Resource use by three juvenile scarids (Cryptotomus roseus, Scarus iseri, Sparisoma radians) in Caribbean seagrass beds

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ABSTRACT

The bucktooth parrotfish *Sparisoma radians*, the striped parrotfish *Scarus iseri* and the bluelip parrotfish *Cryptotomus roseus* are three herbivorous fishes commonly found at juvenile stages in Caribbean seagrass beds. While the diet of the three species as adults is relatively well known, few studies have been conducted on the feeding patterns of juveniles. In this study, the resource use of the juveniles of three scarid species were studied using two complementary methods: gut content and stable isotope analyses (^{13}C:^{12}C and ^{15}N:^{14}N ratios). Bayesian mixing model approaches were used to calculate the contribution of each food item to fish diets (SIAR, mixing models). The three parrotfish species appeared to rely essentially on the consumption of fleshy macrophytes. *Cryptotomus roseus* consumed more benthic invertebrates and presented a higher trophic level than the two other scarid species. *Scarus iseri* presented a higher assimilation of benthic biofilm, in accordance with the high percentage of sediment in its gut content, and *Sparisoma radians* assimilated more *Thalassia testudinum* leaves. This research highlighted a food resources partitioning among the juveniles of the three herbivorous fishes, probably to avoid inter-specific competitive interactions for the most palatable food at a critical stage of their life.

Keywords: Gut content analyses, trophic niche, stable isotopes analyses.

1. Introduction

Herbivorous fishes have been widely studied on coral reefs due to their major role in the control of algae and are known to mitigate their competitive interactions with corals (Burkepile and Hay, 2010 and references therein). On tropical seagrass beds, herbivorous fishes, such as parrotfishes (Scarinae), have received less attention (Del Moral *et al*., 2016). In the Caribbean, three species of herbivorous fishes are commonly found on seagrass beds: the bucktooth parrotfish *Sparisoma radians* (Valenciennes, 1840), the bluelip parrotfish *Cryptotomus roseus* (Cope, 1870) and the striped parrotfish *Scarus iseri* (Bloch, 1789). The first two species are exclusively found on seagrass beds, whereas *Scarus iseri* can also be found on coral reefs at all life stages. During their juvenile stages, these three species cohabit in shallow seagrass meadows which represent their primary nursery habitat (Bouchon-Navaro *et al.*, 2004; Kopp *et al*., 2010; Layman and Silliman, 2002; Weinstein and Heck, 1979). Indeed, studying seagrass meadows is particularly relevant due to their role as nursery areas for nearshore fishes (Tuya *et al*., 2014).

Possibly due to anthropic pressures on seagrass meadows, the stocks of these three juveniles have dramatically decreased over the last ten years in Guadeloupe, although they were formerly common in this habitat (Y. Bouchon-Navaro, Pers. com. 2016).

Information on the diet of these herbivorous fishes in the literature is principally available for adults. *Sparisoma radians* mainly ingests turtlegrass *Thalassia testudinum* Banks & Sol. ex König, 1805, fleshy macroalgae like *Dictyota* sp. or *Acanthophora spicifera* (M. Vahl) Børgesen, 1910 and calcified macroalgae such as *Halimeda* sp. (Lobel and Ogden, 1981; Randall, 1967; Targett and Targett, 1990). Depending on the region, *Scarus iseri* is described as a feeder on microalgae from dead coral pavements, fleshy macroalgae or epiphytic filamentous microalgae (McAfee and Morgan, 1996; Nagelkerken *et al.*, 2006; Randall, 1967). *Cryptotomus roseus* is described as strictly herbivorous, feeding on seagrass

(Carpenter, 2002), but its diet has not been studied in detail. However, little information is available for the juveniles of these three parrotfish species, although ontogenetic shifts in diet are common for tropical fishes (Cocheret de la Morinière *et al*., 2003) or temperate herbivorous fishes (Havelanche *et al*., 1997). Dietary changes are useful to understand their ecological role in seagrass beds, such as the regulation of the algal biomass.

Trophic niches of fishes can be described on the basis of gut content analyses (Ogden, 1976; Randall, 1967) or direct observation of their feeding behaviour in the field by the counting of "bites" (Cardoso *et al*., 2009; Lobel and Ogden, 1981; McAfee and Morgan, 1996; Overholtzer and Motta, 1999). However, these methods provide a description of a species' diet at a specific time and present several practical problems (Bearhop *et al*., 2004). With gut content analyses, the principal difficulty results from the ability of herbivorous fishes, such as Scarinae, to grind the ingested matter into small fragments with their fused beak and their pharyngeal mills (Bellwood and Choat, 1990; Randall, 1967). The relative proportions of food items in gut contents are estimated with varying degrees of accuracy by different authors and on the basis of different parameters (occurrence, weight or volume percentages). With field observations, it is difficult to discriminate between potential dietary targets (e.g. between seagrass leaves and epiphytes) and the ingestion of some food items, such as detritus, is difficult to quantify. For these reasons, determining the trophic niches of herbivorous fishes remains challenging.

More recently, stable isotope ratios of fish muscles (^{13}C:^{12}C and ^{15}N:^{14}N) have been used to determine their trophic niche. Isotopic ratios measured in consumer tissues are closely linked to those of their diet and increase in a stepwise fashion with each trophic level.

The mean trophic isotopic enrichment, or fractionation factor ($\Delta^{15}N$ and $\Delta^{13}C$), was estimated at + 3.4 ± 1.1‰ for nitrogen (Minagawa and Wada, 1984) and + 0.4 ± 1.3‰ for carbon (Post, 2002). However, Mill *et al.* (2007) demonstrated a higher $\Delta^{15}N$ for herbivorous fishes (4.7‰

for *Acanthurus sohal* and 4.1‰ for *Sparisoma* spp.) and Sweeting *et al.* (2007) recommended a $\Delta^{13}C$ ranging between 1.5‰ and 2‰ for marine fishes.

