Abstract.—The chemistry of calcified tissues has been suggested as a source of useful information on the population structure and environmental histories of fishes. We evaluated this possibility by examining in detail regional and ontogenetic variability in the chemical composition of sagittae of juveniles and adults of the temperate marine groundfish *Nemadactylus macropterus*. Six elements (in order of decreasing abundance, Ca, Na, Sr, K, S, and Cl) were consistently detected in the sagittae at concentrations greater than 200 ppm; all exhibited levels of individual, ontogenetic, and regional variability well in excess of their respective scales of measurement error. Comparisons of juveniles and adults from different sites indicate that composition of the otolith is most alike in fish from adjacent sites, that most juveniles are similar to adults collected from the same site, and that the differences in composition that characterize sites are manifest during most, if not all, of the fish's ontogeny. These results are consistent with the hypotheses that otolith composition reflects population structure and that these populations are largely self-recruiting. However, the results also suggest that the chemical composition of otoliths is much less sensitive to environmental conditions than previously thought. Rather, it appears that regional differences in composition either have a genetic basis or are set by environmental influences early in life and are then maintained throughout subsequent life history.

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An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae)

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Knowledge of geographic structure is fundamental to understanding the dynamics of marine fish populations (e.g. Sinclair, 1987). Nonetheless, even for the small number of species thus far investigated, there remains considerable uncertainty regarding population structure. This is due to the lack of a widely applicable, direct means of mapping how far and in what directions larvae disperse. A variety of indirect techniques have been applied to the problem, e.g. modelling larval advection from oceanographic features, analyzing parasite loads, mapping phenotypic characters, and locating and enumerating discrete spawning areas. However, all are limited in scope and in the strength of the inferences that can be drawn from them. For this reason, the geographic structure of marine populations is usually inferred from genetic studies (e.g. Avise et al., 1987; Waples and Rosenblatt, 1987; Smith et al., 1990). But genetic techniques are also far from ideal for this task: they will not detect differences in the face of even low levels of larval or adult mixing among populations (Hartl and Clark, 1989); they cannot directly measure rates of individual exchange among sites or, usually, specify the origin of individuals; and results from even a successful study can be ambiguous, i.e. a genetic difference between sites suggests little dispersal, but the lack of any difference is largely uninformative. Such difficulties have prompted continuing research into alternative, and perhaps more definitive, techniques for evaluating population structure.

One alternative technique is the analysis of the chemical composition of calcified structures. As early

as 1967 (Fisheries Agency of Japan, 1967), preliminary studies suggested that the quantitative analysis of the microconstituents and trace elements in otoliths, vertebrae, and scales could provide information on population structure and the movements of individual fish. This suggestion was based on two assumptions and a hypothesis. The assumptions were that 1) the calcified structures are not susceptible to dissolution or resorption and 2) the growth of these tissues continues throughout life. If these assumptions are correct, calcified structures are permanent records of the influence of endogenous and exogenous factors on their calcium-protein matrices. The hypothesis is that differences in the environments to which fish in each population are exposed affect the incorporation of elements in calcified structures, which results in chemical compositions specific to each population. An extensive fisheries literature supports the assumptions for otoliths, if not perhaps for scales and vertebrae (e.g. Sauer and Watabe, 1989). The working hypothesis also appears reasonable, given an extensive literature on invertebrates that relates differences in the composition of, for example, mollusc shells, and coral skeletons to a range of environmental and physiological conditions (Thompson and Livingston, 1970; Weber, 1973; Houck et al., 1977; Buchardt and Fritz, 1978; Smith et al., 1979; Rosenberg, 1980; Schneider and Smith, 1982).

Since 1967, several studies have investigated whether the composition of calcified structures indicates stock or subpopulation identity in fishes (e.g. Klokov and Frolenko, 1970; Calaprice, 1971, 1985; Calaprice et al., 1971, 1975; Bagenal et al., 1973; Gauldie and Nathan, 1977; Behrens Yamada et al., 1987; Lapi and Mulligan, 1981; Mulligan et al., 1983, 1987; Edmonds et al., 1989; Calaprice[1]), using a variety of analytical techniques (see reviews by Coutant, 1990; Gunn et al., 1992). The results have been mixed. In part, this is because most techniques used required a relatively large amount of material for analysis. Otoliths or bones from many individuals often had to be pooled to reach the minimum sample mass required. Because individual and ontogenetic variability could not be addressed, it has been difficult to assess the potential of the approach.

In 1987, we began experiments with a view to using fine-scale, ontogenetic variation in the composition of fish otoliths as an indicator of movement and migration patterns. The results of the first step — an investigation of the operating characteristics of probe microanalyzers as they affect data quality and the development of reliable techniques for 'life history scans' across otoliths — are reported in Gunn et al. (1992) and Sie and Thresher (1992). In this paper, we evaluate the extent to which otolith composition in a test species varies ontogenetically, among individuals within sites, and among sites, in order to assess whether such variation is of sufficient magnitude for, and contributes to, resolving population structure in the species.

The species chosen for study was the jackass morwong, *Nemadactylus macropterus* (Cheilodactylidae), a moderate-sized (maximum about 70 cm standard length), bottom-dwelling fish common on the middle and outer continental shelf off southern Australia, New Zealand, South Africa, and the Pacific coast of South America (Robertson, 1978). The species was chosen for two reasons. First, the population structure of the species in Australian waters is contentious. On the one hand, regional declines in catch rates suggest localized stocks, which is consistent with work in New Zealand, where the species has three geographically discrete populations (Gauldie and Nathan, 1977; Robertson, 1978) . On the other hand, a small amount of tagging data for adults (Smith, 1989), allozyme data for specimens collected in southeast Australia (Richardson, 1982), and recent allozyme and mitochondrial DNA analyses for the entire Australian range (Elliott and Ward, 1994; Grewe et al., in press) suggest a single, broadly distributed population (Smith, 1989; Tilzey et al., 1990). This interpretation also appears to be consistent with the early life history of the species: *N. macropterus* spawns along the middle continental shelf, has a planktonic duration of 9–12 months, and has a morphologically specialized late-stage larva ('paper fish') that is neustonic and generally caught offshore of the continental shelf (Vooren, 1972). This combination is taken to imply high rates of local mixing during the larval stage.

