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REPORT

Identifying carbon sources and trophic position of coral reef fishes using diet and stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analyses in two contrasted bays in Moorea, French Polynesia

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Abstract Stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) and diet of three fish species, *Stegastes nigricans*, *Chaetodon citrinellus* and *Epinephelus merra*, were analyzed on the fringing coral reefs of two bays that are differentially exposed to river runoff on Moorea Island, French Polynesia. *S. nigricans* and *C. citrinellus* relied mostly on turf algae and presented similar trophic levels and $\delta^{15}N$ values, whereas *E. merra* fed on large invertebrates (crabs and shrimps) and had higher trophic levels and $\delta^{15}N$ values. Discrepancies existed between stomach content and stable isotope analyses for the relative importance of food items. Bayesian mixing models indicated that sedimented organic matter was also an important additional food for *S. nigricans* and *C. citrinellus*, and fishes for *E. merra*. The main sources of organic matter involved in the food webs ending with these species were algal turfs and surface sediments, while water particulate organic matter was barely used. Significant spatial differences in C and N isotopic ratios for sources and fishes were found within and between bays. Lower ^{13}C and higher ^{15}N values were observed for various compartments of the studied trophic network at the end of each bay than at the entrance. Differences were observed between bays, with organic sources and consumers being, on average, slightly more ^{13}C-depleted and ^{15}N-enriched in Cook's Bay than in Opunohu Bay, linked with a higher mean annual flow of the river at Cook's Bay. Our results suggest that rivers bring continental material into these two bays, which is partly incorporated into the food webs of fringing coral reefs at least close to river mouths. Thus, continental inputs can influence the transfer of organic matter within coral reef food webs depending on the diet of organisms.

Keywords Food webs · Stable isotopes · Continental inputs · French Polynesia · Bayesian mixing model

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Introduction

In a global context of multiple anthropogenic threats on coral reef ecosystems (Hughes et al. 2003), it has become increasingly urgent to better assess how organic matter circulates within these ecosystems. Thus, more information on trophic pathways is needed to fill this critical knowledge gap. However, information on this topic remains rare,

possibly due to the diversity and complexity of specific interactions that generate difficulties in the assessment of energetics on coral reefs (Harmelin-Vivien 2002). One way to fill this gap is to study trophic networks that constitute the main pathways of organic matter transfer on coral reefs. The high species diversity in coral reef ecosystems makes it very difficult to obtain information on the diet of all fish species (Carassou et al. 2008). In addition, diet studies do not usually provide information on prey assimilation rates. Furthermore, they identify ingested food at a given time only and so may underestimate the extent of generalist and detritus feeding.

Since diet studies do not provide a comprehensive view of food webs, it is necessary to use complementary methods, such as stable isotopes analyses. Diet analyses compounded with stable isotopes have been successfully developed for more than three decades (Kneib and Stiven 1980; Nichols et al. 1985; Wells et al. 2008). Stable isotopes have the great advantage of providing information on time-integrated assimilated food (Fry 1988; Vander Zanden and Rasmussen 1999). Carbon and nitrogen stable isotope ratios have been successfully used to understand the trophic dynamics in marine systems and to trace the transfer of organic matter of different origins through various aquatic food webs (Fry and Scherr 1984; Kaehler et al. 2000; Pinnegar and Polunin 2000). Carbon isotope composition in living animals usually provides clues to the initial origin of the ingested organic matter through a low increase in $\delta^{13}C$ per trophic level of about 1–1.4 ‰ on average (De Niro and Epstein 1978; Wada et al. 1991; Sweeting et al. 2007a). Nitrogen isotope ratio can be used to define the trophic level of organisms, as $\delta^{15}N$ usually increases to about 3.2–3.4 ‰ from food to consumer (Minagawa and Wada 1984; Post 2002; Sweeting et al. 2007b). Thus, combined measurements of both isotopes can provide information on source material and trophic level, allowing the construction of trophic relationships within the food web structure. Despite a few notable exceptions (Cocheret de la Morinière et al. 2003; McMahon et al. 2010, 2011; Wyatt et al. 2010), this tool remains largely unused in coral reefs and variability in trophic parameters between locations or species remains little studied (Greenwood et al. 2010; Wyatt et al. 2012). An additional issue specific to coral reefs is that these ecosystems support multiple primary production sources and highly variable trophic assemblages.

The main goal of this study is to gain an understanding of how different marine and continental organic matter sources are incorporated into coral reef food webs using a simplified food web model. The various compartments of a trophic network (i.e., potential carbon sources and their potential users) ending with three reef fish species were analyzed and combined with a study of fish diet. This limited number of fish species was chosen from key feeding groups, i.e., an herbivore, an omnivore and a carnivore. This choice was made to reduce the complexity of coral reef fish food webs and to establish a simple model that would still allow for the quantification of differences in trophic position and differences in the type and origin (marine or continental) of used organic matter. Moorea is a high island with permanent rivers of small catchment areas widely used for agricultural practices. As terrestrial primary producers most often have lower $\delta^{13}C$ values than marine producers, the origin of carbon in the particulate organic matter pools of coastal environments can be traced (Haines and Montague 1979; Riera and Richard 1996; Bouilllon et al. 2000). The demonstration of the trophic linkages between river runoffs and coastal food webs has mainly concerned large rivers with strong mean annual flows (Riera et al. 2000; Darnaude et al. 2004), and this point remains largely unknown in coral reefs ecosystems. Usually, coral reefs flourish in oligotrophic waters. However, sporadic rainy events and hurricanes can have a strong impact on river runoffs on coastal coral reefs. In addition, land-use practices, such as urbanization and/or deforestation for agricultural purposes, most likely increase quantities and change the 'quality type' of continental inputs on coral reefs. It is thus necessary to understand how organic matter from continental origin may enter coral reef food webs. The questions addressed in this study were (1) to determine whether or not organic matter of continental origin was incorporated into the fringing coral reef food webs and transferred up to the selected fishes, (2) to assess the pathway of this potential incorporation by comparing fish diets and isotopic ratios and (3) to assess the spatial variability in these processes in two contrasted bays subject to different levels of anthropogenic activities.

