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Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish

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ABSTRACT: Estuaries and associated seagrass habitats are thought to be important nursery areas for many fishes. There is, however, no direct evidence for movement of fish from estuaries to reefs. The aim of this study was to determine if populations of *Achoerodus viridis* (Labridae) on rocky reefs were sustained by (1) recruitment to estuarine seagrass habitat followed by movement to rocky reefs, (2) direct recruitment to rocky reefs, or (3) a combination of the two. Recruits were collected from estuarine seagrass and rocky reef habitats and elements in their otoliths analysed by inductively coupled plasma-mass spectrometry (ICP-MS) to determine if different 'elemental fingerprints' could be found. Higher concentrations of Zn, Al, Pb, Mn, Ba and Co were found in otoliths of recruits from estuarine seagrass habitat than in otoliths of recruits from coastal reefs, the latter 3 elements showing significant differences. Strontium occurred in significantly higher concentrations in otoliths of recruits from coastal reefs. Differences in concentrations of some elements in the otoliths of recruits allowed fish from the 2 environments to be distinguished with a high degree of accuracy, enabling the contribution of estuarine recruitment to sustaining reef populations to be determined. Elemental composition of the juvenile core of otoliths from adults on reefs was related to the composition of otoliths of recruits from each environment to identify historical recruitment environments. Discriminant function analysis showed that 41% of adults had recruited to estuaries and 59% had recruited to reefs, but these figures may be overestimated because adults must be assigned to 1 of the 2 groups. There was evidence to suggest that some adults may form a third intermediate group. Further validation (e.g. comparison with laser or probe based methods and tagging techniques) of our approach is warranted. Elemental techniques may have great potential for resolving fisheries problems and identifying broader scale effects of environmental degradation.

KEY WORDS: Reef-fish · Otoliths · Trace elements · Movement · ICP-MS Recruitment · Nursery habitat

INTRODUCTION

Estuaries and associated seagrass habitats are thought to be important nursery areas for many fishes whose adults are found on reefs (Bell & Pollard 1989, Parrish 1989). It is generally argued that juvenile fish recruit to estuarine nursery habitats and then move to coastal reefs at greater sizes and ages (Bell & Worthington 1993). However, direct evidence of this process is lacking, because of the difficulties of quantifying movement (Jones 1991). The best evidence for movement is observing recognisable or tagged fish shifting from one place to another; however, this is difficult because of problems with tagging delicate juveniles by conventional methods and high rates of mortality at early life history stages. Alternative methods for determining the origins and movement of fish are needed; for example, analysing trace elements in calcified structures.

Calcified tissues are deposited in layers over time and differences in microchemistry between the layers can be resolved to within days or years of their deposition (Coutant 1990). The calcium carbonate and trace elements that make up 90% of the otolith are thought to be derived primarily from the water (Simkiss 1974), although they may not necessarily track ambient water

chemistry directly (Kalish 1989, Fowler et al. 1995). There is likely to be a closer relationship between ambient water chemistry and concentration in the otolith for some elements than for others, due to the properties of the element itself Differences in water chemistry between environments (e.g. estuaries and rocky reefs) may occur, enabling calcified tissues to retain a chemical record that can be related to the age of the fish and the physical and chemical properties of its environment (Radtke et al. 1990, Gunn et al. 1992, Radtke & Shafer 1992, Kalish 1993). Differences in chemical components between layers of calcified tissues may therefore indicate past environments inhabited by individual fish and possible patterns of movement.

Chemical constituents in calcified tissues (otoliths and scales) have allowed individual diadromous fish to be related to periods of freshwater and marine residence, and enabled freshwater and saltwater fish within the same species to be distinguished (e.g. Belanger et al. 1987, Kalish 1990, Secor 1992, Coutant & Chen 1993). Chemical analyses have also been used to distinguish stocks or sub-populations within marine species (e.g. Mulligan et al. 1987, Edmonds et al. 1989, 1991, 1992, Campana & Gagne 1994, Campana et al. 1994, Proctor et al. 1995), but only recently have differences in microchemistry been used to detect movement in non-diadromous fish (e.g. Campana et al. 1995).

