Trace metals in four structures of fish and their use for estimates of stock structure

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Abstract—Trace elements in calcified tissues have been suggested as one of the most powerful means for stock discrimination yet developed. The structure of choice for determining elemental composition or fingerprints is the otolith, although other structures also incorporate trace elements into their matrix. The aim of this study was to compare the elemental fingerprints of four structures (otoliths, scales, eye lenses, and spines) of a territorial reef fish to determine whether there were correlations between otoliths and each of the other structures. Elemental fingerprints of juvenile (<3 years of age) and adult fish (which may reach a maximum age of 37 years) were also compared for each structure to determine whether there may be differences between size classes of fish. All structures were analyzed by solution-based inductively coupled plasma-mass spectrometry (ICP-MS). Otoliths, scales, spines, and eye lenses differed in composition. Calcium dominated otoliths, scales, and spines but was not detected in eye lenses. Some elements, for example barium, showed significant correlations between the otolith data and that of scales and spines of both juvenile and adult fish. A multivariate test of matrix correspondence (Mantel's test) detected significant relationships between the otolith data and the data matrices for each of scales and spines of both juvenile and adult fish. Significant relationships between the otolith data and the eye lens data were detected only for juvenile fish. Significant differences were also found between juvenile and adult fish for all structures. The BIOENV multivariate analyses showed that the highest rank correlation was found between the otolith data and the scale or spine data for both juvenile and adult fish. These data suggest that the use of scales and spines may provide a nonlethal alternative to the use of otoliths for future stock discrimination studies.

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Studies of the population dynamics of marine fishes depend on the ability to distinguish separate "stocks" that may make up the total population of a single fish species. Although much of fisheries management assumes that a single population is being monitored, measures of growth, natural mortality, recruitment, and reproduction are often made for the total population because data on movement or stock separation or fidelity (or the combination of all three) are inadequate to allow separation of stocks. A variety of methods exist for identifying stocks (e.g. population parameters, capture-mark-recapture studies, physiological and behavioral characters, morphometric and meristic characters, calcareous characters, cytogenic characters, and biochemical characters; Ihssen et al., 1981), but few methods can provide a reliable measure of stock identity. Genetic techniques are frequently used but fail to differentiate stocks because a small amount of larval or adult mixing among populations makes differences undetectable (Hartl and Clark, 1989). Recently, many studies have focused on the use of "elemental fingerprints" (*sensu* Campana et al., 1994), or the elemental composition of the otoliths, as a measure of stock identity.

Initial microchemistry studies tended to focus on diadromous species and distinguished between freshwater and marine life history phases of fish (e.g. Bagenal et al., 1973; Belanger et al., 1987; Kalish, 1990; Coutant and Chen, 1993; Rieman et al., 1994). The electron microprobe was widely used for such studies but because of the "typical" concentrations of Ca, Na, Sr, K, S, and Cl found in otoliths, electron probes were effectively limited to detecting these six elements. Recently, there have been dramatic advances in analytical techniques and studies now indicate that other elements also exist in otoliths (e.g. Edmonds et al., 1989, 1991, 1992; Sie and Thresher, 1992), many of which are thought to be reflective of the environment (Fowler et al., 1995). Stock discrimination capabilities have improved with the sensitivity of recent instrumentation and the inductively coupled plasma-mass spectrometer (ICP-MS) has now become the instrument of choice for simultaneously quantifying the concentration of multiple elements and isotopes (Houk, 1986; Date, 1991).

ICP-MS has recently been used for assays of various fish tissues including otoliths (e.g. Edmonds et al., 1991; Campana and Gagne, 1995; Campana et al., 1994, 1995; Dove et al., 1996; Gillanders and Kingsford, 1996), scales (e.g. Coutant and Chen, 1993, Wells et al., 2000), eye lenses (e.g. Dove and Kingsford, 1998) and soft tissues (e.g. Beauchemin et al., 1988; Ishii et al., 1991; Hellou et al., 1992).

Sample-specific differences in elemental concentrations in tissues other than otoliths have also been reported (e.g. vertebrae—Mulligan et al., 1983; Behrens Yamada et al., 1987; scales—Bagenal et al., 1973; Lapi and Mulligan, 1981; eye lenses—Dove and Kingsford, 1998; soft tissue—Hellou et al., 1992). Such sample-specific differences in trace elements provide evidence for the nonmixing and segregation of populations. Otoliths are an ideal natural marker of elemental composition because they grow throughout the life of the fish and once the elements are de-

