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REPORT

Carbon isotopes in otolith amino acids identify residency of juvenile snapper (Family: Lutjanidae) in coastal nurseries

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Abstract This study explored the potential for otolith geochemistry in snapper (Family: Lutjanidae) to identify residency in juvenile nursery habitats with distinctive carbon isotope values. Conventional bulk otolith and muscle stable isotope analyses (SIA) and essential amino acid (AA) SIA were conducted on snapper collected from seagrass beds, mangroves, and coral reefs in the Red Sea, Caribbean Sea, and Pacific coast of Panama. While bulk stable isotope values in otoliths showed regional differences, they failed to distinguish nursery residence on local scales. Essential AA δ^{13}C values in otoliths, on the other hand, varied as a function of habitat type and provided a better tracer of residence in different juvenile nursery habitats than conventional bulk otolith SIA alone. A strong linear relationship was found between paired otolith and muscle essential AA δ^{13}C values regardless of species, geographic region, or habitat type, indicating that otolith AAs recorded the same dietary information as muscle AAs. Juvenile snapper in the Red Sea sheltered in mangroves but fed in seagrass beds, while snapper from the Caribbean Sea and Pacific coast of Panama showed greater reliance on mangrove-derived carbon. Furthermore, compound-specific SIA revealed that microbially recycled detrital carbon, not water-column-based new phytoplankton carbon, was the primary carbon source supporting snapper production on coastal reefs of the Red Sea. This study presented robust tracers of juvenile nursery residence that will be crucial for reconstructing ontogenetic migration patterns of fishes among coastal wetlands and coral reefs. This information is key to determining the importance of nursery habitats to coral reef fish populations and will provide valuable scientific support for the design of networked marine-protected areas.

Keywords Mangroves · Seagrass · Migration · Diet · Coral reefs

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Introduction

Many commercially and ecologically important coral reef fishes, including species of Lutjanidae (snappers), Serranidae (grouper), Haemulidae (grunts), and Scaridae (parrotfish), use coastal mangroves and seagrass beds as juvenile nursery areas before migrating to coral reef habitats as adults (see reviews by Beck et al. 2001; Adams et al. 2006; Faunce and Serafy 2006; Nagelkerken et al. 2008a). Nearshore habitats provide a number of benefits to resident juvenile reef fishes, including an abundance of food, refuge from predators, and shelter from physical disturbance (Laegdsgaard and Johnson 2001; Cocheret de la Moriniere

et al. 2004; Manson et al. 2005; Verweij et al. 2006). These benefits may result in higher growth and survival rates leading to locally elevated juvenile densities within habitats and, presumably, the movement of significant numbers of individuals from these nursery areas to adult habitats (Beck et al. 2001; Sheridan and Hays 2003; Adams et al. 2006).

Few researchers would dispute that mangroves and seagrass beds typically harbor higher densities of many juvenile reef fish species compared to reef habitats (Jackson et al. 2001; Manson et al. 2005). However, do juveniles within these coastal habitats successfully recruit to the adult populations on reefs? Most studies to date have either assumed successful migration from nursery habitats to reef environs or inferred movements based upon differential size class distributions among habitats (Nagelkerken 2007). Only a handful of studies have provided direct evidence for such ontogenetic movements (Chittaro et al. 2004; Nakamura et al. 2008; Mateo et al. 2010), and even fewer have quantified the relative contribution of different juvenile habitats to adult populations on coral reefs.

Tracking movement of fishes between juvenile and adult habitats requires the ability to either follow individuals between habitats over long time scales or retrospectively identify juvenile habitat associations in adult fishes. Conventional mark–recapture approaches suffer a number of limitations when applied to early life-history stages of marine fishes, including tagging effects on mortality and behavior, difficulties tagging a high proportion of the individuals within a location, and low recapture rates of tagged fish (Thorrold et al. 2002). More recently, fish ecologists have used otolith geochemistry to overcome many of the problems associated with conventional tagging (reviewed by Elsdon et al. 2008). Otoliths are composed almost invariably of aragonite deposited on a proteinaceous matrix that together form daily and annual increments visible throughout the life of a fish (Campana 1999). These increments preserve a chronological record of the fish's metabolic activity and the physical and chemical characteristics of the water in which the fish resided during the time of deposition (Campana 1999). Stable isotope analysis (SIA) relies on spatial variation in the abundance of ambient stable isotope ratios (isoscapes, West et al. 2010) that are recorded in otoliths as a fish lives and feeds in different habitats. Tropical seascapes, including mangroves, seagrass beds, and coral reefs, provide an excellent isoscape for examining residence in and movement among isotopically distinct habitats (Fry 1981; Nakamura et al. 2008). For instance, mangroves, coral reefs, and seagrass beds have distinct carbon isotope signatures at the base of the food web ($\delta^{13}C_{Base}$) resulting from the $\delta^{13}C$ values of the dominant primary producers in those habitats (Marguillier et al. 1997; Layman 2007). Similarly, estuarine environments often exhibit unique dissolved inorganic carbon (DIC) $\delta^{13}C$ and $\delta^{18}O$ values of ambient water ($\delta^{18}O_{H2O}$) associated with freshwater inputs that create isotopic gradients from coastal wetlands to offshore coral reefs (Dansgaard 1964; Siegenthaler and Oeschger 1980; Stewart and Taylor 1981).

McMahon et al. (2011) recently developed a technique for analyzing $\delta^{13}C$ values of essential amino acids (AAs) in otoliths that may provide a new source of information on habitat use by juvenile reef fishes. Essential AAs are excellent tracers of dietary carbon sources because animals typically cannot synthesize essential AAs de novo and must incorporate them into tissues directly from their diet with little or no isotope fractionation (Hare et al. 1991; Howland et al. 2003; Jim et al. 2006; McMahon et al. 2010). Therefore, $\delta^{13}C$ values in essential AAs of otoliths provide a way of distinguishing residence among habitats with different $\delta^{13}C_{Base}$ values. The approach is complementary to traditional bulk analysis of $\delta^{13}C$ and $\delta^{18}O$ in otolith aragonite, where isotope composition is a function of physico-chemical properties of ambient water (McMahon et al. 2011).

