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Stable isotope records from otoliths as tracers of fish migration in a mangrove system

M. Huxham*†, E. Kimani‡, J. Newton§ and J. Augley*

*School of Life Sciences, Napier University, Edinburgh EH10 5DT, Scotland, U.K.,
‡Kenya Marine and Fisheries Research Institute, P. O. Box 81651, Mombasa, Kenya and
§S.U.E.R.C., Scottish Enterprise Technology Park, Rankine Avenue, East Kilbride, Glasgow G75 OQF, U.K.

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The ratios of stable isotopes ^{18}O:^{16}O and ^{13}C:^{12}C were measured in otolith carbon taken from nine species of fishes caught within mangroves and on the reef at Gazi Bay, Kenya. Before analysis, otoliths were divided into 'larval' 'post-larval' and 'adult' sections using a drill. Fishes were putatively classified as 'mangrove residents' 'offshore residents' or 'migrants' on the basis of information from the literature, and depending on where they were caught (mangroves only, offshore only or both mangroves and offshore) in the present study. Eight of the species exhibited an increase in otolith ^{13}C:^{12}C with age, but this was significant only in the two migrant species *Lethrinus harak* and *Lutjanus fulviflammus*. There were no consistent patterns in ^{18}O:^{16}O with age, or between migrants and non-migrants. These results suggest that comparing absolute values of otolith oxygen and carbon isotope signatures between fish species is not a useful way of determining migration patterns at this site, because of species-specific differences in carbon metabolism and insufficiently steep gradients in temperature and salinity. Changes in carbon isotope signatures between life stages within a species, however, do hold promise as migration tracers.

Key words: fish migration; habitat use; mangroves; reef; stable isotopes.

INTRODUCTION

Many authors emphasize the importance of mangroves as nurseries for fishes, with this function providing a key argument for mangrove conservation (Nagelkerken *et al.*, 2000; Laegdsgaard & Johnson, 2001; Mumby *et al.*, 2004). Most studies that claim to show a nursery role for mangroves have recorded densities of juvenile fishes in mangal habitats, and compared these densities with those found outside mangroves. Whilst such studies can provide important information, they are not sufficient on their own to establish a nursery role for mangroves, since higher densities of juveniles may indicate 'sink' habitats caused by hydrodynamics. Compelling evidence for this requires the demonstration that

†Author to whom correspondence should be addressed. Tel.: +44(0) 131 4552514; fax: +44(0) 131 4552291; email: m.huxham@napier.ac.uk

mangrove habitats contribute proportionally more individuals to an adult stock than comparable areas of other juvenile habitat (Beck *et al.*, 2001; Gillanders *et al.*, 2003). Such a demonstration represents a major challenge, since it implies the need to trace the fate of juveniles originating in different habitats.

The most convincing studies of dispersal and migration in marine organisms involve mark–recapture techniques. For example, Jones *et al.* (1999) used tetracycline to mark the otoliths of over 10 million larval damselfish *Pomacentrus amboinensis* Bleeker, and were able to demonstrate substantial self-recruitment in their study population. Artificial physical markers such as plastic discs have proved extremely useful in many studies of juvenile fishes (Nash *et al.*, 1994; Gillanders *et al.*, 2003). Whilst such studies are impressive, the use of mark–recapture techniques is likely to remain too costly and laborious for most situations, and physical tagging may compromise fish survival and is limited to fishes of suitable size (Arnason & Mills, 1981, 1987; Gillanders *et al.*, 2003; McDonald *et al.*, 2003).

An alternative approach, that may overcome bias due to tag-loss and tag-induced mortality, and which does not involve the work and expense of capturing and then releasing fishes, is to use naturally occurring markers that can leave a signature unique to the juvenile habitat. For example, Swearer *et al.* (1999) used five metallic trace elements, the concentrations of which differ between coastal waters and the open ocean, as natural markers in a study of reef fish migration. The stable isotopes of oxygen and carbon provide other promising markers, which may be of use in cases when metals would not differentiate between habitats; data on metal concentrations in the Gazi Bay, Kenya (the site of the current study), mangal and adjacent seagrass system are not available, while evidence of a strong carbon stable isotope signal has been published (Marguillier *et al.*, 1997). Analyses of tissue ^{13}C:^{12}C ratios ($\delta^{13}C$) have been used to investigate marine food webs, including mangrove systems (Marguillier *et al.*, 1997), and also to infer migration and habitat connectivity (Gillanders *et al.*, 2003; Harrod *et al.*, 2005; Herzka, 2005). $\delta^{13}C$ measurements in muscle and other tissues reflect the main source of carbon to consumers. Organic material derived from mangroves has much lower $\delta^{13}C$ values than that of most marine carbon. For example, $\delta^{13}C$ signals for mangrove leaves taken from Gazi Bay, averaged $-26{\cdot}8‰$, compared with $-16{\cdot}2‰$ for the adjacent seagrass beds (Marguillier *et al.*, 1997). Thus, $\delta^{13}C$ may serve as a marker of fish migration here.

