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## Stable-isotope analyses reveal the importance of seagrass beds as feeding areas for juveniles of the speckled worm eel *Myrophis punctatus* (Teleostei: Ophichthidae) in Florida

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The feeding habits and habitats of the speckled worm eel *Myrophis punctatus* were studied on the mangrove edge of the Indian River Lagoon (IRL, Florida) using gut-content and stable-isotope analyses of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ). Four taxa were identified through analyses of gut contents, and the index of relative importance suggested that amphipods, microphytobenthos and annelids are the most important food sources in the fish's diet. To assess the feeding habits of the fish after their recruitment to the IRL, these food sources were collected from mangroves and nearby seagrass beds for isotope analyses. Stable isotopes constituted a powerful tool for discriminating fish prey items from mangroves (mean  $\pm$  s.d.  $\delta^{13}\text{C} = -20.5 \pm 0.6\text{‰}$ ) and those from seagrass beds (mean  $\pm$  s.d.  $\delta^{13}\text{C} = -16.9 \pm 0.6\text{‰}$ ), thus providing good evidence of food source origins. The 56 *M. punctatus* collected [ $10.0 < \text{total length } (L_T) < 16.2$  cm] had average isotopic signatures of  $\delta^{13}\text{C} = -16.7 \pm 0.2\text{‰}$  and  $\delta^{15}\text{N} = 8.2 \pm 0.1\text{‰}$ . A significant depletion in  $^{13}\text{C}$  was observed for larger juveniles ( $15.0 < L_T < 16.2$  cm), suggesting that they found a portion of their food in mangroves. Estimation of the trophic level from stable isotopes ( $T_{\text{Liso}}$ ) was similar among different size groups of juvenile fish ( $T_{\text{Liso}} = 3.2\text{--}3.5$ ); therefore, *M. punctatus* was considered a secondary consumer, which is consistent with its zoobenthic diet. The concentration-dependent mixing Stable Isotope Analysis in R (SIAR) model revealed the importance of food sources from seagrass beds as carbon sources for all the fish collected, with a significant increase in mangrove prey contributions, such as annelids, in the diet of larger juveniles. This study highlights the importance of seagrass beds as feeding habitats for juveniles of *M. punctatus* after their recruitment to coastal waters.

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### INTRODUCTION

The speckled worm eel *Myrophis punctatus* Lütken 1852 lives on sandy and muddy substrata of shallow coastal waters (depth < 20 m) such as mangrove forests, seagrass

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beds, intertidal bays or channels (Barletta *et al.*, 1999). This species occurs in the western Atlantic Ocean from Bermuda and North Carolina to south-east Brazil, including the Gulf of Mexico, West Indies and the coast of Central America (Froese & Pauly, 2010).

*Myrophis punctatus* migrates offshore to spawn, and the elopomorph leptocephali migrate to shallow estuaries and bays where they transform to juveniles (Warlen & Burke, 1990; Harnden *et al.*, 1999). In the Florida Bay, Harnden *et al.* (1999) observed a seasonal recruitment of *M. punctatus* leptocephali during autumn and winter with peaks in abundance during November and January. The development and transformation of the fish from leptocephali [total length ( $L_T$ ) of 48–59.8 mm] to elvers ( $L_T$  of 50.9–59 mm) and juveniles ( $L_T$  of 76–134 mm) coincide with a reduction in the body depth and fins, differentiation of the nostrils and modification of the teeth (Eldred, 1966; Fahay & Obenchain, 1978). The recruitment from pelagic to shallow coastal waters and the metamorphosis from leptocephali to juveniles coincide with a change of feeding habits and habitats. Leptocephalus larvae are planktivores and consume harpacticoid copepods, ostracods, diatoms and protozoa such as aloricate ciliates (Duque, 2004; Govoni, 2010). In the coastal waters of Louisiana, *M. punctatus* elvers rely primarily on organic matter from salt marshes (Duque, 2004). Research on other Ophichthidae, *e.g.* goldspotted eel *Myrichthys ocellatus* (LeSueur 1825) and finned worm eel *Echelus uropterus* (Temminck & Schlegel 1846), showed that larger juveniles ( $L_T > 20$  cm) are zoobenthic feeders that forage on small crustaceans (crabs and amphipods) and polychaetes (Randall, 1967; Harmelin-Vivien, 1979). Despite previous studies describing the diet of larvae and elvers, there is still scarce data on the foraging habits and habitats of *M. punctatus* after their recruitment to coastal waters.

Previous research on the ecological behaviour of *M. punctatus* in coastal habitats revealed that this species has developed strategies to live in macrotidal habitats and to resist stressful environmental conditions such as high variation in salinity, temperature and dissolved oxygen (Barletta *et al.*, 1999, 2000). In Brazil, Barletta *et al.* (2000) observed that *M. punctatus* remains in mangrove habitats during low tide by inhabiting crab holes, and lives most of the time in the vicinity of these burrows. By assuming similar behaviours between *M. punctatus* and other Ophichthidae species, juvenile and adult fish remain in crab burrows for *c.* 18 h each day and leave their holes only to feed at night (Barletta *et al.*, 1999). Despite this apparent association between *M. punctatus* and mangrove habitats, the dependence of the fish on mangrove food sources has not been established. In the western Atlantic Ocean, mangroves are often interlinked with seagrass beds, which appear to represent the main foraging grounds for nocturnally active zoobenthivorous fish species (Nagelkerken & van der Velde, 2004). Therefore, an assessment of the relative importance of food sources from mangroves and seagrass beds in the diet of juvenile *M. punctatus* is necessary to enhance the understanding of the role of these coastal habitats as feeding areas for this species.