Nemadactylus macropterus was also chosen because of uncertainty about the location of its nursery areas in Australia. To date, the only place in Australia where large numbers of juveniles have been found is the shallow bays and inlets of southeastern Tasmania. As a result, it has been suggested that this area is a critical habitat supporting the entire Australian population (Tilzey et al., 1990). Given continuing coastal development in this area, if the hypothesis is correct, conservation measures need to be developed and implemented to ensure the continued viability of the fishery.

Analysis of otolith composition could help resolve both questions. With regard to population structure, we hypothesized that if there are discrete spawning populations in Australian waters, then the composition of the central, first-forming portion of the otolith

[1] Calaprice, J. R. 1983. X-ray fluorescence study of stock variation in bluefin tuna. Status report submitted to NMFS, Miami, March 1983, 60 p.

would differ geographically. With regard to the number and location of nursery areas, we further hypothesized that if there is only one nursery area, then the composition of that part of the otolith deposited during residence in the nursery ground would be similar for all adults, irrespective of where they were caught, and would match that of juveniles caught in southeastern Tasmania.

Methods

Collection details for juvenile and adult *N. macropterus* are provided in Figure 1 and Table 1. Recently settled (0+) juveniles were collected by hand-lining and trawling at six sites off Tasmania and southern Victoria. Adult specimens were also obtained at six sites, from commercial and scientific trawls on the southeastern Australian continental shelf. To minimize possible effects of interannual variation in otolith composition, we minimized the number of year classes in the sample by using only adults in the size range of 30–35 cm fork length. Otolith macrostructure and published length-at-age keys for the species (Smith, 1982) suggest that the specimens were a mixture of the 1980 to 1984 year classes and that year-class distributions overlapped broadly among sites. The juveniles were from the 1987 and 1988 year classes.

All specimens were frozen at –20°C shortly after collection and remained frozen (up to 30 days) until the otoliths were removed. After extraction, each otolith was cleaned of adhering tissue with fine forceps and a soft-bristled brush in millepore-filtered distilled water. They were then dried in an oven at 40–45°C for at least 6 hours, after which they were stored in polyurethane capsules in a desiccating cabinet.

Procedures for embedding, sectioning, and preparing otoliths for probe microanalysis are detailed in Gunn et al. (1992). Only sagittae were used in this study, because of their larger size. Prior to embedding, a scaled diagram of the distal surface of each otolith was made in order to guide subsequent sectioning. The otolith was then fixed upright on its ventral edge to the base of an embedding mold with a drop of Araldite. The mold was then filled with a harder-setting resin. After hardening, the otolith was sectioned with a diamond-edged saw blade (350 μm thick) on a rotary saw. Grinding to the plane of the primordium was done by hand with 2400-grade silicon carbide wet/dry paper. Final polishing was done by using progressively finer grades of diamond paste (6–3 μm) and aluminum oxide powder (Linde B) on a lapping machine. After polishing, the section was ultrasonically cleaned and stored in a moisture-free environment. Prior to probe microanalysis, the section was heated on a hot-plate at 80°C for 10 min-

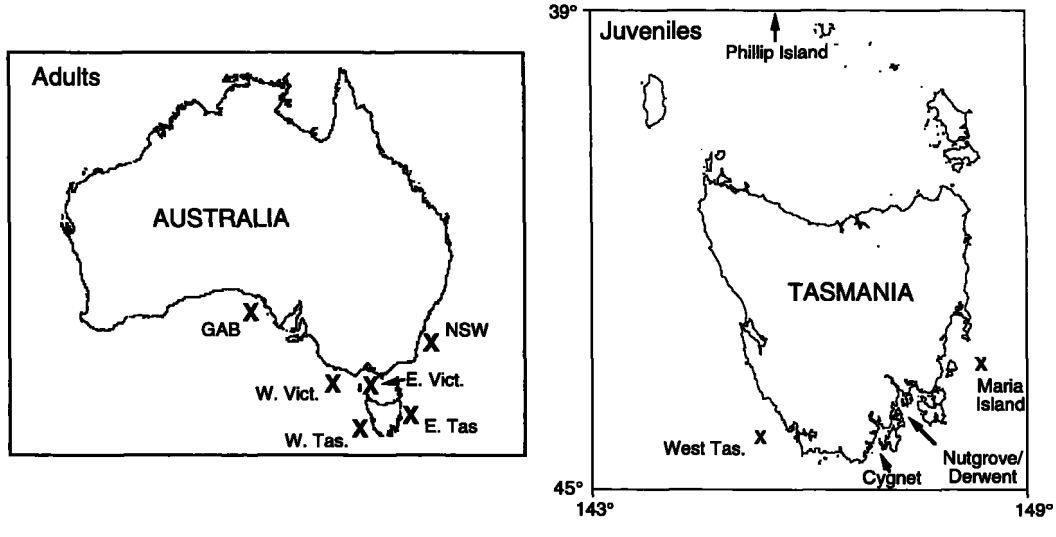


Figure 1

Source locations of samples of adult and juvenile *Nemadactylus macropterus* examined by electron probe microanalysis. Sample details are provided in Table 1. Adult sites: GAB=Great Australian Bight, W. Vict.=western Victoria; E. Vict.=eastern Victoria; W. Tas.=western Tasmania; E. Tas.=eastern Tasmania; and NSW=New South Wales. Nutgrove and Derwent are two inshore sites sampled for juveniles, both located in the Storm Bay (SE Tasmanian) estuary. Phillip Island is an inshore site off Victoria.

utes to remove any residual moisture, coated with a 250–300 Å (measured by color on brass) coat of carbon with a sputter coater, and then stored under vacuum until insertion into the probe.

The procedures used to analyze otolith composition are detailed in Gunn et al. (1992). Damage to the specimens under the electron beam is inevitable. The amount of damage, and hence quality of the data, is proportional to beam-power density (i.e. beam current × accelerating voltage/ target area). In Gunn et al. (1992), we concluded that beam power densities greater than 3.0 $\mu W \cdot \mu m^{-2}$ resulted in unacceptable levels of specimen damage, data precision, and accuracy. Hence, data for the current study were acquired by using the following beam conditions: 25 nA current, 15 kV accelerating voltage, a 14 μm diameter 'defocused' beam (and hence a beam power density of 2.44 $\mu W \cdot \mu m^{-2}$) and a total acquisition time of 3 minutes, 42 seconds per point. Comparisons of parallel scan lines (see Fig. 6) included some analyses at a 6 μm beam diameter, 5.5 nA current, and 15 kV accelerating voltage; despite the small beam diameter, beam power density for this series (2.92 $\mu W \cdot \mu m^{-2}$) is within our 'safe' limit. The electron probe microanalyzer used was a Cameca Camebax fitted with three wave-length dispersive detectors. The concentrations (weight-fractions) of Na (sodium), K (potassium), Ca (calcium), S (sulphur), and Cl (chlorine) were calculated based on the count rates measured for their respective K_α lines, and for Sr (stron-

Table 1

Collection details for *Nemadactylus macropterus* adults and juveniles analyzed in this study.

