Coastal stocks of fish: from which estuaries are most adults derived?

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The University of Sydney

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Table of contents

Non-technical summary

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OBJECTIVES:
1. To solve a pressing problem for the fishery by determining what proportion of the commercial catch is from different estuaries.
2. To use methods being developed on juvenile fish to establish a chemical "fingerprint" for each estuary so that in future years the proportion of adults from each estuary can be determined.

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED
Differences in elemental chemistry of otoliths among estuaries suggested that elemental fingerprints can be used as a natural tag of the recruitment or nursery habitat. Significant spatial and temporal variation in elemental fingerprints does however mean that it is necessary for adults that are subsequently sampled to originate from one of the year classes of juveniles that are sampled. The results provide a model of replenishment for adult populations. Adults on reefs outside estuaries have come from the estuaries closest to them with little transfer from other estuaries, at least for the 2+ year class of snapper.

The origins of many stocks of fish are unknown. As juveniles, many fish are found in estuarine regions. After a period in these habitats they may leave estuaries for coastal reefs and shelf waters where they may be commercially fished. Currently, we do not know the proportion of individuals in harvested populations that may have spent time in different estuaries as juveniles and whether one or a few estuaries are making substantial contributions to maintaining local stocks. This study used elemental chemistry of ear bones (otoliths) of fish to determine natal or nursery estuaries of adult fish.

Spatial variation in elemental fingerprints of ear bones
The elemental fingerprints of otoliths of three species of sparids were determined to investigate their utility as a natural tag of the nursery habitat. Juvenile snapper (*Pagrus auratus*), tarwhine (*Rhabdosargus sarba*) and bream (*Acanthopagrus australis*) were collected from two sites in each of 15, 6 and 3 estuaries respectively and their otoliths analysed to determine concentrations of elements. Significant differences in otolith chemistry were found for all three species of juveniles collected from different estuaries. The same patterns among estuaries were not seen for all species, although it was not possible to sample the same sites within an estuary for all species. For bream, significant differences in otolith chemistry were found among all three estuaries, whereas for tarwhine the six estuaries were separated into three groups. For snapper, a number of estuaries could be separated, but there was some overlap for other estuaries. All three species were collected from the same site within one estuary and their otoliths analysed. Significant differences were found among species, but the implication of this finding remains unclear as the three species show differences in microhabitat use and may also differ in age. Overall, the differences in

elemental fingerprints among estuaries suggested that they could be used as a natural tag of the natal or nursery habitat.

Temporal variation in elemental fingerprints of ear bones of snapper
The elemental fingerprints of ear bones of snapper were determined for fish collected in each of three recruitment years (1998/1999, 1999/2000 and 2000/2001) from 12-15 estuaries to determine temporal variation in elemental fingerprints for each estuary and to examine whether there may be overlap in elemental fingerprints of fish collected in different years and from different estuaries that may confound subsequent spatial comparisons. Significant differences in otolith chemistry were found among years for individual elements and for multielement fingerprints. Some estuaries showed large variation in multielement fingerprints among years whereas others showed little variation among years. There was some overlap of elemental fingerprints of different estuaries, but these were not always for fish collected in the same year. The significant spatial and temporal variation in elemental fingerprints meant that it was possible to confound spatial differences with temporal differences. This does not prevent determination of natal estuaries of adult fish, but it does suggest that a library of elemental fingerprints needs to be built-up over time for each estuary rather than a single year class of juveniles being used as the elemental fingerprint for a number of year classes of adults. For example, the age of adult fish needs to be determined and elemental fingerprints of juvenile fish from the adult fish's birth year used to determine its natal estuary.

Determining natal estuaries of adult snapper from the commercial fishery
Because the elemental fingerprints of juveniles vary among estuaries or groups of estuaries, the "nursery" estuary of adult fish could now be determined by analysing the juvenile region of adult otoliths. Adult fish from the commercial fishery in the vicinity of Sydney were aged and fish with birth dates during 1998/1999 selected for microchemical analysis. Otoliths of recruits from 1998/99 and 2+ adults with birth dates in 1998/1999 were sectioned and elements within the otoliths analysed using a laser based approach. Maximum likelihood analyses were then used to determine the proportion of juvenile fish and adult fish from different regions (Wallis Lake, Sydney estuaries, Eden, and other estuaries). For juvenile fish, the actual composition ranged from 7 to 53% depending on the estuary or group of estuaries and the estimate of proportion of recruits from the different estuaries ranged from 7.24 to 48.21% suggesting an error rate of <1 to 4.79%, although this error rate has not taken into account the variability in the simulation runs. The majority (89%) of adult fish caught as part of the snapper fishery in the Sydney region originated from estuaries in the vicinity of Sydney, although about 9% of fish had originated from Eden and 2% had come from the remaining estuaries excluding Wallis Lake. After taking into account the variability for the estimated proportions, all fish from the Sydney region may have recruited to Sydney estuaries suggesting that adults on reefs outside estuaries have come from the estuaries closest to them with little transfer from other estuaries, at least for this age class of snapper.

Further work should continue to sample adults from the dominant year classes in the fishery to determine whether models of replenishment between estuaries (where juveniles occur) and open coastal populations (where adults occur) change with different year classes and over time. To determine the recruitment estuary of adults it will also be necessary to sample juveniles to ensure that a baseline data set of elemental signatures exists.

KEYWORDS: Snapper, otolith chemistry, spatial variation, temporal variation, estuary

Acknowledgments

The study was conducted while BMG held ARC postdoctoral fellowships (APD and QEII). We especially thank Julian Hughes (University of Sydney) for his help with many aspects of the project. In addition, we thank Robert McQuilty (RPA hospital) and John Tsiros (Monash University) for help with analysing samples by ICP-MS, Doug Ferrell (NSW Fisheries) for providing and ageing adult otoliths, Megan Lidelow, and Ben Stewart (University of Sydney) for help with collecting juveniles, Travis Elsdon (University of Adelaide) for help preparing and analysing samples, Russell Millar (University of Auckland) for use of his mixed stock composition estimation program (HISEA), and both Russell Millar and Malcolm Haddon (TAFI) for discussions regarding maximum likelihood-based analyses. Comments by Cynthia Jones and Sean Connell significantly improved sections of this report.

1. General introduction

1.1. Background

"Nursery" areas are thought to be of crucial importance to sustaining commercial and recreational stocks of fish. It is generally argued that juvenile fish recruit to estuarine habitats and move to coastal reefs at greater sizes and ages (e.g. Bell & Worthington, 1993). This idea stems from the fact that juveniles of many species have been observed in large numbers in estuarine habitats, but their adults have only been found on coastal reefs. Despite the occurrence of species on coastal reefs and in estuaries, there is little unequivocal evidence that estuarine habitats sustain populations of coastal reef fishes. Currently, we do not know the proportion of individuals in harvested populations that may have spent time in different estuaries as juveniles and whether one or a few estuaries are making substantial contributions to maintaining local stocks.

1.2. Need

The origins of many stocks of fish are unknown. As juveniles, many fish are found in estuarine regions. After a period in these habitats they may leave estuaries for coastal reefs and shelf waters. Degradation of habitats within estuaries (from pollution, land reclamation, marinas etc) and death of fish as by-catch in commercial and recreational fishing may affect abundances of adults on coastal reefs. The contribution of each estuary to total stock size in coastal waters is unknown. It is difficult to determine which estuary adult fish may have come from using conventional tagging methods, because of the small size of fish in estuaries. Alternative methods for determining the origins of adult fish are needed.

Molecular genetics provides one possibility, but these methods are in their infancy. An alternative method may utilise chemical elements in bones to show origins of fish. Chemical analyses have proven useful in distinguishing between periods of freshwater and marine residence within individual fish (e.g. Kalish, 1990) and have also been used to distinguish stocks or sub-populations within marine species (e.g. Edmonds *et al.*, 1989, 1991, 1992, 1995). Recently, we (Gillanders & Kingsford, 1996) have used chemical analyses of ear bones to distinguish recruits that settled to seagrass from those that settled to reef habitats. We then analysed the centre region of adult ear bones to determine the origin (estuary or reef) of adult fish showing that this approach is possible.

Many studies have documented important estuarine "nursery" areas in terms of numbers of fish, but if few fishes from these areas reach coastal reefs, such habitats may be relatively unimportant to sustaining populations of adults. The research focused on snapper (*Pagrus auratus*) because it is the most important species (in terms of production) that has individuals leaving estuaries for coastal reefs or shelf waters (Bell & Worthington, 1993).

1.3. Objectives

(1) To solve a pressing problem for the fishery by determining what proportion of the commercial catch is from different estuaries.

(2) To use methods being developed on juvenile fish to establish a chemical "fingerprint" for each estuary so that in future years the proportion of adults from each estuary can be determined.

2. Spatial variation in elemental composition of otoliths of three species of sparid

2.1. Introduction

Otoliths or ear bones are located within the inner ear of teleost fish. Their primary function is the detection of sound, but there is a long history of using them as a structure for ageing of fish by counting annual growth zones, analysis of microstructure to determine life-history events (e.g., settlement) and rates of growth, and more recently, the analysis of elemental composition to identify environments fish have experienced (Campana & Neilson, 1985; Beamish & McFarlane, 1987; Campana, 1999). Many of these applications are possible because otoliths form prior to hatching, grow continuously throughout the life of the fish and are composed of alternating layers of calcium carbonate (usually in the mineral form aragonite) and protein, which are deposited on a daily basis (Campana & Neilson, 1985; Jones, 1986). Once the material in otoliths is deposited it is generally not resorbed or reworked, because otoliths are acellular (Campana & Neilson, 1985).

As otoliths are formed, minor and trace levels of many elements may be incorporated into either the organic or inorganic part. The concentrations of these minor and trace elements are thought to be influenced primarily by environmental conditions, although physiological processes may also be important, especially in adult fish (see Kalish, 1989, 1991). Changes in the Sr:Ca ratio across the otolith have been used to demonstrate migratory patterns of anadromous fishes [e.g., detect periods of freshwater (low Sr) and marine (high Sr) residence] and to distinguish freshwater and saltwater fish from within the same species (e.g., Kalish, 1990; Secor, 1992). Elements in otoliths, therefore, provide considerable interest in that they may form a natural biological tag (e.g., Gillanders & Kingsford, 1996).

Recent studies have shown that otolith chemistry of juvenile fish varies between different juvenile habitats (Gillanders & Kingsford, 1996; Thorrold *et al.*, 1998b). Gillanders and Kingsford (1996) showed that differences in otolith chemistry occurred between groups of juvenile fish collected from estuarine seagrass and reef habitats outside estuaries. Differences in microchemistry of adult fish collected from coastal reefs were then used to determine the proportion of fish moving from estuarine to reef habitats. In this study, we investigate the ability of trace elements in otoliths of three species of fish (snapper, *Pagrus auratus*; tarwhine, *Rhabdosargus sarba* and bream, *Acanthopagrus auratus*; Sparidae) to act as natural tags of estuarine nursery habitats. If differences in elemental composition of otoliths are

found among estuaries, then adult fish collected outside estuaries as part of the fishery could then be tracked to their natal estuary. Such tracking of adults to their natal estuary would allow the contribution of different estuaries to the adult population to be determined and could have major implications for fisheries management.

Snapper, bream and tarwhine all recruit to estuarine habitats (bare substrata or beds of seagrass) and remain associated with estuaries for at least 12 months (Bell & Worthington, 1993). Snapper and tarwhine then leave estuaries for coastal reefs and other habitats over the continental shelf, whereas adult bream are common in estuaries but can also be abundant on coastal reefs (Bell & Worthington, 1993). Commercial fishers within New South Wales, Australia, take all three species. Snapper is taken as part of the inshore trap and line fishery (average age of fish 3 years), whereas bream and tarwhine are taken by meshing and hauling within estuaries, but also as part of the ocean haul fishery outside estuaries (NSW Fisheries, 2001). The latter fishery targets pre-spawning aggregations (age 2-12^{+} years; Gray, NSW Fisheries pers. comm.). The specific objectives of this study were (1) to quantify variation among estuaries in the otolith chemistry of each species of fish, and (2) compare whether similar patterns were found among estuaries for the three species, as well as determine whether the elemental fingerprints of the three species of fish are similar. If similar patterns of elemental fingerprints are found among species, then it may be possible to track adults of any of the three species to their natal estuary using the elemental fingerprints of one species.

2.2. Materials and methods

2.2.1. Sample collection

Juvenile snapper (*Pagrus auratus*, Sparidae) were collected by hand line from two sites in each of 15 estuaries spanning 600 km along the east coast of New South Wales, Australia to determine variation in elemental fingerprints among estuaries (Fig. 2.1). Of the 15 estuaries in which snapper were collected, bream (*Acanthopagrus australis*, Sparidae) and tarwhine (*Rhabdosargus sarba*, Sparidae) were also collected from multiple sites within three and five estuaries respectively. In addition, tarwhine were collected from one other estuary. For bream and tarwhine, sample sizes were not adequate to stratify by site, thus enabling stratification by estuary only. Each species was collected from different sites within the estuaries, corresponding to their habitats. All three species were collected from one site within Lake Illawarra and this site was used for the comparison among species. Tarwhine and bream were collected by either hand line or beach seine nets. All fish were stored on ice in the field before being dissected on return to the laboratory (Table 2.1).

FIGURE 2.1. New South Wales coast, showing estuaries in which fish were collected.

TABLE 2.1. Summary information for bream (*Acanthopagrus australis*), tarwhine (*Rhabdosargus sarba*) and snapper (*Pagrus auratus*) collected for comparisons of otolith chemistry. Shown are the estuaries and sites (Snapper only) that fish were collected, dates of collection (month and year), mean standard length (SL ± SE [mm]) and mean otolith weight (OW ± SE [mg]). Sample sizes were *n*=10 fish per estuary for bream and tarwhine and *n*=10 fish per site for snapper.

