportation to the lab. SPM samples were obtained after passing the water through a

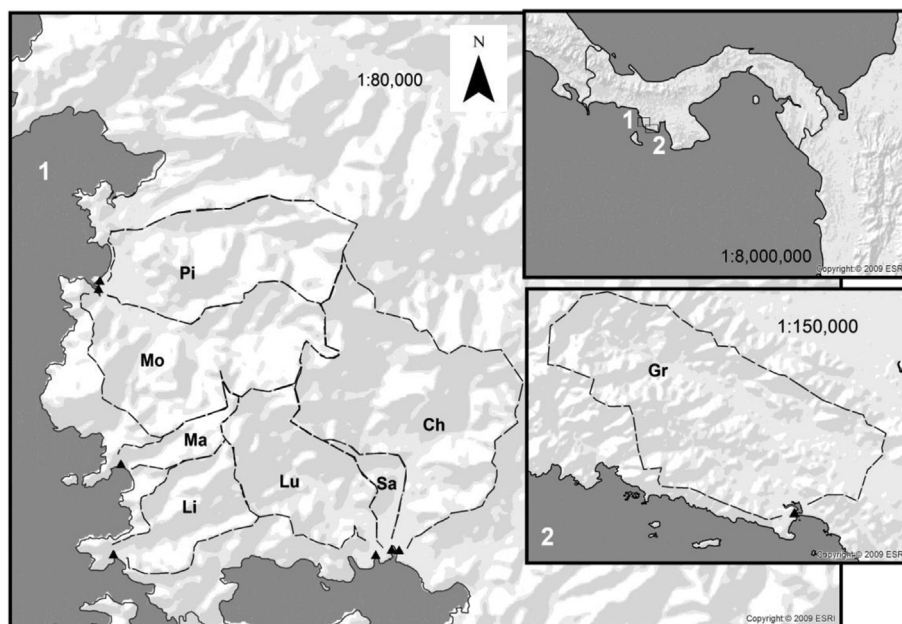


Fig. 1. Map of study areas. Inset on top right: Map of Panama, smaller boxes labeled 1 and 2 indicate location of study areas. Enlarged Box 1 shows location of seven watershed-estuary systems (Pi: Rio Pixvae, Mo: Rio de la Mona, Ma: Rio Manglarito, Li: Rio Limon, Lu: Rio Luis, Sa: Rio Salmonete, and Ch: Rio Chamuscado) and Box 2 shows location of Rio Grande (Gr). Watershed bounds are shown with dashed lines; triangles show location of the sampling sites at each of the watershed-estuary systems.

210 μm mesh to remove zooplankton that would have compromised stable isotope measurements. The material obtained was dried at 60 °C. POM samples were then obtained by filtering 0.5–1.5 L of water through pre-ashed and pre-weighed 0.7 μm GF/F filters. Filters were rinsed, dried in a drying oven for 2 days at 60 °C and then reweighed. To sample POM, plankton nets (mesh size of 250 μm) fixed with two stakes were installed at the mouth of the estuaries in a shallow place (<1.5 m) during ebb tide, thus collecting the material transported down estuary to the ocean.

Mangrove forest sediments were sampled by taking 1-cm deep, 1 cm diameter cores in the same stations down-estuary where we sampled mangrove leaves. The samples were revised and large detritus and macrofauna (if any) removed. Sediment samples were treated with 1.0 N HCl to remove bicarbonate. No acid treatment effects on the $\delta^{15}\text{N}$ signature were test, as despite some effect on the N signature have been found and reported in the literature for biota, $\delta^{15}\text{N}$ signature in sediment samples is quite constant to acid treatment (Harris et al., 2001).

To find evidence of the organic matter sources that could not been sampled in the Panama estuaries, we sought through the literature for additional potential sources. One primary producer, microphytobenthos, and one source of organic matter, coralline algae, were selected. They were not sampled, but they are present in the region. Summaries of nitrogen and carbon isotopic values of these two sources were gathered through the literature (Microphytobenthos, from Lee, 2000; Bouillon et al., 2002, 2004; Kon et al., 2007; Nyunja et al., 2009; Kruitwagen et al., 2010; Vaslet et al., 2012; coralline algae from Swart et al., 2005; Lamb et al., 2012).

2.2.2. Consumers

Several species of macro-invertebrates were collected at ebbing tide at the estuary mouth. At least three individuals were pooled to make a composite sample that could capture individual variation. Invertebrates were kept during 24 h in small aquaria containing filtered seawater to allow evacuation of gut contents. Invertebrate samples were acidified, and shells of gastropods and bivalves were removed to avoid carbonate contamination. We sampled fish by towing hand-held seines at the estuary mouth. Fish samples contained white muscle tissue of 1–3 individuals of similar total length. As in the case with primary producer samples, all consumer samples were rinsed with double-distilled water, dried until constant weight at 60 °C, ground to a fine powder, and stored at room temperature in glass vessels until analysis.

2.3. Stable isotope analysis

N, C, and S stable isotope and elemental analyses were performed at The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA, USA. Solid samples were analyzed for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ using an Europa 20–20 continuous-flow isotope ratio mass spectrometer interfaced with an Europa ANCA-SL elemental analyzer. Replicate analyses of isotopically homogeneous NIST Standard Reference Materials, IAEA N-A and IAEN-2 for $\delta^{15}\text{N}$, NBS-21 for $\delta^{13}\text{C}$ and IAEA S-1, IAEA S-2 and IAEA S-3 for $\delta^{34}\text{S}$, were made. The analytical precision based on those standards was $\pm 0.1\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ measurements, and $\pm 0.3\text{‰}$ for $\delta^{34}\text{S}$. Stable isotope ratios were reported as the deviation from the standard by δ notation in units per mil (‰):

$$\delta^{15}\text{N}, \delta^{13}\text{C} \text{ or } \delta^{34}\text{S}(\text{‰}) = \left[\left(R_{\text{sample}} - R_{\text{standard}} \right) / R_{\text{standard}} \right] \times 10^3,$$

where R was $^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$, or $^{34}\text{S}/^{32}\text{S}$. The standards used were the air, Pee Dee Belemnite (PDB) and Canon Diablo Troilite for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ respectively.