In trophic studies, gut content analyses provide taxonomic resolution of prey items ingested, but they only reveal a snapshot of the consumer's diet within the last few hours (Hyslop, 1980). Erroneous or incomplete pictures of diets can be obtained due to difficulties in prey identification and differences in digestion rates of ingested materials (Hyslop, 1980; Cocheret de la Morinière *et al.*, 2003). Combining stable-isotope analyses (SIA) of carbon and nitrogen with dietary analyses may provide

the most comprehensive information on trophic transfers and food-web structures (Fry & Sherr, 1984). Due to isotopic enrichment during assimilation processes, there is a consistent enrichment of stable isotopes at each trophic level, on average *c.* 0.0–1.3‰ for  $\delta^{13}\text{C}$  and 2.0–3.5‰ for  $\delta^{15}\text{N}$  (DeNiro & Epstein, 1978, 1981; Post, 2002; McCutchan *et al.*, 2003). Stable isotopes of carbon enable determination of food sources consumed by an organism and the habitat where the consumer found its food. Primary producers use different photosynthetic pathways and inorganic carbon sources, which explains the wide range of  $\delta^{13}\text{C}$  signatures observed among terrestrial and aquatic macrophytes (Hemminga & Mateo, 1996; Bouillon *et al.*, 2008). Terrestrial  $\text{C}_3$  mangrove trees, which use  $\text{CO}_2$  as inorganic carbon, are characterized by  $\delta^{13}\text{C}$  signatures more depleted in  $^{13}\text{C}$  ( $\delta^{13}\text{C}$  generally between  $-30$  and  $-24$ ‰) than submerged seagrasses ( $\delta^{13}\text{C} > -15$ ‰), which use the bicarbonate ion  $\text{HCO}_3^-$  (Fry & Sherr, 1984; Hemminga & Mateo, 1996; Bouillon *et al.*, 2008). The abundance of  $^{15}\text{N}$  in the tissues of consumers is enriched by 2–3.5‰ relative to their food; therefore, nitrogen signatures give some insights into the trophic position of food sources and consumers in the food web (Post, 2002). Stable isotopes, which accumulate in body tissues of consumers, integrate food-source signatures from weeks to months following consumption and can be used as proxies for the origin of organic matter assimilated by a consumer (Post, 2002; Nagelkerken & van der Velde, 2004).

Combining gut content and stable-isotope analyses, the feeding habits of juvenile *M. punctatus* recruiting to the coastal waters of the Indian River Lagoon (IRL), Florida, were examined in this study and the dependence of the *M. punctatus* on mangrove and seagrass food sources was quantitatively determined. The objectives of this article were to (1) characterize the foraging habits of *M. punctatus* after their recruitment near shore, (2) identify the feeding habitats of juvenile *M. punctatus* and (3) determine the relative importance of food sources from mangroves and seagrass beds in their diet.

## MATERIALS AND METHODS

### STUDY AREA

The study was conducted along Big Starvation Cove, a 122 ha stand of coastal mangroves located on the west shore of North Hutchinson Island in the IRL ( $27^\circ 33' \text{N}$ ;  $80^\circ 20' \text{W}$ ; Fig. 1). The estuarine IRL is 260 km long and located on the Florida's east central coast. It is separated from the Atlantic Ocean by a series of barrier islands. Water salinity is on average between 20 and 35 but varies to brackish conditions due to rainfall and freshwater inputs from creeks and rivers located in the northern part of the estuary (Gilmore, 1995). The IRL is considered a microtidal lagoon, with semi-diurnal tidal amplitudes varying between 0.1 and 0.7 m near inlets (Smith, 1987). In the study area, water depth varies between 0.5 and 3.0 m above a muddy–sandy substratum. The National Wetlands Inventory estimated that the IRL harbours 20% of the 202 000 ha of the Florida's mangroves and contains three main mangrove species: red mangrove *Rhizophora mangle*, black mangrove *Avicennia germinans* and white mangrove *Laguncularia racemosa* (Lewis *et al.*, 1985). The Saint Johns River Water Management District (SJRWMD) estimated that seagrass beds, mainly represented by the turtlegrass *Thalassia testudinum*, shoalweed *Halodule wrightii* and manatee grass *Syringodium filiforme*, cover an area of 30 000 ha in the IRL and occupy *c.* 16 ha in the vicinity of the Fort Pierce (around the present study site, Virnstein *et al.*, 2007). The canopy height of the seagrass beds sampled in this study averages 10 cm (SJRWMD, unpubl. data). Sampling in this study was conducted along 400 m of mangrove shoreline dominated by *R. mangle*

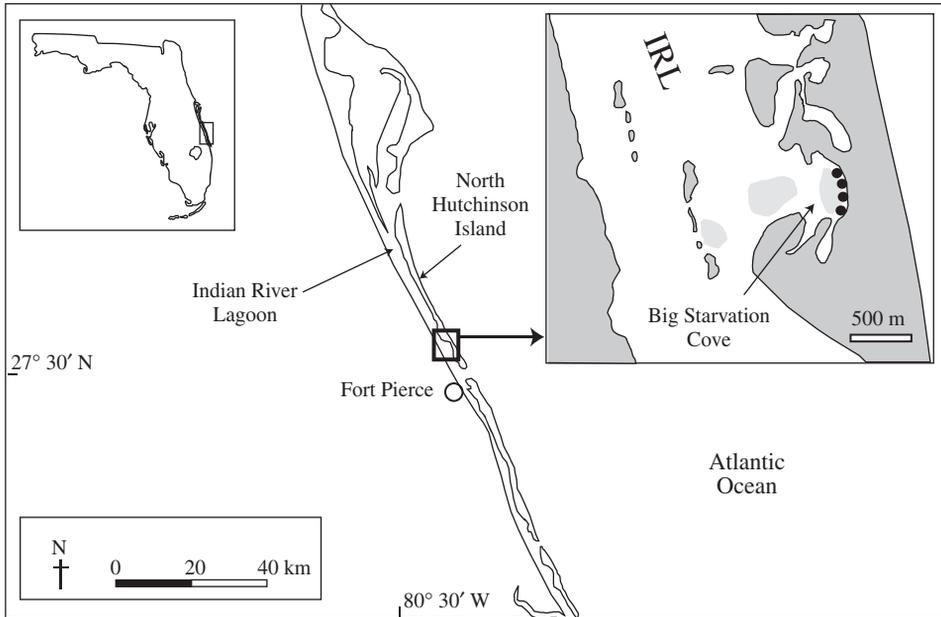


FIG. 1. Location of mangroves and seagrass beds sampled in the Indian River Lagoon (IRL), Florida [mangrove sites (●), seagrass sites (■)].

and in 4 ha seagrass beds located in three positions relative to the mangroves: immediately adjacent to the mangrove fringe, 500 m and 1 km from the mangrove shoreline (Fig. 1).

