

## Partitioning of Trophic Resources Allows Multi-specific Foraging Groups of Acanthurid Fishes (Guadeloupe, Lesser Antilles)

### La Partición de los Recursos Tróficos Permite la Formación de Grupos Multi-Específicos de Acanthuridae (Guadeloupe, Antillas Menores)

### Le Partage des Ressources Trophiques Permet la Formation de Groupes Multi-Spécifiques d'Acanthuridae (Guadeloupe, Lesser Antilles)

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#### ABSTRACT

Herbivorous fishes control the algal dynamic on coral reefs and are widely exploited by Caribbean fisheries. Among this guild, acanthurids are routinely seen in multi-specific foraging groups and are globally considered as a homogenous functional group. In fact, the different species can display various dietary patterns. In this study, we stated the hypothesis that the formation of such multi-specific groups can be explained by a difference of trophic niche among the acanthurid species. To investigate this fact, a study was conducted on three species of Acanthuridae (*Acanthurus coeruleus*, *A. chirurgus* and *A. bahianus*), common on the reefs of Guadeloupe. Stomach content analyses were coupled with stable isotope analyses (<sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N ratios) to determine their trophic niches. Contributions of sources in fish diet were estimated using a mixing model. Among the three species, *A. coeruleus* showed the most diversified diet and was the only species to ingest benthic invertebrates. Food items were assimilated proportionally to the quantity ingested. *A. chirurgus* ingested an important amount of calcareous macroalgae that was not assimilated proportionally to the quantity ingested. Finally, *A. bahianus* presented an important amount of unidentified matter in its stomach contents and assimilated a high proportion of fleshy macroalgae. These three acanthurids could then coexist in the same foraging group because they ingest and assimilate the food items in different proportions, avoiding competitive interactions for food resources.

KEY WORDS: Acanthuridae, trophic niche, Caribbean reefs

#### INTRODUCTION

On coral reefs, surgeonfishes (Acanthuridae) represent a common and abundant family of herbivorous fishes. They play a major ecological role on the dynamics of benthic communities (Bellwood and Choat 1990, Mumby et al. 2006) and are commonly exploited by Caribbean fisheries (Polunin and Robert 1993). Compared to other tropical regions, the diversity of herbivorous species is relatively low in the Caribbean. Among 38 species of Acanthuridae (Froese and Pauly 2012), only three species are found in the Caribbean: *Acanthurus coeruleus* (Bloch and Schneider, 1801), *A. bahianus* (Castelnau, 1855) and *A. chirurgus* (Bloch, 1787). These three species are routinely observed in multi-species foraging associations (Robertson et al. 1976), defined as “multi-species shoaling association” by Lukoschek and McCormick (2000).

Most studies on multi-species associations or single species fish shoal are focused on the benefits of this feeding strategy that are principally based on two axes: foraging benefits and predator avoidance (Barlow 1974, Morse 1977, Ogden and Lobel 1978, Pitcher et al. 1982, Lukoschek and McCormick 2000). Group feeding can increase individual's ability to catch otherwise unobtainable prey. For example, fish shoals permit the access to defended algal resources of territorial herbivores, like damselfishes, that are not available to individuals (Barlow 1974, Robertson et al. 1979, Foster 1985, Reinthal and Lewis 1986). Foraging in groups may also facilitate earlier detection of predators. Alarmed conspecifics trigger a change in behaviour of other group members to increase vigilance. Fish interpret conspecific feeding as a sign that it is safe to feed, thus spend more time feeding and less maintaining antipredator vigilance (Magurran and Pitcher 1983).

In ecology, the coexistence of several species in a same foraging group raises the question of the resources use by the different species. Feeding behaviours can be described by the definition of the “trophic niche” of each fish species (Elton 1927). As the three fish species share the same environment on reefs, the description of their trophic niche may be important to understand how they coexist without competitive interactions. The trophic niche can be described by digestive contents analyses (Randall 1967, Tilghman et al. 2001, Ferreira and Gonçalves 2006) or direct observations of feeding behaviours on the field, by the counting of “bites” (Francini-Filho et al. 2010, Kopp et al. 2010). However, even if field observations give important information on feeding behaviours, an accurate description of the diet is not allowed by this method. To the contrary, digestive contents analyses identify and quantify the ingested food items with more certainty but present several practical problems (Bearhop et al. 2004). The principal difficulty lies in the ability of herbivorous fishes to triturate and grind their food, due to the adaptation of their digestive anatomy. Indeed, surgeonfishes can have a thick-walled, gizzard-like stomach with an important musculature that allows them to triturate the ingested matter (Ogden and Lobel 1978).