Stable isotope analysis is considered to be a powerful tool to reflect the feeding behaviour of individuals over long periods (approximately three months in the muscles of adult fishes) for fish muscles in adults), corresponding to the turnover of the tissues of consumers (Bearhop *et al*., 2004).

Several methods using C and N stable isotopes ratios, expressed as $\delta^{13}C$ and $\delta^{15}N$, have been developed to understand and interpret fish ecology. Isotopic signatures of the consumer and those of its potential food sources can be used in mixing models to calculate and estimate the contribution of several food sources to the diet of a consumer (see Phillips, 2012 and Phillips *et al*., 2015 for reviews). Results of mixing models can be used to describe and compare trophic niches between fish species (Dromard *et al*., 2015; Plass-Johnson *et al.*, 2013). Despite the fact that numerous potential organic matter sources occur in seagrass beds, mixing models have been used before for fish species living in this habitat (Benstead *et al.*, 2006; Hyndes and Lavery, 2005; Loneragan *et al.*, 1997). However, few studies have been done in the Caribbean seagrass beds (Nagelkerken and van der Velde, 2004).

In the present study, we analysed the diet of juveniles of three parrotfish species in a Caribbean seagrass bed to describe and compare their trophic niche, combining stable isotope and gut content analyses.

2. Materials and methods

The study was carried out in the Bay of the Grand Cul-de-Sac Marin, located in the northern part of Guadeloupe Island (Lesser Antilles) (Fig. 1). In the south, the bay is bordered by mangroves and the northern part is partially enclosed by a coral barrier reef. Shoals of the bay are colonized by seagrass meadows dominated by the turtlegrass, *Thalassia testudinum*.

Sampling was performed in a shallow seagrass bed (< 2 m depth), at mid-distance between the coast and the barrier reef, far from the influence of mangroves, at the end of the wet season (October to December 2010). The sampling area covers 1 km^2 approximately.

2.1. Sampling protocol

Three herbivorous fish species (*C. roseus, S. iseri* and *S. radians*) were sampled during 6 purse seine samples (Table 1). The total length of fish was measured to the nearest millimetre and individuals were weighed to the nearest milligram (Table 2). All specimens sampled were below their minimum size at first maturity (Bouchon-Navaro *et al*., 2006). The main potential organic matter (OM) sources in the seagrass beds were sampled and treated for stable isotope analysis (Table 1). The dominant species or genera of fleshy macroalgae occurring in seagrass beds were collected and cleaned with distilled water: *Anadyomene stellate* (Wulfen) C. Agardh, 1823, *Caulerpa cupressoides* (Vahl) C. Agardh, 1817, *Chaetomorpha* sp., *Dictyota* cf *pulchella* Hörnig & Schnetter, 1988 and *Padina* sp., along with the calcified macroalgae *Halimeda incrassata* (J. Ellis) J.V. Lamouroux, 1816 and *Udotea flabellum* (J. Ellis & Solander) M.A. Howe, 1904. Two seagrasses were collected, *Thalassia testudinum* and *Syringodium filiforme* Kützing, 1860. Samples of *T. testudinum* were sorted into two categories: old leaves (O) and young leaves (Y), both selected without epiphytes. Around 100g (wet weight) of each species of macroalgae and seagrass were collected on field. Epiphytes colonising old leaves of *Thalassia* were gently scrapped with a scalpel blade and stored apart. Benthic invertebrates (amphipods, copepods, decapods, gastropods) collected with seagrass and macroalgae samples were sorted. When collected, macroalgae and *Thalassia* leaves were preserved in plastic bags in order to retain the detritus, composed of organic matter and bacteria (Crossman *et al*., 2001), deposited on algal thalli and seagrass leaves. Plastic bags were then opened in plastic boxes, macroalgae and *Thalassia* were gently

shacked on water and detritus settled were collected at the bottom of the boxes. Due to the small amounts, invertebrates were pooled together to constitute another type of food source. Gastropods were acidified to remove their hard shells before analyses. Particulate organic matter (POM) present in the water column above seagrasses was collected by filtering subsurface water on preweighed Whatman GF/F filters precombusted for 4 hours at 450°C. The first centimetre of surface sediment was collected to analyse the biofilm mainly composed of benthic diatoms, bacteria, detritus and settling POM (Belicka *et al.*, 2012). To ensure a sufficient quantity of material for the analyses, around 10g (wet weight) of epiphytes, invertebrates, detritus, POM and biofilm were sampled.

2.2. Gut content analyses

Ten individuals of each species had full stomach and were studied for gut content analyses. Two methods were used according to the fish species. For *C. roseus*, food categories were sorted, oven dried and weighed separately as described by Hyslop (1980), because it was possible to isolate the different food items. Diets of the two other fish species were studied using the method of point-intercepts, described by Jones (1968). Food categories were expressed as percentages of the total dry-weight for *C. roseus* and as percentages of point-intercepts for *S. iseri and S. radians*.

