

Seagrass nurseries contribute to coral reef fish populations

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Abstract

We here provide strong evidence that seagrass beds act as nurseries providing fish to adult populations of a coral reef fish. We studied this habitat connectivity by tracing life history movements of the Caribbean reef fish *Ocyurus chrysurus* (yellowtail snapper). Carbon- and nitrogen-stable isotope variations in muscle tissue and otoliths (ear bones) record former food sources and show that 98% of the *O. chrysurus* reef population has likely passed through seagrass nurseries as juveniles during their first 2 yr of life. Our findings indicate a significant degree of habitat connectivity and stress; in order to conserve healthy reefs and sustainable fisheries of *O. chrysurus*, marine protected areas and fisheries reserves that traditionally focus on protecting only the coral reef habitat should be expanded to include seagrass nurseries.

Marine protected areas (MPAs) and fishery reserves are essential to protect fish populations from intensive exploitation and to prevent habitat loss. Coral reefs are heavily overfished and threatened by loss of biodiversity (Bellwood et al. 2004; Newton et al. 2007). Most strategies for conservation, management, and sustainable fishing concentrate on the reef environment. However, a significant proportion of reef fish biomass consists of commercially important species whose juveniles occur in high densities not on the reef, but in mangroves and/or seagrass beds (Nagelkerken et al. 2000; Mumby et al. 2004). This spatial segregation of juveniles and adults has led to the hypothesis that mangrove and seagrass nurseries sustain coral reef fish populations (Adams et al. 2006). For decades this has been inferred from density data (Beck et al. 2001; Gillanders et al. 2003), but the genuine contribution of these nurseries to the reef population has never been proven.

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Mangrove and seagrass habitats are supposedly attractive settlement habitats to larval reef fish from the open ocean because of high food abundance and low predation pressure (Parrish 1989). It remains unclear whether these fish later restock the adult reef population because their movement to the reef has almost never conclusively been shown. Artificial tagging, although a direct measure of habitat connectivity, cannot be applied in most cases to small juvenile fish. Naturally occurring chemical and isotopic markers in bone and tissue samples are more promising for tracing migration patterns (Elsdon and Gillanders 2003; Rubenstein and Hobson 2004). Habitat differences such as temperature and salinity are recorded by trace element and oxygen isotope ratios in fish otoliths (ear bones) (Campana 1999). However, ambient water chemistry in nonestuarine tropical regions is relatively uniform, making these tracers nondiscriminating (Chittaro et al. 2006). In these regions, carbon-stable isotopes are potentially more valuable tracers because $\delta^{13}\text{C}$ (the ratio of $^{13}\text{C}:^{12}\text{C}$) in both bone and tissue records food sources, which are typically enriched in (i.e., show higher values of) $\delta^{13}\text{C}$ in seagrass beds, compared to more depleted offshore food webs (Boutton 1991; Fry et al. 1999).

In this study we link $\delta^{13}\text{C}$ recorded in muscle tissue and otoliths to life history movements of the coral reef fish *Ocyurus chrysurus* (yellowtail snapper) collected from two nonestuarine embayments harboring seagrass beds (Spanish Water Bay and Piscadera Bay) and from their adjacent coral reefs on the Caribbean island of Curaçao. A $\delta^{13}\text{C}$ enrichment has been previously found in food sources in the seagrass beds that are enclosed by inland embayments in our study area (Cocheret de la Morinière et al. 2003). These are spatially separated from nearby fringing coral reefs where food sources are depleted in $\delta^{13}\text{C}$. For additional discriminating power between habitat types we used $\delta^{15}\text{N}$ (the ratio of $^{15}\text{N}:^{14}\text{N}$) in muscle tissue, because this value has been shown to be distinctively high in areas