More recently, stable isotope analyses have been proposed to reflect the feeding behaviours of individuals over long periods, corresponding to the period during which the tissues of consumers are synthesized (Bearhop et al. 2004). Thus, it has been argued that niche axes may be determined using stable isotope ratios (Bearhop et al. 2004) and have been formalized in the concept of the “isotopic niche” (Newsome et al. 2007) according to the fact that values measured in consumer tissues are linked to those of their diet with a constant enrichment at each trophic level (Minagawa and Wada 1984). Although isotopic niche is likely to be tightly correlated to the trophic niche, these are not the same and should not be confused (Jackson et al. 2011). Over the last two decades, a number of isotope mixing models have been proposed to identify the relative contributions of food resources to a consumer’s diet (Layman et al. 2012). In this study, we used a concentration-dependant mixing model because of the presence of animal and vegetal items in diets (Phillips and Koch 2002). This model, performed with Stable Isotope Analysis with R (SIAR), is based on a series of related linear equations that utilize Bayesian statistics techniques to identify proportional contributions of sources pools (Parnell et al. 2010).

Thus, while digestive contents gave a snapshot of the diet, stable isotope analyses draw the isotopic niche of an organism and give information on the long-term assimilations of sources. Coupling these two methods provides a powerful tool to determine the trophic niche of fish species. To our knowledge, these two approaches have never been used to describe specific trophic niche among acanthurids. The principal aim of this study was to determine and compare the trophic niche of *Acanthurus coeruleus*, *A. bahianus* and *A. chirurgus*. To do so, we stated the hypothesis that the coexistence of acanthurids fishes in same multi-species foraging associations can be explained by a difference of trophic niche among the three species.

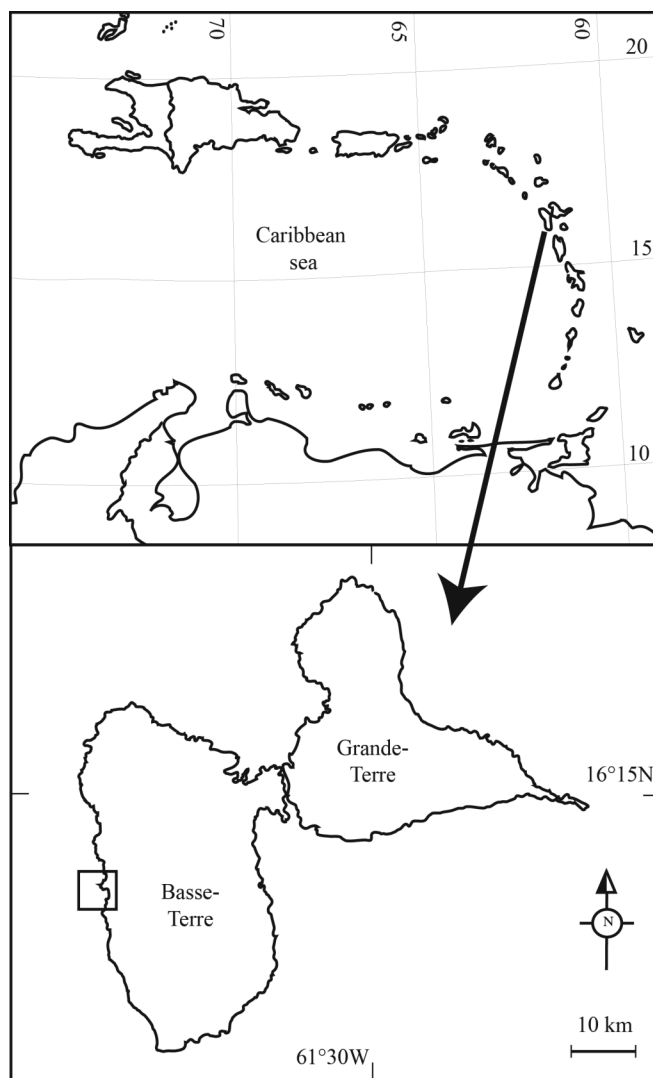
## MATERIAL AND METHODS

### Study Site and Field Samplings

This study was carried out in Guadeloupe, Lesser Antilles (16°30'N; 61°30'W). The studied site was located on the leeward side of the island and represented a surface of approximately 500 m x 100 m approximately (Figure 1). Maximum depth was 15 meters. The substratum was composed of rocky blocks colonized by a non reef-building coral community dominated by *Montastraea annularis* (Ellis and Solander, 1786) and *M. faveolata* coral species (Ellis and Solander, 1786).