Estuary & site	Date	Mean SL (mm)	Mean OW (mg)
Bream			
Wallis Lake	12/98	73.9 ± 2.0	9.74 ± 0.72
Tuggerah Lakes	1/99	58.7 ± 3.4	4.76 ± 0.62
Lake Illawarra	1 & 4/99	59.0 ± 2.5	3.79 ± 0.27
Tarwhine			
Port Stephens	2/99	47.1 ± 1.3	2.85 ± 0.18
Tuggerah Lakes	1 & 2/99	49.9 ± 3.0	3.05 ± 0.36
Botany Bay	2 & 3/99	54.6 ± 2.5	3.31 ± 0.31
Georges River	2-4/99	57.8 ± 1.7	4.05 ± 0.26
Port Hacking	1& 4/99	47.5 ± 2.1	2.41 ± 0.29
Lake Illawarra	1 & 4/99	54.3 ± 1.9	3.16 ± 0.14
Snapper			
Wallis Lake			
1	1/99	72.6 ± 0.8	10.04 ± 0.37
2	1/99	69.5 ± 2.7	8.49 ± 0.67
Port Stephens			
1	1/99	72.3 ± 1.4	10.15 ± 0.34
2	1/99	68.4 ± 1.0	9.20 ± 0.42
Lake Macquarie			
1	1/99	68.5 ± 2.0	7.92 ± 0.44
2	1/99	63.6 ± 2.6	8.33 ± 0.62
Tuggerah Lakes			
1	1/99	59.9 ± 2.6	6.95 ± 0.58
2	1/99	62.2 ± 3.0	7.87 ± 0.69
Hawkesbury Est			
1	11/98 & 1/99	70.9 ± 4.0	9.32 ± 0.95
2	1/99	66.5 ± 3.0	8.26 ± 0.82

Middle Harbour			
1	11/98	57.8 ± 0.6	5.39 ± 0.18
2	12/98	68.3 ± 1.5	8.01 ± 0.41
Port Jackson			
1	12/98 & 1/99	55.5 ± 2.8	5.81 ± 0.66
2	12/98	63.7 ± 2.3	7.85 ± 1.15
Botany Bay			
1	1/99	73.8 ± 2.1	10.82 ± 0.54
2	1/99	74.0 ± 0.6	9.18 ± 0.22
Port Hacking			
1	12/98 & 1/99	69.2 ± 2.2	8.07 ± 0.58
2	1/99	69.2 ± 1.6	8.36 ± 0.32
Lake Illawarra			
1	1/99	68.4 ± 2.0	8.67 ± 0.51
2	1/99	69.3 ± 2.0	8.66 ± 0.58
Jervis Bay			
1	2/99	76.0 ± 1.9	11.27 ± 0.62
2	3/99	72.7 ± 1.8	9.23 ± 0.55
Burrill Lake			
1	2/99	69.4 ± 1.9	8.67 ± 0.47
2	2/99	68.1 ± 2.1	8.16 ± 0.48
Batemans Bay			
1	2/99	70.6 ± 2.0	9.68 ± 0.41
2	2/99	56.5 ± 2.3	6.82 ± 0.42
Wagonga Inlet			
1	2/99	65.4 ± 1.7	7.72 ± 0.34
2	2/99	58.7 ± 1.8	7.01 ± 0.36
Eden			
1	2/99	69.6 ± 1.6	8.05 ± 0.46
2	2/99	66.6 ± 3.0	7.40 ± 0.77

In the laboratory, the standard length (SL) of each fish was measured, and the sagittal otoliths removed, cleaned of adhering tissue in Milli-Q water, air-dried and placed in eppendorf microcentrifuge tubes. Otoliths were weighed, cleaned in 1% nitric acid for 5-10 s to remove any possible contamination resulting from weighing, rinsed in Milli-Q water and placed in acid-washed polycarbonate tubes ready for analysis. The average weight loss between weighing and cleaning of otoliths in 1% acid was 0.2 mg (n = 10 otoliths), which is considered negligible given the average weight (8.03 ± 0.09 mg) of the otoliths.

2.2.2. Sample preparation and analysis

Samples were dissolved overnight in nitric acid inside a laminar flow cabinet. They were then diluted with Milli-Q water to 1% HNO_3. Blank samples were prepared in a similar manner, but no otolith was present; these were used for blank corrections and to calculate limits of detection. Spiked samples were also analysed every 10 samples to assess instrument drift and recovery. A lab standard, consisting of *Pagrus auratus* otoliths ground to micron-sized particles, was analysed during each session of analysis to assess repeatability of measurements. Samples were analysed by solution-based inductively coupled plasma-mass spectrometry (ICP-MS; Perkin Elmer SCIEX ELAN 5000). We consider that collecting and/or handling effects are likely to be minimal for all elements in our study based on similar treatment of fish after capture, chemical similarity (e.g., Sr and Ba) and the use of Milli-Q water for rinsing otoliths (but see Milton & Chenery, 1998; Proctor & Thresher, 1998).

Preliminary analyses indicated that 5 elements (Li, Mn, Sr, Ba and Pb) were detectable in otoliths of juvenile sparids using ICP-MS. These elements were chosen for subsequent analyses. Detection limits, which were calculated from the concentration of analyte yielding a signal equivalent to three times the standard deviation of the blank signal, for each of the elements were: 0.042 μg g^{-1} (Li), 0.009 μg g^{-1} (Mn), 0.097 μg g^{-1} (Sr), 0.006 μg g^{-1} (Ba), and 0.007 μg g^{-1} (Pb). Lead was frequently below detection limits for samples of otoliths and was, therefore, removed from subsequent analyses. Mean estimates of precision (%RSD, relative standard deviation) based on replicate measurements of (1) our lab standard (n=54) were: 10.1% (Li), 10.5% (Mn), 8.9% (Sr), and 9.1% (Ba), and (2) within otolith samples were: 16.4% (Li), 2.2% (Mn), 1.9% (Sr), and 2.3% (Ba). Mean recovery of spiked samples was: 93% (Li), 96% (Mn and Sr), and 90% (Ba).

2.2.3. Statistical analyses

Univariate and multivariate techniques were used to test hypotheses concerning individual elements and multi-element fingerprints. Spatial differences in otolith chemistry and differences among species were analysed by analyses of variance (ANOVA) for each element (Li, Mn, Sr and Ba). One factor (bream & tarwhine) and two factor nested (snapper) sampling designs were used to determine whether significant differences were found among estuaries and for the two factor design between sites within an estuary. All analyses were performed on ln(×+1) transformed data. The assumption of homogeneity of variance was tested prior to each analysis using Cochran's *C* test. When data remained heterogeneous after transformation, analyses were still performed, as ANOVA is robust to departures from assumptions where data are balanced and sample sizes are relatively large (Underwood, 1997). Means were compared using Student-Newman-Keuls (SNK) tests, when significant differences were detected. The variance components at each level of variation were estimated from the ANOVA model on untransformed data (Kingsford, 1998).

Multielement fingerprints were analysed using parametric and non-parametric multivariate analysis of variance (MANOVA), using the same designs to the univariate ANOVA's. Assumptions of parametric MANOVA (e.g., multivariate normality and equal variance-covariance matrices) are unlikely to be met in most ecological data sets, are rarely tested in a formal sense despite MANOVA being very sensitive to departures, and individual variables meeting the assumptions of univariate ANOVA does not mean that they satisfy multivariate assumptions. Within-group scatterplot matrices for individual elements were examined to provide an indication of how groups covary and Box's M test was used to test for equality of covariance matrices. Parametric MANOVA tests were performed to aid comparison with a new method of non-parametric MANOVA (NP-MANOVA).

The NP-MANOVA uses permutations to test multivariate hypotheses (Anderson, 2001a). The data were ln(×+1) transformed before euclidean distances between each pair of samples were calculated to obtain a distance matrix. The test statistic was then calculated as the ratio of the sum of squared euclidean distances among groups divided by the sum of squared distances within groups, and is thus essentially an *F*-ratio (Anderson, 2001a; McArdle & Anderson, 2001). For bream and tarwhine (one-way designs) permutation of the raw data was used and for snapper (two-way nested design) permutation of residuals under the full model was used (ter Braak, 1992; Manly, 1997; Anderson & Legendre, 1999). The methods of permutation (i.e., permutation of raw data, permutation of residuals under a reduced model and permutation of residuals under a full model) are described in detail elsewhere (Manly, 1997; Anderson & Legendre, 1999).

Maximum likelihood-based analyses were used to determine the ability of elemental fingerprints to record correctly the proportion of recruits of the juvenile sparids from different estuaries. Such analyses provide estimates of the proportion of fish from different estuaries as opposed to individual identifications. The maximum likelihood estimator was chosen because it performs best in practice and provides maximum discriminatory power in mixed stock situations (Millar, 1987, 1990a). Results were obtained from a multipurpose simulation/bootstrap/analysis program (Millar, 1990b). The program was run in simulation mode with 100 simulations being made. Classification errors were estimated from the difference between the actual or known composition and the estimated composition, although these errors do not take into account the variability associated with estimating the composition. The data were not resampled, and therefore the classification rule remained fixed throughout the 100 simulations (see Millar, 1990a). Data from the 100 simulations provide an estimate of the variability of the estimator.

2.3. Results

2.3.1. Spatial variability among estuaries – individual elements

Significant differences were found in the otolith chemistry of all three species of juveniles collected from different estuaries. The amount of lithium in otoliths of bream and snapper showed a significant difference among estuaries (Fig. 2.2, 2.3; Table 2.2a). For bream, all three estuaries showed significant differences with increasing amounts in otoliths of fish from estuaries moving progressively south (SNK results; Fig. 2.2). For snapper, Wallis Lake, Jervis Bay and Wagonga Inlet had significantly greater amounts of Li in otoliths than some of the other estuaries (Fig. 2.3).

Significant differences in concentrations of Mn were found among estuaries for tarwhine and snapper (Fig. 2.2, 2.3; Table 2.2a). Although otoliths of tarwhine from Tuggerah Lakes and Lake Illawarra had similar amounts of Mn, these two estuaries had significantly greater amounts than the other four estuaries (Fig. 2.2). For snapper, the amount of Mn in otoliths of fish was significantly greater for fish collected from Lake Illawarra than for fish collected from Lake Macquarie. Fish from both of these estuaries showed significant differences in amount of Mn to all other estuaries, suggesting that fish from Lake Illawarra and Lake Macquarie could be distinguished on the basis of Mn alone.

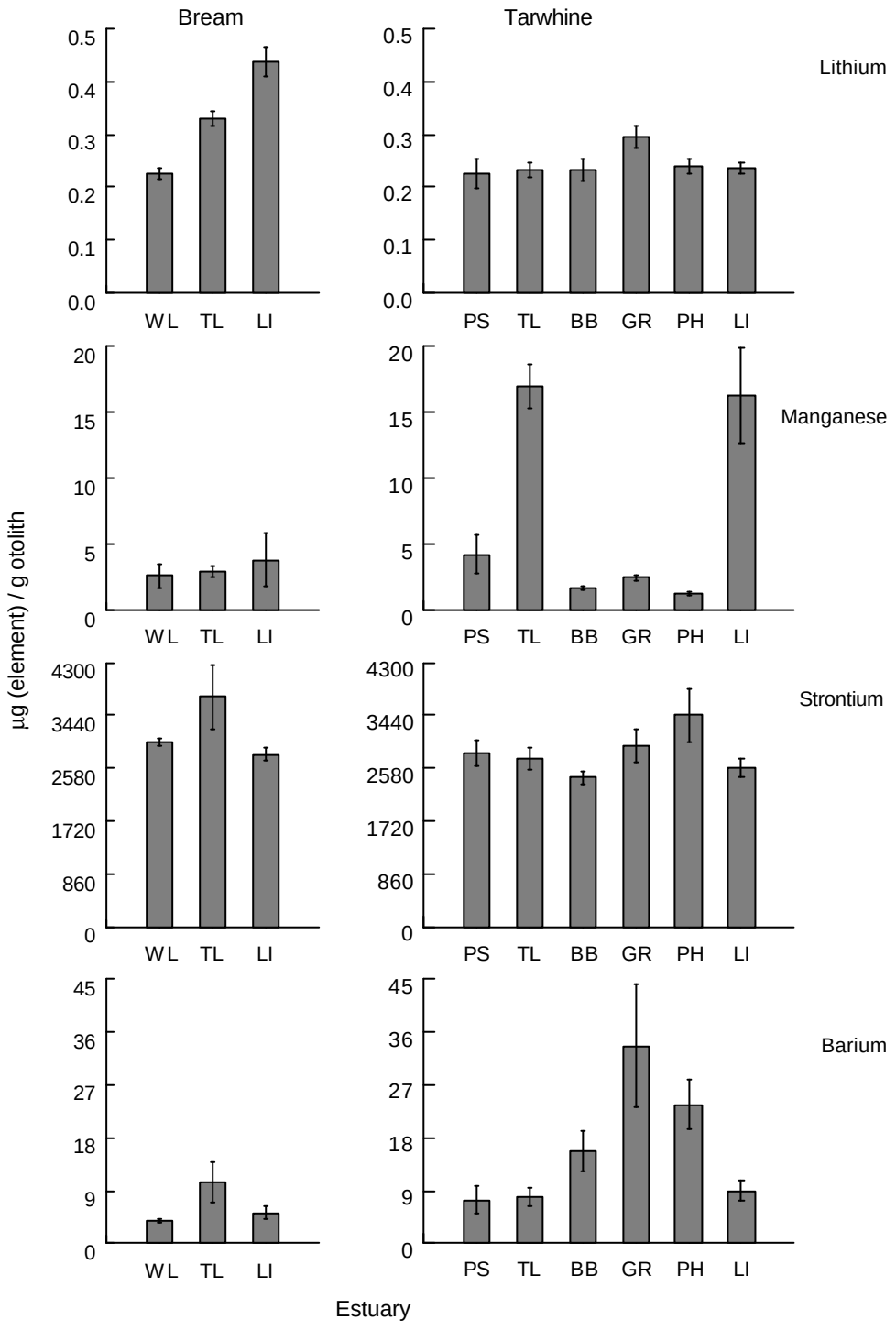

FIGURE 2.2. Mean concentration (±SE) of Li, Mn, Sr and Ba in otoliths of juvenile bream and tarwhine collected from three and six estuaries along the coast of New South Wales, Australia. The estuaries were Wallis Lake (WL), Port Stephens (PS), Tuggerah Lakes (TL), Botany Bay (BB), Georges River (GR), Port Hacking (PH) and Lake Illawarra (LI).

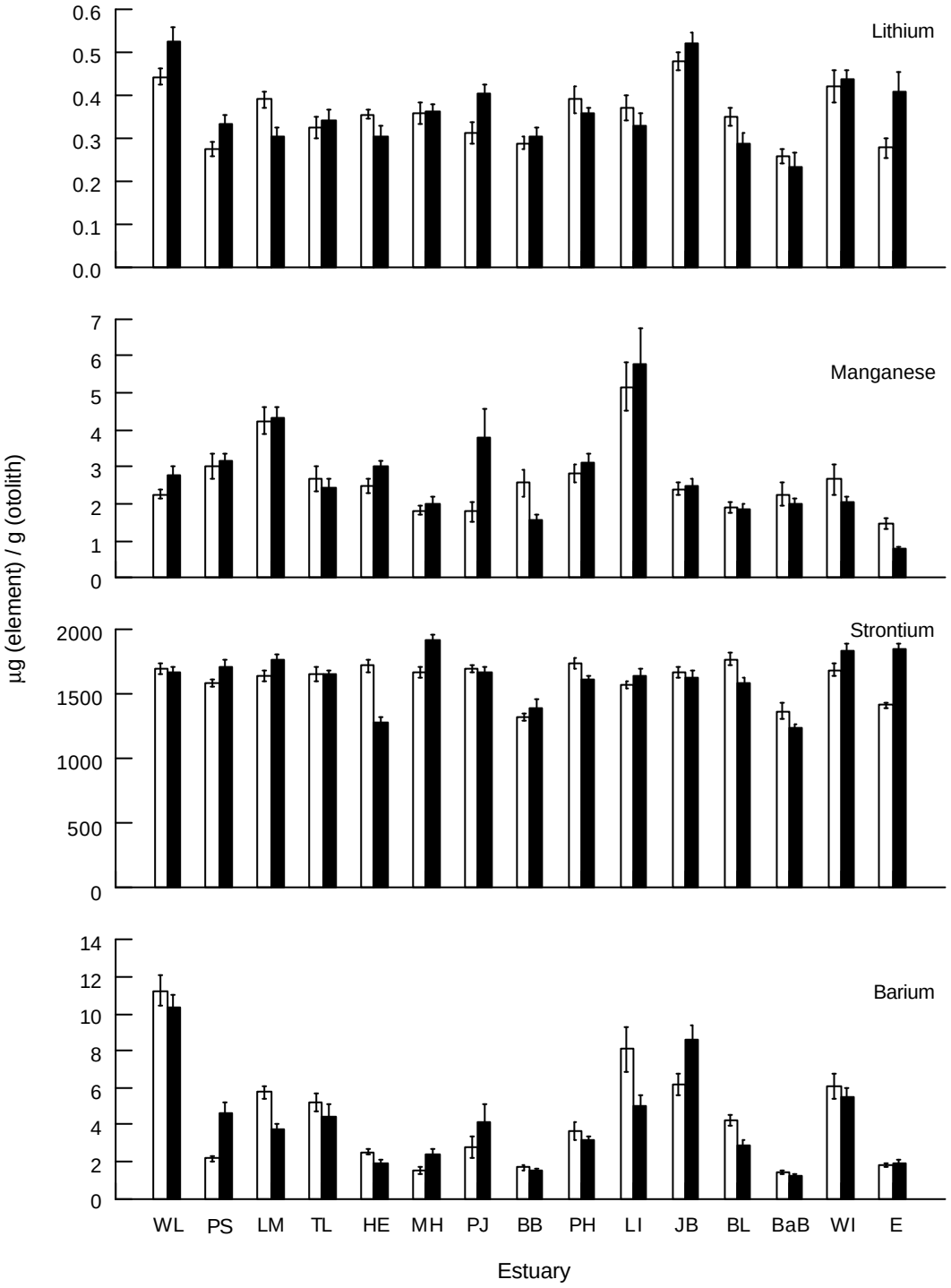

FIGURE 2.3. Mean concentration (±SE) of Li, Mn, Sr and Ba in otoliths of juvenile snapper collected from two sites within each of 15 estuaries along the coast of New South Wales, Australia. The estuaries were Wallis Lake (WL), Port Stephens (PS), Lake Macquarie (LM), Tuggerah Lakes (TL), Hawkesbury Estuary (HE), Middle Harbour (MH), Port Jackson (PJ), Botany Bay (BB), Port Hacking (PH), Lake Illawarra (LI), Jervis Bay (JB), Burrill Lake (BL), Batemans Bay (BaB), Wagonga Inlet (WI) and Eden (E). Site 1 and 2 are indicated by open and closed bars respectively.