N and C stable isotopes were determined for all samples considered. To constrain costs of isotopic analysis and because in some cases there was not enough material, a subset of samples of some consumers were selected for S stable isotope analysis.

2.4. Data analysis

To determine whether watershed deforestation influenced N and C stable isotopes signatures found in consumers, two approaches were made. First, to assess differences in values of stable isotopes in consumers along the gradient of % forest cover on contributing watersheds, we regressed these variables. Second, we stratified the estuaries into three forest cover bins (23–29, 47–73, and 91–92 % forested), and compared isotopic values of consumers among pairs of bins (t -test for independent samples). Only stable isotopic data from taxa sampled within all estuaries were included in this analysis. Sulfur stable isotopes were not used in these approaches, as there was not enough data to compare the different watersheds. All the above-mentioned tests were performed with SPSS Statistical Software (version 11.5).

The contributions of the producers and organic matter for selected consumer taxa were estimated applying the Bayesian mixing model SIAR v4.0 (Parnell et al., 2010) in R (R Development Core Team, 2013). N and C stable isotopes were used for this estimation, as not enough data of S stable isotopes was available. The fractionation factors considered were 3.2 and 1‰ for N and C stable isotopes respectively (Minagawa and Wada, 1984; Peterson and Fry, 1987; Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003). No concentration dependent model was realized. The model was based on 500,000 iterations and 50,000 initial iterations were discarded.

3. Results

3.1. Effect of degree of watershed deforestation on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in mangrove estuary food webs

In a first exploratory analysis of the data, no differences between wet and dry seasons, or among the different years considered (2009, 2010, 2011) were found within samples from the same estuary (analysis not shown). Therefore the data from each estuary, sampled at different seasons and years, were pooled together for the comparison among different estuaries.

There were no significant relationships between $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ of fish or invertebrates and the percentage of forest cover on the contributing watersheds. There was some variation in stable isotopic values of the consumers, but the links to watershed deforestation were not clearly evident (Table 2).

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of consumers collected from estuaries whose watersheds were 23–29, 47–73, and 91–92 % forested were remarkably similar (Fig. 2). There were no significant differences among bins.

3.2. Food sources that fuel mangrove estuary food webs

The lack of significant watershed-related influence on consumer stable isotope values (Table 2 and Fig. 2), together with the absence of the influence of the year or season, suggested that we could pool data of different sites, years and seasons to examine the food sources and structure of the mangrove estuary food web at a regional scale. Here we use the pooled stable isotopic N, C, and S data to first identify possible producers or organic sources that supported the estuarine food web. Second, we use mixing models to better quantify likely sources of organic matter that support consumers.

Table 2
Variables, *F* values and *p*-values of the regression lines comparing isotopic composition in fish and invertebrates and % forest cover of the watersheds. *P*-values in bold correspond to the significant regressions. *Uca* sp. b is one of the two species of this genus that could not be identified to species level.

Variable	Species	Regression	d.f.	b0	b1	<i>F</i>	<i>p</i> -value
Fish							
$\delta^{15}\text{N}$	<i>Lutjanus argentiventris</i>	Linear	28	11.45	0.009	2.45	0.13
	<i>Atherinella</i> sp. & <i>Atherinopsidae</i>	Linear	4	10.71	0.009	1.80	0.25
	<i>Bathygobius andrei</i>	Linear	14	10.45	0.0001	0.0001	0.99
$\delta^{13}\text{C}$	<i>Lutjanus argentiventris</i>	Linear	28	-17.03	-0.002	0.03	0.86
	<i>Atherinella</i> sp. & <i>Atherinopsidae</i>	Linear	4	-16.57	0.015	5.41	0.08
	<i>Bathygobius andrei</i>	Linear	14	-19.43	0.016	0.44	0.52
Invertebrates							
$\delta^{15}\text{N}$	<i>Callinectes</i> sp. & <i>Muricidae</i> sp.	Linear	4	10.65	-0.016	0.29	0.62
	<i>Saccostrea palmula</i>	Linear	11	7.18	0.001	0.003	0.96
	<i>Littorina zebra</i>	Linear	15	5.91	-0.007	0.34	0.57
	<i>Cerithidea</i> spp.	Linear	12	4.23	0.001	0.02	0.90
$\delta^{13}\text{C}$	<i>Uca</i> sp. b	Linear	24	4.31	-0.012	2.67	0.12
	<i>Callinectes</i> sp. & <i>Muricidae</i> sp.	Linear	4	-14.10	-0.049	10.67	0.03
	<i>Saccostrea palmula</i>	Linear	11	-16.45	-0.007	0.31	0.59
	<i>Littorina zebra</i>	Linear	15	-25.66	0.089	5.69	0.03
	<i>Cerithidea</i> spp.	Linear	12	-14.41	-0.020	0.39	0.54
	<i>Uca</i> sp. b	Linear	24	-16.88	0.033	1.09	0.31

3.2.1. N, C and S stable isotopes in consumers and producers in the estuarine food web

The aggregate $\delta^{15}\text{N}$ data collected in the Panama estuaries span a large range (Fig. 3, gray bars and Table 3). Mean $\delta^{15}\text{N}$ of primary producers and organic matter found within the estuaries varied between 3.3 for macroalgae to 4.7‰ for SPM and POM (Fig. 3 and Table 3). While N isotopic values of organic matter were around 4.5‰, primary producers were constrained to mean values lower than 3.7‰, which were observed in mangrove trees. These latter species did not show a large variation among species, varying from 1.4 to 5.5‰. Summaries of nitrogen isotopic values of the two sources from literature are included in Fig. 3 (as white bars). Microphytobenthos collected from other mangrove estuaries had mean values of 2.3‰, while the $\delta^{15}\text{N}$ values of coralline algae were 4.9‰ (Table 3). Microphytobenthic $\delta^{15}\text{N}$ fall within a range that

could reasonably support the $\delta^{15}\text{N}$ of primary consumers, as $\delta^{15}\text{N}$ values of consumers ranged from about 2.7 to 12.3‰.