Samples were collected along the studied site between September and November 2010. In this study, ten individuals of the three species of surgeonfishes were collected: *Acanthurus coeruleus*, *A. bahianus* and *A. chirurgus* (Table 1). Fish were immediately placed in an icebox to stop enzymatic activities and preserve gut and stomach contents. Mature erect macroalgae, called “macroalgae” in

this study, were hand collected and preserved in a close box. On the studied site, six species of macroalgae were collected: *Dictyota cf pulchella* (Hörnig and Schnetter, 1988), *Acanthophora spicifera* (Vahl, Børgesen 1910), *Tricleocarpa fragilis* (L.), Huisman and Townsend, 1993), *Amphiroa fragilissima* (L.), Lamouroux, 1816), *Udotea flabellum* (Ellis and Solander, Howe, 1904) and *Ventricaria ventricosa* (Agardh), Olsen and West 1988). In addition, five replicates of algal turf, defined as a multi-specific assemblage of algae at a juvenile stage, mixed with small size species (Carpenter 1986, Hay 1981), were scraped and collected with an air sucker connected to a 500 µm meshed collector bag. This method allowed us to sort benthic invertebrates from turf samples and to keep them as a potential food source.



**Figure 1.** Location of Guadeloupe Island, in the Caribbean region and location of the studied area in Guadeloupe.

**Table 1.** Studied fish species, mean total length  $T_L$  in cm (range) and mean wet body mass  $M$  in g (range) of fish.  $n_{DIET}$  is the number of samples used for stomach contents analysis and  $n_{ISO}$  is the number of sample used for isotopic analysis.

Fish species	$n_{DIET}$	$n_{ISO}$	$T_L$ (cm)	$M$ (g)
<i>Acanthurus bahianus</i>	10	7	17.4 (17–19)	107 (90–145)
<i>Acanthurus coeruleus</i>	10	7	22.0 (19–26)	234 (167–289)
<i>Acanthurus chirurgus</i>	10	7	19.0 (16–23)	148 (99–231)

### Digestive Content Analysis

At the laboratory, total length of fish ( $L_T$ ) was measured to the nearest mm. Wet body mass ( $M$ ) was also measured to the nearest g. All individuals were speared at a maturity size (Table 1) (Froese and Pauly 2012). Fish were dissected, and stomachs were placed in a formaldehyde solution until analysis. Diets were determined by the method of point-intercept, originally described by Jones (1968). Stomach contents were spread in a Petri dish and placed under a stereomicroscope. Ten points on each Petri dish were randomly chosen and photographed (10x magnification). A grid was superimposed to the digitized photographs, and the nature of food items found under each point-intercept was recorded (Jones 1968). By this method, 1,000 points were reported per individual or 10,000 points per fish species. The results were then expressed as percentage of each food category ingested.

### Stable Isotope Analysis

A small piece of the dorsal white muscle was cut on seven individuals of each fish species. Each sample of algal turf was sorted under a binocular microscope to exclude all benthic invertebrates that were preserved independently as a potential food source. The thallus of macroalgae was cleaned and scraped to collect detritus, principally constituted by detrital organic deposits and bacteria (Crossman et al. 2001). All samples were cut into small pieces and oven dried at 50°C to a constant weight, before being ground into an homogenous fine powder. Carbon and nitrogen stable isotope ratios of fish muscles and sources were determined on the same sample. Analyses were performed on two subsamples for food sources that might contain carbonates: calcified macroalgae, algal turf, detritus and invertebrates. For  $\delta^{13}C$ , a subsample was acidified drop by drop with 1N HCl to remove calcified material that presents a less negative  $\delta^{13}C$  than organic material (De Niro and Epstein 1978). For  $\delta^{15}N$ , a non-acidified subsample was used, as acidification can modify  $\delta^{15}N$  (Pinnegar and Polunin 1999). Nitrogen and carbon isotope ratios were determined by a continuous flow mass spectrometer (Thermo Fisher™, delta V Advantage). Elemental concentrations of carbon and nitrogen ([C]% and [N]%) were measured with an elementary analyser (Thermo Fisher™, Flash EA 1112). Isotopic ratios were expressed in standard delta notation ( $\delta$  values (‰)) according to the following formula:  $\delta = (R_{sample}/R_{standard} - 1) \times 1000$ , where  $R$  is the ratio of the heavy to light isotope (i.e.  $^{15}N:^{14}N$  or  $^{13}C:^{12}C$ ),  $R_{sample}$  is measured for fish and sources and  $R_{standard}$  is an international standard (Vienna Pee Dee belemnite limestone carbonate for carbon and atmospheric air for nitrogen).