This study explored the potential for otolith geochemistry in snapper (Family: Lutjanidae) to identify residency in juvenile nursery habitats with distinctive $\delta^{13}C_{Base}$ values. We compared the ability of conventional bulk otolith $\delta^{13}C$ and $\delta^{18}O$ analysis and otolith essential AA $\delta^{13}C$ analysis, to assess residence of Ehrenberg's snapper, *Lutjanus ehrenbergii*, among three potential nursery habitats along the Red Sea coast of Saudi Arabia. A common snapper species in the Indo-west Pacific, *L. ehrenbergii* is abundant as juveniles in coastal wetlands and is thought to undergo an ontogenetic migration to coral reefs as adults (Unsworth et al. 2009). Bulk and compound-specific SIA were also conducted on otoliths from juvenile schoolmaster snapper (*L. apodus*) collected in seagrass beds and mangrove lagoons around the islands of St. Croix and Puerto Rico in the Caribbean Sea and from juvenile yellow snapper (*L. argentiventris*) sampled in mangrove lagoons along the Pacific coast of Panama. It was hypothesized that essential AA $\delta^{13}C$ values in otoliths would vary as a function of habitat type while bulk otolith $\delta^{13}C$ and $\delta^{18}O$ values would vary regionally due to different coastal water mass properties at each of the locations, but not at smaller spatial scales within regions. Analysis of stable isotope values in the organic component of otoliths will allow, for the first time, retrospective studies of diet from fish otoliths. These data will facilitate reconstructions of fish movements among isotopically distinct habitats and provide critical information necessary to establish effective management plans for marine fisheries.

Materials and methods

Field collections

Ehrenberg's snapper, *L. ehrenbergii* (Peters 1869), were collected at five locations along the coast of Saudi Arabia in the Red Sea in November 2008, March 2009, and June 2010 (Fig. 1). Juvenile *L. ehrenbergii* (total length [TL] = 85 ± 17 mm) were collected with cast nets from three coastal wetland systems: Al Lith Bay, Khor Al Kharrar Bay, and Cape Al-Askar Bay. All three coastal wetland sites were large, semi-enclosed bays with submerged aquatic vegetation dominated by *Halodule uninervis* and with fringing white mangroves (*Avicennia marina*). Adult *L. ehrenbergii* were speared from a coastal reef 2 km off the coast of Al Lith (Coast Guard Reef; fish TL = 188 ± 41 mm) and a shelf reef 14 km offshore (Ron's Reef; fish TL = 217 ± 18 mm). Adult *L. ehrenbergii* were used to characterize $\delta^{13}C$ values of reef residents, as no juvenile *L. ehrenbergii* were observed on any reefs outside of Al Lith Bay. To constrain $\delta^{13}C_{Base}$ values of coastal food webs, we collected seagrass blades (*H. uninervis*) and mangrove leaves (*A. marina*) by hand from Al Lith Bay. Detritus-feeding crabs, a major component of *L. ehrenbergii* diet, were collected by hand from Al Lith Bay (*Metopograpsus thukuhar*), Coast Guard Reef (*Trapezia tigrina*), and Ron's Reef (*T. tigrina*). Zooplankton samples, consisting predominantly of calanoid copepods, were collected with a 1-m diameter, 333-μm mesh net in Al Lith Bay, and in open water adjacent to Coast Guard Reef and Ron's Reef. Crab and zooplankton samples served as proxies for detritus and phytoplankton food web end members in the system, respectively. Triplicate samples were collected for all food web analyses.

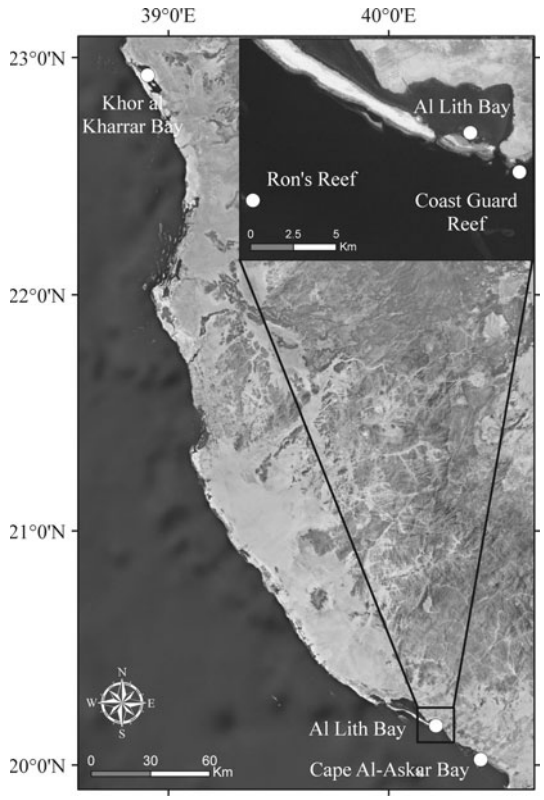

Fig. 1 Ehrenberg's snapper, *Lutjanus ehrenbergii*, collection sites from three coastal wetland habitats (Khor Al Kharrar Bay, Al Lith Bay, and Cape Al-Askar Bay), a coastal coral reef (Coast Guard Reef) and a shelf coral reef (Ron's Reef) near Al Lith, Saudi Arabia in the Red Sea

Juvenile snapper in the genus *Lutjanus* were also collected from four sites in the Caribbean Sea and three sites on the Pacific coast of Panama to examine regional variability in juvenile nursery habitat signatures. Juvenile schoolmaster snapper, *L. apodus* (Walbaum 1792) (fish TL = 75 ± 40 mm), were collected with seine nets and wire traps at two mangrove sites in Puerto Rico (N 17.9908°, W 66.7528°; N 17.9636°, W 66.9856°), a mangrove site in St. Croix (N 17.775°, W 64.76°), and a seagrass site in St. Croix (N 17.7231°, W 64.6458°). Juvenile yellow snapper, *L. argentiventris* (Peters 1869) (fish TL = 83 ± 7 mm), were collected with seine nets from three mangrove sites near Bahia Honda, Panama (N 7.7639°, W 81.5489°; N 7.7656°, W 81.5128°: N 7.7653°, W 81.4986°) on the Pacific coast of Panama.