If we solely use the benchmark of about 3.2‰ per trophic step suggested by Peterson and Fry (1987), we find that the consumers of the food web (Fig. 3) would include three trophic levels. A continuous gradient in trophic position as proxied by $\delta^{15}\text{N}$ was observed. This result implies that the taxa we sampled consumed mixed diets that blurred trophic distinctions, and erased presumed trophic level boundaries (Fig. 3).

The isotopic discrepancy between potential foods available in the Panamanian estuary and isotopic values in consumers also appears in the aggregate data on $\delta^{13}\text{C}$ (Fig. 4). Producers and organic matter $\delta^{13}\text{C}$ ranged between -25.4 and -30.7‰, but $\delta^{13}\text{C}$ of primary consumers fell between -9.1 and -22.9‰ (Fig. 4).

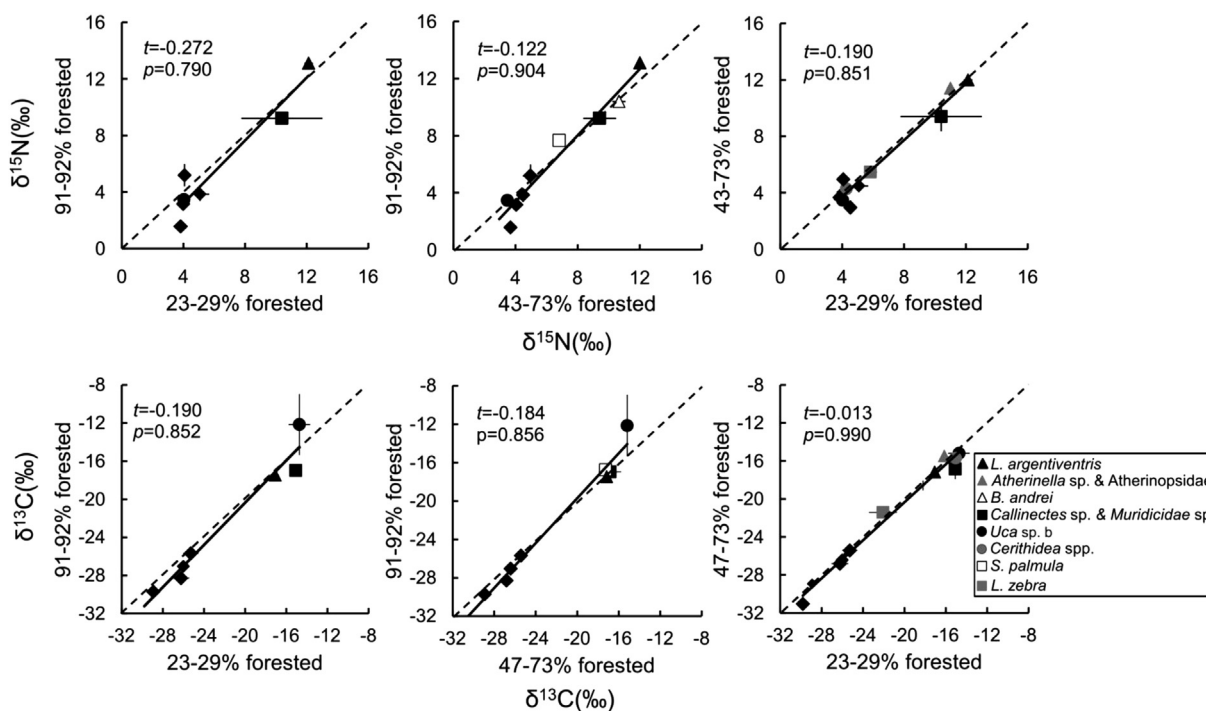


Fig. 2. Comparison of $\delta^{15}\text{N}$ in invertebrate and fish taxa collected from sets of estuaries binned into three groups of % forest cover on watersheds. Left: 91–92 vs. 23–29% forested; center: 91–92 vs. 47–73% forested, and right: 47–73 vs. 23–29% forested. Results of the *t*-test are shown on each figure. *Uca* sp. b is one of the two species of *Uca* that could not be identified to species level.