TABLE 2.2. (a) Comparisons of mean amount of individual elements in otoliths of juvenile bream, tarwhine and snapper. All factors were treated as random. Cochran's tests were used to test homogeneity of variances. All data were transformed to ln(×+1). Variances were not homogeneous at $P<0.05$ for Li in bream and snapper, and Sr in snapper and $P<0.01$ for Ba in all three species, Sr in bream, and Mn in both tarwhine and snapper. For this and the following tables: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. (b) Percentage of variation estimated from analyses of variances for *Pagrus auratus*.

(a) Source	df	MS Li	MS Mn	MS Sr	MS Ba
Bream					
Estuary	2	0.0624***	0.1010	0.1313	0.9501*
Error	27	0.0018	0.3107	0.0512	0.2619
Tarwhine					
Estuary	5	0.0041	6.9763***	0.1099	2.3493**
Error	54	0.0023	0.2253	0.0635	0.6459
Snapper					
Estuary	14	0.0484**	1.3024***	0.1659	4.2310***
Site (Estuary)	15	0.0097***	0.1691***	0.0766***	0.3268***
Error	270	0.0032	0.0522	0.0072	0.0627

(b) Element	Estuary	Site	Residual
Li	33.8	11.2	55.0
Mn	42.6	5.4	52.1
Sr	19.6	39.0	41.4
Ba	65.0	8.6	26.4

Significant differences in the amount of Sr in otoliths of fish among estuaries were not detected for any of the three species examined (Fig. 2.2, 2.3; Table 2.2a). In contrast, Ba showed a significant difference among estuaries for all three species (Fig. 2.2, 2.3; Table 2.2a). For Ba in bream, fish from Tuggerah Lakes were significantly different from those collected in Wallis Lake, but could not be distinguished from those collected from Lake Illawarra. For tarwhine, fish collected from the Georges River showed similar amounts of Ba to those collected from Port Hacking, but could be distinguished from fish collected in all other estuaries. For snapper, fish collected from one estuary (Wallis Lake) could be

distinguished, based on the amount of Ba in their otoliths, from fish collected in all other estuaries. Fish collected from Wagonga Inlet, Lake Illawarra and Jervis Bay had significantly greater amounts of Ba in their otoliths than fish collected from four, five and nine other estuaries respectively (Fig. 2.3).

2.3.2. Within estuary variation – snapper

All four elements showed significant variation between sites within an estuary (Fig. 2.3, Table 2.2). With the exception of Sr, however, the level of variability associated with sites was always the lowest (Table 2.2b), suggesting that variation among estuaries was greater than variation between sites within an estuary. With the exception of Ba, there was also large variation among replicate fish.

2.3.3. Spatial variability among estuaries – multielement fingerprints

Significant differences in elemental fingerprints of otoliths of the three sparid species were found (Fig. 2.4, Table 2.3). For bream, the 95% confidence ellipses around the mean values for each estuary showed that none of the ellipses overlapped, suggesting significant variation in elemental fingerprints among all three estuaries (Fig. 2.4a, Table 2.3). Greatest variation was seen along the first canonical variate and this reflected differences in the amount of Li in otoliths of fish from the different estuaries (Fig. 2.4a). The pattern for tarwhine was less clear than that for bream (Fig. 2.4). Elemental fingerprints for tarwhine from Port Stephens suggested that this estuary differed from the other estuaries (Fig. 2.4b). The remaining five estuaries were separated into two groups: estuaries in the vicinity of metropolitan Sydney (Botany Bay, Georges River & Port Hacking) and estuaries with coal-fired power stations (Tuggerah Lakes & Lake Illawarra). Greatest variation was again seen along the first canonical variate reflecting differences in Mn and Ba (Fig. 2.4b). Within Lake Illawarra, there was large variability between fish collected from the two sites, one of which was well inside the lake and the other was near the entrance to the lake. Two of the estuaries that showed differences in elemental fingerprints for bream (Tuggerah Lakes & Lake Illawarra), showed some overlap for tarwhine (cf. Fig. 2.4a & 2.4b).

The 95% confidence ellipses around the mean values for each estuary for snapper indicated that a number of estuaries showed significant variation in elemental fingerprints (Fig. 2.4c, Table 2.3). There were, however, a group of 5-6 estuaries that showed some overlap in elemental fingerprints, mainly with Port Jackson. Estuaries were separated along the first canonical variate based primarily on differences in the amount of Li and Ba, whereas along the second canonical variate estuaries were separated based on differences in the amount of

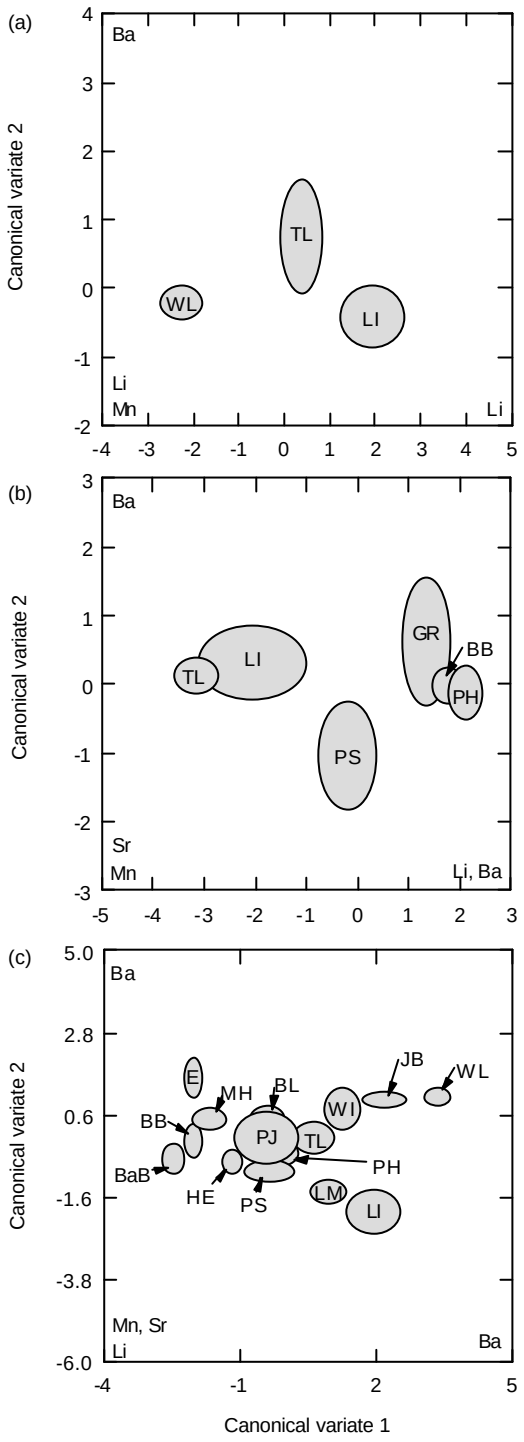

FIGURE 2.4. Plot summarising variation in elemental fingerprints of otoliths of juvenile (a) bream, (b) tarwhine and (c) snapper collected from different estuaries along the coast of New South Wales, Australia. Shaded areas represent bootstrapped 95% confidence ellipses (n=1000) around the means of each estuary for each canonical variate. Elements that contributed most variation to the data set (canonical loadings) are indicated.

TABLE 2.3. Comparison of mean amounts of multielement fingerprints (Li, Mn, Sr & Ba) in otoliths of juvenile bream, tarwhine and snapper using parametric and non-parametric MANOVA. All data were ln (×+1) transformed prior to analysis. df represents the numerator and denominator respectively. The parametric MANOVA used the Pillai's trace statistic for significance tests.

	Parametric MANOVA			**NP-MANOVA**	
Source	**df**	**Value**	***F***	**df**	***F***
Bream					
Estuary	8, 50	0.961	5.777***	2, 27	2.851***
Tarwhine					
Estuary	20, 216	1.222	4.750***	5, 54	10.703***
Snapper					
Estuary	56, 60	3.093	3.651***	14, 15	9.873***
Site(Estuary)	60, 1080	0.831	4.723***	15, 270	4.649***

Ba and Mn or Sr (Fig. 2.4c). Tuggerah Lakes and Lake Illawarra showed significant differences in elemental fingerprints. This pattern was in contrast to that found for tarwhine, but similar to the results for bream. Elemental fingerprints of otoliths of snapper from Botany Bay and Port Hacking were significantly different, which differs from that found for tarwhine (Fig. 2.4). Although multiple sites were sampled for all species, the same sites within an estuary may not necessarily have been sampled for all three species due to differences in microhabitat distribution, which may account for some differences / similarities among species.

Maximum likelihood-based estimation was used to determine the ability of elemental fingerprints to classify correctly juvenile fish to their natal estuaries. The true composition, estimated composition and variability in estimates based on simulations are shown in Table 2.4. Previous microchemistry studies have not included estimates of variability in classification-type analyses. For bream, the actual composition was 33% for each estuary and the estimate of contribution ranged from 31.05 to 35.64% suggesting an error rate of less than 3%, although this error rate has not taken into account the variability in the simulation runs (Table 2.4a). The variability in the classification from the simulations for bream ranged from 8.34 to 11.27% being greatest for Tuggerah Lakes and least for Wallis Lake. For tarwhine,

the estimated composition ranged from 14.35 to 17.63% (actual composition was 17%; error rate was less than 3%), although the variability was relatively large compared to the actual composition (range 9.5 to 15.5%) suggesting that there may be some similarity among estuaries (Table 2.4b). Tarwhine from Botany Bay, Georges River and Port Hacking (all estuaries in the vicinity of Sydney) were grouped together, as well as Tuggerah Lakes and Lake Illawarra (estuaries containing coal-fired power stations) and the maximum likelihood-based estimators recalculated resulting in reduced variability (Table 2.4c). For snapper, 15 estuaries was considered too great a number of estuaries to discriminate amongst given that there were only four discriminatory variables (Li, Mn, Sr, Ba) and therefore the estuaries were grouped into the five meteorological districts (see Fig. 2.1). Rates of error at classifying the fish to their natal estuaries ranged from 3 to 6%, although the simulation results suggested variability in the classification ranged from 9 to 22% (Table 2.4d).

TABLE 2.4. Results of maximum likelihood analyses where juvenile sparids are classified to recruitment estuary based on elemental chemistry (Li, Mn, Sr, Ba) of otoliths. The estimated contribution to each estuary is shown for (a) bream, (b) tarwhine for the six estuaries, (c) tarwhine for 3 groups of estuaries, and (d) snapper. See caption to Figures 2.2 and 2.3 for details of abbreviations to estuaries.

Estuary	True contribution	Estimated contribution	SD
(a) Bream			
WL	0.333	0.3564	0.0834
TL	0.333	0.3105	0.1127
LI	0.333	0.3331	0.1070
(b) Tarwhine			
PS	0.170	0.1750	0.1175
TL	0.170	0.1683	0.1070
BB	0.170	0.1435	0.1069
GR	0.170	0.1763	0.0953
PH	0.170	0.1762	0.0994
LI	0.170	0.1608	0.1553
(c) Tarwhine			
PS	0.170	0.1920	0.0853

TL & LI	0.330	0.2960	0.0873
Sydney	0.500	0.5121	0.0976
(d) Snapper			
Manning	0.070	0.1209	0.0727
Hunter	0.200	0.2298	0.1437
Metropolitan	0.330	0.3654	0.2163
Illawarra	0.130	0.0773	0.0924
South Coast	0.270	0.2066	0.1839

2.3.4. Comparison among three species

Fish used in the comparison among species were of a similar size (F=1.36, df=2,12, P>0.5), but otoliths from snapper were significantly larger than those from bream or tarwhine (F=39.5, df=2,12, P<0.05). Significant differences were found among species for individual elements and for multielement fingerprints (Fig. 2.5, 2.6; Table 2.5). Although similar trends were found for Mn and Ba, these patterns did not match those found for Li and Sr (Fig. 2.5). For Li, Mn and Sr the species with the lowest concentration of the element was significantly different to the other two species (SNK tests; P<0.05; Fig. 2.5). For Ba, however, snapper had significantly higher concentrations of Ba in their otoliths than bream, but did not differ from tarwhine, which was similar to bream (SNK tests, P<0.05; Fig. 2.5). Multielement fingerprints also showed significant differences among species with all three species being clearly distinguished (Fig. 2.6, Table 2.5).

TABLE 2.5. Comparisons of mean amount of individual elements and multielement fingerprints in otoliths of juvenile bream, tarwhine and snapper. Data were transformed to ln(×+1). Cochran's tests were not significant at P=0.05.

