Linking habitat mosaics and connectivity in a coral reef seascape

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Tropical marine ecosystems are under mounting anthropogenic pressure from overfishing and habitat destruction, leading to declines in their structure and function on a global scale. Although maintaining connectivity among habitats within a seascape is necessary for preserving population resistance and resilience, quantifying movements of individuals within seascapes remains challenging. Traditional methods of identifying and valuing potential coral reef fish nursery habitats are indirect, often relying on visual surveys of abundance and correlations of size and biomass among habitats. We used compound-specific stable isotope analyses to determine movement patterns of commercially important fish populations within a coral reef seascape. This approach allowed us to quantify the relative contributions of individuals from inshore nurseries to reef populations and identify migration corridors among important habitats. Our results provided direct measurements of remarkable migrations by juvenile snapper of over 30 km, between nurseries and reefs. We also found significant plasticity in juvenile nursery residency. Although a majority of individuals on coastal reefs had used seagrass nurseries as juveniles, many adults on oceanic reefs had settled directly into reef habitats. Moreover, seascape configuration played a critical but heretofore unrecognized role in determining connectivity among habitats. Finally, our approach provides key quantitative data necessary to estimate the value of distinctive habitats to ecosystem services provided by seascapes.

amino acid | *Lutjanus ehrenbergii* | mangroves and seagrass | otoliths | Red Sea

The ecological integrity of tropical marine habitats, including mangroves, seagrass beds, and coral reefs, is coming under increasing pressure from human activities (1–3). Habitat destruction and unsustainable exploitation, including mangrove deforestation and overfishing, have led to declines in the function and resilience of these ecosystems on a global scale (4). Efforts to promote ecological integrity and sustainable harvest have traditionally focused on protecting coral reefs. More recently, attention has been directed at the issue of preserving critical seascape functions as well as habitat types, with particular emphasis on seascape connectivity (5). For example, many commercially and ecologically important coral reef fishes, including species of Lutjanidae (snappers), Serranidae (grouper), and Scaridae (parrotfish), use mangroves and seagrass beds as juvenile nursery areas before presumably migrating to coral reef habitats as adults (see reviews in refs. 6–8). Preserving seascape connectivity is therefore likely necessary to maintain coral reef ecosystem function and healthy fisheries (9). However, it has proved remarkably difficult to develop quantitative assessments of habitat use and movements among different habitat types for any reef fish species (10). This lack of quantitative data on seascape connectivity represents a major obstacle to marine spatial management (5) and attempts to value ecosystems services provided by coral reef habitats (11–13).

A number of studies have demonstrated a strong relationship between the presence of coastal wetlands and offshore fish abundance and fisheries yield (14, 15). These studies formed the basis for the nursery hypothesis (6–8), and subsequently, the economic valuation of coastal wetlands (13). The use of coastal wetlands as nursery habitats may, however, be facultative and spatially complex (16). Studies identifying mangroves and seagrass beds as nurseries have noted higher densities of juvenile fishes in those habitats relative to other habitats where juveniles could reside (16, 17), and have documented size-frequency differences among habitats that are consistent with ontogenetic movements of juvenile fishes from mangrove nurseries to adult reef habitats (14, 15). The conclusions of these studies rely, nonetheless, on the assumption that the increased density of juveniles in nursery habitats will result in increased recruitment into adult populations on coral reefs. To accurately parameterize reserve selection models for the development of effective marine reserves (12), we need to identify specific migration corridors between nursery habitats and reef environs.

Determining movement corridors between juvenile and adult habitats requires the ability to either track individuals between habitats or to retrospectively identify juvenile habitat residency of adult fishes. Natural geochemical tags provide an approach that allows for the reconstruction of habitat residency while avoiding the logistic problems inherent with artificial tagging (10). We recently described a unique method for quantifying fish movements in coral reef ecosystems by analyzing amino acid $\delta^{13}C$ values in otoliths (ear-bones) (18–20). The technique relies on natural geographic variations in $\delta^{13}C$ at the base of food webs among mangrove habitats, coral reefs, and seagrass beds that are permanently recorded by otolith amino acids. Compound-specific stable isotope analysis (SIA) provides more robust tracers of residency bulk SIA and trace element geochemistry, which have met with mixed results in previous attempts to reconstruct nursery use in coral reef fishes (20).

Here we use amino acid $\delta^{13}C$ values to quantify seascape connectivity for a commercially important snapper species (Ehrenberg's snapper, *Lutjanus ehrenbergii*, Peters 1869) in a coral reef ecosystem from the Red Sea (Fig. 1). Our approach allows for reconstruction of juvenile habitat associations by those fish that have successfully recruited to adult populations on reefs. We characterized unique $\delta^{13}C$ signatures from habitats within the study seascape by analyzing five essential amino acid $\delta^{13}C$ values from *L. ehrenbergii* collected from five potential juvenile habitats: coastal wetlands consisting of seagrass bays with fringing mangroves, coastal reefs within 2 km of shore, shelf reefs on the continental shelf, the continental island of Abu Latt at the shelf break, and oceanic reefs surrounded by deep open water (Fig. S1). We then surveyed densities of *L. ehrenbergii* and collected fish for otolith analysis from two replicate reefs at six distances along a