Sagittal otoliths and white muscle tissue were dissected from each fish in the field, with the exception of *L. argentiventris*, where only otoliths were retained. Otoliths were cleaned of residual surface tissue with water and stored dry in 1.5 ml vials. White muscle samples from the dorsal surface of each fish and food web samples were frozen on the boat prior to transport to an onshore laboratory. In the laboratory, white muscle and food web samples were frozen at −20°C and then lyophilized (freeze-dried) for 48 h. Samples were transferred to the Woods Hole Oceanographic Institution, Woods Hole, MA, USA for further preparation and analysis. Ten *L. ehrenbergii* were collected from each of the five sites in the Red Sea (except Al Lith Bay where $n = 9$), four *L. apodus* were collected from each of the four sites in the Caribbean Sea, and five *L. argentiventris* were collected from each of the three sites on the Pacific coast of Panama.

Sample preparation and analysis

A single, randomly selected sagittal otolith from each fish was used for bulk and compound-specific SIA. All otolith samples were scrubbed and rinsed in ultra-pure water, cleaned ultrasonically for 5 min in ultra-pure water, and then air-dried under a class-100 positive-flow fume hood for 24 h. Whole otoliths from juvenile *Lutjanus spp*. were used for SIA. For adult *L. ehrenbergii*, a Merchantek

MicroMill (Electro Scientific Industries, Portland, OR, USA) was used to collect otolith powder after the last annulus, corresponding to the most recent several months of growth and thus the most recent location of residence.

Otolith material was homogenized with a mortar and pestle and then subdivided into two portions for bulk inorganic and compound-specific SIA. Approximately 50 μg of otolith material was analyzed for bulk $\delta^{13}C$ and $\delta^{18}O$ values on a Thermo Finnigan Mat 253 isotope ratio monitoring-mass spectrometer (irm-MS) with a Kiell III carbonate device at the Woods Hole Oceanographic Institution following the methods of Ostermann and Curry (2000). External precision of the mass spectrometer for $\delta^{13}C$ measurements in carbonate standards was ±0.03‰. Approximately 10 mg of otolith material from each fish was acid hydrolyzed with 0.1 ml of 6 N HCl mg^{-1} otolith under a N_2 atmosphere at 110°C for 20 h to isolate individual AAs according to McMahon et al. (2011). Samples were neutralized with ultrapure water and evaporated to dryness under a gentle stream of N_2 to remove HCl. Samples were stored frozen until they were derivatized just prior to compound-specific SIA.

Freeze-dried, homogenized white muscle samples from each fish and food web samples from the Red Sea were also subdivided into two portions. Approximately 1 mg of each sample was weighed into tin cups and analyzed for bulk $\delta^{13}C$ and $\delta^{15}N$ values using a Europa Hydra 20/20 irm-MS at the UC Davis Stable Isotope Facility, Davis, CA, USA. A second portion of each sample (approx. 500 μg for muscle and 1 mg for plant matter) was acid hydrolyzed with 1 ml of 6 N HCl mg^{-1} freeze-dried tissue in the same manner as the otolith samples. Dried, neutralized samples were stored frozen until derivatization.

Acid hydrolyzed otolith, muscle, and food web samples were derivatized prior to SIA following the methods of McMahon et al. (2011) as modified from Silfer et al. (1991) and Johnson et al. (1998). Briefly, samples underwent an acid-catalyzed esterification with 0.8 ml of a 2-propanol and acetyl chloride solution (4:1 by volume) under an atmosphere of N_2 at 110°C for 1 h. Samples were then acylated with 0.5 ml of trifluoroacetic anhydride (TFAA) and 0.5 ml of dichloromethane (DCM) under an atmosphere of N_2 at 110°C for 10 min. Samples were brought up in DCM and injected on column in splitless mode at 260°C and separated on a forte SolGel-1 ms column (60 m length, 0.25 mm inner diameter, and 0.25 μm film thickness; SGE Analytical Science, Sydney, Australia) in an Agilent 6890 N Gas Chromatograph (GC) at the Woods Hole Oceanographic Institution. The separated AA peaks were combusted online in a Finnigan gas chromatography-combustion continuous flow interface at 1,030°C and then measured as CO_2 on a Thermo Finnigan Mat 253 irm-MS (hereafter GC-C-irm-MS). Standardization of runs was achieved using intermittent pulses of a CO_2 reference gas of known isotopic value. All compound-specific SIA samples were analyzed in duplicate. Amino acid standards of known isotopic value were concurrently derivatized and analyzed with each batch of samples to correct for exogenous carbon and potential kinetic fractionation introduced during derivatization. We focused on five essential AAs with sufficient peak size and baseline GC separation: threonine, isoleucine, valine, phenylalanine, and leucine. Overall external precision of the $\delta^{13}C$ measurements after correcting for the fractionation associated with derivatization was 0.5 ± 0.2‰ (SD), averaged across all five essential AAs.

Data analysis

Stable isotope ratios were expressed in standard delta (δ) notation:

$$\delta^{13}C_{sample} = \left(\frac{{}^{13}C/{}^{12}C_{sample}}{{}^{13}C/{}^{12}C_{std}} - 1 \right) \times 1,000,$$

where the standard for carbon was Vienna PeeDee Belemnite (VPDB). Differences in bulk otolith $\delta^{13}C$ and $\delta^{18}O$ values, bulk muscle $\delta^{13}C$ and $\delta^{15}N$ values, and bulk tissue C:N ratios among *L. ehrenbergii* and food web components from different habitats were assessed using separate one-way analyses of variance (ANOVAs) with Tukey's honestly significant difference (HSD) post hoc tests ($\alpha = 0.05$). In addition, 95% confidence ellipses were calculated for the bulk otolith $\delta^{13}C$ and $\delta^{18}O$ values from fish collected in the Red Sea, the Caribbean Sea, and the Pacific coast of Panama. Differences in essential AA stable isotope values from *L. ehrenbergii* and food web components were visualized using principal component analysis (PCA). The relationships between $\delta^{13}C$ values of individual essential AAs from paired otolith and muscle samples were determined by linear regression analyses with 95% confidence intervals for each AA from *L. ehrenbergii* and *L. apodus*, separately.

