Linking habitat mosaics and connectivity in a coral reef seascape

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Abstract

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. While 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. While 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.

Introduction

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 instance, 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 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. In order 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 (AA) $\delta^{13}C$ 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 AAs. Compound-specific SIA provides more robust tracers of residency bulk stable isotope analysis (SIA) and trace element geochemistry, which have met with mixed results in previous attempts to

89 reconstruct nursery use in coral reef fishes (20).

90 Here we use AA $\delta^{13}C$ values to quantify seascape connectivity for a commercially
91 important snapper species (Ehrenberg's snapper, *Lutjanus ehrenbergii*, Peters 1869) in a
92 coral reef ecosystem from the Red Sea (Fig. 1). Our approach allows for reconstruction of
93 juvenile habitat associations by those fish that have successfully recruited to adult
94 populations on reefs. We characterized unique $\delta^{13}C$ signatures from habitats within the
95 study seascape by analyzing five essential AA $\delta^{13}C$ values from *L. ehrenbergii* collected
96 from five potential juvenile habitats: coastal wetlands consisting of seagrass bays with
97 fringing mangroves, coastal reefs within 2 km of shore, shelf reefs on the continental
98 shelf, the continental island of Abu Latt at the shelf break, and oceanic reefs surrounded
99 by deep open water (Fig. S1). We then surveyed densities of *L. ehrenbergii* and collected
100 fish for otolith analysis from two replicate reefs at six distances along a 50 km cross-shelf
101 transect from the coast to oceanic reefs off the continental shelf. Finally, we isolated the
102 juvenile cores from adult *L. ehrenbergii* otoliths, analyzed their essential AA $\delta^{13}C$ values,
103 and then classified fish to one of the five potential juvenile habitats based on these
104 multivariate isotope values (see SI text). The multivariate approach allowed us to
105 accurately distinguish residence patterns among source habitats that were not possible
106 using conventional bulk stable isotope analysis (20).
107
108 **Results**
109 We found significant variability in *L. ehrenbergii* densities across the continental
110 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 (see SI text). 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 AA $\delta^{13}C$ 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, while 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 AA $\delta^{13}C$ values. Jackknifed reclassification success rate to each potential juvenile habitat averaged 95% compared to a random reclassification success expectation of 20%.

Essential AA $\delta^{13}C$ 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 post-

settlement lives. These juveniles were likely either highly cryptic, residing inside the reef matrix during daylight hours, or inhabiting depths that were beyond the limits of open circuit SCUBA equipment. Regardless of their whereabouts, the otolith AA 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 as 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. While 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 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 inter-reef movement compared to the deep open water between oceanic reefs. The shelf break was not a hard barrier, however, as 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 due to 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

177 mangrove cays and reef sites in Belize. Acoustic tracking of adult coral reef fishes has
178 revealed within-reef migrations to spawning aggregation sites over distances of up to 20
179 km (22), and inter-reef movements of up to 16 km (23). The fact that significant numbers
180 of juvenile *L. ehrenbergii* were migrating up to 30 km among reefs on the continental
181 shelf and across oceanic waters beyond the shelf break highlights how little we know
182 about seascape connectivity of tropical marine fishes (24).

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

197 Our description of juvenile coral reef fish movements represents a unique direct
198 estimation of seascape connectivity for any reef fish species. The functioning and