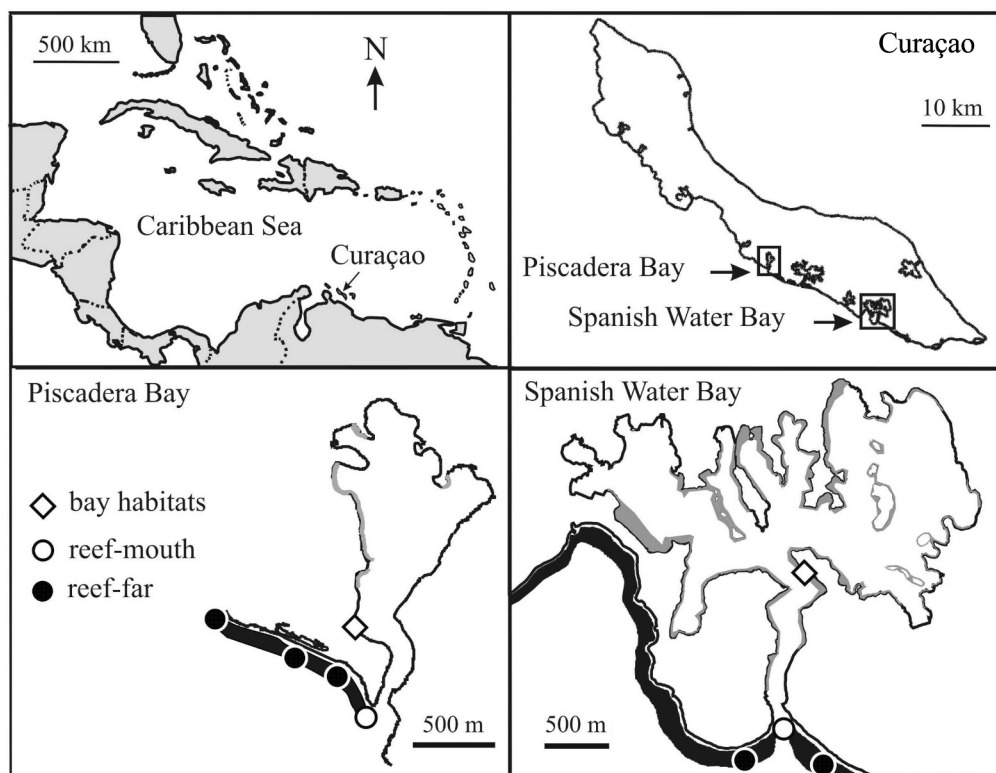


Fig. 1. Study area and collection sites. Gray shading indicates seagrass beds and black shading indicates coral reefs. Direction as indicated in upper left panel is the same for all other panels.

polluted with sewage (Hansson et al. 1997). The inland bays around Curaçao are expected to be much more influenced by anthropogenic activity than the coral reefs.

O. chrysurus was selected for this study because size-frequency data strongly suggest that it grows up in coastal embayments with mangroves and seagrass beds (Nagelkerken et al. 2000) with feeding predominantly taking place in seagrass beds (Nagelkerken and van der Velde 2004). Inside Spanish Water Bay *O. chrysurus* ranges in size between 2.5 and 25 cm, whereas it has been observed only at sizes above 15 cm at the coral reef of Curaçao (Nagelkerken et al. 2000). This suggests that migration takes place when the fish range between 15 and 25 cm in length, which corresponds to ages of about 1–2 yr (using equations of Garcia et al. 2003). Another benefit of studying *O. chrysurus* is that it seems to be highly dependent on nonreef nurseries. This is suggested by lower population densities, or even complete absence of this species, on reefs of Caribbean islands without mangroves or seagrass beds, as opposed to those with these habitats, including Curaçao (Nagelkerken et al. 2002). Thus, the majority of *O. chrysurus* individuals collected from the coral reef habitat should hypothetically contain tissue or other body parts that record feeding within seagrass beds during earlier life stages. Unfortunately, stable isotope signatures remain in muscle tissue for as little as several weeks or months after food consumption (Rubenstein and Hobson 2004). This means that muscle tissue data alone cannot identify reef fish that have passed through seagrass nurseries, because these fish typically spend a significant

part of their adult lives on the coral reef. Therefore, we expanded our study to include otoliths, which are metabolically inert and should thus permanently record the initial seagrass feeding signature. This process is irrespective of fish size, as newly grown CaCO_3 does not equilibrate with earlier mineralized and older core otolith zones (Campana 1999). Stable isotopes in otoliths, such as those of carbon and oxygen, have already successfully traced migration patterns of temperate and cold-water fishes (see references in Campana 1999). By combining muscle and otolith stable isotope data, our study provides highly plausible evidence of ontogenetic migration of coral reef fish between tropical coastal habitats.

Materials and methods

Study area—Spanish Water Bay and Piscadera Bay are nonestuarine inland embayments (Fig. 1). Both bays have narrow mouths (70–100 m wide) and entrance channels of up to 1 km in length. The channels lead to shallow (mostly <6 m deep) inland bay areas with seagrass beds, algal flats, submerged mudflats, and rocky shorelines (fossilized reef terraces) with fringing mangroves. A fringing coral reef starts at the mouth of both bays and extends outwards, following the coastline both eastwards and westwards. This reef is spatially separated from seagrass beds and other bay habitats, and consists of a reef flat of up to 150 m wide that leads to a drop-off at a depth of about 5–8 m. Here the reef slopes down to at least 30 m deep.

Table 1. Number of replicates of *O. chrysurus* samples of muscle tissue, otoliths, stomach contents, and potential food items. Bay collection sites consisted mainly of seagrass beds. Additionally, some fish and food items from the bay area were collected in mangroves, mudflats, and rocky shoreline habitats.