Source	**df**	**MS Li**	**MS Mn**	**MS Sr**	**MS Ba**
Individual elements					
Species	2	0.0474**	0.8565**	0.3556**	0.5376*
Error	12	0.0047	0.0778	0.0305	0.0996

	Parametric MANOVA			NP-MANOVA	
	df	**Value**	***F***	**df**	***F***
Multielement fingerprints					
Species	8, 20	1.651	11.814***	2, 12	9.266***

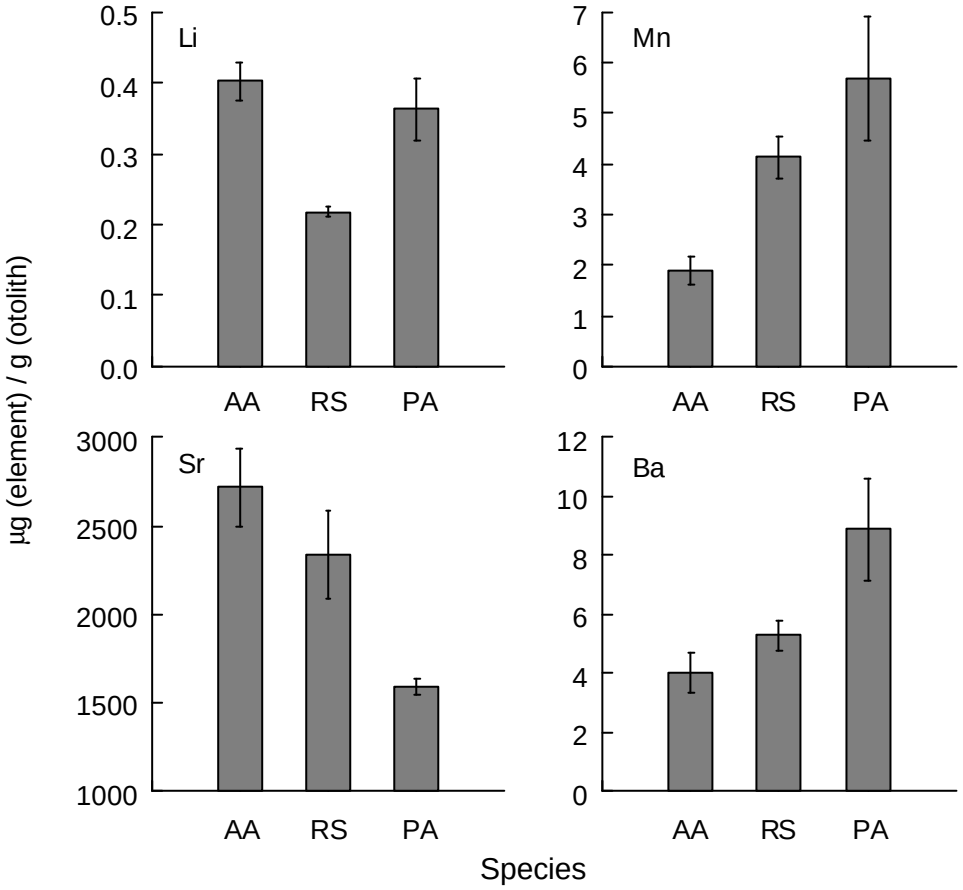

FIGURE 2.5. Mean concentration (±SE) of Li, Mn, Sr and Ba in otoliths of three species of sparid collected from the same site within Lake Illawarra. The species were bream (AA), tarwhine (RS) and snapper (PA).

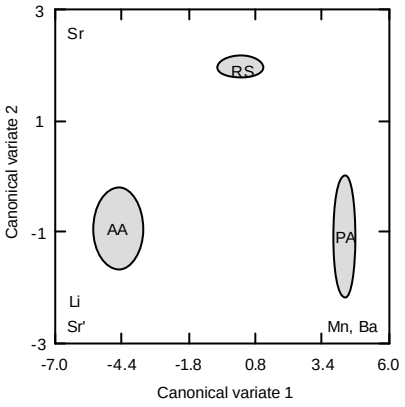

FIGURE 2.6. Plot summarising variation in elemental fingerprints of otoliths of three species of juvenile sparids [bream (AA), tarwhine (RS) and snapper (PA)]. Shaded areas represent bootstrapped 95% confidence ellipses (n=1000) around species means for each canonical variate. Elements that contributed most variation to the data set (canonical loadings) are indicated.

2.4. Discussion

The elemental composition of otoliths of juvenile bream, tarwhine and snapper showed significant variation among estuaries. Although it was not always possible to separate fish from all estuaries that were sampled, some individual estuaries and/or groups of estuaries could be separated. Overlap in elemental fingerprints among estuaries was greater where an increasing number of estuaries were sampled (e.g., for snapper). Other studies have also found variation in elemental fingerprints among different geographic areas (e.g., Edmonds *et al.*, 1989, 1991, 1992; Campana *et al.*, 1994, 1995; Begg *et al.*, 1998), including among estuaries (Thorrold *et al.*, 1998b; Gillanders & Kingsford, 2000) and between estuaries and coastal reefs (Gillanders & Kingsford, 1996). Such differences are frequently used to infer differences in stock structure, but among-estuary and estuary-coastal reef differences have also been used as natural tags of area of origin for juveniles. Our data suggest that there were variations in elemental fingerprints in otoliths of all three species of juvenile sparids that allowed separation of a number of estuaries.

Despite differences in elemental composition of otoliths among estuaries the mechanisms generating these differences are not well understood. Land use in catchments of the different estuaries varied from intense urban and industrial (e.g., Port Jackson, Botany Bay) to low

intensity rural activities. This provided a range of potential levels of anthropogenic input that may influence the concentration of elements in the water, as well as the temperature and salinity of the water. Elements in the water, temperature and salinity have all been found to influence microchemistry of otoliths in previous studies (Fowler *et al.*, 1995a; Gallahar & Kingsford, 1996; Chesney *et al.*, 1998; Bath *et al.*, 2000; Milton & Chenery, 2001; Elsdon & Gillanders, unpublished data). A previous study that investigated concentrations of metals in oysters from estuaries along the coast of New South Wales found that oysters from estuaries with primarily agricultural or forested catchments had a distinctly different metal fingerprint than oysters from estuaries surrounded by urban and industrial inputs (Scanes & Roach, 1999). For tarwhine, estuaries with coal-fired power stations showed very different elemental fingerprints to estuaries in the vicinity of Sydney (highly urbanised and industrial); both of these groups of estuaries showed significant differences to an estuary with a predominantly forested catchment. In the case of at least one of the estuaries with a coal-fired power station (Tuggerah Lakes), the coal is known to contain high levels of manganese and the cooling water is then released into the lake (Gibbs, NSW Fisheries, pers. comm.). Otoliths of tarwhine from both estuaries with coal-fired power stations contained significantly greater amounts of Mn in their otoliths than the other estuaries. Such elevated levels of Mn were not found in bream and snapper, although samples of these two species were collected closer to the entrance of the estuary than samples of tarwhine and thus the sites might be influenced by flushing of water with tidal cycles. Elevated levels of Mn in otoliths of fish from Tuggerah Lakes have also been found for another species in each of two consecutive years (Gillanders & Kingsford, 2000). Possible anthropogenic influences are also confounded by natural variation in catchments. Most of the estuaries in our study have different catchments, which may have different levels of rainfall, as well as types of rock, soil and vegetation.

Previous studies have found a range of other factors (e.g., diet, growth rates, ontogenetic and physiological effects) may also influence otolith composition in some species (Kalish, 1989, 1991; Sadovy & Severin, 1992; Fowler *et al.*, 1995b; Limburg, 1995). Differences in mean size at age of adult snapper have been observed along the coast of NSW (Ferrell & Sumpton, 1997) and a positive relationship was found between sea surface temperature and growth of juvenile snapper in NZ (Francis, 1994). Since sea surface temperature is likely to vary among estuaries along the coast of NSW, differences in rates of growth may be found among estuaries. In addition, temperature is likely to have a direct effect on some elements (e.g., Sr). While we can not discount differences in growth rates and diets among estuaries as possible determinants of otolith composition in sparids, ontogenetic and physiological effects were minimised by analysing otoliths of juvenile fish (0^+ years) of similar sizes. In addition, significant differences were not found in the length of snapper among estuaries. Although,

the length of bream and tarwhine did vary among estuaries, we believe that the effect sizes and the differences in spatial variation of otolith chemistry for each element suggested that the length of fish would not confound patterns of otolith chemistry.

Significant differences were found in otolith composition among the three species, which were collected from the same site. Although collections were made in the same area and all fish were collected using the same method (hand line) there are differences in microhabitat use of the three species. Tarwhine and bream recruit to *Zostera* seagrass habitat, whereas snapper recruits to bare substrata (Bell & Worthington, 1993). Despite tarwhine and bream being found over similar habitat, some elements (e.g., Li, Mn) showed significant differences. The different species may also have different growth rates and therefore may have spent different lengths of time in the nursery habitat thus they may have experienced differences in salinity, temperature or concentration of elements in the water. Alternatively, they may also have different diets, which could account for differences in elemental composition of otoliths among species, although differences in diet are not thought to be a major determinant of otolith chemistry (e.g., Gallahar & Kingsford, 1996; Milton & Chenery, 2001). Other studies have also found differences among species collected from the same area, but these studies have focused on species from different families (Edmonds *et al.*, 1995; Dove *et al.*, 1996). Whatever the mechanism for the differences, these results suggest that elemental fingerprints of each species of sparid (rather than one species) need to be determined if we want to track all three species of adults on coastal reefs to their respective natal estuaries.

Differences in otolith composition of juvenile sparids among estuaries should allow patterns of movement from estuaries to be determined and thus the proportion of stock in a coastal fishery originating from each estuary to be calculated. In some cases movement from individual estuaries may be determined, but in other cases it may be necessary to group some estuaries that show similar elemental fingerprints. To determine movement the juvenile region of adult otoliths (which would correspond to the natal or nursery habitat) could be analysed for fish that were collected as part of a fishery operating outside of estuaries. This could be done using a probe-based approach (e.g., laser ablation ICP-MS) or by extracting the juvenile region and analysing using a solution-based approach. Using the latter approach, Gillanders & Kingsford (1996) determined the proportion of adult fish on coastal reefs that recruited to seagrass habitat versus reef habitat. Proportions of adults from reef (59%) versus seagrass (41%) habitats could be determined because recruits that settled into reef habitat could be clearly distinguished from those that settled into seagrass habitat (95% accuracy).

We caution that this approach is likely to be complicated by the inclusion of time (see Chapter 3). Juvenile snapper of the size used in our study would be approximately three-four months old based on previous studies of otolith microstructure (Bell *et al.*, 1991; Kingsford & Atkinson, 1994), and bream and tarwhine a maximum of 6 months old based on cohort analysis (Worthington *et al.*, 1992). All three species, therefore, represent fish from the same year class. Although Thorrold et al. (1998a) found little variation in otolith composition over a two month period (i.e., within a recruitment season), a recent study found significant variation in otolith composition of juvenile fish collected from two consecutive years (i.e., between recruitment seasons, Gillanders & Kingsford, 2000). Significant variation in otolith composition among recruitment seasons does not prevent determining natal estuaries of adults based on otolith composition of the juvenile core, but it does suggest that adult fish need to be matched to juveniles of the appropriate year class. This also means that it would be necessary to collect juveniles of all the year classes of adults of interest.

In conclusion, an understanding of connectivity between different estuaries and adult populations has considerable implications for fisheries management and the effective conservation of fishes. The relative abundance and year class strength of recruits in different estuaries needs to be determined over a similar time scale to which elemental fingerprints of recruits are determined. Knowledge of abundance of recruits in different estuaries, the area of suitable nursery habitat in each estuary, as well as estimates of numbers of fish that spent their juvenile life in different estuaries will then allow survival rates among estuaries to be calculated. In addition, knowledge of important estuaries in terms of nursery habitats will be important in predicting the impacts of estuarine habitat degradation and by-catch of fish stocks thus allowing conservation efforts (e.g., protected areas) to be focused on estuaries of greatest concern.

3. Temporal variation in elemental composition of otoliths of snapper

3.1. Introduction

Estuaries and other sheltered habitats are assumed to be important "nursery" areas for many fishes whose adults are found on reefs (Bell & Pollard, 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. Thus, the extent to which juveniles occurring in estuarine habitats contribute to adult stocks on coastal reefs is unknown.

Environmental and physiological information is incorporated into the growing surface of the ear bone of fish. The metabolically inert nature of otoliths ensures that deposition of trace elements remains unaltered through time (Campana & Neilson, 1985). Deposition occurs in layers over time and differences in microchemistry among the layers can be estimated by reference to the growth zones within the otolith. Such growth zones are routinely used for daily and/or annual ageing of fish. Trace elements in otoliths can be used as a natural tag to determine past environmental history and to trace movements of fish (e.g. Gillanders & Kingsford, 1996).

Differences in elemental fingerprints of recruits have been found among different estuaries, among different streams and between both estuarine and open coastal, and offshore and inshore reef habitat (e.g. Gillanders & Kingsford, 1996, 2000; Thorrold *et al.*, 1997, 1998a, 1998b). Such differences in chemical fingerprints among areas provide evidence that the otolith does act as a natural tag (Campana, 1999). This means that chemical fingerprints from the juvenile portion of adult otoliths can then be used to classify these fish to their natal or "nursery" habitat. To date, only one study has attempted to determine the "nursery" habitat of adults of a marine species (Gillanders & Kingsford, 1996). This study, however, did not use adult fish that were collected after sampling of the juveniles and therefore assumes that chemical fingerprints of juveniles are the same among different years. Recent research suggests that elemental fingerprints of otoliths of juvenile fish in different estuaries may vary among years, largely because there are differences in temperature, salinity and freshwater input between years (Gillanders & Kingsford, 2000).

The general objective of this part of the research was to determine spatial and temporal variation in elemental fingerprints of otoliths of juvenile fish to examine whether the elemental fingerprints of fish from one year are representative of fish from other years. The specific objectives of this study were to (1) determine spatial variation in elemental fingerprints of otoliths of juvenile snapper collected in different estuaries and across three years of sampling, (2) determine temporal variation in elemental fingerprints for each estuary, and (3) examine whether there may be overlap in elemental fingerprints of fish collected in different years and from different estuaries that may confound subsequent spatial comparisons. If there is little temporal variability in elemental fingerprints among estuaries then fish collected in one year from a number of estuaries could be used to establish an elemental fingerprint for each estuary so that in future years the proportion of adults from each estuary could be determined based on elemental fingerprints of juvenile fish collected in one year. If, however, there is temporal variability in elemental fingerprints among estuaries, then a library of fingerprints may need to be built-up encompassing a range of year classes of juveniles and the birth year of adult fish estimated. The natal estuary of adult fish could then be determined with elemental fingerprints of juveniles from the appropriate birth year.

3.2. Materials and Methods

3.2.1. Sample collection

Juvenile snapper (*Pagrus auratus*, Sparidae) were collected in each of three recruitment seasons (Nov 1998 – Feb 1999, Dec 1999 – Feb 2000, Dec 2000 – Jan 2001, hereafter referred to as 1998/1999, 1999/2000, 2000/2001) to determine variation in elemental fingerprints among estuaries and years. In each year, ten fish were collected from each of two sites (separated by hundreds of metres to kilometres) in each of 15 estuaries (separated by tens to hundreds of kilometres) spanning 600 km along the coast of New South Wales, Australia. No juvenile snapper were found in Jervis Bay and Batemans Bay during 1999/2000 and 2000/2001 and in Eden during 1999/2000 recruitment seasons. All fish were collected by hand line and were stored on ice in the field before being dissected on return to the laboratory.

In the laboratory, the standard length (SL) of each fish was measured (range 43 to 83 mm SL, average 66.66 ± 0.30 mm SL), and the sagittal otoliths removed, cleaned of adhering tissue in Milli-Q water, air-dried and placed in eppendorf microcentrifuge tubes.

3.2.2. Sample preparation and analysis

Otoliths were weighed, cleaned in 1% nitric acid for 5-10 s to remove any possible contamination resulting from weighing, rinsed in Milli-Q water and placed in acid-washed polycarbonate tubes ready for analysis. The average weight loss between weighing and cleaning of otoliths in 1% acid was 0.2 mg (n = 10 otoliths), which is considered negligible given the average weight of the otoliths (8.03 ± 0.09 mg).

Samples were dissolved overnight in concentrated nitric acid inside a Class 100 laminar flow cabinet. They were then diluted with Milli-Q water to 1% HNO_3. Blank samples were prepared in a similar manner, but no otolith was present; these were used for blank corrections and to calculate limits of detection. Spiked samples were also analysed every 10 samples to assess instrument drift and recovery. A lab standard, consisting of *Pagrus auratus* otoliths ground to micron-sized particles, was analysed during each session of analysis to assess repeatability of measurements. Elemental concentrations were determined by solution-based inductively coupled plasma-mass spectrometry (ICP-MS; Perkin Elmer SCIEX ELAN 5000). All elements were quantified using standards that were matrix-matched (i.e. addition calibration), except Sr, which was determined using standards made up in 1% HNO_3 (i.e. external calibration).