2.3. Stable isotope analysis

Dorsal fish muscles cut into small pieces and all seagrass carbon sources were oven dried at 50°C to a constant weight and ground into a homogenous fine powder. Carbon and nitrogen stable isotope ratios were performed on two subsamples for food sources that may contain carbonates: POM, biofilm, detritus, epiphytes, invertebrates and calcified macroalgae. For $\delta^{13}C$, a subsample was acidified with 1N HCl to remove calcified material that presents a less

157 negative $\delta^{13}C$ than organic material (De Niro and Epstein, 1978). For $\delta^{15}N$, a non-acidified
158 subsample was used, because acidification can distort $\delta^{15}N$ values (Pinnegar and Polunin,
159 1999). For each sample (and each subsample), 1mg of dry weight was used for analysis.
160 Nitrogen and carbon isotope ratios were determined by a continuous flow mass spectrometer
161 (Thermo Fisher™, delta V Advantage). Elemental concentrations of carbon and nitrogen
162 ([C]% and [N]%) were measured with an elementary analyser (Thermo Fisher™, Flash EA
163 1112). Isotopic ratios were expressed in standard delta notation [δ values (‰)] according to
164 the following formula: $\delta_X = [(R_{sample}/R_{standard} - 1)] \times 1000$, where X is ^{13}C or ^{15}N and R the
165 ratio ^{15}N:^{14}N or ^{13}C:^{12}C of samples or international standards (Vienna Pee Dee belemnite
166 limestone carbonate for carbon and atmospheric air for nitrogen). The measurement precision
167 was <0.1‰ for $\delta^{13}C$ and $\delta^{15}N$. Percentages of organic C and organic N were measured on
168 acidified sub-samples for those treated by HCl, using the elemental analyser and were used to
169 calculate the sample C/N ratio. Low C/N ratio indicated higher nutritional value and
170 digestibility of food sources.
171
172 *2.4. Mixing models*
173 The Bayesian stable isotope mixing model SIAR v4.0 (Stable Isotope Analysis in R)
174 developed by Parnell *et al.* (2010) was used to estimate the proportional contribution of food
175 sources to the diet of the three herbivorous fishes. As there were too many sources of carbon
176 in seagrass beds, we reduced their number to eight. Some were not taken into account in
177 mixing models, as they were not or hardly consumed by the juvenile scarids: MOP,
178 calcareous macroalgae (*Halimeda incrassata* and *Udotea flabellum*) and the seagrass
179 *Syringodium filiforme*. Even if some juvenile scarids scraped calcified *Halimeda* (Overholzer
180 and Motta, 1999), they probably mainly ingest epiphytes. Other prey were combined when
181 presenting not significantly different C and N stable isotopic values. Epiphytes, detritus and

182 *Dictyota* were considered as one food source, as were old and young leaves of *Thalassia*
183 combined. Three models were run according to each fish species. In each model, we entered
184 the individual isotopic values of fish muscles and the mean carbon and nitrogen signatures (±
185 SD) of the eight potential food sources. Mixing models took into account carbon and nitrogen
186 fractionation factors ($\Delta^{13}C$ and $\Delta^{15}N$). In this study, we fixed mean enrichments (± SD) of 1.5
187 ± 0.5‰ for carbon and 3.9 ± 0.5‰ for nitrogen as the mean difference between $\delta^{15}N$ values
188 of all sources taken into account and juvenile scarids. This $\Delta^{15}N$ is slightly lower than those
189 given for adult herbivorous fishes by Mill *et al*. (2007) (4.3 to 5.7‰), and higher than those
190 indicated by Wyatt *et al.* (2010) (1.7 to 2.5‰). Mean contributions (Bayesian Credibility
191 Interval 95%) of food sources to fish diets were expressed as percentages.
192
193 *2.5. Statistical analyses*
194 Data were tested for normality with Shapiro tests. When the data were normal, nitrogen and
195 carbon signatures among fish muscles and among food sources were compared using one-way
196 analyses of variance (ANOVA), followed by *post hoc* Tukey tests. Otherwise a non-
197 parametric Kruskall-Wallis analysis of variance was used. Hierarchical clustering based on
198 normalized Euclidean distance and Ward's criterion was performed on mean isotopic ratios to
199 identify groups of species with similar C and N isotopic ratios. All tests were performed using
200 the program R.
201
202 **3. Results**
203
204 *3.1. Gut content analyses*
205 Due to the difficulty of accurately identifying all fragments of seaweeds and seagrasses in gut
206 contents, it was not possible to calculate the proportion of each plant species ingested by the

three fish species. Gut contents were sorted into four categories: sediment, vegetal matter (including microalgae, turfing algae, macroalgae and seagrass), benthic invertebrates and unidentified organic matter (Table 3). Benthic invertebrates (amphipods, isopods and decapods) were found only in the gut contents of *Cryptotomus roseus*. *Scarus iseri* and *Sparisoma radians* presented similar diets with a dominance of vegetal matter (Table 3).

3.2. Stable isotope analyses

Nitrogen and carbon signatures of fish muscles and food sources are represented as a bi-plot (Fig. 2, Table 1). *Cryptotomus roseus*, *Scarus iseri* and *Sparisoma radians* presented the most enriched nitrogen isotopic signatures ($\delta^{15}N \pm SD$), with values of 7.0 ± 0.7‰, 5.7 ± 0.8‰ and 5.7 ± 0.6‰, respectively. $\delta^{15}N$ of fish muscles were significantly different between *C. roseus* and the two other fish species ($F = 12.30$, $p < 0.001$), while *S. radians* differed significantly from *C. roseus* and *S. iseri* by its higher $\delta^{13}C$ value (-9.6 ± 0.8‰ *vs* -11.5 ± 1.6‰ and -11.9 ± 0.9‰ respectively; $F = 14.01$, $p<0.001$). Nitrogen signatures of OM sources ranged from 5.1 ± 0.2‰ for invertebrates to 1.2 ± 0.1‰ for *Udotea flabellum* and their carbon signatures ($\delta^{13}C \pm SD$) varied between -17.2 ± 0.7‰ for *Chaetomorpha* sp. and -4.7 ± 0.1‰ for *Syringodium filiforme* (Fig. 2, Table 1). Significant differences in C and N isotopic signatures were found among OM sources ($\delta^{13}C$: $X^2 = 69.7$, $p < 0.001$; $\delta^{15}N$: $X^2 = 58.3$, $p < 0.001$). However, no significant difference was observed between old and young leaves of *Thalassia,* and between epiphytes, *Dictyota* and detritus ($p > 0.05$ for both groups). They were thus pooled together into two food categories for mixing models.

