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Evidence of ontogenetic migration from mangroves to coral reefs by black-tail snapper *Lutjanus fulvus*: stable isotope approach

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ABSTRACT: Mangroves are often considered to be important nurseries for coral reef fishes, yet this assumption has rarely been tested. At Ishigaki Island, southern Japan, black-tail snapper *Lutjanus fulvus* juveniles often occur in mangroves, whereas subadults and adults are usually found on coral reefs. To test the hypothesis that *L. fulvus* uses mangroves as a nursery, we conducted stomach content and stable isotope analyses of *L. fulvus* collected from mangroves and an adjacent coral reef. Stomach content analysis showed that specimens from mangroves fed on mangrove-associated prey, whereas those from the coral reef took coral reef-associated prey, indicating that the species undergoes ontogenetic changes in resource use from the mangroves to the coral reef, i.e. coral reef individuals did not migrate to the mangroves to feed. Stable isotope analysis showed that potential prey and mangrove red snapper *L. argentimaculatus* (control fish for mangroves) collected from the mangroves had ^{13}C-depleted values of –23 to –17‰, distinct from the –16 to –8‰ values of potential prey and humpback red snapper *L. gibbus* (control fish for coral reef) collected from the coral reef. $\delta^{13}C$ values of *L. fulvus* in the mangroves had a mangrove signature, whereas individuals on the coral reef gradually shifted from a mangrove signature to a coral reef signature with growth, indicating that small individuals on the coral reef were recent migrants from the mangroves. Based on the $\delta^{13}C$ values of the subadult population of *L. fulvus* on the coral reef, 36 of 41 individuals were estimated to have inhabited the mangroves during their juvenile stage, demonstrating that *L. fulvus* used the mangroves as a nursery.

KEY WORDS: Mangrove · Coral reef · Lutjanidae · Stable isotope analysis · Ontogenetic habitat shift

INTRODUCTION

Assessing connectivity among populations of marine organisms is vital for understanding population dynamics, managing fisheries stocks, and designing marine protected areas. Many ecologically and commercially important marine organisms have life histories in which juvenile stages use different habitats than adults (Adams et al. 2006). Following Beck et al. (2001), a habitat is identified as a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater,

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on average, than production from other habitats in which juveniles occur. Although several aquatic habitats, such as seagrass beds, marshes, and mangroves, have been frequently cited as nursery habitats, very few studies have addressed whether these juvenile habitats successfully transfer a higher juvenile biomass to the adult populations, thus being the vital missing link in our understanding of nurseries. This is largely due to the difficulty of tracking the movements of organisms between aquatic habitats and identifying the nursery habitats that adults have occupied.

A variety of artificial tagging methods have been used to determine movement from juvenile to adult habitats, although such traditional techniques pose difficulties because of the small size and high mortality rate of juveniles, and the large numbers that must be tagged in order to recover a sufficient sample size (Gillanders et al. 2003). Recently, the interpretation of biological markers such as isotopic and elemental composition has been used to determine connectivity between populations (e.g. Gillanders 2005, Herzka 2005). Stable isotopes can be used to trace the origin or movement of organisms, because isotopic signatures in animal tissues reflect those of local food webs or of the habitat in which they have grown (Hobson 1999). Several studies have used a variety of stable isotopes to investigate movements among habitats, including Fry et al. (1999), who examined $\delta^{13}C$ values in tissues of pink shrimp *Farfantepenaeus duorarum* as they moved from inshore seagrass beds and mangrove-lined bays to offshore areas. Shrimps from seagrass beds had ^{13}C-enriched values that were distinct from the values of individuals from mangrove-lined bays. However, smaller individuals collected offshore had $\delta^{13}C$ values typical of individuals in seagrass beds rather than mangrove-lined bays, suggesting that most pink shrimp had moved from seagrass beds to offshore regions.

Mangroves often cover extensive areas surrounding coral reefs and are considered to be important nurseries for coral reef fishes (Parrish 1989). This nursery concept is largely supported by the abundance of juveniles of common reef fish species within mangroves (e.g. Nagelkerken & van de Velde 2002) and higher adult densities on coral reefs adjacent to mangroves than on coral reefs without adjacent mangroves (Nagelkerken et al. 2002, Halpern 2004, Mumby et al. 2004). Typically, juveniles will eventually move to a reef habitat at maturation, although this assumption has rarely been tested (but see Chittaro et al. 2004). In recent years, mangrove systems have been under stress due to inadequate management and their vulnerability to human-related activities (Valiela et al. 2001, Duke et al. 2007). Under such circumstances, an assessment of biological connectivity between mangroves and coral reefs is urgently required, being a basic requirement for effective management of tropical coastal ecosystems as well as regional fisheries resources (Sheridan & Hays 2003, Adams et al. 2006).

Black-tail snapper *Lutjanus fulvus* are widely distributed along the coasts of the subtropical/tropical Indo-Pacific region and are an important component of reef-based fisheries (Allen 1985). At Ishigaki Island, southern Japan, Shibuno et al. (2008) found that juvenile *L. fulvus* (30 to 120 mm in total length, TL) predominantly occurred in mangrove estuaries but sometimes occurred over reef flats, while subadults and adults (>120 mm TL) inhabited coral reefs, based on a quantitative visual census across multiple habitats (mangrove estuary, sand area, seagrass bed, coral rubble area, branching coral area on the reef flat, and tabular coral area on the reef slope). During the daytime, *L. fulvus* swim actively within each habitat, while at night they are found around mangrove prop roots, rocks on river bottoms, or under corals. Although the distribution patterns of individuals of different sizes support an ontogenetic habitat shift in *L. fulvus* from mangroves to coral reefs, the former functioning as a nursery, direct evidence of population connectivity between the 2 habitats is still lacking. If *L. fulvus* exhibit a distinct a clear ontogenetic shift from mangroves to coral reefs, individuals feeding in each habitat must consume local prey. Moreover, if *L. fulvus* use the mangrove habitat as a nursery, the most recent migrants to coral reefs should have stable isotope ratio values typical of individuals in mangroves.