Materials and methods

Study area

This work was carried out on the fringing coral reefs of the two bays of the north shore of Moorea Island, French Polynesia (Fig. 1). In each bay, three zones were sampled along an increasing gradient of confinement: the entrance (seawards), the middle and the end (nearshore) of the bay (Fig. 1). These two bays are subject to contrasted anthropogenic influences. Opunohu Bay is characterized by a low human impact. However, a small shrimp farm is located at the mouth of Opunohu River, providing nitrogen enrichment at the end of the bay through its waste outputs (Lin and Fong 2008), and scattered agricultural activities occur further upstream in the valley. The river flow is highly variable depending on seasonal and sporadic rainy events

(average: $\sim$30 l s^{-1}; Lafforgue and Robin 1986). Cook's Bay is subject to a stronger human impact with a higher human population density and reduced sewage treatment capacity (houses have septic tanks, at best). In addition, the valley supports large and intensive pineapple fields and the Cook River generates relatively high terrigenous inputs (mean flow of $\sim$130 l s^{-1}; Lafforgue and Robin 1986).

Sampling

Sampling was performed in March 2009. In all zones of both bays, fish were collected by spear that enabled the capture of small fish. The targeted fish species were *Stegastes nigricans* (Pomacentridae; herbivore), *Chaetodon citrinellus* (Chaetodontidae; omnivore) and *Epinephelus merra* (Serranidae; carnivore). These species are sedentary, site attached and, for *S. nigricans*, strongly territorial. The probability of fish migration among sites was thus assumed to be very low. The fish chosen were all large adults to avoid possible variations in isotopic ratios due to fish size. About 5–10 g of the dorsal white muscle of each fish caught was collected and frozen for stable isotope analysis (see below). Their stomachs were extracted and immediately frozen for further diet analyses. The study of prey eaten, as identified through stomach content analysis, was preferred over collecting fresh prey items in the field, for two reasons: (1) the study of the entire macrobenthic communities in both bays was not feasible, and (2) the feeding selectivity of these fish species in Moorea is not known potentially generating significant gaps between potential food and real diet. Prey found in fish stomach contents were sorted by taxonomic group, rinsed with distilled water and frozen for stable isotope analysis. As digestive process occurs differentially for different component of prey (carbohydrates are digested faster than proteins), undigested prey (i.e., recently consumed) only were retained for stable isotope analysis.

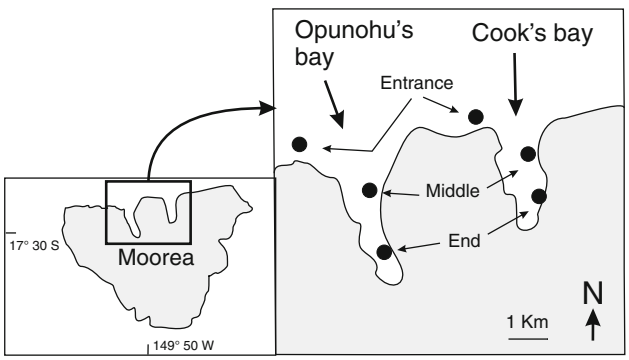

Fig. 1 Location of the sampled sites (*dots*) in the two bays of Moorea Island, French Polynesia

The main potential carbon sources in the bays were sampled and treated for stable isotope analysis. These sources are the particulate organic matter (POM), including 'phytoplankton,' present in marine subsurface seawaters, and the widely dominant algal 'turfs' growing on fringing reefs. Subsurface seawater was collected in each zone (three samples per zone) and filtered using preweighed Whatman GF/F filters precombusted for 4 h at 450 °C. According to previous studies (Rau et al. 1990; Rolff and Elmgren 2000), the 63–200-µm-sized fraction was considered to be the best proxy for analyzing the main phytoplankton components of the community. Because POM progressively sinks to the sea floor, where benthic primary production also possibly occurs, the surface sediments were sampled and treated for stable isotope analysis (sediment organic matter: SOM). Three samples of algal turfs and sediments were collected at each zone in both bays. In addition, continental inputs (i.e., terrestrial plant detritus floating in the river and freshwater POM) were sampled in March 2012 in both rivers. They were assumed to be representative of these freshwater sources in 2009 as well, as other studies have indicated that interannual variability in river POM isotopic ratios is low compared with seasonal variations when the level of rainfall is similar between years and land-use practices in catchments areas have not changed (Hélie and Hillaire-Marcel 2006; Harmelin-Vivien et al. 2010), as was the case in the present study.

Diet analysis

Stomach contents of *Stegastes nigricans* ($n = 60$), *Chaetodon citrinellus* ($n = 48$) and *Epinephelus merra* ($n = 9$) were analyzed. Prey items were identified and counted under a binocular microscope. They were sorted to class or order levels and weighed to the nearest 0.01 mg (wet mass). Diet composition allowed the calculation of the trophic level (TRL) for all species. The TRL value expresses the position of a given organism within the food web of an ecosystem (Pauly and Christensen 1995):

$$\mathrm{TRL}_i = 1 + \sum_{j=1}^{n} \mathrm{DC}_{ij} \times \mathrm{TL}_j$$

where i is the fish species (*S. nigricans*, *C. citrinellus* or *E. merra*), j the nth prey, and DC_{ij} the diet composition expressing the fractions (mass) of each j in the diet of i and TRL_j the trophic level of the jth prey (Pauly et al. 2001). The variance of the TRL estimate, referred to as the omnivory index (OI), is calculated as follows (Pauly et al. 2001):

$$\mathrm{OI}_i = 1 + \sum_{j=1}^{n} \mathrm{DC}_{ij} \times (\mathrm{TRL}_j - \mathrm{TRL}_{\mathrm{preys}})^2$$

where TRL_{prey} is the average TRL of the n prey groups of the i fish species. The standard error (SE) of TRL is the square root of OI. Mean TRL of prey were defined according to values found in the literature (Bautista-Vega et al. 2008).

We also calculated TRL of each fish species with the formulae adapted from Baladamenti et al. (2002): $TRL_i = [(\delta^{15}N_i - \delta^{15}N_{ref})/\Delta\delta^{15}N_c] + a$, where i is the fish species, $\delta^{15}N_i$ is the isotopic ratio (nitrogen) for species i, $\delta^{15}N_{ref} = [\Sigma\ (\delta^{15}N_{prey} \times DC(mass\%)_{prey})]/100$, $\Delta\delta^{15}N_c$ is the average increase in $\delta^{15}N$ between the prey and its consumer (the conventional value of 3.4 ‰ is used), and a is the trophic level of the main prey type for the i fish species. We replaced %IRI used by Badalamenti et al. (2002) by DC(mass%), as food item like turf and filamentous algae cannot be counted.