Location	Date collected	Collection method	Sample size	Size range (cm FL)
Adults				
NSW (Lat.34°40'S Long.151°10'E)	12 Jan 90	Trawl	11	36.0–38.0
W. Victoria (Lat.38°34'S Long.141°10'E)	30 Aug 87 –6 Sep 87	Trawl	6	34.6–37.1
E. Victoria (Lat.37°48'S Long.139°45'E)	6 Dec 89	Trawl	17	27.7–35.5
E. Tasmania (Lat.42°40'S Long.148°15'E)	16 April 89 15 Nov 89	Trawl Trawl	2 11	30.7–33.0 32.0–38.0
W. Tasmania (Lat.43°07'S Long.145°32'E)	21 Mar 89	Trawl	5	30.3–35.4
GAB (Lat.33°25'S Long.125°56'E)	15 Oct 89	Trawl	16	28.0–35.0
Juveniles				
Cygnet (Lat.43°10'S Long.147°05'E)	19 Dec 87 3 Dec 88	Hand-line Hand-line	27 13	8.6–11.6 8.3–13.6
Derwent (Lat.42°53'S Long.147°20'E)	25 Sep 87 9 Nov 87	Hand-line Hand-line	9 11	13.4–17.8 15.8–16.7
W Tasmania (Lat.43°07'S Long.145°32'E)	21 Mar 89	Trawl	26	9.8–18.1
Maria Island (Lat.42°31'S Long.148°10'E)	7 April 88	Trawl	11	12.0–15.8
Nutgrove (Lat.42°55'S Long.147°21'E)	29 Mar 88	Trawl	9	14.6–16.1
Phillip Island (Lat.38°35'S Long.145°10'E)	13 Dec 87	Trawl	10	9.0–18.0

tium), the L_α line. S and Cl were measured on Spectrometer 1 (PET), K and Ca on Spectrometer 2 (PET), and Na and Sr on Spectrometer 3 (TAP). Matrix corrections were made by using the "PAP" (Pichou and Pichoir, 1984) matrix conversion software. Minimum detection limits and confidence intervals for the concentration estimates are based on equations provided by Ancey et al. (1978).

Ontogenetic variation in composition was assessed by analyzing a series of points along the longest growth axis of each otolith section—a "life history scan." The finished section of the sagitta of *N. macropterus* exposes a nearly straight, uninterrupted growth axis, through which we ran a series of programmed scan lines that tracked the slight curvature of the axis (Fig. 2). We duplicated this axis as closely as possible in each specimen in order to maximize comparability of the data sets. The life history scan line for each fish ran from the primordium to the posterior ventral tip of the otolith. The size of the scan points and their spacing were determined by logistic considerations and, in part, by the results of experimental trials. In practice, routine spacing (center to center) between points was 25 μm. In the case of the parallel scans (see Fig. 6), we used a beam diameter of 14 μm spaced 16 μm apart and 6 μm diameter beams spaced 8 μm apart. Reproducibility of life history scan data was evaluated by comparing left and right otolith pairs from the same fish.

Even if the data are free from conspicuous distortions due to irregular surface features, the use of data from a single-point probe analysis for evaluation of stock structure still risks high error rates due to measurement noise (Gunn et al., 1992). Consequently, we analyzed stock structure in two ways: based on comparisons of single-point data collected adjacent to the primordium (point 2); and based on mean concentrations of the first five probe points adjacent to the primordium (points 2–6 inclusive). The latter method filters out high-frequency measurement noise but risks low discriminant power by including information from relatively late in larval life. Point 6 is about 125 μm from the primordium; increment counts suggest this corresponds to a larval age of about 45–55 days.

Statistical procedures in general follow Sokal and Rohlf (1981). Test for normality were made by using Lilliefors *K-S* test. Differences in mean concentrations among sites were tested by means of a Kruskal-Wallis nonparametric ANOVA. Groupings of sites and specimens were tested and quantified by linear discriminant function analysis (LDFA) with the SYSTAT statistical software package. General procedures for and assumptions underlying LDFA are described by Klecka (1980); Cameron (in press) reviews the application of discriminant analysis to studies of otolith and skeletal composition.

Results

Composition of *N. macropterus* otoliths; data quality and reproducibility

Six elements could be reliably detected in *N. macropterus* otoliths by wavelength-dispersive electron probe microanalysis (WD-EPMA): Ca, Na, Sr, K, S, and Cl, in order of decreasing mean concentration. The elements in *N. macropterus* sagittae constitute three distinct sets separated in concentration from other less abundant elements by a difference of one to three orders of magnitude (Fig. 3): Ca, carbon, and oxygen (the last two are not routinely measured because of methodological difficulties) constitute the 'macro-constituents' present in concentrations >10% (10^5 ppm) by weight; Na, Sr, K, S, and Cl constitute the 'micro-constituents,' which occur in mean con-

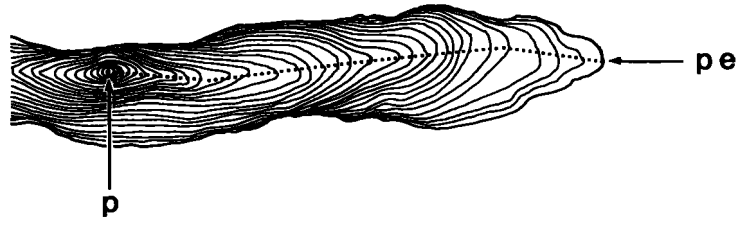


Figure 2

Diagram of section of a sagitta of an adult *Nemadactylus macropterus*, as prepared for electron probe microanalysis, depicting programmed transects used to analyze points along the main growth axis from the primordium (p) to the otolith's posterior edge (pe).

