

# Ontogenetic dietary changes of coral reef fishes in the mangrove-seagrass-reef continuum: stable isotopes and gut-content analysis

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**ABSTRACT:** Juveniles of a number of reef fish species develop in shallow-water 'nursery' habitats such as mangroves and seagrass beds, and then migrate to the coral reef. This implies that some reef fish species are distributed over the mangrove-seagrass-reef continuum in subpopulations with different size distributions that are spatially separated for considerable periods of time. To test this assumption, and to determine ontogenetic dietary changes (which may drive fish migrations from nursery habitats to the coral reef), we selected 9 herbivorous and carnivorous reef fish species whose juveniles are highly abundant in nearby nursery habitats.  $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$  ratios were measured in fish muscle tissues and in potential food items collected from each of the 3 habitats, and fish gut contents were identified. All  $\delta^{13}\text{C}$  signatures of fishes sampled from the coral reef were close to the carbon isotope signatures of food items on the reef, and were significantly depleted compared to those of fishes from the nursery habitats (with the exception of *Scarus iserti*). Gut-content analysis of herbivorous fishes entailed problems with identification of the ingested food items, but there was no change in the  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  signatures of the muscle tissue as a function of fish size. Regression analysis showed significant positive relationships between  $\delta^{15}\text{N}$  content and fish size in all carnivorous fish species; this was correlated to the decreasing dietary importance of small crustaceans and the increasing consumption of decapod crabs or prey fishes with increasing size. The combined study of stomach contents and stable isotopes showed that the juveniles and adults of these reef fish species are separated ecologically and spatially for a considerable period of time, and that herbivorous fishes do not change their trophic status with increasing size, whereas carnivorous fishes feed on increasingly larger prey at increasingly higher trophic levels prior to their migration from the nursery habitat to the coral reef.

**KEY WORDS:** Reef fishes · Seagrass beds · Mangroves · Coral reef · Nursery habitats · Diet shift · Fish size · Stable isotopes · Life cycle migrations

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## INTRODUCTION

Seagrass beds and mangroves provide nursery habitats for a number of coral reef fish species in the Indo-Pacific Ocean and in the western Atlantic (Pollard 1984, Parrish 1989). Although this nursery concept is widely acknowledged, a quantitative description of the

relative importance of nursery habitats to the various size classes of coral reef fish species in the mangrove-seagrass-coral reef continuum has only begun recently (Nagelkerken et al. 2000b, 2001, Adams & Ebersole 2002, Cocheret de la Morinière et al. 2002). Moreover, the assumptions on which the nursery concept is based are rarely tested (Beck et al. 2001).

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The term 'nursery' implies settlement of post-larvae in nursery habitats where they grow to juveniles, followed by a directional migration of the sub-adults from the nursery habitat to the adult habitat. Such life-cycle migration patterns can be inferred from the spatial size frequency distributions of fish populations (e.g. Weinstein & Heck 1979, Yáñez-Arancibia et al. 1988, Appeldoorn et al. 1997, Blaber 1997, Cocheret de la Morinière et al. 2002), since the spatial separation of the various size classes of a species suggests movement from one habitat to another (Werner & Gilliam 1984, Ross 1986). In our previous studies in Spanish Water Bay (Curaçao, Netherlands Antilles), a number of reef fish species which indeed use habitats such as mangroves and seagrass beds as nurseries were identified using a single technique (visual census) (Nagelkerken et al. 2000b, 2001, Cocheret de la Morinière et al. 2002). The spatial size distributions over different habitats in the mangrove-seagrass-reef continuum were identical in 2 consecutive years for each of the selected reef fish species (Cocheret de la Morinière et al. unpubl. data). The adult subpopulations of these species on the reef thus appeared to be spatially separated from their juvenile subpopulations in the nursery, and post-settlement nursery-to-reef migrations must occur over a species-specific size range (Cocheret de la Morinière et al. 2002). Other types of migrations, such as tidal, seasonal, spawning, or diurnal feeding migrations between the coral reef and Spanish Water Bay have not been observed (Nagelkerken et al. 2000a,b, Cocheret de la Morinière et al. 2002), which makes this an excellent area for testing the assumption that the juveniles in the nursery are spatially and ecologically separated from the adult subpopulation on the reef. Such separation should be reflected in the differences in resource use of the different habitats.

The timing of nursery-to-reef migrations (and hence the amount of time spent in nursery areas) is determined by factors related to ontogeny. The ontogeny of resource partitioning may directly influence life-cycle migration patterns of coastal fishes (Livingstone 1982, Hyndes et al. 1997), and nursery-to-reef migrations could be instigated or promoted by diet shifts (Cocheret de la Morinière et al. unpubl. data). Stomach-content analysis of different size classes of these reef fish species in different nursery and coral reef habitats therefore provides insight into the changing interaction with habitats during ontogeny.

The analysis of naturally occurring stable isotopes has been used as a complementary tool for animal diet analysis in many food-web studies (France 1994, 1996, Michener & Schell 1994). Carbon isotope ratios of animals reflect those of their diet, within about 1‰ variation (e.g. Rau et al. 1983, Michener & Schell 1994). Both laboratory studies (e.g. DeNiro & Epstein 1978,