Stable isotope analysis

Carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were analyzed on dorsal white muscle for all fishes collected, as this tissue gives the most reliable values (Pinnegar and Polunin 1999). Water POM samples collected on GF/F filters were freeze-dried and cut into small pieces. Animal and SOM samples were freeze-dried and ground into a fine powder (<6 μm) using a mortar and pestle. Samples (1 mg) of soft tissues of small invertebrates and large crustaceans (shrimps and brachyurans) and fish were analyzed with no prior treatment. The same samples were analyzed for lipid contents in another study, yielding low values that fluctuated between 0.25 % for *S. nigricans* and 0.32 % for *E. merra* (YL pers comm). In addition, C/N ratios did not vary significantly between fish species (average: 3.19 ± 0.07) or between the other components (POM, SOM, algae and invertebrates). So, lipid content did not play a role in the carbon isotope differences between species and sites and did not influence their variability. For small crustaceans and polychaetes, individuals were pooled to obtain enough material for analysis. For crustaceans having hard calcareous structures (shrimps and crabs) as well as for POM and SOM, two subsamples were analyzed. One was treated for $\delta^{13}C$ analysis, after acidification by 1 % HCl solution to remove carbonates, rinsed with distilled water and oven-dried at 40 °C for 24 h, as carbonates present higher $\delta^{13}C$ than organic carbon (De Niro and Epstein 1978). The other subsample for nitrogen isotope analysis was not acidified because acidification results in enrichment in $\delta^{15}N$ (Pinnegar and Polunin 1999).

The ^{13}C:^{12}C and ^{15}N:^{14}N ratios were measured by continuous-flow isotope-ratio mass spectrometry. The spectrometer (Delta V Advantage stable isotope analyzer, Thermo Scientific, Bremen, Germany, with Flash EA-1112 elemental analyzer, Thermo Scientific, Milan, Italy) was operated in dual isotope mode. The analytical precision, estimated from standards analyzed along with the samples, was <0.1 ‰ for $\delta^{13}C$ and <0.15 ‰ for $\delta^{15}N$. Reference gas and the internal standard used (acetanilide, Thermo Scientific) were calibrated against reference materials (USGS-24, IAEA-CH6, IAEA-600 for carbon; IAEA-N1, -N2, -N3, -600 for nitrogen). Isotope ratios were expressed as parts per 1000 (‰) differences from a standard reference material:

$\delta X = [(R_{sample} \times R^{-1}{}_{standard}) - 1] \times 10^3$; where X is ^{13}C or ^{15}N, R is the corresponding ratio (^{13}C:^{12}C or ^{15}N:^{14}N) and δ is the proportion of heavy to light isotope in the sample. The international standard references are Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N_2 for nitrogen.

Data analysis

Variation in stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of fish muscle, POM, SOM and algal turfs were tested by nonparametric two-way (bay × zone) Kruskal–Wallis ANOVAs. When a significant difference was demonstrated, Mann–Whitney post hoc U tests were run to identify groups without significant differences. Variation in stable isotope ratios of freshwater POM and river detritus between rivers were tested by non-parametric U tests.

Influence of organic matter (OM) sources on POM and consumption of prey by fish was assessed using Bayesian mixing models. These models calculate the most feasible solutions that could explain isotopic ratios measured for POM or fishes and allow the integration of all uncertainties linked to sources of OM or consumers and with trophic enrichment factors. The package SIAR (Parnell et al. 2010) was used. For POM, local river POM and marine POM values were computed to assess the differential influence of OM from continental and marine origin in each bay and location. The values used for marine POM were $\delta^{13}C = -20.89 \pm 0.34$; $\delta^{15}N = 7.23 \pm 0.44$ (YL pers comm). Due to the complexity of SOM, no successful calculation could be performed on this pool.

A Bayesian mixing model was also used to confirm the importance of food items (algal turf, filamentous algae, invertebrates and fishes) in the diet of the three fish species. All isotopic ratios measured for each species were pooled regardless of the sampling site before applying the model. For *C. citrinellus* and *S. nigricans*, only food items observed in stomach content were incorporated into the model. For *E. merra*, mean $\delta^{13}C$ and $\delta^{15}N$ values representative of *C. citrinellus* and *S. nigricans* were calculated ($\delta^{13}C = -12.14 \pm 2.22$; $\delta^{15}N = 9.32 \pm 1.20$) and added to the model, as *E. merra* is known to occasionally feed on these species. Even if some digestible prey could be missed

out in the stomach contents, the use of only observed or theoretical food items allows the constraint of the mixing model to a reasonable number of actually consumed end-members. A major issue with the use of mixing models lies in the choice of trophic enrichment factors (TEFs), as the outputs of the models can be strongly influenced by the use of incorrect TEFs (Bond and Diamond 2011). While the average trophic fractionation for carbon is around 1 ‰, it can vary between −3 and +5 ‰. It also tends to be higher for herbivores than carnivores (Mill et al. 2007). Similarly, it can be very challenging to decipher differences in consumer carbon isotope values due to changes in diet/trophic position from those due to changes in the base of the food web signature. This is particularly true for omnivores and systems with multiple or variable basal food web carbon sources. An alternative is to move toward compound-specific stable isotope analyses that provide direct evidence of the carbon isotopes at the base of the food web as well as more robust trophic positions (McClelland and Monotya 2002; McMahon et al. 2010, 2011). Previous works reported different TEFs between trophic groups (Wyatt et al. 2010), with higher TEFs for herbivorous fish (Mill et al. 2007). These differences were considered by using specific TEF for each species and isotope, in relation to fish feeding habits (*S. nigricans*: 2.0 ‰ for C, 4.6 ‰ for N; *C. citrinellus*: 2.0 ‰ for C, 3.4 ‰ for N; *E. merra*: 1.0 ‰ for C, 2.0 ‰ for N). The default 0.5 standard error value, associated with TEFs in SIAR, was maintained, as no specific value could be found.