This study investigates potential movement between estuarine seagrass and rocky reef environments for blue groper *Achoerodus viridis* (Labridae) using an application of otolith microchemistry. Large numbers of newly settled *A. viridis* have been found in seagrass habitats in estuaries (Bell et al. 1987, McNeill et al. 1992) and smaller numbers have been found on rocky reefs (Gillanders & Kingsford 1993), yet adults are only found on rocky reefs. Juveniles disappear from seagrass habitat after 3 to 4 mo (Worthington et al. 1992), which is thought to be the result of movement to coastal reefs, although mortality could also account for the disappearance of fish (Gillanders & Kingsford 1993). The objective of this study was to determine if populations of *A. viridis* on rocky reefs are sustained by recruitment to estuarine seagrass habitat followed by movement to rocky reefs, direct recruitment to rocky reefs, or by a combination of the two. The specific aims were to (1) determine if otoliths of recruits in seagrass and rocky reef environments have different 'elemental fingerprints' (sensu Campana et al. 1994) or composition and (2) determine the possible source (i.e. seagrass or rocky reef) of adult reef fish by analysing the juvenile core (i.e. portion of otolith that was laid down when the fish was a juvenile) of otoliths from adults and relating this to the composition of otoliths of recruits from each environment.

MATERIALS AND METHODS

Collection of fish. ***Collection of recruits:*** Twenty-eight recruits from rocky reef and 27 recruits from estuarine seagrass (*Zostera capricorni*) environments were collected between November 1993 and February 1994 to determine whether elemental composition of their otoliths could be related to their environment. Recruits from both seagrass and rocky reef environments were collected from the shallow fringe of the subtidal zone (<2 m) at 4 sites in each environment (n = 5 to 10 fish per site; Fig. 1). A beach seine was used to collect fish from seagrass areas, whereas a handspear was used to collect fish from rocky reefs; all fish were frozen after collection. Size frequency distributions of collected fish were similar between rocky reef and seagrass environments [mean length (range): rocky reef fish 59 (37.5-80.8) mm SL; seagrass fish 48 (33.7-86.9) mm SL]. Labrids spend approximately 2 to 6 wk as pelagic larvae before they settle (Victor 1986) and therefore part of the juvenile core of the otolith represents the pre-settlement phase. The lower size limit of fish collected for analyses was, therefore, chosen so that fish would have settled approximately 4 to 8 wk before they were collected (based on cohort analysis). It was assumed that this amount of time was sufficient for the incorporation of the elemental fingerprint from the estuarine or reef environment. The

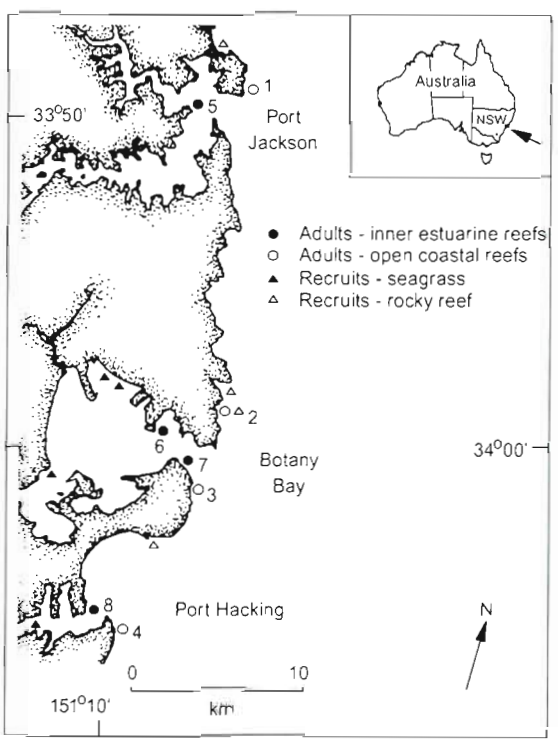

Fig. 1. Location of collection sites for adults and recruits of *Achoerodus viridis*

mean weight of otoliths used in the analyses (0.335 mg) was approximately 8 times the mean weight of otoliths from fish that had recently settled (0.043 mg), again suggesting that there was sufficient incorporation of elemental fingerprint since time of settlement. The upper size limit was the largest fish found in seagrass habitat.