3.3. Mixing models

Three mixing models were performed, i.e. one per fish species. Eight types of food sources were introduced into each model: invertebrates, biofilm, *Thalassia testudinum* (O and Y

232 leaves combined), the group epiphytes-detritus-*Dictyota* and four macroalgae likely to be
233 consumed by juvenile scarids (*Anadyomene stellata*, *Caulerpa cupressoides*, *Chaetomorpha*
234 sp. and *Padina* sp.). The results of the mixing models were largely undetermined, with a
235 broad overlap in the 95% confidence intervals, due to the number of sources and the wide
236 range of isotopic values in the macroalgae (Table 4). However, macroalgae constituted the
237 largest part of the food assimilated by juvenile scarids (mean contribution: 42%, 53% and
238 57% in *C. roseus*, *S. iseri* and *S. radians* respectively), followed by the group epiphytes-
239 detritus-*Dictyota* between 10% and 15% (Fig. 3). The three other food sources discriminated
240 better the three fish species. *C. roseus* assimilated a higher proportion of invertebrates (mean
241 contribution: 21%) than the two other species (*Scarus iseri S.i*: 4% and *Sparisoma radians*
242 *S.r.*: 5%), *S. iseri* a higher proportion of biofilm (mean contribution: 22% vs *Cryptotomus*
243 *roseus C.r.*:15% and *S.r.*: 10%) and *S. radians* a higher proportion of *Thalassia testudinum*
244 (mean contribution: 17% vs *C.r.*: 8% and *S.i.*: 11%) (Fig. 3, Table 4). While seagrass,
245 macrophytes and epiphytes presented high C/N ratios (10 – 38), invertebrates, biofilm and
246 detritus had lower C/N values (6 - 9), testifying their higher nutritional quality (Table 1).
247 These high quality food items contributed around 50% of the food assimilated by *C. roseus*,
248 36% by *S. iseri* and 25% by *S. radians*, epiphytes and *Dictyota* being included.
249

250 **4. Discussion**

251 Coupling gut contents and stable isotope analyses allows the description of fish diets with
252 greater accuracy. These two complementary methods have been used before in *Thalassia*
253 seagrass beds to study fish feeding behaviours (Harrigan *et al.*, 1989; Vaslet *et al*., 2015), but
254 few such studies have been conducted on fish juveniles (Lugendo *et al*., 2006; Nagelkerken
255 and van der Velde, 2004) or on herbivorous fishes (Cocheret de la Morinière *et al*., 2003).
256

4.1. Gut content and isotopic mixing model problems

Observations of gut contents showed that the three species principally ingested vegetal matter (including micro- and macroalgae, seagrass and their epiphytes). Gut content analyses give interesting results but present several limitations, as previously discussed, especially for Scarinae which grind their food. As these three parrotfish species differed significantly in their stable isotopic values, this suggested that they might have different feeding patterns. Results of stable isotope mixing models (SIAR) showed that juveniles of these three species indeed assimilated mainly macroalgae, but presented nevertheless some food partitioning, which was in accordance with the results of gut content analysis. *C. roseus* presented a more enriched $\delta^{15}N$ than the two other species and assimilated more benthic invertebrates, such as small crustaceans, in accordance with gut content analysis. *S. iseri* had a more depleted $\delta^{13}C$ and assimilated a higher proportion of biofilm, which might explain the higher proportion of sediment in the gut content of this species. *S. radians* presented an enriched $\delta^{13}C$ and a higher assimilation of *Thalassia testudinum* leaves. However, the results of the mixing models were largely undetermined, with a wide range of mean contributions (95% CI, Table 4) and should be taken with caution. This lack of resolution was due, firstly, to the wide range of food sources taken into account in the mixing models, even in selecting the most susceptible ones to be eaten by juvenile scarids and in combining those with close isotopic values; and secondly to the wide range of $\delta^{13}C$ in macroalgae and their rather close $\delta^{15}N$ (cf Table 1). *Chaetomorpha* presented a highly negative $\delta^{13}C$ value close to the biofilm, while *Caulerpa* and *Padina* exhibited enriched $\delta^{13}C$ values close to those of *Thalassia* leaves. Grouping these food categories together to reduce the number of sources used in the mixing models would have resulted in more determined models, but would not have had any ecological meaning. Juvenile parrotfishes consume a wide range of food resources on Caribbean coral reefs (Overholtzer and Motta, 1999) and seagrass beds (this study). Taking into account too many