Accordingly, this study was designed to address the following questions, using an application of stomach content and stable isotope analyses: (1) Is there an ontogenetic shift in habitat use from mangroves to coral reefs in *Lutjanus fulvus*? (2) Does the mangrove-based food web have $\delta^{13}C$ signatures that are distinct from the coral reef-based food web? (3) Do $\delta^{13}C$ values of *L. fulvus* collected from coral reefs gradually shift from a mangrove signature to a coral reef signature? (4) Do *L. fulvus* use mangroves as a nursery? The stomach contents and isotopic signatures of several sizes of 2 other species of snappers, humpback red snapper *L. gibbus* and mangrove red snapper *L. argentimaculatus*, were also investigated (as habitat control fishes), the former residing on coral reefs during the juvenile and adult life stages (control species for coral reef habitat), and the latter inhabiting mangroves until the body length reaches around 300 mm TL (control species for mangrove habitat; Shibuno et al. 2008).

MATERIALS AND METHODS

Study site. The study was carried out on the Itona coast of Ishigaki Island, southern Ryukyu Islands (24°29′N, 124°13′E; Fig. 1). Despite the high latitude,

well developed fringing coral reefs and mangrove habitats are present at Ishigaki Island, due to the Kuroshio Warm Current, which flows along the Ryukyu Islands. The mangrove estuary habitat was located near the mouth of the Fukido River (500 m long, 10 to 40 m width up to 300 m upstream of the river mouth, total water surface area ca. 15 000 m^2), where riverbanks were densely occupied by undisturbed mature mangrove trees, dominated by *Rhizophora stylosa*, *Bruguiera gymnorrhiza*, *Kandelia candel*, and *Lumnitzera racemosa*. Water depth in the center of the river mouth was 0.5 to 1 m at low tide (the deep channel was ca. 1.5 m) and 1 to 2 m at high tide (average tidal range 50 cm), with mangrove prop roots alternately inundated and partially exposed during the tidal cycle. The river mouth was 18 m wide and marked by an extensive shallow tidal flat stretching (water depth 60 cm at low tide) approximately 200 m seaward. The fringing reef (0.5 to 5 m depth) extended seaward for approximately 1 km from the Fukido River to the reef edge. Beyond the reef edge, the outer slope (reef front) was steeply inclined to a sandy floor (depth ca. 15 to 20 m). The salinity in the Fukido River ranged from 25 (low tide) to 35 (high tide), while that in reef habitats ranged from 34 to 36, and water temperatures ranged from 26°C (May) to 29°C (August) in each habitat.

Sample collection. Collection of fishes and potential food items took place from late May through early October in 2004, 2005, and 2006 in the Fukido River (mangrove estuary, hereafter mangroves) and an adjacent coral reef (inner-reef habitats and outer reef slope; Fig. 1). Fishes were collected using seine (mesh size 5 mm) and gill nets (mesh size 20 and 35 mm) for juveniles (< ca. 120 mm standard length, SL) and harpoons for young and adults (> ca. 120 mm SL). On the coral reef, most specimens of *Lutjanus fulvus* and *L. gibbus* were collected from inner-reef habitats, although large individuals (>200 mm SL) were collected from the outer reef slope. On the coral reef, potential food items were collected from live/dead corals and coral rubble in the inner-reef coral habitats and on the reef slope, whereas prey from the mangrove habitat were collected from under rocks/stones or mangrove prop roots. Epifaunal (e.g. crabs, shrimps) and infaunal invertebrates (e.g. errant polychaetes) were collected using a hand-net (net mesh 1 mm) and a cylindrical core sampler (13 cm in diameter and 10 cm in height), respectively. Because juvenile *L. gibbus* were also collected around small coral patches in the seagrass bed, potential food items (crabs, shrimps, amphipods, and isopods) were also collected from the latter. All samples were immediately frozen after collection. In the laboratory, SLs of fishes were measured to the nearest mm, and the stomachs were preserved in 10% formalin for subsequent analysis.

Stomach content analysis. Food items in the stomach contents of each fish specimen were identified to the lowest possible taxon. The percentage volume of each food item in the diet was visually estimated under a