Preliminary analyses indicated that 4 elements (Li, Mn, Sr, and Ba) were detectable in otoliths of juvenile snapper using ICP-MS, and these elements were chosen for further analyses. Detection limits, which were calculated from the concentration of analyte yielding a signal equivalent to three times the standard deviation of the blank signal, for each of the elements were: 0.040 $\mu g\ g^{-1}$ (Li), 0.014 $\mu g\ g^{-1}$ (Mn), 0.082 $\mu g\ g^{-1}$ (Sr) and 0.015 $\mu g\ g^{-1}$ (Ba). Mean estimates of precision (%RSD, relative standard deviation) based on replicate measurements of (1) our lab standard (n=120) were: 12.2% (Li), 16.8% (Mn), 11.5% (Sr), and 12.1% (Ba), and (2) within otolith samples were: 12.2% (Li), 2.8% (Mn), 2.4% (Sr), and 2.7% (Ba). Mean recovery of spiked samples was: 95% (Li), 97% (Mn), 98% (Sr), and 84% (Ba).

3.2.3. Statistical analyses

Univariate and multivariate techniques were used to test hypotheses concerning individual elements and multi-element composition of otoliths of snapper. Temporal and spatial variation was analysed by three-factor analyses of variance for each element. Additional ANOVAs (two-factor) were also conducted on each year of data. All factors were treated as random. Site was nested within the interaction of year and estuary for the three-factor design because the same sites could not always be sampled in all years. All analyses were performed

on ln(×+1) transformed data. The assumption of homogeneity of variance was tested prior to each analysis using Cochran's *C* test. When data remained heterogeneous after transformation, analyses were still performed, as ANOVA is robust to departures from assumptions where data are balanced and sample sizes are relatively large (Underwood, 1997). To ensure balanced data in the three-factor analyses, only estuaries in which fish were collected in all three years were used. Means were compared using Student-Newman-Keuls (SNK) tests, when significant differences were detected.

The Friedman non-parametric test was used to determine whether there was a systematic response or pattern across the estuaries for each year. The Friedman test transforms the data for each year to ranks and then sums the ranks for each estuary. The calculated statistic is approximately distributed as X^2, and can be compared to X^2 critical values to determine significance.

Multielement fingerprints were analysed using non-parametric multivariate analysis of variance (NP-MANOVA), using a similar design to the univariate ANOVA's. The NP-MANOVA uses permutations to test multivariate hypotheses (Anderson, 2001a). The data were ln(×+1) transformed before Euclidean distances between each pair of samples were calculated to obtain a distance matrix. The test statistic was then calculated as the ratio of the sum of squared Euclidean distances among groups divided by the sum of squared distances within groups, and is thus essentially an *F*-ratio (Anderson, 2001b; McArdle & Anderson, 2001). For the three-factor design, unrestricted permutation of raw data was used, whereas for the two-factor design permutation of residuals under the full model was used because the number of levels of the nested factor (site) was 2 and therefore the number of possible permutations could not give *P*-values less than 0.05 (Anderson & Legendre, 1999). The units being permuted depend on the term being tested; further details on methods of permutation can be found in Manly (1997) and Anderson and Legendre (1999).

3.3. Results

3.3.1. Temporal and spatial variation across three years – individual elements

Variation in otolith chemistry of snapper differed depending on the element. Lithium and barium both showed a significant year × estuary interaction (Fig. 3.1, Table 3.1). For lithium, some estuaries varied among all years (e.g. Wallis Lake) or two of the three years differed (e.g. Port Stephens), whereas other estuaries showed no significant variation among years (e.g. Lake Macquarie and Tuggerah Lakes). Although there was variation in Li of otoliths

among estuaries in each year, the magnitude of variation differed among the different estuaries. For example, large differences were seen among years for Wallis Lake, but relatively minor differences were found for other estuaries (e.g. Lake Macquarie). For barium, significant variation among years was largely due to differences found in Wallis Lake and Burrill Lake.

For manganese, significant differences were found among years and estuaries (Fig. 3.1, Table 3.1). Concentrations of manganese in otoliths of fish were consistently higher in fish collected during 1999/2000 than in fish collected in the other two years (SNK tests). Fish from Lake Illawarra had consistently higher levels of Mn than fish from the other estuaries. Strontium only showed significant variation among years, where concentrations were lower in 1998/1999 than in the other two years.

TABLE 3.1. Results of ANOVA for concentrations of individual elements in otoliths of juvenile snapper collected across three years. Cochran's tests were used to test homogeneity of variances. All data were transformed to ln(×+1). For this and the following tables: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Time and estuary were tested over the time × estuary interaction, the time × estuary interaction was tested over the site (time × estuary) term and the site (time × estuary) term was tested over the residual.

Source	**df**	**MS Li**	**MS Mn**	**MS Sr**	**MS Ba**
Time	2	0.2738**	3.8823**	0.4215**	9.5728*
Estuary	11	0.2201***	3.1682***	0.0633	15.7153***
Site (time × estuary)	36	0.0158***	0.2903***	0.0379***	0.8309***
Time × estuary	22	0.0418**	0.5127	0.0537	1.8547*
Residual	648	0.0068	0.0564	0.0107	0.0818
Cochran's C		0.0656**	0.0626**	0.0352	0.0510

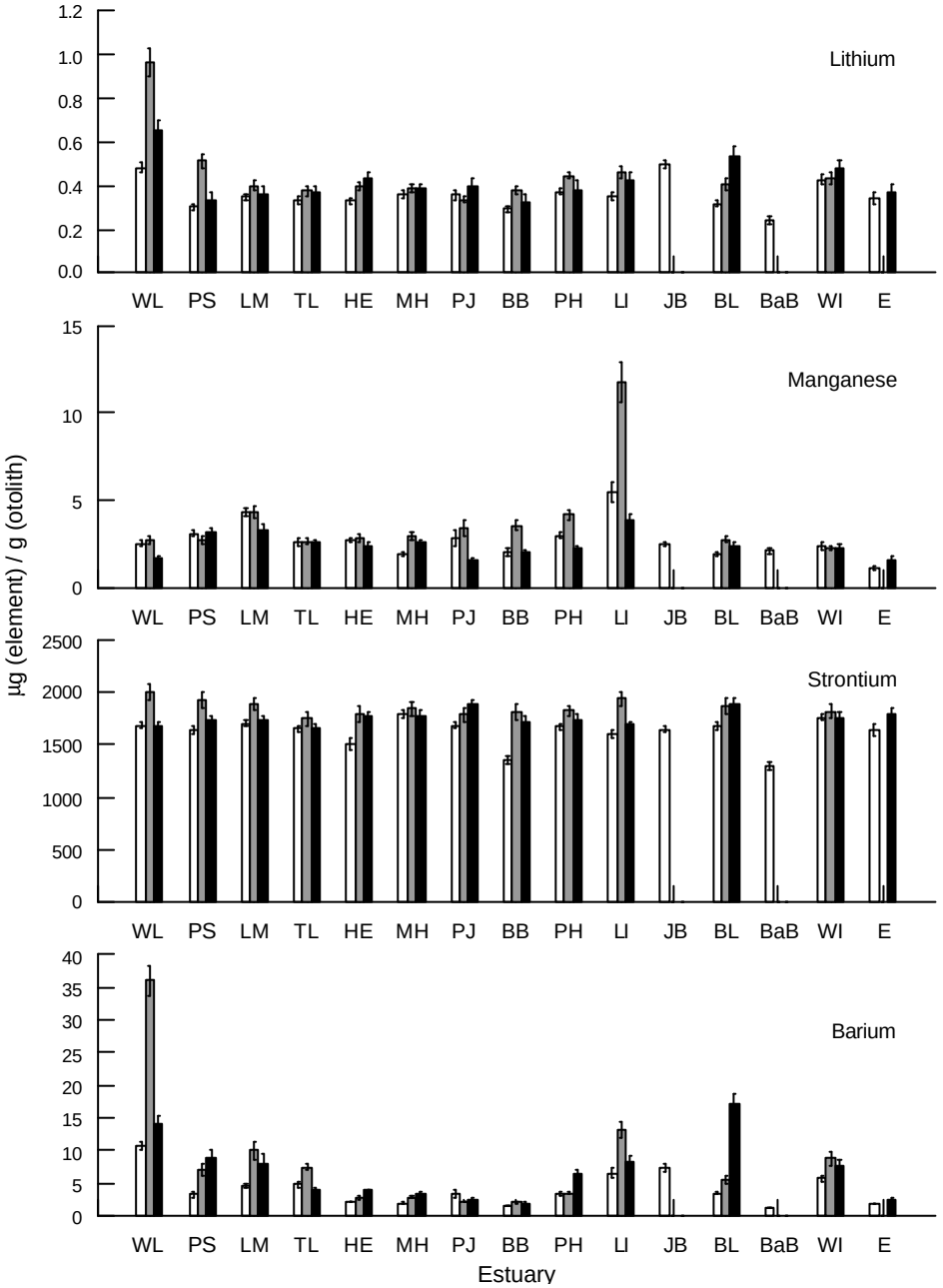

FIGURE 3.1. Mean concentrations (±SE) of Li, Mn, Sr and Ba in otoliths of juvenile snapper collected from three years in each of two sites within 15 estuaries along the coast of New South Wales, Australia. Data were pooled by estuary. Open bars are 1998/1999, hatched bars are 1999/2000 and solid bars are 2000/2001 recruitment seasons. The estuaries were Wallis Lake (WL), Port Stephens (PS), Lake Macquarie (LM), Tuggerah Lakes (TL), Hawkesbury Estuary (HE), Middle Harbour (MH), Port Jackson (PJ), Botany Bay (BB), Port Hacking (PH), Lake Illawarra (LI), Jervis Bay (JB), Burrill Lake (BL), Batemans Bay (BaB), Wagonga Inlet (WI) and Eden (E). No fish were collected from Jervis Bay and Batemans Bay in 1999/2000 and 2000/2001, and Eden in 1999/2000.

3.3.2. Spatial variability among estuaries for each of three years – individual elements

Significant differences were found in the otolith chemistry of snapper collected from the different estuaries for all years (Fig. 3.1, Table 3.2). Lithium, manganese and barium all showed significant differences among estuaries for each year. In contrast to these results, strontium showed no significant difference among estuaries for any of the years examined.

For lithium, otoliths of fish from Wallis Lake had consistently higher levels for all years, although in 1998/1999 fish from Jervis Bay also had high levels. The twelve estuaries in which fish were collected in all three years were ranked for each year according to mean concentration of Li in otoliths of snapper and Friedman's statistic calculated. Although Wallis Lake had the highest levels of Li in each year (rank = 1 for each year), the twelve estuaries differed in their rank across years (Friedman's X^2 statistic = 19.513, df = 11, ns indicating sums of ranks across years were equal for each estuary and therefore the years differed in the way they ranked estuaries).

For manganese, otoliths of fish collected from two estuaries (Lake Illawarra and Lake Macquarie) had higher levels of Mn than fish from all other estuaries in 1998/1999. These same two estuaries were ranked 1 and 2 in terms of concentration of Mn for each of the three years, although in 1999/2000 only Lake Illawarra had higher concentrations of Mn than the other estuaries and in 2000/2001 Lake Illawarra only differed from three other estuaries (Fig. 3.1). The twelve estuaries differed in their ranks across years, suggesting that for most estuaries it was difficult to predict where they would be ranked and therefore whether higher or lower concentrations of Mn may be found.

For barium, otoliths of fish collected from Wallis Lake had consistently higher concentrations than those of fish collected from the other estuaries, although in 2000/2001 Burrill Lake also had high concentrations. The Friedman X^2 statistic was significant (27.4615, df = 11, $P<0.005$) suggesting that ranks of estuaries within each year were similar among years. For example, Wallis Lake was ranked 1 or 2 in all three years (highest concentrations) and Botany Bay had the lowest concentrations in all three years.

3.3.3. Spatial variability among estuaries for each of three years – multielement fingerprints

Significant differences were found in multielement fingerprints of otoliths of snapper among estuaries for each of the three years (Fig. 3.2, Table 3.3). For all years the 95% confidence ellipses around the mean value for each estuary suggested that a number of estuaries showed significant variation in elemental fingerprints (Fig. 3.2).

TABLE 3.2. Results of ANOVA for concentrations of individual elements in otoliths of juvenile snapper collected during the (a) 1998/1999, (b) 1999/2000 and (c) 2000/2001 recruitment seasons. Estuary and site were random factors. The degrees of freedom vary across years because in 1999/2000 and 2000/2001 no juvenile snapper were found in Jervis Bay and Batemans Bay and in 1999/2000 no juvenile snapper were found at Eden.

Source	**df**	**MS Li**	**MS Mn**	**MS Sr**	**MS Ba**
(a) 1998/1999					
Estuary	14	0.0484**	1.3024***	0.1659	4.2310***
Site(estuary)	15	0.0097***	0.1691***	0.0766***	0.3268***
Residual	270	0.0032	0.0522	0.0072	0.0627
Cochran's C		0.1045*	0.1624**	0.0995*	0.1128**
(b) 1999/2000					
Estuary	11	0.1924***	2.4260**	0.0188	10.0851***
Site(estuary)	12	0.0166***	0.5345***	0.0266*	1.0509***
Residual	216	0.0054	0.0557	0.0133	0.0733
Cochran's C		0.1723**	0.1157	0.0854	0.1247*
(c) 2000/2001					
Estuary	12	0.0768*	0.7723*	0.0170	5.9669**
Site(estuary)	13	0.0213	0.1972***	0.0172	1.0148***
Residual	234	0.0122	0.0570	0.0111	0.0975
Cochran's C		0.1018	0.0976	0.0700	0.1184*

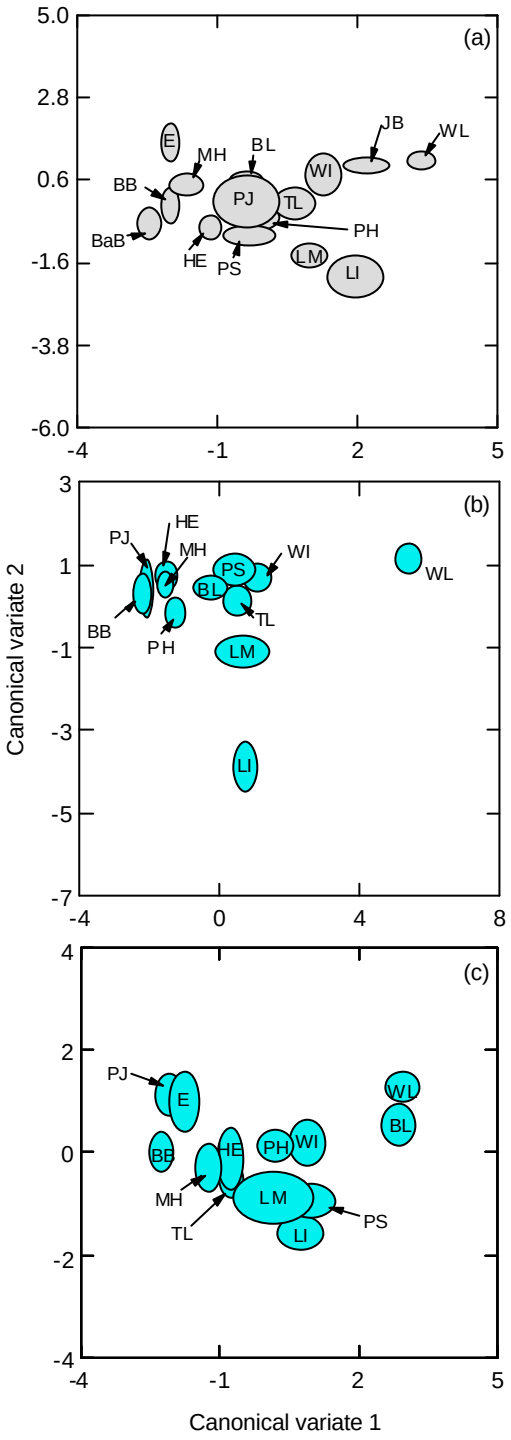

FIGURE 3.2. Plots summarising variation in elemental fingerprints of otoliths of juvenile snapper collected in (a) 1998/1999, (b) 1999/2000 and (c) 2000/2001 from different estuaries along the coast of New South Wales, Australia. Shaded areas represent 95% confidence ellipses around the means for each estuary for each canonical variate. See Fig. 3.1 for estuary abbreviations.