199 resilience of coral reefs and the fisheries they support are directly linked to connectivity,
200 both by dispersal and ontogenetic movement, within tropical seascapes (26). The ability
201 to quantify the contributions of different nurseries to reef fish populations and identify
202 important migration corridors is critical to identify management priorities (5) and
203 parameterize models of habitat value (11-12) and metapopulation persistence (27). Our
204 results are particularly timely given the increasing use of spatial management approaches,
205 including networks of marine protected areas, in coral reef ecosystems (21, 28-29). While
206 at least some of these efforts, including the recent rezoning of the Great Barrier Reef
207 Marine Park, have explicitly recognized the importance of maintaining links among
208 habitats (30), zoning decisions have necessarily been based on imprecise rules of thumb
209 rather than empirical data on seascape connectivity (5). More time is needed before the
210 effectiveness of these rules can be evaluated. Nonetheless, the lack of a mechanistic
211 understanding of the role that seascape configuration plays in determining connectivity
212 significantly hinders the ability to predict the influence of extrinsic factors including
213 climate change on reef fish populations (31). It is clear, however, that to effectively
214 maintain functioning ecosystems and sustainable fisheries in structurally complex ocean
215 ecosystems, management plans must conserve the functional integrity of ecosystems at
216 the seascape level rather than focusing solely on individual habitat types. More generally,
217 our approach provides a quantitative method for estimating the value of ecosystem
218 services provided by distinctive habitats to fisheries yields within a seascape (11-13).
219 This will, in turn, allow for more accurate accounting of these services, including the

220 assessment of suitable remediation requirements when these habitats are removed during
221 tourism or aquaculture developments.
222
223 **Materials and Methods**
224 Ehrenberg's snapper, *Lutjanus ehrenbergii* (Peters 1869), were collected from five
225 distinct habitats, 1) coastal wetlands (n = 2 sites), 2) coastal reefs (n = 2), 3) shelf reefs (n
226 = 4), 4) offshore island patch reefs (n = 1) and 5) oceanic reefs (n = 4), along a 50 km
227 cross-shelf transect from coastal Saudi Arabia in the Red Sea in November 2008, March
228 2009 and June 2010 (Fig. 1and Fig. S2). Densities of *L. ehrenbergii* were estimated by
229 visual survey on SCUBA. Individual fish were counted along four replicate 100 m by 10
230 m transects at 5 and 15 m depth from each reef and then averaged per distance. We
231 visualized the separation of potential juvenile habitats using a quadratic discriminant
232 function analysis (32) on the muscle essential AA $\delta^{13}C$ data of *L. ehrenbergii* grouped
233 into five regions according to their collection location across the continental shelf. See
234 Table S1 for variance and loadings of quadratic discriminant function analysis on
235 juvenile snapper habitat signatures. Briefly, total free AAs were isolated by acid
236 hydrolysis and then converted to isopropyl-TFAA derivatives (19), prior to individual
237 isotopic analysis on an Agilent 6890N Gas chromatograph coupled via continuous flow
238 interface to a Thermo Finnigan Mat 253 isotope ratio monitoring-mass spectrometer (see
239 SI Material and Methods).
240 In order to retrospectively identify where each adult *L. ehrenbergii* spent its
241 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 in order 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 AA $\delta^{13}C$ analyses and data processing, please see the SI Materials and Methods. Raw data are available in McMahon (34).

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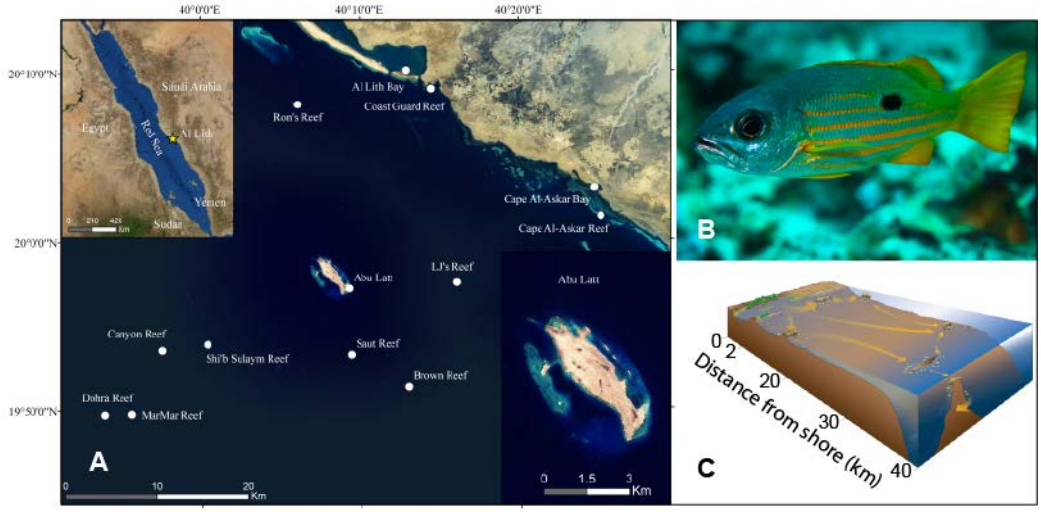
Figure legends