posited, they are unlikely to be resorbed or altered (Campana and Neilson, 1985). Recently, Dove and Kingsford (1998) found that eye lenses may be suitable for differentiating populations of fish because the eye lens has no efficient mechanism for removing ions from the tissue. Eye lenses also grow by the addition of protein-rich cells to their outer surface. Bone, scales, and other soft tissues (e.g. gills, liver, muscles) will reflect a variety of factors including composition during growth, but resorption and remineralization may occur in some species (Gauldie and Nelson, 1990). The temporal stability of the elemental concentrations in other tissues is therefore questionable (Campana and Gagne, 1995). In some species, however, the market value of the fish is likely to be reduced by removing otoliths and eye lenses; therefore, structures such as scales or spines may be easier to obtain. If scales and spines show similar elemental fingerprints (for fish caught at different locations) to otoliths and eye lenses, then their use in stock discrimination studies may be warranted. The use of scales and spines would also allow sampling without the need to kill the fish, with the result that broodstock could be kept alive and individuals from rare or endangered stocks could be sampled. With the exception of two studies (Dove and Kingsford, 1998; Wells et al., 2000), who compared elemental fingerprints of otoliths with eye lenses and scales, respectively, there have been no comparative studies of more than two structures.

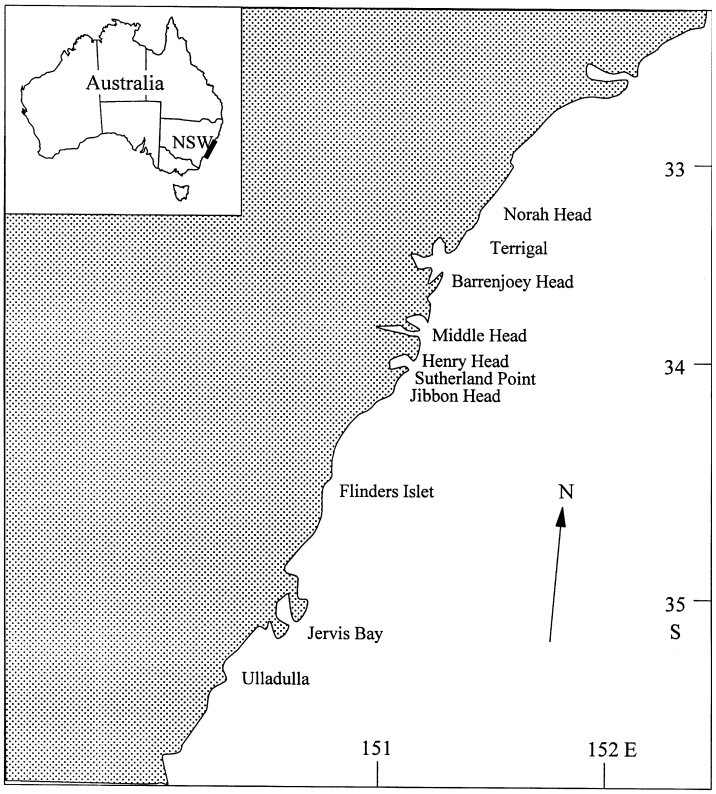

Figure 1

Map showing sampling locations along the coast of New South Wales, Australia.

The aim of my study was to compare the elemental fingerprints obtained from different structures (otoliths, scales, eye lenses, and dorsal spines) to determine if there were correlations among structures. Because otoliths are not subject to resorption (but see Mugiya and Uchimura, 1989), their use in studies of stock discrimination appears justified; therefore all comparisons were made between otoliths and the other three structures. These four structures chosen for the present study are also very different in terms of their composition. Otoliths are composed primarily of calcium carbonate, whereas scales and spines are composed primarily of calcium phosphate, and eye lenses, largely of water and structural proteins. There may, therefore, be differences in the affinities of ions for the different structures and this difference could have implications for the elemental chemistry of each structure.

The damselfish *Parma microlepis* is a territorial species, both juveniles (Moran and Sale, 1977) and adults (territory sizes 2–17 m^2, Tzioumis, 1995) remain attached to their natal sites. Therefore it is an excellent model species for this kind of research. Otoliths and eye lenses of *P. microlepis* have been shown to contain microconstituents that can be detected with ICP-MS, some elements of which may be site-specific (Dove et al., 1996; Dove and Kingsford, 1998). Juvenile fish are distinguished from adults on the basis of coloration and are generally less than 3 years of age (Tzioumis and Kingsford, 1999). By comparison adults reach a maximum age of 37 years, and fish larger than 120 mm SL may represent a wide range of age classes (Tzioumis and Kingsford, 1999). Adults will therefore have longer and potentially more variable age-integrated elemental fingerprints than those of juvenile fish.