Minagawa & Wada 1984) and field studies (Fry 1983, Schoeninger & DeNiro 1984, Owens 1987, Fry 1988, Cabana & Rasmussen 1994) show an average 3‰  $^{15}\text{N}$  enrichment between the animal and its diet. The simultaneous measurement of carbon and nitrogen stable isotopes can thus provide information on source materials and trophic relationships (Peterson et al. 1985, Ehleringer et al. 1986, Owens 1987, Peterson & Fry 1987, Nyssen et al. 2002), even though significant temporal and spatial within-group variation in the stable isotope composition of food sources has been found (Cooper & DeNiro 1989, Durako & Hall 1992, Boon & Bunn 1994, Hemminga & Mateo 1996) and trophic levels must be assigned and interpreted with great care (Gannes et al. 1997, Jennings et al. 1997, France et al. 1998, O'Reilly et al. 2002). Moreover, animal migrations can be inferred from studies of stable isotopes (e.g. Fry 1983, Hesslein et al. 1991, Newel et al. 1995, France 1996, Hansson et al. 1997, Hobson 1999). The stable isotope ratios in animal tissues are based on actual food assimilation and reflect, on average, their diet over the previous weeks to months (Hobson 1999), whereas stomach-content analysis is based on ingested prey and usually represents the animal's diet over the last few hours. Also, food studies based on stomach-content analysis alone may require vast numbers of samples and sometimes entail problems with the identification of prey items. The analysis of stable isotopes is therefore a useful tool for verifying conclusions from animal diet studies and for gaining additional insight into feeding relationships and changes therein (Michener & Schell 1994).

The combination of fish gut-content analysis and the measurement of stable isotopes of reef fishes in the mangrove-seagrass-reef continuum at different life stages thus provides insight in both short- and long-term dietary changes that occur during ontogeny and with change of habitat. However, trophic studies on seagrass and mangrove fish communities are scarce (Marguillier et al. 1997). Few authors have distinguished separate size classes of fishes in nursery habitats (Livingstone 1982, Harrigan et al. 1989, Heck & Weinstein 1989, Muñoz & Ojeda 1998), and simultaneous studies of gut contents and multiple stable isotopes of different size classes of fishes in the mangrove-seagrass-reef continuum are rare as well.

In this study, we collected a wide size range of juveniles and adults of 4 herbivorous species (Acanthuridae and Scaridae) and 5 carnivorous species (Haemulidae and Lutjanidae), to study their feeding habits in nursery habitats in a marine bay as well as on the adjacent coral reef. Stable carbon and nitrogen isotopes were measured in the fish muscle tissues and in potential food items collected from these habitats. The main research questions in this study were: (1) Can the sep-

aration of juveniles in nursery habitats from their adult subpopulation on the coral reef be confirmed from fish diets and stable isotope ratios? (2) Do ontogenetic changes in resource use and trophic level, which may possibly drive nursery-to-reef migrations, occur in these reef fish species?

## MATERIALS AND METHODS

**Study area.** The present study was carried out in Spanish Water Bay in Curaçao, Netherlands Antilles

(Fig. 1). This 3 km<sup>2</sup> bay is shallow (mostly <6 m deep), harbours extensive seagrass meadows, and is fringed by mangroves *Rhizophora mangle*. The seagrass beds are dominated by monospecific stands of *Thalassia testudinum*, sometimes mixed with *Halimeda* spp. algae (Kuenen & Debrot 1995).

There is no freshwater input into the bay other than rain, and salinity (mean 35.4) is slightly higher than on the reef (mean 34.6). The water surface temperature averaged  $30.1 \pm 0.8^\circ\text{C}$  in the bay and  $28.4 \pm 0.9^\circ\text{C}$  on the reef. Underwater visibility was high at all sites and varied between an average of  $6.5 \pm 1.8$  m in the bay