Table 2

Minimum detection limits (MDL), mean and ranges of estimated concentrations, and measurement error for *Nemadactylus macropterus*, of the six elements that could be assayed reliably with a WD electron probe. The values are based on a random subset of our data (n = 478 points), including numerous individuals and positions along the scanned axis of points analyzed. Concentrations are given in ppm (by weight), except for Ca which is in percent of the target mass. Note that values below the minimum detection limit are effectively zero. 'Minimum significant difference' is based on comparison of 'replicate' points in parallel life history scans (see text). CI = Confidence interval.

Element	MDL	Mean (range) concentrations	Measurement error (absolute, %)	Minimum significant difference	
				Mean (range)	99% CI
Ca	—	38.8% (35.3–44.5)	—	—	—
Sr	311	2240 (1430–3860)	±157 (7%)	210 (12–964)	331
Na	159	3331 (2680–4240)	±122 (3.7%)	160 (10–450)	235
K	136	729 (280–1630)	± 72 (10%)	77 (0–320)	118
S	149	421 (220–1220)	± 76 (18%)	80 (4–247)	121
Cl	157	255 (0–1230)	± 72 (28%)	48 (10–210)	73

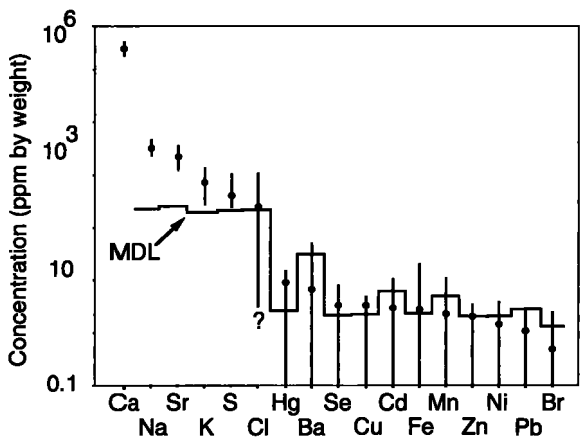


Figure 3

Mean (solid circle) and ranges (vertical line) of concentrations of the elements detected in the sagittae of *Nemadactylus macropterus* by means of electron probe (for Ca, Na, Sr, K, S, and Cl) and proton probe (for mercury (Hg), barium (Ba), selenium (Se), copper (Cu), cadmium (Cd), iron (Fe), manganese (Mn), zinc (Zn), nickel (Ni), lead (Pb), and bromine (Br)) microanalyses. For proton probe methodology and results, see Sie and Thresher (1992). Data are a compilation of >500 points across numerous individuals and positions along the growth axis. The minimum detection limit (MDL) for each element is indicated by the irregular horizontal line and is based on the standard output of the respective probe microanalyzers at our standard operating conditions (see Gunn et al., 1992; Sie and Thresher, 1992). The minimum concentration of Cl is below the detection limit of the electron-probe microanalyzer but could not be determined with the more sensitive proton-probe microanalyzer because of our operating conditions (see Sie and Thresher, 1992).

centrations of 100–5000 ppm; and a variety of 'trace elements' (e.g. iron, copper, and bromine) occur at concentrations <10 ppm. Only the micro-constituents and Ca can be measured accurately by WD-EPMA. Absolute ranges of concentrations, measurement error (absolute and 95% confidence intervals), and minimum detection limits (MDL's) for each of these elements are given in Table 2. Measurement error is inversely correlated with mean concentration, ranging from 3.7% in Na to 28% in Cl. Of the six elements measured, only Cl occurred occasionally at less than its respective MDL (157 ppm). Although the microanalyzer reports values less than the MDL, these values were considered noise and set equal to zero for analyses of population structure.

Life history scans for three fish chosen randomly from the data set (Fig. 4) illustrate several points typical of our data. First, all six elements vary ontogenetically in concentration well in excess of the uncertainty associated with measurement. Second, concentrations at any given position correlate strongly with those at neighboring points; for all six elements, autocorrelations are highly significant at scales <100 μm (e.g. Fig. 5), which suggests that in *N. macropterus* this is the typical scale of ontogenetic variability in composition. Comparison of 'small spot-closely spaced' analyses (6 μm beam diameter spaced at 8 μm intervals) with 'large spot-widely spaced' analyses (14 μm diameter at 16 μm spacing) suggests that a sampling scale finer than our standard analysis reveals few, if any, major variations in otolith composition that would not be detected at the coarser sampling scale (Fig. 6). Third, absolute variability is highest for Sr, which can vary within specimens over half an order of magnitude. However, relative variability is as high in S and Cl; coefficients of