Materials and methods

Parma microlepis was collected from each of ten locations along the coast of New South Wales, Australia (Fig. 1). Fish were not collected from multiple sites within each location because small-scale differences in trace elements of otoliths and eye lenses (sites separated by 50 to 200 m) were not found in a previous study (Dove and Kingsford, 1998). All fish were collected by divers using SCUBA and a hand-spear between January and April 1998. Between five and seven replicate adult and juvenile fish were collected from each location. Juveniles and adults were distinguished from each other on the basis of differences in patterns of coloration.

Fish were stored on ice during transportation to the laboratory. In the laboratory, the standard length of each fish was measured (Table 1). Otoliths, eye lenses, and approximately five non-regenerated scales were removed from each fish and rinsed in MilliQ water. Otoliths and scales were stored dry at room temperature, and eye lenses were stored at –70°C in Eppendorf microcentrifuge tubes in preparation for ion analysis. Dorsal spines were removed and frozen (–4°C) prior to removal of surrounding flesh. After flesh was removed, spines were also stored dry at room temperature.

Table 1

Summary of collections of *Parma microlepis*. Sample locations are shown in Figure 1. Sample sizes were n=5 for juveniles and adults at all locations, except for adult fish at Henry Head where n=7. Lengths refer to standard length in mm.

Sample no.	Location	Date of collection	Juvenile mean length (range)	Adult mean length (range)
1	Norah Head	Mar 1998	72.0 (56–89)	125.6 (117–133)
2	Terrigal	Mar 1998	83.0 (61–95)	122.6 (105–132)
3	Barrenjoey Head	Feb 1998	64.0 (49–89)	118.8 (106–129)
4	Middle Head	Feb 1998	88.6 (83–94)	125.6 (115–136)
5	Henry Head	Jan 1998	83.6 (71–94)	122.6 (114–136)
6	Sutherland Point	Jan 1998	85.4 (76–93)	118.6 (107–125)
7	Jibbon Head	Mar 1998	83.2 (65–95)	126.4 (114–136)
8	Flinders Islet	Mar 1998	76.0 (73–80)	110.4 (105–124)
9	Jervis Bay	Apr 1998	81.2 (75–95)	117.8 (112–125)
10	Ulladulla	Apr 1998	80.8 (76–90)	111.2 (102–120)

All samples and standards were prepared for ion analysis in a laminar flow cabinet. Otoliths and eye lenses, and scales and spines were individually weighed on an analytical balance (to 0.0001 g) and a microbalance (to 0.001 mg), respectively. All samples were rinsed in 1% nitric acid for 15 s prior to dissolution. Structures were digested in nitric acid (Aristar) either overnight (otoliths and scales) or for 36 h (eye lenses and spines). For eye lenses, sulphuric acid (Aristar) was added following dissolution and each sample was heated to ~90°C for 1 hour. For all structures varying amounts of MilliQ water were then added followed by additional dilution with 1% nitric acid where necessary. This was necessary so that concentrations of the sample solutions were standardized before analysis with ICP-MS.

Samples were analyzed by solution-based ICP-MS (Perkin Elmer SCIEX ELAN 5000). Initially, the four structures of fish collected from one location (Henry Head, Fig. 1) were analyzed in "totalquant II" mode (n=7 fish). This procedure provided a rapid-survey-analysis to semiquantitatively determine the elements present (and their range) within each of the four structures. The instrument was calibrated at six points spanning the mass range from 9 to 209. Accuracy and drift of the machine were determined from spiked samples.

Samples were then analyzed in "quantitative analysis" mode after the instrument was calibrated for either external standardization or addition calibration, depending on the structure and expected concentration range of each element. Measurement parameters for the ICP-MS were as detailed in Gillanders and Kingsford (1996). Seven blank solutions were run at the beginning of each session to determine detection limits, which were calculated from the concentration of analyte, yielding a signal equivalent to three times the standard deviation of the blank signal. Blank solutions were 1% HNO_3 for otoliths, scales, and spines, and 2% acid (1%HNO_3 and 1%H_2SO_4) for eye lenses. Standards were run through the machine at the beginning of each session. Spiked samples were run every ten samples to measure sensitivity changes to the machine through use and for recovery verification.

All samples were initially analyzed by using addition calibration mode. The data were then reprocessed in external calibration mode to obtain concentrations of microelements. Each sample took around 3 to 4 min to analyze, including a delay before starting to read a sample (70 s) and the rinse (1% HNO_3, 0.1% Triton X) between samples of 60 s. Samples from each structure (otoliths, scales, spines, and eye lenses) and size class (juveniles and adults) were analyzed together in blocks, but all samples within each block were randomized to ensure that resulting patterns did not reflect variation in the performance of the instrument.

Univariate and multivariate techniques were used to test hypotheses concerning individual elements and multielement fingerprints of *Parma microlepis*. Pearson's correlations were used to determine the relation between otoliths and each of the other structures for individual elements (Mn, Sr, Ba, and Pb). Separate analyses were performed for each size class and structure.