Figure 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.

Figure 2. Underwater visual census estimates. Adult *Lutjanus 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), Lg = lagoon habitat and Fr = fringing reef habitat.

Figure 3. Discrimination of juvenile *Lutjanus ehrenbergii* habitats based on $\delta^{13}C$ values of essential amino acids (AAs). 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.

Figure 4. Relative contribution (mean ± SD) of *Lutjanus 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.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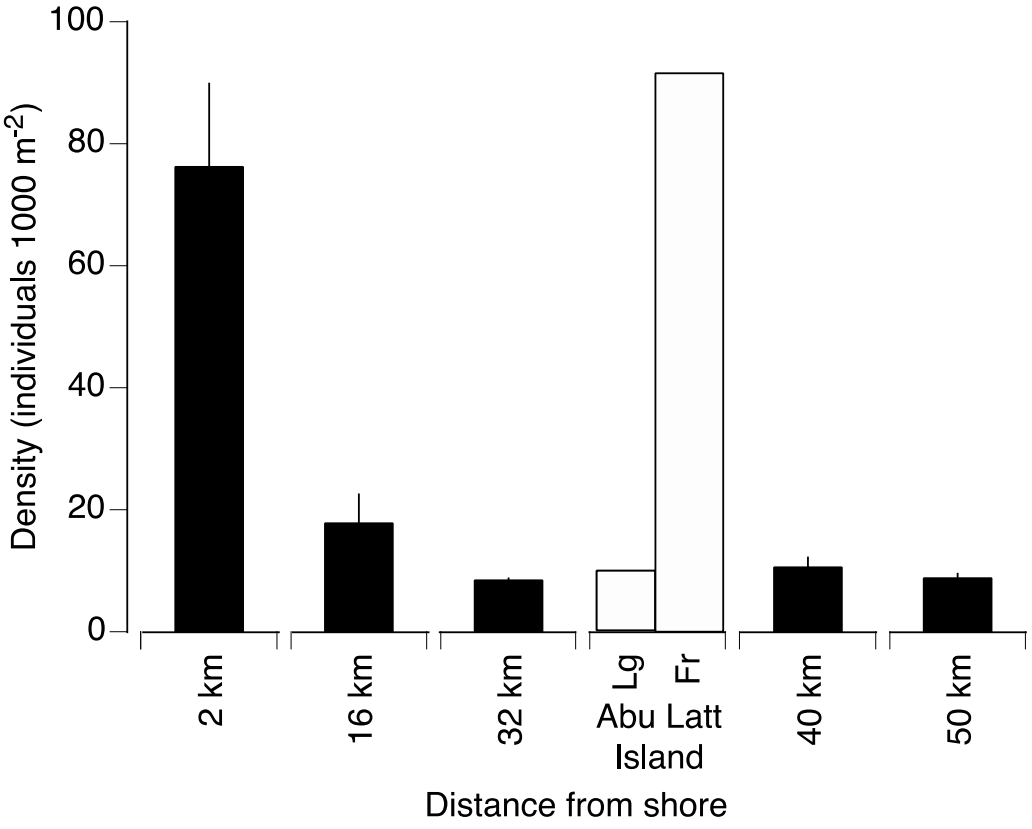
Saudi Arabia
Egypt
Red Sea
Al Lidi
Yemen
Sudan
Al Lith Bay
Coast Guard Reef
Ron's Reef
Cape Al-Askar Bay
Cape Al-Askar Reef
Abu Latt
LJ's Reef
Canyon Reef
Shi'b Sulaym Reef
Saut Reef
Brown Reef
Dohra Reef
MarMar Reef
A
0 10 20 Km
Abu Latt
0 1.5 3 Km
B
C
0 2 20 30 40
Distance from shore (km)