Collection of adults: A total of 58 adult fish were collected by spearfishing (at depths <12 m) from 8 sites (n = 7 to 8 fish per site) to determine the composition of their juvenile core and the environment they had recruited to (Fig. 1). Sites represented inner estuarine and open coastal reefs. The sagittal otoliths were removed, rinsed and stored dry in acid-washed glass vials for later analyses. Fish ranged in length from 140 to 250 mm SL and were 0–4$^+$ years of age (Gillanders 1995).

Sample preparation. Milli-Q water and nitric acid (AristaR, BDH chemicals) were used throughout this study for otolith cleaning and digestion and for preparation of standards. Multi-element standards (see below) were prepared from individual stock solutions (100 µg ml^{-1} or 10 µg ml^{-1}) in 1% nitric acid (Plasmachem, ICP-MS standard). All plasticware/glassware was washed in 0.5% EDTA for at least 4 h, soaked in 10% HNO_3 for at least 24 h and repeatedly rinsed in Milli-Q water prior to use to avoid contamination. All standards and samples (except diamond saw cutting) were prepared in a laminar flow cabinet to minimise possible sources of contamination.

Otoliths from recruits: Sagittal otoliths from recruits were dissected using glass probes in a laminar flow cabinet, thereby avoiding potential contamination from metal instruments, and then cleaned and washed in Milli-Q water. One otolith was placed in a 10 ml polycarbonate tube and used for analysis, the other was placed in a clean 0.5 ml microcentrifuge tube and used for weighing. Otoliths used for analysis were not used for weighing in order to prevent possible contamination, especially of smaller otoliths. A preliminary study indicated that there was a 1:1 relationship between the weights of sagittal otoliths within a given fish ($r = 0.995$, $p < 0.001$, $n = 20$). Otoliths were removed from the microcentrifuge tube and weighed using a Cahn 25 automatic electrobalance (precision ±0.0007 mg).

Otoliths from adults: An otolith from each fish was embedded in thermoplastic cement and the juvenile core extracted by cutting the bulk of the otolith material away with a low speed diamond saw, so that the core region was approximately the same length and width as otoliths from the largest recruit collected in seagrass. The thermoplastic cement was removed from the juvenile core with 100% acetone (Rhone Poulenc Chemicals, AR grade) and the juvenile core further dissolved in 10% HNO_3, to give a more 3-dimensional approach. These techniques did not cause increases in concentration of elements that may result from contamination (see Dove et al. in press). The remaining juvenile core of each otolith was then weighed using a Cahn 25 automatic electrobalance, cleaned in 1% HNO_3 for 15 s to remove any possible contamination resulting from weighing, rinsed in Milli-Q water and placed in a 10 ml polycarbonate tube.

Samples for inductively coupled plasma-mass spectrometry (ICP-MS) were processed in liquid form (i.e. solution-based). Samples were left to dissolve in 10% HNO_3 for at least 1 h inside a laminar flow cabinet. Samples were then diluted with Milli-Q water to 1% HNO_3. Blank samples were prepared in the same manner, but no otolith was present.

Analysis procedure. The inductively coupled plasma-mass spectrometer was a Perkin-Elmer SCIEX ELAN 5000. A detailed description of measurement parameters is given in Table 1. All analyses were carried out in 'quantitative analysis mode', which provides accurate quantitative measurements on samples over a wide range of concentrations (Perkin-Elmer 1992). Within this mode, 2 methods of machine calibration were used: 'external standardisation' and 'addition calibration' (Perkin-Elmer 1992).