The match between the otolith microchemistry data and the microchemistry data for each of the other three structures was examined by using two types of nonparametric multivariate analysis. For both types of analysis, the data were standardized by subtracting the mean and by dividing by the standard deviation of the variable, so that each element would have equal weight. A matrix of Euclidean distances among all pairs of samples was then calculated. Mantel's test (from the R package) and the BIOENV procedure (included in the PRIMER computer program; copyright M.R. Carr and K.R. Clarke, Marine Biological Laboratory, Plymouth, UK) were used to determine the degree of correlation between pairs of distance matrices (otoliths and each of the other three structures).

Mantel's test describes the relation between two distance matrices with Pearson's correlation coefficient, r (Mantel, 1967; Legendre and Fortin, 1989). A test of significance for r is obtained by random permutations of the replicate sample units for one of the matrices. A total of

999 permutations were performed for each test and the probability calculated as the number of values equal to, or larger than, the observed value of r divided by the total number of permutations.

If significant relationships were found with Mantel's test, the BIOENV procedure was then used to determine which elements were likely to be important in describing the correlation between the distance matrices (Clarke and Ainsworth, 1993; Clarke and Warwick, 1994). BIOENV calculates rank correlations between a dissimilarity matrix derived from the otolith microchemistry data and matrices derived from various subsets of the elements for either the scale, spine, or eye lens data matrices, thereby defining suites of elements that "best explain" the otolith microchemistry data (Clarke and Ainsworth, 1993). The harmonic or weighted Spearman correlation ρ_w was used.

Results

Otoliths, scales, spines, and eye lenses of *Parma microlepis* differed in composition, both in terms of the actual elements present and the concentration of individual elements. Otoliths, scales, and spines were dominated by calcium, whereas no calcium was detected in eye lenses. The microelement Sr was present in otoliths, scales, and spines at concentrations of 100's to 1000's µg/g of structure, whereas it occurred at low concentrations in eye lenses. Other elements (e.g. Mn, Ba, Pb) were typically found in low-to-trace levels in all structures. Mercury was found in detectable concentrations only in eye lenses.

Univariate analyses

Differences in the concentration of manganese among structures varied over several orders of magnitude (range: 0.05 for eye lenses to 100 µg Mn/g structure for spines). Concentrations of Mn in otoliths showed significant correlations with concentrations of Mn in both eye lenses and scales of juvenile fish, but only with scales from adult fish (Fig. 2, Table 2). For juveniles, fish from one site may have influenced the correlation analyses; therefore fish from this site were removed and the correlations recalculated. This adjustment resulted in a significant correlation between Mn in otoliths and Mn in scales, as found previously. The relation between Mn in otoliths and Mn in spines was also significant when fish from this one site were removed (r=0.302, P<0.05). However, there was no longer a significant correlation for eye lenses: fish from one site may therefore have influenced the correlation result for this structure.

Concentrations of Sr in otoliths showed significant correlations with concentrations of Sr in all the other structures for juvenile fish; however the relation was only positive for otoliths and spines (Fig. 3, Table 2). For scales and spines, fish from one site did not unduly influence correlations; when these fish were removed from analyses, correlations were still significant. However, as found for Mn, the correlation between Sr in otoliths and Sr in eye lenses was no longer significant when fish from this site were removed. The only significant relationship for Sr in adult fish was found between otoliths and spines (Fig. 3, Table 2).

Table 2

Correlations between concentration of elements in otoliths and concentration of elements in eye lenses, scales, and spines of juvenile and adult fish collected from ten locations along the coast of New South Wales. Shown are Pearson's correlation coefficients. Significance levels are indicated by * (P<0.05) and ** (P<0.01), df=48. No corrections were made for experiment-wise error rate; therefore 1 in 20 tests would be expected to be significant by chance alone.

	Otolith			
	Mn	Sr	Ba	Pb
Juveniles				
Eye lenses	0.3410*	–0.3787**	0.1260	
Scales	0.4018**	–0.6237**	0.8810**	
Spines	0.2466	0.6586**	0.9001**	
Adults				
Eye lenses	0.0429	0.2353	0.0239	–0.2564
Scales	0.4717**	–0.0051	0.8839**	–0.1487
Spines	0.2696	0.8446**	0.9121**	–0.0732

Significant positive relationships were found between the amount of Ba in otoliths and the amount of Ba in scales and spines of both juvenile and adult fish (Fig. 4, Table 2). Lead was usually at or below detection limits of the instrument for juvenile fish and therefore correlations were not made. Correlations between the amount of Pb in otoliths of adult fish and the other structures showed no significant relationship (Fig. 5, Table 2).