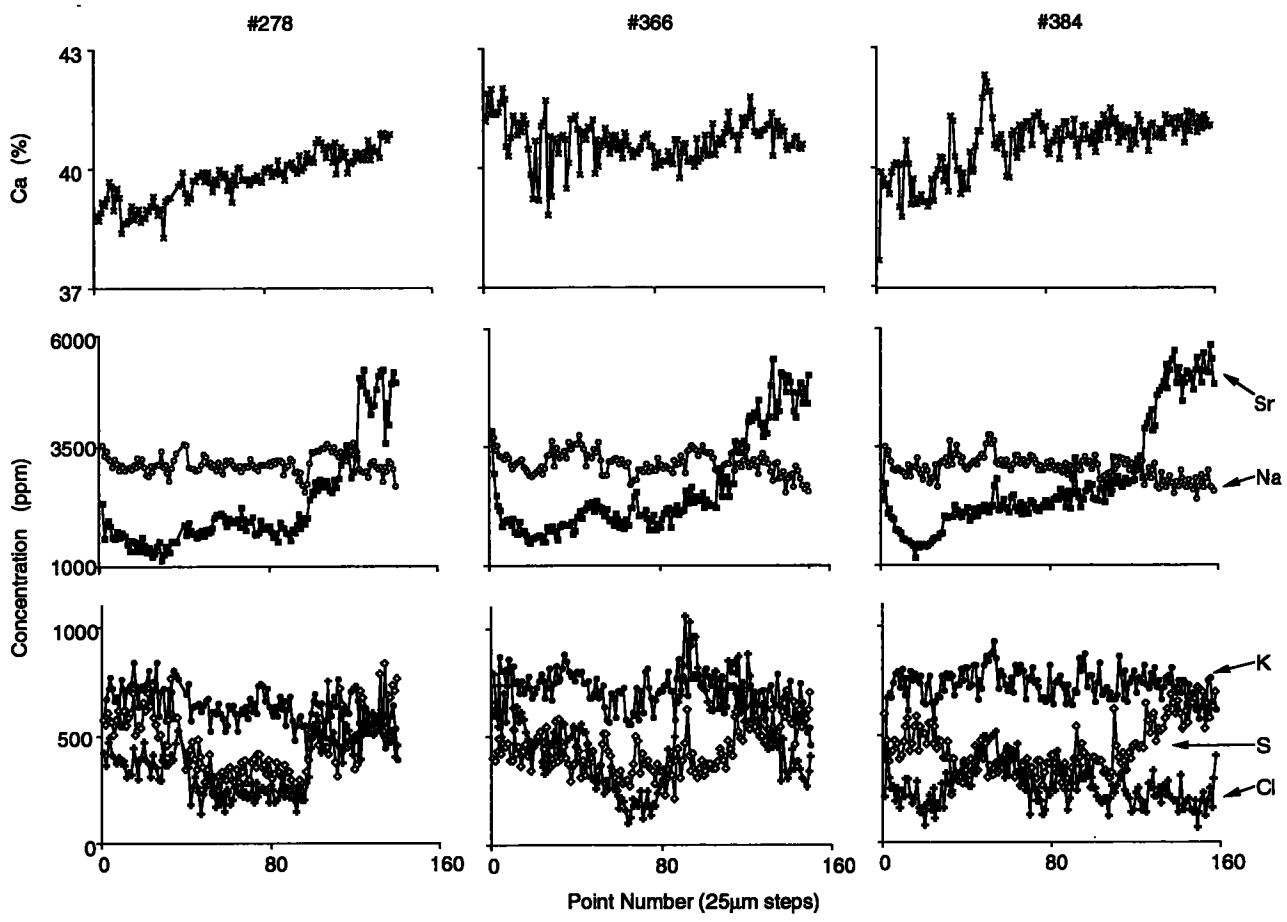


Figure 4

Cross-otolith (primordium to posterior margin) variation in the measured concentrations of the six elements (Ca, Na, Sr, K, S, and Cl) that could be reliably measured by WD electron-probe microanalysis in *Nemadactylus macropterus* sagittae. Concentrations are reported in units of ppm by weight for all elements other than Ca, which is reported as percent by weight. Data are adjusted to standards and cleaned of effects of surface pitting and cracks but are otherwise unfiltered. Specimens were chosen randomly from the adult data set and appear to be representative of the variation observed.

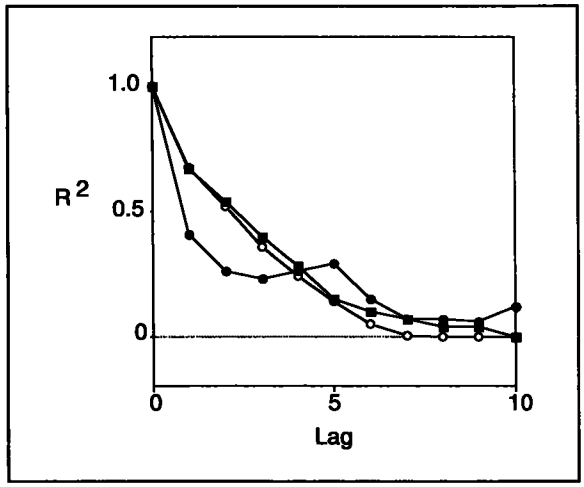


Figure 5

Auto-correlation analysis of the unfiltered Sr data for the three adult *Nemadactylus macropterus* of Figure 3, at lags (intervals between points correlated) of 0 to 10 points. The correlations become insignificant ($P>0.05$) at lags of 4 to 6 points, varying slightly among specimens. Points are spaced 25 µm center-to-center.

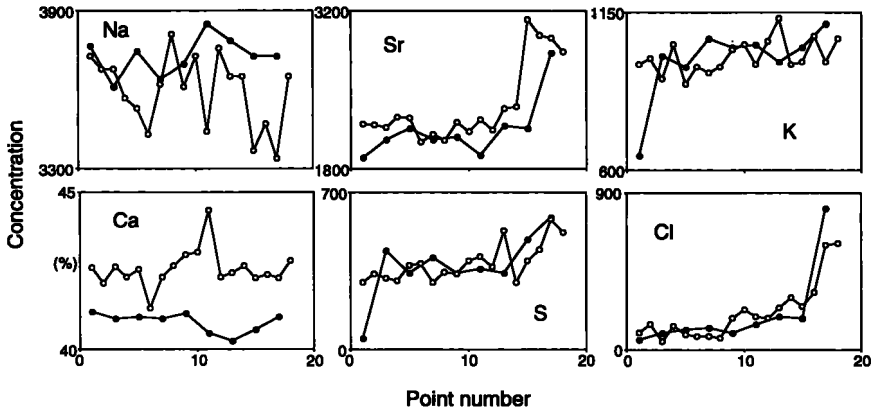


Figure 6

Comparison of parallel fine-scale (open circle) and coarse-scale (solid circle) transects of the outer margin of an adult *Nemadactylus macropterus* sagitta. Distance between the parallel scans was approximately 20 μm. Fine-scale analysis was done with a 6-μm diameter beam at 8-μm intervals, center to center, and with a beam-power density of 2.92 $\mu W \cdot \mu m^{-2}$; coarse-scale analysis was done with a 14-μm beam at 16-μm intervals and with a beam-power density of 2.44 $\mu W \cdot \mu m^{-2}$. Concentrations are reported in units of ppm by weight for all elements other than Ca, which is reported as percent by weight. The large disparity between the two analyses in Na and Ca concentrations is an expected consequence of the different beam-power densities used in the two runs (see Gunn et al., 1992).

variation in the three specimens depicted range up to 45.4 for Sr, 43.4 for Cl, and 31.3 for S (as opposed to, at the other extreme, 1.8 for Ca). Fourth, ontogenetic patterns in the variation are often consistent across specimens. All *N. macropterus* that we have analyzed, for example, show steep gradients in Sr levels in the region immediately around the primordium. Similarly consistent, though less pronounced, patterns are evident in Na and Ca.