Because otoliths, the carbonate structures found in the internal ear sacs of fishes, are acellular and therefore metabolically inert after deposition, they have the potential to preserve chemical signals specific to particular periods (possibly even to particular days) in a fish's life. This gives otoliths an advantage over other fish tissue in migration research; carbon isotope ratios of muscle, for example, will reflect only what the fish has eaten for the previous months, rather than years, since turnover times in muscle are of the order of 6 months (Tieszen *et al.*, 1983). The relationship between carbon isotope signatures in food and those found in otoliths, however, is complex. An estimated 10–30% of otolith carbon is derived from metabolic sources, with the remainder coming from dissolved inorganic carbon (DIC) (Campana, 1999); thus, whilst diet is an important control on otolith $\delta^{13}C$ it is not the dominant factor.

$\delta^{13}C$ (otolith) can respond to lifetime changes in metabolic rate (Weidman & Millner, 2000; Stephenson *et al.*, 2001) and this may also complicate interpretation. Given these factors (which are likely to vary in importance between species), $\delta^{13}C$ values are often of limited value as migration markers. They may, however, be useful at sites with a steep $\delta^{13}C$ gradient where the environmental signal may be strong enough for detection regardless of other sources of variability; indeed, Dufour *et al.* (2005) successfully demonstrated this technique in Lake Michigan alewives *Alosa pseudoharengus* (Wilson).

In contrast to carbon, oxygen isotopes in otoliths are deposited in, or very near to, equilibrium with ambient water, with lower $\delta^{18}O$ values indicating higher temperatures and lower salinity (Campana, 1999). For example, Weidman & Millner (2000) investigated stable isotope ratios in North Sea cod *Gadus morhua* L. They found a good correlation between $\delta^{18}O$ in the otolith and ambient water temperature at the time of aragonite formation in the otolith. Hence, otolith $\delta^{18}O$ does not have the problems of interpretation associated with carbon. Its value as a migration marker will depend therefore on the extent of differences in temperature and salinity within the proposed range of the species investigated. For example, Weidman & Millner (2000) found that individual cod contained a clear oxygen isotope mark of their geographic origin when they considered North Atlantic sites with a range of 20° latitude. The slight temperature and salinity gradients reported between inshore and offshore waters at Gazi Bay (Kitheka, 1997) might, therefore, allow the use of $\delta^{18}O$ values as additional migration tracers.

The present study had two main aims: 1) to test the utility of using stable oxygen and carbon isotope ratios in fish otoliths to trace migrations between mangrove and coral reef habitat at Gazi Bay and 2) to use evidence from otolith isotope ratios to determine reef fish use of mangroves as a nursery site.

MATERIALS AND METHODS

FIELD SITE

All fishes were caught at Gazi Bay (4°22′ S; 39°30′ E), 60 km south of Mombasa, Kenya. The bay covers *c.* 1·5 km^2 and has a tidal range of *c.* 3·5 m. A small, permanent river, the Kindongoweni, flows into the bay from the north. There is relatively little freshwater influence, however, and salinity within the bay fluctuates between 32 and 35, except during the rainy season between May and July when it can reduce to 15 (Kimani *et al.*, 1996). Sea surface temperature varies between 29 and 30° C, except between May and September when it can drop to 26–28° C (Kimani *et al.*, 1996). Hence, temperature in the bay as a whole is similar to that offshore (Kimani *et al.*, 1996), although sea temperature in shallow intertidal areas can often exceed 30° C (M. Huxham, pers. obs.). There is a clear hydrodynamic separation between contiguous biomes at the site; the $\delta^{13}C$ (mangrove) signal is known to fade rapidly with increasing distance from the shore, so that by 2 km distance from the shore there is little influence of mangrove carbon on $\delta^{13}C$ of organic matter in the benthic sediment (Hemminga *et al.*, 1994).