TABLE 3.3. Results of NP-MANOVA for concentrations of elements in otoliths of juvenile snapper collected during the (a) 1998/1999, (b) 1999/2000 and (c) 2000/2001 recruitment seasons. All data were transformed to ln(×+1). See Table 3.2 for further details.

Source	**df**	***F***	**df**	***F***	**df**	***F***
	(a) 1998/1999		**(b) 1999/2000**		**(c) 2000/2001**	
Estuary	14	9.8727***	11	7.8118***	12	5.4647***
Site(estuary)	15	4.6485***	12	11.0303***	13	7.0327***
Residual	270		216		234	

In 1998/1999 there were, however, a group of 5-6 estuaries, which showed some overlap in elemental fingerprints, mainly with Port Jackson (Fig. 3.2a). Despite this overlap of some estuaries, there were a number of estuaries that could be distinguished suggesting that we can track adult fish back to these estuaries. In 1999/2000 two groups of estuaries showed some overlap (Fig. 3.2b). One of these groups included estuaries in the vicinity of Sydney (e.g. Hawkesbury Estuary, Port Jackson, Middle Harbour, Botany Bay). Lake Macquarie, Wallis Lake and Lake Illawarra were clearly differentiated from the other estuaries (Fig. 3.2b). In 2000/2001, a number of estuaries showed significant variation (e.g. Wallis Lake, Burrill Lake, Botany Bay) and others showed some overlap (e.g. Hawkesbury Estuary and Tuggerah Lakes) (Fig. 3.2c).

3.3.4. Consequences of ignoring temporal differences – multielement fingerprints

Six estuaries were randomly selected from the twelve estuaries in which fish were collected in all three years and used to investigate spatial and temporal variation and the consequences of ignoring temporal variation if it exists. Some estuaries showed large variation among years (e.g. Wallis Lake and Lake Illawarra), others showed variation among years, but less than that seen for Wallis Lake and Lake Illawarra (e.g. Port Stephens and Lake Macquarie), while others showed some overlap for two of the three years sampled (e.g. Botany Bay and Wagonga Inlet) (Fig. 3.3a, b, c).

When the individual years were distinguished, many of the ellipses did not overlap ellipses from other years (Fig. 3.3d). However, some ellipses did overlap those from other years and this overlap did not always involve samples collected in different years from the same estuary. For example, fish collected from Wagonga Inlet in 1998/1999 showed some overlap with those collected from Port Stephens in 1999/2000 (Fig. 3.3). Likewise, fish collected

from Lake Macquarie in 1999/2000 showed some overlap with those collected from Lake Illawarra in 2000/2001. Overlap among years for different estuaries is likely to have consequences for assigning adult fish to natal estuaries especially if juvenile fish are collected from different estuaries in different years and adult fish are not assigned to natal estuaries using elemental fingerprints from the year class of recruits in which they were juveniles (see discussion).

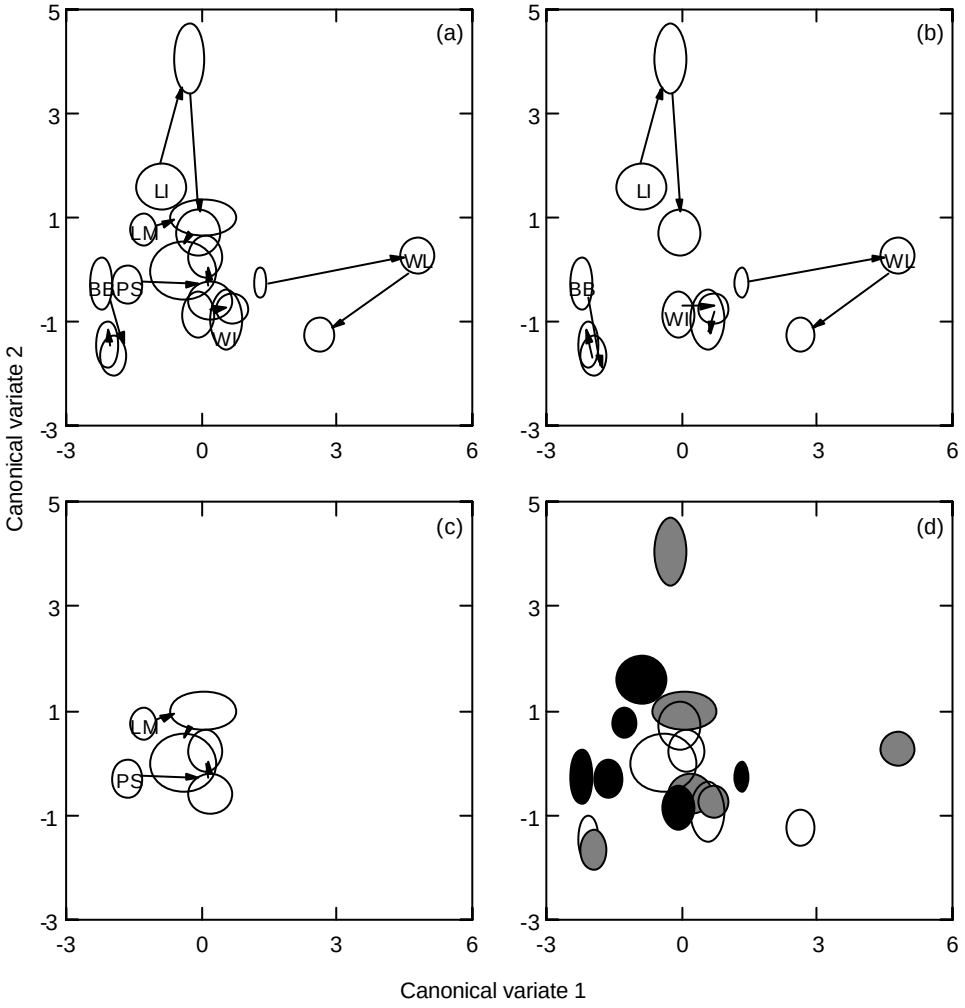

FIGURE 3.3. Plots summarising variation in elemental fingerprints of otoliths of juvenile snapper collected from six of the 15 estuaries in each of three years. (a) All six estuaries and years are shown with arrows representing the change from one year to the next for each individual estuary; (b) and (c) the same plot as that shown in (a) with four and two estuaries shown respectively to clarify the changes over time for each individual estuary; and (d) the same plot as that shown in (a) but distinguishing among years rather than among estuaries. Shaded areas represent 95% confidence ellipses around the means for each estuary for each

canonical variate. See Fig. 3.1 for estuary abbreviations. In (d) solid ellipses represent 1998/1999, hatched ellipses 1999/2000 and open ellipses 2000/2001 recruitment seasons.

3.4. Discussion

The elemental composition of otoliths of juvenile snapper showed significant spatial and temporal variation, such that it was possible to confound spatial differences with temporal differences (see for example Fig. 3.3). This does not prevent determination of natal estuaries of adult fish, but it does suggest that a library of elemental fingerprints needs to be built-up over time for each estuary rather than a single year class of juveniles being used as the elemental fingerprint for a number of year classes of adults (as was done in Gillanders & Kingsford, 1996). The natal estuary of adult fish would then need to be determined based on elemental fingerprints of juveniles from the year class in which the adults of interest recruited to the estuaries (see Chapter 4). This may not necessarily be the case for all regions, for example, elemental fingerprints of juvenile fish from Port Phillip Bay (Victoria, Australia) are consistently different from other estuaries, at least over a two-year period (Hamer, MAFRI, pers. comm.).

Significant temporal variation in elemental fingerprints was found for individual estuaries, suggesting that either the water chemistry (e.g. elements in the water, temperature and salinity) or ontogenetic and physiological effects may vary among years. Juvenile snapper of the size used in the current study would be approximately three-four months old based on previous studies of otolith microstructure (Bell *et al.*, 1991; Kingsford & Atkinson, 1994), therefore, different cohorts have been sampled each year. Although fish were collected at similar times (summer) each year, differences in water chemistry may occur among years. Rainfall and subsequent freshwater input to estuaries may vary substantially each year, especially along the coast of NSW, Australia. For example, rainfall to the different coastal meteorological regions varied two to three-fold for the November-February period of the years 1997/98 to 2000/01. The northern regions including the Sydney metropolitan region had the highest rainfall in the 1999/2000 period, whereas the southern regions had the highest rainfall in 1998/99 (South Coast) or in 2000/01 (Lake Illawarra) suggesting that freshwater effects may vary not only among catchments but also among years. In addition, all catchments did not always have the highest rainfall in the same year.

Many past studies have only sampled at one time or sampled different locations at different times and have ignored possible temporal effects. In regions where there is little variation in

rainfall and freshwater input to coastal waters, there may be little variation in elemental fingerprints among years, but this should not be assumed. Sampling different locations in different years may confound spatial differences with temporal differences. For example, if Lake Macquarie had been sampled in 1999/2000 and Lake Illawarra had been sampled in 2000/2001, it would be concluded that there were no significant differences between the two estuaries in terms of elemental fingerprints, whereas if samples were taken from both estuaries in 1999/2000 significant differences would have been found (Fig. 3.3). Thus, it is necessary to sample all locations at similar times.

A number of studies have found significant variation within a year, although the main elements examined in most of these studies were Sr, Na, K and S (Table 3.4). In addition, most studies have only examined one site, therefore it is unknown whether some sites may show variation in otolith chemistry over time whilst others show little or no variation as was found in the current study. Of the trace elements examined in the present study Mn has been found to show temporal variability in otolith chemistry over time periods of less than a year (2-6 month period), whilst Ba showed significant variability over a 2 month period in one study, but not over a 6 month period in another study (Thorrold *et al.*, 1998a; Thorrold & Shuttleworth, 2000). No studies have examined variability of Li in otoliths within a year.

Significant variation in elemental composition of otoliths of juvenile snapper among estuaries was found for all years. There have been few studies that have investigated temporal variability compared to the number examining spatial variability in otolith chemistry. Campana *et al.* (2000) found that elemental concentrations varied little over 2 year intervals, but more substantive differences were noted for some elements (e.g. Li, Mg, Ba) and some locations over 4-13 year intervals. However, a number of other studies have found significant variation in otolith chemistry over 2-3 year intervals (Table 3.4, Milton *et al.*, 1997; Dove & Kingsford, 1998; Patterson *et al.*, 1999; Gillanders & Kingsford, 2000; Rooker *et al.*, 2001). These results suggest that it will not be easy to predict whether there will be inter-annual variability in elemental fingerprints, but it should not be assumed that there will not be inter-annual variability. Variability in elemental fingerprints over time may have major implications for determining stock mixing when whole otoliths are used since temporal variability implies that the marker may not remain stable between characterisation of stocks and mixing of stocks (see also Campana *et al.*, 2000).

TABLE 3.4. Review of studies examining temporal variation in elemental chemistry of otoliths. Details of the sampling design, elements analysed and differences over time are given. Within each temporal scale (within years and among years), the table is order by year of reference.

Temporal scale	Elements analysed	Differences	Source
Within years			
2 collections within 6 months at 1 site	Mn, Sr, Ba	Ba & Sr ns Mn significant	Thorrold & Shuttleworth, 2000
August & October collections in 1 year	Mg, K, Mn, Sr, Ba	Significant differences among sampling occasions	Thorrold *et al*., 1998a
4 collections at 3 month intervals over 1 year at 1 site	Mg, Na, P, S, Sr	Seasonal variation observed in canonical variate plot	Edmonds *et al*., 1995
8 collections over approx. 1 year from 1 site	Sr, Na, K, S	Significant seasonal variation for all elements ratioed to Ca	Kalish, 1991
Monthly collections over 1 year period at 1 site	Sr, Na, K, S	Significant variation during the year for all elements	Kalish, 1989
Among years			
3 collections over 3 years at 1 site	Li, Mg, Mn, Sr, Ba	Mg, Mn and Ba showed significant interannual trends	Rooker *et al*., 2001
2 collections pooled per year over 2-3 years at 4 sites	Ba, Li, Mg, Mn, Sr	Relatively similar	Campana *et al*., 2000
Approx. 5 collections over 13 year period at 4 areas	Ba, Li, Mg, Sr	Concentrations differed relatively little over 2-year intervals, more substantive differences noted for some elements and some locations after 4-13 year intervals	Campana *et al*., 2000
Sampled twice approx. 1 year apart; 2-5 sites in each of 7 estuaries	Ba, Mn, Sr	Significant temporal effects for some or all estuaries	Gillanders & Kingsford, 2000
2 collections in summer of each of 2 years at one site	Zn, Sr, Ba, Pb	Significant differences for Ba	Patterson *et al*., 1999
2 collections approx. 1 year apart; 2 sites sampled at each of 6 locations	Al, Ba, Co, Cu, Hg, Mg, Mn, Pb, Sr, Rb, Ti, Zn	Significant temporal differences for Ba and Mn. Results for other elements not reported	Dove & Kingsford, 1998
2 collections approx. 1 year apart at 5 sites	Li, Na, Mg, Cu, Zn, Sr, Ba	Significant difference between years for all elements except Na	Milton *et al*., 1997

2 collections separated by about 2 yr at 3 sites	Mg, Na, P, S, Sr, K	S and P showed temporal variation; no statistical analyses for any elements	Edmonds *et al*., 1995
3 collections over 3 years	Mg, Na, P, S, Sr	Temporal variation observed in CV plot but elements contributing to differences not mentioned	Edmonds *et al*., 1995
2 collections approx. 2 years apart at 3 sites	B, K, Mg, Mn, Na, Sr	Temporal variation observed in CV plot but elements contributing to differences not mentioned	Edmonds *et al*., 1992

The elemental fingerprints allowed some estuaries to be individually distinguished in each year (e.g. Wallis Lake). However, other estuaries could only be distinguished in some years (e.g. Lake Illawarra, Lake Macquarie, Eden), while others showed overlap with some estuaries in all years (e.g. estuaries in the vicinity of Sydney). Although there was some overlap in elemental fingerprints among some estuaries, groups of estuaries could usually be distinguished. Other studies have also found variation in elemental fingerprints among different geographic areas (e.g., Edmonds *et al.*, 1989, 1991, 1992; Campana *et al.*, 1994, 1995; Begg *et al.*, 1998), including among estuaries (Thorrold *et al.*, 1998b; Gillanders & Kingsford, 2000) and between estuaries and coastal reefs (Gillanders & Kingsford, 1996). Such differences are frequently used to infer differences in stock structure, but among-estuary and estuary-coastal reef differences have also been used as natural tags of area of origin for juveniles. For each year of sampling, the current data suggest that there were variations in elemental fingerprints in otoliths of snapper that allowed separation of a number of estuaries. Hence, these differences provide a natural tag or area of origin for snapper.