From September to December 2009, 56 specimens of *M. punctatus* were collected on the edge of the mangrove shoreline and inside the mangrove prop roots using light traps, rotenone and hand-nets. The specimens ranged between 10.0 and 16.2 cm in total length ( $L_T$ ) and, based on morphological characters, they can be characterized as juveniles (Eldred, 1966; Fahay & Obenchain, 1978). To assess any ontogenetic differences in foraging habits, the juveniles were sorted into three size groups: small juveniles ( $10.0 < L_T < 11.5$  cm;  $n = 15$  specimens), medium-sized juveniles ( $13.0 < L_T < 13.6$  cm;  $n = 11$ ) and large juveniles ( $15.0 < L_T < 16.2$  cm;  $n = 30$ ).

## GUT CONTENT ANALYSES

Gut content analyses were performed on each specimen to identify prey items to the lowest possible taxonomic level. To estimate the relative contribution of a prey item in the fish diet, the index of relative importance ( $I_{RI}$ ) was calculated by combining the percentage of number ( $\%N_b$ ), gravimetric measures ( $\%M$ , dry mass) and frequency of occurrence ( $\%F$ ) of different prey items following the equation:  $I_{RI} = (\%N_b + \%M)\%F$  (MacDonald & Green, 1983). The  $I_{RI}$  index is expressed as a percentage ( $\%I_{RI}$ ) and enables the separation of different prey items according to their importance such as main prey ( $\%I_{RI} > 50\%$ ), secondary prey ( $25\% < \%I_{RI} < 50\%$ ) or rare prey ( $\%I_{RI} < 25\%$ ).

To assess *M. punctatus* feeding habits and habitats, the main prey species identified in gut contents were then collected in mangroves and seagrass beds and subjected to stable-isotope analyses. Primary producers from mangroves (litter, algae and microphytobenthos) and seagrass beds (seagrass leaves and their epiphytes and microphytobenthos) were also sampled to assess the origin of the organic matter supporting fish prey items. Epiphytes on *T. testudinum* seagrasses were removed by scraping the seagrass leaves. Microphytobenthos, composed of benthic diatoms, were sampled by collecting the top centimetres of the sediment

following the methods of Couch (1989) and Riera & Richard (1996). Plankton samples were collected using a plankton net towed in the top 2 m of water and zooplankton (mainly comprising copepods) was separated from tripton in the laboratory. Particulate organic matter (POM) was sampled by filtering surface water following the method of Riera & Richard (1996). Crustaceans and annelids were collected with light traps and hand-nets.

## STABLE-ISOTOPE ANALYSES AND ISOTOPIC MIXING MODELS

Stable-isotope analyses of carbon ( $^{13}\text{C}:^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}:^{14}\text{N}$ ) were performed on *M. punctatus* and prey species to identify the carbon sources and the trophic levels of the organisms. Isotopic analyses were performed on *M. punctatus* muscle tissue and on entire prey items. The samples were rinsed with distilled water and dried at 60°C for at least 48 h. Carbon and nitrogen stable-isotope compositions were measured with a Thermo Scientific Delta V Advantage mass spectrometer (www.thermo.com) coupled to a C-N-S Costech ECS 4010 Elemental Analyser for combustion of organic material to  $\text{CO}_2$  and  $\text{N}_2$  gases. The analyses were performed at the Smithsonian Institution, Office of the Undersecretary for Science Museum Conservation Institute (OUSS/MCI), Stable Isotope Mass Spectrometry Laboratory. Isotopic ratios are expressed in standard delta notation according to the following formula:  $\delta = 1000 [(R_{\text{sample}} - R_{\text{standard}}) R_{\text{standard}}^{-1}]$ , where  $R$  is the ratio of heavy to light isotope (*i.e.*  $^{15}\text{N}:^{14}\text{N}$  or  $^{13}\text{C}:^{12}\text{C}$ ),  $R_{\text{sample}}$  is  $R$  measured for fish and prey samples and  $R_{\text{standard}}$  is an international standard (Vienna Pee Dee belemnite limestone carbonate for carbon and atmospheric air for nitrogen). Average reproducibilities based on replicate measurements on standards are 0.2‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

The variation of lipid content among tissues of an organism can affect  $\delta^{13}\text{C}$  values and thus ecological interpretations (Post *et al.*, 2007). The  $\delta^{13}\text{C}$  values of *M. punctatus* muscle tissues were normalized for lipid content according to the mathematical normalization technique of Post *et al.* (2007). Those authors proposed a correction factor for  $\delta^{13}\text{C}$  based on C:N values (*i.e.* C:N ratios >3.5 for lipid-rich tissue) and lipid content in animal samples.

On the basis of the model developed by Post (2002),  $\delta^{15}\text{N}$  values of *M. punctatus* were converted to trophic level ( $T_{\text{Liso}}$ ) under the assumption that enrichment in  $\delta^{15}\text{N}$  per trophic level is 2.2‰ and the trophic position of the baseline is 1 (Froese & Pauly, 2010):  $T_{\text{Liso}} = 1 + (\delta^{15}\text{N}_{\text{Eel}} - \delta^{15}\text{N}_{\text{Base}})2.2^{-1}$ , with  $\delta^{15}\text{N}_{\text{Eel}}$  the  $\delta^{15}\text{N}$  values of *M. punctatus* specimens and  $\delta^{15}\text{N}_{\text{Base}}$  the  $\delta^{15}\text{N}$  signatures of microphytobenthos from mangroves (the representative baseline in this study).