	Bay	Reef-mouth	Reef-far
Spanish Water Bay			
Muscle tissue	20	24	28
Otoliths	17	—	30
Stomach contents	5	2	7
Plankton	11	15	8
Crustacea (pooled)	19	6	13
Amphipoda	1	—	—
Crabs	3	3	8
Shrimp	15	3	5
Piscadera Bay			
Muscle tissue	16	22	30
Otoliths	19	—	21
Stomach contents	12	4	7
Plankton	23	13	12
Crustacea (pooled)	47	15	12
Amphipoda	9	—	—
Crabs	9	11	9
Mysidacea	19	—	—
Shrimp	10	4	3

Species collection—Fish and potential food sources were collected in bay (mainly in seagrass) and reef habitats (see Fig. 1 and Table 1). Reef fish were collected in two areas: 300–1,500 m away from the bay mouth (reef-far) and in the bay mouth where the reef started (reef-mouth). Among reef fish, mostly the smaller specimens were captured, hypothetically recent immigrants from the bay. We collected fish using hook and line. Muscle tissue was analyzed for fish collected during the year of 2006 and for some additional reef-far fish collected during 2002. Otoliths were analyzed for bay and reef-far fish collected during 2002 and 2006.

The diet of *O. chrysurus* has been identified in earlier studies of stomach contents, and consists mainly of benthic crustaceans and zooplankton (Randall 1967; Cocheret de la Morinière et al. 2003). Potential prey items from these species groups were collected at the three collection areas (i.e., bay, reef-mouth, and reef-far) of both bays during 2006. Plankton was collected at a water depth of approx. 1 m, using a plankton net with a mesh size of 55 μm and a gape diameter of 50 cm. Crustaceans were collected with tweezers or small hand nets while snorkeling (bay) or SCUBA diving (coral reef). Each group of potential prey consisted of different species, which showed some variations in $\delta^{13}\text{C}$. Unfortunately, it is difficult to prove which of the selected prey samples were actually eaten by *O. chrysurus*, because the details of diet have never been accurately identified down to prey species level. Therefore, potential food items were pooled into species groups (see Table 1, Fig. 2), and we also analyzed the stable isotope ratios of fish stomach contents. It is unclear how much carbon in the prey tissue in a stomach may be assimilated into muscle or otolith carbon. However, this approach of analyzing fish stomach contents does give more confidence

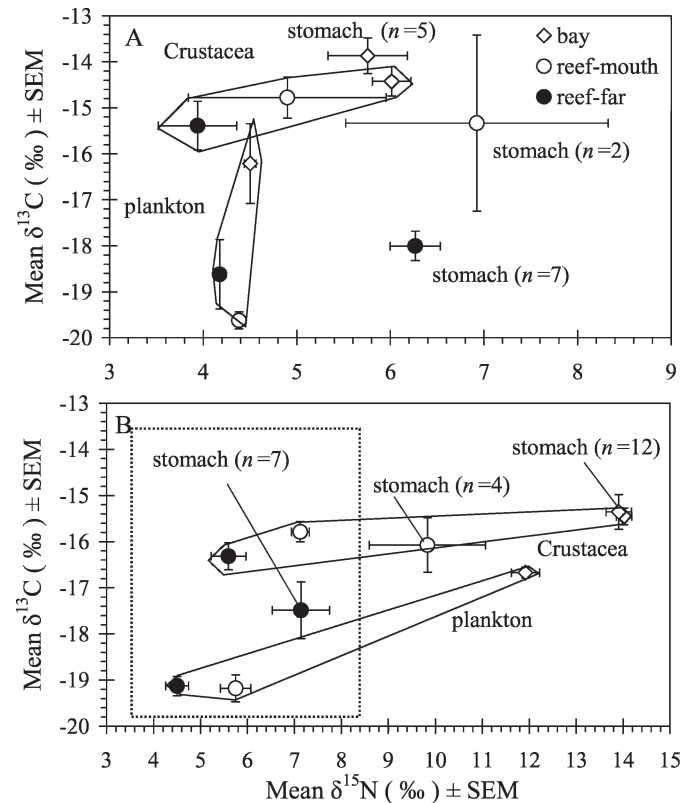


Fig. 2. C and N isotope ratios (mean $\text{‰} \pm \text{SEM}$) of potential food items and of *O. chrysurus* stomach contents from (A) Spanish Water Bay and (B) Piscadera Bay. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are defined in the text. The dashed line in (B) indicates the data range of Spanish Water Bay. Crustacea consisted of Amphipoda, Mysidacea, and crabs and shrimp (Decapoda). The number of replicates per food group varied between 6 and 47 (mean $n = 16$; for exact n , see Table 1). Note that the number of stomach content samples of the reef-mouth area of Spanish Water Bay was very low.

about which food items are actually ingested and thus likely to be important. Moreover, the main reason for analyzing stomach contents was to examine whether fish living on the reef had ingested seagrass-derived food sources, which would indicate short-term feeding migrations into the bay. All specimens were stored in a freezer before further preparation. Exact sample sizes of fish and food items are given in Table 1.