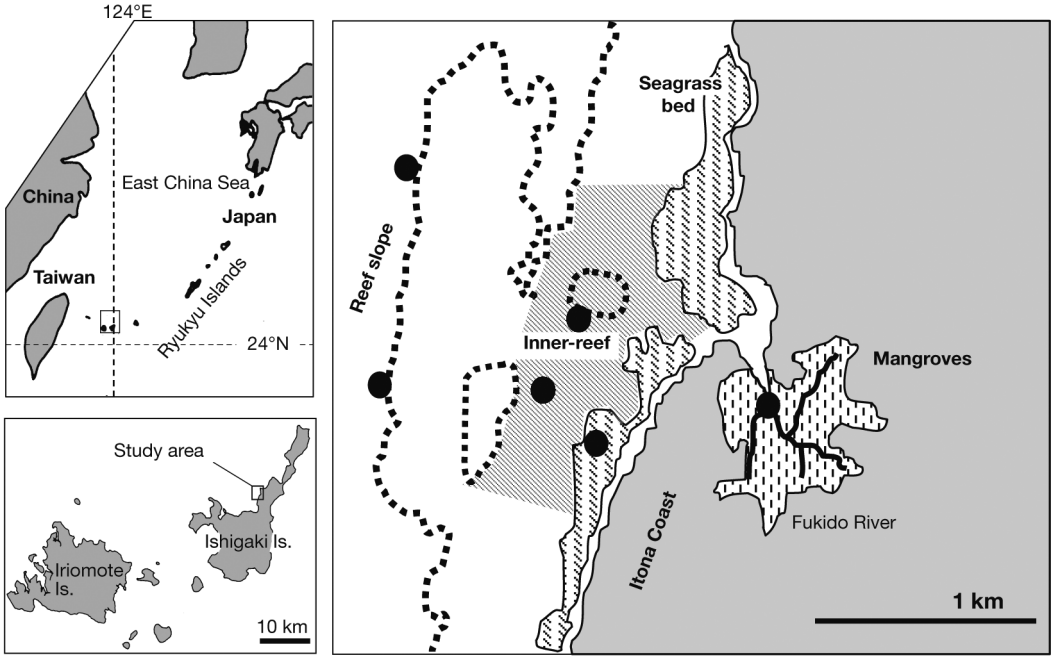

Fig. 1. Study site. Dotted line indicates reef margin. Black circles, sampling site; shaded area, assumed juvenile habitat area for *Lutjanus fulvus* within the coral reef (see Table 4)

binocular microscope. Food resource use was expressed as mean percentage composition of each item by volume, calculated by dividing the sum total of the individual volumetric percentages for the item by the number of specimens examined. Specimens with empty stomachs were excluded from the analysis.

Stable isotope analysis. Fish tissues and potential food items were dried at 60°C for 24 to 40 h to a constant weight and then ground to a fine powder. Fish white muscle tissue was used for isotope analysis because of its slow turnover rate, which should reflect the isotopic composition of food assimilated over periods of several weeks to months (Herzka 2005). Because of the small size of gammaridean amphipods, isopods, crabs (carapace width <1 cm), and shrimp juveniles, whole individuals were processed, with 4 to 10 individuals pooled to make a single sample. Larger specimens (1 to 2 cm) were each treated as single samples. To eliminate lipid effects on muscle $\delta^{13}C$ measurements, lipids were removed from all samples by adding 3 ml of chloroform:methanol (2:1) and extracting for 3 h. The mixture was then centrifuged (4°C, 760 × *g* for 10 min), the supernatant discarded, and the pellet dried in a vacuum desiccator for 1 h. All samples (fish tissues and invertebrates) were then treated by fuming under 12 M HCl for 10 h to remove inorganic carbonates, and excess acid was subsequently removed in a vacuum desiccator with some pellets of NaOH for 3 h. The samples were dried at 60°C before analysis. Carbon and nitrogen stable isotope compositions were measured with an elemental analyzer connected on-line to an isotope-ratio mass spectrometer (Thermo-electron, FLASH EA-Conflo III-DELTA plus XP). Isotopic compositions of C and N were expressed in δ notation as ‰ differences from an international standard (Vienna Pee Dee Belemnite for carbon, atmospheric N_2 for nitrogen):

$$\delta X = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$$

where X is ^{13}C or ^{15}N, and R is the corresponding ratio, i.e. $^{13}C/^{12}C$ or $^{14}N/^{15}N$. Average reproducibility of $\delta^{13}C$ and $\delta^{15}N$ values was about 0.1‰.

Otolith analysis. To estimate the time required for an individual to complete the shift from an isotopic signature reflective of a mangrove habitat to one reflective of the prey available in the reef habitat, otolith analysis of *Lutjanus fulvus* was conducted for age determination. Left sagittal otoliths were removed from 80 individuals, mounted in epoxy, and then sectioned transversely (1 mm thick) using a Buehler Isomet low-speed jewelry saw. Sections were mounted on glass slides with Crystalbond thermoplastic cement, and the transverse plane of both sides was ground with 400–600 grit wet-dry sand paper until they were 0.05 mm thick. Type A alumina powder (0.3 µm) and a Buehler polishing cloth were used for final otolith preparations. The otolith micro-increments were then observed under a microscope with reflected light, and opaque rings were counted using an online computer screen. In this study, otolith rings were considered to represent daily increments, following Szedlmayer (1998). Moreover, increment deposition was considered to have begun at the time of hatching, because incubation times of lutjanids are relatively short (Thresher 1984). Two independent counts were made on each otolith by the same reader, and counts that differed by no more than 10% were averaged (Szedlmayer & Conti 1999).

Data analysis. To assess whether dietary and isotopic changes occurred in *Lutjanus fulvus* in response to an ontogenetic habitat shift, individuals were sorted into 6 main size categories: (1) small juveniles in mangroves (42–73 mm SL), (2) large juveniles in mangroves (75–110, and 125 mm SL), (3) large juveniles on the coral reef (86–119 mm SL), (4) young on the coral reef (120–149 mm SL), (5) small adults (150–179 mm SL) on the coral reef, and (6) large adults (180–205 mm SL) on the coral reef. The control species, *L. gibbus* and *L. argentimaculatus*, were sorted into 2 main size categories; small individuals (54–73 mm SL for *L. gibbus* and 66–92 mm SL for *L. argentimaculatus*), and large individuals (142–275 mm SL for *L. gibbus* and 120–189 mm SL for *L. argentimaculatus*).