Multivariate analyses

Mantel's test detected a significant relationship between the Euclidean distances among replicates based on the otolith data and the distances based on data from eye lenses, scales, or spines for juvenile fish (Table 3). For adult fish, however, a significant relation was detected between the otolith data and the scale and spine data (Table 3); thus there may be either changes in assimilation of elements with age or resorption and remineralization may occur in some structures, for example in eye lenses.

The BIOENV analyses showed that the highest rank correlation was found between the otolith data and the scale data for juvenile fish and involved Sr and Ba (Table 3). For adult fish the highest correlation was between the otolith data and the spine data and involved three elements (Mn, Sr, and Ba; Table 3).

Comparisons of juvenile and adult fish for each structure showed no significant relationships (Table 3). The Mantel test, however, was marginally nonsignificant for otoliths (Mantel r statistic 0.2000, P=0.060).

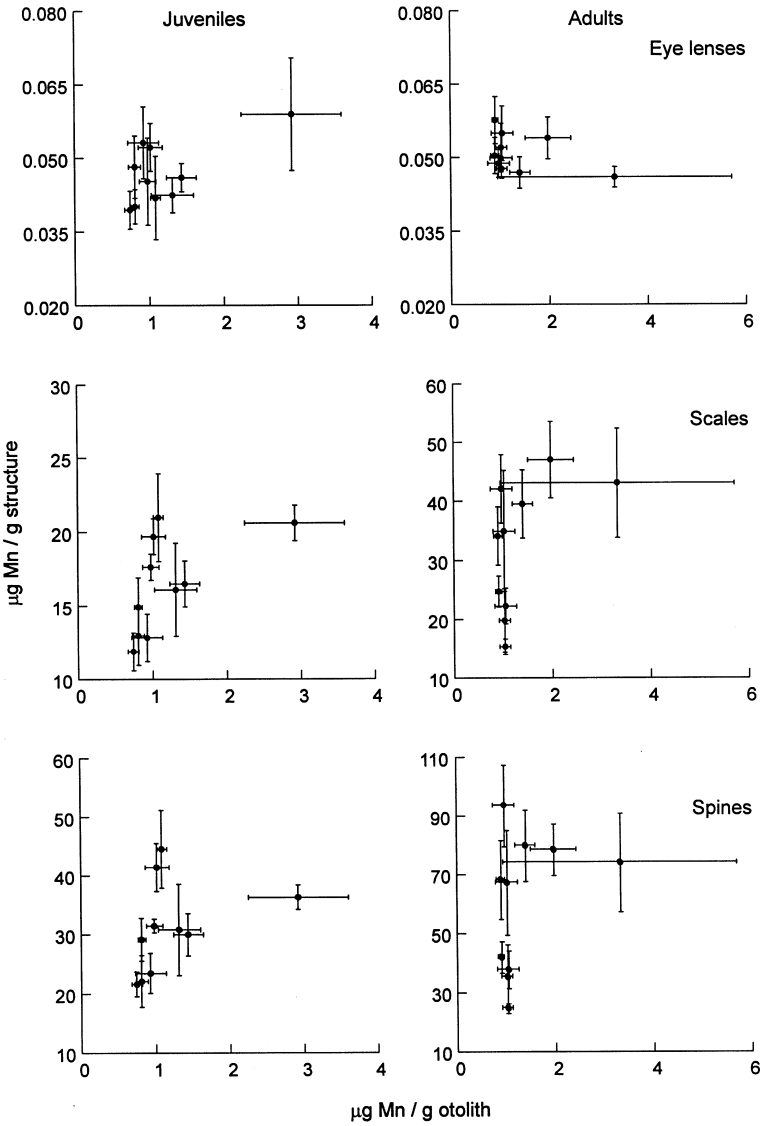

Figure 2

Relation between concentration of manganese in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

Discussion

Otolith elemental fingerprints are reported to be among the most important tools for stock discrimination for some species of fish (Campana et al., 1995). In the current study, adult fish showed a significant relationship between the otolith elemental fingerprints and the scale and spine elemental fingerprints. In contrast, juvenile fish showed significant relationships between comparisons of otoliths and each of scales, spines, and eye lenses. Relationships among some structures were also seen for some individual elements. There have been two other studies involving comparisons between elemental fingerprints of otoliths and other structures (Dove and Kingsford, 1998; Wells et al., 2000). Wells et al. (2000) compared trace elements in otoliths and scales of juvenile weakfish by using correlation analyses on individual elements. They found similar results to those in the current study, namely a significant correlation between Mn, Sr, or Ba in otoliths and that in scales. Dove and Kingsford (1998) compared elemental fingerprints of otoliths and eye lenses. In their study differences between locations were compared by using analysis of similarity (ANOSIM) permutation tests. Where comparisons between locations gave the same response