FISHES SAMPLED

Fishing occurred from February to November 2002. Fishes were sampled from two areas; 'inshore' (within the mangrove forests) and 'offshore' (from the reef 3–5 km

offshore). Fishing in the mangroves was conducted at five sites (which included natural and replanted forest) using a stake-netting method modified from Vance *et al.* (1996). A square path, 6 × 6 m, was cleared through the mangroves to allow placement of a 2 m high, 24 m long, 1 mm mesh-size net. The lead line of the net had a rope attached that was buried in the sediment to prevent the bottom of the net lifting up at high tide. The net was deployed at low tide along the cleared path. It was rolled down to the level of the sediment and left until high water, when the top of the net was lifted onto wooden stakes, placed at *c.* 3 m intervals, such that it cleared the water. At the next low water, the net was inspected for fishes. Although laborious and limited to small catches, this method allows the capture of fishes that actually enter the mangrove forests at high tide. Other approaches, such as seining in nearby creeks, leave open the possibility that fishes caught are simply entering the bay but not using the mangroves. Hence it was possible to identify with confidence species that used mangrove habitats at Gazi Bay. Huxham *et al.* (2004) provide a more detailed description of the field site and catch methodology. 'Offshore' specimens were obtained from local fishermen who caught them on the reef. All fishes caught were placed into one of three putative categories: a) 'mangrove residents', b) 'migrants' and c) 'offshore residents'. Mangrove residents were those species that were found in this study (and reported in the literature) as both adults and juveniles in inshore samples. Migrants were those found as juveniles in inshore samples and as adults in offshore samples. Offshore residents were those found only offshore, and which were reported in the literature not to use mangrove habitats.

OTOLITH PREPARATION AND ANALYSIS

Sagittal otoliths were removed and submerged in a sodium hypochlorite (bleach) bath for 15 min to remove any organic material adhered to the surface. They were processed [using methods similar to Kalish (1991*a*)] to separate three sections: the outer edge, the larval nucleus and the region just beyond the larval section (post-larval) for phosphoric acid decomposition to liberate CO_2 for stable isotope analysis. The outer section was separated using a Hobbycraft© drill and the powdered otolith collected on GF filter paper using a vacuum pump. The filter paper was heated at 400° C for 4 h to remove any organic material before powder collection. The otolith sides were then ground using 1200 grade (20–30 μm grit size) wet-dry sandpaper until the sides were flat. They were then immersed in 1 M HCl until the radius was approximately halved. Increments were used to help estimate the area corresponding to the first year of life (post-larval section); this was powdered using the drill and collected with the vacuum pump. The larval section was identified as a very dark spot in the centre of the otolith, using a light microscope. This was powdered using a pestle and mortar and the powder collected with the vacuum pump.

The otolith powders were weighed into quartz buckets and plasma ashed. Each sample in turn was subjected to a common bath of 100% H_3PO_4 to produce CO_2, which was cryogenically purified and analysed for $\delta^{13}C$ and $\delta^{18}O$ on a VG Prism II mass spectrometer. Values are reported with respect to V-PDB; internal standards run over the entire sample set give a S.D. of 0·13 and 0·20 for $\delta^{13}C$ and $\delta^{18}O$ respectively.

Intrafish variation in $\delta^{13}C$ and $\delta^{18}O$ was examined by comparing the left and right otoliths of seven fishes. The relationship between $\delta^{13}C$ and $\delta^{18}O$ from the same otoliths was explored using correlation and regression. The mean $\delta^{13}C$ and $\delta^{18}O$ values for larval and adult stages of residents, migrants and offshore species were compared using paired *t*-tests (pairing within individuals). Cohen's *d*, a measure of 'effect size', was also calculated for each within-fish comparison; this approach is recommended by Nakagawa (2004) in cases where statistical power is low. The procedure was used for correlated designs (or paired groups in the present study) proposed by Dunlap *et al.* (1996): $d = (M_e - M_c)\ S^{-1}$, where, *d* is Cohen's effect size, M_e is the mean of the outer otolith sections, M_c is the mean of larval otolith sections and *S* is S.D. Cohen (1988) cautiously suggests classification of 'effect size' into the following categories: $d < 0{\cdot}2$ suggests a small 'effect size', $0{\cdot}2 < d < 0{\cdot}5$ suggests a medium 'effect size' and $d > 0{\cdot}5$ suggests

a large 'effect size'. The sign of the 'effect sizes' calculated shows the direction of the change; positive d means $M_e > M_c$ and *vice versa* for negative d.