Results

Ehrenberg's snapper, *L. ehrenbergii*, collected from five sites along the coast of Saudi Arabia had significantly different bulk otolith $\delta^{13}C$ values (one-way ANOVA, df = 4, 48, $F = 38.2$, $P < 0.05$) and bulk muscle $\delta^{13}C$ values (one-way ANOVA, df = 4, 48, $F = 163.4$, $P < 0.05$) (Table 1). *L. ehrenbergii* from the three coastal wetland sites, Al Lith Bay, Khor Al Kharrar Bay, and Cape Al-Askar Bay, had statistically similar bulk muscle and otolith $\delta^{13}C$ values that were higher than *L. ehrenbergii*

Table 1 Bulk otolith $\delta^{13}C$ and $\delta^{18}O$ values (mean ± SD), bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD), and bulk tissue C:N ratios (mean ± SD) of *Lutjanus ehrenbergii* and selected food web components collected from coastal wetlands (Khor Al Kharrar Bay, Al Lith Bay, and Cape Al-Askar Bay), a coastal coral reef (Coast Guard Reef) and a shelf coral reef (Ron's Reef) along the coast of Saudi Arabia in the Red Sea

	Bulk otolith		Bulk muscle		
	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{15}N$	C:N
Lutjanus ehrenbergii					
Khor Al Kharrar Bay	-0.5 ± 0.6^{a}	-0.2 ± 0.4^{a}	-10.2 ± 0.3^{a}	7.0 ± 0.3^{a}	3.1 ± 0.1^{a}
Al Lith Bay*	-0.7 ± 0.6^{a}	-0.8 ± 0.4^{b}	-10.5 ± 0.8^{a}	8.6 ± 0.5^{b}	3.4 ± 0.3^{b}
Cape Al-Askar Bay	-1.4 ± 0.7^{a}	$-0.5 \pm 0.4^{a,b}$	-10.6 ± 0.6^{a}	8.2 ± 0.5^{b}	3.2 ± 0.4^{a}
Coast Guard Reef	-2.6 ± 0.5^{b}	-0.7 ± 0.2^{b}	-14.9 ± 0.8^{b}	8.6 ± 0.6^{b}	3.3 ± 0.1^{a}
Ron's Reef	-3.8 ± 0.9^{c}	$-0.5 \pm 0.3^{a,b}$	-16.9 ± 1.1^{c}	8.3 ± 0.4^{b}	3.4 ± 0.2^{a}
Ribbon Seagrass (*Halodule uninervis*)					
Al Lith Bay			-7.9 ± 0.7	-0.3 ± 1.5	22.8 ± 4.9
White Mangrove (*Avicennia marina*)					
Al Lith Bay			-27.7 ± 0.6	1.4 ± 0.6	36.0 ± 3.8
Zooplankton					
Al Lith Bay			-18.8 ± 0.3^{a}	5.1 ± 0.5^{a}	$5.0 \pm 0.3^{a,b}$
Coast Guard Reef			-16.9 ± 1.0^{b}	$4.6 \pm 0.4^{a,b}$	5.6 ± 0.3^{a}
Ron's Reef			-20.0 ± 0.2^{a}	4.1 ± 0.1^{b}	4.7 ± 0.3^{b}
Crab					
Al Lith Bay (*Metopograpsus thukuhar*)			-12.8 ± 0.4^{a}	5.0 ± 0.2^{a}	3.4 ± 0.2^{a}
Coast Guard Reef (*Trapezia tigrina*)			-15.2 ± 0.2^{b}	5.7 ± 0.8^{a}	3.8 ± 0.4^{a}
Ron's Reef (*Trapezia tigrina*)			-17.5 ± 0.2^{c}	5.7 ± 0.5^{a}	3.4 ± 0.2

Means with the same superscript letter were not significantly different from one another by one-way ANOVA and Tukey's HSD post hoc test ($\alpha = 0.05$). ($n = 10$ *L. ehrenbergii* per site except for Al Lith Bay* where n = 9, and n = 3 for food web components)

from either Coast Guard Reef or Ron's Reef. Bulk otolith $\delta^{18}O$ values were similar among sites, with the exception of high $\delta^{18}O$ values for *L. ehrenbergii* from Khor Al Kharrar Bay (one-way ANOVA, df = 4, 48, $F = 3.8$, $P < 0.05$). Bulk muscle $\delta^{15}N$ values were similar among sites with the exception of *L. ehrenbergii* from Khor Al Kharrar Bay and Cape Al-Askar Bay, which had low $\delta^{15}N$ values (one-way ANOVA, df = 4, 48, $F = 23.3$, $P < 0.05$) (Table 1). There were significant differences in zooplankton bulk $\delta^{13}C$ values (one-way ANOVA, df = 2, 8, $F = 19.5$, $P < 0.05$) and bulk $\delta^{15}N$ values (one-way ANOVA, df = 2, 8, $F = 5.4$, $P < 0.05$) among sites (Table 1; Fig. 2). There were also significant differences in crab bulk $\delta^{13}C$ values (one-way ANOVA, df = 2, 8, $F = 205.9$, $P < 0.05$), but not bulk $\delta^{15}N$ values (one-way ANOVA, df = 2, 8, $F = 1.7$, $P < 0.05$) among sites (Table 1; Fig. 2). Tissue C:N ratios were, however, similar among all samples (Table 1).

Almost all of the variation in $\delta^{13}C$ values of five essential AAs in *L. ehrenbergii* muscle and food web components was captured in principal components (PC) one (92%) and two (5%) of the PCA analysis (Fig. 3). The first PC clearly separated carbon produced by mangroves, zooplankton (a proxy for phytoplankton), and seagrass from each other. Alternatively, the second PC distinguished stable isotope values of detritivores from those of the primary producers and their proxies. Variate loadings for the first PC were all positive and of similar magnitude for all five essential AAs. However, loadings for the second PC were positive for isoleucine (0.50) and leucine (0.47), and negative for valine (−0.67), threonine (−0.26), and phenylalanine (−0.05).

Essential AA $\delta^{13}C$ values from *L. ehrenbergii* muscle samples were generally within the PC coordinate space delineated by zooplankton, detritivores, and seagrass (Fig. 3). Juvenile *L. ehrenbergii* in the coastal wetland habitats fell along a continuum between seagrass and either zooplankton or detritivores. Conversely, adult *L. ehrenbergii* had PC values similar to local detritus-feeding crabs, which were quite different from those of the juvenile *L. ehrenbergii* in the coastal wetlands.