Results

Spatial variation in $\delta^{15}N$ and $\delta^{13}C$ values of organic matter sources

Continental inputs and marine POM

Freshwater POM displayed N-enriched and C-depleted values in Cook river compared with those found in Opunohu River (Table 1), although statistical significance was found only for $\delta^{15}N$ (*U* test, $p = 0.016$) but not for $\delta^{13}C$ (*U* test, $p = 0.063$). A similar pattern was found for terrestrial plant detritus brought by rivers, with significant differences for both $\delta^{15}N$ and $\delta^{13}C$ (*U* tests, $p = 0.038$ and $p = 0.042$, respectively). For marine POM, $\delta^{15}N$ values ranged from 6.76 ± 0.41 to 7.25 ± 0.10 ‰ between bays and zones (Table 2). No significant difference was found between bays, zones or bays × zones, although higher values were found at the entrance of each bay (Table 2). The $\delta^{13}C$ values ranged from -23.21 ± 0.55 to -20.58 ± 0.05 ‰, with no significant difference between bays and bays × zones. However, a significant difference was found ($p = 0.0043$) between zones, with $\delta^{13}C$ values decreasing from the entrance to the end of each bay (Table 2). The mixing model demonstrated a decrease in the influence of river POM from the end to the entrance (Fig. 2). However, this trend differed between bays, as about 50 % of POM originated from the river at the end of Cook's bay, while only about 20 % of POM was river derived at the end of Opunohu Bay (Fig. 2).

Table 1 Mean (±SD) $\delta^{15}N$ and $\delta^{13}C$ values in freshwater POM and macrodetritus (tree leaves, etc.) found in rivers ending in the studied bays in Moorea

	Opunohu' river	Cook' river	*p*
Freshwater POM			
$\delta^{15}N$ (‰)	4.20 (1.17)	7.06 (0.65)	*
$\delta^{13}C$ (‰)	−26.67 (0.47)	−24.11 (1.81)	ns
Plant detritus			
$\delta^{15}N$ (‰)	1.31 (1.24)	5.02 (1.99)	*
$\delta^{13}C$ (‰)	−30.85 (0.84)	−27.42 (2.04)	*

$n = 3$ for each case

Statistical significance: *ns* nonsignificant, * $p < 0.05$

Surface sediments

The $\delta^{15}N$ values ranged from 4.22 ± 0.92 to 5.42 ± 0.39 ‰ between bays and zones (Table 2). The $\delta^{15}N$ values were higher in Cook's Bay at all zones ($p < 0.05$ in all cases) and displayed a significant ($p < 0.01$) increase from the entrance to the end of each bay. The $\delta^{13}C$ values ranged from -22.55 ± 1.11 to -15.75 ± 0.32 ‰ (Table 2); this latter value was found at the entrance of Cook's Bay and was high compared with all other values. The $\delta^{13}C$ values were similar to those found in POM, although the decrease from the entrance to the end of the bay was significant only in Opunohu (Table 2).

Algal turfs

The $\delta^{15}N$ values of algal turfs ranged from 3.47 ± 0.18 to 5.65 ± 1.21 ‰ (Table 2). These values were significantly different depending on zones ($p = 0.0043$) and bays × zones ($p = 0.0009$), but not between bays ($p > 0.05$). In particular, the $\delta^{15}N$ values were higher at the end of the bay on Opunohu, but no clear trend appeared in Cook's Bay (Table 2). The $\delta^{13}C$ values ranged from -18.29 ± 2.02 to -13.49 ± 2.48 ‰ depending on the bay and the zone, with a significant ($p = 0.0061$) decrease from the entrance to the end of each bay (Table 2). Differences found between bays and zones × bays were not statistically significant ($p > 0.05$).

Table 2 Mean (±SD) $\delta^{15}N$ and $\delta^{13}C$ values in water POM, SOM and algal turfs in the two studied bays in Moorea

Position in the bay		POM			SOM			Algal turfs		
		Opunohu	Cook	*p*	Opunohu	Cook	*p*	Opunohu	Cook	*p*
Entrance	$\delta^{15}N$ (‰)	7.21 (0.69)	7.25 (0.20)	ns	4.22 (0.92)	4.40 (0.46)	*	3.47 (0.18)	5.65 (1.21)	**
	$\delta^{13}C$ (‰)	−21.19 (0.11)	−20.58 (0.05)	*	−20.34 (1.43)	−15.75 (0.32)	***	−13.95 (1.89)	−13.49 (2.48)	ns
Middle	$\delta^{15}N$ (‰)	6.76 (0.41)	6.99 (0.22)	ns	4.68 (0.71)	4.72 (0.33)	*	4.97 (0.86)	4.18 (0.72)	ns
	$\delta^{13}C$ (‰)	−21.72 (0.27)	−20.93 (0.44)	ns	nd	−20.56 (3.91)		−14.05 (1.88)	−15.42 (0.88)	ns
End	$\delta^{15}N$ (‰)	6.83 (0.27)	6.93 (0.59)	ns	4.77 (0.49)	5.42 (0.39)	*	5.60 (0.14)	5.38 (0.39)	ns
	$\delta^{13}C$ (‰)	−22.05 (0.18)	−23.21 (0.55)	ns	−22.55 (1.11)	−20.07 (1.54)	ns	−18.29 (2.02)	−16.09 (2.65)	ns
Significance of position	$\delta^{15}N$ (‰)	ns	ns		**	**		*	ns	
	$\delta^{13}C$ (‰)	**	**		*	ns[a]		**	**	

$n = 3$ for each case

nd no data

Statistical significance: *ns* nonsignificant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