282 food resources leads to inconclusive mixing model results (cf. Brett, 2014; Fry, 2013 and
283 Philipps *et al.*, 2015, for critical reviews on stable isotope mixing models). Thus the selection
284 of a reduced number of food sources improve the results of mixing models but may lead to an
285 oversimplification of fish diets that would be inconsistent with field observations. Combining
286 gut content and stable isotope analyses with DNA analysis of the food ingested, field
287 observations and feeding trial experiments may be necessary to fully resolve the problem of
288 parrotfish feeding.
289
290 *4.2. Interspecific differences*
291 Few data are available on the diet of *Cryptotomus roseus*, which is assumed to feed mostly on
292 *Thalassia testudinum* (Carpenter, 2002). However, the morphology of its teeth (separated
293 teeth) could explain its greater consumption of invertebrates compared to the other species.
294 The use of benthic invertebrates by young herbivorous fishes could also result from a relic of
295 their previous carnivorous stage (early post-settlement stage). Ontogenetic shifts in diet from
296 carnivorous to herbivorous have been described before for Scarinae in the Pacific region
297 (Bellwood, 1988; Chen, 2002).
298 Adult individuals of *Scarus iseri* are described as feeders on microalgae and consumers of
299 algal turf, especially when found on coral reefs (Dromard *et al*., 2015; McAfee and Morgan,
300 1996; Nagelkerken *et al.*, 2006; Randall, 1967; van Rooij *et al*., 1996), on macroalgae
301 (Cardoso *et al*., 2009; Randall, 1967; Wolf, 1985), or epiphytes from seagrass leaves
302 (Nagelkerken *et al*., 2006). In Guadeloupean seagrass beds, *S. iseri* juveniles were
303 characterized by a higher assimilation of biofilm compared to the two other fish species. The
304 shift of diet between juvenile and adult stages could result from the ontogenetic change of
305 habitat, from seagrass beds to coral reefs. Algal turf, constituted by filamentous macroalgae
306 heavily grazed by adults, was not found in the studied seagrass bed because of the lack of

307 dead pavement support. The greater quantity of sediment in *S. iseri* gut content could be due
308 to the consumption of biofilm developing at sediment surface.
309 Previous studies showed that adult *Sparisoma radians* consume between 88 and 95% of
310 *Thalassia testudinum,* including epiphytes from leaves (Lobel and Ogden, 1981; Montague *et*
311 *al.*, 1995; Randall, 1967), because this fish is undeterred by chemical and physical defences of
312 *T. testudinum* (Goecker *et al.*, 2005; Targett and Targett, 1990). In the present study, the
313 proportion of *T. testudinum* assimilated by juveniles of *S. radians* was estimated to be 17%,
314 most of the vegetal matter ingested coming from macroalgae. *S. radians* seems thus to exhibit
315 an ontogenetic diet shift, possibly due to a gradual physiological adaptation allowing *S.*
316 *radians* to consume and digest *T. testudinum*. Several authors have shown the importance of
317 epiphytes of *Thalassia* leaves in the diet of *S. radians* and *S. iseri* (Cocheret de la Morinière *et*
318 *al.*, 2003; Montague *et al*., 1995; Nagelkerken *et al.*, 2006). In the present study, the
319 respective assimilation of detritus, epiphytes and *Dictyota* could not be estimated because of
320 their close isotopic values. The high proportion of detritus in the diets of herbivorous fishes
321 has been underlined by several authors (e.g. Crossman *et al.* 2001; Dromard *et al*., 2013) and
322 related to their high nutritional quality estimated by their low C/N ratio, as also observed in
323 this study. The proportion of food sources with low C/N ratios (invertebrates, biofilm, detritus
324 and epiphytes) differed among the three juvenile parrotfishes, in the following decreasing
325 order, *C. roseus* (50%), *S. iseri* (36%) and *S. radians* (25%). These differences suggested that
326 they differed in their ability to consume, process and assimilate plant material. Further studies
327 focussed on the physiological processes involved in nutrient extraction and utilisation in
328 herbivorous fishes, as highlighted by Clements *et al*. (2009), should be conducted in order to
329 fully understand interspecific differences in scarid diets.
330 Thus, the juveniles of the three scarid species seem to present a high overlap in their use of
331 food resources, with high consumption of macroalgae, but nevertheless displayed different

preferences for specific food sources: invertebrates for *C. roseus*, biofilm for *S. iseri* and *Thalassia* leaves for *S. radians*. These results indicated some early food partitioning among juvenile scarids of different species, differences which would increase with individual growth.

5. Conclusions

The lack of dietary redundancy observed for adult scarids on Indian Ocean coral reefs (Plass-Johnson *et al.*, 2013) seems to be also observed for juvenile scarid species in Caribbean seagrass beds. At a juvenile stage, the three fish species live together in seagrass meadows, their primary nursery habitat, forming multispecific shoals. The present study indicated that, while macroalgae formed a large part of their diets, some differences in the use of food resource occur between these juveniles, in spite of the lack of robustness of mixing model results. The difference of diet between the three herbivorous scarid species could contribute to their coexistence in seagrass beds without inter-specific competitive interactions during their juvenile stage, as observed among other juvenile parrotfishes on coral reefs in Florida Keys (Overholtzer and Motta, 1999). The comparison between juveniles (present study) and adults (from the literature) also suggested that ontogenetic dietary changes occurred in these three scarid fish species, resulting in higher trophic niche differentiation in adults.

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List of figures

503 Figure 1. Location of the study site in the Bay of the Grand Cul-de-Sac Marin in Guadeloupe

504 (Lesser Antilles).