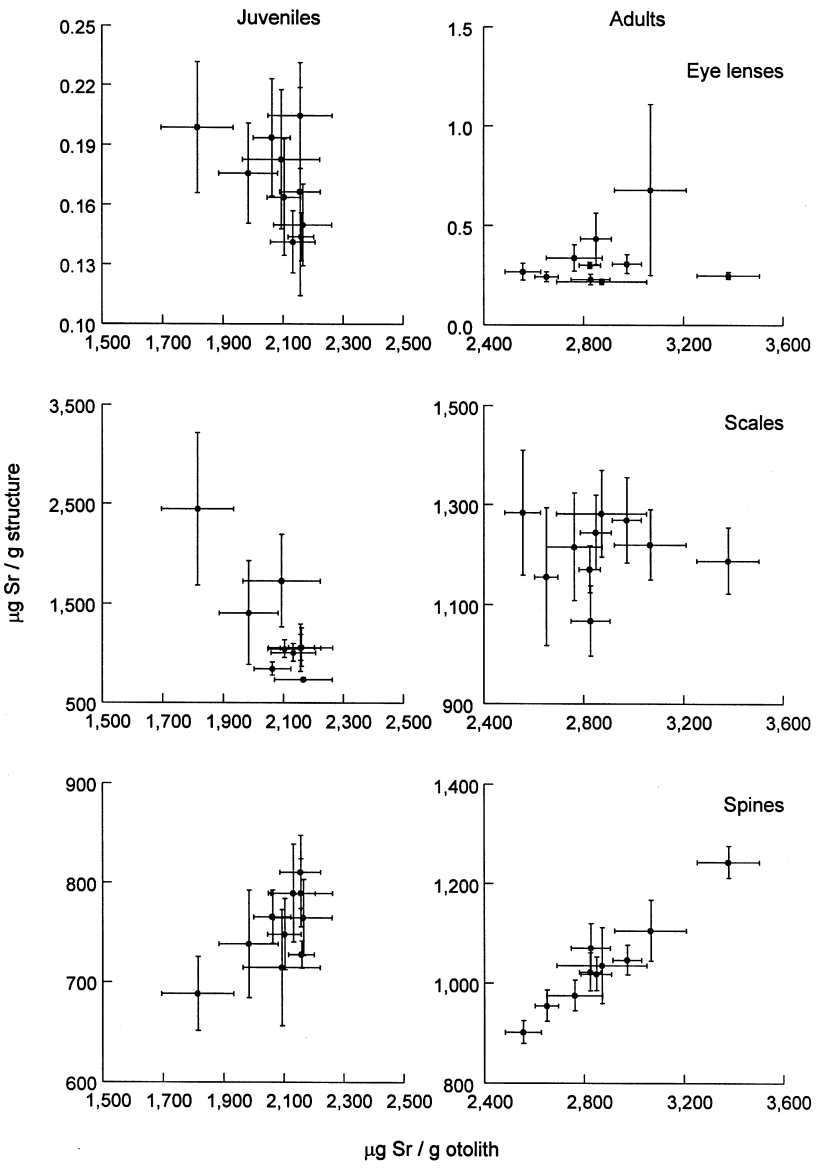

Figure 3

Relation between concentration of strontium in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

for both otoliths and eye lenses (either both significant or both nonsignificant), then the two structures were viewed as being similar. With this approach, approximately 73% (11/15) of pair-wise comparisons showed agreement (Dove and Kingsford, 1998). Their approach is, however, indirect and does not show evidence of correlation between data matrices for otoliths and those for eye lenses. I am aware of no other studies that have compared multiple structures for fish obtained from a number of locations.

Otoliths have been considered the structure of choice for determining elemental fingerprints and for relating these to movements or stock structure of fish because otoliths grow throughout the life of the fish and the material of the annual growth increment, once deposited, is unlikely to be resorbed or altered (Campana and Neilson, 1985). Otoliths therefore have the potential to record endogenous and exogenous factors permanently within their calcium-protein matrix. Other structures may also grow throughout the life of the fish (e.g. eye lenses—Dove, 1997; scales—Mitani, 1955; spines—Hill et al., 1989). Although other structures have been used for chemical analyses and for aging fish, some structures are susceptible to resorption and remin-

Table 3

Summary of results from Mantel's test and the BIOENV procedure where otolith microchemistry data were compared with the microchemistry data obtained from eye lenses, scales, and spines. Comparisons are also made between juvenile and adult fish for each structure. The BIOENV procedure (see general text) was done only if significant correlations were found with Mantel's test. Only the combination of elements that contributed to the maximum ρ overall, as measured by the weighted Spearman correlation, are shown for each structure comparison. The significance of the Mantel test statistic is shown by * (P<0.05) or ** (P<0.01).