The quality of these data were assessed by comparing life history scans from the left and right otoliths from the same fish. The quality of the match within each otolith pair differs markedly (Fig. 7). The comparisons suggest two principal sources of error. First, there is consistent evidence of the difficulty of tracking identical growth trajectories even within a pair of otoliths from the same individual. In all three pairs, the match between left and right otoliths deteriorates as the otolith margin is approached. We attribute this to the decline in the growth rate of the otolith with age, a corresponding compression of ontogenetic variability and, therefore, a larger effect of errors in tracking through the growth axis on the apparent ontogenetic pattern of composition. Slight differences in the shape of the otoliths also give rise to differences in the length of each section, and hence the spacing of scan points relative to the distance along the growth axis. Most of the left and right differences in specimens #304 and #312 appear to result from these tracking errors; that is, the same ontogenetic patterns and mean concentrations are generally evident but variously expanded or compressed along the growth axis. Second, in four of the six elements examined (Na, K, S, and Cl), mean concentrations occasionally differ between left and right otoliths over relatively large portions of the otoliths. This second source of error is difficult to assess. The mismatch is most evident for Cl in #339 and S in #312, where the scale of the mismatch greatly exceeds machine-induced measurement error. The pattern of the mismatch varies widely and inconsistently among the samples: for example, Cl levels match well in #304, match intermittently and poorly in #312, and differ markedly near the margin of #339, whereas S matches well in #304 and #339 but very poorly in #312. Comparisons of parallel life history scans across a single otolith (e.g. Fig. 6) suggest that differences between otolith pairs of the magnitude observed cannot easily be attributed to either measurement error or slight differences between otoliths in the position of the scan line relative to the main growth axis. We conclude, therefore, that the differ-

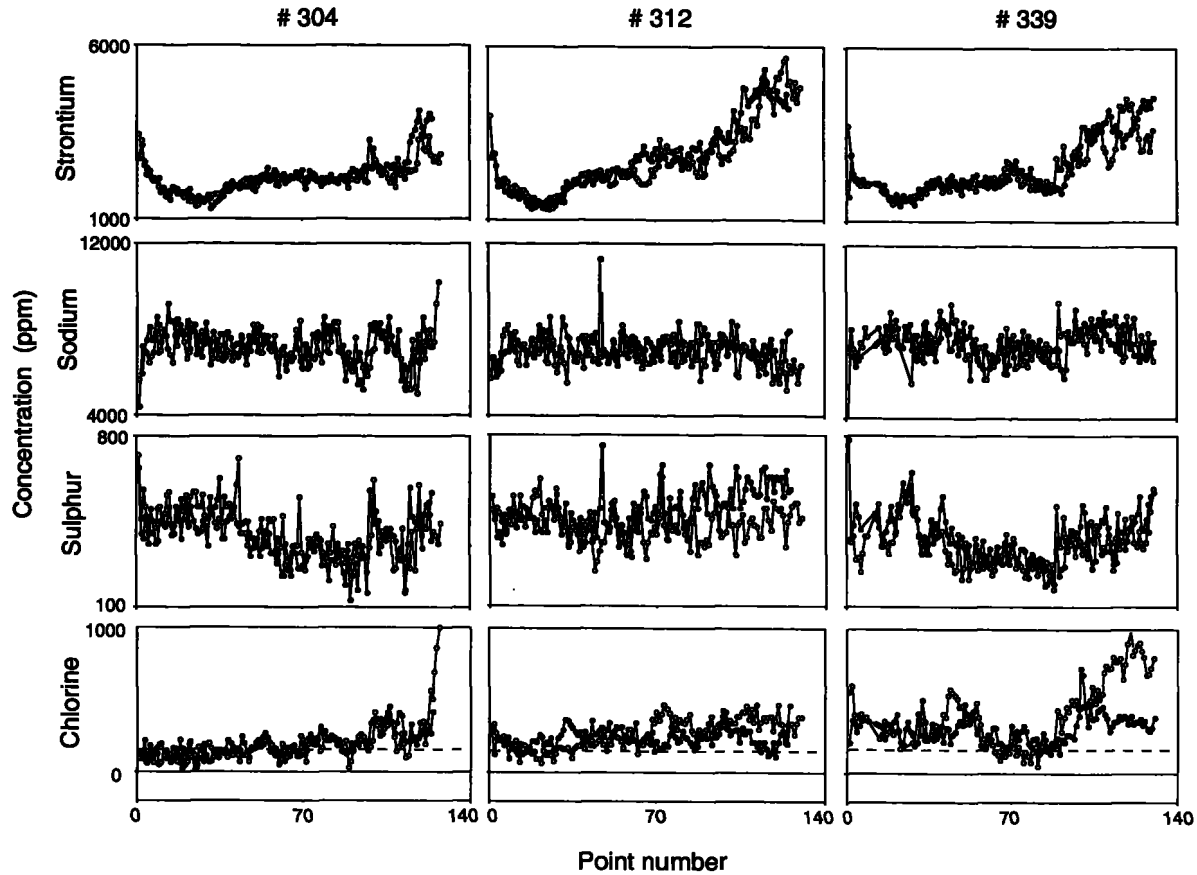


Figure 7

Comparisons of life history transects of the left and right sagittae of three adult *Nemadactylus macropterus* based on unfiltered data for four representative elements (Sr, Na, S, and Cl). The dashed horizontal line in the Cl plots is the minimum detection limit for the element. The comparison is based on identical analytical conditions and an attempt to track as closely as possible the same growth axis in the two otoliths in each pair.

ences between otolith pairs are real, appear to be more common in some elements than in others (e.g. evident in Cl, but not in Sr), and may be more common near the otolith margin than closer to the primordium. The data are limited but clearly indicate that slight differences in elemental concentrations, particularly Cl and S, should be used with caution for stock delineation.