Analysis of samples with calibration by external standardisation: The macroelement Ca, and microelement Sr, were analysed in external calibration mode. Calibration was accomplished by using a blank and 4 or 5 standard solutions (spanning the appropriate concentration ranges) run at the beginning of each session with spikes added to additional blank samples for recovery verification. The matrix of both the blank and the standard solutions was 1% HNO_3. The blank intensity, observed while running a 1% HNO_3 solution, was subtracted from both the standards and the sample intensities to obtain net intensities. ^{44}Ca was used for analyses due to major isobaric interferences on the most abundant isotope (from ^{40}Ar); ^{86}Sr was analysed be-

Table 1. Inductively coupled plasma-mass spectrometry (ICP-MS) measurement parameters

Measurement parameters	
Resolution mode	Normal (peak width at 10% peak height of 0.7–0.9 atomic mass units)
Measurement mode	Sequential
Scanning mode	Peak hop (to masses of interest)
Replicate time	1000 ms
Dwell time	100 ms
Sweeps/reading	10
Readings/replicate	1
Number of replicates	3
Points/spectral peak	1
Omni range settings	+20 Ca, 0 other elements

cause ^{88}Sr gave off-scale readings. Analysis of samples showed results for Ca and Sr were orders of magnitude above detection limits (DL, which were calculated from the concentration of analyte yielding a signal equivalent to 3 times the standard deviation of the blank signal; n = 7) and were precise [mean relative standard deviation (RSD) <1.1%; %RSD = SD/mean × 100, n = 3] and that known concentrations were recovered to acceptable levels (105 to 119%).

Analysis of samples with addition calibration: Eleven elements (Mg, Al, Mn, Ni, Co, Cu, Zn, Sn, Ba, Hg, and Pb) were analysed in addition calibration mode to minimise sample-specific matrix interferences (Perkin-Elmer 1992). Calibration was achieved by running a blank, an unspiked solution and 5 spiked solutions (0.01, 0.1, 1, 5 and 10 µg l^{-1}) at the beginning of each session; further spiked solutions (0.5 µg l^{-1}) were run regularly for recovery verification. The matrix of the blank was the same as for external calibration (i.e. 1% HNO_3), that of the unspiked and spiked solutions was otolith. The major isotope of each element was analysed, except for Ni and Sn. The ^{60}Ni isotope was chosen in preference to ^{58}Ni to avoid the ^{58}Fe isobaric interference, and the ^{118}Sn isotope was used in preference to ^{120}Sn to avoid the possible ^{120}Te isobaric interference. Elements were selected from those analysed based on whether or not they met 3 criteria: element occurred in concentrations greater than the DL, precision was less than 10.5% RSD, and percent recovery of spiked sample was between 75 and 125%. Sample analysis of otoliths from recruits showed that 3 elements could not be determined, either because levels fell below detection limits (Sn and Hg) or because of poor precision (Cu). These elements were not analysed for juvenile cores of adult otoliths. Eight elements (excluding Ca and Sr) showed acceptable precision (<10.5% RSD) and accurate recovery of spiked samples (79 to 125%) for otoliths from recruits. Results for juvenile cores of otoliths from adults showed that concentrations of a further 2 elements (Al and Zn) fell below DL, whereas concentrations of Mg, Ni and Pb gave poor recovery of spiked samples (either >125% or <75%) and therefore were not considered accurate measures, but were used as a relative measure. Three elements (Mn, Co and Ba) from juvenile cores of otoliths from adults met the above criteria.

All samples were initially analysed using addition calibration mode. The data were then reprocessed in external calibration mode to obtain concentrations for Ca and Sr. Each sample took around 3 to 3.5 min to analyse, including a sample read delay of 50 s, and rinse (0.5–1% HNO_3, 0.1% Triton X) between samples of 60 s.

Statistical analysis of data. One factor analyses of variance (ANOVAs) were used to determine whether significant differences occurred between otoliths of seagrass and rocky reef recruits for each element. Discriminant function analysis (DFA) was used to develop an algorithm to classify fish according to whether they recruited to rocky reef or seagrass environments based on the elemental composition of their otoliths (Seber 1984). The apparent error rate (i.e. the proportion of the data set that were misclassified) was calculated using both the resubstitution method and the cross-validation (leave-one-out) method (Seber 1984). Adult fish were then categorised, using the algorithm, as having recruited either to rocky reef or seagrass environments, based on the elemental composition of their juvenile core.