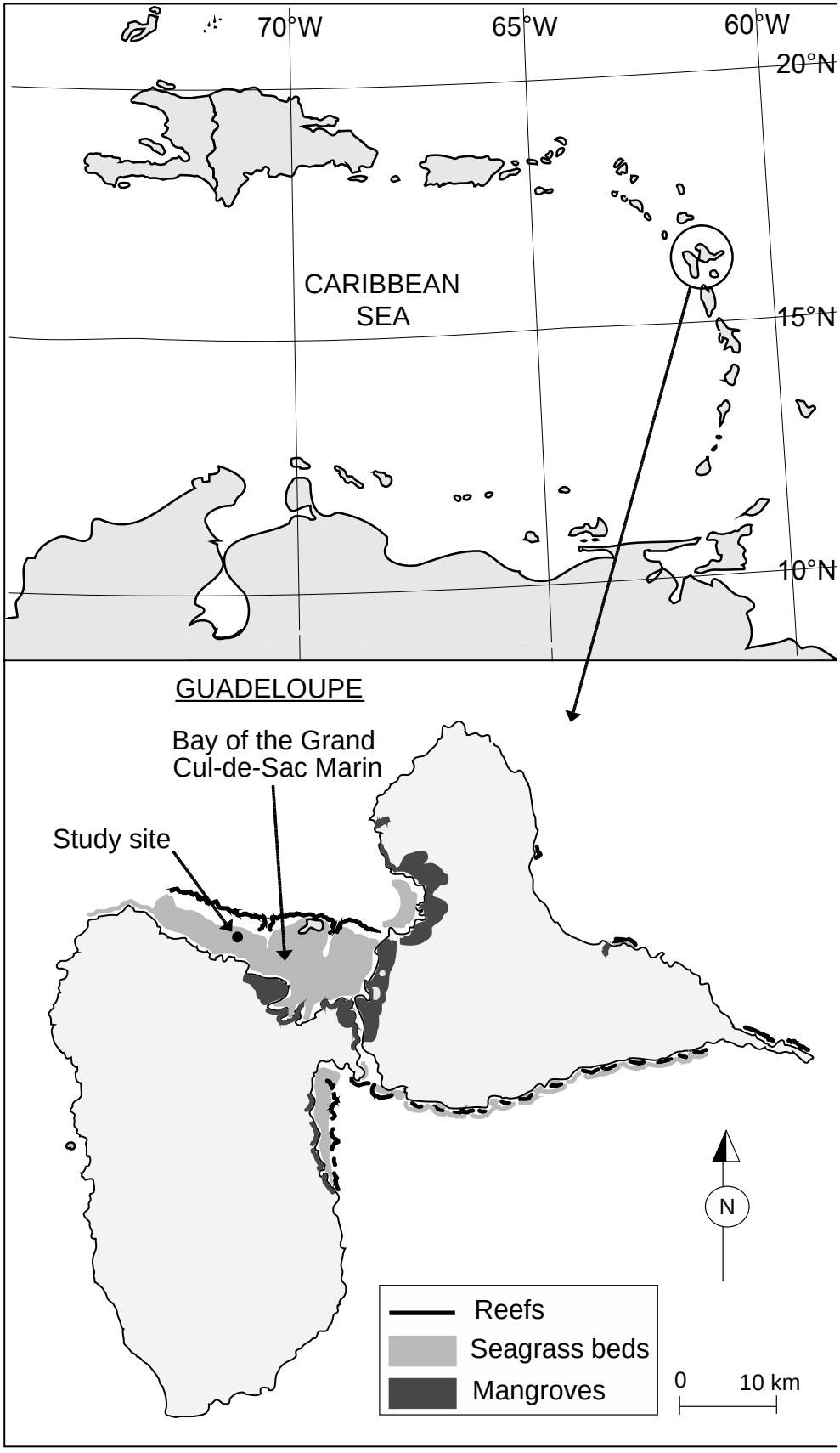

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Figure 2. Mean isotopic signatures of carbon ($\delta^{13}C \pm SD$) and nitrogen ($\delta^{15}N \pm SD$) of the three juvenile fish species and their potential food sources in Guadeloupean seagrass beds. *Thal.*-O: *Thalassia*-old leaves; *Thal.*-Y: *Thalassia*- young leaves; * indicates sources not taken into account in the mixing models.