Evaluation of stock structure

We hypothesized that stocks of *N. macropterus* would differ in either or both spawning grounds or times and that these differences would result in diagnostic patterns of composition in the first forming part of the otolith. However, in practice, compositional data for the primordium itself proved of low quality because of the specimen preparation required for EPMA and the incremental structure of otoliths. In most specimens, minute cracks or a pit several microns in diameter developed at the primordium during preparation, the latter because of the 'plucking' of the primordium from the otolith center during polishing. Our previous work (Gunn et al., 1992) indicated that topographic irregularities degrade EPMA data because of unpredictable patterns of x-ray absorption. A comparison of data for point 1 (on and immediately around the primordium) and point 2 (25 μm from the primordium) for the adults supports this conclusion: for all elements except Cl the variance in estimated concentrations is 36 (Na) to 270 % (S) higher for point 1 than for point 2 (Fig. 8). Nevertheless, for all elements, concentrations at point 1 are significantly correlated with those at point

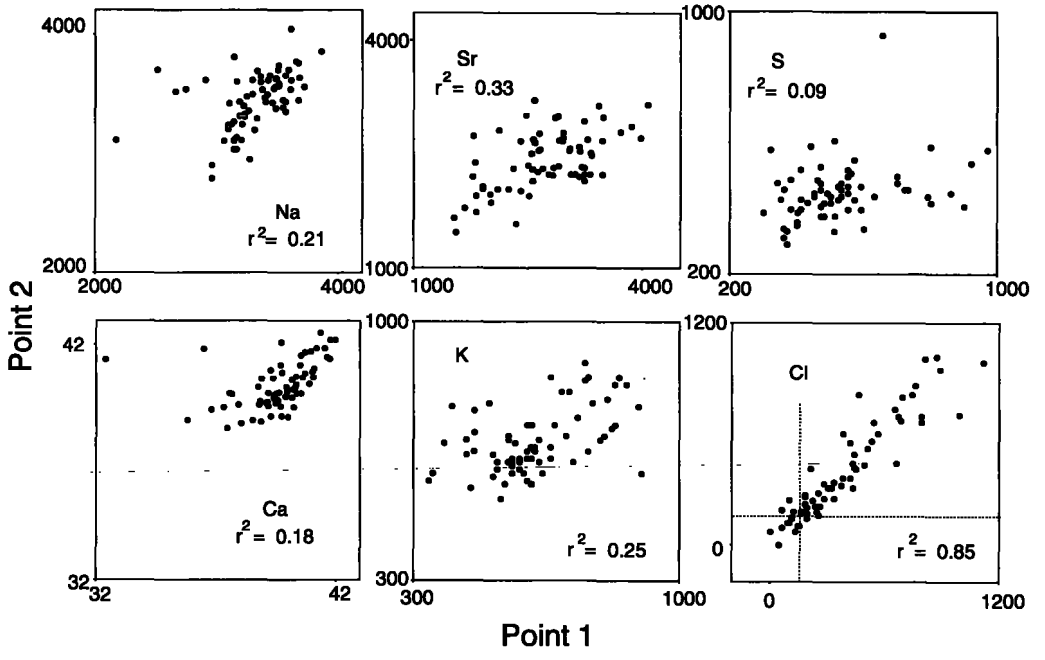


Figure 8

Scattergrams of the relationship between concentrations of the six detected elements (Ca, Na, Sr, K, S, and Cl) as measured on the otolith primordium (point 1) and as measured at a point 25 μm from the primordium (point 2) along the long growth axis of 64 adult *Nemadactylus macropterus*. Concentrations are reported in units of ppm by weight for all elements other than Ca, which is reported as percent by weight. Correlations between the two estimated concentrations are significant for all elements. The slope of the regressions differ significantly from 1.0 for all elements except Cl, although for several elements (Na, K, and Ca) the trend line is very close to a slope of 1 and the calculated regression shifted mainly because of relatively few very low values at point 1. The latter are expected consequences of pitting and cracking on the primordium. Dashed lines in the Cl scattergram indicate minimum detection limits.

2 (r^2 values from 0.09 (S) to 0.85 (Cl)). Slopes for regressions between point 1 and point 2 data are typically not equal to 1, which for most elements probably reflects the highly proteinaceous nature of the primordium. Nonetheless, we conclude that differences among specimens evident in the primordium are also evident adjacent to the primordium, where they can be measured more precisely.

The distributions of mean concentrations of the six elements for the 68 adult *N. macropterus* analyzed are depicted in Figure 9. Three of the six (K, S, and Cl) are significantly skewed to higher concentrations. Ca also shows evidence of a weak skew, and Sr evidence of a weak bimodality. Mean concentrations of four of the six elements differ significantly among sites (Fig. 10), the exceptions being Sr and S. The differences are manifest in both the point 2 and point 2–6 data and are of similar pattern and comparable magnitude in both data sets. For most elements, error bars are smaller in the filtered data, which presumably reflects the reduced effect of random measurement errors. Differences among sites are greatest for Cl: the mean values for three sites (eastern and western Tasmania and the Great Australian Bight) do not differ significantly from the minimum detection limit (MDL), whereas means for the Victorian and New South Wales (NSW) samples are well above the MDL and do not differ significantly from each other. There are suggestions of a similar, though less pronounced grouping of sites in Na and K (concentrations in the Victorian and NSW fish higher than in those from Tasmania and the Bight) and Ca (lower concentrations in Victorian and NSW adults).

The grouping of sites was examined further by plotting Na/Cl and Sr/Ca ratios for specimens from each of the six sites (Fig. 11). These ratios were chosen on the basis of a preliminary survey of the data as likely to separate sites. As expected, the scatter of points

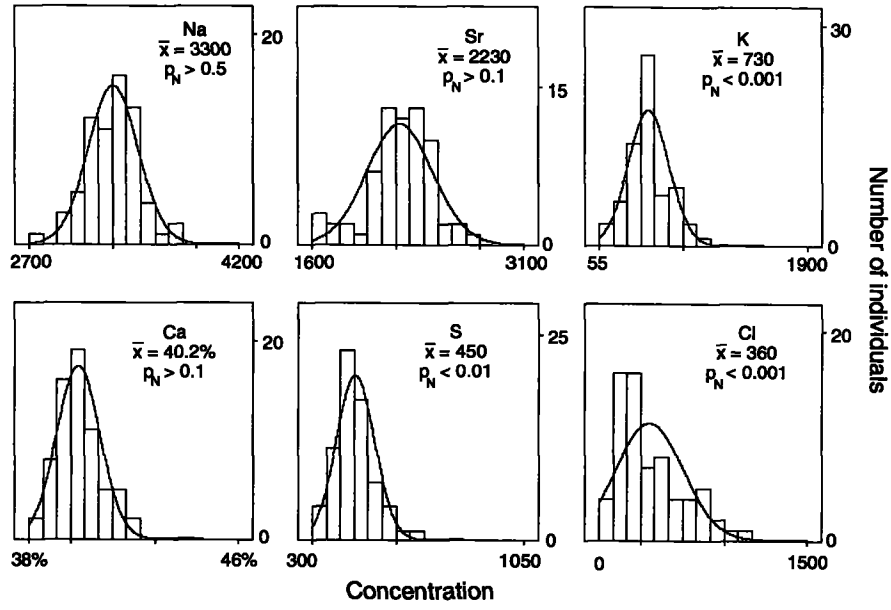


Figure 9

Frequency distributions of concentrations of the six detected elements (Ca, Na, Sr, K, S, and Cl) at point 2 for 64 adult *Nemadactylus macropterus* pooled across sites in SE Australia. P_N=probability the distribution is normal. Concentrations are reported in units of ppm by weight for all elements other than Ca, which is reported as percent by weight.

for individual fish is greater for the single-point data than for the filtered data, but the patterns of regional groupings and the relationships between the concentrations of elements are much the same for the two data sets. Victorian and NSW samples appear to group based on both Na/Cl and Sr/Ca ratios. The two Tasmanian samples group with specimens from the Bight on the basis of Na/Cl ratios but appear to differ based on mean Sr/Ca ratios.