Biplots of $\delta^{13}C$ and $\delta^{15}N$ values were used to examine the proximity of fish from different habitat types to potential source signatures. The main focus was on $\delta^{13}C$ because the carbon baseline isotopic signatures differ among habitats, and $\delta^{13}C$ signatures of consumers closely follow source values (e.g. Fry et al. 1999). Carbon isotope ratio values of fishes and prey animals in each month and year did not differ significantly (*t*-test or analysis of variance [ANOVA], $p > 0.05$), enabling data for all sampling events to be combined. Mean values for $\delta^{13}C$ values were compared among ontogenetic stages of the 3 lutjanid species by a Tukey-Kramer multiple comparison test. All data were log(x) transformed to improve homogeneity of variances.

To evaluate whether mangroves serve as a nursery habitat for *Lutjanus fulvus* on the Itona coast, the carbon isotopic signatures of large juveniles and young (hereafter, subadult) *L. fulvus* on the reef were examined. Here, we followed the nursery-role hypothesis of Beck et al. (2001): a nursery is a habitat for a particular species that contributes a greater than average number of individuals to the adult population on a per-unit area basis in comparison to other habitats used by juveniles. In this study, 'contribution' indicated the percentage of *L. fulvus* juveniles enter-

ing the subadult population on the reef. The adult population (>150 mm SL) on the reef was not included in this analysis because all of the latter had reached isotopic equilibrium with the reef signatures, i.e. it was impossible to determine whether *L. fulvus* had migrated from the mangroves or grown on the reef. 'Area of mangroves' represented the total water surface area of the Fukido River and that of the coral reef represented by the inner-reef area on the Itona coast (Fig. 1). 'Nursery habitat' indicated whether contribution/area for a habitat was greater than the average contribution/area for the mangroves and coral reef.

RESULTS

Stomach contents of fishes

Of 80 *Lutjanus fulvus*, 37 (46%) contained food items within their stomachs (Table 1). Those caught in mangroves (42–100 mm SL analyzed) contained predominantly estuary-associated crabs and shrimps, including *Metopograpsus thukuhar*, *Utica gracilipes* and *Gaetice* spp. (13 of 23 individuals), and *Alpheus* spp., followed by small fishes, including *Lutjanus* sp. (25 mm TL) and *Eviota* spp. Individuals collected from the coral reef contained mainly coral reef-associated crabs, such as Xanthidae spp. (e.g. *Chlorodiella cytherea*; large juveniles, 1 of 4 individuals; young, 3 of 6 individuals; small adults, 1 of 3 individuals), and *Galathea* spp. Estuary-associated crabs were not observed in the stomach contents of the coral reef individuals.

Of 18 *Lutjanus argentimaculatus* individuals, 14 (78%) contained food items (66–174 mm SL) (Table 1), with the major item being estuary-associated grapsid crabs (*Metopograpsus* spp. and *M. thukuhar*; all individuals in the small-size class and 6 of 8 individuals in the large-size class). Three large *L. argentimaculatus* had also fed on fishes, including *Ophiocara* sp. (65 mm TL) and *L. monostigma* (40 mm TL). All *L. gibbus* examined contained food items; small individuals (54–73 mm SL) consumed shrimps and isopods, whereas larger fish (142–275 mm SL) consumed predominantly coral reef-associated crabs, such as Xanthidae (e.g. *Chlorodiella cytherea*, *Etisus* sp., *Liomera* spp., and *Pilumnus* sp.; 11 out of 18 individuals) and *Menaethius monoceros* (3 individuals).

Isotopic signatures of potential food items and fishes

The $\delta^{13}C$ values of potential food items collected in the mangrove habitat ranged between –23 and –17‰, and those of food items from the coral reef ranged between –16 and –8‰ (Fig. 2). Most invertebrate samples showed relatively uniform $\delta^{13}C$ values within each taxon (Table 2). Pooled samples of each taxon (crabs, shrimps, and hermit crabs) from the mangroves exhibited significantly lower $\delta^{13}C$ values compared to those from the coral reef (*t*-test, $p < 0.05$; Fig. 2). The $\delta^{13}C$ values of pooled samples of shrimps showed no differences between the coral reef and the seagrass bed (*t*-test, $p = 0.79$).

The $\delta^{13}C$ values of *Lutjanus fulvus* caught in the mangroves ranged from –23 to –17‰, with no difference observed between the 42–73 and 75–125 mm SL size

Table 1. Size range, number of fish samples used for stable isotope analysis, and stomach content analysis for each species per habitat. Food items comprising mean percentage volume of food items of each species. Cr: crabs, Sh: shrimps, He: hermit crabs, Is: isopods, My: mysids, Gm: gammaridean amphipods, Fh: fishes, Pl: errant polychaetes, Op: ophiuroids, Bi: bivalves, Others: food items composing <5% of the gut content volume

Species/habitat	Size class		Samples analyzed		Food items (%)										
	Range (mm)	Symbol	Isotopes	Stomachs	Cr	Sh	He	Is	My	Gm	Fh	Pl	Op	Bi	Others
Lutjanus fulvus															
Mangrove	42–73	a	18	18	60	13				10	16				1
	75–125	b	12	5	73	27									
Coral reef	86–119	c	11	4	50		25					13			12
	120–149	d	30	6	67	5	17	5				6			
	150–179	e	6	3	76		7		17						
	180–205	f	3	1		80	20								
Lutjanus gibbus															
Coral reef	54, 73	g	2	2		50		50							
	142–275	h	18	18	35	7	11				6	20	9	8	4
Lutjanus argentimaculatus															
Mangrove	66–92	i	7	6	100										
	120–189	j	11	8	66	13					20				1