RESULTS

Elemental composition of otoliths collected from recruits

Significant differences in the elemental composition of otoliths were found between recruits collected from seagrass and rocky reef environments. Strontium occurred in significantly greater concentrations in otoliths of fish collected from rocky reefs than in otoliths of fish collected from seagrass, whereas 3 other elements (Ba, Mn, and Co) occurred in significantly greater concentrations in otoliths of fish collected from seagrass (ANOVA, $p < 0.05$; Fig. 2). Although Zn, Pb and Al occurred in greater concentrations in otoliths of fish from seagrass than rocky reefs (Fig. 2), significant differences were not detected (ANOVA, $p > 0.05$). Three elements that showed significant differences (Sr, Mn and Ba) were used as variables in a 2 group (seagrass and rocky reef) DFA. This analysis resulted in a significant separation of recruits between rocky reef and seagrass environments (Wilk's lambda = 0.301, $F_{3,51}$ =

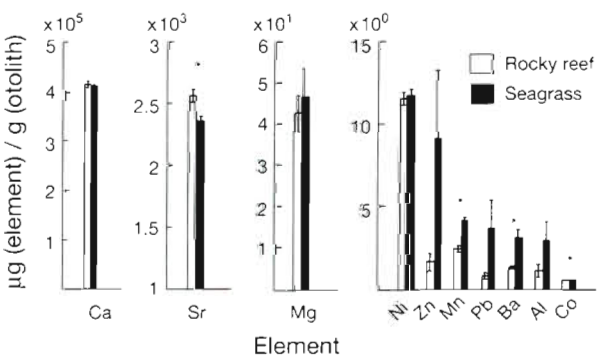

Fig. 2. Mean (±SE) concentration of elements in otoliths from recruits of *Achoerodus viridis* collected from seagrass and rocky reef environments. *significant difference between otoliths of recruits from seagrass and rocky reef using ANOVA ($p < 0.05$)

Table 2. Classification of recruits into rocky reef or seagrass origin by discriminant function analysis (DFA)

Actual site fish collected	Predicted group membership (%) Rocky reef (open coast)	Seagrass (within estuary)
Rocky reef (n = 28)	96	4
Seagrass (n = 27)	7	93

39.57, $p < 0.001$; Table 2); the function was also statistically significant (chi-squared statistic = 61.9, df = 3, $p < 0.001$). There was an error rate of 5.5% when classifying fish using the cross-validation method, which was only marginally greater than when using the resubstitution method (3.6%). The discriminant function coefficients standardised by the within-group standard deviations were 0.855 for Mn, 0.672 for Ba and −0.802 for Sr. A frequency plot of discriminant function scores showed a bimodal distribution enabling rocky reef and seagrass recruits to be separated (Fig. 3a). Recruits from seagrass and rocky reef environments could therefore be distinguished with a high degree of accuracy using otolith microchemistry.