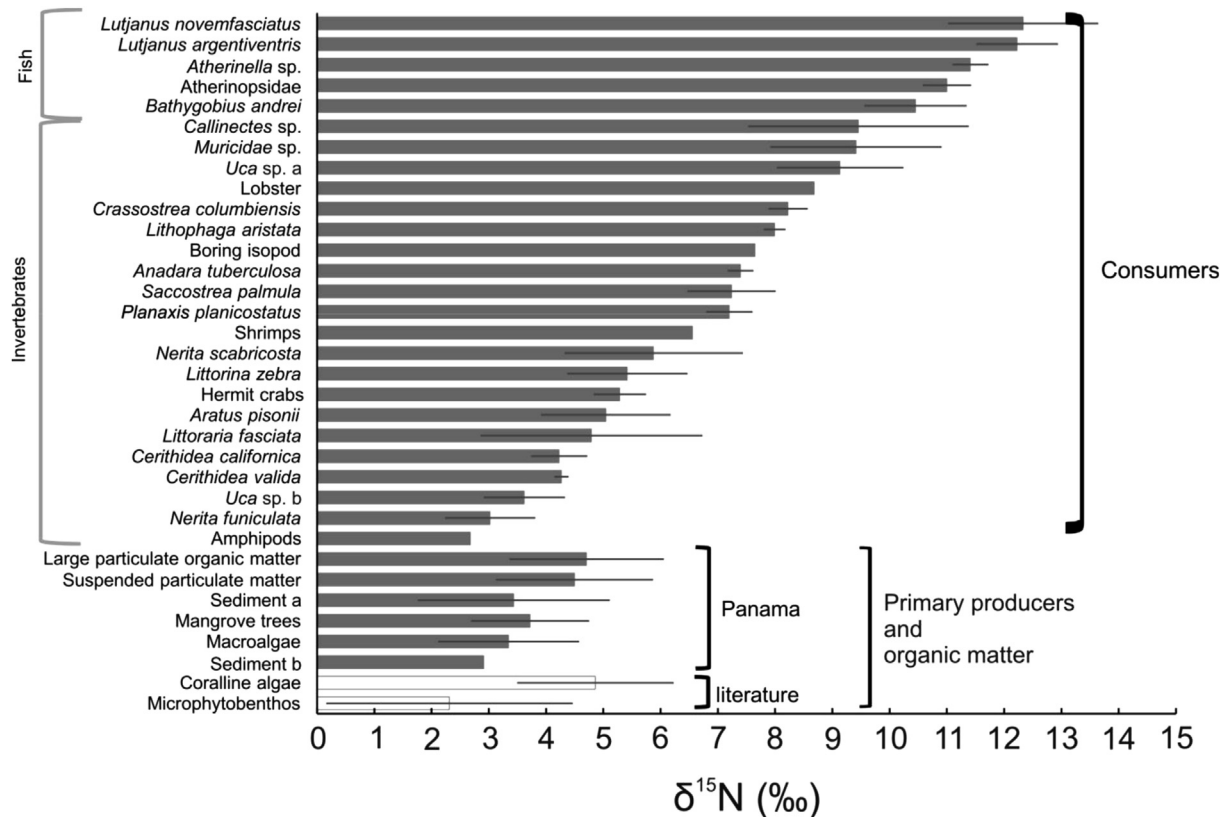


Fig. 3. $\delta^{15}\text{N}$ (‰) values (mean \pm sd) of the species sampled in this study, including fish and invertebrates, primary producers and organic matter collected from the Panama estuaries (gray bars), and from the literature (white bars, sources cited in Table 3, Fig. 4 and in the text). Sediment **a** and **b** correspond to samples with potentially different sources (based on the C isotopic signature). *Uca* sp. **a** and **b** are two species from the same genus that could not be identified to species level.

Summaries of carbon isotopic values of the two sources from bibliography are included in Fig. 4 (gray squares). Microphytobenthos collected from other mangrove estuaries had mean values of $\delta^{13}\text{C}$ around -18.2‰ , while the $\delta^{13}\text{C}$ values of coralline algae were -15.1‰ (Table 3).

$\delta^{34}\text{S}$ values had a wide range of variation in producers and organic matter at the estuary mouth (Fig. 5). Macroalgae had $\delta^{34}\text{S}$ mean values of 19.7‰ , and the range of variation of the other sources reached similar values; while fish and invertebrates had mean values ranging between 16.5 and 20‰ (Fig. 5, Table 3). As with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, one sediment sample (SED b, Fig. 4) had $\delta^{34}\text{S}$ value that fell within the range observed for invertebrate and fish (SED b, Fig. 5).

3.2.2. Application of mixing models to estimate the contribution of different food sources for consumers

Four species present in all estuaries were used to estimate the possible contribution of the different food sources. The mixing model results confirmed that to some degree, all the seven sources selected contributed to the consumer diets, but coralline algae or some organic matter with similar isotopic signature, and more importantly, microphytobenthos made the most important contributions to diet of the consumers (Fig. 6), if literature values for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are suitable for use in Panama.

4. Discussion

4.1. Role of mangrove estuaries in land–sea coupling

The results confirmed that the stable isotopic values of the food web found at the mouth of the mangrove estuaries were not

affected by the degree of deforestation of the contributing watershed.

The finding that land cover of watersheds had no imprint on the stable isotope values of the food web at the mouth of receiving estuaries contrasted with what has been well-established in temperate latitude estuaries. Land covers of watersheds, particularly in those affected by human activities, are powerfully coupled to producers and consumers found within the receiving estuaries and wetlands (McClelland et al., 1997; Martinetto et al., 2006).

In earlier papers, we showed that degree of deforestation in these same Panamanian estuaries did significantly affect amounts of dissolved (Valiela et al., 2013b) and particulate materials (Valiela et al., 2013a) released into the fresh reaches of the estuaries studied here.

The biogeochemical transformations that take place within mangrove estuaries, however, were apparently powerful enough as to erase the watershed imprint during transit of the waters and materials down-estuary. Down-estuary profiles of dissolved (Valiela et al., 2013a) and particulate (Valiela et al., 2013b) nitrogen, for example, reflected influence of watershed land cover, but midway through the estuaries, that linkage disappeared, and near the estuary mouth (where we sampled the mangrove food web), exports were not affected by watershed land cover. This confirms what we found in mangrove trees where nitrogen and carbon stable isotopic measurements on the various species also showed no evidence of watershed land cover influence (Valiela et al., unpublished data).

Land cover on the Panamanian watersheds only differed in the ratio of forest to pasture. There were very few people living in these remote watersheds, and agricultural activities were trivial. If population density were much higher, wastewater and agriculture

Table 3

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ signatures (mean \pm sd) for consumers and producers and organic matter of the 8 watersheds from the Pacific coast of Panama in Fig. 1 and from literature references (Microphytobenthos, from Lee, 2000; Bouillon et al., 2002, 2004; Kon et al., 2007; Nyunja et al., 2009; Kruitwagen et al., 2010; Vaslet et al., 2012; coralline algae from Swart et al., 2005; Lamb et al., 2012). Blank spaces: No data. Sediment **a** and **b** correspond to samples with potentially different sources (based on the C isotopic signature). *Uca* sp. **a** and **b** are two species from the same genus that could not be identified to species level.