CONTRIBUTION OF METABOLIC CARBON TO OTOLITH SIGNATURE

Where data were available on the signature of metabolic carbon in a species, the contribution of this carbon to the otolith signature was calculated using the following mass balance model taken from Kalish (1991*b*): $\delta^{13}C_{oto} = (\delta^{13}C_{b,sw} + \varepsilon_{s\text{-}b})\ [(1 - M\%(\delta^{13}C)\ 0{\cdot}01)] + (\delta^{13}C_{meta} + \varepsilon_{s\text{-}a})[(M\%(\delta^{13}C)\ 0{\cdot}01)]$, where $M\%(\delta^{13}C)$ is the percentage of metabolically derived carbon in the otolith, and $\delta^{13}C_{meta}$, $\delta^{13}C_{oto}$ and $\delta^{13}C_{b,sw}$ are the isotopic ratios of metabolically derived CO_2, otoliths and seawater bicarbonate respectively; this last value was taken as 2, following Kalish (1991*b*), for all calculations. $\varepsilon_{s\text{-}b}$ is the calcium carbonate–bicarbonate enrichment factor, with a value of 2·5 for the present study, following the equation given in Kalish (1991*b*) and assuming a sea temperature of 28° C. $\varepsilon_{s\text{-}a}$ is the fractionation factor between aqueous CO_2 and $CaCO_3$. Using a modified version of the equations given in Kalish (1991*b*) this was calculated as 11·1, again assuming 28° C. This model assumes there are only two sources of carbon ($\delta^{13}C_{meta}$ and $\delta^{13}C_{b,sw}$) with different $\delta^{13}C$ values, that contribute to otolith carbonate ($\delta^{13}C_{oto}$). The difference between the carbon sources' isotopic compositions, in conjunction with the measured otolith carbon isotopic composition, can be used in this model to calculate the relative contributions to otolith carbon from each source. Kalish (1991*a*,*b*) provides a full discussion of the logic supporting the use of the two fractionation factors ($\varepsilon_{s\text{-}b}$ and $\varepsilon_{s\text{-}a}$), and cites empirical studies used to calculate these. To use this model, the otolith $\delta^{13}C$ values were measured during the present study and fish muscle $\delta^{13}C$ ($\delta^{13}C_{meta}$) values were taken from Marguillier *et al.* (1997), who sampled fishes in Gazi Bay for isotope analysis. Therefore, the equation had to be rearranged in order to calculate $M\%[\delta^{13}C]$ in the otolith, assuming a $\delta^{13}C_{b,sw}$ value of 2. This method is also similar to two-source mixing models used by Darnaude *et al.* (2004).

RESULTS

Two hundred and fifty-eight fishes, of 20 different species, were caught within the mangroves. The majority of individuals were juveniles. Forty-eight adult fishes were collected by fishermen on the offshore reef. Fishes were grouped as mangrove residents (two species), migrants (five species) and offshore residents (two species) (Table I). Otoliths from a total of 59 fishes were removed and processed. Because of suspected impurities and difficulty with obtaining sufficient material (particularly in the case of *Scomberoides commersonnianus* Lacepède, a species with very small and brittle otoliths) isotope signatures were recorded from a total of 50 individuals.

PAIRED OTOLITHS

$\delta^{13}C$ and $\delta^{18}O$ values were taken from paired left and right otoliths of five *Lutjanus sanguineous* Cuvier. Since technical problems resulted in a smaller sample size from this species than intended, the data were supplemented with values from two *Valamugil seheli* (Forsskål) individuals. Plots showed some deviation from 1:1 correspondence, especially in the case of $\delta^{18}O$ (Fig. 1; this deviation was increased by removal of the outlier in this plot). There were smaller mean differences within pairs for $\delta^{13}C$ than for $\delta^{18}O$ (mean ± S.D.: 0·03 ± 0·6‰, and 0·31± 0·15‰ respectively).

TABLE I. Stable isotope values for fish otoliths

	Species (*n*)	$\delta^{13}C$ (‰) mean ± S.D. by otolith section			$\delta^{18}O$ (‰) mean ± S.D. by otolith section			*d*	
		Outer	Post-larval	Larval	Outer	Post-larval	Larval	$\delta^{13}C$	$\delta^{18}O$
Migrants caught offshore	*Sphyraena jello* (2)	−3·49 ± 0·24	−2·39 ± 0·91	−5·96 ± 1·29	−0·81 ± 0·00	0·77 ± 0·31	−0·93 ± 0·22	2·65	0·80
	Lutjanus fulviflammus (8)*	−3·37 ± 0·80	−3·06 ± 0·73	−4·09 ± 0·77	−0·97 ± 0·90	−0·56 ± 0·99	−0·82 ± 0·71	0·91	−0·18
	Lethrinus harak (6)*	−3·15 ± 1·02	−3·83 ± 1·00	−4·83 ± 1·03	−0·58 ± 0·62	−0·99 ± 0·37	−1·05 ± 0·16	1·64	1·02
	Sphyraena putnamiae (8)	−3·26 ± 0·55	−3·05 ± 0·35	−3·73 ± 0·72	−0·97 ± 0·50	−0·59 ± 0·65	−0·59 ± 0·58	0·64	−0·66
	Lutjanus ehrenbergi (2)	−8·03 ± 0·85	−7·36 ± 1·71	−7·04 ± 2·36	−1·12 ± 0·12	−1·56 ± 0·04	−0·96 ± 0·85	−0·56	−0·26
Offshore residents	*Scomberoides commersonnianus* (3)	−5·18 ± 1·39	−5·92 ± 0·33	−5·51 ± 0·34	−1·01 ± 0·63	−1·38 ± 0·12	−0·95 ± 0·42	0·33	−0·11
	Lutjanus sanguineus (10)	−3·56 ± 0·74	−3·64 ± 0·90	−3·92 ± 0·89	−1·09 ± 0·15	−1·09 ± 0·27	−0·90 ± 1·07	0·47	−0·26
Mangrove residents	*Sphaeramia orbicularis* (7)	−5·41 ± 1·36	−5·58 ± 0·91	−5·53 ± 0·98	−0·56 ± 0·92	−0·57 ± 0·43	−1·06 ± 0·45	−0·07	0·69
	Valamugil seheli (4)	−0·55 ± 3·01	−0·67 ± 4·45	−3·45 ± 4·25	−0·72 ± 0·97	−0·38 ± 0·85	−0·17 ± 0·56	0·58	−0·51