Bulk otolith $\delta^{13}C$ and $\delta^{18}O$ values varied little among sites in the Red Sea relative to the large regional variability in values among congeneric snapper species from locations in the Red Sea, Caribbean Sea, and Pacific coast of Panama (Fig. 4). Locations were clearly separated in isotope space, although *L. apodus* samples from seagrass and mangrove sites in the Caribbean Sea were not significantly different.

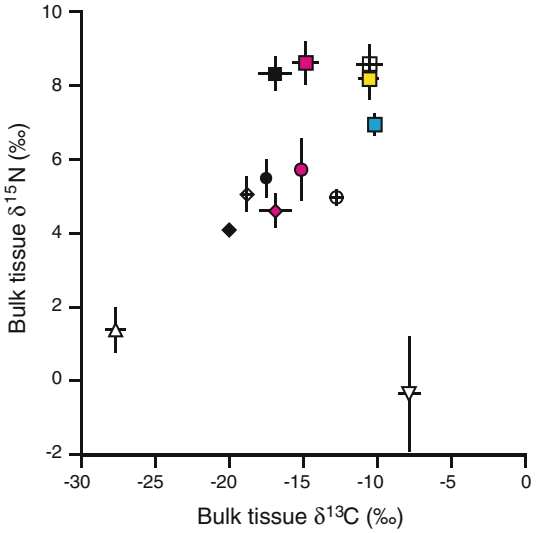

Fig. 2 Bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) of *Lutjanus ehrenbergii* (*square symbols*, $n = 10$ fish per site except Al Lith Bay where $n = 9$), crabs (*circles*, $n = 3$), zooplankton (*diamonds*, $n = 3$), seagrass blades (*inverted triangles*, $n = 3$), and mangrove leaves (*triangles*, $n = 3$) collected from three coastal wetland habitats: Khor Al Kharrar Bay (*Cyan*), Al Lith Bay (*white*), and Cape Al-Askar Bay (*yellow*), a coastal coral reef: Coast Guard Reef (*magenta*), and a shelf coral reef: Ron's Reef (*black*) near Al Lith, Saudi Arabia in the Red Sea

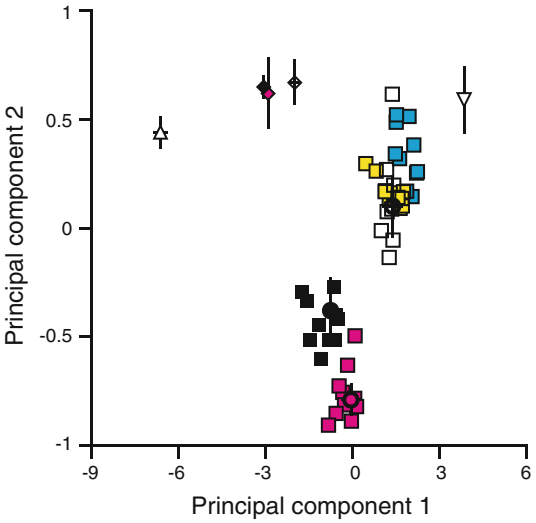

Fig. 3 First and second principal components generated from a principal component analysis of five essential amino acid $\delta^{13}C$ values from *Lutjanus ehrenbergii* (*square symbols*, $n = 10$ fish per site except Al Lith Bay where $n = 9$), and selected food web components (mean ± SD): crabs (*circles*, $n = 3$), zooplankton (*diamonds*, $n = 3$), seagrass blades (*inverted triangles*, $n = 3$), and mangrove leaves (*triangles*, $n = 3$). Samples were collected from three coastal wetland habitats: Khor Al Kharrar Bay (*Cyan*), Al Lith Bay (*white*), and Cape Al-Askar Bay (*yellow*), a coastal coral reef: Coast Guard Reef (*magenta*), and a shelf coral reef: Ron's Reef (black) near Al Lith, Saudi Arabia in the Red Sea

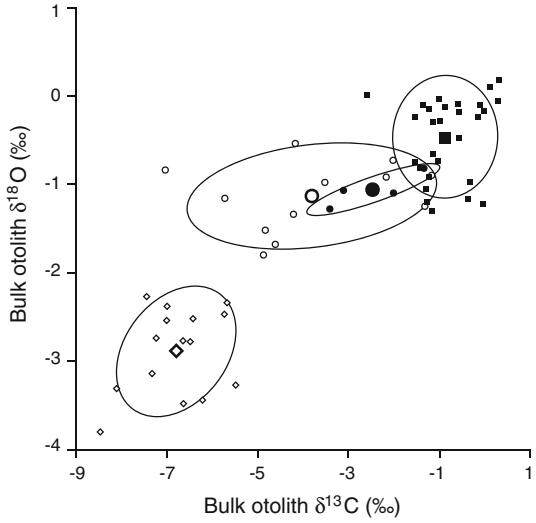

Fig. 4 Bulk otolith $\delta^{13}C$ and $\delta^{18}O$ values (with 95% confidence ellipses) from *Lutjanus ehrenbergii* collected from seagrass bays (*black square symbols*, $n = 29$ fish) in the Red Sea, *L. apodus* collected from seagrass bays (*black circles*, $n = 4$ fish) and mangrove lagoons (*white circles*, $n = 12$ fish) in the Caribbean Sea, and *L. argentiventris* collected from mangrove lagoons (*white diamonds*, $n = 15$ fish) on the Pacific coast of Panama

Essential AA $\delta^{13}C$ from the same samples showed a very different pattern. First, there was a strong linear relationship between paired otolith and muscle essential AA $\delta^{13}C$ values for *L. ehrenbergii* ($y = [0.89 \pm 0.10]x - [1.16 \pm 1.55]$, $R^2 = 0.81 \pm 0.12$) and *L. apodus* ($y = [1.03 \pm 0.04]x - [1.38 \pm 1.07]$, $R^2 = 0.98 \pm 0.09$) (Fig. 5). Unlike the bulk otolith $\delta^{13}C$ and $\delta^{18}O$ results, essential AA stable isotope values distinguished residence of fishes in mangrove and seagrass habitats regardless of the species or geographic region (Fig. 6).