Figure 1



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412 Figure 2

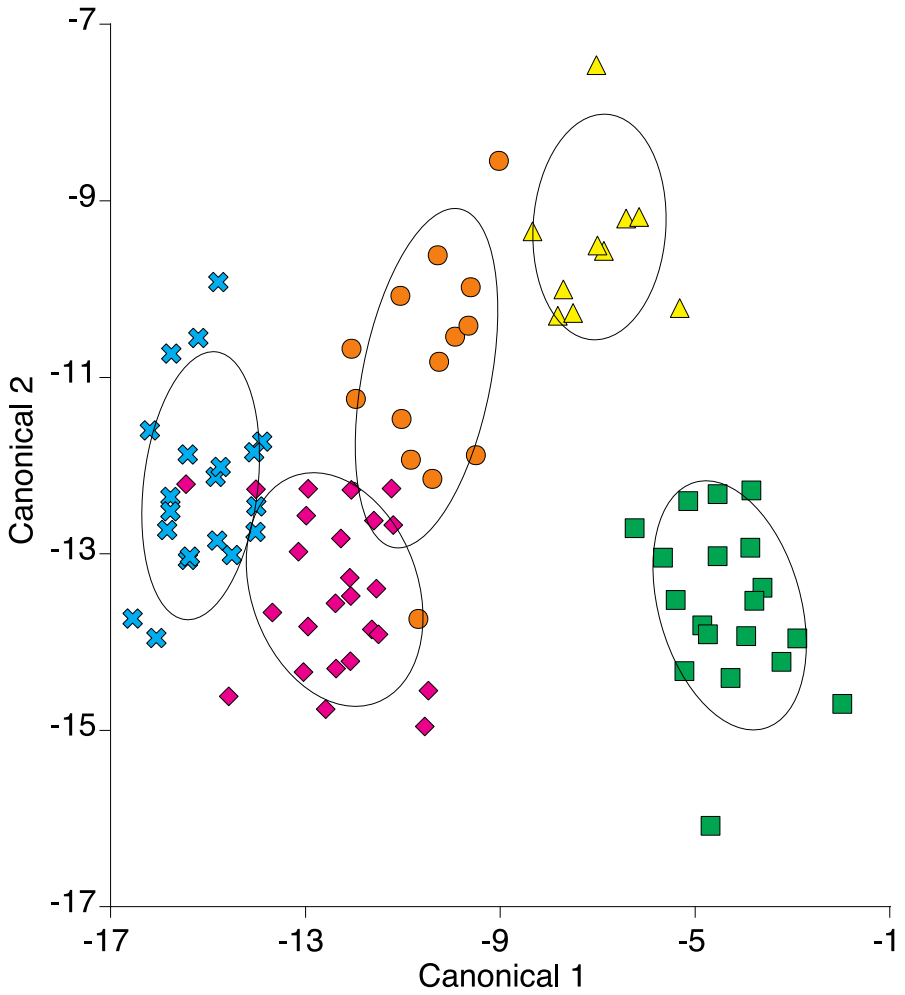


Figure 3

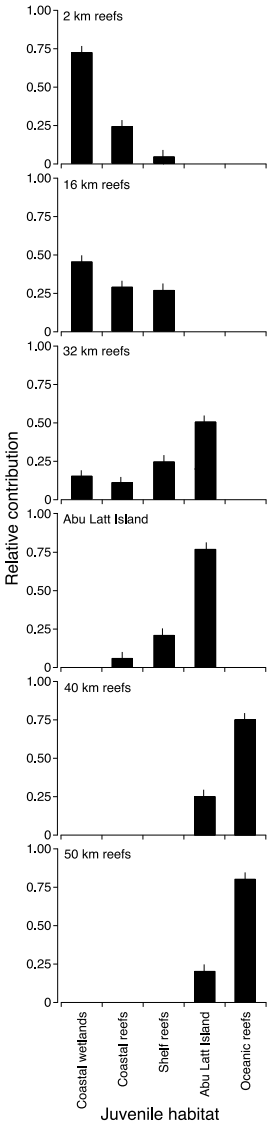

2 km reefs
16 km reefs
32 km reefs
Abu Latt Island
40 km reefs
50 km reefs
1.00
0.75
0.50
0.25
0
Relative contribution
Coastal wetlands
Coastal reefs
Shelf reefs
Abu Latt Island
Oceanic reefs
Juvenile habitat

Figure 4

Supporting Information

Methods

Field Collections

Ehrenberg's snapper, *Lutjanus ehrenbergii* (Peters 1869), were collected from five distinct habitats, 1) coastal wetlands, 2) coastal reefs, 3) shelf reefs, 4) offshore island patch reefs and 5) 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, semi-enclosed 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 approximately 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: 1) coastal reefs within 2 km of shore: Coast Guard Reef and Cape Al-Askar Reef, 2) shelf reefs 16 km offshore: Ron's Reef and LJ's Reef, 3) an offshore island 24 km offshore: Abu Latt Island, 4) shelf reefs 32 km offshore: Saut Reef and Brown Reef, 5) oceanic reefs 40 km offshore: Canyon Reef and Shi'b Sulaym Reef, and 6) oceanic reefs 50 km offshore: MarMar Reef and Dohra Reef.

449 Sagittal otoliths and white muscle tissue were dissected from each fish in the field.
450 Otoliths were cleaned of residual surface tissue with water and stored dry in 1.5 ml vials.
451 White muscle samples from the dorsal surface of each fish were frozen on the boat prior
452 to transport to an onshore laboratory. In the lab, white muscle samples were frozen at -
453 20°C and then lyophilized (freeze-dried) for 48 hours. Samples were transferred to the
454 Woods Hole Oceanographic Institution, Woods Hole, MA, USA for further preparation
455 and analysis. Muscle tissue from *L. ehrenbergii* at each site was used to identify local
456 habitat signatures because muscle has a very fast turnover rate and its isotopic signature