Despite differences in elemental composition of otoliths among estuaries and over time the mechanisms generating these differences are not well understood. Estuaries show differences in land use varying from intense urban and industrial (e.g. Port Jackson, Botany Bay) to low intensity rural activities (e.g. list), which may provide a range of potential levels of anthropogenic input that could influence the concentration of elements in the water. There is also likely to be natural variation among estuaries due to different catchments, which may have varying levels of rainfall, rock, soil, and vegetation types. Besides factors influencing trace elements in the water, temperature and salinity are also likely to vary among estuaries. For example, temperatures in December and January 1998/1999 were approximately 4 °C lower in Eden than in Botany Bay waters (Gillanders, unpublished data). Elements in the water, temperature and salinity may also vary not only among estuaries, but also among years.

Past studies have shown that these factors can influence microchemistry of otoliths (Fowler *et al.*, 1995a; Gallahar & Kingsford, 1996; Chesney *et al.*, 1998; Bath *et al.*, 2000; Milton & Chenery, 2001).

In addition to environmental factors influencing otolith chemistry, a range of other factors (e.g. diet, growth rates, ontogenetic and physiological effects) may also influence otolith composition in some species (Kalish, 1989, 1991; Sadovy & Severin, 1992; Fowler *et al.*, 1995b; Limburg, 1995). Differences in mean size at age of adult snapper have been observed along the coast of NSW (Ferrell & Sumpton, 1997) and a positive relationship was found between sea surface temperature and growth of juvenile snapper in NZ (Francis, 1994). Since sea surface temperature varies among estuaries along the coast of NSW (see above), differences in rates of growth may be found among estuaries. In addition, temperature is likely to have a direct effect on some elements (e.g. Sr, Ba). While differences in growth rates and diets among estuaries can not be discounted as possible determinants of otolith composition in snapper, ontogenetic and physiological effects were minimised by analysing otoliths of juvenile fish (0^+ years) of similar sizes. In addition, a recent study found that water chemistry had a greater effect on otolith chemistry than similar levels of trace elements within the diet (Milton & Chenery, 2001).

Although the mechanisms generating spatial and temporal differences in otolith chemistry are not well understood, spatial differences in otolith chemistry suggest that the elemental fingerprints of fish from different estuaries do provide a natural tag of their juvenile habitat. The natural tag for individual estuaries does however vary over time and such temporal differences need to be considered when determining natal estuaries of adult fish. For example, the age of adult fish needs to be determined and elemental fingerprints of juvenile fish from the adult fish's birth year used to determine its natal estuary. A similar region of the otolith to that analysed for the juvenile should also be analysed for the adult fish. Numbers of adult fish originating from different estuaries could then be determined for different year classes of adults. Thus, spatial models of population replenishment could be developed and patterns of movement of different year classes established (e.g. Allison *et al.*, 1998). Spatial models of population replenishment would help determine whether adults on reefs outside estuaries have come from the estuary closest to them with little transfer from other estuaries or whether the predominant movement is from estuaries close by with few fish remaining on the closest reef to their natal estuary and some moving long distances or whether one estuary essentially sustains coastal reefs of all other estuaries. Results from such models could have major implications for fisheries management and conservation of estuarine habitats.

4. Which estuaries did snapper caught as part of the coastal fishery spend their juvenile lives in?

4.1. Introduction

Determining connectivity between spatially segregated juvenile and adult populations is critical, but remains poorly understood. Rates of exchange among populations of marine organisms are vital for studying population dynamics, management of fishery stocks and the design of marine protected areas. Quantifying rates of exchange of marine organisms using conventional tagging techniques is difficult because of the small size of larvae and juveniles, high rates of mortality at early life history stages and the large numbers that need to be tagged in order to get any recoveries. Recently, the interpretation of biological markers such as isotopic and elemental composition has been developed to determine connectivity between populations (e.g. Gillanders & Kingsford, 1996; Thorrold *et al.*, 2001).

The major calcified structure used as a natural tag has been the ear bones or otoliths of fish. The acellular and metabolically inert nature of otoliths means that any elements accreted onto the growing surface of the otolith are permanently retained (Campana, 1999). The otolith continues to grow through time ensuring that the entire lifetime of the fish has been recorded and differences in microchemistry between layers can be resolved to within days or years of their deposition. The calcium carbonate and trace elements that make up 90% of the otolith are derived primarily from the water, although in practice there are likely to be physiological filters that ensure that there is not a simple linear relationship between the water and the otolith (Campana, 1999). Analysis of either whole or points within the otolith have been used to distinguish stocks or sub-populations within marine species (Edmonds *et al.*, 1989, 1991, 1992; Campana *et al.*, 1994; Campana & Gagne, 1995; Proctor *et al.*, 1995), to reconstruct temperature history (Patterson *et al.*, 1993) and to detect anadromy (Kalish, 1990; Secor, 1992; Coutant & Chen, 1993).

This study investigates potential movement between estuarine and open coastal habitats for snapper *Pagrus auratus* (Sparidae) using an application of otolith chemistry. Within New South Wales, Australia, snapper recruit to bare substrata of estuarine habitats where they remain for 12-18 months, after which time they move to coastal reefs and other habitats over the continental shelf (Bell & Worthington, 1993). Commercial fishers take snapper as part of the inshore trap and line fishery, where the average age of fish taken is 3 years. The general objective of this study was to determine the nursery estuary of snapper collected in the inshore trap and line fishery. The specific aims were to (1) determine the elemental

fingerprints of juvenile snapper collected from different estuaries along the coast of New South Wales, Australia, and (2) determine the nursery estuary of snapper of the 2 year age class by analysing the region of the otolith that was laid down when the fish was a juvenile. Because the chemical signal laid down in the otolith varies both among estuaries and amongst years there is potential to confound temporal and spatial differences (see Chapter 3). Consequently, here it was necessary to determine the natal estuary of 2^{+} adult fish based on the chemical signal of otoliths from juveniles that were collected 2 years earlier.

4.2. Materials and Methods

4.2.1. Sample collection

Juvenile snapper (*Pagrus auratus*, Sparidae) were collected between November 1998 and February 1999 to determine variation in elemental fingerprints among estuaries. Ten fish were collected from each of two sites (separated by hundreds of metres to kilometres) in each of 15 estuaries (separated by tens to hundreds of kilometres) spanning 600 km along the coast of New South Wales, Australia. All fish were collected by hand line and were stored on ice in the field before being dissected on return to the laboratory. For each fish, the standard length (SL) was measured (range 44 to 83 mm SL, average 67 ± 0.5 mm SL), and the sagittal otoliths removed, cleaned of adhering tissue in Milli-Q water, air-dried and placed in eppendorf microcentrifuge tubes.

Adult snapper were sampled from catches at the Sydney Fish Markets, as part of the NSW Fisheries snapper monitoring program. The sagittal otoliths were removed, rinsed and stored dry in paper envelopes for latter analysis. One sagittal otolith was prepared for ageing by sectioning through the focus with a low speed saw. The resulting section was polished and mounted on a standard microscope slide. Thin sections were viewed with reflected light at a magnification of 20× on a compound microscope and age estimated from the count of opaque increments. In addition, the edge type was noted as opaque or translucent. Using the estimated age and whether the edge was opaque, the birth summer of the fish was calculated. Fish with a birth summer of 1998/99 were then used for subsequent microchemical analysis.

4.2.2. Sample preparation

Otoliths from recruits and adults were sectioned through the primordium with a low speed saw. The resulting section was polished on an abrasive sheet with Milli-Q water and sonicated for 5 min before being rinsed several times in Milli-Q water and being placed in a plastic laminar flow cabinet to dry overnight. Sections were then mounted on microscope

slides and individually placed in plastic bags ready for analysis using laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS).

4.2.3. Analytical methods

The LA ICP-MS system at the Department of Earth Sciences, Monash University consists of a Merchantek LUV266 petrographic ultraviolet laser (Nd:YAG) microprobe connected to a Finnigan MAT ELEMENT high resolution ICP-MS. The ICP-MS instrument was operated in low resolution ($m/\Delta m$ = 300 mode). The sample to be analysed was placed in the ablation chamber and viewed remotely on a computer screen where the area for ablation was selected. The laser was focused on the sample surface and was fired through the microscope objective lens. The ablated material was entrained in argon and helium gas for analysis of Mg, Ca, Mn, Sr and Ba isotopes by ICP-MS. Calcium was used as an internal standard to correct for variations in ablation yield. Calcium concentration was assumed from the stoichiometry of calcium carbonate as 400 000 μg g^{-1} and the concentration of other elements were estimated against the Ca concentration (Ludden *et al.*, 1995).

At the beginning and end of each day of analysis, background counts were collected for 60 s and the variation amongst these was used to calculate limits of detection. Before each sample, 20-30 s of blank counts were determined, the average of which was used for subtraction from that sample. After about 20-30 s of blank counts, the sample was then ablated using a spot size of 100 μm. Only one crater was ablated for each sample because preliminary analyses showed that there was greater variation between otoliths of different fish than within otoliths of the same fish. To correct for instrument drift NIST 612 standards were analysed every 10-12 samples and a linear interpolation between the two consecutive sets of standards made. A pressed powder snapper standard was also analysed prior to any otolith samples and at the end of each day of analysis.

4.2.4. Statistical methods

To determine whether there were significant differences among estuaries for individual elements and multielement fingerprints, analysis of variance and multivariate analysis of variance were used. Both types of analyses involved two factor designs and both terms (estuary and site) were considered random factors. Site was nested within the estuary term. All analyses were performed on $\ln(\times+1)$ transformed data. The MANOVA used a non-parametric technique incorporating permutation tests (Anderson, 2001a). The method of

permutation involved permuting the raw data; further details on methods of permutation can be found elsewhere (Manly, 1997).

A maximum likelihood-based analysis was used to determine (1) the ability of elemental fingerprints to record correctly the proportion of recruits from different estuaries, and (2) the proportion of adult fish originating from the different estuaries/groups of estuaries. The maximum likelihood estimator was chosen because it performs best in practice and provides maximum discriminatory power in mixed stock situations (Millar, 1987, 1990a). Results were obtained from a multipurpose simulation/bootstrap/analysis program (Millar, 1990b). The program was initially run in simulation mode using the juvenile elemental data. Fifteen estuaries were too many to discriminate amongst based on only four discriminatory variables (Mg, Mn, Sr, Ba) and therefore the estuaries were grouped into four regions (Wallis Lake, Sydney region, Eden and others). Classification errors were estimated from the difference between the actual or known composition of juveniles and the estimated composition, although these errors do not take into account the variability associated with estimating the composition. The data were not re-sampled, and therefore the classification rule remained fixed throughout the simulations (see Millar, 1990a, b). In simulation mode, 100 simulations were made and the data used to provide an estimate of the variability of the estimator. The second analysis then involved using the juvenile elemental fingerprints from 1998/1999 as the baseline data for which estuarine affinity was known and running the program in bootstrap mode to estimate the proportion of adults from each estuary/groups of estuaries. Thus, the adult elemental fingerprints were used as the mixed fishery data. Again, 100 simulations provided an estimate of variability associated with the proportion of adult fish estimated to be from each estuary/group of estuaries.

4.3. Results

4.3.1. Juvenile fish

Manganese and barium both showed significant variation among estuaries (Fig. 4.1, Table 4.1). Otoliths of fish from Eden had significantly lower amounts of Mn than those from the other estuaries. Greater amounts of Mn were found in otoliths of fish from Lake Macquarie, Tuggerah Lakes and Lake Illawarra. For Ba, otoliths of fish from Wallis Lake had significantly greater levels than fish from elsewhere. Several estuaries (e.g. Hawkesbury Estuary, Middle Harbour, Batemans Bay and Eden) produced low readings of Ba in otoliths of fish. No significant differences among estuaries were found for Mg and Sr.

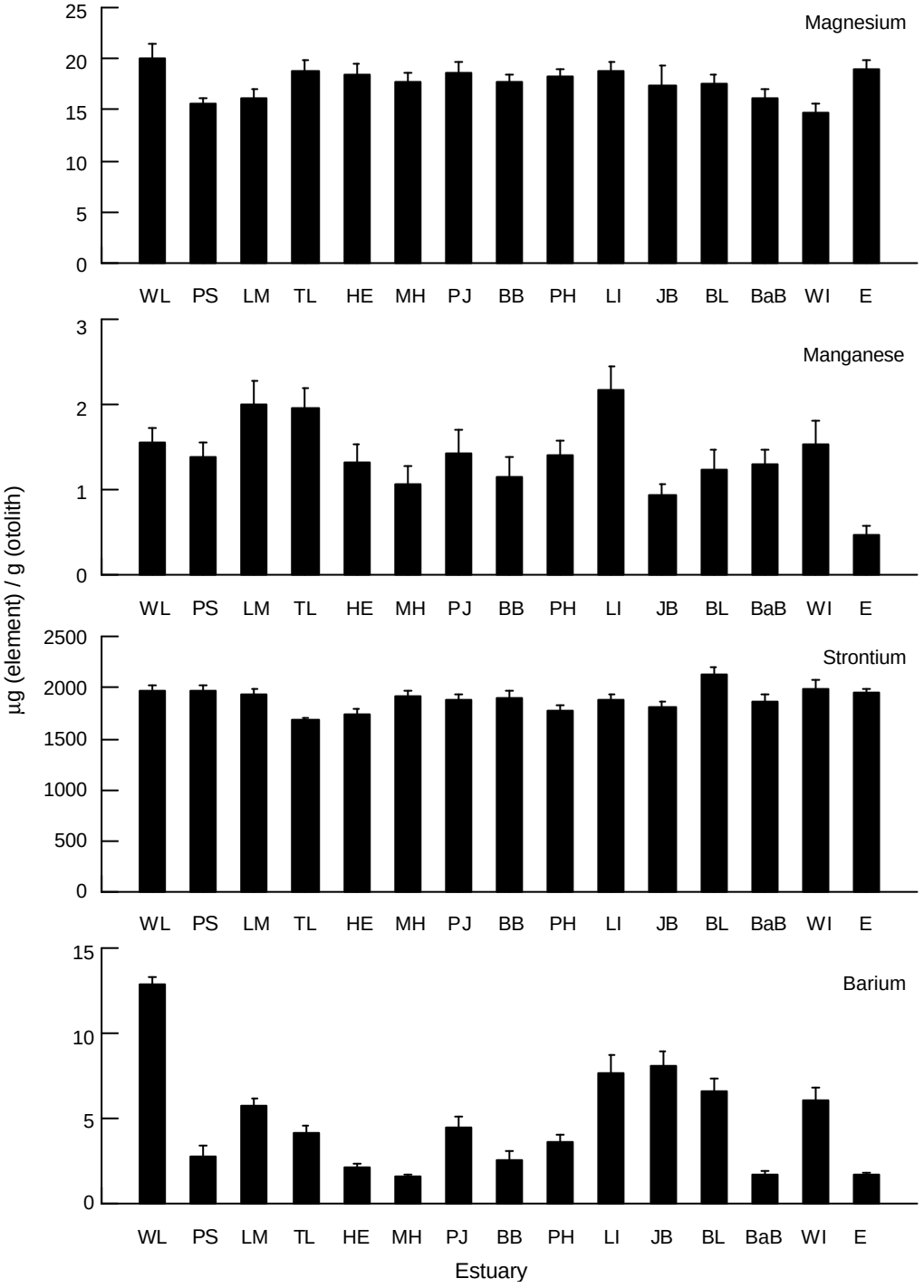

FIGURE 4.1. Mean concentration (±SE) of Mg, Mn, Sr and Ba in otoliths of juvenile snapper collected from 15 estuaries along the coast of New South Wales, Australia. The data were pooled for the 2 sites within each estuary. The estuaries were Wallis Lake (WL), Port Stephens (PS), Lake Macquarie (LM), Tuggerah Lakes (TL), Hawkesbury Estuary (HE), Middle Harbour (MH), Port Jackson (PJ), Botany Bay (BB), Port Hacking (PH), Lake Illawarra (LI), Jervis Bay (JB), Burrill Lake (BL), Batemans Bay (BaB), Wagonga Inlet (WI) and Eden (E).