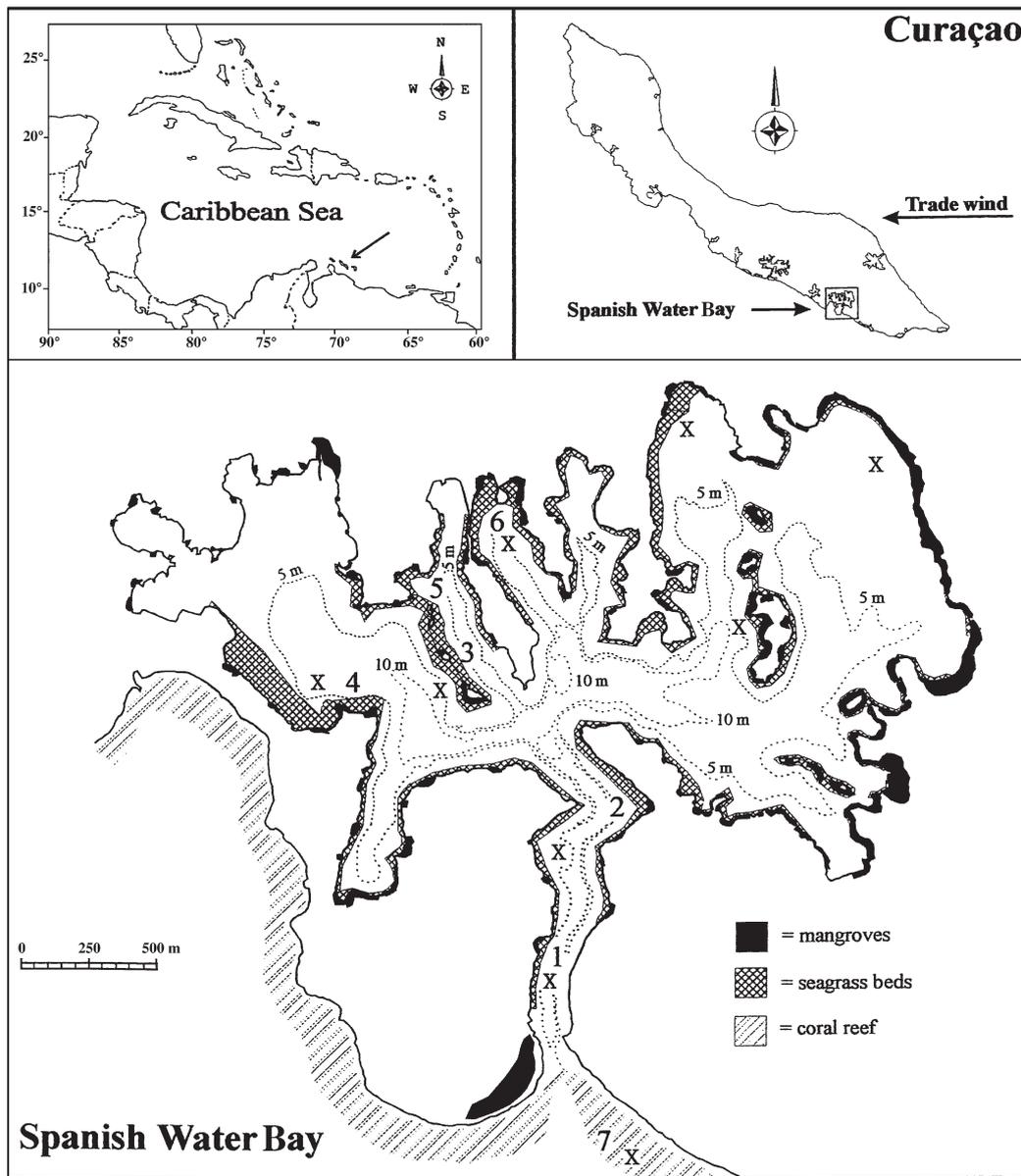


Fig. 1. Spanish Water Bay. Habitats studied, and 5 and 10 m isobaths. 1–7: sites at which potential food items were collected from seagrass, mangrove and coral reef ecosystem for stable isotope analysis; plankton was collected at Sites 1–6. X: sites of fish collection

and  $21.4 \pm 3.1$  m on the reef, as measured by horizontal Secchi disk distance. The average tidal amplitude in the area is 30 cm (De Haan & Zaneveld 1959). The bay has a long (1 km) and narrow (70 m) entrance that connects it to the adjacent fringing reef. This reef is part of a marine park that stretches to the southeast tip of the island. A detailed description of the reefs in the Netherlands Antilles can be found in Bak (1975).

**Sampling design.** In various parts of Spanish Water Bay, beach seine nets were used during daytime to collect fishes inhabiting seagrass beds ('X' in Fig. 1). Antillean fish traps were used overnight in mangrove habitats and on the coral reef. The smallest individuals were captured from isolated patches of seagrass, mangroves or boulders by use of the ichthyocide rotenone. Data on gut contents of fishes from mangroves were pooled with data on fish guts from seagrass bed communities, since the fish species in Spanish Water Bay mainly feed in the seagrass beds and not in the mangroves (Nagelkerken et al. 2000b). Fish collection took place from May through September 1998.

Of the total number of fishes that had sufficient gut contents for analysis, 338 were Haemulidae and

Lutjanidae and 95 were Acanthuridae and Scaridae (Table 1). The gut contents were preserved in ethanol (70% v:v) and dyed with Rose Bengal before identification and quantification of the food items. All fishes were measured (fork length, FL) and weighed. Food components in the digestive tracts were classified to class level (Oligochaeta, Polychaeta, Bivalvia, Gastropoda, Echinoidea), subclass (Ostracoda, Copepoda) or order level (Tanaidacea, Mysidacea, Isopoda, Amphipoda, Decapoda), while prey fishes, sediment and plant material (seagrass or algae) found in the guts were categorised as fish, sediment, foraminifers, seagrass, filamentous algae, calcareous algae or macroalgae. Using a stereomicroscope, the relative volumetric quantity of the food items was estimated, i.e. the volume of the contents of the digestive tract was set at 100%, and the food items found were estimated by eye as volumetric percentage of the total stomach volume (Nielsen & Johnson 1992). A volumetric measure was chosen because it is an estimation of biomass, whereas gravimetric methods would produce large errors in these small volumes because of water content (blotting would damage the samples in some cases), and methods involving frequencies would underestimate large food items and overestimate small food categories (Hyslop 1980).

Muscle tissue was sampled from 270 individuals of Haemulidae and Lutjanidae, and from 74 individuals of Acanthuridae and Scaridae for stable isotope analysis (Table 1). In addition, various potential food items were sampled from seagrass, mangrove and coral reef habitats at Sites 1 to 7 (Fig. 1). Seagrass leaves and seagrass epiphytes were collected at each site in the bay (Sites 1 to 6; Fig. 1). Planktonic invertebrates were collected at night using a plankton net with a 55  $\mu$ m mesh size and a gape diameter of 25 cm. All samples were dried immediately after collection (70°C, 48 h) and ground in liquid nitrogen. Subsamples for the measurement of carbon isotopes were decalcified (Nieuwenhuize et al. 1994), while subsamples for nitrogen isotope measurements were not decalcified. The carbon and nitrogen isotopic composition was determined using a Fisons element analyser coupled on-line via a Finnigan Conflo II, with a Finnigan Delta-S mass-spectrometer. Carbon and nitrogen isotope ratios are expressed in the delta notation ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) relative to Vienna PDB standard and atmospheric nitrogen. Average reproducibilities based on replicate measurements for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were about 0.1‰.