The Bayesian mixing model SIAR v4.0 (Stable Isotope Analysis in R) developed by Parnell et al. (2010) was used to estimate the proportional contribution of food sources to the diet of fish species. As the elemental concentrations varied substantially among sources, the mixing model incorporated concentration dependence as recommended by Phillips and Koch (2002). This model deals with unequal assimilation of carbon and nitrogen, and assumes that for each element, the contribution of a source is proportional to the assimilated biomass times the elemental concentrations in that source. Three models were run according to each fish species. In each model, we entered the mean carbon and nitrogen signatures ( $\pm$  CI) of food sources and mean signatures of fish muscles, the mean elemental concentrations ( $\pm$  CI) of the sources ([C]% and [N]%) and carbon and nitrogen fractionation factors ( $\Delta^{13}C$  and  $\Delta^{15}N$ ). Fractionation factors were calculated with the algorithm described by Caut et al. (2009) and the same  $\Delta^{13}C$  and  $\Delta^{15}N$  were used for the three mixing models. We fixed mean enrichments ( $\pm$  S.D.) of  $1.5 \pm 0.2\text{‰}$  for the carbon and  $4.5 \pm 0.1\text{‰}$  for the nitrogen, according to the data calculated with the algorithm (Caut et al. 2009) and the data given in the previous literature on herbivorous fishes (Sweeting et al. 2007, Mill et al. 2007, Wyatt et al. 2010).

### Statistical Analysis

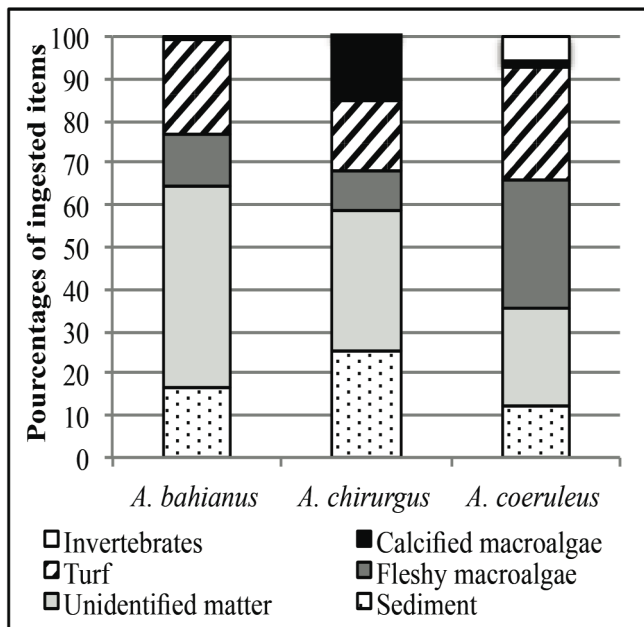
Data were tested for normality with the Shapiro-Wilks test and for homogeneity of variance with Levene's test. When all these assumptions were verified, we used analyses of variance (MANOVA and ANOVA) to compare the proportions of food items between fish species, and isotopic carbon and nitrogen signatures between food sources. Analyses of variance were combined with Tukey's honestly significant difference (HSD) post hoc tests to perform multiple comparisons. As data were not normal for fish muscles, isotopic signatures were compared with a Kruskal-Wallis test. Contributions of sources to fish diet (calculated with mixing models) have been compared between fish species with a Chi-square test. A redundancy analysis (RDA) was used to relate the proportions of sources that were assimilated by fish (calculated with mixing models) and the proportions of source that were ingested by fish (measured with stomach content analysis). RDA is a constrained ordination analysis and represents multivariate data in a reduced number of axes of the greatest variability (Legendre and Anderson 1999). The variables that contributed most to explain the variation in the dependent variables were selected using a forward selection procedure available in CANOCO program (ter

Braak 1989). All statistical analyses were performed using the program R version 12.2, excepted for RDA, which were performed with CANOCO program (ter Braak 1989).

## RESULTS

### Stomach Content Analysis

Five categories of items were identified in stomach content: calcified algae, algal turf, fleshy macroalgae, benthic invertebrates and sediment (Figure 2). A large amount of unidentified material was observed in the digestive contents but was not regarded as a full-fledged type of source because of its uncertain origin. Unidentified material could represent ingested detritus, or result in the digestion on the other ingested sources as algae. Ingestion of sediment had been considered as incidental ingestion and resulting from the type of feeding. The proportions of ingested items were significantly different between the three fish species (MANOVA, Wilks' lambda = 0.002,  $F_{2,23} = 23.8$ ,  $p < 0.0001$ ). The mean proportion of fleshy macroalgae ( $\pm$  CI) ingested by *A. coeruleus* represented  $30.2 \pm 7.5\%$  of its stomach contents and was significantly higher than in the two other fish species (Figure 2, Table 2). Similarly, *A. coeruleus* was the only species to ingest benthic invertebrates, even if their mean percentage was low ( $6.0 \pm 3.2\%$ ). The stomach content of *A. chirurgus* showed the highest proportions of calcareous algae and sediment ( $15.0 \pm 2.8\%$  and  $25.4 \pm 2.8\%$ , respectively) and the lowest proportions of algal turf ( $17.1 \pm 2.0\%$ ) among the three fish species (Figure 2; Table 2).