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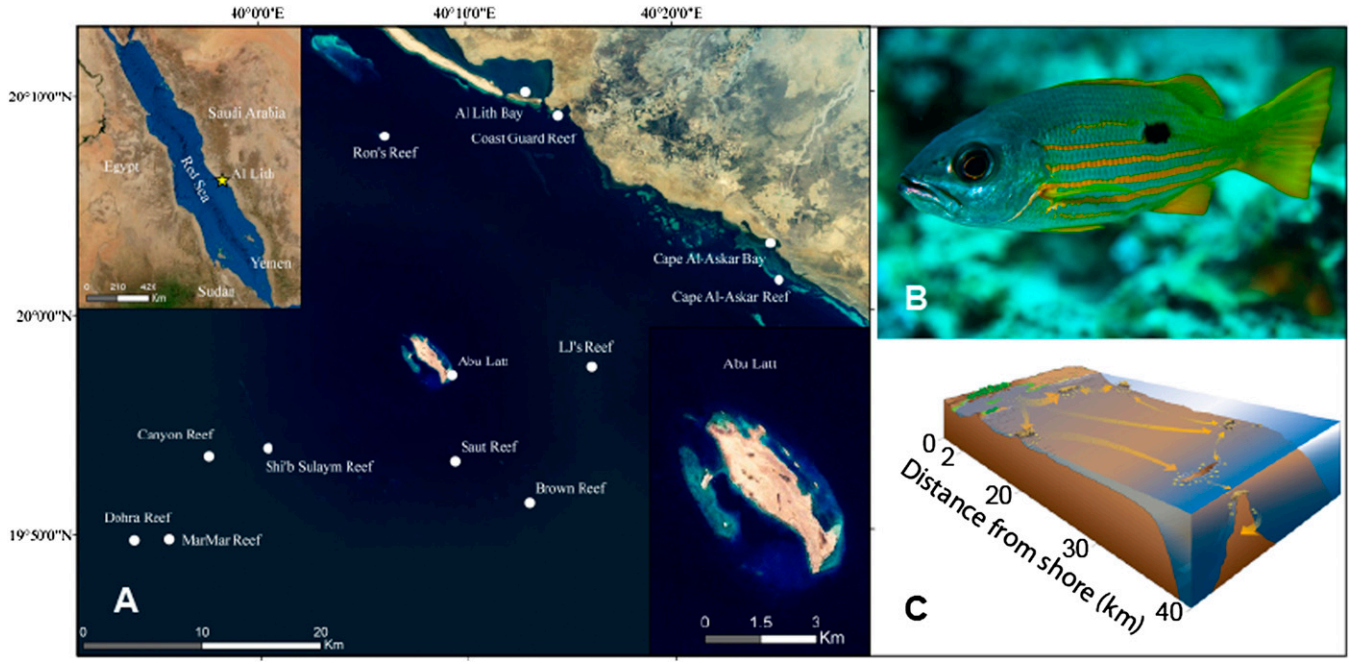

Fig. 1. Study site and species. (*A*) Collection sites from coastal wetlands (Al Lith Bay and Cape Al-Askar Bay), coastal reefs (Coast Guard Reef and Cape Al Askar Reef), shelf reefs (Ron's Reef, LJ's Reef, Saut Reef, and Brown Reef), a continental island (Abu Latt), and oceanic reefs (Shi'b Sulaym Reef, Canyon Reef, MarMar Reef, and Dohra Reef) near Al Lith, Saudi Arabia in the Red Sea. (*B*) Ehrenberg's snapper (*Lutjanus ehrenbergii*, Peters 1869) is a commercially important reef-associated snapper species in the Indo-West Pacific. (*C*) Conceptual diagram of habitat configuration and potential seascape connectivity of *L. ehrenbergii* in the study area.

50-km cross-shelf transect from the coast to oceanic reefs off the continental shelf. Finally, we isolated the juvenile cores from adult *L. ehrenbergii* otoliths, analyzed their essential amino acid $\delta^{13}C$ values, and then classified fish to one of the five potential juvenile habitats based on these multivariate isotope values (see *SI Materials and Methods*). The multivariate approach allowed us to accurately distinguish residence patterns among source habitats that were not possible using conventional bulk stable isotope analysis (20).

Results

We found significant variability in *L. ehrenbergii* densities across the continental shelf (Fig. 2). Highest densities were found on nearshore reefs and on the fringing reef surrounding the continental island of Abu Latt. These patterns were consistent with our observations of recently settled juveniles in mangrove and seagrass habitats along the coast and in the lagoon at Abu Latt (*SI Materials and Methods*). We have, however, never seen juvenile *L. ehrenbergii* on coastal, shelf, or oceanic reefs despite several years of regular work in this area. Moreover, the sharp drop in densities of adult *L. ehrenbergii* from nearshore reefs and fringing reefs around Abu Latt Island to shelf and oceanic reefs suggested that the majority of juveniles were moving relatively short distances (~2 km) from juvenile nursery habitats.

Discriminant function analysis on the muscle essential amino acid $\delta^{13}C$ data of *L. ehrenbergii* showed that each of the five regions was clearly separated in multivariate space (Fig. 3). The first discriminant function identified a gradient from coastal wetlands to oceanic reefs, and the second discriminant function separated coastal wetlands from the shelf island habitat of Abu Latt Island. Moreover, we were able to assign individuals to each of these habitats with a high degree of accuracy based on the multivariate essential amino acid $\delta^{13}C$ values. Jackknifed reclassification success rate to each potential juvenile habitat averaged 95% compared with a random reclassification success expectation of 20%.