**Statistical analysis.** Relationships between fish size (FL) and volumetric percentage of food categories or isotope ratios in muscle tissues were described using least-squares linear regressions. Spatial differences in the stable isotope contents of seagrass leaves and seagrass epiphytes at different sites were also described

Table 1. Size range, number of fish guts used for stomach-content analysis, and number of fish muscle-tissue samples used for stable isotope analysis for each species per habitat

Species, Habitat	Size-range (cm)	Guts	Isotopes
<i>Haemulon flavolineatum</i>			
Bay	2.0–16.3	69	57
Reef	12.6–18.0	12	11
<i>Haemulon sciurus</i>			
Bay	4.6–25.9	76	70
Reef	28.7–29.0	4	3
<i>Lutjanus apodus</i>			
Bay	5.6–27.5	71	32
Reef	18.8–25.4	8	9
<i>Lutjanus griseus</i>			
Bay	6.5–18.8	19	18
Reef	23.8–27.9	3	3
<i>Ocyurus chrysurus</i>			
Bay	3.2–17.1	71	51
Reef	13.1–27.2	5	16
<i>Acanthurus bahianus</i>			
Bay	2.9–17.2	20	8
Reef	13.2–18.2	5	8
<i>Acanthurus chirurgus</i>			
Bay	3.0–18.6	7	6
Reef	18.0–22.5	4	7
<i>Scarus iserti</i>			
Bay	3.0–11.0	44	28
Reef	19.5–26.5	9	11
<i>Scarus taeniopterus</i>			
Bay	–	0	0
Reef	22.1–27.3	6	6

using linear regressions. Differences between average stable isotope composition of fishes were tested with a Student's *t*-test for independent samples (Sokal & Rohlf 1995).

Some of the smallest individuals (<4 cm FL) of *Haemulon flavolineatum*, *Ocyurus chrysurus* and *Acanthurus bahianus* had carbon stable isotope compositions that are common in biota with oceanic signatures (e.g. Fry & Sherr 1984, Peterson & Fry 1987). The  $\delta^{13}\text{C}$  signatures of these small fishes were between  $-19.53$  and  $-16.59\%$ , and were much lower than for other samples of the same species. The stable isotope ratios in these juvenile individuals probably reflect the larval planktivorous phase in the open ocean before settlement to the nursery habitats (Herzka & Holt 2000, Herzka et al. 2001). For the purpose of this study, the data of the smallest juveniles with 'oceanic' pre-settlement signatures were not included in the averages and regression analyses as they would cloud the interpretation of habitat use and ontogenetic shifts.

## RESULTS

### Isotope ratios for bay versus reef

Stable isotope ratios in potential food items in the bay and on the coral reef ranged from  $-28.0$  to  $-9.4\%$  for  $\delta^{13}\text{C}$ , and from  $-1.0$  to  $8.9\%$  for  $\delta^{15}\text{N}$  (Fig. 2). Seagrass leaves were richest in  $^{13}\text{C}$  stable isotopes, mangrove leaves were poorest in  $^{13}\text{C}$ , while algae showed intermediate  $\delta^{13}\text{C}$  values. Primary producers were lighter in  $^{15}\text{N}$  than invertebrates and zooplankton (Fig. 2). Primary producers on the coral reef were depleted in  $^{13}\text{C}$  compared to seagrass leaves and seagrass epiphytes, while their  $\delta^{15}\text{N}$  values were similar

(Fig. 2).  $\delta^{13}\text{C}$  values of seagrass leaves significantly decreased progressively deeper into the bay (linear regression,  $r^2 = 0.85$ ,  $p < 0.05$ ), but with a maximum difference between sites of only  $1.2\%$ . Such a trend was not significant for the spatial distribution of  $^{13}\text{C}$  in seagrass epiphytes or for any of the  $\delta^{15}\text{N}$  values.

Fig. 3 shows the stable isotope ratios in muscle tissues of the reef fishes collected from coral reef habitats and from bay habitats. The stable isotope ratios of the 'oceanic juveniles' (see last paragraph of 'Materials and methods') were not included in the averages and regression analyses reported below, but are shown in Fig. 3. For all species (except *Scarus iserti*), individuals which were caught in bay habitats were significantly richer in  $^{13}\text{C}$  than individuals of the same species caught in coral reef habitats (Student's *t*-test,  $p < 0.05$ ). Carbon isotope values for *S. iserti* collected from the coral reef were high compared to those of the other fish species from the reef, and were in the range of biota associated with the seagrass food web. All individual fishes caught in bay habitats showed  $\delta^{13}\text{C}$  values close to those of biota collected from the seagrass system (Fig. 2).

*Haemulon sciurus*, *Acanthurus bahianus*, *A. chirurgus* and *Scarus iserti* from bay habitats had  $\delta^{15}\text{N}$  values that were similar (Student's *t*-test,  $p > 0.05$ ) to those of conspecifics collected from the coral reef. *H. flavolineatum*, *Lutjanus apodus*, *L. griseus*, and *Ocyurus chrysurus* from the coral reef had significantly higher  $\delta^{15}\text{N}$  values than their conspecifics from bay habitats (Student's *t*-test,  $p < 0.05$ ). The zoobenthivorous and piscivorous fish species (*H. flavolineatum*, *H. sciurus*, *L. apodus*, *L. griseus*, and *O. chrysurus*) had higher average  $\delta^{15}\text{N}$  values than the herbivorous species (*A. bahianus*, *A. chirurgus*, *S. iserti*, *S. taeniopterus*) on the reef as well as in the bay, but there was some overlap between the feeding guilds was present.