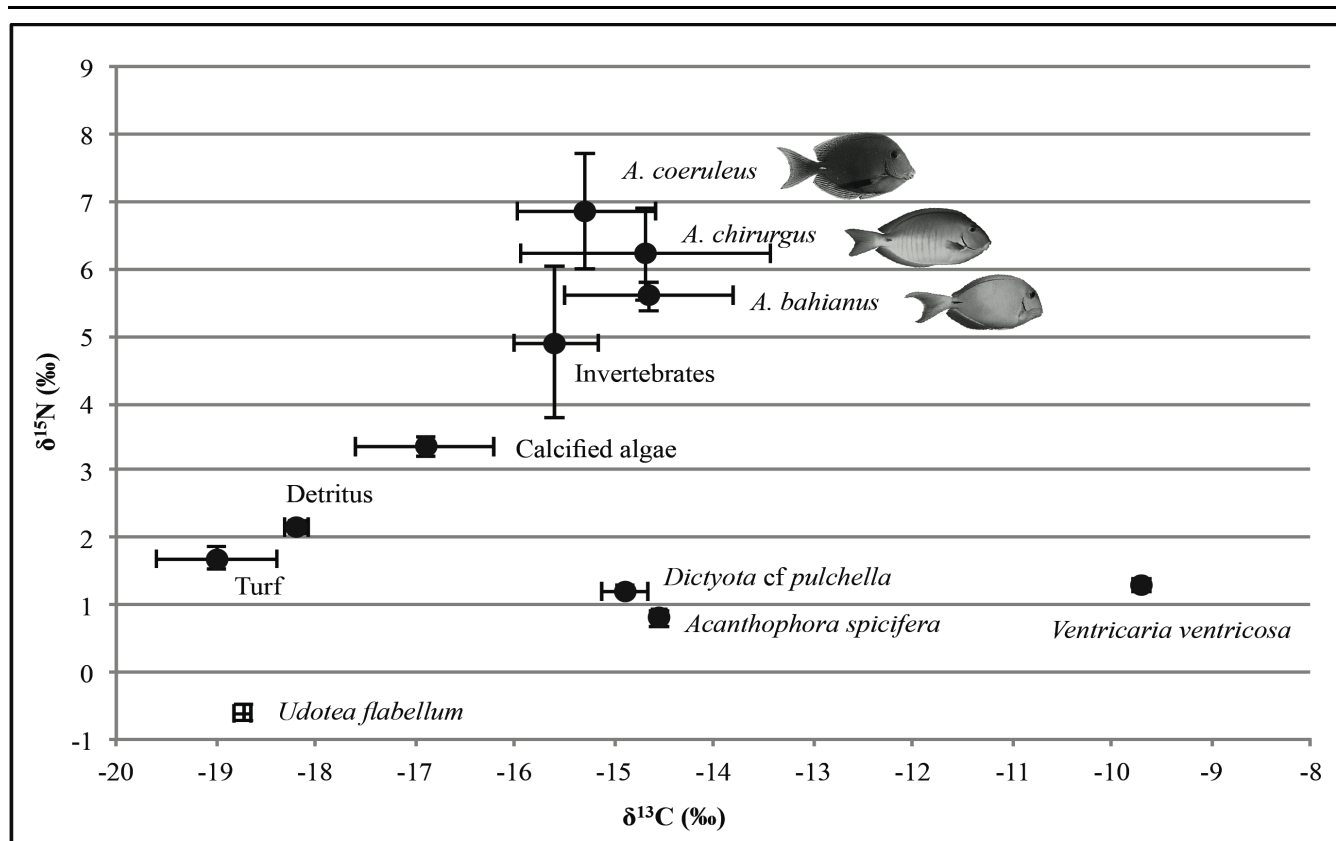
**Figure 2.** Proportions of food categories (% of point-intercept) measured in the stomach contents of *Acanthurus bahianus*, *A. chirurgus* and *A. coeruleus*.

### Stable Isotope Analysis

Isotopic signatures of fish muscles and food sources were presented as a bi-plot in Figure 3. Among food sources, algal turf showed the lowest carbon signatures (mean  $\pm$  CI =  $-19.0 \pm 0.6\text{‰}$ ) whereas the macroalgae *Ventricaria ventricosa* presented the highest  $\delta^{13}\text{C}$  ( $-9.7 \pm 0.04\text{‰}$ ). Benthic invertebrates displayed the highest  $\delta^{15}\text{N}$  value, with a mean value ( $\pm$  CI) equal to  $4.9 \pm 1.1\text{‰}$  and the macroalgae *Udotea flabellum* had the lowest nitrogen signatures ( $-0.6 \pm 0.1\text{‰}$ ). Carbon and nitrogen signatures of food sources were significantly different from each other (MANOVA, Wilks' lambda = 0.004,  $F_{9,36} = 60.8$ ,  $p < 0.0001$ ). However, multiple comparisons showed that *Dictyota* cf. *pulchella* and *Acanthophora spicifera*, presented similar isotopic signatures of carbon and nitrogen (Tukey's HSD tests, both  $p > 0.99$ ). Carbon signatures of the fish muscles were similar between the three species (Kruskal-Wallis,  $X^2 = 2.26$ ,  $p = 0.32$ ). However, a significant difference was found between the highest  $\delta^{15}\text{N}$  value, measured in *A. coeruleus* ( $6.9 \pm 0.9\text{‰}$ ) and the lowest  $\delta^{15}\text{N}$  value recorded in *A. bahianus* ( $5.6 \pm 0.2\text{‰}$ ) (Kruskal-Wallis,  $X^2 = 7.9$ ,  $p = 0.02$ ).