	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)
Panama primary producers and organic matter			
Mangrove trees	−29.2 \pm 1.2	3.7 \pm 1.0	6.4 \pm 5.9
Macroalgae	−30.7 \pm 1.2	3.3 \pm 1.2	19.7 \pm 1.4
Particulate organic matter	−25.4 \pm 1.5	4.7 \pm 1.4	10.9 \pm 5.1
Suspended particulate matter	−26.5 \pm 1.4	4.5 \pm 1.4	11.0 \pm 7.9
Sediment a	−26.9 \pm 1.5	3.4 \pm 1.7	0.8 \pm 6.7
Sediment b	−8.6	2.9	16.7
Literature primary producers and organic matter			
Coralline algae	−15.2 \pm 2.8	4.9 \pm 1.4	
Microphytobenthos	−18.2 \pm 2.4	2.3 \pm 2.2	
Bivalvia			
<i>Anadara tuberculosa</i>	−16.6 \pm 0.3	7.4 \pm 0.2	
<i>Crassostrea columbiensis</i>	−18.1 \pm 0.7	8.2 \pm 0.3	
<i>Lithophaga aristata</i>	−16.9 \pm 0.8	8.0 \pm 0.1	
<i>Saccostrea palmula</i>	−16.9 \pm 0.7	7.2 \pm 0.8	
Gastropoda			
<i>Cerithidea californica</i>	−15.7 \pm 2.3	4.2 \pm 0.5	17.9
<i>Cerithidea valida</i>	−15.1 \pm 0.1	4.3 \pm 0.1	
<i>Littoraria fasciata</i>	−21.9 \pm 1.3	4.8 \pm 1.9	20.2 \pm 0.3
<i>Littorina zebra</i>	−20.8 \pm 3.7	5.4 \pm 1.1	19.5 \pm 3.1
<i>Muricidae</i> sp.	−16.8 \pm 1.5	9.4 \pm 1.5	
<i>Nerita funiculata</i>	−9.1 \pm 0.5	3.0 \pm 0.8	21.6
<i>Nerita scabricosta</i>	−14.0 \pm 2.1	5.9 \pm 1.6	22.5
<i>Planaxis planicostatus</i>	−18.0 \pm 1.2	7.2 \pm 0.4	
Malacostraca			
Amphipods	−22.8	2.7	
<i>Aratus pisonii</i>	−22.9 \pm 1.4	5.0 \pm 1.1	
Boring isopod	−15.2	7.6	
<i>Callinectes</i> sp.	−16.3 \pm 1.8	9.5 \pm 1.9	17.3 \pm 0.3
Hermit crabs	−22.1 \pm 3.1	5.3 \pm 0.5	16.4 \pm 0.1
Lobster	−18.6	8.7	
Shrimps	−16.0	6.6	
<i>Uca</i> sp. a	−16.2 \pm 1.4	9.1 \pm 1.1	17.9
<i>Uca</i> sp. b	−14.7 \pm 2.9	3.6 \pm 0.7	18.9 \pm 0.8
Teleostei			
<i>Bathygobius andrei</i>	−18.5 \pm 1.7	10.5 \pm 0.9	16.6
Atherinepsidae	−16.2 \pm 0.5	11.0 \pm 0.4	
<i>Atherinella</i> sp.	−15.5 \pm 0.1	11.4 \pm 0.3	
<i>Lutjanus argentiventris</i>	−17.2 \pm 0.9	12.2 \pm 0.7	19.0 \pm 1.7
<i>Lutjanus novemfasciatus</i>	−16.8 \pm 1.4	12.3 \pm 1.3	16.6 \pm 4.9

would surely provide stronger land cover signals, and the down-estuary trends might be more prominent. In sum, mangrove estuaries in this region of Panama act as transformers that uncouple human activities on land—in this case, conversion of forests to pastures—from food webs in coastal receiving waters.

Therefore, mangroves in this region are providing important dual ecological services, in maintaining water quality as well as subsidizing coastal food webs. These considerations might seem inconsistent with the many papers that convey the notion that mangrove estuaries export materials that in fact support coastal food webs (Dittmar and Lara, 2001a among others). The importance of these exports follows from two aspects. First, most often, the receiving coastal waters are quite depauperate in nutrients, so even small estuarine contributions are biologically meaningful. Second, mangrove-derived particles, although initially reasonably refractory to decay, eventually are decomposed by bacteria and fungi once in the sea floor, and the resulting regeneration of nutrients, as well as the production of microbial biomass on sediment particles can, in time, support secondary production by other consumers in coastal ecosystems. Conservation of mangrove estuaries therefore

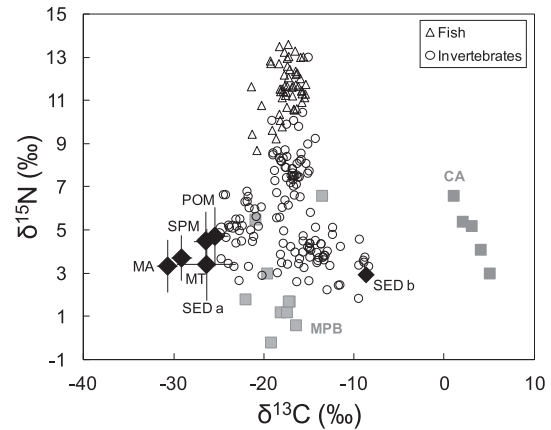


Fig. 4. Plot of $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) (mean \pm sd) for various elements of the mangrove estuary food web. Values shown in black diamonds are primary producers and organic matter from the Panama estuaries, which are further identified as large particulate organic matter (POM), suspended particulate matter (SPM), macroalgae (MA), mangrove trees (MT), and sediment (SED a and SED b). Sediment a and b correspond to samples with potentially different sources (based on the C isotopic signature). Open symbols show Panama estuary fish (triangles), and invertebrates (circles). Gray squares show literature values for microphytobenthos (MPB, from Lee, 2000; Bouillon et al., 2002, 2004; Kon et al., 2007; Nyunja et al., 2009; Kruitwagen et al., 2010; Vaslet et al., 2012), and coralline algae (CA, from Swart et al., 2005; Lamb et al., 2012).

should be a high priority for management of water quality and coastal biological resources.