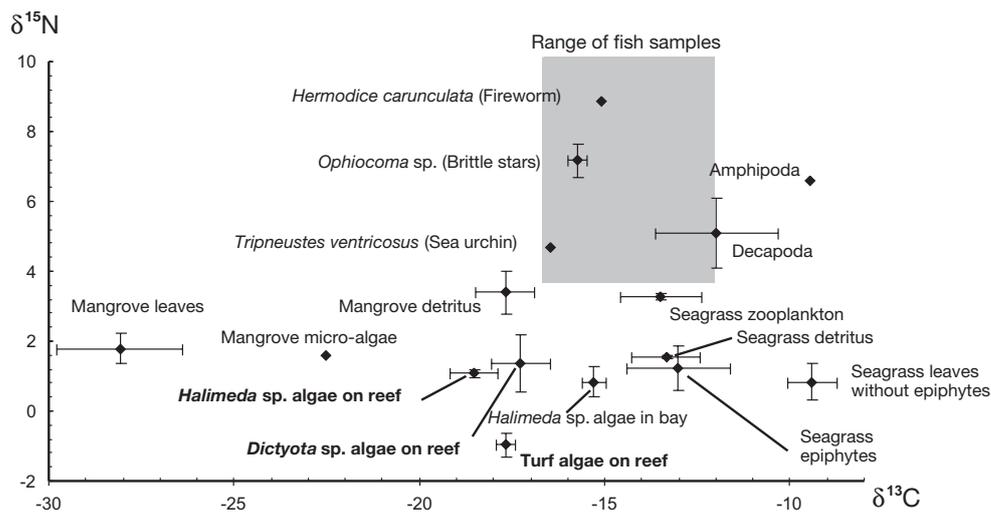


Fig. 2. Average ( $\pm$ SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios of potential food items collected from the coral reef (bold) or from seagrass and mangrove habitats. Shaded area indicates range of average  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios of fish muscle-tissue samples (excluding 'oceanic juveniles') shown in Fig. 3

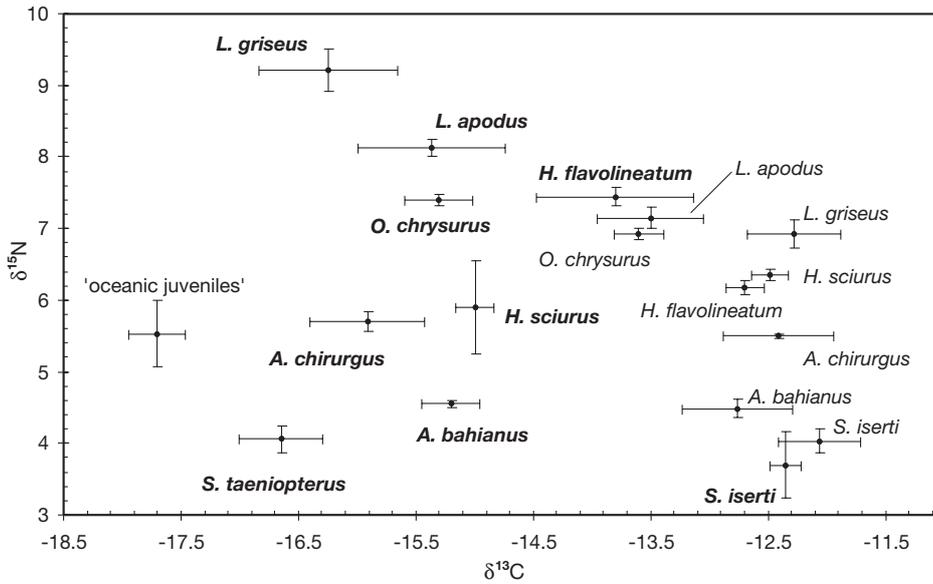


Fig. 3. Average ( $\pm$ SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios of fish samples taken from the coral reef (bold) and the bay habitats (seagrass beds and mangroves). 'oceanic juveniles': separate group of very small juveniles in seagrass beds which reflected presettlement signatures (see 'Materials and methods'). Full species names in Table 1

**Herbivorous fishes**

The stomach contents of Acanthuridae and Scaridae of all sizes consisted of plant material, with the exception of fishes <4 cm FL, which also fed on copepods. However, due to problems with identification of the partly digested epiphytic algae and macroalgae (seagrass leaf fragments were rarely observed in these guts), the data on the stomach contents of the Acanthuridae and Scaridae are not reported. Average  $\delta^{15}\text{N}$  values for the herbivorous fish species were between 3.69 and 5.70‰, while the average  $\delta^{13}\text{C}$  values were between -12.06 and -16.65‰. These  $\delta^{13}\text{C}$  values are in the range of samples from the seagrass ecosystem

(Fig. 2). None of the herbivorous species in the bay showed significant ontogenetic change in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values in their muscle tissues within this feeding guild (see Fig. 5), nor within separate species (linear regression,  $R^2 < 0.10$ ,  $p > 0.05$ ).

**Carnivorous fishes**

The reef-inhabiting individuals of the carnivorous fish species *Haemulon flavolineatum*, *H. sciurus*, *Lutjanus apodus*, *L. griseus* and *Ocyurus chrysurus* mainly fed on decapod crabs and prey fishes (Table 2). In the bay habitats, smaller individuals of these carni-

Table 2. Haemulidae and Lutjanidae species. Diet analysis based on average percent volume of each food category. Rest: food items in bay fish stomachs that never exceeded 2% estimated volume of gut contents (Oligochaeta, Polychaeta, Echinoidea, Ostracoda, seagrass, Foraminifera, filamentous algae, calcareous algae, macroalgae); Unid.: unidentified

Species, Habitat	Tanai- dacea	Cope- poda	Iso- poda	Amphi- poda	Mysi- dacea	Bivalvia	Gastro- poda	Deca- poda	Fish	Sedi- ment	Rest	Unid.
<i>Haemulon flavolineatum</i>												
Bay	40	25	0	3	0	0	1	1	0	2	2	26
Reef	6	0	1	0	0	3	8	17	13	4	40	8
<i>Haemulon sciurus</i>												
Bay	33	8	2	1	0	4	3	17	0	8	12	12
Reef	0	0	0	0	0	0	0	88	0	0	0	12
<i>Lutjanus apodus</i>												
Bay	23	3	1	4	3	0	0	47	16	0	0	3
Reef	0	0	0	0	0	0	0	31	69	0	0	0
<i>Lutjanus griseus</i>												
Bay	8	0	0	0	2	0	0	75	6	0	0	9
Reef	0	0	0	0	0	0	0	33	67	0	0	0
<i>Ocyurus chrysurus</i>												
Bay	30	19	0	0	10	1	1	24	8	2	3	2
Reef	0	0	0	0	6	0	0	44	40	10	0	0