*Significant difference ($P < 0·05$) between outer and larval sections using paired *t*-tests. *d* is Cohen's effect size for differences between larval and outer sections.

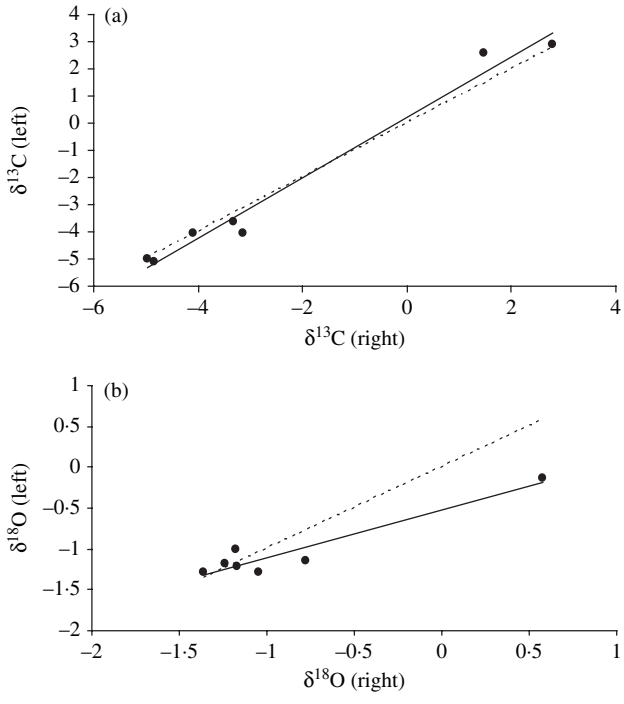

FIG. 1. (a) δ^{13}C and (b) δ^{18}O values from seven pairs of otoliths, analysed to explore intrafish variability. The curves were fitted by: (a) $-y = 1{\cdot}113x + 0{\cdot}225$ and (b) $-y = 0{\cdot}582x - 0{\cdot}521$. The broken line represents a 1:1 relationship.

CHANGES BETWEEN AGE CLASSES

Mean δ^{13}C and δ^{18}O values for the different otolith sections of the nine species, along with 'effect sizes', are given in Table I. Since separate *t*-tests were performed on each species, there is a danger of inflated type I error due to multiple testing (although correct interpretation might depend on whether each species is seen as testing a separate hypothesis, or whether all species are part of the same hypothesis). The small sample sizes, however, mean that statistical power for some species is low; adopting Bonferroni corrections in such circumstances results in unacceptable type II error rates (Nakagawa, 2004). Hence, the original *P*-values were used, and should be interpreted in the context of the relevant sample and 'effect sizes'.

Mangrove residents

Sphaeramia orbicularis (Cuvier) and *V. seheli* were the two species identified as mangrove residents. δ^{13}C values for *S. orbicularis* were amongst the most negative recorded in this study, and showed little variation between the three sections of the otoliths (at 0·12‰, the range was the lowest for any species investigated; Table I). δ^{18}O values of the larval sections were more negative than for both of the outer sections, implying that larvae may have been exposed to higher temperatures or lower salinities than juvenile and adult fish.

In contrast, *V. seheli* otoliths had the highest average $\delta^{13}C$ recorded, and showed a large (2·9‰) increase in $\delta^{13}C$ and a decrease in $\delta^{18}O$ moving from larval to adult sections, possibly implying a reduced reliance on mangrove habitat with age. The differences in $\delta^{13}C$ between larval and adult sections for both species were non-significant (paired *t*-tests: *S. orbicularis*: d.f. = 6, $P > 0{\cdot}05$ and *V. seheli*: d.f. = 3, $P > 0{\cdot}05$). Cohen's *d* for $\delta^{13}C$ showed a very small difference in *S. orbicularis* and a large difference in *V. seheli*. Comparisons of $\delta^{18}O$ values for these species, and all others, also showed no significant differences between life stages. Cohen's *d* for $\delta^{18}O$ showed large differences for both species, a positive difference for *S. orbicularis* and a negative difference for *V. seheli*.