4.2. Food sources of mangrove estuary food webs

The ranges of N and C stable isotopes within the food web in Panama estuaries were similar to those in other mangroves estuaries elsewhere (Abrantes and Sheaves, 2009; Giarrizzo et al., 2011). Although in other mangrove estuaries, the reason of these high isotopic values was that consumers used seagrasses extensively (Rodelli et al., 1984), and the right-ward skew of the consumers reflected the range of seagrass $\delta^{13}\text{C} \approx -20$ to -10 ‰ (Marguillier et al., 1997; Bouillon et al., 2002; Guest et al., 2004). Pacific Panamanian mangrove estuaries are devoid of seagrasses, so they are not a likely carbon source in our region. Instead, the coast

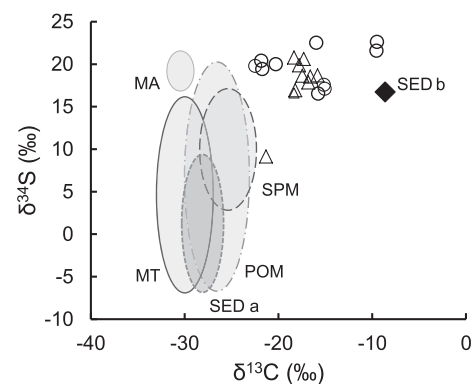


Fig. 5. Plot of $\delta^{13}\text{C}$ (‰) vs. $\delta^{34}\text{S}$ (‰) (individual samples and ranges of variation) for various elements of the mangrove estuary food web. Values shown as gray shading ellipses are the ranges of variation of primary producers and organic matter from the Panama estuaries, which are further identified as large particulate organic matter (POM), suspended particulate matter (SPM), macroalgae (MA), mangrove trees (MT), sediment (SED a). Black diamond represents the sediment sample with significant differences with the other (SED b). Open symbols show individual samples of Panama estuary fish (triangles) and invertebrates (circles).

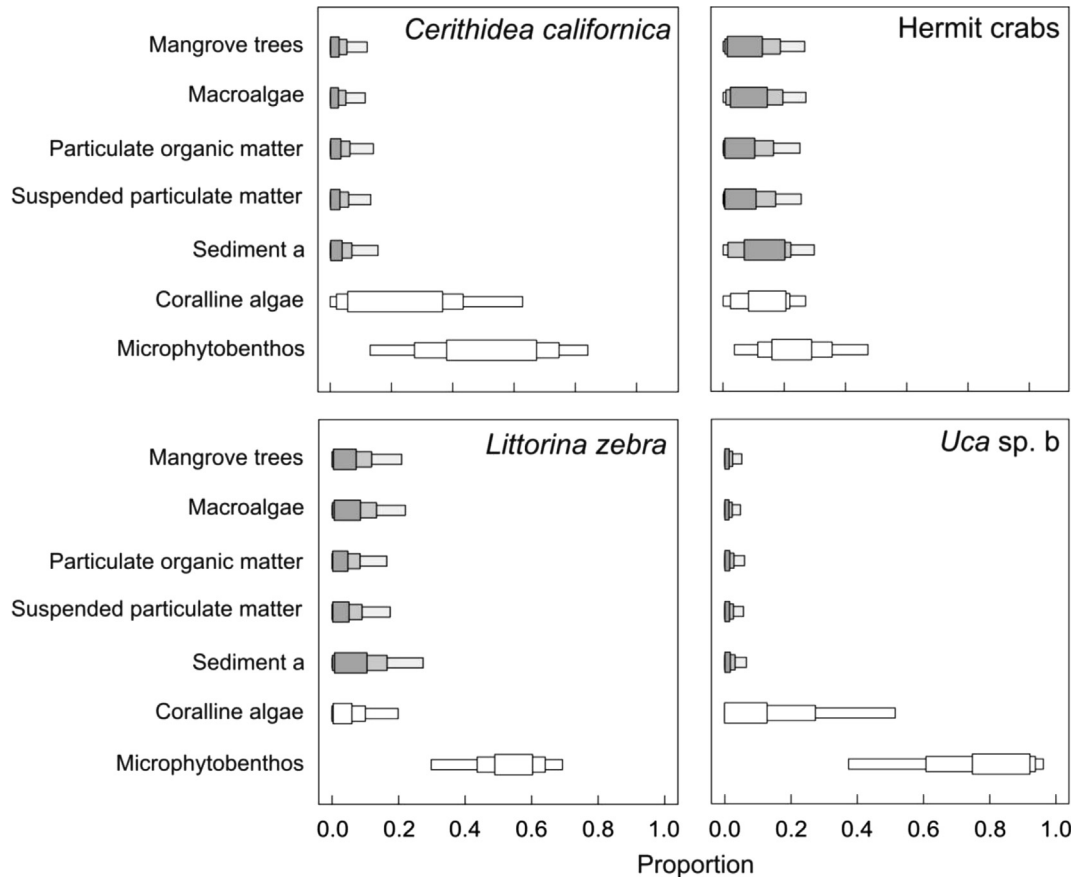


Fig. 6. Mean proportional contributions of primary producers and organic matter from Panama estuaries (gray bars) and literature (white bars) to the four most common primary consumers in terms of biomass (Fig. 3). Boxplots and the proportional contributions were obtained using the Bayesian mixing model SIAR in R.