Essential amino acid $\delta^{13}C$ values in otoliths revealed a complex pattern of habitat use by juvenile *L. ehrenbergii* (Fig. 4). Our data also showed that many *L. ehrenbergii* larvae had apparently settled directly into adult reef habitats. Although we never saw juvenile *L. ehrenbergii* on offshore reefs, as much as 50% of the adults on coastal and shelf reefs and nearly 80% of adults on oceanic reefs had resided in these habitats for their entire postsettlement lives. These juveniles were likely either highly cryptic, residing inside the reef matrix during daylight hours, or

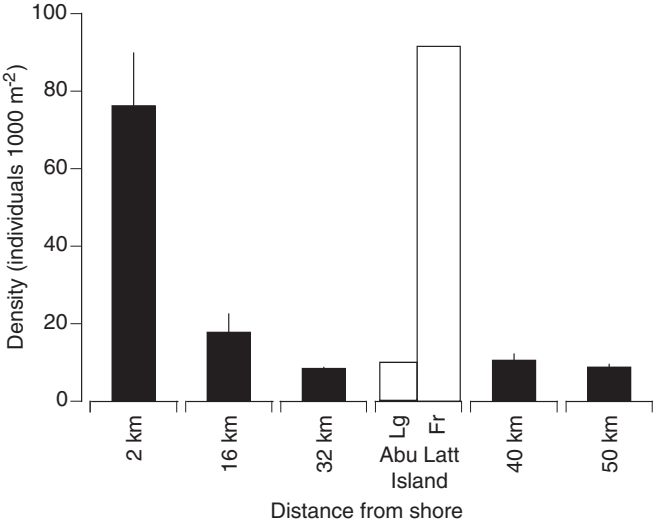

Fig. 2. Underwater visual census estimates. Adult *L. ehrenbergii* densities (mean ± SD) on reefs at five distances offshore ($n = 2$ reefs per distance) and two habitats at Abu Latt Island (24-km offshore). Fr, fringing reef habitat; Lg, lagoon habitat.

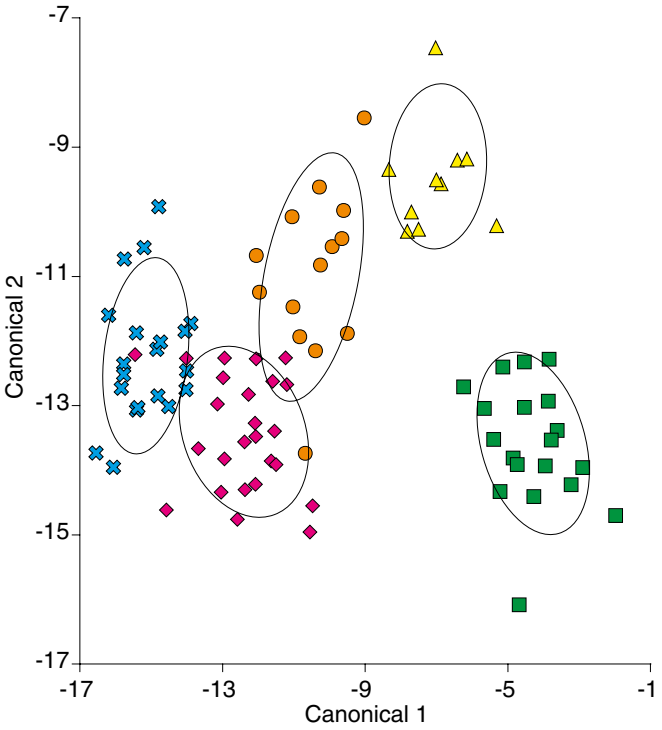

Fig. 3. Discrimination of juvenile *Lutjanus ehrenbergii* habitats based on $\delta^{13}C$ values of essential amino acids. Multivariate separation of habitats visualized after discriminant function analysis of five essential amino acid $\delta^{13}C$ values from *L. ehrenbergii* collected from five potential juvenile habitats: coastal wetlands (green squares: $n = 19$ fish), coastal reefs (orange circles: $n = 15$), shelf reefs (magenta diamonds: $n = 25$), Abu Latt Island lagoon and fringing reefs (yellow triangles: $n = 10$), and oceanic reefs (cyan crosses: $n = 20$). Colored symbols represent individual fish surrounded by 95% confidence ellipses.

inhabiting depths that were beyond the limits of open-circuit SCUBA equipment. Regardless of their whereabouts, the otolith amino acid technique allowed us to definitively quantify the proportion of each adult population that had resided in different nursery habitats as juveniles.

Our results confirmed the importance of mangrove and seagrass systems to inshore fish populations. Over 70% and 45% of adult *L. ehrenbergii* at the 2-km and 16-km reefs, respectively, had migrated from these coastal wetland habitats as juveniles. A number of individuals had also moved at least 30 km from inshore nurseries to reefs on the edge of the continental shelf. The shelf break did, however, act as a barrier for inshore juveniles because no adults on oceanic reefs beyond the continental shelf had resided in mangrove or seagrass environments.