[a] Comparison between middle and end of the Cook's bay

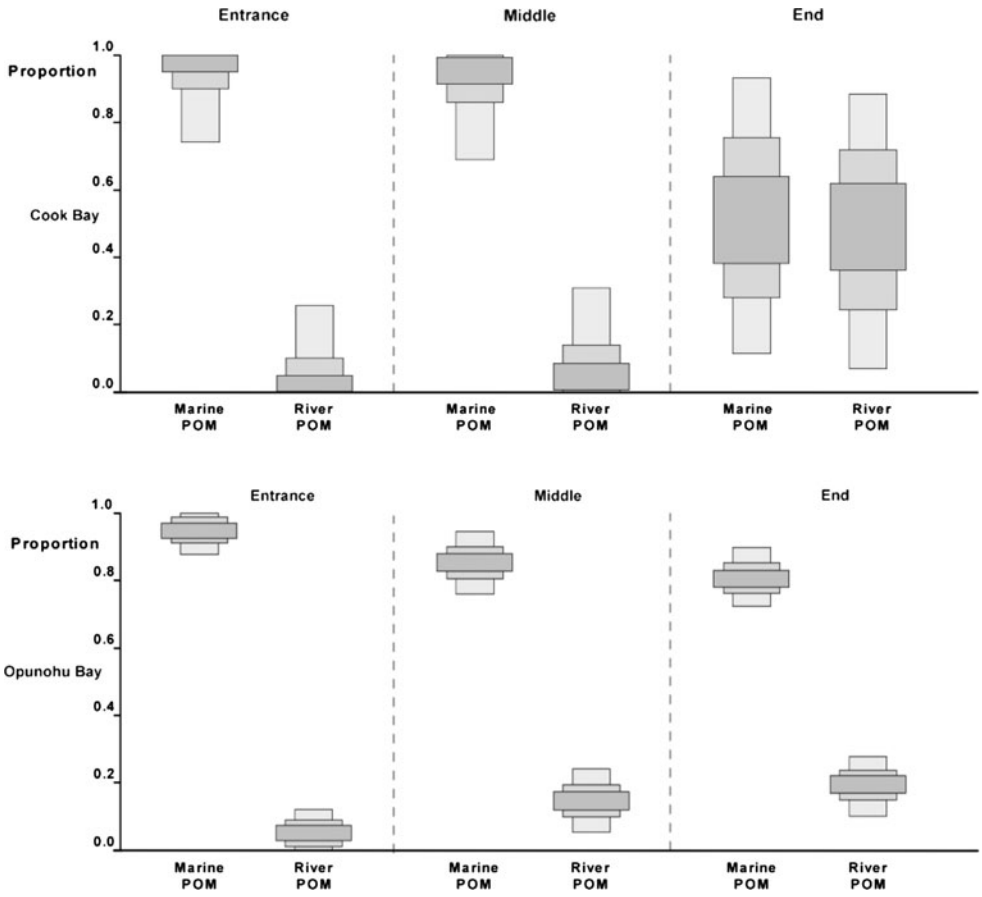

Fig. 2 Relative importance of freshwater and marine particular organic matter (POM) in the POM isotopic signature at the entrance, middle and end of each bay. *Shaded boxes* represent 50, 75 and 95 % Bayesian credibility intervals from *dark* to *light gray*

Overall, when all zones and bays were considered together, the $\delta^{15}N$ values of POM were significantly higher than those of SOM and algal turfs (*U* test; $p < 0.01$), while higher $\delta^{13}C$ values were found in algal turfs than in POM and SOM (*U* test; $p < 0.01$) (Table 2).

Isotopic ratios of fish prey

The main prey consumed by the fish species exhibited different stable isotope ratios (Table 3). Polychaetes and small crustaceans (copepods, mysids and isopods together)

displayed lower $\delta^{15}N$ values than large crustaceans (shrimps and brachyurans) and fish eggs, although significance could not be tested due to the absence of replicates. An opposite trend was found for $\delta^{13}C$ values, with small invertebrates having higher $\delta^{13}C$ values than larger ones (Table 3). Filamentous algae, eaten only by *C. citrinellus*, were separated from 'turf algae' during stomach content analysis. Their isotopic ratios differed from the isotopic ratios of the algal turf, exhibiting significantly higher $\delta^{15}N$ values ($p = 0.0187$) and higher (although not significantly) $\delta^{13}C$ mean values (Table 3).

The mixing model confirmed the relative importance of various prey items in the isotopic signatures of fish species, compared with stomach contents (Fig. 3). However, there is no direct equivalence between percentages in mass of prey eaten (Table 4) and their contributions to isotopic signatures. In *S. nigricans,* turfs accounted for ~85 % of stomach contents, but only ~30 % of isotopic signatures (Fig. 3). By contrast, sediments accounted for ~11 % of stomach contents and ~40 % of isotopic signatures. In *C. citrinellus*, filamentous algae accounted for ~75 % of stomach contents but only for ~50 % of fish isotopic signatures, whereas fish eggs represented ~15 % of stomach contents and accounted for ~35 % of isotopic signatures (Fig. 3). For *E. merra*, the role of small fish species such as *C. citrinellus* or *S. nigricans* was demonstrated as their mean contribution in the diet was ~20 %.

Fish diet and trophic level

A Kruskal–Wallis two-way ANOVA did not reveal any statistical difference in diet between bays and zones for *Stegastes nigricans* or *Chaetodon citrinellus*. Fish diets at all sampling sites were thus pooled for subsequent analyses. This test was not run for *Epinephelus merra* because this species was caught only at the entrance of each bay. The diets of *S. nigricans* and *C. citrinellus* were dominated by fleshy algae (Table 4). The only other important prey item for *S. nigricans* was sediment plus undetermined material, whereas *C. citrinellus* exhibited a relatively high percentage of fish eggs. For both species, the amount of invertebrate matter in the diet was low. The trophic levels calculated for both species were close, irrespective of the method used (Table 4). The prey items of *Epinephelus*

Table 3 Mean (±SD) $\delta^{15}N$ and $\delta^{13}C$ values in some fish preys in the two studied bays in Moorea (data pooled all sites together)

Type of prey	*N*	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Filamentous algae	9	6.23 (1.24)	−13.90 (0.83)
Polychaetes	1	3.68	−9.47
Nudibranchs	1	6.28	−2.61
Small crustaceans	1	4.86	−8.90
Crabs	4	5.76 (0.31)	−12.53 (1.43)
Shrimps	4	6.54 (0.38)	−16.18 (0.65)
Fish eggs	3	7.93 (0.20)	−12.01 (0.17)