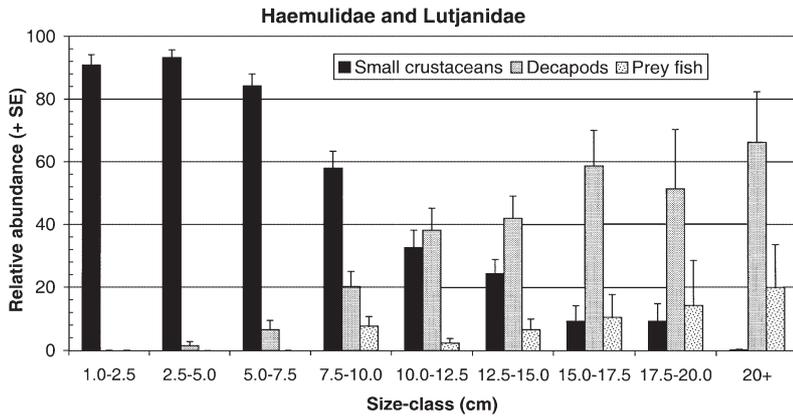


Fig. 4. Diet composition per size class of pooled Haemulidae and Lutjanidae collected from bay habitats (seagrass beds or mangroves), based on average percent volume of each food category ( $\pm$ SE). 'Small crustaceans' consisted mainly of Tanaidacea and Copepoda

vorous species fed predominantly on tanaids, copepods and decapods (Table 2). The average  $\delta^{13}\text{C}$  content of the bay-inhabiting carnivores (between  $-12.3$  and  $-13.6\text{‰}$ ; Fig. 3) was in the range of the carbon ratios in seagrass zooplankton and seagrass detritus (Fig. 2:  $-13.5 \pm 1.1$  and  $-13.3 \pm 0.9\text{‰}$ , respectively). Average  $^{15}\text{N}$  enrichment of the grunts and snappers in the bay relative to seagrass detritus was  $+4.6$  to  $+5.6\text{‰}$ , and  $+2.9$  to  $+3.9\text{‰}$  relative to seagrass zooplankton. The individuals of the haemulid and lutjanid species collected in the bay generally showed a diet shift with decreasing importance of small crustaceans and increasing importance of decapods and prey fishes with increasing fish size (Fig. 4). This coincided with the significantly positive relationship between  $\delta^{15}\text{N}$  in fish muscle tissue and body size within the feeding guild of carnivores (Fig. 5), as well as for separate fish species (Table 3). In the case of *H. flavolineatum* and *H. sciurus*, however, the positive relationship between fish size and decapod crabs was negligible ( $R^2 < 0.10$ ). The 2 snapper species *L. apodus* and *O. chrysurus* displayed a significant ontogenetic increase in prey fish consumption that coincided with  $\delta^{15}\text{N}$  accumulation. Only *L. apodus* showed a statistically significant (negative) relationship between fish size and  $\delta^{13}\text{C}$  values in its muscle tissue (Table 3).

**DISCUSSION**

**Isotope ratios for bay versus reef**

Generally, stable isotope ratios of the fishes and the potential food items in the bay and on the coral reef were in the range of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values reported by other authors for similar ecosystems (e.g. McMillan et

al. 1980, Fry et al. 1982, Harrigan et al. 1989, Lin et al. 1991, Hemminga & Mateo 1996). Mangrove leaf carbon was strongly depleted compared to biota collected from seagrass beds and from the coral reef. Primary producers on the coral reef were carbon-depleted relative to  $\delta^{13}\text{C}$  values for the seagrass system, while their  $\delta^{15}\text{N}$  ratios were similar to nitrogen ratios in seagrass leaves. The  $\delta^{15}\text{N}$  content of samples from the various habitats generally increased with increasing trophic level. Carbon stable isotopes were therefore a