TABLE 4.1. ANOVA (and MANOVA) table showing differences among estuaries and between sites for Mg, Mn, Sr and Ba (and for the multielement fingerprint) in otoliths of juvenile snapper.

Source	df	MS	*F*	P
Mg				
Estuary	14	0.1316	1.80	0.1349
Site (estuary)	15	0.0730	1.55	0.0885
Residual	270	0.0472		
Mn				
Estuary	14	06979	3.79	0.0075
Site (estuary)	15	0.1842	1.32	0.1876
Residual	270	0.1392		
Sr				
Estuary	14	0.0645	1.53	0.2125
Site (estuary)	15	0.0422	2.45	0.0022
Residual	270	0.0172		
Ba				
Estuary	14	5.0265	11.44	0.0000
Site (estuary)	15	0.4394	3.26	0.0001
Residual	270	0.1346		
Mg, Mn, Sr & Ba				
Estuary	14	5.9123	8.0161	0.0002
Site (estuary)	15	0.7375	2.1870	0.0030
Residual	270	0.3372		

Significant differences were found in multielement fingerprints among estuaries (Fig. 4.2, Table 4.1). The 95% confidence ellipses around the mean value for each estuary suggested that some estuaries such as Wallis Lake and Eden had quite different elemental fingerprints compared with the rest (Fig. 4.2). There were, however, a number of estuaries that overlapped with others particularly those in the vicinity of Sydney: Hawkesbury Estuary, Port

Jackson, Middle Harbour, Botany Bay and Port Hacking. This suggests that these estuaries can be grouped to determine nursery estuaries of adult fish.

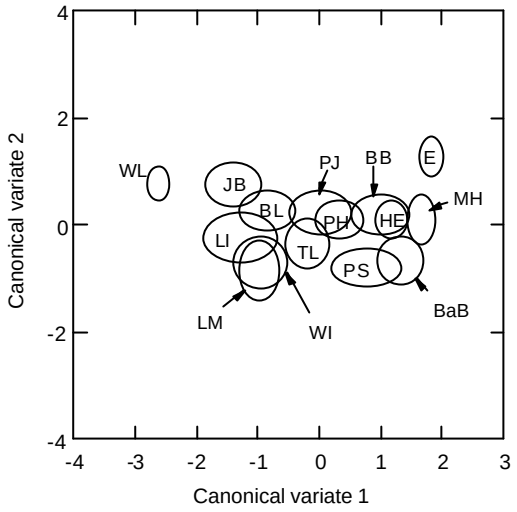

FIGURE 4.2. Plot summarising variation in elemental fingerprints of otoliths of juvenile snapper collected from different estuaries along the coast of New South Wales, Australia. Shaded areas represent bootstrapped 95% confidence ellipses (n=1000) around the means of each estuary for each canonical variate.

Maximum likelihood-based estimation was used to determine the ability of elemental fingerprints to classify correctly the proportion of recruits from different estuaries. The actual composition ranged from 7 to 53% depending on the estuary or group of estuaries (Table 4.2a). The estimate of proportion of recruits from the different estuaries ranged from 7.24 to 48.21% suggesting an error rate of <1 to 4.79%, although this error rate has not taken into account the variability in the simulation runs (Table 4.2a). The greatest error was associated with the grouping of estuaries labelled "Other", whilst the estimates of error for Wallis Lake, Eden and the Sydney estuaries was less than 3%. The variability in the classification from the simulations for juvenile snapper ranged from 7.64 to 19.39% being greatest Sydney and "Other" estuaries.

4.3.2. Adult fish

Maximum likelihood-based estimation was used to determine the proportion of adult fish that originated from the different estuaries. Most fish (89%) caught in the Sydney region originated from local nurseries (e.g. Hawkesbury Estuary, Middle Harbour, Port Jackson, Botany Bay or Port Hacking) (Table 4.2b). About 9% of fish had Eden as their natal estuary

and 2% had come from the remaining estuaries, excluding Wallis Lake which contributed no fish. These estimates of the proportion of adult fish originating from the various estuaries do not take account of potential variability in the maximum likelihood-based estimator. Results from 100 simulations of the data showed that the variability ranged from 0% (Wallis Lake) to 12.85% (Sydney estuaries). After taking into account the variability for the estimated proportions, all fish from the Sydney region may have recruited to Sydney estuaries. This suggests that adults on reefs outside estuaries originated from the estuaries closest to them, with little input from other estuaries.

TABLE 4.2. Maximum likelihood analysis results for (a) juvenile snapper and (b) unknown adult snapper.

Estuary	**Actual contribution**	**Estimated contribution**	**SD**
(a) Juveniles			
Wallis Lake	0.07	0.1000	0.0864
Other	0.53	0.4821	0.1930
Sydney	0.33	0.3455	0.1939
Eden	0.07	0.0724	0.0764
(b) Adults			
Wallis Lake		0.0000	0.0000
Other		0.0199	0.0352
Sydney		0.8944	0.1285
Eden		0.0857	0.1243

4.4. Discussion

The concentration of some elements in the otoliths of recruits of snapper varied among fish collected from different estuaries. For example, barium was significantly greater in otoliths of fish collected from Wallis Lake than the other estuaries, whereas manganese occurred in greater concentrations in otoliths of fish from Lake Illawarra, Lake Macquarie and Tuggerah Lakes. This meant that elemental fingerprints that were representative of a number of estuaries could be used to distinguish amongst snapper recruits. Although the causal factors

responsible for these spatial differences are not known, these differences have enabled the use of trace and micro-elements to reconstruct past life history.

Use of otolith elemental fingerprints as natural tags must meet three assumptions, namely (1) there are significant differences in the elemental fingerprints of the groups of interest, (2) all possible groups contributing to the group mixture have been characterised and (3) the fingerprint remains stable over the interval between characterisation and mixing (Campana, 1999, 2000). The elemental fingerprints of juvenile snapper showed significant differences among estuaries. However, fifteen estuaries was considered too great a number to discriminate amongst given that there were only four discriminatory variables (Mg, Mn, Sr, Ba) and therefore fish were grouped into four categories (Wallis Lake, Eden, Sydney estuaries and other) for determining the proportion of fish from different estuaries. While many studies have found group-specific variation in elemental composition (Edmonds *et al.*, 1989, 1991, 1992; Campana & Gagne, 1995; Campana *et al.*, 1995; Thorrold *et al.*, 1997), including among estuaries (Thorrold *et al.*, 1998b; Gillanders & Kingsford, 2000) and between estuaries and coastal reefs (Gillanders & Kingsford, 1996), this is not always the case (Kalish *et al.*, 1996; Gillanders *et al.*, 2001) and needs to be assessed empirically.

To satisfy the second assumption, juvenile fish were collected from as many estuaries as possible in order to characterise all potential groups. If some estuaries remain uncharacterised then it is possible that adult fish are characterised to an incorrect natal estuary. While there are over 80 estuaries along the coast of New South Wales (Bucher & Saenger, 1991), many are open for relatively short periods of time (or not at all) and therefore may not be available as recruitment habitats for snapper. Once snapper have recruited, the estuary must be open for subsequent movement to the adult population. In addition, many estuaries along the coast of New South Wales are relatively small (area of water <10 km2 West *et al.*, 1985) and may not contribute greatly to adult populations. Of the estuaries between Wallis Lake and the NSW/Victoria border that have a greater surface area than 10 km^2, fish were collected and elemental fingerprints determined for 70%. In addition, some smaller estuaries were also sampled. In past studies, it is not clear whether all possible groups have been characterised, however, it is vital that as many groups as possible are characterised if mistakes about nursery origin are to be avoided.

Elemental fingerprints of some species vary amongst different recruitment seasons (see also Chapter 3, Gillanders & Kingsford, 2000). Campana *et al.* (2000) found that elemental concentrations varied little over 2 year intervals, but more substantive differences were noted for some elements (e.g. Li, Mg, Ba) and some locations over 4-13 year intervals. However, a

number of other studies have found significant variation in otolith chemistry over 2-3 year intervals (See Chapter 3, Milton *et al.*, 1997; Dove & Kingsford, 1998; Patterson *et al.*, 1999; Gillanders & Kingsford, 2000; Rooker *et al.*, 2001). Variation over short time periods has significant implications for solution-based analyses of whole otoliths as this implies that the elemental fingerprint may not remain stable over time. For laser-based analyses, variation in elemental fingerprints over time suggests that similar regions of juvenile and adult otoliths need to be sampled, as was done in the current study (see below). In addition, variation in elemental fingerprints among years does not preclude determining natal estuaries of adults but it does suggest that adults need to be tracked back to natal estuaries using elemental fingerprints developed on recruits from the year class in which the adults of interest were in the estuary. Thus in the current study, since the elemental fingerprints were determined for juveniles that originated in 1998/99, it was necessary that the adults that were subsequently sampled originated as part of the year class.

A laser-based approach was used to sample the otoliths of juveniles and adults. The area of each otolith sampled was that which would have formed during the 20-30 day period in the estuarine habitat. Based on the approximate distance from the core to the region sampled in juveniles, the corresponding region of the adult otolith was then sampled. A similar approach was used by Thorrold *et al.* (2001) to determine homing of spawning weakfish (*Cynoscion regalis*) to natal estuaries. Gillanders & Kingsford (1996) used a solution-based approach to analyse whole juvenile otoliths from two types of habitat (estuarine seagrass and algal reef) and cores of adult blue groper (*Achoerodus viridis*) otoliths and thereby determine natal habitats of adult fish collected on the reef. Analyses of whole otoliths of cod (*Gadus morhua*) collected from known spawning grounds were also used as the baseline data from which to characterise mixed over-wintering schools (Campana *et al.*, 2000).

Within NSW, more than 97% of commercial landings of snapper are from the trap and line fishery, which operates in nearshore and continental shelf waters (NSW Fisheries, 2001). Although there are no signs of the large variation in recruitment to the fishery evident in some other snapper populations (e.g. South Australia, McGlennon *et al.*, 2000), year-to-year variation in landings has often been high (Ferrell & Sumpton, 1997). Two and 3 year old fish dominate all landings in NSW with fewer than 2% of fish landed being older than 10 years despite a potential longevity of in excess of 30 years (NSW Fisheries, 2001). Knowing the estuaries from which adult fish originate and their potential movement patterns as 2-4+ year olds is extremely valuable information for the management of the fishery. The recruitment estuaries of adult snapper collected in the vicinity of Sydney were predominantly local, i.e. from Hawkesbury Estuary to Port Hacking. Few fish recruited from other estuaries, although

there was some indication that some fish may have moved considerable distances (e.g. from Eden north to Sydney). In addition, when the variability in the estimates of proportions originating from different estuaries was taken into account, there was evidence to suggest that all fish from the Sydney region may have recruited to Sydney estuaries suggesting little or no movement for 2+ fish.

By analysing multiple year classes of snapper over time it will be possible to construct spatial models of population replenishment that identify the distance that fish have moved from their recruitment estuary, and also the number of estuaries that contribute to a certain adult population. For example, evidence from 2+ fish suggests that adults in nearshore waters have come from the estuaries closest to them with little input from other estuaries suggesting short distance movements. In addition, it does not appear that there is only one estuary replenishing all adult populations in nearshore and shelf waters. Whether 3+ and 4+ fish show similar patterns is unknown, although older fish may be expected to move further from their recruitment estuaries given that snapper are relatively mobile. In addition, it is not known whether similar patterns would be found for 2+ fish sampled in different years, but given that these fish may have left estuaries 6-12 months previously they may have had relatively little time to move long distances. Differences in spatial models of population replenishment between 2+ fish sampled in one year and 3+ fish sampled in the following year may also indicate predominant movement patterns of fish once they have left estuaries.

In conclusion, elemental fingerprints of fish show great promise in determining the origins or recruitment habitats of fish. Determining origins of multiple year classes of adults will allow spatial models of population replenishment to be established and provide estimates of connectivity among habitats. An understanding of connectivity between juvenile (e.g. estuary) and adult populations has considerable implications for fisheries management and the effective conservation of fishes.

5. Benefits

Although the research focused on snapper in New South Wales, the methods developed in this study are of use for many estuarine species around Australia. A similar study is currently underway in Victoria (Migratory dynamics and recruitment of snapper, *Pagrus auratus*, in Victorian waters, Jenkins, MAFRI, Victoria). Knowledge of the nursery habitats of snapper in New South Wales directly benefits the commercial and recreational fisheries in this state by providing information on estuaries that supply juveniles to the adult population. Since both the Queensland and Victorian fisheries have estuaries where juvenile snapper are found there will be some benefits to these states from this research.

6. Further Development

Since the elemental fingerprints of juvenile fish vary among years a "library" of elemental fingerprints needs to be built-up over time for the birth years of adults of interest. Thus, in the current study juvenile fish were collected in 1998/99, 1999/2000 and 2000/2001 and therefore it was only possible to determine the natal habitats of 2+ fish (birth year 1998/1999). However, the average age of fish in the fishery is 3 years. Further research should focus on determining natal habitats of 3 and 4 year old fish and determining whether there is variation in natal habitats of different year classes of 2-4+ fish.

7. Outcomes

Results from this project showed that there were differences in elemental chemistry of otoliths among estuaries ensuring that elemental fingerprints can be used as a natural tag of the recruitment or nursery habitat. However, significant spatial and temporal variation in elemental fingerprints was found. For adults that are subsequently sampled it is therefore necessary that they must originate from one of the year classes of juveniles that have previously been sampled. In addition, future researchers must ensure that spatial variations in elemental signatures are not confounded by temporal variation. The results also provide a model of replenishment for adult populations. Adults on reefs outside estuaries have come from the estuaries closest to them with little transfer from other estuaries, at least for the 2+ year class of snapper.

8. Conclusion

Significant differences in otolith chemistry were found for three species of juvenile sparids collected from different estuaries. The same patterns among estuaries were not seen for all species, although it was not possible to sample the same sites within an estuary for all species. For snapper, a number of estuaries could be separated, but there was some overlap for other estuaries. Spatial differences in otolith chemistry suggested that the elemental fingerprints of fish from different estuaries do provide a natural tag of their juvenile habitat. The natural tag for individual estuaries does however vary over time and such temporal differences need to be considered when determining natal estuaries of adult fish. For example, the age of adult fish needs to be determined and elemental fingerprints of juvenile fish from the adult fish's birth year used to determine its natal estuary. Thus, the results from juvenile fish suggest that chemical "fingerprints" need to be determined for each estuary and for each year. Information on the spatial and temporal variability of the elemental fingerprints was necessary before the proportion of fish in the commercial catch from different estuaries could be determined.

Using a laser based or solid sampling approach the region of adult otoliths corresponding to their juvenile estuarine habitat was sampled and maximum likelihood methods used to determine proportions of adult fish from different estuaries. The natal estuaries of adult 2+ snapper collected in the vicinity of Sydney were predominantly estuaries in the Sydney region (Hawkesbury Estuary to Port Hacking). Few fish recruited to other estuaries, although there was some indication that some fish may have moved considerable distances (e.g. from Eden north to Sydney). In addition, when the variability in the estimates of proportions originating from different estuaries was taken into account, there was evidence to suggest that all fish from the Sydney region may have recruited to Sydney estuaries suggesting little or no movement for 2+ fish. Thus, using otolith chemistry the proportion of the commercial catch from different estuaries was determined.

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Appendix 1:

Intellectual property

No patents have emerged from this research. There is no economic value arising from this project. The information is however relevant to other researchers studying snapper and to researchers using otolith microchemistry as a tool to trace origins and movements of fish.

Appendix 2:

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