N number of samples analyzed

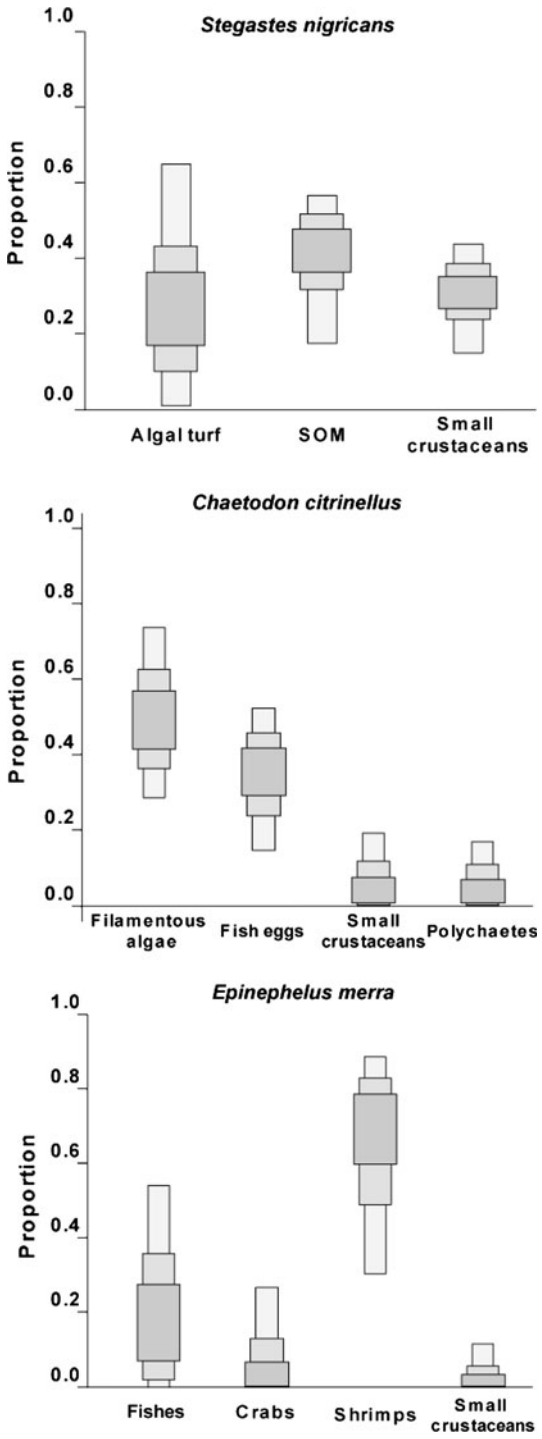

Fig. 3 Relative importance of the various prey items in the isotopic signatures of the three fish species studied (all sites and bays pooled together). *Shaded boxes* represent 50, 75 and 95 % Bayesian credibility intervals from *dark* to *light gray*

Table 4 Importance of prey types in the mean diet of *Stegastes nigricans*, *Chaetodon citrinellus* and *Epinephelus merra*, all sampled sites pooled together, expressed as per cent mass (±SD)

	Stegastes nigricans ($n = 48$)	*Cheatodon citrinellus* ($n = 21$)	*Epinephelus merra* ($n = 4$)
Fleshy algae	86.4 (24.6)	75.2 (38.7)	
Sipunculids	0.1 (0.1)	0.2 (0.3)	
Polychaetes		0.8 (1.5)	
Plathelminths	0.1 (0.1)	0.7 (1.6)	
Gastropods	0.2 (0.4)		
Nudibranchs	0.1 (0.3)	0.1 (0.3)	
Copepods	0.8 (0.4)	0.1 (0.2)	
Mysids	0.1 (0.1)		
Isopods	0.1 (0.1)		
Shrimps			79.0 (38.5)
Brachyurans			21.0 (10.1)
Fish eggs	0.1 (0.1)	14.6 (12.8)	
Fish scales	0.3 (0.4)	8.3 (2.7)	
Undetermined + sand	11.7 (25)		
Mean (s.e.) TRL(a) (Pauly's method)	2.11 (1.05)	2.24 (1.18)	3.50 (0.10)
TRL(b) (Badalamenti' method)	2.21	2.24	3.99

n number of individuals having a nonempty stomach, *TRL* trophic level

merra were shrimp (79.0 % in mass) and crabs (21.0 %), thus yielding a higher trophic level than for the other species and with a higher difference between methods of calculation than for the two other species (Table 4).

Spatial variation in fish $\delta^{15}N$ and $\delta^{13}C$ values

In *S. nigricans*, $\delta^{15}N$ values ranged from 7.57 ± 0.46 to 9.74 ± 0.45 ‰, with significant differences depending on bay, zone and bay × zone (Kruskal–Wallis ANOVA; $p < 0.0001$ in each case) (Table 5). Differences between bays were significant at the entrance and end of the bays, but not in the middle. $\delta^{15}N$ ratios significantly increased (post hoc *U* test; $p < 0.001$) from the entrance to the end of Opunohu Bay, but this pattern was less defined at Cook's Bay (Table 5). The $\delta^{13}C$ values, ranging from −15.09 ± 0.31 to −10.72 ± 0.72 ‰, differed significantly between bays (Kruskal–Wallis ANOVA; $p = 0.0014$), zones and bays × zones ($p < 0.0001$ in both cases). These differences were significant (post hoc *U* test; $p < 0.001$), whereas the difference between the entrance and the middle of the bay was not significant in Cook's Bay (Table 5).

In *C. citrinellus*, $\delta^{15}N$ values ranged from 8.84 ± 0.34 to 11.86 ± 0.43 ‰. $\delta^{15}N$ was higher in *C. citrinellus* than in *S. nigricans* except at the entrance of Cook's Bay (averaged value per species: 10.11 ± 1.06 vs. 9.03 ± 0.96 ‰; *t* test, $p = 0.0002$). The $\delta^{15}N$ values differed significantly depending on zone (Kruskal–Wallis ANOVA; $p < 0.0001$), bay ($p = 0.0441$) and bay × zone ($p = 0.0001$). In addition, there was an increasing trend from the entrance to the end of the bay at Opunohu (post hoc *U* test; $p < 0.0001$) (Table 5). The $\delta^{13}C$ values, ranging from −14.51 ± 0.99 to −8.51 ± 0.86 ‰, exhibited significant differences between bays, zones and bays × zones (Kruskal–Wallis ANOVA; $p < 0.01$), with a significant (post hoc *U* test; $p < 0.001$) decrease in values from the entrance to the end of each bay (Table 5). In *C. citrinellus,* $\delta^{13}C$ values were higher than in *S. nigricans* except in the middle of Cook's Bay (averaged value per species: −11.12 ± 2.13 vs. −13.12 ± 1.66 ‰, respectively; *U* test, $p < 0.0001$).

For *E. merra*, $\delta^{15}N$ values were significantly higher in Cook's Bay than in Opunohu Bay ($p = 0.0478$), with 12.78 ± 1.25 ‰ ($n = 4$) and 11.14 ± 0.52 ‰ ($n = 5$), respectively. Conversely, $\delta^{13}C$ values were significantly higher ($p = 0.0459$) in Opunohu (−13.77 ± 0.76 ‰) than in Cook's Bay (−15.07 ± 1.06 ‰). The overall mean values ($n = 9$) were 11.96 ± 1.16 ‰ for $\delta^{15}N$ and −14.03 ± 1.07 ‰ for $\delta^{13}C$ and were significantly different from those of the other two species (at the entrance zones where the only samples of *E. merra* were collected) (*U* test; $p < 0.001$ for both $\delta^{15}N$ and $\delta^{13}C$ values).