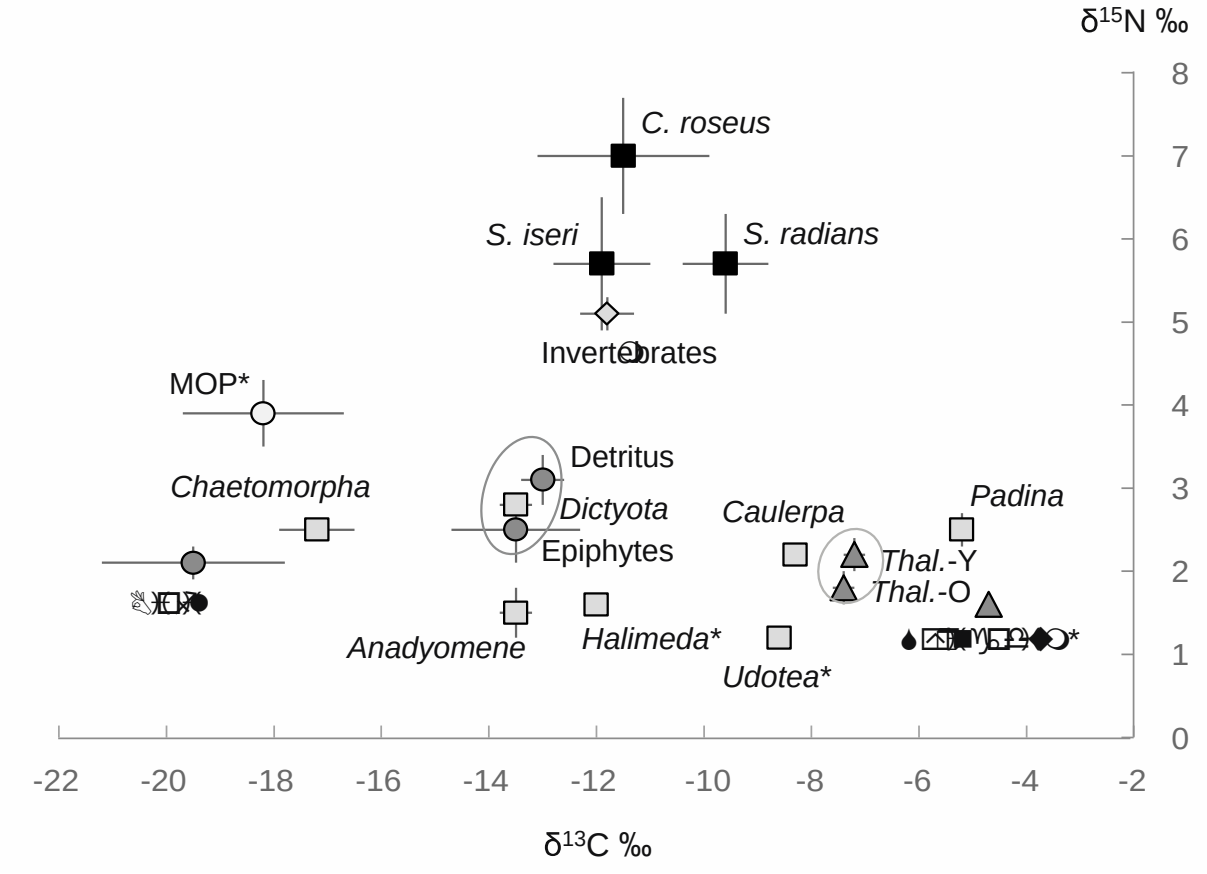
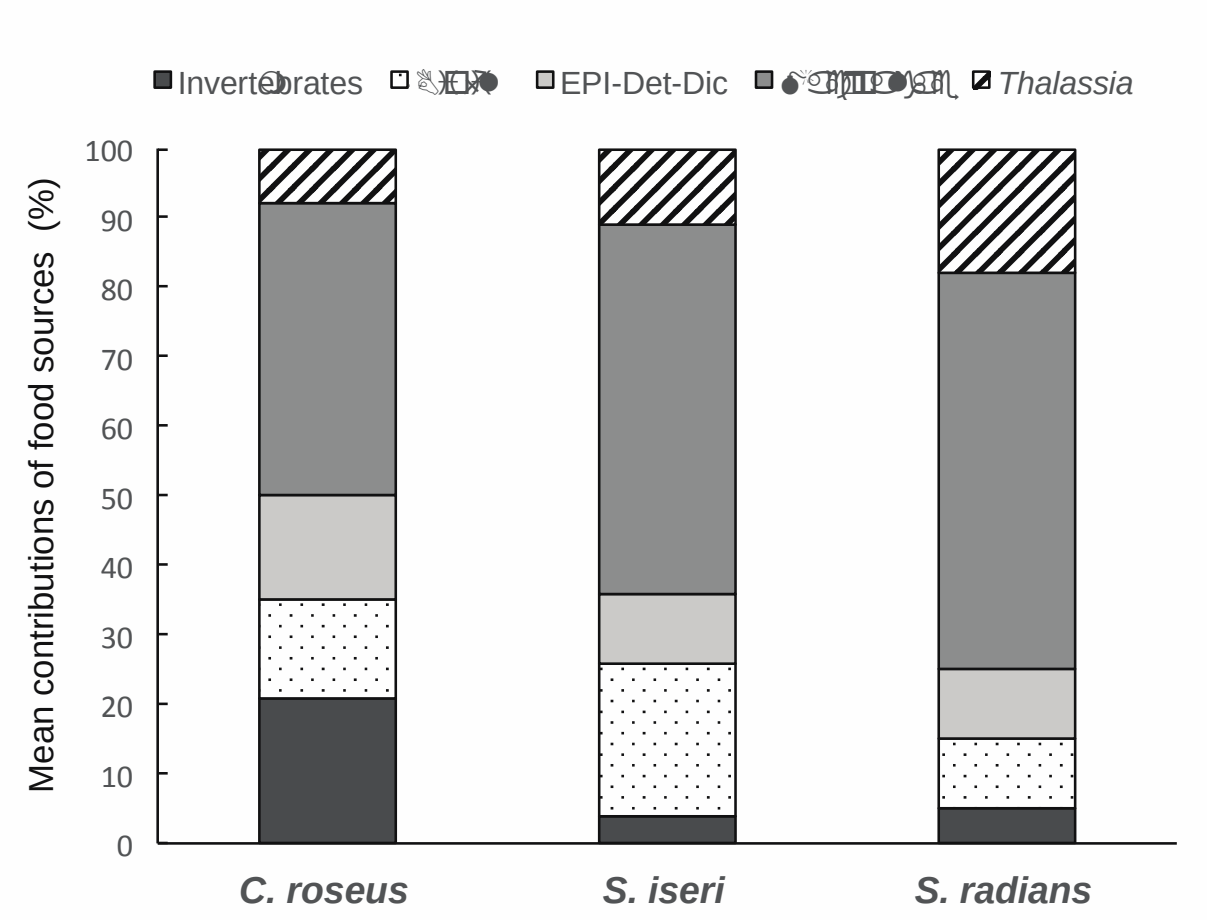


Figure 3. Mean contribution of food sources to the diet of fishes calculated with SIAR mixing models. 95% Bayesian Credibility Intervals of these values are given in Table 3. Fleshy macroalgae: *Anadyomene stellata*, *Caulerpa cupressoides*, *Chaetomorpha* sp. and *Padina* sp. EPI-Det-Dic: epiphytes, detritus and *Dictyota* sp.