### Mixing Models

To determine the contribution of food sources in the diet of each fish species, five potential sources were used in mixing models. Due to their close isotopic signatures, the two macroalgae species *Dictyota* cf. *pulchella* and *Acanthophora spicifera* were grouped in a same food category, called "fleshy algae". *Tricleocarpa fragilis* and *Amphiroa fragilissima* were grouped in a same food category "calcified algae". Algal turf and detritus were equally used as potential resource in the three models. Benthic invertebrates were only used as potential sources for *A. coeruleus* because no invertebrates were found in the digestive contents of the other species. *Udotea flabellum* and *Ventricaria ventricosa* were excluded from the mixing model analyses because of their distant position in the diagram (Figure 3) and because these two macroalgae species are rarely described as potential food sources for herbivorous fishes (Randall 1967, Hay and Fenical 1988). The elemental concentrations ([C]‰ et [N]‰) and isotopic signatures of sources used in mixing models are presented in Table 3. The contributions of food sources to the fish diet were significantly different between fish species (Chi-square test,  $X^2 = 50.3$ , d.f. = 8,  $p < 0.001$ ; Table 4). According to the mean contributions of sources and the range of contributions (Bayesian 95% CI), fleshy macroalgae were preferentially assimilated by *Acanthurus bahianus*, while *A. coeruleus* and *A. chirurgus*, assimilated both algal turf and fleshy macroalgae (Table 4). On the contrary, detritus and calcified macroalgae showed a large range of contributions for the three fish species, indicating that acanthurids fishes occasionally assimilated these sources. In agreement with the low proportion of invertebrates ingested by *A. coeruleus*, invertebrates were also assimilated in a minor proportion by this species (Table 4).



**Figure 3.** Mean isotopic signatures ( $\pm$  CI) of carbon ( $\delta^{13}\text{C}$  ‰) and nitrogen ( $\delta^{15}\text{N}$  ‰), measured in fish muscles and food sources collected on the reef.

**Table 2.** Results of ANOVAs and Tukey's HSD post hoc tests, comparing the proportions of fleshy and calcified macroalgae, algal turf, sediment and invertebrates in the stomach contents of *A. coeruleus* (Aco), *A. chirurgus* (Ach) and *A. bahianus* (Aba). Bold values are significant ( $p < 0.05$ ).

	Fleshy algae	Calcified algae	Algal turf	Sediment	Invertebrates
ANOVA	$F_{(2,23)} = 16.9$ <b><math>p &lt; 0.001</math></b>	$F_{(2,23)} = 63.9$ <b><math>p &lt; 0.001</math></b>	$F_{(2,23)} = 4.0$ <b><math>p = 0.03</math></b>	$F_{(2,23)} = 20.8$ <b><math>p &lt; 0.001</math></b>	$F_{(2,23)} = 12.2$ <b><math>p &lt; 0.0001</math></b>
Tukey's tests					
Ach - Aba	$p = 0.73$	<b><math>p &lt; 0.001</math></b>	$p = 0.30$	<b><math>p = 0.002</math></b>	$p = 0.99$
Aco - Aba	<b><math>p &lt; 0.001</math></b>	$p = 0.82$	$p = 0.53$	$p = 0.17$	<b><math>p = 0.001</math></b>
Aco - Ach	<b><math>p &lt; 0.0001</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p = 0.03</math></b>	<b><math>p &lt; 0.0001</math></b>	<b><math>p &lt; 0.001</math></b>

**Table 3.** Mean isotopic signatures ( $\pm$  CI) of carbon ( $\delta^{13}\text{C}$  ‰) and nitrogen ( $\delta^{15}\text{N}$  ‰) and mean elemental concentrations ( $\pm$  CI) of carbon ([C]%) and nitrogen ([N]%), measured in consumers and food sources and used in mixing models. Fleshy macroalgae: *Dictyota cf pulchella* and *Acanthophora spicifera*; Calcified macroalgae: *Tricleocarpa fragilis* and *Amphiroa fragillissima*. n is the number of samples collected on the reef.