Reconstruction of simplified food webs ending with the three fish species

The major sources of organic matter in these food webs (algal turfs, SOM and POM) had different isotopic ratios (Fig. 4; Table 2). The position of some invertebrates (polychaetes, small crustaceans and nudibranchs) in the trophic network is ambiguous, possibly due to the lack of replication. Their $\delta^{15}N$ values were low compared with those of the potential sources. Based on an averaged enrichment in N of about 3.4 ‰ and of about 1–1.5 ‰ for C, the C and N isotopic ratios of invertebrates and fish

Table 5 Mean (±SD) $\delta^{15}N$ and $\delta^{13}C$ values in *Stegastes nigricans*, *Chaetodon citrinellus* and *Epinephelus merra* in the two studied bays in Moorea

Position in the bay		*Stegastes nigricans*			*Chaetodon citrinellus*			*Epinephelus merra*		
		Opunohu	Cook	*p*	Opunohu	Cook	*p*	Opunohu	Cook	*p*
Entrance	$\delta^{15}N$ (‰)	7.57 (0.46)	9.57 (0.55)	***	8.84 (0.34)	9.33 (0.87)	***	11.14 (0.52)	12.78 (1.25)	*
	$\delta^{13}C$ (‰)	−10.72 (0.72)	−13.02 (1.19)	*	−8.51 (0.86)	−9.94 (0.90)	**	−13.77 (0.76)	−15.07 (1.06)	*
Middle	$\delta^{15}N$ (‰)	9.28 (0.57)	9.15 (0.67)	ns	10.39 (0.41)	11.02 (0.51)	**	nd	nd	
	$\delta^{13}C$ (‰)	−12.71 (0.96)	−12.99 (0.61)	ns	−11.01 (0.68)	−14.51 (0.99)	**	nd	nd	
End	$\delta^{15}N$ (‰)	9.41 (0.70)	9.74 (0.45)	*	11.86 (0.43)	nd		nd	nd	
	$\delta^{13}C$ (‰)	−15.09 (0.31)	−14.52 (0.67)	*	−14.42 (0.54)	nd		nd	nd	
Significance of position	$\delta^{15}N$ (‰)	***	*		***	***				
	$\delta^{13}C$ (‰)	**	**		***	***				

$n = 10$ for each case, except for *C. citrinellus* Opunohu-end ($n = 7$) and Cook-middle ($n = 5$) and for *E. merra* ($n = 5$ for both bays)

nd no data

Statistical significance: *ns* nonsignificant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

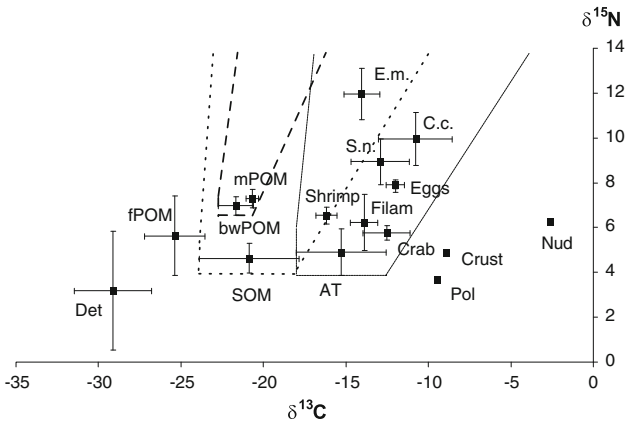

Fig. 4 Plot of $\delta^{13}C$ versus $\delta^{15}N$ (mean ± SD) for the various compartments and taxa on the fringing reefs at Moorea. fPOM = freshwater particulate organic matter, Det = freshwater macrodetritus, SOM = sediment organic matter, bwPOM = particulate organic matter of bay water, mPOM = marine particulate organic matter, AT = algal turf, Pol = polychaetes, Crust = small crustaceans, Nud = nudibranchs, Filam = filamentous algae, Eggs = fish eggs, S.n. = *Stegastes nigricans*, C.c. = *Chaetodon citrinellus* and E.m. = *Epinephelus merra*. *Dotted lines* [corresponding to trophic increases of +1 ‰ in $\delta^{13}C$ and +4.5 ‰ in $\delta^{15}N$ (*left line*) and of +2 ‰ in $\delta^{13}C$ and +2.5 ‰ in $\delta^{15}N$ (*right line*)] show the limits of the isotopic signature ranges expected for trophic transfer of the three major sources of organic matter

suggested that the food webs on these fringing coral reefs are mainly based on algal turfs and on SOM, whereas the direct use of seawater POM appeared to be of low importance (Fig. 4).

Discussion

There is only a small body of existing work using stable isotope ratios in studies on trophic pathways of coral reefs (Cocheret de la Morinière et al. 2003; Greenwood et al. 2010; Wyatt et al. 2012). Isotopic ratios (C and/or N) have usually been determined for a given and limited trophic compartment such as algae (Lin and Fong 2008), seagrass (Yamamuro et al. 2003) or fish (Greenwood et al. 2010; McMahon et al. 2011). Conversely, little has been done at the scale of a coral reef ecosystem, except a recent paper by Wyatt et al. (2012) across a fringing coral reef. Thus, the present study of a simplified food web is an important step in filling the gap of assessing more complex coral reef food webs using stable isotope analyses.

Sources of organic matter and their incorporation into benthic food webs

The subsurface seawater POM exhibited a high $\delta^{15}N$ value compared with SOM and algal turfs and did not appear to be directly used by the organisms studied here. As the sampling was carried out during the rainy summer season, continental inputs within both bays may have generated an unusual signal. Indeed, agricultural or aquaculture activities may have enriched waters in nitrogen (Lin and Fong 2008). The 'rainy summer signal' requires ~3 months for integration into the food web, so this $\delta^{15}N$ value may have become measurable in March, when we collected our samples. High $\delta^{15}N$ values in POM may also have resulted from microzooplankton as the inclusion in samples of small primary consumers feeding partly on POM (Carassou et al. 2008) usually increases $\delta^{15}N$ values. This does not mean that POM is not used by unsampled organisms on the studied coral reefs. For instance, phyto- and zooplankton-feeders may feed widely on this source of OM, and future research on these feeding groups will likely support this prediction.