Invertebrates
EPI-Det-Dic
Thalassia
Mean contributions of food sources (%)
100
90
80
70
60
50
40
30
20
10
0
C. roseus
S. iseri
S. radians

515

516 **Tables**

517

518 Table 1. Mean ± SD values of $\delta^{13}C$ and $\delta^{15}N$ signatures and C/N ratio of juvenile scarids and
519 organic matter sources collected in Guadeloupe seagrass beds. N: number of samples. *
520 Sources not taken into account in mixing models

Sample types	**N**	**$\delta^{13}C$ ‰ ± SD**	**$\delta^{15}N$ ‰ ± SD**	**C/N**
Fish species				
Cryptotomus roseus	13	-11.5 ± 1.6	7.0 ± 0.7	3.3 ± 0.1
Scarus iseri	11	-11.9 ± 0.9	5.7 ± 0.8	3.3 ± 0.03
Sparisoma radians	13	-9.6 ± 0.8	5.7 ± 0.6	3.1 ± 0.02
Invertebrates	5	-11.8 ± 0.5	5.1 ± 0.2	5.6 ± 0.5
MOP*	9	-18.2 ± 1.5	3.9 ± 0.4	7.1 ± 1.2
Biofilm	6	-19.5 ± 1.7	2.1 ± 0.2	9.4 ± 0.2
Detritus	5	-13.0 ± 0.04	3.3 ± 0.03	8.6 ± 0.1
Fleshy macroalgae				
Anadyomene stellata	3	-13.5 ± 0.3	1.5 ± 0.3	20.0 ± 0.4
Caulerpa cupressoides	3	-8.3 ± 0.1	2.2 ± 0.03	13.5 ± 0.2
Chaetomorpha sp.	3	-17.2 ± 0.7	2.5 ± 0.03	19.5 ± 0.7
Dictyota cf *pulchella*	3	-13.5 ± 0.3	2.8 ± 0.1	23.8 ± 0.5
*Halimeda incrassata**	3	-12.0 ± 0.04	1.6 ± 0.04	9.9 ± 0.1
Padina sp.	3	-5.2 ± 0.1	2.5 ± 0.2	38.0 ± 0.9
*Udotea flabellum**	3	-8.6 ± 0.03	1.2 ± 0.1	10.8 ± 0.1
Epiphytes (*Thalassia*)	6	-13.5 ± 1.2	2.5 ± 0.4	11.8 ± 2.9
Seagrass				
Thalassia testudinum (old)	10	-7.4 ± 0.1	1.8 ± 0.2	23.8 ± 2.1
Thalassia testudinum (young)	5	-7.2 ± 0.1	2.2 ± 0.1	19.7 ± 0.2
*Syringodium filiforme**	5	-4.7 ± 0.1	1.6 ± 0.07	23.0 ± 0.3

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525 Table 2. Mean and range of fish total length (TL) and wet weight (W) of the three fish
526 species. Lengths at maturity (Lm) are taken from Bouchon-Navaro *et al.* (2006).
527

Fish species	**TL (cm)**	**W (g)**	**Lm (cm)**
Cryptotomus roseus	5.7 (4.6 – 6.8)	2.5 (1.3 – 4.2)	8.6
Scarus iseri	5.0 (4.5 – 5.5)	2.0 (1.5 – 2.6)	15.9
Sparisoma radians	6.4 (6.0 – 6.7)	5.1 (4.5 – 5.7)	12.0

528
529
530 Table 3. Mean proportion of food items (± SD) ingested by *Cryptotomus roseus, Scarus iseri*
531 and *Sparisoma radians.*
532

Species	**Principal food items**	**Secondary food items**	**Method**
Cryptotomus roseus	Vegetal matter (72 ± 11%)	Invertebrates (28 ± 5%)	Gravimetric
Scarus iseri	Vegetal matter (58 ± 10%)	Sediment (12 ± 7%) and unidentified matter (30 ± 6%)	Point-intercept
Sparisoma radians	Vegetal matter (60 ± 8%)	Sediment (7 ± 4%) and unidentified matter (33 ± 8%)	Point-intercept

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536

537 Table 4. Mean contribution of food sources (%) and Bayesian Credibility Interval 95% to the
538 diet of *Cryptotomus roseus*, *Scarus iseri* and *Sparisoma radians* calculated with SIAR mixing
539 models.
540

Food sources	***C. roseus***	***S. iseri***	***S. radians***
	%	***%***	***%***
Invertebrates	21.1 (7.8 - 33.9)	3.5 (0.0 – 9.9)	4.9 (0.0 – 13.5)
Biofilm	14.7 (0.1 - 26.6)	22.4 (1.0 - 33.8)	10.1 (0.0 - 21.3)
Epiphytes+Detritus+*Dictyota*	14.7 (0.7 - 26.6)	9.8 (0.0 - 23.9)	10.4 (0.0 - 24.1)
Fleshy Macroalgae			
Anadyomene stellata	10.2 (0.0 - 23.0)	21.9 (0.1 - 43.0)	16.8 (0.1 - 33.1)
Caulerpa cupressoides	9.0 (0.0 - 20.8)	11.1 (0.0 - 25.2)	16.1 (0.0 - 31.0)
Chaetomorpha sp	14.8 (0.1 - 28.1)	13.3 (0.0 - 28.2)	9.2 (0.0 - 21.4)
Padina sp.	8.4 (0.0 - 19.0)	6.9 (0.0 - 17.1)	15.3 (0.1 - 28.1)
Seagrass			
Thalassia testudinum	8.4 (0.0 - 19.1)	11.1 (0.0 - 24.8)	17.2 (1.0 - 31.6)

541