Comparison	Mantel r statistic	BIOENV-maximum ρ	Combination of elements contributing to maximum ρ
Juveniles			
Otoliths and eye lenses	0.3402*	0.127	Mn, Ba, Hg
Otoliths and scales	0.5757**	0.587	Sr, Ba
Otoliths and spines	0.4097**	0.403	Sr, Ba
Adults			
Otoliths and eye lenses	–0.0646		
Otoliths and scales	0.3680**	0.454	Mn, Ba
Otoliths and spines	0.4440**	0.512	Mn, Sr, Ba
Adults and juveniles			
Otoliths	0.2000		
Eye lenses	–0.1357		
Scales	–0.0136		
Spines	0.0676		

eralisation (Simkiss, 1974). Despite the possibility of resorption of some elements, otoliths and scales, and otoliths and spines showed a significant correspondence between data matrices for both juvenile and adult fish, which may indicate that scales and spines provide estimates of stock structure that are similar to those obtained from otoliths.

Differences in elemental fingerprints among structures may be due to different routes of ion uptake and differential abilities of each structure to incorporate elements into the organic and inorganic matrix. Calcium and strontium in otoliths are primarily taken up by the gills (Simkiss, 1974), but the route of uptake of trace elements has not been identified (Campana and Gagne, 1995). There may also be some Sr uptake in otoliths through the diet because fish that were fed a Sr-enriched diet showed a detectable increase in Sr/Ca ratios (Gallahar and Kingsford, 1996). Other structures, such as scales and bone, may incorporate ions by diffusion across the gills and through the skin or by ingestion of food and water (Simkiss, 1974). Whether some structures show differential abilities to incorporate ions through different methods of absorption and whether ions are resorbed from different structures in equal proportions is not known.

Otoliths and eye lenses showed the greatest differences in elemental fingerprints between structures. Otoliths are predominantly $CaCO_3$ (Ca constitutes from 30% to 39% of otoliths, Thresher et al., 1994; Dove et al., 1996), although small amounts of protein also occur (Degens et al., 1969) and therefore otoliths are likely to incorporate ions that are able to substitute for Ca in the $CaCO_3$ matrix or bind to proteins in the organic matrix (Gunn et al., 1992; Sie and Thresher, 1992). The spaces between these matrices may also trap ions. In contrast, eye lenses are composed largely of water and structural protein, the latter of which may be soluble or insoluble crystallin (Nicol, 1989). The proteins of eye lenses are rich in sulfhydryl groups that may covalently bind with metals and the proteins also have specific sites for binding with cations (Sharma et al., 1989). Scales and bone are more similar to otoliths in that the dominant ion is Ca (e.g. in bone Ca may constitutes from 24% to 37%; Hamada et al., 1995), but they vary considerably in that otoliths are primarily carbonate structures, whereas scales and bone are primarily phosphate (or hydroxyapatite) structures. Some differences in elemental composition of otoliths and scales or spines may therefore be expected. Because otoliths, scales, and bone are composed predominantly of a mineral, rather than an organic matrix, it is not surprising that these structures were similar in their elemental fingerprints and that eye lenses presented a different fingerprint.

Differences in elemental fingerprints between juvenile and adult fish were also found for all structures. Eye lenses are thought to have no efficient mechanism for removing ions, but there may be changes in structural proteins with age that possibly alter affinities of proteins for specific ions (Dove, 1997). Such ontogenetic effects may result in different elemental fingerprints between juvenile and adult fish for eye lenses. Both scales and spines show some evidence of resorption or remineralization over time in some species. For example, early growth increments in spines of several species are known to be destroyed as the core-matrix expands (Hill et al., 1989; Gillanders et al., 1997) and there is evidence for resorption in scales of fish living in a stressed environment (Sauer and Watabe, 1989).

If an alternative structure to otoliths is required for stock identification, as it may be for broodstock, and for rare or endangered species, or if removing otoliths decreases