Discussion

Our results provided direct measurements of remarkable movements by juvenile snapper from coastal wetlands to coral reefs at least 30 km from the coast, and from a shelf island to oceanic reefs across deep open water. Although connectivity was high among coastal wetland and reef environs on the shallow continental shelf, we found no evidence of wetland use in adults from oceanic reefs. Juveniles from near shore areas were apparently reluctant to move beyond the continental shelf. However, juveniles that settled around Abu Latt Island, on the shelf edge, were able to swim across deep open water to the oceanic reefs. These results reveal complex patterns of ontogenetic movement that we were unable to detect using conventional SCUBA-based surveys. We were able to quantify the relative contributions from each nursery habitat to adult populations and to identify specific corridors used by

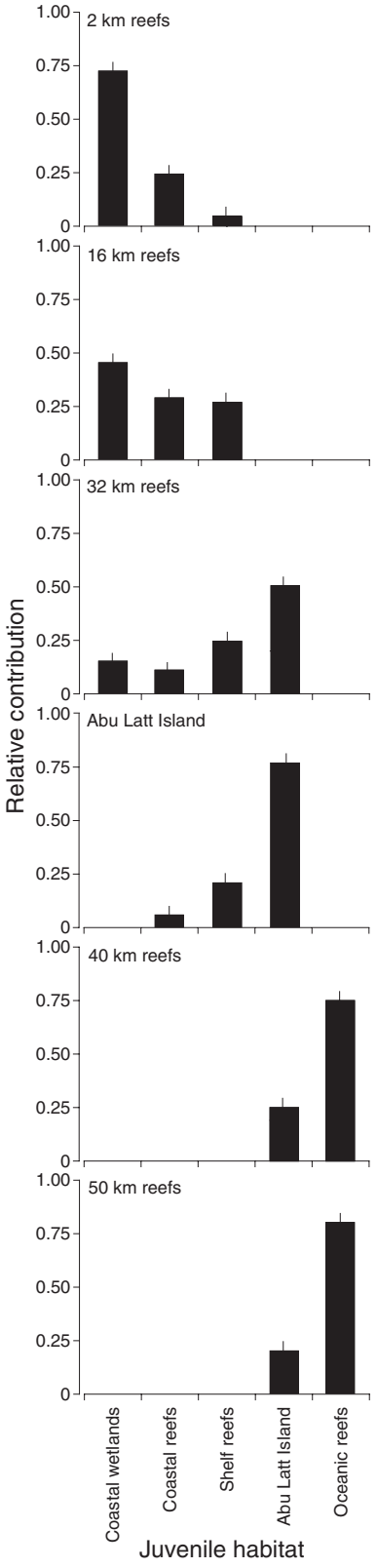

Fig. 4. Relative contribution (mean ± SD) of *L. ehrenbergii* from five potential juvenile habitats to adult populations on offshore coral reefs. Adult *L. ehrenbergii* were collected from reefs at six distances from the coast along a 50-km cross-shelf transect from Al Lith, Saudi Arabia in the Red Sea (2-km reefs, $n = 25$ fish; 16-km reefs, $n = 20$; 32-km reefs $n = 20$; Abu Latt Island $n = 20$; 40-km reefs $n = 20$; and 50-km reefs $n = 20$) and classified to one of five potential juvenile nursery habitats by otolith essential amino acid $\delta^{13}C$ values.

juvenile fish to migrate across the shelf to reef environments. These data are, in turn, critical to parameterize reserve selection algorithms for the development of effective networked marine reserves (12, 21).

Compound-specific SIA data revealed a high degree of plasticity in nursery habitat use. These findings have important implications, both for understanding coral reef fish population biology as well as designing well-informed management strategies. Coastal and shelf reefs appeared to have greater functional connectivity within the seascape than the oceanic reefs. At least three different juvenile source habitats contributed to adult *L. ehrenbergii* populations on coastal and shelf reefs. Conversely, the oceanic reefs were primarily locally recruiting. Coastal and shelf-reef habitats may, therefore, have a greater source redundancy and thus be less vulnerable to fluctuations in juvenile supply from individual habitats. It appears likely that the shallow continental shelf, typically less than 50-m deep, facilitated enhanced interreef movement compared with the deep open water between oceanic reefs. The shelf break was not a hard barrier, however, because juveniles from Abu Latt Island, located on the edge of the continental shelf, were able to move across open waters to oceanic reefs.

There is little movement data on juvenile coral reef fishes to compare with our results because of the difficulties associated with tagging small fish (10). Mumby (21) constrained the maximum distance fish migrate between mangroves and reefs in their reserve selection algorithm to 10 km based upon the maximum distance between offshore mangrove cays and reef sites in Belize. Acoustic tracking of adult coral reef fishes has revealed within-reef migrations to spawning aggregation sites over distances of up to 20 km (22), and interreef movements of up to 16 km (23). The fact that significant numbers of juvenile *L. ehrenbergii* were migrating up to 30 km among reefs on the continental shelf and across oceanic waters beyond the shelf break highlights how little we know about seascape connectivity of tropical marine fishes (24).

We used a direct method to identify juvenile nurseries that retrospectively determined habitat use during juvenile stages of adult fish on reefs. The approach allowed us to quantify relative contributions of individuals from nursery habitats to reef populations, and to categorize additional important juvenile habitats that we had been unable to adequately identify using conventional techniques. For example, individuals that settled directly onto reefs contributed at least 70% to *L. ehrenbergii* populations on oceanic reefs. However, reefs with the highest connectivity to coastal wetlands also had the highest adult *L. ehrenbergii* densities. Densities of adult *L. ehrenbergii* on coastal reefs were fourfold higher than those on the outer shelf and oceanic reefs. This correlation supports previous studies showing higher adult abundance of fishes on reefs closer to nursery sources (14, 15, 25). However, we were able to demonstrate that a higher proportion of individuals on coastal reefs had indeed resided in mangrove and seagrass nurseries before moving out to adult habitats compared with populations on reefs further offshore.