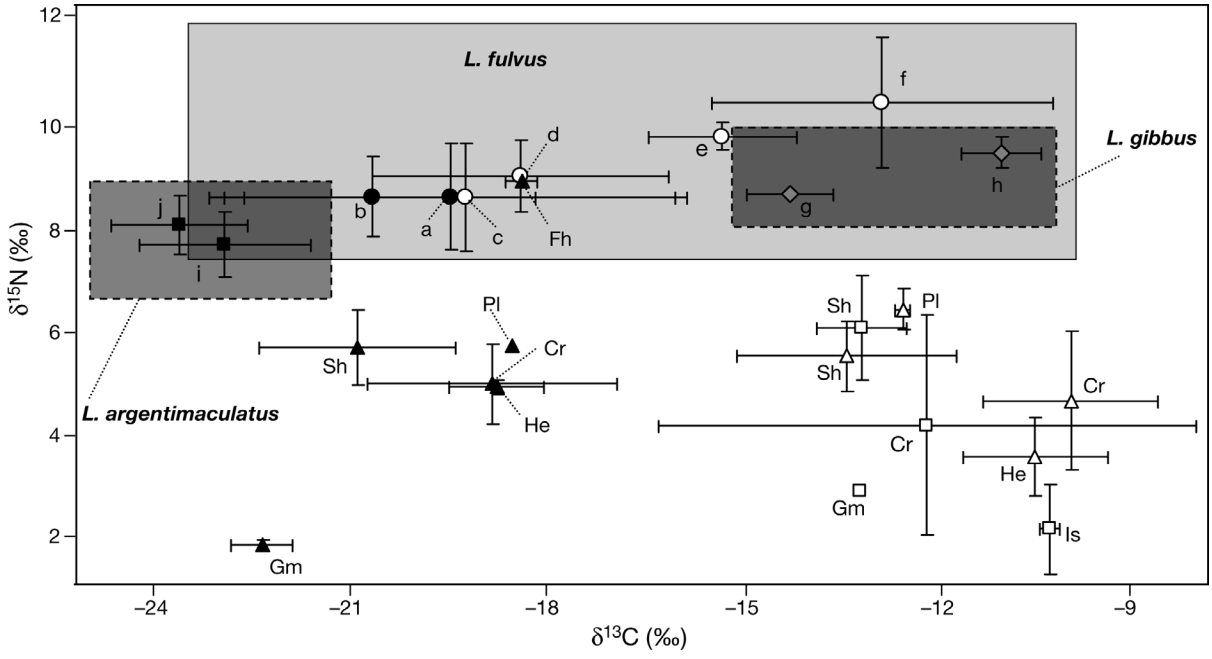

Fig. 2. Mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ values of *Lutjanus fulvus*, *L. gibbus*, and *L. argentimaculatus* and their potential prey, collected from the mangroves (▲), coral reef (△), and seagrass bed (□). Abbreviations and alphabetical symbols, see Table 1

classes (Table 3). *L. fulvus* collected from the coral reef (86–205 mm SL), on the other hand, showed increasingly enriched $\delta^{13}C$ values with an increase in fish size class (Fig. 2), the latter ranging from –23 to –11‰. Whereas the $\delta^{13}C$ values of the 86–119 and 120–149 mm SL size classes were similar to those of individuals collected from the mangroves (42–125 mm SL; <–16‰), they were significantly lower than those of the 180–205 mm SL size class (–16 to –11‰). *L. gibbus* $\delta^{13}C$ values ranged from –15 to –14‰ for the small size class (54–73 mm SL) and –12 to –10‰ for the large size class (142–275 mm SL), values similar to those of the large adult individuals of *L. fulvus* (Fig. 2, Table 3). The $\delta^{13}C$ values of *L. argentimaculatus* ranged between –25 and –22‰ (66–189 mm SL; Fig. 2), which were marginally lower than those of *L. fulvus* from the mangroves (Table 3). All *Lutjanus* species had $\delta^{15}N$ values enriched by approximately 3 to 5‰ over those of prey items, indicating that the former fed on prey caught in the same habitat (Fig. 2).

Fig. 3 shows the relationship between $\delta^{13}C$ values and SL of *Lutjanus fulvus* collected from the mangroves and coral reef. Individuals caught in the mangroves generally had $\delta^{13}C$ values below –16.3‰ (lowest $\delta^{13}C$ value of prey samples on the coral reef), although 4 individuals (52, 53, 55, and 66 mm SL; square symbols in Fig. 3) had markedly high $\delta^{13}C$ values (–15 to –13‰). On the other hand, individual fish collected from the coral reef showed increasingly enriched $\delta^{13}C$ values with an increase in fish size, from –16‰ for most individuals <150 mm SL to over –16‰ (highest value of prey samples in the mangroves) in individuals >150 mm SL. Three individuals had very high $\delta^{13}C$ values (–15 to –13‰) even though they were juveniles (86, 98, and 100 mm SL; see Fig. 3).

Otolith analysis of *Lutjanus fulvus*

Daily otolith increments of *Lutjanus fulvus* were usually difficult to interpret for specimens over 500 d old (ca. 150 mm SL). Moreover, isotopic analysis showed that all *L. fulvus* had carbon isotope ratio values typical of the coral reef when their body length reached around 150 mm SL. Therefore, individuals < ca. 152 mm SL were subjected to otolith analysis in order to estimate the time required for an individual to reach isotopic equilibrium with the food resources available on the coral reef after migrating from the mangrove habitat. Otoliths from 61 of 80 individuals collected were sufficiently clear for interpretation and analysis.

Fig. 4 shows the relationship between age and SL of 61 *Lutjanus fulvus* individuals collected from the mangroves and coral reef. The largest individuals collected from the mangroves were about 80 to 90 mm SL and approximately 180 to 200 d old. The age of 150 mm SL specimens was estimated as ca. 500 d old, indicating that about 300 d were required for migrated individuals to reach equilibrium with the foods available in the coral reef habitat.