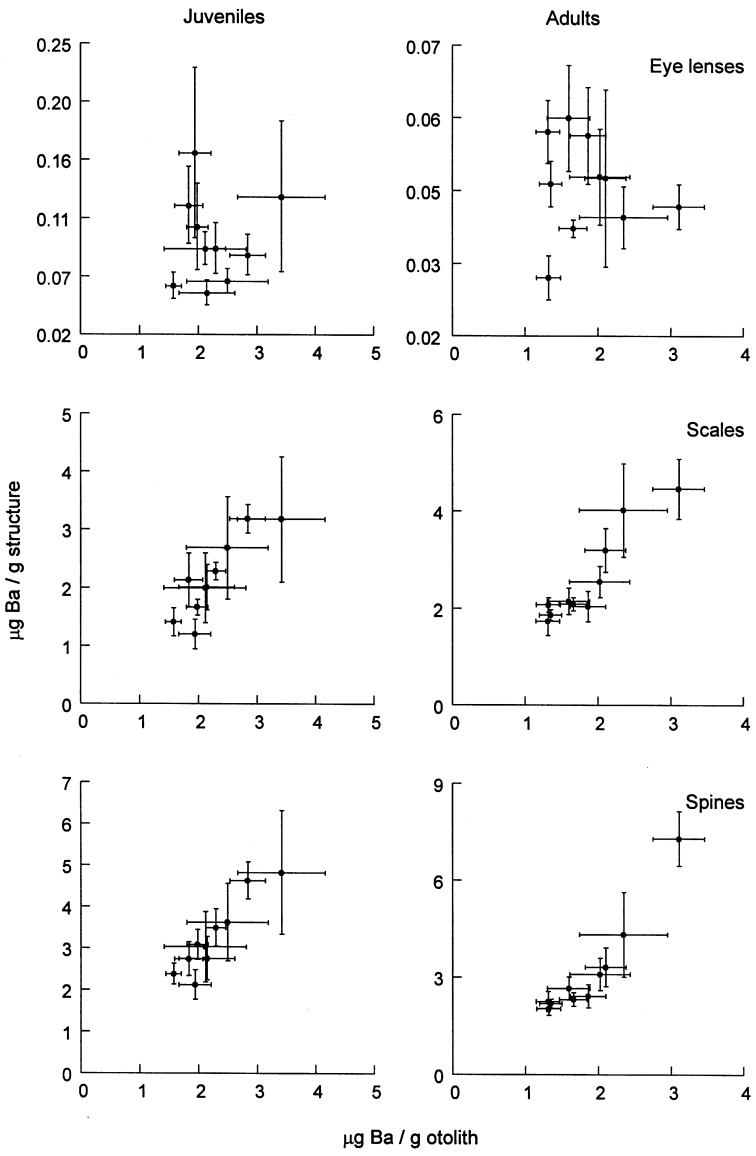

Figure 4

Relation between concentration of barium in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

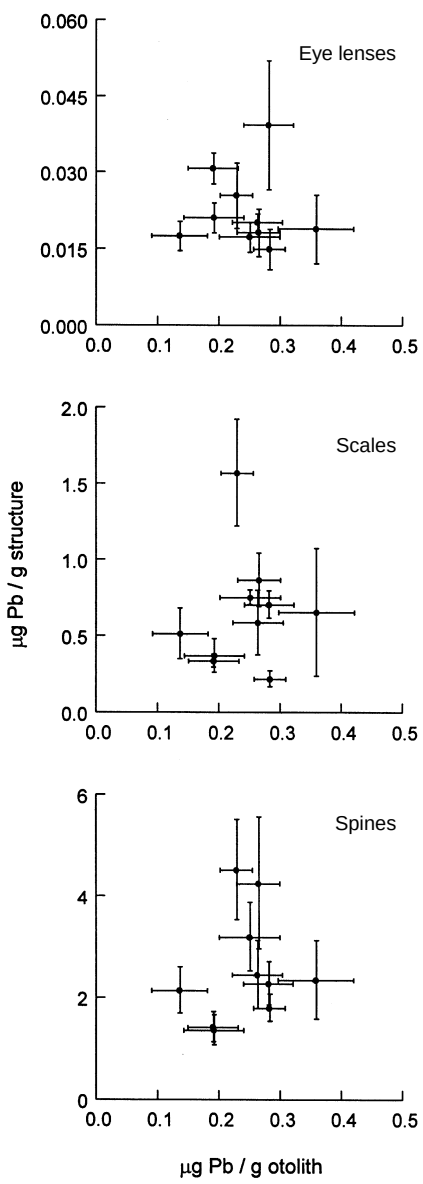

Figure 5

Relationship between concentration of lead in otoliths (mean ±SE) and in eye lenses, scales, and spines of adult fish (mean ±SE) collected from ten locations along the coast of New South Wales. Juveniles are not shown because concentrations of lead were below detection limits.

the market value of the fish, then it is recommended that either scales or spines are the best alternative structure because these were the structures that were significantly correlated with the otolith elemental data for both juvenile and adult fish. However, before using either of these structures, a number of fish (e.g. 30–50) should be collected from three to four locations and the alternative structure, as well as otoliths, should be analyzed. Classification models should then be developed by using data from each structure and the error rates should be compared. This was beyond the scope of the present study because sample sizes from each location were relatively small.

Scales and spines may offer certain advantages over otoliths because they provide a nonlethal alternative. In addition, they can be collected relatively quickly and easily. Scales may also require less preparation for analyses than either spines or otoliths. Thus, scales and spines may provide an alternative structure for determining stock identity.

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