Offshore residents

Lutjanus sanguineus and *S. commersonnianus* were identified as offshore residents. Both species showed little variation in $\delta^{13}C$ between age classes, with no significant differences recorded (Table I). 'Effect sizes' calculated for $\delta^{13}C$ were in the medium category and positive for both species. Contrary to expectations, $\delta^{13}C$ values for *S. commersonnianus* were more negative than for both of the mangrove resident species.

$\delta^{18}O$ values were slightly higher in the larval than the outer sections in both species; 'effect sizes' were small for *S. commersonnianus* and medium for *L. sanguineus* and both 'effect sizes' were negative. This suggests that the fish are exposed to slightly lower water temperatures as larvae, and move to waters with higher mean temperature as they mature; the high S.D. recorded for larval *L. sanguineus* might also suggest a widespread distribution of juveniles compared with adult fish. The $\delta^{18}O$ values for these two species were generally more negative than those for the mangrove resident species. This is contrary to expectations, given the higher seawater temperatures and lower salinities inshore.

Migrants

Five species were identified as migrants: *Sphyraena jello* Cuvier, *Sphyraena putnamiae* Jordan & Seale, *Lutjanus ehrenbergi* (Peters), *Lethrinus harak* (Forsskål) and *Lutjanus fulviflammus* (Forsskål).

Sphyraena jello $\delta^{13}C$ values showed a wide range between age classes (Table I), with larval sections of otolith being much more negative than post-larval and adult. The non-significance of this difference may be due to the low power of the test caused by the small sample size. $\delta^{18}O$ values showed the largest range recorded between different age classes. Whilst the larval and outer sections were similar, the post-larval section $\delta^{18}O$ was positive, indicating the fish had been exposed to relatively low temperatures at this point.

Lutjanus fulviflammus showed a significant increase in mean $\delta^{13}C$ values between larval and outer sections (paired *t*-test, d.f. = 7, $P < 0{\cdot}05$). $\delta^{18}O$ values were similar for both outer and larval sections, whilst post-larval sections were slightly less negative, again indicating the fish had been exposed to lower temperatures during the post-larval period.

Lethrinus harak $\delta^{13}C$ values showed an increase with age, with a significant difference between larval and outer sections (d.f. = 5, $P < 0{\cdot}05$). $\delta^{18}O$ values

showed a similar pattern to $\delta^{13}C$; larval sections were the most negative, post-larval less negative and outer sections least negative. This suggests these fish were exposed to relatively high water temperatures (or low salinity) as larvae and then migrated to lower temperature waters as they matured.

Lutjanus ehrenbergi $\delta^{13}C$ values were similar for all three otolith sections and were the most negative of all species studied. $\delta^{18}O$ values from this species were most negative for post-larval sections, less negative for outer sections and least negative for larval sections. This suggests exposure of the larval fish to the lowest temperatures and exposure of the post-larval fish to the highest temperatures.

Sphyraena putnamiae also had similar $\delta^{13}C$ values for all three otolith sections. $\delta^{18}O$ values were most negative for outer otolith sections and less negative for the other two sections, indicating lower temperatures as juveniles and higher temperatures as adults.

Although only two of the five species categorized as migrants showed significant differences in $\delta^{13}C$, all showed large effects sizes for changes between outer and larval otoliths. Four of the five species in this category showed the four largest effect sizes recorded for all species; all of these were positive. *Lutjanus ehrenbergi* showed the smallest 'effect size' for fishes in this category, and the only one that was negative.

A scatterplot of $\delta^{13}C$ and $\delta^{18}O$ values for all species and all age categories showed a positive relationship between the two variables, but with considerable scatter (Fig. 2). These data contain a mix of results including individuals from