Discussion

A number of species, including many examples from the family Lutjanidae (snapper), use coastal wetlands as nursery habitats prior to moving offshore to join adult populations on coral reefs. Identifying essential habitats for coral reef fishes and the functional linkages among these habitats necessitates understanding habitat use by juveniles that successfully recruit to adult populations. Analysis of essential AA $\delta^{13}C$ values from otoliths provides a powerful new tool to access archival dietary information from individual fish. Essential AA $\delta^{13}C$ values from otoliths successfully distinguished residence of juvenile snappers in mangroves and seagrass beds where conventional $\delta^{13}C$ and $\delta^{18}O$ analyses in otolith aragonite were inconclusive. The technique opens new doors for reconstructing diet, habitat use, and migration of fishes.

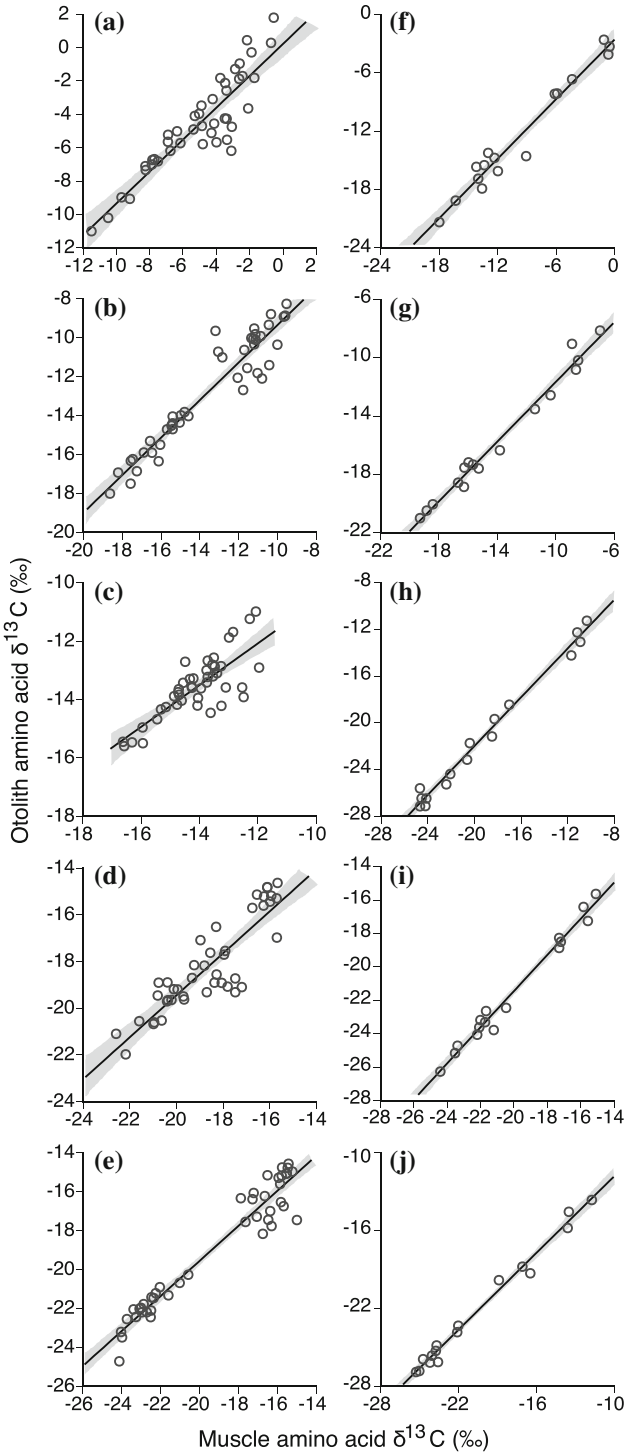

Fig. 5 Linear relationship (*solid line*) of paired otolith and muscle essential amino acid δ^{13}C values from *Lutjanus ehrenbergii* (**a**–**e**) collected from the Red Sea ($n = 43$ fish) and *L. apodus* (**f** –**j**) collected from the Caribbean Sea ($n = 16$ fish): **a** and **f** threonine, **b** and **g** isoleucine, **c** and **h** valine, **d** and **i** phenylalanine, and **e** and **j** leucine. *Shaded areas* represent 95% confidence intervals

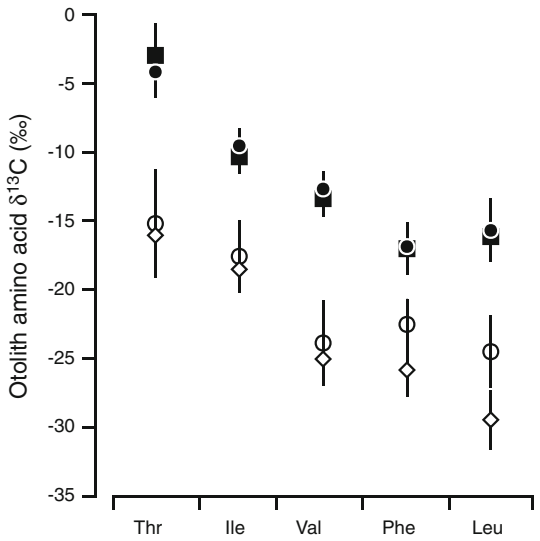

Fig. 6 Essential amino acid δ^{13}C values (mean ± SD) from juvenile *Lutjanus ehrenbergii* collected from seagrass bays (*black square symbols*, n = three sites, 10 fish per site) in the Red Sea, juvenile *L. apodus* collected from seagrass bays (*black circles*, n = four fish) and mangrove lagoons (*white circles*, n = three sites, four fish per site) in the Caribbean Sea, and juvenile *L. argentiventris* collected from mangrove lagoons (*white diamonds*, n = three sites, four fish per site) on the Pacific coast of Panama

This study expanded the relationship between muscle and otolith AA δ^{13}C values first presented by McMahon et al. (2011) to include two species of Lutjanid snapper (*L. ehrenbergii* and *L. apodus*) in mangroves, seagrass beds, coastal reefs, and shelf reefs from the Red Sea and Caribbean Sea. Linear regressions between paired muscle and otolith essential AA δ^{13}C values had a slope near one for both species and geographic regions, indicating that otolith essential AAs provide an excellent tracer of dietary stable isotope values in an archival tissue. The *y*-intercept of this relationship deviated from 0 by approximately 1‰ and showed higher variability than the slopes for both species. The mechanism generating this difference remains unclear; however, it was likely sufficiently small to have few, if any, ecological implications. The fact that a strong relationship between muscle and otolith AA δ^{13}C values exists across multiple species from a variety of habitats suggests that this technique will be useful in a wide range of systems.