Identification of nursery habitat for *Lutjanus fulvus*

Of the 30 *Lutjanus fulvus* individuals caught in the mangroves, 4 showed much higher $\delta^{13}C$ values (–15 to –13‰) than the others (Fig. 3), suggesting that they had recently migrated from the coral reef. On the other hand, 3 juveniles (86, 98, and 100 mm SL) collected from the coral reef showed enriched $\delta^{13}C$ signatures indicative of growth on the reef. Two young individuals (133 and 138 mm SL) had enriched $\delta^{13}C$ signatures, but of equivocal status, i.e. the isotopic signatures were intermediate between a mangrove- and a coral reef-based food web. Of the large juvenile and young individuals of *L. fulvus* collected from the coral reef (subadults), at least 36 of 41 had a carbon isotopic signature (<–16‰) indicative of previous growth in the mangrove habitat. Because the coral reef area (340 000 m^2) covered 96% of the Itona coast and contributed a maximum of 12.2% (5 individuals) of juveniles to the subadult population on the reef, whereas the mangrove area (total water surface area of Fukido River) comprised 4% of the coast and contributed 87.8% of juveniles (36 individuals) to the subadult coral reef population, the mangroves can be classified as a nursery habitat (per-unit area contribution of mangroves = 22.0 > mean of mangroves and coral reef = 11.1; Table 4), following the definition of Beck et al. (2001).

DISCUSSION

On tropical coasts, mangroves often serve as important feeding grounds for fishes from adjacent habitats (Sheaves 2005). In the present study, stomach content analysis of *Lutjanus fulvus* showed that individuals in the mangroves fed predominantly on mangrove-associated crabs and shrimps, whereas

Table 2. Overview of stable isotope data (mean ± 1 SD) for prey samples

Potential prey	$\delta^{13}C$	$\delta^{15}N$	n
Mangroves			
Crabs			
Grapsidae spp.	–19.0 ± 1.4	5.0 ± 0.9	10
Xanthidae spp.	–19.3 ± 5.1	4.9 ± 0.0	2
Portuninae spp.	–17.3 ± 0.7	5.1 ± 0.8	3
Unidentified	–20.1 ± 0.8	4.9 ± 1.1	2
Shrimps			
Macrobrachium spp.	–20.5 ± 1.7	6.2 ± 1.5	3
Palaemon spp.	–22.0 ± 1.3	5.6 ± 0.4	7
Alpheopsis spp.	–19.8 ± 0.8	5.5 ± 0.5	6
Hermit crabs			
Diogenidae spp.	–18.8 ± 0.7	4.9 ± 0.2	4
Errant polychaetes	–18.6	5.7	1
Gammaridean amphipods	–22.4 ± 0.5	1.8 ± 0.1	2
Fishes			
Eviota spp.	–18.4 ± 0.2	8.9 ± 0.0	2
Coral reef			
Crabs			
Trapezia spp.	–10.8 ± 1.0	5.4 ± 1.0	18
Tetralia spp.	–10.1 ± 2.2	5.5 ± 0.7	3
Cymo spp.	–8.1 ± 0.7	3.0 ± 0.8	2
Xanthidae spp.	–9.2 ± 1.0	3.4 ± 1.1	10
Unidentified	–9.8 ± 0.9	3.9 ± 0.2	3
Shrimps			
Palaemonidae spp.	–12.8 ± 1.0	5.5 ± 0.8	4
Hippolytidae sp.	–16.0	5.5	1
Hermit-crabs			
Galathea spp.	–10.6± 1.1	3.5 ± 0.8	2
Errant polychaetes	–12.6 ± 0.1	6.4 ± 0.4	3
Ophiuroids	–3.6 ± 0.6	5.4 ± 0.2	4
Seagrass bed			
Crabs			
Unidentified	–12.2 ± 4.1	4.1 ± 2.2	3
Shrimps			
Leander spp.	–13.3 ± 0.8	7.2 ± 0.1	2
Hippolyte spp.	–13.9 ± 0.8	5.0 ± 1.4	2
Latreutes spp.	–13.0 ± 0.6	6.0 ± 0.6	5
Gammaridean amphipods	–13.3	2.9	1
Isopods	–10.4 ± 0.2	2.1 ± 0.9	2

Table 3. Tukey-Kramer multiple comparison test between $\delta^{13}C$ values of each size class of *Lutjanus fulvus* (a–f), *L. gibbus* (g, h), and *L. argentimaculatus* (i, j). For individual numbers in each size class of 3 lutjanid species, see Table 1. Habitat: habitats in which collections were made, Mg: mangroves, Reef: coral reef, ns: $p > 0.05$

Habitat	Size class		*L. fulvus*						*L. gibbus*		*L. argentimaculatus*	
	Range (mm)	Symbol	a	b	c	d	e	f	g	h	i	j
Mg	42–73	a	–	ns	ns	ns	<0.05	<0.01	ns	<0.01	ns	<0.01
Mg	75–125	b	–	–	ns	ns	<0.01	<0.01	<0.05	<0.01	ns	ns
Reef	86–119	c	–	–	–	ns	ns	<0.01	ns	<0.01	ns	<0.01
Reef	120–149	d	–	–	–	–	ns	<0.01	ns	<0.01	<0.01	<0.01
Reef	150–179	e	–	–	–	–	–	ns	ns	<0.01	<0.01	<0.01
Reef	180–205	f	–	–	–	–	–	–	ns	ns	<0.01	<0.01
Reef	54–73	g	–	–	–	–	–	–	–	ns	<0.01	<0.01
Reef	142–275	h	–	–	–	–	–	–	–	–	<0.01	<0.01
Mg	66–92	i	–	–	–	–	–	–	–	–	–	ns