Our description of juvenile coral reef fish movements represents a unique direct estimation of seascape connectivity for any reef fish species. The functioning and resilience of coral reefs and the fisheries they support are directly linked to connectivity, both by dispersal and ontogenetic movement, within tropical seascapes (26). The ability to quantify the contributions of different nurseries to reef fish populations and identify important migration corridors is critical to identify management priorities (5) and parameterize models of habitat value (11, 12) and metapopulation persistence (27). Our results are particularly timely given the increasing use of spatial management approaches, including networks of marine protected areas in coral reef ecosystems (21, 28, 29). Although at least some of these efforts, including the recent rezoning of the Great Barrier Reef Marine Park, have explicitly recognized the importance of maintaining links among habitats (30), zoning decisions have necessarily been based on imprecise rules of thumb rather than empirical data on seascape connectivity (5). More time is needed before the effectiveness of these rules can be evaluated. Nonetheless, the lack of a mechanistic understanding of the role that seascape configuration plays in determining connectivity significantly hinders the ability to predict the influence of extrinsic factors, including climate change on reef fish populations (31). It is clear, however, that to effectively maintain functioning ecosystems and sustainable fisheries in structurally complex ocean ecosystems, management plans must conserve the functional integrity of ecosystems at the seascape level rather than focusing solely on individual habitat types. More generally, our approach provides a quantitative method for estimating the value of ecosystem services provided by distinctive habitats to fisheries yields within a seascape (11–13). This method will, in turn, allow for more accurate accounting of these services, including the assessment of suitable remediation requirements when these habitats are removed during tourism or aquaculture developments.

Materials and Methods

Ehrenberg's snapper, *Lutjanus ehrenbergii* (Peters 1869), were collected from five distinct habitats: (*i*) coastal wetlands ($n = 2$ sites), (*ii*) coastal reefs ($n = 2$), (*iii*) shelf reefs ($n = 4$), (*iv*) offshore island patch reefs ($n = 1$), and (*v*) oceanic reefs ($n = 4$), along a 50-km cross-shelf transect from coastal Saudi Arabia in the Red Sea in November 2008, March 2009, and June 2010 (Fig. 1 and Fig. S2). Densities of *L. ehrenbergii* were estimated by visual survey on SCUBA. Individual fish were counted along four replicate 100-m by 10-m transects at 5- and 15-m depth from each reef and then averaged per distance. We visualized the separation of potential juvenile habitats using a quadratic discriminant function analysis (32) on the muscle essential amino acid $\delta^{13}C$ data of *L. ehrenbergii* grouped into five regions according to their collection location across the continental shelf. See Table S1 for variance and loadings of quadratic discriminant function analysis on juvenile snapper habitat signatures. Briefly, total free amino acids were isolated by acid hydrolysis and then converted to isopropyl-TFAA derivatives (19), before individual isotopic analysis on an Agilent 6890N gas chromatograph coupled via continuous flow interface to a Thermo Finnigan Mat 253 isotope ratio monitoring-mass spectrometer (see *SI Materials and Methods*).

To retrospectively identify where each adult *L. ehrenbergii* spent its juvenile period, we isolated the juvenile core of adult *L. ehrenbergii* otoliths (see *SI Materials and Methods* and Fig. S3) from fish collected on reefs at six distances offshore along a 50-km cross-shelf transect (2 km, 16 km, 32 km, Abu Latt Island, 40 km, and 50 km). We analyzed the $\delta^{13}C$ values of the same five essential amino acids as used to develop the nursery habitat signatures described above (Fig. S4). We used a maximum-likelihood estimator (33) to classify the juvenile cores of adult otoliths to one of the five potential nursery habitats to calculate the relative contribution of each of the five potential juvenile habitat regions to the adult populations on coral reefs at six distances along the 50-km cross-shelf transect from Al Lith, Saudi Arabia. For more details on the amino acid $\delta^{13}C$ analyses and data processing, please see the *SI Materials and Methods*. Raw data are available in McMahon (34).

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Supporting Information

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SI Materials and Methods

Field Collections. Ehrenberg's snapper, *Lutjanus ehrenbergii* (Peters 1869), were collected from five distinct habitats: (*i*) coastal wetlands, (*ii*) coastal reefs, (*iii*) shelf reefs, (*iv*) offshore island patch reefs, and (*v*) oceanic reefs, along a 50-km cross-shelf transect from coastal Saudi Arabia in the Red Sea in November 2008, March 2009, and June 2010 (Fig. 1). Al Lith Bay and Cape Al-Askar Bay are shallow, semienclosed bays that are dominated by ribbon seagrass, *Halodule uninervis* (Forsk.), with fringing white mangroves, *Avicennia marina* (Forsk.). The offshore island, Abu Latt Island, is a partially vegetated island located ~24 km offshore at the edge of the continental shelf that is fringed by patch reefs and seagrass-lined channels. The oceanic reefs are primarily steep vertical walls surrounded by open water greater than 300-m deep (Fig. S1). Juvenile *L. ehrenbergii* [total length (TL) = 75 ± 11 mm) (Fig. S2) were collected with cast nets from two coastal wetland systems near Al Lith, Saudi Arabia. Adult *L. ehrenbergii* (TL = 195 ± 32 mm) (Fig. S2) were collected with spearguns from 11 reef systems at six distances along the 50-km cross-shelf transect near Al Lith, Saudi Arabia: (*i*) coastal reefs within 2 km of shore: Coast Guard Reef and Cape Al-Askar Reef; (*ii*) shelf reefs 16-km offshore: Ron's Reef and LJ's Reef; (*iii*) an offshore island 24-km offshore: Abu Latt Island; (*iv*) shelf reefs 32-km offshore: Saut Reef and Brown Reef; (*v*) oceanic reefs 40-km offshore: Canyon Reef and Shi'b Sulaym Reef; and (*vi*) oceanic reefs 50-km offshore: MarMar Reef and Dohra Reef.