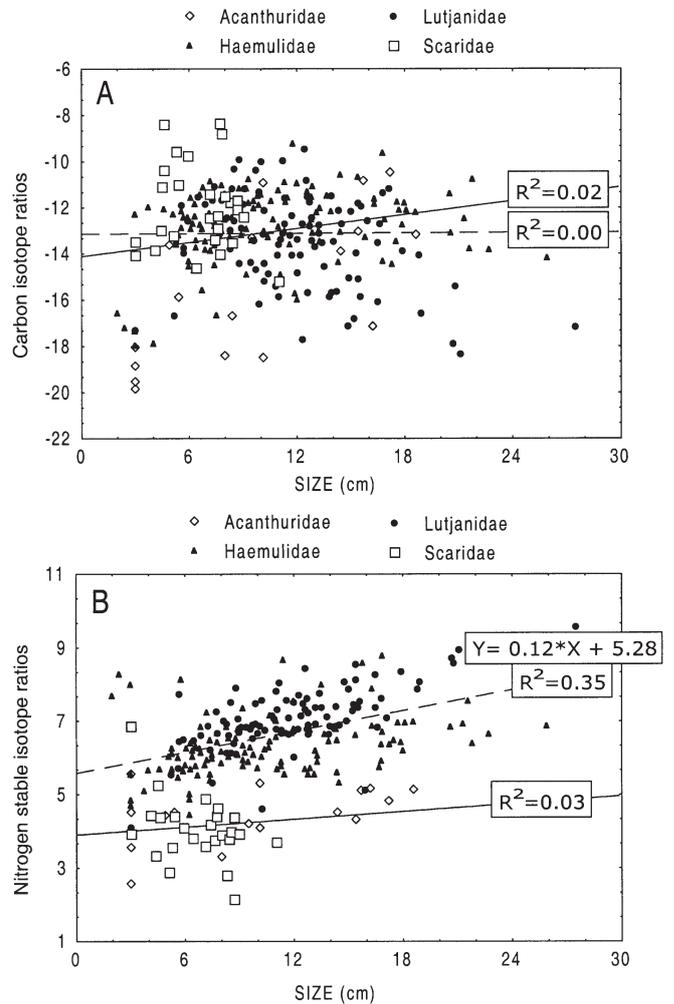


Fig. 5. (A) Carbon and (B) nitrogen stable isotope ratios of fishes collected from bay habitats in relation to fish size. Dotted lines represent linear relation with fish size for carnivorous fishes (Haemulidae and Lutjanidae), continuous lines represent linear relation with fish size for herbivorous fishes (Acanthuridae and Scaridae). Equation of linear regression is only shown when  $R^2$  values were significant

Table 3. Linear regression ( $R^2$ ) of negative (–) or positive (+) relationships between fish size and dietary components, and between fish size and stable isotopes in bay habitats. The average volumetric percentages of small crustaceans, decapod crabs and prey fishes in fish guts were calculated for each fish size class of 2.5 cm, and correlated to the consecutive fish size classes. The stable isotope data were correlated to individual fish sizes. ns: regression not significant ( $p > 0.05$ ); no: food item not observed in guts of that species. Statistically significant  $R^2$ -values are **bold**

Species	Small crustaceans		Decapod crabs		Prey fish		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	$R^2$	p	$R^2$	p	$R^2$	p	$R^2$	p	$R^2$	p
<i>Haemulon sciurus</i>	<b>0.98</b> (–)	<0.01	<b>0.10</b> (+)	0.03	no		0.04 (+)	ns	<b>0.62</b> (+)	<0.01
<i>Haemulon flavolineatum</i>	<b>0.62</b> (–)	0.04	0.02 (+)	ns	0.01 (+)	ns	0.05 (+)	ns	<b>0.72</b> (+)	<0.01
<i>Lutjanus apodus</i>	<b>0.96</b> (–)	<0.01	<b>0.40</b> (+)	0.04	<b>0.77</b> (+)	0.02	<b>0.52</b> (–)	<0.01	<b>0.86</b> (+)	<0.01
<i>Lutjanus griseus</i>	<b>0.81</b> (–)	0.04	<b>0.70</b> (+)	<0.01	0.07 (+)	ns	0.18 (–)	ns	<b>0.84</b> (+)	0.02
<i>Ocyurus chrysurus</i>	<b>0.88</b> (–)	<0.01	<b>0.88</b> (+)	0.00	<b>0.86</b> (+)	0.02	0.10 (+)	ns	<b>0.64</b> (+)	0.04

good discriminant between biota from the mangrove, seagrass and coral reef habitats, while nitrogen stable isotopes were indicative of trophic level.

Differences in the resource use of fishes collected from the coral reef versus that of fishes collected from nursery habitats were reflected in dietary differences and stable isotopic composition of fish muscle tissues. For each of the herbivorous or carnivorous fish species, average carbon isotope ratios of individuals caught on the reef were significantly lower than averages of individuals of that species collected from the bay, with the exception of *Scarus iserti*. Problems with identification of the stomach contents of herbivores prevented detailed interpretation of their feeding habits, but gut-content analysis of the carnivorous Haemulidae and Lutjanidae revealed a difference in resource use between reef habitats and seagrass habitats. Carnivorous fishes generally had higher  $\delta^{15}\text{N}$  contents than the herbivorous fish species, reflecting their higher trophic level. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope data for *Scarus iserti* were similar in the bay and on the reef, and in the range of those in the seagrass system.

Adult *Scarus iserti* were rarely observed in seagrass beds and mangroves in Spanish Water Bay in our previous studies (Nagelkerken et al. 2000b, 2001, Cocheret de la Morinière et al. 2002), which makes consumption of food items from the bay habitats by adults unlikely, and the stable isotope data on *S. iserti* therefore imply that reef-inhabiting adults consume food with stable isotope signatures similar to those found in seagrass-associated biota. Some coral polyp tissues and benthic microalgae do have carbon isotope values similar to the carbon signature of the seagrass ecosystem (e.g. Fry et al. 1982, Yamamuro et al. 1995). All the other fish species apparently do not regularly execute diurnal feeding migrations from the coral reef to nearby seagrass or mangrove habitats (based on the different  $\delta^{13}\text{C}$  values of individuals sampled in the bay versus individuals sampled on the reef), and such diurnal migrations to and from Spanish Water Bay have not been detected in visual surveys during the day and at

night in any of the selected species (Nagelkerken et al. 2000a), although some of these species execute such diurnal migrations in other Caribbean locations (e.g. Ogden & Ehrlich 1977, McFarland et al. 1979, Helfman et al. 1982).

Our findings suggest that the reef-inhabiting (adult) individuals of these nursery species no longer depend on nursery carbon from the bay habitats (seagrass beds and mangroves) from which they have migrated. The same conclusions can be drawn for the bay-inhabiting individuals of these species, which apparently all feed on seagrass beds and do not frequent the coral reef habitats. This confirms the spatial separation of adult reef fish subpopulations from their juveniles in nursery habitats (Cocheret de la Morinière et al. 2002), and implies that post-settlement life cycle migrations from the nursery habitat to the coral reef do occur.