The Bayesian mixing model SIAR v4.0 (Stable Isotope Analysis in R) developed by Parnell *et al.* (2010) was used to estimate the extent to which *M. punctatus*' food sources were supported by organic matter from mangroves and seagrass beds and subsequently the relative contribution of food sources in the fish diet. Besides being able to work with underdetermined systems [where the number of food sources is greater than the number of isotopic signatures plus one (Phillips & Gregg, 2003)], SIAR accounts for uncertainties associated with sample variability and trophic enrichment. The SIAR mixing model assumes that variability associated with food sources and trophic enrichment is normally distributed (Parnell *et al.*, 2010). Bayesian models modify prior estimates of source contributions based on new observed data, which results in posterior distributions of source contributions. In the absence of any specific prior estimates, non-informative priors were used and assumed that all source contributions were equal. To assess the origin of the organic matter supporting *M. punctatus*' food sources, the SIAR mixing model was performed on annelids, amphipods and on several primary producers from mangroves (litter, algae and microphytobenthos) and seagrass beds (seagrass leaves and their epiphytes and microphytobenthos). To evaluate fish feeding habits, SIAR was performed on four different food sources collected in both mangroves and seagrass beds: microphytobenthos, zooplankton, amphipods and annelids. Due to the omnivorous diet of *M. punctatus*, the model incorporated concentration dependence (Phillips & Koch, 2002) in the results to consider the different digestibility of sources (plants and animals). The assimilated carbon and nitrogen concentrations of each source were estimated with the source's digestibility reported in the literature and the elemental concentrations ([C] and [N]) measured in this study following the methods outlined by Koch & Phillips (2002). *A posteriori* combinations

of source proportions were determined as described by Phillips *et al.* (2005) to provide the composite contributions of sources from mangroves and seagrass beds to the diet. Prior to modelling, isotope signatures were adjusted for trophic enrichment using published values determined for invertebrates,  $\Delta C$  of  $1.0 \pm 0.3\text{‰}$  and  $\Delta N$  of  $2.0 \pm 0.3\text{‰}$ , and for omnivorous fish species,  $\Delta C$  of  $1.0 \pm 0.3\text{‰}$  and  $\Delta N$  of  $2.2 \pm 0.3\text{‰}$  (mean  $\pm$  s.d.) (Vander Zanden & Rasmussen, 2001; McCutchan *et al.*, 2003). The SIAR mixing model was utilized on the three size groups of juvenile *M. punctatus*.

## STATISTICAL ANALYSES

Data were tested for normality using Shapiro–Wilk tests and for homogeneity of variances with Levene’s tests. Differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were tested using parametric and non-parametric analyses as appropriate. Potential disparities among isotopic values of food sources were tested using *t*-tests, as these variables were normally distributed and showed homogeneous variances. Because isotopic values of *M. punctatus* samples were not normally distributed and did not show homogeneous variances, non-parametric tests of Mann–Whitney and Kruskal–Wallis were used to test for differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures among specimens (Legendre & Legendre, 1998). To describe similarities and differences in the fish feeding habits, a hierarchical agglomerative cluster analysis with complete linkages was performed on the  $I_{\text{RI}}$  data set using Bray–Curtis similarity (Legendre & Legendre, 1998). A significant level of  $P < 0.05$  was used in all tests and all statistical analyses were performed using the programme R (R Development Core Team; www.r-project.org).

## RESULTS

### *MYROPHIS PUNCTATUS* DIET

A total of 56 specimens of *M. punctatus* were studied, 8 of which had empty stomachs. Four types of food sources belonging to three taxa were identified in gut contents: crustaceans (zooplankton and amphipods), annelids and benthic diatoms (microphytobenthos). Among the variety of zooplanktonic organisms, copepods were the main items observed.

The  $I_{\text{RI}}$  of the different taxa identified in gut contents varied among the three size groups of juvenile *M. punctatus* (Fig. 2). The most important prey consumed by small and medium-sized *M. punctatus* ( $10.0 < L_{\text{T}} < 13.6$  cm) were amphipods (55.1 and 47.8%, respectively) and microphytobenthos (37.5 and 32.9%, respectively), whereas large juveniles ( $15.0 < L_{\text{T}} < 16.2$  cm) consumed a higher percentage of

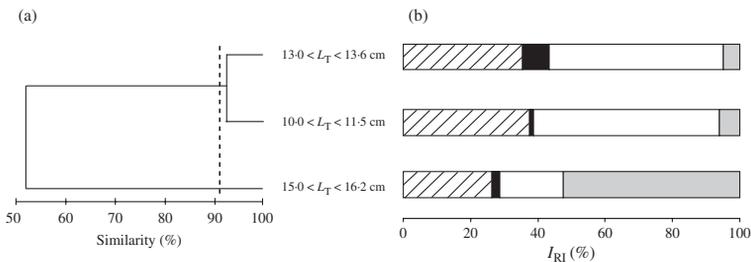


FIG. 2. (a) Cluster analysis of similarity in *Myrophis punctatus* feeding habits among the three size groups of juveniles. (b) Per cent of the index of relative importance ( $I_{\text{RI}}$ ) of the dietary analyses performed on the three size groups of juvenile *M. punctatus* [microphytobenthos (▨), zooplankton (■), amphipods (□), annelids (◼)].

annelids (48.6%) followed by microphytobenthos (24.5%) [Fig. 2(b)]. Cluster analysis performed on the  $I_{RI}$  data obtained for the three size groups of *M. punctatus* differentiated two clusters at a similarity of 90% [Fig. 2(a)]. These clusters corresponded to the different dietary habits observed between small–medium juveniles and large juveniles [Fig. 2(b)]. Gut content dietary analyses showed that all juveniles of *M. punctatus* feed on zoobenthos and plant material (microphytobenthos) and could be characterized as omnivores.