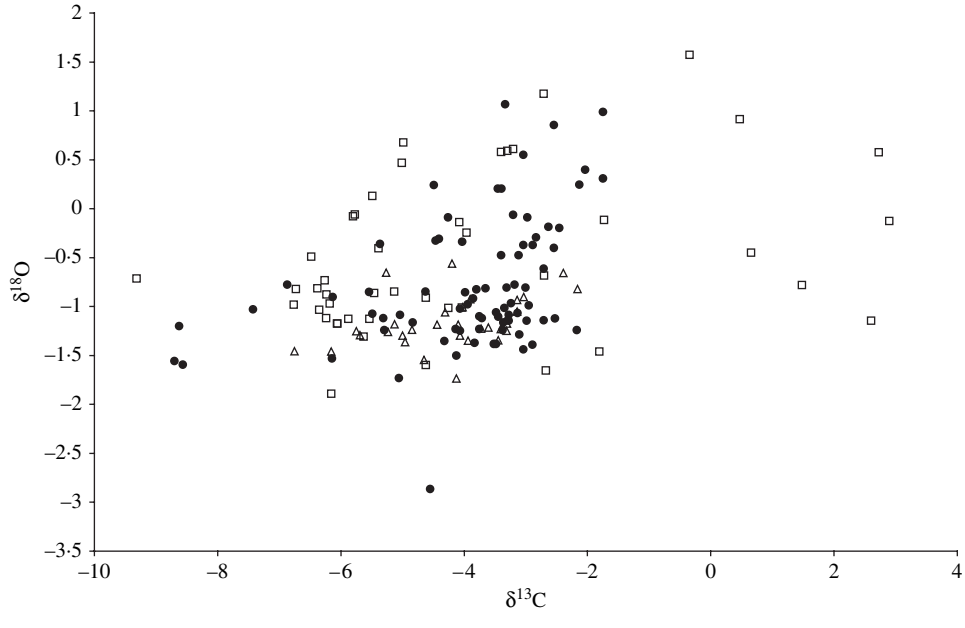

FIG. 2. $\delta^{18}O$ plotted against $\delta^{13}C$ for all otolith samples analysed: offshore residents (△), mangrove residents (□), and migrants (●). The figure includes data from all three age categories.

within the same species and the same genus, hence were not treated as independent because of the possible confounding effects of phylogeny. Therefore no formal test of correlation was performed.

METABOLIC CONTRIBUTION TO OTOLITH CARBON

Marguillier *et al.* (1997) provide data on tissue $\delta^{13}C$ for three species in common with the current study: *S. orbicularis*, *L. fulviflamma* and *V. seheli,* with values of −18·85, −14·17 and −19·31‰ respectively. Assuming that they sampled adults (and hence using the outer otolith values recorded for these species in the present study), this gives estimated per cent contributions of metabolic carbon to the otolith signatures of 29 for *S. orbicularis,* 26 for *L. fulviflamma* and 14 for *V. seheli*.

DISCUSSION

The absolute values for otolith $\delta^{13}C$ reported here are not powerful indicators of habitat use. There was no general pattern of enrichment going from mangrove resident to offshore species, probably because of the species-specific metabolic effects just discussed. Changes in $\delta^{13}C$ between inner and outer sections of the otolith, however, show more promise as a migration marker. Eight of the nine species investigated show enrichment in ^{13}C with age, which could be caused by a slowing of metabolism or re-allocation of resources to reproductive processes (Weidman & Millner, 2000). This change was significant, however, in only two of the proposed migrants. One of the migrants, *S. jello*, showed the largest enrichment recorded for any species; the statistical non-significance of this change is likely to be due to the small sample size (two) and resultant low power. Accordingly, the Cohen's *d* 'effect size' was the largest of any of the species sampled (Table I). Hence changes in otolith $\delta^{13}C$ should be further researched as a tool for migration studies. *Lutjanus ehrenbergi* was anomalous in being the only species that showed depletion in ^{13}C with age. Although the small sample size means that this result must be treated cautiously, this might imply that this species was incorrectly classed as a migrant (despite having juveniles found in mangrove habitats).

Values for $\delta^{18}O$ ranged from −1·56 to 0·77‰, and showed no clear pattern between fish groups. Kalish (1991*b*) reported a significant, negative regression between otolith $\delta^{18}O$ and the temperature at which fishes were reared. Using the regression equation from that paper ($\delta^{18}O = 6{\cdot}69–0{\cdot}326$ T° C) gives a temperature range for the fishes in this study of 18·2–25° C. An alternative relationship reported by Kalish (1991*b*) [and coming originally from Grossman (1982)] has a higher intercept, and would add *c.* 1·6° C to these temperatures. Although closer to the sea surface temperatures, of *c.* 28° C, recorded at Gazi Bay, these figures are still low for the present study site, suggesting that a different relationship might apply. It is possible that changes in the average depth inhabited by fishes at different life-history stages could also have contributed to the variability found. For example, *S. commersonnianus* juveniles may inhabit relatively deeper water, thus causing the higher value for $\delta^{18}O$ recorded in their sub-adult otolith sections; little is known about the depth distribution of the