Sagittal otoliths and white muscle tissue were dissected from each fish in the field. 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 were frozen on the boat before transport to an onshore laboratory. In the laboratory, white muscle 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 for further preparation and analysis. Muscle tissue from *L. ehrenbergii* at each site was used to identify local habitat signatures because muscle has a very fast turnover rate and its isotopic signature represented the most recent residence signature. We did not find any juvenile *L. ehrenbergii* on offshore coral reefs; however, we wanted to know the potential contribution of individuals from these coral reefs to the adult population. Therefore, muscle samples from adult *L. ehrenbergii* were used to characterize the habitat signatures of the offshore reefs where no juveniles were collected. We justified this in two ways. Despite a large range in TL across juvenile and adult *L. ehrenbergii* in this study, there was no significant trend in muscle $\delta^{15}N$ values with TL ($y = 0.004x + 8.04$, $R^2 = 0.15$) (Fig. S2). This finding indicates that juvenile and adult *L. ehrenbergii* were feeding at the same trophic level. Thus, we are confident that adult muscle signatures provided an accurate reflection of the values we would find for juvenile muscle in the same habitat.

Sample Preparation and Analysis. Approximately 1 mg of freeze-dried, homogenized white muscle tissue from each fish was weighed into a tin cup and analyzed for bulk $\delta^{15}N$ with a Europa Hydra 20/20 isotope ratio monitoring-mass spectrometer (irm-MS) at the University of California at Davis Stable Isotope Facility. A second portion of each muscle sample (~1 mg) was acid hydrolyzed to isolate free amino acids by refluxing samples in 6N HCl at 110 °C for 20 h, neutralizing in ultrapure water and evaporating to dryness under a gentle stream of N_2 gas. These samples were used to characterize the geochemical signature of the five juvenile habitats (discussed below). To retrospectively identify where each adult *L. ehrenbergii* spent its juvenile period, we isolated the juvenile core of adult *L. ehrenbergii* otoliths (Fig. S3) from fish collected on reefs at six distances offshore along a 50-km cross-shelf transect. A single, randomly selected, sagittal otolith from each adult *L. ehrenbergii* was scrubbed and rinsed in ultrapure water, cleaned ultrasonically for 5 min in ultrapure water, and then air-dried under a class-100 positive-flow fume hood for 24 h. We then isolated a core from each adult otolith, representing the first year of growth. To do this, we cut along the first annulus using a Buehler Isomet Low Speed Saw with a diamond wafering blade (Buehler) and then ground down the resulting core from the top and bottom with a Buehler Ecomet3 variable speed grinder-polisher to remove postfirst year material deposited in the vertical plane. Next, we contoured the shape of the juvenile core to match the mean 3D shape (4–5 mm by 2–3 mm) and mass (10–15 mg) of otoliths from juvenile *L. ehrenbergii* (TL ~75 mm) collected in the coastal wetlands using a Buehler Ecomet3 variable speed grinder-polisher. Each juvenile core was homogenized with a mortar and pestle and acid hydrolyzed in the same manner as the muscle samples.

Acid-hydrolyzed samples were derivatized before stable isotope analysis according to McMahon et al. (1). Samples were brought up in dichloromethane (DCM) and injected on the column in splitless mode at 260 °C and separated on a forte SolGel-1ms column (60-m length, 0.25-mm inner diameter, and 0.25-μm film thickness; SGE Analytical Science) in a Agilent 6890N Gas Chromatograph (GC) at the Woods Hole Oceanographic Institution, Woods Hole, MA. The separated amino acid peaks were combusted online in a Finnigan gas chromatography-combustion (GC-C) continuous-flow interface at 1,030 °C, then measured as CO_2 on a Thermo Finnigan Mat 253 irm-MS. Standardization of runs was achieved using intermittent pulses of a CO_2 reference gas of known isotopic composition. All compound-specific stable isotope analysis samples were analyzed in duplicate along with amino acid standards of known isotopic composition. We focused on five essential amino acids with sufficient peak size and baseline GC separation: threonine, isoleucine, valine, phenylalanine, and leucine (Fig. S4).

Data Analysis. Stable isotope ratios were expressed in standard δ notation:

$$\delta^{13}C_{sample} = \left(\frac{{}^{13}C/{}^{12}C_{sample}}{{}^{13}C/{}^{12}C_{std}} - 1 \right) \times 1000, \qquad \textbf{[S1]}$$

where the standard for carbon was Vienna Peedee Belemnite. Differences in total length of *L. ehrenbergii* among the five potential juvenile habitat regions were assessed using a one-way ANOVA, with Tukey's honestly significant difference post hoc test ($\alpha < 0.05$). The relationship between TL and bulk muscle $\delta^{15}N$ values was determined by linear regression. We visualized the separation of potential juvenile habitats using a quadratic discriminant function analysis on the muscle essential amino acid $\delta^{13}C$ data of *L. ehrenbergii* grouped into five regions according to their collection location across the continental shelf. These regions were as follows: coastal wetlands ($n = 2$ sites), coastal reefs ($n = 2$), shelf reefs ($n = 4$), Abu Latt Island ($n = 1$), and oceanic reefs ($n = 4$). The first and second canonical variables accounted for 96% of the total variance in canonical space (Table S1). The jackknife reclassification success rate of the discriminant func-