457 represented the most recent residence signature. We did not find any juvenile *L.*
458 *ehrenbergii* on offshore coral reefs; however, we wanted to know the potential
459 contribution of individuals from these coral reefs to the adult population. Therefore,
460 muscle samples from adult *L. ehrenbergii* were used to characterize the habitat signatures
461 of the offshore reefs where no juveniles were collected. We justified this in two ways.
462 Despite a large range in TL across juvenile and adult *L. ehrenbergii* in this study, there
463 was no significant trend in muscle $\delta^{15}N$ values with TL ($y = 0.004x + 8.04$, $R^2 = 0.15$;
464 Fig. S2). This indicates that juvenile and adult *L. ehrenbergii* were feeding at the same
465 trophic level. Thus, we are confident that adult muscle signatures provided an accurate
466 reflection of the values we would find for juvenile muscle in the same habitat.
467
468 ***Sample preparation and analysis***
469 Approximately 1 mg of freeze-dried, homogenized white muscle tissue from each
470 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 UC Davis Stable Isotope Facility, Davis, CA, USA. A second portion of each muscle sample (~1 mg) was acid hydrolyzed to isolate free AAs by refluxing samples in 6N HCl at 110°C for 20 hrs, neutralizing in ultra-pure 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). In order 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 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 hrs. 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, Lake Bluff, Illinois, USA) and then ground down the resulting core from the top and bottom with a Buehler Ecomet3 variable speed grinder-polisher to remove post first year material deposited in the vertical plane. Next, we contoured the shape of the juvenile core to match the mean 3D shape (4 to 5 mm by 2 to 3 mm) and mass (10 to 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.