#### Herbivore diet during ontogeny

The selected herbivorous fish species are consumers of (macro)algae, with very little direct grazing on seagrass leaves (Randall 1967). The fact that seagrass leaf fragments were rarely observed in the guts of the herbivorous fishes matched our field observations; herbivorous fishes were observed to consume epiphytes from the seagrass leaves repeatedly, and were rarely seen biting off parts of the seagrass leaf itself. Moncreiff & Sullivan (2001) suggested that epiphytic microalgae are of major trophic importance in seagrass beds, and that in some cases they are the primary source of organic matter for higher trophic levels. The average trophic enrichment ( $\delta^{13}\text{C}_{\text{animal}} - \delta^{13}\text{C}_{\text{food}}$  or  $\delta^{15}\text{N}_{\text{animal}} - \delta^{15}\text{N}_{\text{food}}$ ) of the herbivores in the bay relative to seagrass epiphytes was +0.61‰  $\delta^{13}\text{C}$  and +3.34‰  $\delta^{15}\text{N}$ ; this fits well with the expected enrichment values of 0 to 1‰  $\delta^{13}\text{C}$  and 3 to 4‰  $\delta^{15}\text{N}$  per trophic level (DeNiro & Epstein 1981, Fry 1983, Minagawa & Wada 1984), while the carbon enrichment relative to seagrass leaves does not (–3.01‰  $\delta^{13}\text{C}$  enrichment).

Variation in isotopic composition within the samples of herbivorous fishes collected from the bay was small, and no significant change in the carbon or nitrogen isotope composition in their muscle tissues was detected with increasing fish size. This indicates that no major diet shift occurs after settlement in the nursery habitats for these herbivorous fish species.

### Carnivore diet during ontogeny

Both dietary analysis and stable isotope composition of fish muscle tissue showed ontogenetic changes in the carnivorous grunts (Haemulidae) and snappers (Lutjanidae). The relative volumetric importance of small crustaceans in the fish stomachs significantly decreased with increasing fish size in all carnivorous species, and in some species this concurred with a significant increase in the dietary importance of decapod crabs or prey fishes. Regression analysis also showed a significant increase in  $\delta^{15}\text{N}$  with increasing fish size for each of the carnivorous fishes. A significant (negative) relationship between fish size and  $\delta^{13}\text{C}$  values was only found in *Lutjanus apodus*. Positive relationships between fish size and prey size have been observed by some authors (e.g. Edgar & Shaw 1995a,b, Hyndes et al. 1997), while others have reported an ontogenetic relationship between  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  and animal size (e.g. Fry 1983, Rau et al. 1983, Sholto-Douglas et al. 1991, France 1996, France et al. 1998, Hentschel 1998, Marguillier 1998, Overman & Parrish 2001, Jennings et al. 2002). Relationships between stable isotope signatures and body size could theoretically be the result of changing allocation of the isotope or changes in tissue turnover rates during ontogeny, and little is known about such physiological changes (Fry & Arnold 1982, Tieszen et al. 1983, Doucett et al. 1999, Herzka et al. 2001). It is generally assumed, however, that these relationships are not the result of changing physiology during ontogeny unless the growth rate of the subject animal is low (Hesslein et al. 1993, Overman & Parrish 2001). The herbivorous fish species in the present study have growth rates close to or higher than those of the carnivores, and yet they do not display any ontogenetic change in stable isotope composition in their muscle tissue with increasing size. The  $\delta^{15}\text{N}$  accumulation with increasing size in the carnivorous fish species in the present study is therefore not regarded as a phenomenon caused by physiological changes during ontogeny, and can be attributed to dietary changes. The combined data of the stomach contents and  $\delta^{15}\text{N}$  accumulation therefore shows that, in the bay, these Haemulidae and Lutjanidae feed on ever larger prey of ever higher trophic levels with increasing size.

The diet shift to ever larger prey items during ontogeny could, hypothetically, lead to expansion of the

foraging distance with increasing fish size in these mobile fish species, since the biomass per unit area of larger food items (such as decapod crabs and prey fishes) is much less than the biomass of smaller macroinvertebrates (Nagelkerken et al. 2000a, Cocheret de la Morinière et al. unpubl. data). Such an expansion of foraging distance could enable the (sub-)adults to find the coral reef and could, therefore, drive nursery-to-reef migrations. Also, zoobenthivorous fishes which gradually become piscivorous will increasingly require good visibility, and this is better on the coral reef (Cocheret de la Morinière et al. 2002). Such considerations explain how nursery-to-reef migrations can be related to ontogenetic dietary changes.

### Conclusions

The  $\delta^{13}\text{C}$  signatures were useful discriminants between food items from the nursery habitats and samples from the coral reef, while the  $\delta^{15}\text{N}$  signatures were indicators of trophic level within a habitat. All but 1 fish species showed a difference in carbon isotope signatures between adult individuals collected from reef habitats versus juvenile individuals collected from nursery habitats, indicating that these juveniles use nursery habitats for a considerable period of time, and that diurnal feeding migrations between the nursery grounds and the coral reef (as reported for other Caribbean locations) do not regularly occur. Stable nitrogen isotopes proved a good indicator of diet change during ontogeny in the carnivorous fish species.  $\delta^{15}\text{N}$  accumulation with increasing fish size was not apparent in the herbivorous species. The combined study of stomach content and stable isotope analysis shows that the juveniles and adults of these reef fish species are ecologically separated for a considerable period of time, and that the herbivorous fish species do not change trophic status during ontogeny while, with increasing size, the carnivorous fishes feed on increasingly larger prey at increasingly higher trophic levels prior to migration from their nursery habitat to the coral reef.

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