### STABLE-ISOTOPE ANALYSES OF FOOD SOURCES

No difference was observed between the isotopic values of primary producers and food sources from the three seagrass-bed sites, therefore the isotopic values were pooled (Fig. 3). A gradient in  $\delta^{13}C$  signatures was discerned for primary producers and *M. punctatus*' food sources from mangroves and seagrass beds (Table I and Fig. 3). Mangrove food sources were more depleted in the heavier carbon  $^{13}C$  ( $\delta^{13}C_{\text{mean}} \pm \text{s.d.} = -21.8 \pm 1.1\text{‰}$ ) compared with those collected in seagrass beds ( $\delta^{13}C_{\text{mean}} \pm \text{s.d.} = -16.9 \pm 0.7\text{‰}$ , pair-wise *t*-test, d.f. = 49,  $P < 0.001$ ). This trend was also observed while considering only *M. punctatus*' food sources (microphytobenthos, zooplankton, amphipods and annelids) from mangroves ( $\delta^{13}C_{\text{mean}} \pm \text{s.d.} = -20.5 \pm 0.6\text{‰}$ ) and seagrass beds ( $\delta^{13}C_{\text{mean}} \pm \text{s.d.} = -16.9 \pm 0.6\text{‰}$ , pair-wise *t*-test, d.f. = 38,  $P < 0.001$ ). Primary producers from mangroves and seagrass beds had the lowest  $\delta^{15}N$  signatures ( $\delta^{15}N_{\text{mean}} \pm \text{s.d.} = 3.4 \pm 0.5\text{‰}$ ) compared with animal prey items ( $\delta^{15}N_{\text{mean}} \pm \text{s.d.} = 5.9 \pm 0.5\text{‰}$ , pair-wise *t*-test, d.f. = 49,  $P < 0.001$ ). The C:N ratios provided insights on the digestibility of fish prey and

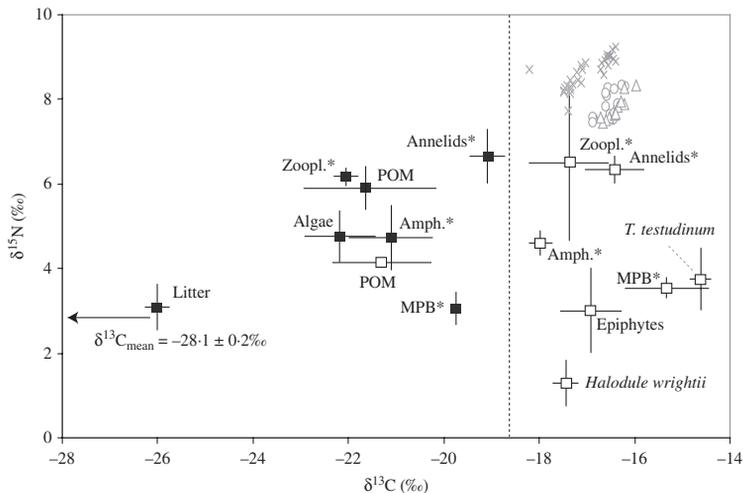


FIG. 3. Mean  $\pm$  s.d.  $\delta^{13}C$  and  $\delta^{15}N$  signatures of primary producers and *Myrophis punctatus*' food sources collected in mangroves (■) and seagrass beds (□). Isotopic values of the three size groups of *M. punctatus* are shown: small juveniles (○, total length ( $L_T$ ) 10.0–11.5 cm,  $n = 15$ ), medium-sized juveniles ( $\Delta$ ,  $L_T$ : 13.0–13.6 cm,  $n = 11$ ) and large juveniles ( $\times$ ,  $L_T$ : 15.0–16.2 cm,  $n = 30$ ). *Myrophis punctatus* prey items considered in this study are shown by \*. The dotted line refers to the separation of  $\delta^{13}C$  values between mangrove and seagrass food sources. Amph., amphipods; MPB, microphytobenthos; POM, particulate organic matter; *T. testudinum*, *Thalassia testudinum*; Zoopl., zooplankton.

TABLE I. Mean  $\pm$  S.D. values of carbon and nitrogen signatures and elemental concentrations of *Myrophis punctatus* food sources and the three total length groups of *M. punctatus* collected in the mangroves and seagrass beds of the Indian River Lagoon

Samples	<i>n</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%C (wt)	%N (wt)	C:N	$T_{\text{Liso}}$
Mangrove sources							
Microphytobenthos	3	$-19.8 \pm 0.1$	$3.1 \pm 0.4$	7.7	0.8	8.9	
Zooplankton	4	$-22.1 \pm 0.2$	$6.2 \pm 0.2$	37.6	8.2	4.6	
Amphipods	3	$-21.1 \pm 0.9$	$4.7 \pm 0.8$	29.3	5.8	5.1	
Annelids	4	$-19.1 \pm 0.4$	$6.7 \pm 0.6$	48.1	12.2	3.9	
Seagrass sources							
Microphytobenthos	2	$-15.3 \pm 0.9$	$3.5 \pm 0.2$	4.4	0.9	4.9	
Zooplankton	3	$-17.4 \pm 0.8$	$6.5 \pm 1.4$	32.4	7.5	4.4	
Amphipods	3	$-18.0 \pm 0.2$	$4.6 \pm 0.3$	16.0	3.6	4.5	
Annelids	3	$-16.4 \pm 0.6$	$6.4 \pm 0.3$	27.7	5.9	4.7	
<i>M. punctatus</i>							
Group 1: 10.0–11.5 cm	15	$-16.5 \pm 0.1$	$7.9 \pm 0.1$	43.3	11.7	3.7	$3.2 \pm 0.1$
Group 2: 13.0–13.6 cm	11	$-16.4 \pm 0.1$	$7.8 \pm 0.2$	43.8	11.3	3.9	$3.2 \pm 0.1$
Group 3: 15.0–16.2 cm	30	$-17.0 \pm 0.1$	$8.6 \pm 0.1$	43.5	11.3	3.9	$3.5 \pm 0.1$

*n*, number of samples;  $T_{\text{Liso}}$ , trophic levels of the fish calculated from stable-isotope values.

revealed differences among the plant and animal food sources (Table I). Animal sources were more digestible (lower C:N values,  $\text{C:N}_{\text{mean}} \pm \text{s.d.} = 4.5 \pm 0.3$ ) compared to microphytobenthos (higher C:N ratios,  $\text{C:N}_{\text{mean}} \pm \text{s.d.} = 7.3 \pm 1.9$ ) (pair-wise *t*-test, d.f. = 23,  $P < 0.001$ ).