Algal turfs appeared to be a major source of organic matter for two of the three fish species studied, although

the role of SOM as a complementary source for herbivores and omnivores is also plausible. However, the results from the mixing model indicated that turfs, although an important OM source, might be overestimated through a classical diet analysis. Algal turfs might have a low nutritional value that could be complemented by SOM comprising fish feces, microphytobenthos and/or small algal fragments. Sedimented detritus can be an additional food source for these species as observed in other coral reef fishes (Wilson and Bellwood 1997; Crossman et al. 2001). The discrepancy between the importance of SOM in the results of the mixing model and its low contribution to the diet could be because some nutritive components of SOM are more easily assimilated. In particular, sediments ingested by *S. nigricans* also contain some essential fatty acids (Hata and Umezawa 2011). Dromard et al. (2013) also found that, while turf algae constitute the main food ingested by *Stegastes adustus*, they represent only 25 % of the assimilated food and sedimented detritus 30 %. Additionally, while *S. nigricans* and *C. citrinellus* feed mainly on fleshy algae (Letourneur et al. 1997; Pratchett and Berumen 2008), mixing models have indicated an important assimilation of animal matter and/or detritus, as observed in other *Stegastes* spp. (Crossman et al. 2001; Dromard et al. 2013).

In contrast, there was less discrepancy between the diet and stable isotope analyses for *E. merra*. *E. merra* preyed on shrimps that are known to be partly detritivorous in estuarine environments (Riera et al. 2000), which might explain the low $\delta^{13}C$ values of this fish species. As *E. merra* is known to feed mainly on large benthic invertebrates such as crabs and shrimps in addition to fish (Kulbicki et al. 2005), its high $\delta^{15}N$ value appears consistent when compared to the other two fish species. Adding a small fish prey category in the mixing model improved the understanding of the isotopic signature of the species, and could explain the discrepancy between TRL issued from stomach contents (Table 1) and its position in Fig. 4.

The discrepancies between stomach contents and stable isotope analyses also highlighted differences between the processes involved. Stomach contents give information on eaten items at a more or less instantaneous time scale, whereas stable isotope signatures result from metabolic processes on a $\sim$3 months temporal scale, i.e., not only what was ingested but also what was incorporated in fish tissue after ingestion and assimilation steps.

Spatial differences within and between the two bays

Significant interactions found in Table 2 suggest that the results may depend on site location. This could potentially be important because factors such as different regimes of runoffs or hydrodynamics according to site or bay might cause differences in the quantity and composition of OM available among bay locations. Consequently, different isotopic values might be found for different runoffs or hydrodynamics conditions at least for sources of OM.

A gradient in C and N isotopic values was demonstrated from the entrance to the end of each bay for the sources of OM and fish species. Lower $\delta^{13}C$ values and higher $\delta^{15}N$ values were measured at the end of each bay, compared with the entrance. This gradient was strong for POM and algal turfs and was also demonstrated for *Stegastes nigricans* and *Chaetodon citrinellus*. Differences were also observed between bays. Cook's Bay was, on average, more C-depleted and N-enriched than Opunohu Bay. Given that terrestrial primary producers often have lower $\delta^{13}C$ values than marine producers, our results suggest that rivers bring continental material into the two bays, which is partly incorporated into the fringing coral reef food webs, at least at the end of the bays. This incorporation is likely due to the incorporation of dissolved nutrients of terrestrial origin by algae at the end of the bay and to the sedimentation of continental POM and terrestrial detritus at the sediment surface. These forms of detritus are then ingested by some herbivorous or omnivorous invertebrates (shrimps) and fishes (*Stegastes*). These rivers are relatively small, their catchment areas are low (7.7 km^2 for Opunohu and 9.7 km^2 for Cook; Lafforgue and Robin 1986) and the averaged yearly quantity of continental material transported into the bays is highly variable, from 0.1 to 3.3 mg l^{-1} (suspended matter) during the dry season and from 9 to 23 mg l^{-1} during the wet season in Opunohu (Morancy et al. 1995). Continental inputs into Cook's Bay are likely higher due to anthropogenic activities and higher river flux (Lafforgue and Robin 1986), and this is consistent with our findings of Cook's Bay being more C-depleted and N-enriched. The demonstration of the trophic linkages between river runoff and coastal food webs have mainly concerned large rivers with strong mean annual flows (Riera et al. 2000; Darnaude et al. 2004). Our study shows that small areas subjected to moderate runoff may also incorporate continental inputs into food webs at least in sites close to the river mouth. Indeed, the difference in water POM between the entrance and the end of the bay was 0.86 ‰ in Opunohu and 2.63 ‰ in Cook's Bay for carbon. In algal turfs, $\delta^{13}C$ value differences were 4.34 and 2.60 ‰, respectively. These examples, plus the C and N isotopic ratios of continental POM and terrestrial plant detritus, provide evidence that the small rivers of Moorea have a perceptible influence on $\delta^{13}C$ and $\delta^{15}N$ values at a small spatial scale and that this influence persists in the trophic food webs ending with *S. nigricans* and *C. citrinellus*. Given that the diet of these species did not change along the entrance-end gradient, the results found can be directly linked to changes in the C and N isotopic ratios of

their food that may be partly related to continental runoff. As it is widely assumed that ~3 months are needed for fish muscle to integrate the isotopic signal (Mac Avoy et al. 2001), the isotopic variations may also be linked to differences in the amount of continental POM supplied to the coral reef system. The higher transfer of the river inputs into the fringing reef food webs in Cook's Bay was reflected by the low $\delta^{13}C$ values recorded in fishes collected at the end of this bay, in contrast to the higher $\delta^{13}C$ values observed at other sites.

River inputs directly influence what is probably a small area of each bay, but this influence can be transferred within the bays by coastal currents (Wolanski and Delesalle 1995). The extension and intensity of the influence of river inputs usually vary with the season, being higher during summer when the river discharge is high (Morancy et al. 1995). The lower $\delta^{15}N$ values in algal turfs and *Stegastes nigricans* collected in the middle of Cook's Bay may be due to a lower influence of continental POM in this zone or to a higher influence of the low $\delta^{15}N$ water POM in the local food web.

Species that incorporate a larger amount of OM of continental origin usually display lower $\delta^{13}C$ values, as has been shown in a Mediterranean flatfish (Darnaude et al. 2004). However, in Moorea, continental OM originating from the rivers is nevertheless partly incorporated into the food webs of the two bays. Its moderate contribution to the isotopic signatures of the three fish species studied is likely linked with their diet. Polychaetes were identified as the main invertebrate group involved in the transfer of POM of continental origin within trophic networks (Darnaude et al. 2004; Carlier et al. 2007). The species studied in Moorea consume large amounts of fleshy algae (*S. nigricans* and *C. citrinellus*) or crustaceans (*E. merra*) that may explain their low dependence on river inputs.

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