surrounding Panamanian estuaries is formed by hard bottom rich in carbonate-enriched species (Fortunato and Schäfer, 2009). The position of the one sediment sample from Panama (SED b, Fig. 4) hints that in fact the presence of some sources similar to isotopic signature of coralline algae might be present in sediments of the region. Sulfur signatures (Fig. 5) suggest the possible influence of reduced estuarine sediment sources, as well as marine sulfate sources are possible (Peterson and Fry, 1987; Newell et al., 1995). There is no fractionation of $\delta^{34}\text{S}$ in food webs (Peterson and Fry, 1987; Hsieh et al., 2002), so consumers and food sources ought to show similar $\delta^{34}\text{S}$.

Another well-established feature that may influence consumers in this Panamanian food web is well-established idea that microphytobenthos can support food webs (Haines, 1976). The contrasting degree to which vascular plants, macroalgae, and microalgae may be used by consumers is closely controlled by differences in presence of unpalatable and refractory compounds in these primary producers. Microalgae are far less protected against consumers than vascular plants, so their greater prominence as support for food webs is not a surprise within mangrove estuaries (Newell et al., 1995; France et al., 1998). In most tropical estuaries grazing rates seem to be sufficiently high as to significantly lower biomass and appearance of micro-producers.

There is a gap of as much as 15‰ between the C stable isotopes of Panamanian food sources and invertebrate herbivore in the food web of Fig. 4. Fractionation of $\delta^{13}\text{C}$ has been traditionally considered as minor, perhaps -1‰ or less (Peterson and Fry, 1987). Nevertheless, a recent study with mangrove leaves and crabs observed $\Delta^{13}\text{C}$ values varying between 5.1 and 4.1‰ in different species

(Herbon and Nordhaus 2003). To resolve these issues, it may be necessary to apply compound-specific isotope analysis, including amino acids, in further studies.

Results from mixing models should be taken with caution, due to the fractionations factors that are necessarily used, but these models suggested that coralline algae and microphytobenthos were a potential source of organic matter sustaining the food web (Fig. 6). Although we sampled the apparent, conspicuous potential sources in the Panama estuaries, perhaps less conspicuous producers are important enough to set the overall $\delta^{13}\text{C}$ of not only many invertebrate consumers but also for the entire upper trophic web, whose $\delta^{13}\text{C}$ ranged between -15 and -22‰ (Fig. 4).