492 Acid hydrolyzed samples were derivatized prior to SIA according to McMahon et
493 al. (1). Samples were brought up in dichloromethane (DCM) and injected on column in
494 splitless mode at 260°C and separated on a forte SolGel-1ms column (60 m length, 0.25
495 mm inner diameter, and 0.25 μm film thickness; SGE Analytical Science, Sydney,
496 Australia) in a Agilent 6890N Gas Chromatograph (GC) at the Woods Hole
497 Oceanographic Institution, Woods Hole, MA, USA. The separated AA peaks were
498 combusted online in a Finnigan gas chromatography-combustion (GC-C) continuous
499 flow interface at 1030°C, then measured as CO_2 on a Thermo Finnigan Mat 253 irm-MS.
500 Standardization of runs was achieved using intermittent pulses of a CO_2 reference gas of
501 known isotopic composition. All compound-specific SIA samples were analyzed in
502 duplicate along with AA standards of known isotopic composition. We focused on five
503 essential AAs with sufficient peak size and baseline GC separation: threonine, isoleucine,
504 valine, phenylalanine, and leucine (Fig. S4).
505
506 ***Data analysis***
507 Stable isotope ratios were expressed in standard delta (δ) notation:

$$\delta^{13}C_{sample} = \left(\frac{{}^{13}C/{}^{12}C_{sample}}{{}^{13}C/{}^{12}C_{std}} - 1 \right) * 1000$$

508 ,
509 where the standard for carbon was Vienna Peedee Belemnite (VPDB). Differences in
510 total length of *L. ehrenbergii* among the five potential juvenile habitat regions were
511 assessed using a one-way analysis of variance (ANOVA), with Tukey's honestly
512 significant difference (HSD) post-hoc test ($\alpha < 0.05$). The relationship between TL and

513 bulk muscle $\delta^{15}N$ values was determined by linear regression. We visualized the
514 separation of potential juvenile habitats using a quadratic discriminant function analysis
515 (DFA) on the muscle essential AA $\delta^{13}C$ data of *L. ehrenbergii* grouped into five regions
516 according to their collection location across the continental shelf. These were as follows:
517 coastal wetlands (n = 2 sites), coastal reefs (n = 2), shelf reefs (n = 4), Abu Latt Island (n
518 = 1) and oceanic reefs (n = 4). The first and second canonical variables accounted for
519 96% of the total variance in canonical space (Table S1). The jackknife reclassification
520 success rate of the DFA was evaluated by leave-one-out cross-validation and compared to
521 the 1/g reclassification success expectation, where g was the number of groups analyzed
522 (2). We used a maximum likelihood estimator (3) to calculate the relative contribution of
523 each of the five potential juvenile habitat regions to the adult populations on coral reefs at
524 six distances (2 km, 16 km, 32 km, Abu Latt Island, 40 km, and 50 km) along the 50 km
525 cross-shelf transect from Al Lith, Saudi Arabia. McMahon et al. (4) showed that muscle
526 and otolith essential AA $\delta^{13}C$ values had a consistent 1:1 correlation and could be used
527 interchangeably. Thus the training data set was comprised of muscle essential AA $\delta^{13}C$
528 data from each potential juvenile habitat region. The otolith essential AA $\delta^{13}C$ data from
529 juvenile cores of adult *L. ehrenbergii* were treated as unknowns to be classified by the
530 training data set. We identified juvenile nurseries as any juvenile habitat that contributed
531 more than the average if all five juvenile habitats had contributed to the adult population
532 evenly (20%).
533
534

References

1. McMahon KW, Fogel ML, Johnson BJ, Houghton LA, Thorrold SR (2011) A new method to reconstruct fish diet and movement patterns from **δ**13C 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.