species considered in the present study. Kalish (1991*b*), however, found a range of >4‰ in $\delta^{18}O$ values from fishes raised at the same temperature. In addition, there was intrafish variation (between whole paired otoliths from the same individual) of up to 0·53 in $\delta^{18}O$, with less intrafish variation for $\delta^{13}C$ values. The present study concurs with this; the regression between paired otoliths deviates markedly from unity in the case of $\delta^{18}O$ (Fig. 1). Kalish (1991*b*) speculated that these differences could arise due to 'metabolic differences at the otolith level' as well as analytical error. An additional source of error in the present study is possible differences in the amounts of material (and therefore slight differences in the life period studied) taken with the drill from paired otoliths. Hence, ambient temperatures derived from small sample sizes of wild fish otoliths may have wide CI intervals. Given this scatter and the absence of obvious trends in the $\delta^{18}O$ data presented here, it is clear that stable oxygen isotopes are unlikely to be powerful markers of small-scale migration in sites such as Gazi Bay. This reflects the rather small recorded differences in salinity and temperature between inshore and offshore areas here, and supports the conclusion in Kalish (1991*b*) that large sample sizes of wild fishes would be needed for accurate temperature reconstruction using these methods.

Like previous studies (Kalish, 1991*a*; Thorrold *et al.*, 1997; Weidman & Millner, 2000), the current work found a positive relationship between $\delta^{13}C$ and $\delta^{18}O$ recorded from the same individual fishes (Fig. 2). Kalish (1991*a*) suggests this arises as a result of a temperature-related mechanism linking $\delta^{18}O$, deposited at equilibrium, with $\delta^{13}C$; the influence of temperature on metabolic rate is an obvious possibility. Another possible cause in these data is that individuals that consume more mangrove-derived material (and hence have relatively low otolith $\delta^{13}C$ values) may also spend relatively more time in shallow, warmer, less saline waters (and thus have relatively low $\delta^{18}O$ values). The large scatter present in the data here is expected given the other sources of variability, such as differences in diet and metabolic kinetics, between the fish species sampled.

The present study concurs with previous work (Kalish, 1991*a*, *b*; Weidman & Millner, 2000) in finding otolith aragonite enriched in ^{13}C compared with metabolic sources of carbon. This was the case for all species of fishes studied. Kalish (1991*b*) calculated that *c.* 30% of otolith carbon in Australian salmon *Arripis trutta* (Forster) came from metabolic sources, whilst Weidman & Millner (2000) estimated a 20% contribution to cod *Gadus morhua* L. otoliths. Hence, the values for percentage contribution of metabolic carbon to *S. orbicularis* and *L. fulviflamma* otoliths lie within the range reported in the literature. The calculated value for *V. seheli*, at 14%, is lower than those usually reported for other species. It is low because of the large difference between the values for otolith and muscle $\delta^{13}C$. *Valamugil seheli* had the highest otolith $\delta^{13}C$ value recorded in the present study, a surprising finding given its assumed status as a mangrove 'resident' and the low tissue value of 19·31‰ reported by Marguillier *et al.* (1997). Two possible explanations for this anomaly are first, that the reported tissue $\delta^{13}C$ value is misleading and second, that *V. seheli* has an unusually low metabolic rate. The first possibility could arise if the fish sampled by Marguillier *et al.* (1997) had recently spent an unusually high proportion of their time feeding in and around mangroves; the otolith signals should provide an integrated picture of feeding not available from other tissues. Although *V. seheli* is not

restricted to mangrove habitats (Huxham *et al.*, 2004), it was captured inside the mangroves in the present study and is regarded as a mangrove-associated species. The second explanation is supported by the fact that *V. seheli* was the only herbivorous species studied. *Sphyraena jello* and *S. putnamiae* are piscivores, *S. commersonnianus*, *L. sanguineus*, *L. ehrenbergi*, *L. fulviflammus* and *L. harak* consume fishes and a range of invertebrates, *S. orbicularis* preys predominantly on crustaceans whilst *V. seheli* consumes algae and detritus (Salini *et al.*, 1990; de Troch *et al.*, 1998; www.fishbase.org). Hence *V. seheli* might be expected to have a lower metabolic rate than faster moving carnivores. Previous studies (Thorrold *et al.*, 1997; Kalish, 1991*a*) have shown evidence that otolith $\delta^{13}C$ is negatively related to metabolic rate.

In conclusion, this work shows that otolith $\delta^{18}O$ and $\delta^{13}C$ values on their own are not reliable indicators of habitat use and migration at Gazi Bay. This is because of uncertainties over the species-specific differences in derivation of carbon from metabolic and non-metabolic sources, and the considerable scatter (including intrafish variation) recorded in the data. Relative changes of $\delta^{13}C$ between inner and outer parts of the otolith, with large 'effect sizes', however, were consistent with the proposed migration behaviour of four of the species studied, despite the variability in the data and the relatively crude methods of otolith preparation. Hence, such changes do show promise as migration tracers and should be further researched.

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