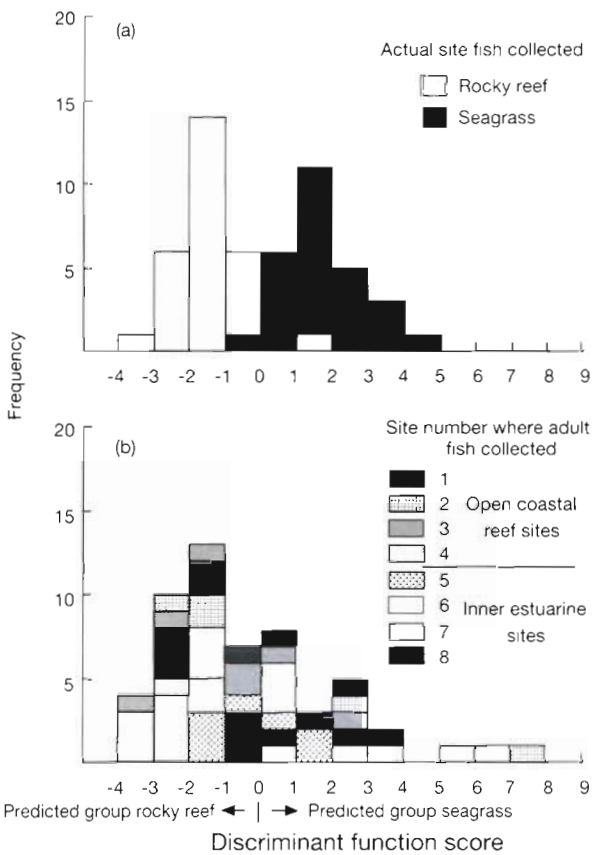

Fig. 3. Frequency distribution of discriminant function scores from DFA showing (a) separation of recruits collected from rocky reef and seagrass environments and (b) results for analyses of the juvenile core of adults that were collected from different sites. Predicted recruitment habitats of adults are also shown (scores > 9, n = 3 fish from Site 2)

Source of adult fish on rocky reefs

Algorithms generated from recruits classified 59% of adult fish as having settled on rocky reefs and 41% in seagrass environments (Fig. 3b), although the number of fish that recruited to the 2 environments varied among sites (rocky reef origin: 37 to 86%, estuarine origin: 14 to 63%; Fig. 3b). Three-dimensional scatterplots of the actual data points showed that recruits from seagrass and rocky reef could easily be distinguished (Fig. 4a), but that some of the adults may actually form a third group (Fig. 4b). The scatterplot suggests that adults have been forced into either group because DFA must assign each individual to 1 of the 2 groups and there is no option for a third group. However, many of the adults clearly fall within the range of values shown by the recruits (Fig. 4b).

DISCUSSION

Constituents of otoliths as a tool for understanding movements among environments

Concentrations of some elements in the otoliths of recruits of *Achoerodus viridis* varied between fish collected from seagrass and rocky reef environments. This enabled elemental fingerprints that were representative of each environment to be used to distinguish recruits of *A. viridis*. A greater amount of Sr was found in otoliths of recruits from rocky reef than in recruits from seagrass habitat. High Sr levels have previously been correlated with high salinity levels (Radtke et al. 1988, Kalish 1990, Secor 1992, Coutant & Chen 1993) and low temperatures (Radtke et al. 1990, Townsend et al. 1992, but see Kalish 1989, Gallahar & Kingsford 1992). Large plumes of low density water are known to extend out of Botany Bay (Kingsford & Suthers 1994), meaning that estuarine seagrass habitats are likely to be exposed to lower salinity waters than coastal rocky reefs. Fish from rocky reefs may therefore be expected to have higher concentrations of Sr. Elements occurring in greater concentrations in otoliths of recruits from estuarine seagrass regions (Al, Ba, Mn, Pb, Co and Zn) occur naturally in freshwater runoff. Furthermore, these elements are also known to be components in paints, fertilisers, etc. which are likely to appear in runoff to rivers and drains through to estuaries and hence be incorporated into estuarine environments (see Beder 1989).