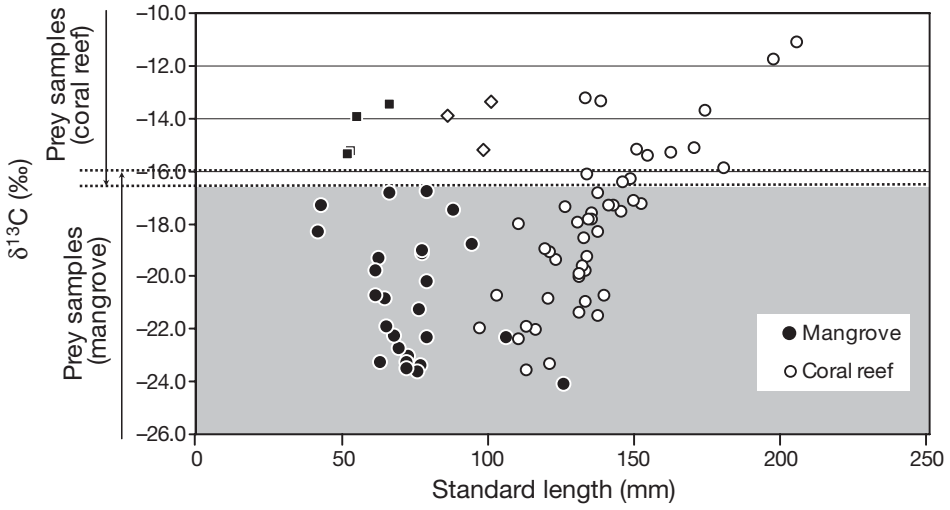

Fig. 3. *Lutjanus fulvus.* Relationship between $\delta^{13}C$ values and standard length of individuals collected from the mangroves (black) and coral reef (white). Dotted horizontal bars indicate highest or lowest range of $\delta^{13}C$ values of prey samples in the mangroves and on the coral reef, respectively. Shaded area shows typical range of $\delta^{13}C$ values for mangrove prey samples. ■, individuals with enriched $\delta^{13}C$ values considered to be recent migrants from the coral reef; ◇, individuals with enriched $\delta^{13}C$ values indicative of growth on the coral reef

individuals on the coral reef fed mainly on coral-associated crabs. Moreover, *L. fulvus* >100 mm SL were rarely observed in the mangroves during the study period (Shibuno et al. 2008). Given that juveniles exhibited a clear ontogenetic habitat shift from mangroves to coral reefs on the Itona coast, feeding migrations of *L. fulvus* into the mangrove habitat was unlikely. The migration patterns inferred from the isotopic data were therefore not confounded by short-term feeding excursions. Stomach content analysis also showed that *L. fulvus* and *L. argentimaculatus* captured in the mangroves fed on fishes, including juvenile snappers. This indicates that lutjanids may themselves be piscivores targeting smaller fish sheltering and feeding in the mangroves, a common phenomenon in mangrove ecosystems (Sheaves 2001).

The $\delta^{13}C$ values of potential food items from the mangroves, seagrass bed, and coral reef on the Itona coast were divisible into 2 groups: one associated with the mangroves (range, –23 to –17‰), and the second associated with the seagrass bed (–17 to –7‰) and coral reef (–16 to –8‰). Similar $\delta^{13}C$ gradients among habitats have been reported from other tropical coasts (Fry et al. 1982, Marguillier et al. 1997, Cocheret de la Morinière et al. 2003), indicating that carbon stable isotopes provided good discrimination between biota from mangroves and seagrass beds/coral reefs.

$\delta^{13}C$ values of the control fish species reflected those of their respective habitats. *Lutjanus gibbus* had coral reef-based food web signatures (–15 to –10‰), whereas *L. argentimaculatus* had mangrove-based food web signatures (–25 to –22‰). $\delta^{13}C$ values of *L. fulvus* collected from the mangroves also indicated mangrove-based food web signatures (–23 to –17‰), although slightly enriched compared to those of *L. argentimaculatus.* This difference might be explained by differences in the sampling localities of the 2 species in the mangroves. *L. fulvus* and potential prey were collected

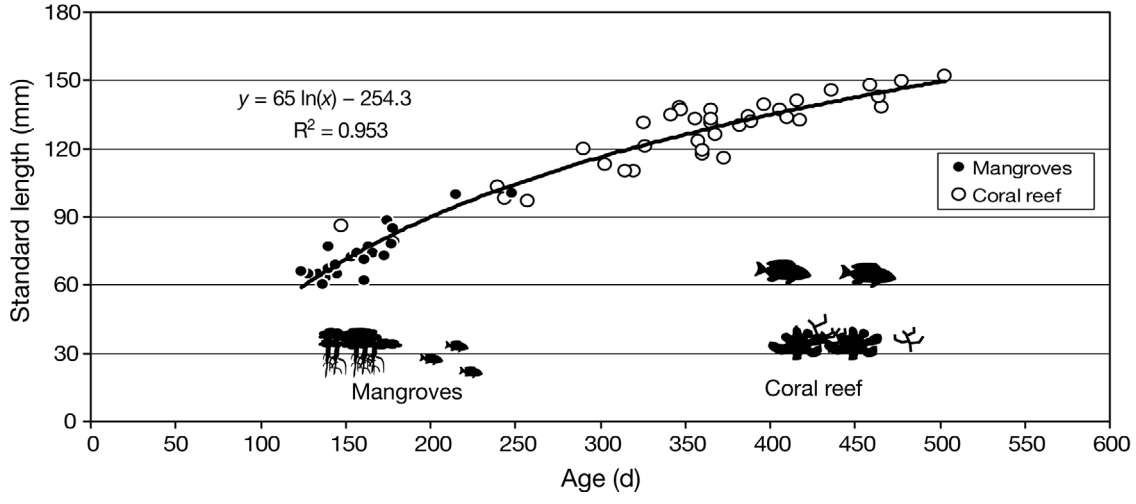

Fig. 4. *Lutjanus fulvus.* Relationship between age and standard length of 61 individuals collected from the mangroves (black) and coral reef (white). Approximate logarithmic curve plotted