Sample preparation and analysis—Fish muscle tissue was taken from the musculature below the dorsal fin, above the lateral line. Fish muscle, stomach contents, and potential food items were oven-dried at 60°C for at least 96 h. Dried samples were ground to a homogenous powder with a mortar and pestle. All inorganic carbon (CaCO_3) from crab samples was removed by adding 20–30% HCl (Jacob et al. 2005). These acidified samples were dried at 90°C during 90 h. Crab subsamples for N isotope analysis were not decalcified. Samples were weighed accurately into tin containers (fish muscle, 0.25–0.35 mg; stomach contents and prey items, 0.25–3.50 mg, depending on the element [C or N] and species). The isotope ratios of ^{12}C and ^{13}C and those of ^{14}N and ^{15}N were measured using a Finnigan EA-

Table 2. Statistical analyses of stable isotopic variations. Dependent variables were isotopic ratios of potential food items, *O. chrysurus* stomach contents, muscle tissue, and otoliths; independent variables were collection area (bay, reef-mouth, reef-far), fish or otolith size, year, and otolith zone. Bold values indicate significance at $\alpha = 0.05$. — means not included in test. Size = fish fork length in case of muscle and otolith width in case of otoliths. A year effect was calculated for muscle and otolith data of fish collected during two different years (see Study area and species collection in the Methods). Zone = the main effect of juvenile vs. adult otolith zones. Post hoc tests or contrasts analyzed pairwise differences between collection areas (col. area) and different letters indicate significant differences. For food sources and stomachs, the post hoc tests used were Gabriel's for ANOVA and Games-Howell for Kruskal-Wallis (see Statistical analyses in the Methods). *p* values represent main effects of independent variables, corrected for additional variables in the model. This means that even though we found a significant year effect in some cases, the significant values for collection area and size were corrected for this temporal difference, and thus represent true effects. For muscle and otolith data, contrast tests made pairwise comparisons between the collection areas. These contrasts show the main effect of collection area, i.e., corrected for the effects of size, year, and zone.

	$\delta^{15}\text{N}$						$\delta^{13}\text{C}$						
	<i>p</i> values			Post hoc or contrasts, col. area			<i>p</i> values				Post hoc or contrasts, col. area		
	Col. area	Size	Year	Bay	Reef-mouth	Reef-far	Col. area	Size	Year	Zone	Bay	Reef-mouth	Reef-far
Spanish Water Bay													
Crustacea	0.002	—	—	A	AB	B	0.239	—	—	—	A	A	A
Plankton	0.001	—	—	A	A	B	0.017	—	—	—	A	B	AB
Stomachs	0.376	—	—	A	A	A	0.013	—	—	—	A	AB	B
Muscle	0.139	0.886	0.428	A	A	A	0.044	<0.001	0.251	—	A	B	AB
Otoliths	—	—	—	—	—	—	0.018	0.001	0.017	0.837	A	—	B
Piscadera Bay													
Crustacea	<0.001	—	—	A	B	C	0.078	—	—	—	A	A	A
Plankton	<0.001	—	—	A	B	C	<0.001	—	—	—	A	B	B
Stomachs	<0.001	—	—	A	B	B	0.020	—	—	—	A	AB	B
Muscle	<0.001	0.006	<0.001	A	B	C	0.008	<0.001	<0.001	—	A	B	B
Otoliths	—	—	—	—	—	—	0.800	0.310	0.127	0.052	A	—	A

IRMS (elemental analyzer–isotope ratio mass spectrometer) with Dynamic Flash Combustion. Carbon and nitrogen isotope ratios are expressed in standard delta notation: $\delta^{13}\text{C} = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1,000\text{‰}$, where *R* is the ratio of $^{13}\text{C}:^{12}\text{C}$ and R_{standard} is Vienna Pee Dee Belemnite; $\delta^{15}\text{N}$ is expressed using the same equation where *R* is the ratio of $^{15}\text{N}:^{14}\text{N}$ and R_{standard} is atmospheric nitrogen. Mean reproducibility of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was within 0.15‰.