#### STABLE-ISOTOPE SIGNATURES AND TROPHIC LEVELS OF FISH SPECIES

The 56 specimens of *M. punctatus* collected along the mangrove shoreline had mean  $\pm$  S.D.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of  $-16.7 \pm 0.2$  and  $8.2 \pm 0.1\text{‰}$ , respectively (Table I and Fig. 3). The overall distributions of  $\delta^{13}\text{C}$  signatures showed that, considering a trophic enrichment of 1‰, *M. punctatus* carbon values were in general quite similar to those of seagrass food sources (Table I and Fig. 3). Kruskal–Wallis tests revealed significant differences in  $\delta^{13}\text{C}$  ( $H = 19.74$ ,  $P < 0.001$ ) and  $\delta^{15}\text{N}$  ( $H = 32.40$ ,  $P < 0.001$ ) among the three groups of *M. punctatus*. Large juveniles ( $15.0 < L_T < 16.2$  cm) were more depleted in  $^{13}\text{C}$  than small and medium-sized juveniles ( $10.0 < L_T < 13.6$  cm), suggesting the assimilation of food sources with lower carbon signatures. The examination of  $\delta^{15}\text{N}$  values among the three size groups showed that large juveniles had more enriched  $\delta^{15}\text{N}$  signatures than small and medium-sized juveniles, with an average difference of 0.8‰ in  $\delta^{15}\text{N}$  between these size groups (Table I and Fig. 3). Even with these differences in  $\delta^{15}\text{N}$  values, trophic levels were similar among the different size groups of *M. punctatus* and ranged between  $3.2 \pm 0.1$  for small and medium-sized juveniles and  $3.5 \pm 0.1$  for larger ones (Table I). The trophic position of *M. punctatus* was *c.* 2.2–2.5‰ higher in  $\delta^{15}\text{N}$  than the baseline (microphytobenthos; Table I); *M. punctatus* is thus considered a secondary consumer.

## ISOTOPIC MIXING MODELS

A mixing model performed between *M. punctatus* food sources and primary producers indicated higher contributions of organic matter from mangroves for invertebrates collected in mangroves. Mean (Bayesian 95% c.i.) proportions of mangrove carbon sources varied between 58.3% (34.8–81.5%) for amphipods and 56.2% (41.4–71.4%) for annelids and were lower for the same invertebrates collected in seagrass beds, with contributions of 41.6% (18.5–65.2%) and 36.8% (15.5–60.0%). Invertebrates from seagrass beds were mainly supported by organic matter from seagrass habitats with mean (Bayesian 95% c.i.) contributions of 57.8% (43.3–72.7%) for amphipods and 63.1% (39.9–84.5%) for annelids. Lower mean (Bayesian 95% c.i.) contributions of mangrove sources were observed for these invertebrates from seagrass beds, with contributions of 42.1% (27.2–56.7%) for amphipods and 36.8% (15.5–60.0%) for annelids. An overlap in Bayesian 95% c.i. was observed between mangrove and seagrass food source contributions, but was <31% for mangrove invertebrates and <21% for seagrass invertebrates. This overlap in mangrove and seagrass contributions in the invertebrate diets is relatively low and may be due to the fact that all invertebrate food sources were not considered in this study. As POM carbon signatures were similar between mangroves and seagrass beds (pair-wise *t*-test, d.f. = 2,  $P > 0.05$ ), their relative contributions to zooplankton diet was not assessed. A good separation in carbon signatures for zooplankton from mangroves ( $\delta^{13}\text{C}_{\text{mean}} \pm \text{s.d.} = -22.1 \pm 0.2\text{‰}$ ) and seagrass beds ( $\delta^{13}\text{C}_{\text{mean}} \pm \text{s.d.} = -17.4 \pm 0.8\text{‰}$ , pair-wise *t*-test, d.f. = 6,  $P < 0.01$ ) suggested, however, the assimilation of carbon sources depleted and enriched in heavier  $^{13}\text{C}$ , respectively, depending on the habitat (Table I and Fig. 3).

Considering fish species, the SIAR mixing model, performed after *a posteriori* grouping of food sources from mangroves and seagrass beds, produced a narrow distribution of possible source contributions to the fish diet with no overlap between mangrove and seagrass source contributions (Fig. 4). Thus, the mean and Bayesian 95% c.i. for biomass proportions of food sources enabled the determination of the relative importance of mangrove and seagrass sources in the fish diet. SIAR results showed that seagrass sources constituted the main diet for small (mean 79.2%; Bayesian 95% c.i. 64.2–92.3%), medium-sized (80.8%; 65.3–94.1%) and large juveniles of *M. punctatus* (64.6%; 52.9–75.5%; Fig. 4). Mangrove source contributions were low for small and medium-sized juveniles with mean (Bayesian 95% c.i.) values of 20.8% (7.6–35.7%) and 19.2% (5.8–34.6%). A slight increase in the percentage of mangrove sources was observed in the diet of large juveniles (mean 35.4%; Bayesian 95% c.i. 24.4–47.1%) (Fig. 4). Results from the isotopic mixing model therefore corroborated the ontogenetic dietary shift observed between small, medium-sized and large juveniles.