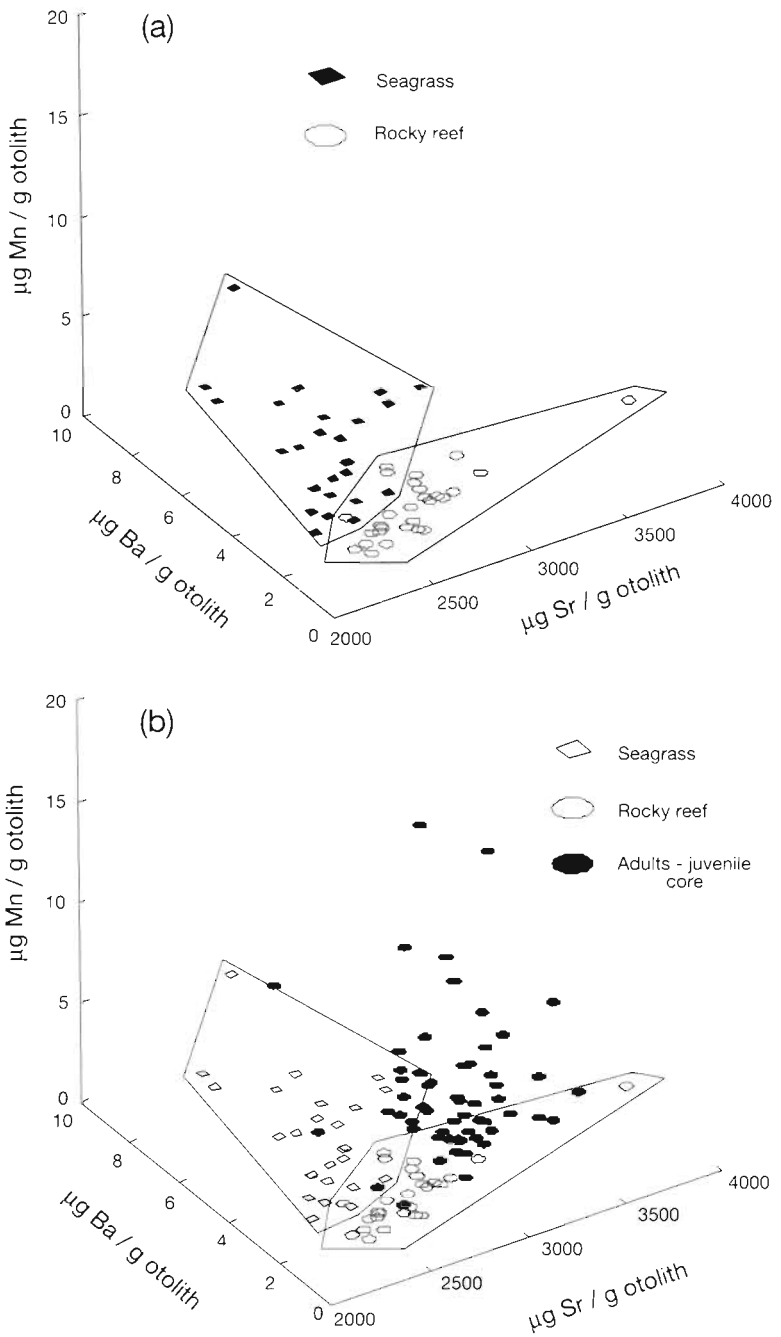

Fig. 4. Concentration of Mn, Ba and Sr in otoliths from (a) recruits and (b) recruits and the juvenile core region of otoliths from adults. Recruits were collected from rocky reef and seagrass environments, adults were collected from rocky reef. Lines around each group of recruits were drawn by eye

Although causal factors contributing to differences in otolith chemistry between recruits collected in seagrass and rocky reef environments are not known, these differences have enabled the use of trace (<15 ppm) and micro- (100 to 5000 ppm) elements in otoliths to reconstruct past life history and, therefore, enabled the determination of possible patterns of movement. Recruits were found in both estuarine seagrass and rocky reef habitats, whereas adults were only found in rocky reef habitat. Analysis of the juvenile core of adult otoliths indicated that some fish could be classified as having originated in either seagrass or rocky reef habitat. Although the DFA suggested that 59% of adults came from rocky reef habitat and that 41% had recruited to seagrass habitat and moved to rocky reef, 3-dimensional scatterplots of the data suggested that some adults may differ from both groups of recruits in terms of amounts of Sr, Mn and Ba in their otoliths. All recruits were collected during 1 recruitment season; therefore, there is no indication of possible inter-annual variability in elemental fingerprints within seagrass or reef habitats. Changes in the amount of freshwater runoff among years may influence elemental fingerprints of juveniles, particularly with respect to concentrations of Sr and Ba. Inter-annual variability (e.g. of Mn) may explain why the elemental concentration of the juvenile core of adult otoliths falls outside the 2 juvenile groups, because adults represent more than 1 age class. Few studies have determined inter-annual variability. Campana et al. (1995) found small differences in elemental fingerprints between samples collected from the same place in 2 different years, and Edmonds et al. (1992) found substantial differences in potassium concentrations in samples taken in 2 different years, but found that other elements showed similar patterns over time. Changing environmental and/or oceanographic conditions have the potential to cause differences in otolith microchemistry; therefore, future studies need to determine the magnitude of changes in elemental fingerprints over time.