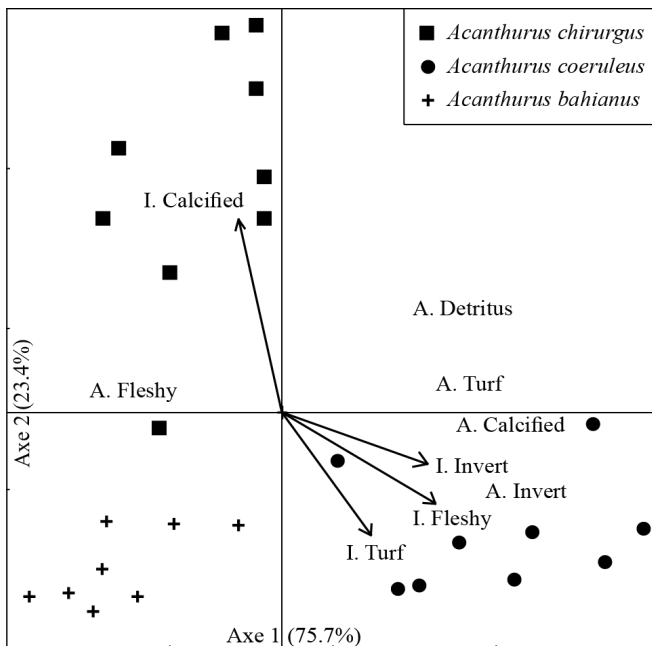
Sample types	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	[C]%	[N]%
<b>Food sources</b>					
Detritus	5	$-18.2 \pm 0.1$	$2.2 \pm 0.1$	$6.8 \pm 0.1$	$0.8 \pm 0.02$
Algal Turf	5	$-19.0 \pm 0.6$	$1.7 \pm 0.2$	$7.6 \pm 1.0$	$2.1 \pm 0.5$
Fleshy algae	6	$-14.7 \pm 0.2$	$1.0 \pm 0.2$	$12.3 \pm 3.0$	$1.9 \pm 0.2$
Calcified algae	9	$-16.9 \pm 0.7$	$3.4 \pm 0.1$	$21.8 \pm 3.6$	$8.8 \pm 0.4$
Invertebrates	5	$-15.6 \pm 0.4$	$4.9 \pm 1.1$	$44.5 \pm 1.1$	$7.5 \pm 0.4$
<b>Consumers</b>					
<i>A. coeruleus</i>	7	$-15.3 \pm 0.7$	$6.9 \pm 0.9$	$45.6 \pm 0.8$	$14.4 \pm 0.2$
<i>A. bahianus</i>	7	$-14.7 \pm 0.9$	$5.6 \pm 0.2$	$48.5 \pm 1.1$	$14.9 \pm 0.5$
<i>A. chirurgus</i>	7	$-14.7 \pm 1.2$	$6.2 \pm 0.7$	$45.8 \pm 2.0$	$14.7 \pm 0.6$

**Table 4.** Mean (Bayesian 95% CI) biomass contributions of food sources to the diet of *Acanthurus coeruleus*, *A. bahianus*, *A. chirurgus* estimated with mixing models.

Fish species	Detritus	Invertebrate	Fleshy algae	Calcified algae	Algal turf
<i>A. coeruleus</i>	22.8 (0.0–43.0)	8.0 (0.0–21.6)	22.5 (1.1–40.3)	20.3 (0.0–40.3)	26.4 (1.6–47.1)
<i>A. bahianus</i>	20.0 (0.0–44.1)	–	57.8 (33.3–84.6)	1.2 (0.0–3.7)	21.0 (0.0–44.1)
<i>A. chirurgus</i>	25.2 (0.0–48.9)	–	39.1 (15.2–64.2)	10.1 (0.0–29.5)	25.6 (0.2–47.9)

### Relation Between Ingestion and Assimilation of Sources

A redundancy analysis (RDA) was performed to relate the proportions of food sources that were ingested and the proportions that were assimilated by fishes (Figure 4). The forward selection procedure of the first RDA retained four variables (ingestions of calcified macroalgae, fleshy macroalgae, turf and invertebrates) to explain the assimilations of food sources by fish. The overall ordination was significant ( $p < 0.001$ ). The first axis explained 75.7% of the total variance and was mostly related to the proportions of ingestion of invertebrates and fleshy macroalgae. This axis was positively correlated with *Acanthurus coeruleus* and negatively correlated with the other fish species (Figure 4). The second axis summarized 23.4% of the variance and was mostly related to variations in the ingestion of calcified macroalgae and algal turf. This second axis was positively correlated with *A. chirurgus* and negatively correlated with *A. bahianus* (Figure 4). While the ingestion of invertebrates and algal turf were correlated with the assimilation of these two food items, no correlations were found between the ingestion and the assimilation for calcified and fleshy macroalgae.