tion analysis was evaluated by leave-one-out cross-validation and compared with the $1/g$ reclassification success expectation, where g was the number of groups analyzed (2). We used a maximum-likelihood estimator (3) to calculate the relative contribution of each of the five potential juvenile habitat regions to the adult populations on coral reefs at six distances (2 km, 16 km, 32 km, Abu Latt Island, 40 km, and 50 km) along the 50-km cross-shelf transect from Al Lith, Saudi Arabia. McMahon et al. (4) showed that muscle and otolith essential amino acid $\delta^{13}C$ values had a consistent 1:1 correlation and could be used interchangeably. Thus, the training dataset was comprised of muscle essential amino acid $\delta^{13}C$ data from each potential juvenile habitat region. The otolith essential amino acid $\delta^{13}C$ data from juvenile cores of adult *L. ehrenbergii* were treated as unknowns to be classified by the training dataset. We identified juvenile nurseries as any juvenile habitat that contributed more than the average if all five juvenile habitats had contributed to the adult population evenly (20%).

1. McMahon KW, Fogel ML, Johnson BJ, Houghton LA, Thorrold SR (2011) A new method to reconstruct fish diet and movement patterns from $\delta^{13}C$ values in otolith amino acids. *Can J Fish Aquat Sci* 68:1330–1340.
2. White JW, Ruttenberg BI (2007) Discriminant function analysis in marine ecology: Oversights and their solutions. *Mar Ecol Prog Ser* 329:301–305.
3. Millar RB (1990) A versatile computer program of mixed stock fishery composition estimation. *Can Tech Rep Fish Aquat Sci* 1753:29–38.
4. McMahon KW, Berumen ML, Mateo I, Elsdon TS, Thorrold SR (2011) Carbon isotopes in otolith amino acids identify residency of juvenile snapper (Family: Lutjanidae) in coastal nurseries. *Coral Reefs* 30:1135–1145.

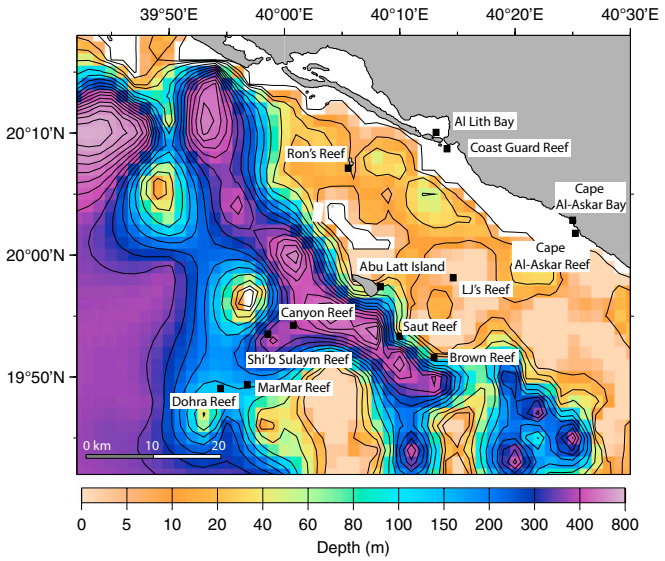

Fig. S1. Study site bathymetry map. Color contours represent one arc-minute gridded bathymetry data for the study region, with gray representing land and white indicating no data (General Bathymetric Chart of the Oceans: http://www.gebco.net/data_and_products/gridded_bathymetry_data/). The continental shelf is consistently shallow (<60 m deep), and the bottom depth increases rapidly at the shelf break to nearly 800 m. Oceanic reefs are surrounded by deep open water.

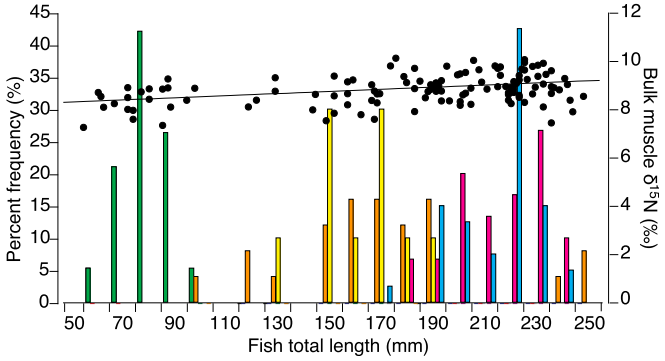

Fig. S2. Frequency distribution of total length (in millimeters; left y axis). *L. ehrenbergii* were collected from five potential juvenile habitats: coastal wetlands (green bars: $n = 19$ fish), coastal reefs (orange bars: $n = 25$), shelf reefs (magenta bars: $n = 40$), Abu Latt Island lagoon and fringing reefs (yellow bars: $n = 10$), and oceanic reefs (cyan bars: $n = 40$) in the Red Sea. Superimposed on the length distribution data are bulk muscle $\delta^{15}N$ values of *L. ehrenbergii* in relation to total length (gray circles; right y axis) ($n = 125$ fish) (black line: $y = 0.004x + 8.04$, $R^2 = 0.15$).