Fish otoliths (sagittae) were removed and stored in a freezer. They were cleaned ultrasonically in 1 mL 100% methanol, and then in Milli-Q. Otoliths were dried overnight at 70°C. Sagittae from the right side of the head were selected for analyses. They were measured, weighed, and mounted on glass plates. After the otoliths had been embedded in resin, they were cross-sectioned in the transverse plane through the core. For fish from bay and reef-far locations, we analyzed the otolith margin (reflecting the current habitat) and additionally, for reef fish, the juvenile zone (possibly reflecting earlier life in bay nurseries) (see inset, Fig. 4A). Juvenile zones targeted in reef fish otoliths were measured to have a similar mean width to those of juvenile bay fish. Otolith material was drilled out with a micromill that sampled a crater with a diameter of about 0.35 mm. Two craters were drilled per sample on opposite sites of the cross section, approximating the same life stage (see Fig. 4A), to provide sufficient otolith material for one analysis. Pulverized otolith material (weight $\geq 10\ \mu\text{g}$) was collected with a scalpel and put into glass tubes. At a temperature of 80°C, a few drops of 100% orthophosphoric acid were added to digest all

CaCO_3 . The isotope ratios of ^{12}C and ^{13}C of the released CO_2 were measured by a Finnigan MAT 252 mass spectrometer equipped with an automated carbonate extraction line (Kiel device). The NIST SRM 8544 (NBS 19) carbonate standard was routinely monitored during sample runs. Mean reproducibility of $\delta^{13}\text{C}$ by this latter technique was within 0.05‰.

Statistical analyses—C and N isotope ratios of potential food items and stomach contents (dependent variables) were tested for differences among collection areas (independent variable) using one-way analysis of variance (ANOVA) (SPSS 14.0). Post hoc tests for ANOVA were Gabriel's (for unequal sample sizes; Field 2005). In case of heterogeneity of residual variances as shown by Levene's test, we used the nonparametric Kruskal-Wallis test followed by the Games-Howell post hoc test (Field 2005).

C and N isotope ratios of muscle tissue and fish otoliths (dependent variables) were tested for differences among collection areas, years, size (i.e., fork length for muscle tissues, otolith width for otoliths), and otolith zone (i.e., juvenile vs. adult zones) (independent variables) using Mixed Linear Models in SAS 8.2 (Verbeke and Molenberghs 1997). For otoliths of reef fish, the juvenile and adult zones of the same fish were treated as a repeated variable.

Results

Food—Isotopic signatures for potential food items contrasted strongly between bay and reef locations.

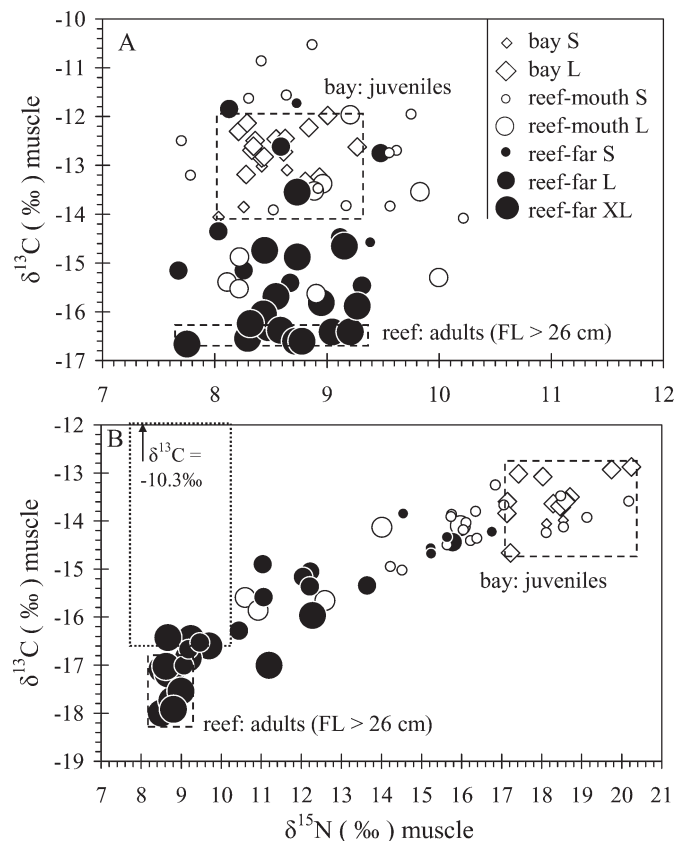


Fig. 3. C and N isotope ratios of *O. chrysurus* muscle tissue from (A) Spanish Water Bay and (B) Piscadera Bay. The dashed line in (B) indicates the data range of Spanish Water Bay. Fish size classes are represented by symbol size: bay small (S) = 6.5–7.5 cm, bay large (L) = 7.5–12 cm, reef-mouth and reef-far S = 10–15 cm, reef-mouth and reef-far L = 15–20 cm, reef-far extra large (XL) = 20–40 cm. Fish were considered adult at fork length >26 cm, the size of sexual maturity (Claro 1983).