Ranges of per cent biomass of each food source to the fish diet help describe the different foraging habits of the three size groups of fish (Table II). In terms of per cent biomass, the diet of small and medium-sized juvenile *M. punctatus* comprised annelids (12.3–36.1%), microphytobenthos (3.2–47.2%) and amphipods (3.0–40.6%) from the seagrass beds. For large juveniles, the results showed a decrease in the consumption of microphytobenthos and amphipods from seagrass beds concurrent with an important increase in the utilization of annelids from mangroves (9.4–32.6%; Table II). This increase in the percentage of annelids from mangroves explains the rise in mangrove source contributions in the diet of large

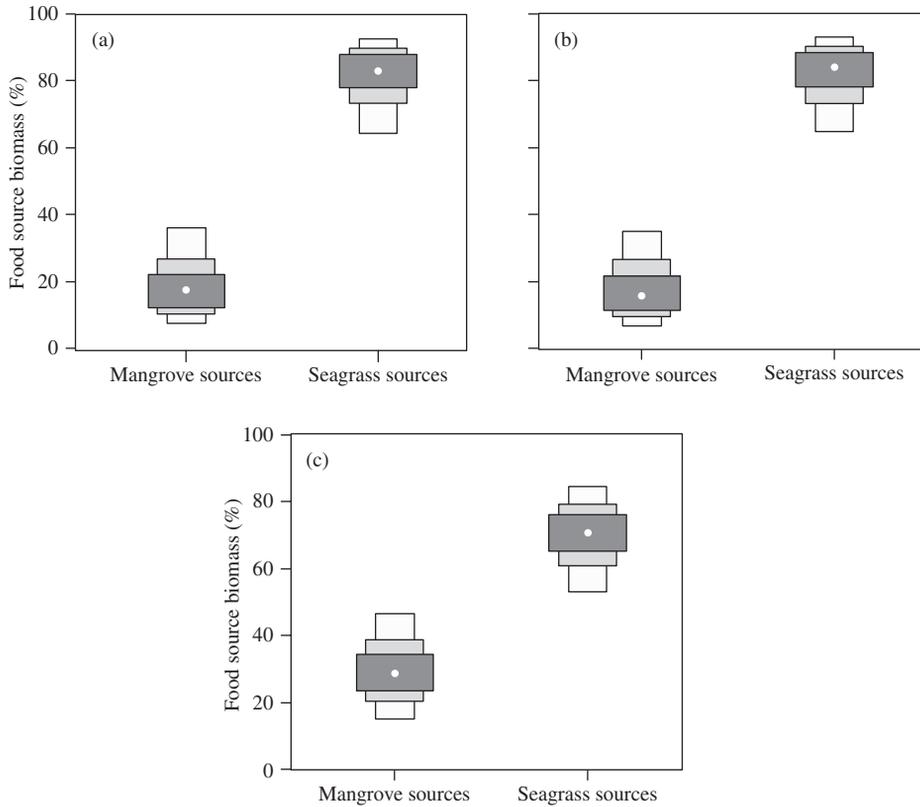


FIG. 4. Per cent biomass of mangrove and seagrass sources in juvenile *Myrophis punctatus* diet estimated with SIAR v4.0 after *a posteriori* grouping of the sources contributions: (a) small juveniles [ $10.0 < L_T < 11.5$  cm], (b) medium-sized juveniles ( $13.0 < L_T < 13.6$  cm) and (c) large juveniles ( $15.0 < L_T < 16.2$  cm). The plot shows the median (O), 50 (■), 95 (□) and Bayesian 99% c.i. (□) of the feasible biomass contributions of each food source to the fish diet.

juveniles of *M. punctatus* (Fig. 4) and the more depleted  $\delta^{13}\text{C}$  values observed for these specimens (Fig. 3 and Table II). The examination of isotopic signatures and results from the SIAR mixing model confirmed the change in the origin and proportions of food sources in the diets of small, medium-sized and large juveniles (Figs 3 and 4). The carbon and nitrogen mean contributions of food sources were quite similar for the three size groups of fish with the exception of microphytobenthos, which showed per cent nitrogen mean contributions (3.0%) lower than carbon (4.3%). This difference is probably due to the very low nitrogen content of microphytobenthos and illustrates the advantage of utilizing a concentration-dependent model; otherwise, carbon and nitrogen contributions would incorrectly have been assumed to be the same when calculating the biomass contributions.

TABLE II. Mean (Bayesian 95% C.I.) biomass contributions of each food source to *Myrophis punctatus* diet estimated with Stable Isotope Analysis in R (SIAR). Important source contributions are shown in bold

	Biomass contributions (%)		
	Small juveniles 10.0 < $L_T$ < 11.5 cm	Medium-sized juveniles 13.0 < $L_T$ < 13.6 cm	Large juveniles 15.0 < $L_T$ < 16.2 cm
Mangrove sources			
Microphytobenthos	12.4 (0.0–28.2)	12.4 (0.0–28.3)	7.0 (0.0–18.4)
Zooplankton	1.7 (0.0–4.3)	1.5 (0.0–3.7)	4.1 (0.0–10.1)
Amphipods	2.9 (0.0–7.3)	2.7 (0.0–7.0)	2.7 (0.0–7.1)
Annelids	3.7 (0.0–7.7)	2.6 (0.0–6.1)	<b>21.6 (9.4–32.6)</b>
Seagrass sources			
Microphytobenthos	<b>25.7 (3.2–47.2)</b>	<b>28.3 (4.3–51.7)</b>	9.6 (0.0–23.8)
Zooplankton	7.0 (0.1–13.7)	6.2 (0.0–13.3)	13.8 (0.2–25.9)
Amphipods	<b>22.5 (3.0–40.6)</b>	<b>22.2 (1.6–41.2)</b>	5.4 (0.0–14.2)
Annelids	<b>23.9 (12.3–36.1)</b>	<b>23.9 (11.4–36.8)</b>	<b>35.7 (19.3–52.0)</b>

$L_T$ , total length.