Juvenile *L. ehrenbergii* in coastal wetland habitats had muscle essential AA δ^{13}C values that were very different from adult *L. ehrenbergii* on coastal and shelf reefs. The distinction was clear even when comparing *L. ehrenbergii* from Al Lith Bay and Coast Guard Reef, which were only 2 km apart. This is in agreement with earlier research suggesting that while mangroves and adjacent seagrass beds were tightly coupled in terms of particulate organic matter flux, adjacent coral reefs appeared to be relatively isolated from the carbon exchange (Rodelli et al. 1984;

Hemminga et al. 1994). Furthermore, data in our study imply that adult *L. ehrenbergii* do not migrate into the coastal wetlands to feed. Nagelkerken et al. (2008b) showed that grunts on coral reefs near semi-enclosed bays had significantly lower $\delta^{13}C$ values compared to grunts on reefs adjacent to open bays. The authors suggested that the restricted width and depth of channels connecting coral reefs to semi-enclosed bays reduced the likelihood of reef fish entering the seagrass beds compared to open seagrass systems. In the Red Sea, the $\delta^{13}C$ values of essential AAs from fish in semi-enclosed coastal wetland nurseries appeared to be both unique and localized, providing an excellent tracer of residence in wetlands compared to coral reefs.

The unique habitat signatures in coastal wetlands and coral reefs can be traced to the local food web values in those habitats. All juvenile *L. ehrenbergii* samples fell within the detritivore-zooplankton-seagrass canonical space, despite being collected within mangrove prop roots during the day. Mangrove carbon contributed little to the $\delta^{13}C$ values of *L. ehrenbergii* in any of the wetland sites sampled along the coast of Saudi Arabia. These data support previous research on a variety of fish and invertebrate species (Sheaves and Molony 2000; Bouillon et al. 2002; Kieckbusch et al. 2004; Abed-Navandi and Dworschak 2005) indicating the limited role of mangrove carbon as a direct and significant source of carbon for these fauna. It appeared that, like many coastal wetland species, *L. ehrenbergii* in the Red Sea used the sparse fringing mangroves as daytime shelter, but vacated the security of mangroves in favor of more food-rich seagrass beds at night (Rooker and Dennis 1991; Nagelkerken et al. 2000; Dorenbosch et al. 2004; Luo et al. 2009).

While seagrass was an important contributor to the $\delta^{13}C$ value of juvenile *L. ehrenbergii* in the coastal wetlands, fresh seagrass carbon was not the primary carbon source supporting *L. ehrenbergii* production. Bulk tissue SIA suggested that zooplankton and crabs were potentially important dietary components of juvenile *L. ehrenbergii*. However, the relative importance of a water-column-based phytoplankton food web versus a benthic detrital food web was difficult to tease apart with bulk muscle $\delta^{13}C$ and $\delta^{15}N$ values alone. Compound-specific $\delta^{13}C$ analyses, on the other hand, clearly distinguished zooplankton and crab contributions to juvenile *L. ehrenbergii* diets. Detritivorous crabs, or at least food web components with similar $\delta^{13}C$ values, appeared to be the dominant food source for both juvenile and adult *L. ehrenbergii* in this system. Moreover, the compound-specific SIA approach provided access to dietary information from otoliths that was not confounded by DIC dilution and variable metabolic carbon contribution that typically hinder dietary reconstructions using conventional otolith aragonite SIA (McMahon et al. 2011).

The distinction between zooplankton and detritivorous crabs in multivariate space likely represented the impact of microbial processing on the $\delta^{13}C$ value of essential AAs. Microorganisms with the enzymatic capabilities to break down the fibrous, often refractory, components of seagrass and mangrove leaves play a crucial role in making the carbon from these dominant primary producers available to consumers (Zieman et al. 1984). The stable isotope value of valine has provided a valuable tool for assessing contributions from microbially reworked detrital pathways to aquatic food webs (Fogel and Tuross 1999; Keil and Fogel 2001; Ziegler and Fogel 2003; McCarthy et al. 2004). Plants use acetolactetate mutase during the first step in the biosynthesis of valine while bacteria use acetohydroxy acid synthase (Gottschalk 1988; Rawn 1989). As a result, valine synthesized by bacteria shows lower $\delta^{13}C$ values compared to valine produced by plants. Both *L. ehrenbergii* and crabs in the Red Sea had lower valine $\delta^{13}C$ values relative to the other essential AAs on both the coastal and shelf reefs compared to mangroves, seagrasses, and zooplankton. This small difference in AA stable isotope profile resulted in clear separation of microbially mediated carbon pools from new production in canonical space. Thus, it appears that the microbially mediated detrital carbon pool was an important source of carbon for higher trophic levels in these reef systems. The ability to distinguish contributions of new, phytoplankton-based carbon versus microbially recycled detrital carbon to coral reef food webs using compound-specific SIA has potentially far reaching implications for understanding carbon flow in coral reef systems and warrants further investigation.

Essential AA $\delta^{13}C$ values in fish muscle provided an accurate tracer of residence in coastal wetlands and coral reefs with unique food web $\delta^{13}C_{Base}$ signatures. However, due to its rapid turnover rate, particularly in fast-growing juvenile fish, muscle is not an ideal tissue for tracking ontogenetic migration of coral reef fish from juvenile nursery habitats to coral reefs over the potentially long temporal scales of such migrations (Herzka 2005). As a result, previous studies have attempted to use inorganic SIA of otoliths to examine the relative contributions of individuals from mangroves and seagrass beds to coral reefs (Huxham et al. 2007; Verweij et al. 2008; Mateo et al. 2010). We found that bulk otolith $\delta^{13}C$ and $\delta^{18}O$ values differed significantly between seagrass-dominated Red Sea coastal wetlands and the mangrove-dominated sites on the Pacific coast of Panama. This was likely due to regional variability in coastal water mass properties impacting DIC $\delta^{13}C$ and $\delta^{18}O_{H2O}$ values (Dufour et al. 1998). The low $\delta^{13}C$ and $\delta^{18}O$ values of *L. argentiventris* from the Pacific coast of Panama sites were due, in large part, to high freshwater inputs at those sites compared to the evaporative high salinity Red Sea system.