Crustaceans and plankton from bay habitats were significantly more enriched in $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ than those from one or both reef areas (Fig. 2; Table 2). Enrichment in $\delta^{15}\text{N}$ of bay food sources was likely caused by high eutrophication, especially in Piscadera Bay. Isotopic variations of fish stomach contents reflected those of the most likely food items: stomachs of bay fish closely resembled isotopic values of bay crustaceans and were significantly enriched compared to stomach contents of reef-far fish (Fig. 2; Table 2). Reef-mouth fish showed values in between these two, although the small sample size may have influenced our interpretation (Fig. 2; Table 2).

Muscle—Isotopic variations of fish muscle tissue followed the same trends as those shown by potential food items and stomach contents. Muscle tissue of bay fish was significantly more enriched in $\delta^{15}\text{N}$ and/or $\delta^{13}\text{C}$ than that of reef-far fish, and values of reef-mouth fish were scattered in between those two (Fig. 3; Table 2). However, a large proportion of reef-mouth fish fell within the bay range of $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ (58% and 27% for Spanish Water and Piscadera, respectively, Fig. 3). Most of these were small

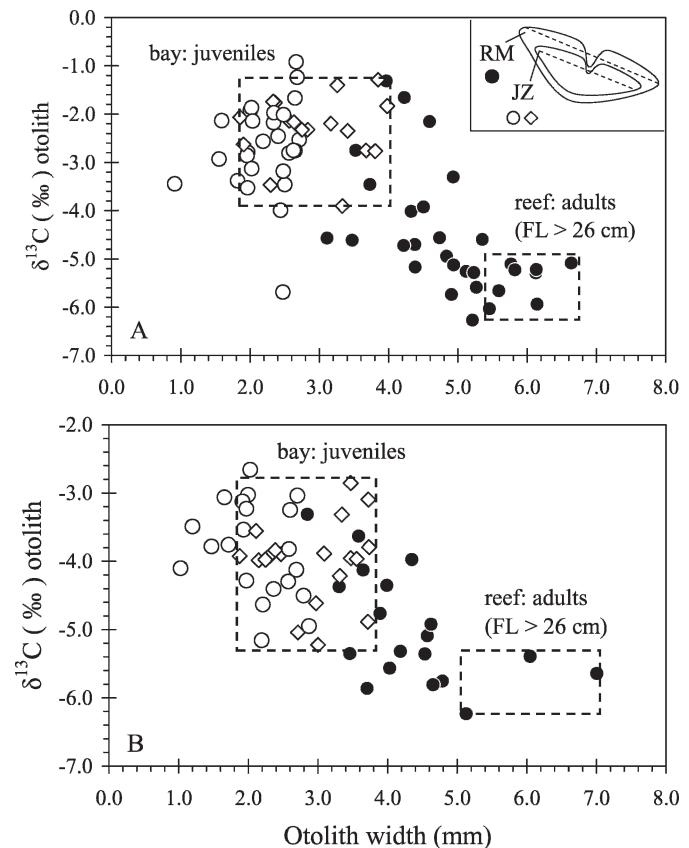


Fig. 4. Otolith width vs. otolith C isotope ratios of *O. chrysurus* from (A) Spanish Water Bay and (B) Piscadera Bay. FL = fish fork length. Analyzed reef fish were those of the reef-far location. Inset (panel A) shows transverse cross sections of reef fish otolith and smaller juvenile (bay fish) otolith. Dashed lines in the inset represent the distance between the two replicate locations that were sampled to collect sufficient material for analysis. This distance is expressed as “otolith width” on the x-axis. JZ = juvenile zone of fish otoliths (diamonds = margins of bay juveniles, white circles = juvenile zones of reef fish), RM = reef fish otolith margin (black circles).

fish, whereas larger ones were typically more $\delta^{15}\text{N}$ and/or $\delta^{13}\text{C}$ depleted and approximated reef-far fish (large and extra large) (Fig. 3; Table 2). Some smaller reef-far fish (21% and 20% of all reef-far fish of Spanish Water and Piscadera, respectively) also showed a bay-like signature (Fig. 3). Statistical analysis confirmed that these stable isotope variations of muscle tissue were explained in large part by fish size (Table 2), which covaried strongly with catch area.