## DISCUSSION

Several studies have examined dietary habits of larvae and elvers of *M. punctatus* and revealed a progressive change from a planktivorous to a zoobenthivorous diet (Mochioka & Iwamizu, 1996; Duque, 2004; Govoni, 2010). This study provides information on the diet of juveniles after their recruitment to coastal habitats of the IRL. The zoobenthic feeding activity observed for juvenile *M. punctatus* is similar to that observed for other Ophichthidae (Randall, 1967; Harmelin-Vivien, 1979). The importance of benthic prey items, such as amphipods and annelids, in the *M. punctatus* diet is consistent with the behaviour of this species, which lives on muddy–sandy sediments and has been observed in fiddler crab *Uca* sp. burrows during low tides (Barletta *et al.*, 2000). Araújo *et al.* (2009) noted that the feeding strategies of *M. ocellatus*, and presumably of other Ophichthidae species, consisted of scanning the substratum surface and searching for buried prey items using their head and tail as searching tools. This behaviour would explain the discovery in this study of microphytobenthos in the guts of juvenile *M. punctatus*: the fish are probably passively ingesting both sediment and benthic diatoms during their stay in crab holes and during their feeding activity. With microphytobenthos forming the baseline of the estuarine benthic food web (Post, 2002), *M. punctatus* is considered a secondary consumer ( $T_{\text{Liso}}$  is *c.* 3), a categorization congruent with its zoobenthic diet.

Stable-isotope data showed a separation in  $\delta^{13}\text{C}$  values for food sources collected in mangroves and seagrass beds of the IRL, reinforcing the value of SIAR in providing reliable insights into the origin of prey items consumed by an organism (Nagelkerken & van der Velde, 2004). The variation of  $\delta^{13}\text{C}$  observed between food sources has been previously documented in the mangroves and seagrass meadows of Florida and the Bahamas (Kieckbusch *et al.*, 2004). The  $\delta^{13}\text{C}$  values of mangrove food sources in this study (–28.1 to –19.1‰) are similar to those reported in the IRL and other mangrove areas in Florida, the Bahamas and Curaçao (Kieckbusch *et al.*,

2004; Nagelkerken & van der Velde, 2004; Kemp, 2008). It is interesting to note that  $\delta^{13}\text{C}$  signatures obtained in this study for food sources from IRL seagrass beds have more depleted carbon values ( $-18.0$  to  $-14.6\text{‰}$ ) than prey items collected in other seagrass meadows in tropical and subtropical areas ( $-14$  and  $-11\text{‰}$ ; Kieckbusch *et al.*, 2004; Nagelkerken & van der Velde, 2004). Carbon signatures of seagrass plants are strongly influenced by the isotopic composition of the dissolved inorganic carbon (DIC) pool available for photosynthesis (Hemminga & Mateo, 1996). Previous studies conducted in Florida reported that  $^{13}\text{C}$ -depleted carbon signatures of the DIC pool, and therefore of seagrasses, may be related to terrestrial inputs from freshwater discharge or to mineralized mangrove organic matter (Lin *et al.*, 1991; Anderson & Fourqurean, 2003). The sites sampled in this study were located near the Fort Pierce inlet and would have been influenced more by marine inputs than freshwater discharges that occur in the northern part of the IRL. Therefore, the depleted carbon signatures observed for seagrass beds are most likely due to the influence of DIC from mangroves (Lin *et al.*, 1991). These relatively depleted  $\delta^{13}\text{C}$  signatures of seagrass sources were also observed in the same area of the IRL by Fry (1984), who found that  $\delta^{13}\text{C}$  values of seagrass epifauna (amphipods and annelids:  $-23.1$  to  $-14.8\text{‰}$ ) are more similar to seagrass epiphytes ( $-22.1$  to  $-16.9\text{‰}$ ) than to seagrass leaves and debris ( $-13.5$  to  $-7.1\text{‰}$ ). These results suggest that in this area of the IRL, seagrass fauna depend more on algal (epiphytes) than seagrass carbon sources (Fry, 1984). Carbon signatures of POM reported here were homogeneous between seagrass and mangrove sites, which may be the result of tidal and current mixing in the IRL. In this estuarine system, carbon signatures of POM exhibited seasonal variation, with lower mean  $\delta^{13}\text{C}$  values in September–October ( $-23.9$  to  $-21.6\text{‰}$ ) than in February (mean  $\pm$  S.D.  $-20.1 \pm 0.8\text{‰}$ ) (Fry, 1984; present study). These differences in the isotopic signatures of POM are probably the results of fluctuations of riverine inflow and tidal currents affecting the inputs of terrestrial and marine organic matter, respectively, in the lagoon (Bouillon *et al.*, 2008).

Previous studies have documented depleted carbon isotopic values in shrimps and fishes inhabiting mangroves that reflect mangrove source contributions in their diet (Rodelli *et al.*, 1984; Marguillier *et al.*, 1997). In this study, large juveniles of *M. punctatus*, which consume higher proportions of annelids from mangroves than small and medium-sized juveniles, had more depleted carbon values. The greater importance of food sources from seagrass beds in the diet of all juvenile *M. punctatus* reported here is, however, consistent with the results of recent studies in which mangrove organic matter was found to have a limited role in coastal food webs (Kieckbusch *et al.*, 2004; Nagelkerken & van der Velde, 2004; Nagelkerken *et al.*, 2008). The contribution of organic matter to diets may vary according to environmental settings and the geomorphology of the area (Nagelkerken *et al.*, 2008). The pattern observed in this study for food source carbon signatures and their contributions to fish diet may therefore be related to the configuration of habitats and the hydrography affecting the export of mangrove-derived carbon sources. Site-to-site variability should be considered in future studies to confirm the findings reported here and to assess the contribution of other organic matter sources for *M. punctatus* occurring in the coastal waters of the IRL. In Curaçao, Cocheret de la Morinière *et al.* (2003) noted an ontogenetic change in the diet of some carnivorous fishes (Haemulidae and Lutjanidae) towards consumption of larger prey items. Gut content and stable isotope analyses also suggest ontogenetic shifts in the dietary habits for three size groups of

juvenile *M. punctatus*. Adult specimens should be included in future studies to assess any additional ontogenetic change in the diet. In conclusion, this work provides the first evidences on the importance of seagrass beds as foraging areas for juveniles of *M. punctatus* after their recruitment to the coastal waters of the IRL.

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