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References

- Abrantes, K., Sheaves, M., 2009. Food web structure in a near-pristine mangrove area of the Australian wet tropics. *Estuar. Coast Shelf Sci.* 82, 597–607.
- Alongi, D.M., 2009. *The Energetics of Mangrove Forests*. Springer, Dordrecht.
- Bouillon, S., Koedam, N., Raman, A.V., Dehairs, F., 2002. Primary producers sustaining macro-invertebrate communities in intertidal mangrove forests. *Oecologia* 130, 441–448.
- Bouillon, S., Moens, T., Overmeer, I., Koedam, N., Dehair, F., 2004. Resource utilization patterns of epifauna from mangrove forests with contrasting inputs of local versus imported organic matter. *Mar. Ecol. Prog. Ser.* 278, 77–88.
- Connolly, R.M., Gorman, D., Guest, M.A., 2005. Movement of carbon among estuarine habitats and its assimilation by invertebrates. *Oecologia* 144, 684–691.
- Dittmar, T., Lara, R.J., 2001a. Do mangroves rather than rivers provide nutrients to coastal environments south of the Amazon River? Evidence from long-term flux measurements. *Mar. Ecol. Prog. Ser.* 213, 67–77.
- Dittmar, T., Lara, R.J., 2001b. Driving forces behind nutrient and organic matter dynamics in a mangrove tidal creek in north Brazil. *Estuar. Coast Shelf Sci.* 52, 249–259.
- Dittmar, T., Lara, R.J., Kattner, G., 2001. River or Mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. *Mar. Chem.* 73, 253–271.
- Fortunato, H., Schäfer, P., 2009. Coralline algae as carbonate producers and habitat providers on the Eastern Pacific coast of Panamá: preliminary assessment. *Neues Jahrb. Geol. Paläontol. Abh.* 253, 145–161.
- France, R., Holmquist, J., Chandler, M., Cattaneo, A., 1998. $\delta^{15}\text{N}$ evidence for nitrogen fixation associated with macroalgae from a seagrass-mangrove-coral reef system. *Mar. Ecol. Prog. Ser.* 167, 297–299.
- Giarrizzo, T., Schwamborn, R., Saint-Paul, U., 2011. Utilization of carbon sources in a northern Brazilian mangrove ecosystem. *Estuar. Coast Shelf Sci.* 95, 447–457.
- 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.
- Haines, E.B., 1976. Stable carbon isotope ratios in the biota, soils and tidal water of Georgia salt marsh. *Estuar. Coast Shelf Sci.* 4, 609–616.
- Harris, D., Horwath, van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Sci. Soc. Am. J.* 65, 1853–1856.
- Herbon, C.M., Nordhaus, I., 2003. Experimental determination of stable carbon and nitrogen isotope fractionation between mangrove leaves and crabs. *Mar. Ecol. Prog. Ser.* 490, 91–105.
- Hsieh, H.-L., Chen, C.-P., Chen, Y.-G., Yang, H.-H., 2002. Diversity of benthic organic matter flows through polychaetes and crabs in a mangrove estuary: $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ signals. *Mar. Ecol. Prog. Ser.* 227, 145–155.
- Igulu, M.M., Nagelkerken, I., van de Velde, G., Mgaya, Y.D., 2013. Mangrove fish production is largely fuelled by external food sources: a stable isotope analysis of fishes at the individual, species, and community levels from across the globe. *Ecosystems* 16, 1336–1352.
- Jennerjahn, T.C., Ittekkot, V., 2002. Relevance of mangroves for the production and deposition of organic matter along tropical continental margins. *Naturwissenschaften* 89, 23–30.
- Kieckbusch, D.K., Koch, M.S., Serafy, J.E., Anderson, W.T., 2004. Trophic linkages among primary producers and consumers in fringing mangroves of subtropical lagoons. *Bull. Mar. Sci.* 74, 271–285.
- Kon, K., Kurokura, H., Hayashizaki, K., 2007. Role of microhabitats in food webs of benthic communities in a mangrove forest. *Mar. Ecol. Prog. Ser.* 340, 55–62.
- Kruitwagen, G., Nagelkerken, I., Lugendo, B.R., Mgaya, Y.D., Wendelaar Bonga, S.E., 2010. Importance of different carbon sources for macroinvertebrates and fishes of an interlinked mangrove-mudflat ecosystem (Tanzania). *Estuar. Coast. Shelf Sci.* 88, 464–472.
- Lamb, K., Swart, P.K., Altabet, M.A., 2012. Nitrogen and carbon isotopic systematics of the Florida reef tract. *Bull. Mar. Sci.* 88, 119–146.
- Lee, S.Y., 2000. Carbon dynamics of Deep Bay, eastern Pearl River Estuary, China. II: trophic relationship based on carbon and nitrogen stable isotopes. *Mar. Ecol. Prog. Ser.* 205, 1–10.
- Marguillier, S., Van der Velde, G., Dehairs, F., Hemminga, M.A., Rajagopal, S., 1997. Trophic relationships in an interlinked mangrove-seagrass ecosystem as traced by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. *Mar. Ecol. Prog. Ser.* 151, 115–121.
- Martinetto, P., Teichberg, M., Valiela, I., 2006. Coupling of estuarine benthic and pelagic food webs to land-derived nitrogen sources in Waquoit Bay, Massachusetts, USA. *Mar. Ecol. Prog. Ser.* 307, 37–48.
- McClelland, J.W., Valiela, I., Michener, R.H., 1997. Nitrogen-stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. *Limnol. Oceanogr.* 42, 930–937.
- McCutchan, J.H., Lewis, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102, 378–390.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim. Cosmochim. Acta* 48, 1135–1140.
- Nagelkerken, I., Blaber, S.J.M., Bouillon, S., Green, P., Haywood, M., Kirton, L.G., Meynecke, J.-O., Pawlik, J., Penrose, H.M., Sasekumar, A., Somerfield, P.J., 2008. The habitat function of mangroves for terrestrial and marine fauna: a review. *Aquat. Bot.* 89, 155–185.
- Newell, R.I.E., Marshall, N., Sasekumar, A., Chong, V.C., 1995. Relative importance of benthic microalgae, phytoplankton, and mangroves as sources of nutrition for penaeid prawns and other coastal invertebrates from Malaysia. *Mar. Biol.* 123, 595–606.
- Nyunja, J., Ntiba, M., Onyari, J., Mavuti, K., Soetaert, K., Bouillon, S., 2009. Carbon sources supporting a diverse fish community in a tropical coastal ecosystem (Gazi Bay, Kenya). *Estuar. Coast. Shelf Sci.* 83, 333–341.
- Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. *PloS One* 5, e9672.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Evol. Syst.* 18, 293–320.
- R Development Core Team, 2013. R: a Language and Environment for Statistical Computing. Version 3.0.1. R Foundation for Statistical Computing, Vienna.
- Rodelli, M.R., Gearing, J.N., Gearing, P.J., Marshall, N., Sasekumar, A., 1984. Stable isotope ratio as a tracer of mangrove carbon in Malaysian ecosystems. *Oecologia* 61, 326–333.
- Schwamborn, R., Ekau, W., Voss, M., Saint-Paul, U., 2002. How important are mangroves as a carbon source for decapod crustacean larvae in a tropical estuary? *Mar. Ecol. Prog. Ser.* 229, 195–205.
- Swart, P.K., Saied, A., Lamb, K., 2005. Temporal and spatial variation in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of coral tissue and zooxanthellae in *Montastraea faveolata* collected from the Florida reef tract. *Limnol. Oceanogr.* 50, 1049–1058.
- Valiela, I., Camilli, L., Stone, T., Giblin, A., Crusius, J., Fox, S., Barth-Jensen, C., Oliveira Monteiro, R., Tucker, J., Martinetto, P., Harris, C., 2012. Increased rainfall remarkably freshens estuarine and coastal waters on the Pacific coast of Panama: magnitude and likely effects on upwelling and nutrient supply. *Glob. Planet. Change* 92–93, 130–137.
- Valiela, I., Barth-Jensen, C., Stone, T., Crusius, J., Fox, S., Bartholomew, M., 2013a. Deforestation of watersheds of Panama: nutrient retention and export to streams. *Biogeochemistry* 115, 299–315.
- Valiela, I., Giblin, A., Barth-Jensen, C., Harris, C., Stone, T., Fox, S., Crusius, J., 2013b. Nutrient gradients in Panamanian estuaries: effects of watershed deforestation, rainfall, upwelling, and within-estuary transformations. *Mar. Ecol. Prog. Ser.* 482, 1–15.
- Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: implications for aquatic food web studies. *Limnol. Oceanogr.* 46, 2061–2066.
- Vaslet, A., Phillips, D.L., France, C., Feller, I.C., Baldwin, C.C., 2012. The relative importance of mangroves and seagrass beds as feeding areas for resident and transient fishes among different mangrove habitats in Florida and Belize: evidence from dietary and stable-isotope analyses. *J. Exp. Mar. Biol. Ecol.* 434–435, 81–93.