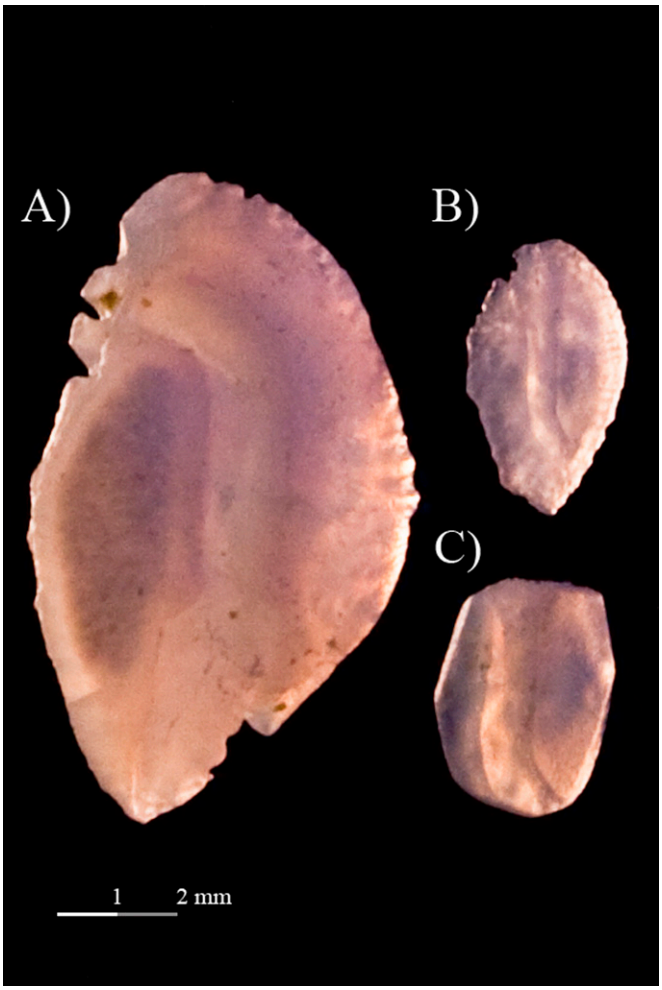

Fig. S3. Otolith preparation diagram. (*A*) The otolith of an adult *L. ehrenbergii* (TL = 230 mm) measuring 9.6 mm by 5.6 mm and weighing 125 mg. (*B*) A juvenile *L. ehrenbergii* otolith (TL = 75 mm) measuring 4.1 mm by 2.4 mm and weighing 8 mg. (*C*) The juvenile core isolated from the adult otolith and contoured to match the mean size and mass of otoliths from juvenile *L. ehrenbergii* (fish TL ~75 mm).

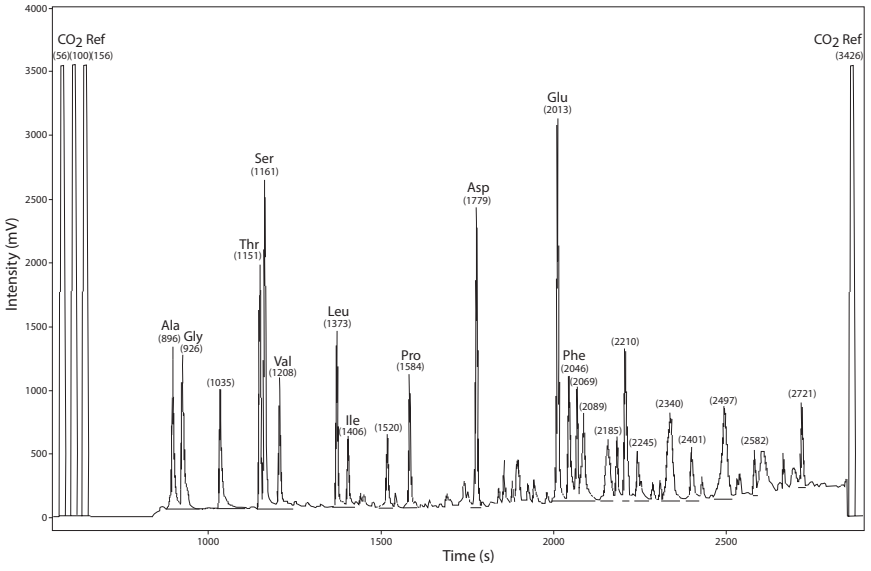

Fig. S4. Otolith amino acid gas chromatogram. A representative gas chromatogram of derivatized individual amino acids from an otolith of *L. ehrenbergii*. Ala, alanine; Asp, aspartic acid; CO_2 ref, Intermittent pulses of a CO_2 gas reference of known isotopic composition; Glu, glutamic acid; Gly, glycine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Val, valine. [Reproduced from McMahon et al. (1)].

Table S1. Variance and loadings of quadratic discriminant function analysis on juvenile snapper habitat signatures

Amino acid	Canonical 1	Canonical 2
Threonine	0.64	−0.02
Isoleucine	0.35	−1.02
Valine	−0.96	1.73
Phenylalanine	−0.21	−0.17
Leucine	0.87	0.32

Almost all of the variation in $\delta^{13}C$ values of five essential amino acids in *L. ehrenbergii* muscle was captured in the first two canonical variables: canonical 1 = 85% and canonical 2 = 11%. The first canonical variable identified a gradient from coastal wetlands to oceanic reefs, and the second canonical variable separated coastal wetlands from the shelf island habitat of Abu Latt.