Recruits were only collected from estuarine seagrass and open coastal reef environments. Had recruits also been collected from estuarine rocky reefs their otolith chemistry may have been intermediate to that of recruits from seagrass and rocky reef environments. This may explain why a bimodal distribution of discriminant function scores (Fig. 3b) was not found for adult fish and why some adult fish appear to form a third group (Fig. 4b). One approach to determining

whether adults come from estuarine rocky reefs or areas other than the ones in which recruits were collected may be to identify fish that have concentrations of some elements outside the range of concentrations found in recruits from seagrass and rocky reef Using this approach, 14 adults had concentrations of Mn greater than those found in recruits, and 1 of these fish also had concentrations of Sr and Ba lower than that found in recruits. Of these 14 adults, almost two-thirds came from the Port Jackson sites (Sites 1 and 5 in Fig 1), this being the only estuary where recruits from seagrass, of the size used in the analyses, were not found.

The use of a low speed saw and dilute acid enabled the core of an otolith to be separated from the edge of an otolith. Although the preparation of cores was relatively time consuming, analysis of the core using solution-based ICP-MS allowed quantitative measurements of trace elements to be made. The technique of obtaining the core region along with ICP-MS has great promise for the reconstruction of large-scale temporal patterns of trace elements over time (e.g when the fish was a recruit vs when it was a juvenile or adult), but the approach warrants further validation. Results obtained with the present approach and laser ablation or other probe-based methods should be compared. Probe-based approaches will allow better spatial resolution than that obtained in the current study and therefore allow smaller-scale temporal differences in trace elements of otoliths to be detected, but the need for analytical sensitivity will remain.

Another approach to validating the methods of the present study and providing definitive support for movement of fish from seagrass to rocky reef environments may be obtained from tagging studies. Although tagging fish by conventional methods is likely to be difficult and expensive, recent innovations suggest that miniature coded tags or fluorescent tags may be useful (Buckley et al. 1994). Tagging of fish in different habitats and their later collection from reefs will provide investigators with adult fish of known recruitment histories. The juvenile core of these fish could then be analysed and the recruitment habitat determined from algorithms based on the elemental composition of otoliths of recruits. Comparison of microchemistry and tagging results will provide additional information on error rates for classifying adult fish of unknown recruitment habitat. The juvenile core of adult fish with known recruitment histories could also be used to develop algorithms for classifying adult fish with unknown recruitment habitats, replacing the need for developing algorithms based on recruits. Tagging studies are, however, likely to produce results less quickly than chemical methods (Edmonds et al. 1989).

Sustainability of reef-associated populations

With further validation of the current approach (e.g. use of laser or probe-based methods and tagging studies) we will be able to assign fish to their recruitment habitat and determine patterns of movement with more confidence. Results of this study show that at least some adult fish have recruited to both types of environment and therefore some have moved from seagrass to reef In the future, it will be possible to determine survival rates of recruits from each environment, provided that information on the area the different types of environment encompass is known.

Conclusion

The method of extracting the core from the rest of the otolith followed by solution-based ICP-MS, as well as laser ablation ICP-MS techniques, offers great promise for answering questions related to habitat use, exposure to pollutants and stock discrimination. With further validation and determination of inter-annual variability, we believe that analyses of trace and microelements in otoliths and an ability to sample the juvenile core of adult fish will allow the environment (estuarine or reef) in which fish had settled to be determined. Relatively strong differences in water chemistry between estuarine and coastal waters probably make this approach possible.

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