In Puerto Rico and St. Croix, where both mangrove and seagrass nursery habitats were abundant, determining residence patterns using bulk SIA was challenging. Mateo et al. (2010) showed small separations in bulk otolith $\delta^{13}C$ values between *L. apodus* residing in mangroves and seagrass beds for some sites in the Caribbean Sea. However, in our study, $\delta^{13}C$ values from *L. apodus* collected in mangroves and seagrass beds in Puerto Rico and St. Croix were not significantly different. Otolith essential AA $\delta^{13}C$ values, on the other hand, were able to clearly distinguish residence in Caribbean mangroves versus seagrass beds. Otolith essential AAs thus provided a reliable tracer of residence in mangroves and seagrass beds that was regionally and taxonomically robust. Generation of accurate geochemical markers of juvenile nursery residency is crucial for examining the relative contribution of individuals from these habitats that successfully recruit to adult populations on reefs

It would be interesting to compare the AA $\delta^{13}C$ values of juvenile fish otoliths with the outer otolith material of co-occurring adults in the same habitat to see how well different portions of the otolith recorded local environmental signatures. That is not possible with the current data set, as we rarely observed juvenile and adult *L. ehrenbergii* co-occurring at the same site in our study area. However, both juvenile and adult *L. ehrenbergii* relied heavily on detritus-feeding crabs with minimal ontogenetic diet shifts, yet their otolith AA $\delta^{13}C$ values were distinct among habitats, suggesting that otolith AA stable isotope values accurately recorded shifts in $\delta^{13}C_{Base}$ throughout the life of the fish.

The unique isotope values in different habitats illustrated in this study can be used to reconstruct ontogenetic migration pathways and examine functional connectivity between coastal wetlands and coral reefs. McMahon et al. (2011) calculated a minimum sample size of 500–1,000 μg for AA $\delta^{13}C$ analysis of *L. ehrenbergii* otoliths with an organic content of 0.6%. Minimum sample sizes will be considerably smaller for species with otolith organic content that may be as high as 10% depending on the species and life-history stage (Degens et al. 1969; Morales-Nin 1986; Jolivet et al. 2008). Temporal resolution was on the order of several months for *L. ehrenbergii*, but this will similarly vary depending on the size and organic content of otoliths in the study species. The sample size necessary for compound-specific analyses using GC-C-irm-MS may never be as small as those necessary for bulk otolith $\delta^{13}C$ measurements. However, subsampling otoliths will allow for retrospective studies of ontogenetic shifts in diet and habitat use that were not always feasible with conventional bulk otolith SIA. This information is crucial for determining the importance of nursery habitats to coral reef fish populations and can provide valuable scientific support for incorporating connectivity into the design of networked marine-protected areas (Grober-Dunsmore et al. 2007; McCook et al. 2009).

Essential AA $\delta^{13}C$ values from *L. apodus* and *L. argentiventris* residing in mangrove habitats were significantly lower than *L. apodus* and *L. ehrenbergii* from seagrass habitats, regardless of region. These data suggest that mangrove-derived carbon may have contributed more to the carbon pool supporting *Lutjanus spp*. in the Pacific coast of Panama and Caribbean Sea systems than for *L. ehrenbergii* in the Red Sea. The limited extent of fringing mangroves at our Red Sea study sites compared to Puerto Rico, St. Croix, and the Pacific coast of Panama likely explains this difference. Frequent exposure of mangroves during low tide in the Red Sea also reduces the foraging time of *L. ehrenbergii* in mangroves. Lugendo et al. (2007) found that the $\delta^{13}C$ values of fishes from mangrove-lined creeks that retained water during low tide indicated feeding within the mangrove habitat, while fish from fringing mangroves that drain completely during low tide had significantly more positive $\delta^{13}C$ values. While few animals are thought to feed directly on mangrove leaves, mangrove-derived carbon does make up a significant portion of the detrital carbon pool in these systems (Sheaves and Molony 2000; Bouillon et al. 2002). Therefore, juvenile fishes that feed extensively on detritivorous invertebrates and have sufficient foraging time in mangroves, such as along the Pacific coast of Panama and in the Caribbean Sea, will likely exhibit distinct mangrove-influenced stable isotope values. Although there is some notable variability in essential AA $\delta^{13}C$ values across mangrove species and regions (Smallwood et al. 2003), the data in the current study suggest that the differences in AA $\delta^{13}C$ within habitat types are small relative to the differences between mangrove and seagrasses.

This study showed that $\delta^{13}C$ values in essential AAs from otoliths provide an accurate tracer of residence in coastal nurseries. Further, the otolith AA $\delta^{13}C$ approach was better able to distinguish habitat use and $\delta^{13}C_{Base}$ contributions of congeneric snapper species than conventional bulk SIA alone. In this study, we also illustrated the first use of compound-specific SIA in otoliths to distinguish the relative importance of water-column-based new phytoplankton carbon versus microbially recycled detrital carbon to higher trophic-level consumers in a coral reef ecosystem. We found that microbially reworked detrital carbon was an important carbon source supporting snapper production not only in coastal wetlands, but also on offshore coral reefs. While this work is still in its infancy, the potential is great for this technique to shed light on the carbon flow that support ecologically and commercially important fisheries in marine ecosystems.

There are of course limitations to the otolith AA approach. As with any stable isotope study, if juvenile

nursery isotope values are not sufficiently distinct, or if fish move through a habitat faster than they can acquire the local signature, then this technique will underestimate the importance of that habitat. However, in this study, we have demonstrated robust coastal wetland habitat signatures using the newly developed otolith AA SIA technique that should provide a valuable new tool to coral reef scientists and managers interested in understanding and protecting coral reef fish populations. Furthermore, the results from this study are not limited to coral reef ecosystems. Stable isotopes of otolith AAs may be used to assess diet and movement patterns for any system with sufficient isoscape structure at the base of the food web. As instrument sensitivity improves and sample size requirements decrease, we will be able to address questions of diet and movement at higher temporal resolution and on smaller otoliths.

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