Otoliths—Otolith width increased linearly with fish fork length at both bays ($n > 40$, $0.949 < R^2 < 0.954$, $p < 0.001$) and thus closely reflected fish size. Otolith $\delta^{13}\text{C}$ (Fig. 4) was more enriched than that for corresponding muscle tissue (Fig. 3) because otolith carbon is only partly (10–30%) metabolically derived (Kalish 1991; Schwarcz et al. 1998; Campana 1999). The majority is taken up from dissolved inorganic carbon (DIC) in the water column, which has a $\delta^{13}\text{C}$ of approximately +1‰ (Schwarcz et al. 1998). Reef-far

fish with depleted (i.e., reef-like) $\delta^{13}\text{C}$ in their muscle tissue also showed a depleted $\delta^{13}\text{C}$ at their otolith margin, but their juvenile otolith zone was relatively enriched (Fig. 4) and was similar to that of the otolith margin of fish from the seagrass bed. In only one case (at Spanish Water) the juvenile otolith zone of a reef-far fish was not enriched (open circle, Fig. 4A). For some reef fish (19% at Spanish Water, 52% at Piscadera), otolith margins showed bay-like signatures: this was the case for smaller reef fish (<22.5 cm). As with the muscle $\delta^{13}\text{C}$ data, most otolith $\delta^{13}\text{C}$ variation was explained by fish size (here reflected by otolith size that covaried with the three collection areas), but significant effects were found only for Spanish Water (Table 2), probably because at Piscadera mainly smaller-sized reef fish were analyzed (see Discussion). Despite this, data of Piscadera showed the same pattern as data of Spanish Water (Fig. 4).

Discussion

The shift in stable isotope composition of muscle tissue coincides with the hypothesized ontogenetic migration of *O. chrysurus* from seagrass to coral reef, but also with the size of the fish. We argue below that this shift is not a main effect of growth, but that these isotopic variations are most likely explained by gradual fading of the enriched seagrass nursery signature after ontogenetic migration to the reef. This fading probably occurs when larger fish reach the reef-like (i.e., depleted) isotopic equilibrium during renewal of muscle tissue. This interpretation also explains why only the smaller reef fish, possibly the most recent immigrants, more closely resembled isotopic signatures of bay fish.

Explanations other than ontogenetic movement to the coral reef can most likely be ruled out. First, the metabolic dilution of the isotopic signal of smaller fish on the reef could hypothetically represent fish originating from the reef, but showing feeding migrations into the bay. The fact that reef fish stomach contents resemble reef food items and not those from the bay leads us to discard this possibility. Secondly, the depletion in muscle $\delta^{15}\text{N}$ and/or $\delta^{13}\text{C}$ with growth may hypothetically be an effect of an ontogenetic diet shift within the reef habitat. However, this cannot explain our data, because the trophic level (indicated by $\delta^{15}\text{N}$) of *O. chrysurus* and other fish species increases with ontogeny. More specifically, for *O. chrysurus*, Cocheret de la Morinière et al. (2003) have shown that fish collected from the Spanish Water Bay displayed a significant increase of $\delta^{15}\text{N}$ with increasing fish size, which coincided with increased prey fish consumption. Thus, if our observed variations were an effect of ontogeny alone, reef fish should show increasing $\delta^{15}\text{N}$ with increasing size and age. Our data of Piscadera Bay are the strongest case against this possibility. Here, muscle tissue of older and larger reef fish at higher trophic levels showed much lower values of $\delta^{15}\text{N}$ than that of smaller reef fish at lower trophic levels. This is more readily explained by movement from the polluted and eutrophicated embayment towards the reef environment with more pristine and lower levels of $\delta^{15}\text{N}$ in the food web.

Likewise, the depletion of otolith $\delta^{13}\text{C}$ with increasing fish size cannot be an effect of somatic growth, because otolith $\delta^{13}\text{C}$ enriches with body size (e.g., Campana 1999; Huxham et al. 2007) when the corresponding decrease in resting metabolism leads to more otolith carbon being derived from relatively enriched DIC (Kalish 1991; Schwarcz et al. 1998). The observed depletion of otolith $\delta^{13}\text{C}$ with increasing fish size followed the same direction as that of the fully food-derived carbon of muscle tissue, and can more readily be explained by ontogenetic migration to the reef with depleted $\delta^{13}\text{C}$ food webs.