Table 4. Determination of nursery habitat of *Lutjanus fulvus*. Contribution: percentage of juveniles entering the subadult population on the reef. Nursery habitat calculated by dividing contribution by area. Nursery habitat: contribution per area is greater than average contribution per area of overall ecosystem. Area of coral reef represented by inner reef area (see Fig. 1)

Habitat	Contribution (%)	Area (%)	Contribution per area	Nursery habitat
Coral reef	12.2	96	0.1	NO
Mangroves	87.8	4	22.0	YES
Total	100	100	22.1	
Mean	50	50	11.1	

throughout the mangrove habitat, whereas *L. argentimaculatus*, especially larger individuals, were collected mainly from the deep channel located in the more upstream zone. Supporting evidence for this has been found in the $\delta^{13}C$ values of invertebrates from upstream mangrove zones showing more depleted $\delta^{13}C$ values than those from downstream zones (Chong et al. 2001). Large juveniles and young *L. fulvus* (86–149 mm SL) caught from the coral reef had a carbon isotopic signature characteristic of the mangrove-based food web, indicating that they had recently migrated from the mangroves. The ^{13}C signature of increasingly larger *L. fulvus* collected from the coral reef was increasingly enriched, suggesting a gradual shift in $\delta^{13}C$ values from the mangrove-based food web to the coral reef-based food web corresponding with isotopic turnover. Large adult *L. fulvus* (>180 mm SL) had enriched $\delta^{13}C$ values similar to those of *L. gibbus*, indicating that the former were close to reaching equilibrium in terms of $\delta^{13}C$ with the coral reef-based food web.

A limited number of studies have estimated isotopic turnover rates for fishes (for review see Herzka 2005). Larvae and early juveniles, which are rapidly growing organisms, reach equilibrium relatively quickly, potentially losing their initial signature within days or weeks (e.g. Herzka & Holt 2000), whereas older or larger fish may take well over 1 yr (e.g. Hesslein et al. 1993). Similarly, *L. fulvus* requires approximately 300 d for a complete turnover of muscle tissue carbon from the lowest mangrove signatures ($\delta^{13}C$ = –24‰) to the lowest coral reef signatures (–16‰; Figs. 3 & 4).

Based on the carbon isotopic signature of subadult *Lutjanus fulvus* collected from the coral reef (Fig. 3) and the nursery definition of Beck et al. (2001), the mangroves rather than the coral reef can be classified as a nursery habitat (Table 4). However, in this study sampling on the reef was only performed on a small fraction of the reef habitats, so an important consideration is how representative subadult samples are of the entire subadult *L. fulvus* population. At Itona coast, we collected subadult *L. fulvus* from 2 inner-reef sites where the latter predominantly occurred. Therefore, the subadult samples collected from the reef might be well representative of the whole subadult *L. fulvus* population on the reef.

The pelagic larval duration of many lutjanid species is 3 to 4 wk (Zapata & Herrón 2002). The sagittal otoliths of *Lutjanus fulvus* exhibited comparable settlement marks (post-settlement increment widths were consistently narrower than those laid down before settlement). Because lutjanid larvae have been captured by light traps in the Fukido River (T. Shibuno unpubl. data), *L. fulvus* larvae may recruit to the mangrove area directly after their pelagic stage. However, because enriched ^{13}C signatures of some juveniles in the mangroves suggested that they were recent arrivals from the coral reef, the juvenile population of *L. fulvus* in the mangroves might therefore have 2 sources, one settling directly to the mangroves and the other migrating to mangroves from reef habitats during the benthic juvenile stage. Although the latter process may be minor and easily missed by underwater observers, it may be important in sustaining mangrove juvenile populations. Further evaluation of the biological connectivity between mangroves and coral reefs is needed. Overall, most *L. fulvus* juveniles were estimated as inhabiting mangroves for some 4 to 6 mo before migrating to adjacent coral reef habitats.

In conclusion, we confirmed population connectivity between the mangrove and coral reef habitats by ontogenetic migration of *Lutjanus fulvus*. Most *L. fulvus* inhabited the mangroves as a nursery for some 4 to 6 mo during their juvenile stage and then migrated to the adjacent coral reef. Such an ontogenetic migration between mangroves and coral reefs is crucial for sustaining coral reef fish populations, because the availability of mangrove nursery habitats strongly influences the assemblage structure and biomass of reef fishes in their adult coral reef habitats (Nagelkerken et al. 2002, Halpern 2004, Mumby et al. 2004). The worldwide declines of mangroves (Valiela et al. 2001) and coral reefs (Bellwood et al. 2004) and related resources call for an urgent reassessment of current management practices. Our findings support the application of management action for tropical coasts to areas of connected habitats, rather than simply identifying representative areas of each habitat in isolation (Mumby et al. 2004). However, we focused on a specific fish species within a relatively small spatial scale (<2 km). Clearly, further work is required to assess the biological connectivity between habitats with different spatial scales using several organisms, relevant to effective management plans for tropical coasts.

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