**Figure 4.** Redundancy analysis (RDA) ordination diagrams on the effect of the ingestion of food items (variables preceded by “I”) on the assimilation of food items (variables preceded by “A”) by the fish. Invert: invertebrates; Fleshy: fleshy macroalgae; Calcified: Calcified macroalgae.

### DISCUSSION

According to stomach contents and stable isotopes analyses, *Acanthurus coeruleus*, *A. chirurgus*, and *A. bahianus* presented different trophic niches. Food categories were ingested in different proportions by the three species and mixing models showed different patterns of food assimilation. Isotopic ratios reflected the assimilation of food sources on a long-term period (corresponding to the time of tissue renewal) and the three fish species exhibited different nitrogen signatures, showing a different use of the resources.

The redundancy analysis (RDA) clearly distinguished the diet of the three fish species, including alimentary patterns of ingestion and assimilation. *A. coeruleus* was characterized by a higher ingestion of fleshy macroalgae and turf than the other species. This species was also characterized by an ingestion of benthic invertebrates (principally small crustaceans) that was not recorded in *A. bahianus* and *A. chirurgus*. According to the mixing models, turf was the most assimilated source by *A. coeruleus*. Kopp et al. (2010) demonstrated a link between the number of “bites” of *A. coeruleus* and an algal facies dominated by turf. Fleshy macroalgae and detritus were the second most assimilated sources whereas invertebrates only complemented the diet with the lower proportion of assimilation. *Acanthurus chirurgus* ingested a large amount of calcified macroalgae, even if the fish did not assimilate this source proportionally. The mixing models indicated a principal assimilation of fleshy macroalgae, and secondary assimilations of turf and detritus with similar proportions. *A. bahianus* presented the particularity to ingest more sediment than *A. coeruleus* and *A. chirurgus*. *A. bahianus* ingested less calcified algae and more fleshy algae than *A. chirurgus* that was reflected in the different assimilations given by the mixing model. Burkepile and Hay (2008) also show a significant effect of *A. bahianus* on upright macroalgae, including *Dictyota* spp. However, due to the deterrent molecules synthesized by the brown macroalgae (Hay and Fennell 1988, Wallim et al. 2005), the consumption and assimilation of macroalgae by surgeonfishes is mostly associated with the species *Acanthopora spicifera* rather than *Dictyota* spp (Reinthal and Macintyre 1994).

While Randall (1967) find an important and similar amount of algal matter, including macroalgae and algal turf, in the stomach contents of the three acanthurids (respectively 92.8%, 93.9% and 91.8%), some authors report a difference of diet that is mostly explained by the

different digestive tract anatomies of these species (Ferreira and Goncalves 2006, Tilghman *et al.* 2001). *A. coeruleus* has a thin-walled stomach while *A. bahianus* and *A. chirurgus* have thick-walled, gizzard-like stomach, which allow them to ingest most sediment and calcified algae. Actually, in this study, the food categories used by fish were similar for the three species (macroalgae, algal turf and detritus) but each category was ingested and assimilated in different proportions.

In this study, we failed to correlate ingestion and assimilation of some food items. For example, *A. chirurgus* ingested a large amount of calcified macroalgae, which were not assimilated by the fish probably due to their low nutritional quality (Montgomery and Gerking 1980). This observation can be explained by three main reasons. Firstly, ingested items are not necessarily assimilated by an organism (Hobson and Clark 1992) and some items are incidentally ingested while they do not represent a nutritional interest for fish. Secondly, stomach contents represent only a snapshot of the diet and give uncertain information in this study due to the high proportion of unidentified organic material. Finally, the method used to evaluate the ingestion of resources was based on visual observations of the stomach content with a stereomicroscope. Thus, calcareous sediment could have been identified as calcified algae, leading to an over-estimation of the proportion of calcified algae ingested by *A. chirurgus*.

In conclusion, the three species of Caribbean surgeonfishes presented different trophic niches. This observation has been reported before and linked to the different digestive anatomies of these species (Ferreira and Goncalves 2006, Tilghman *et al.* 2001). The difference of trophic niche between the three species of surgeonfishes could explain the presence of the three acanthurids fishes in multi-species foraging associations and the different use of resource could justify their coexistence in avoiding competitive interactions for food resources. Moreover, the difference of trophic niche suggests a functional diversity among the Acanthuridae, demonstrated in the Pacific region by Choat *et al.* (2002), even if only three species are found in the Caribbean. This information is particularly important in fisheries management because the functional diversity reflects a complementarity of the three species in terms of impact on benthic communities. Thus, a decrease of one of the three species could have important consequences on the control of the algal communities of the Caribbean coral reefs.

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