Our main purpose for using otoliths was to access an earlier period in the feeding history of the fish than that provided by muscle tissue. It was indeed possible to do this, and reef fish with depleted, reef-like isotopic signatures in their muscle tissue still showed enriched signatures indicative of feeding from the seagrass-based food web in the juvenile zones of their otoliths. The otolith margins of most reef fish were depleted in $\delta^{13}\text{C}$ and confirmed that reef fish had been living and feeding on the reef for a significant period of time, whereas their enriched juvenile zones (true for 50 out of 51 fish) reflected feeding inside seagrass beds at an earlier life stage. Otolith margins of a few smaller (12.8–22.5 cm) reef fish, most abundantly collected at Piscadera, did not appear to support ontogenetic migration because they showed bay-like signatures. However, this may be because these fish were recent reef immigrants that experienced a time lag between migration and the formation of a 0.35-mm-thick increment of CaCO_3 at the otolith margin, which was the otolith portion that was sampled for $\delta^{13}\text{C}$ analysis. Otoliths grow daily, but the daily increments of lutjanids are only several μm thick (e.g., Szedlmayer 1998; Zapata and Herrón 2002). It could therefore take up to several months to grow sufficient CaCO_3 to be seen by the spatial resolution of our sampling technique. Linear regression between the fork lengths of our sampled fish and their otolith widths showed that in theory, reef immigrants should grow at least 2.4 cm in body length from the moment that they arrive on the reef, so that their sampled otolith margin has grown 0.35 mm, in order to show a reef signature. For fish with a fork length between 12.8 and 22.5 cm, growth of 2.4 cm in body length occurs in about 6 months (García et al. 2003). These smaller reef fish had thus probably moved <6 months before capture. The upper size limit of *O. chrysurus* so far observed in Spanish Water Bay is 25.0 cm (Nagelkerken et al. 2000), suggesting that reef fish <22.5 cm in length could indeed be recent immigrants.

Our data strongly suggest that for the *O. chrysurus* population on the coral reef, bay nurseries form an important source of subadults. Although the muscle tissue of larger reef fish no longer resembled the isotope signature of the bay ecosystem, all their juvenile otolith zones (except for that of one fish) matched those of bay fish. Therefore, based on these data, we calculate that 98% (50 out of 51) of the *O. chrysurus* reef population consisted of immigrants that had passed through seagrass nurseries. This means that, even though a seagrass life history phase may not be obligatory to every single individual of *O. chrysurus* on the coral reef, for the great majority this does seem to be the

case. The data do not show whether *O. chrysurus* spends its entire juvenile phase in seagrass beds, because only a part of the juvenile otolith zone was sampled. It could also be possible that other habitat types have an identical isotopic composition to that of the bay or reef ecosystems identified here. This is highly unlikely in our relatively small-scale study area where only few major habitat types occur, but is an important consideration when extrapolating our approach to other sites of greater spatial scales.

Although our results are likely to be applicable to other Caribbean islands with marine embayments, the life history-related patterns of habitat use by *O. chrysurus* may be different for the same species in other geographic areas. Besides *O. chrysurus*, juveniles of 16 other Caribbean reef fish species (most of which are commercially important) have been suggested to be strongly associated with seagrass and mangrove habitats based on size-frequency distributions (Nagelkerken et al. 2000). A study on one of these species, *Haemulon flavolineatum*, using less discriminatory trace element data, suggested that 36% of the reef population could be linked to mangrove nurseries (Chittaro et al. 2004). This is a much weaker association than found in our study, but can be explained by the fact that this species uses shallow coral reef zones (<3 m deep) as a juvenile habitat in addition to mangroves and seagrass beds (Nagelkerken et al. 2000, 2001, 2002; Dorenbosch et al. 2004). *H. flavolineatum* can thus be described as a “facultative nursery species.” Besides *O. chrysurus*, at least 10 of the 16 species identified above were referred to as “obligate nursery species” and are believed to have a high dependence on bays with seagrass and mangrove nurseries (Nagelkerken et al. 2001, 2002; Dorenbosch et al. 2004). If these 10 species show similar migration patterns to those indicated here for *O. chrysurus*, then an important part of the commercial reef fish population is likely to be sustained by seagrass and/or mangrove nurseries.

The global decline in surface area of seagrass beds and mangroves has been estimated to be up to 35% (Shepherd et al. 1989; Valiela et al. 2001). In the light of the present study, this gives great concern as far as vital coral reefs and sustainable fisheries are concerned. Marine reserves are promising management tools with effects extending beyond their boundaries (Roberts et al. 2001), but the effectiveness of coral reef MPAs is heavily criticized (Mora et al. 2006). Worldwide, 247 MPAs include seagrass beds (as opposed to 980 for coral reefs; Mora et al. 2006), many of which are rarely singled out as objects of protection (Spalding et al. 2003). We suggest that establishing combined reef and seagrass MPAs should increase reef *O. chrysurus* biomass, and that of other seagrass-associated species, even stronger than reef MPAs alone, promoting spillover into adjacent fishing grounds. We thus stress the importance of introducing coral reef reserves comprised of a mosaic of interlinked coastal habitats, including seagrass beds.

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