Table S1. Variance and loadings of quadratic discriminant function analysis on juvenile snapper habitat signatures. Almost all of the variation in $\delta^{13}C$ values of five essential AAs 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, while the second canonical variable separated coastal wetlands from the shelf island habitat of Abu Latt.

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

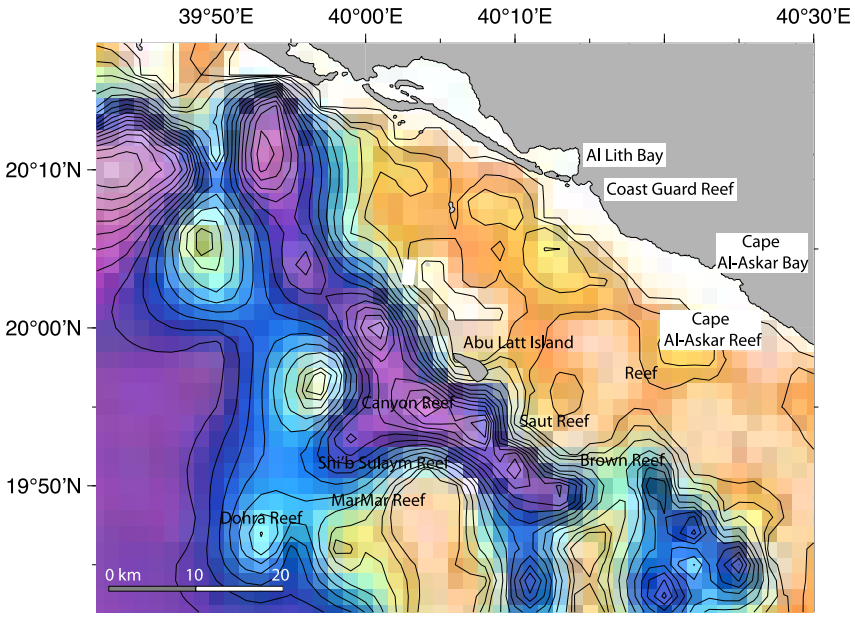
Figure Legends

Figure 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.

Figure S2. Frequency distribution of total length (mm; left y-axis). *Lutjanus 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: $y = 0.004x + 8.04$, $R^2 = 0.15$).

Figure S3. Otolith preparation diagram. A) The otolith of an adult *Lutjanus ehrenbergii* (total length [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, and 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).

Figure S4. Otolith amino acid gas chromatogram. A representative gas chromatogram of derivatized individual amino acids from an otolith of *Lutjanus ehrenbergii*. CO_2 ref: Intermittent pulses of a CO_2 gas reference of known isotopic composition. Gly: glycine, Ser: serine, Asp: aspartic acid, Glu: glutamic acid, Pro: proline, Ala: alanine, Thr: threonine, Ile: isoleucine, Val: valine, Phe: phenylalanine, and Leu: leucine (reproduced from McMahon et al. 19).



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622 Figure S1

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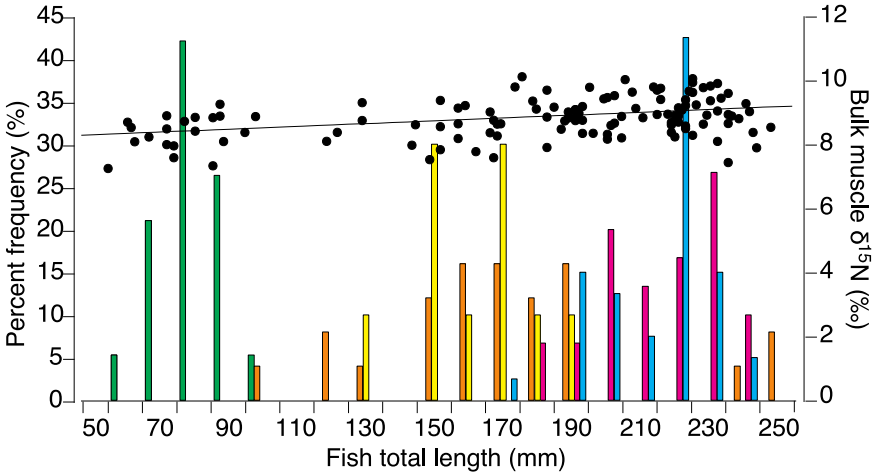

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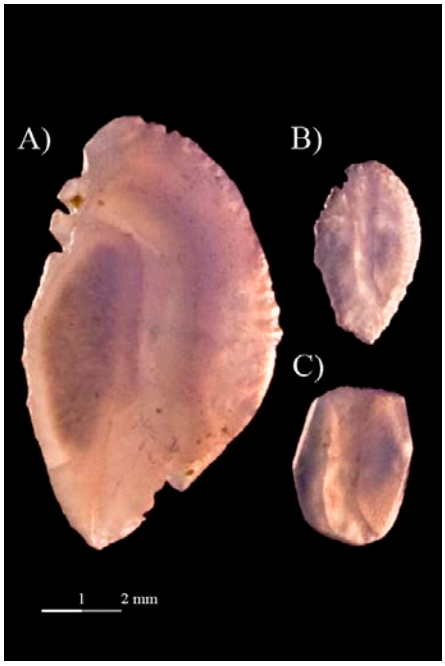
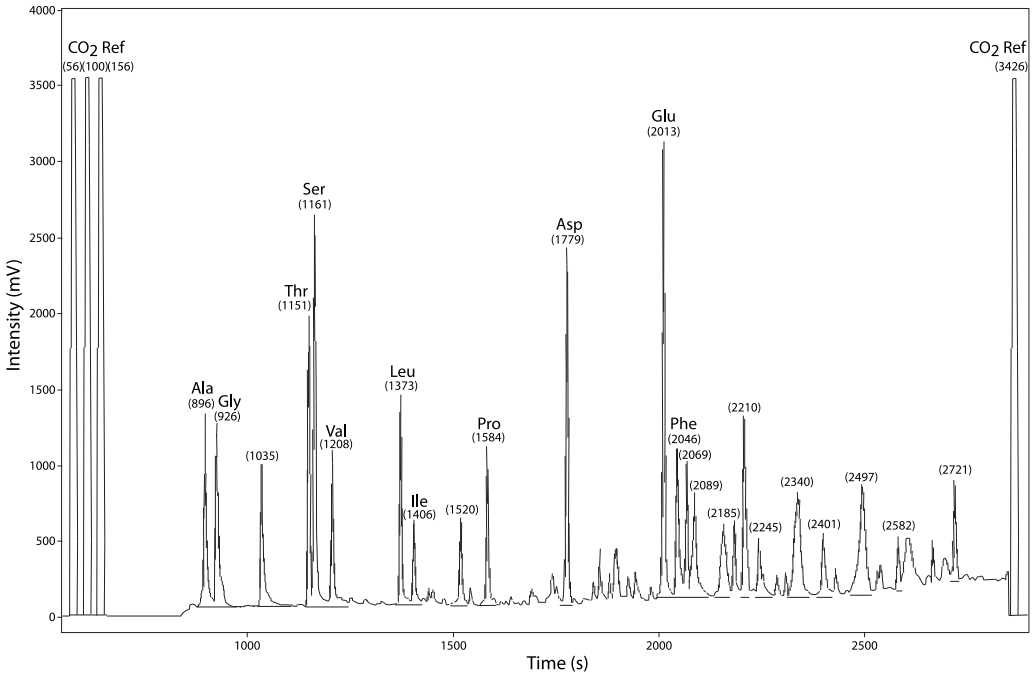


Figure S3



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650 Figure S4