Linear discriminant function analysis (LDFA), for a 3-group discrimination, produces similar results for both single-point and filtered data. For both data sets, the three groups are statistically separable but at a relatively low rate of successful classifications. For the single-point data, 66% of individuals were accurately classified into their three respective 'source populations'; for the filtered data, the success rate increases to 78%. The relatively poor separation is due, in part, to an overlap of the three groups in discriminant space and, in part, to a few individuals located in discriminant space well outside the areas defined by most individuals collected at the same place and time.

For the filtered data, discriminant analysis developed five discriminant functions to classify the six sites. However, only the first three are significant ($P<0.05$), and of these there is a large difference between the first two functions (both at $P<0.01$) and the third ($P=0.02$). Examination of the canonical loadings indicates that discriminant function 1 is correlated with Na, K, and Cl concentrations, and hence represents mainly the initial separation of sites along the Na/Cl axis indicated in Figure 11. The second discriminant function loads heavily only on Sr, whereas the third is mainly a K residual from the first discriminant function. Step-down procedures, in which sites are sequentially pooled, raises the contribution of Ca to the second discriminant function, identifying it with the Sr/Ca axis in Figure 11. The nature of the site separations is indicated in Figure 13. Function 1 separates the two Tasmanian and the Bight (GAB) samples from the two Victorian and the NSW samples; function 2 distinguishes weakly between the GAB sample and the remainder; and function 3 separates the east and west coast Tasmanian samples. The remaining two functions do not clearly distinguish among any sites. The primacy of the first three functions remains in a step-down procedure, as the sites are sequentially pooled based on their degree of overlap. The final step, at which all func-

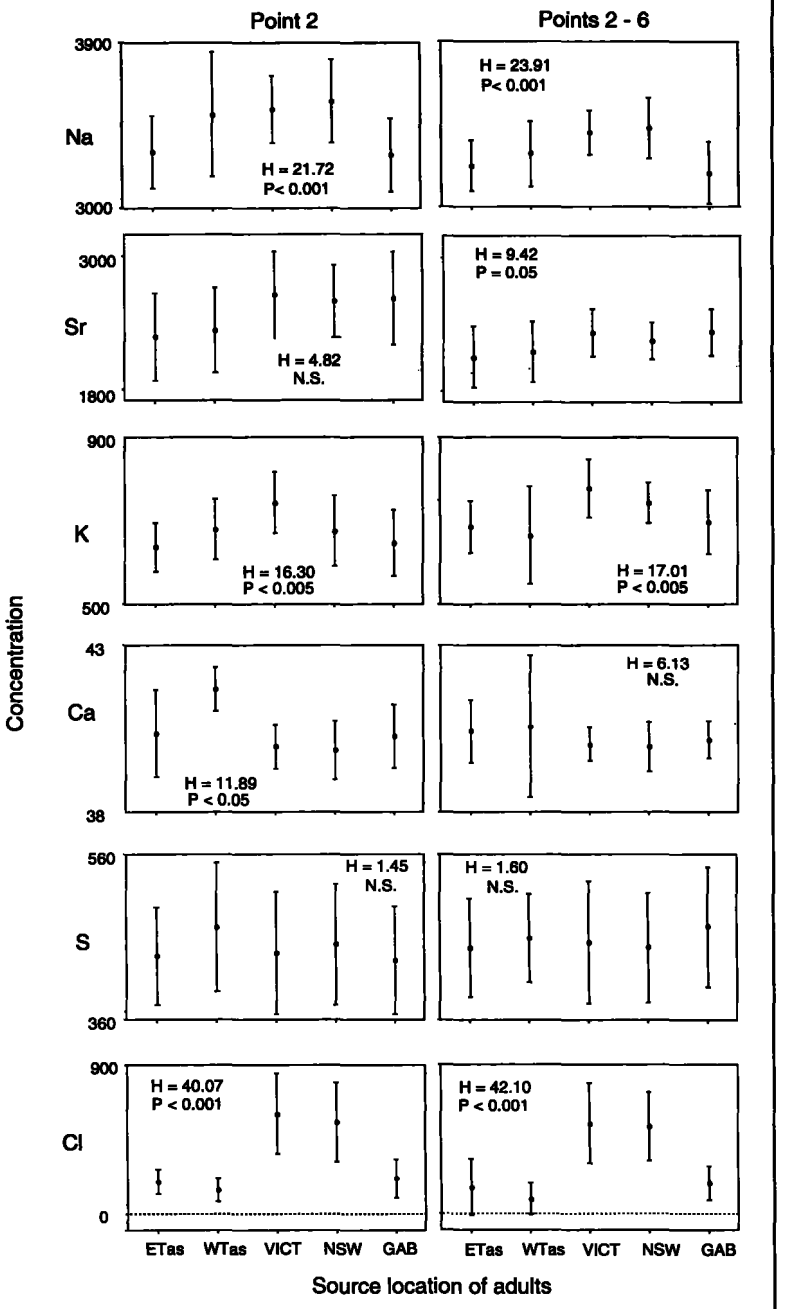


Figure 10

Differences among the six adult *Nemadactylus macropterus* sampling sites in the estimated concentrations (means and standard errors) of the six elements detected by EPMA (Ca, Na, Sr, K, S, and Cl), based on point 2 only and on the mean of points 2–6 inclusive for each individual examined. Concentrations are reported in units of ppm by weight for all elements other than Ca, which is reported as percent by weight. The two Victorian samples were pooled for this comparison. Site labels are defined in Figure 1 and Table 1.

tions contribute significantly ($P<0.001$) to the discrimination, is at the level of three groups and two discriminant functions (i.e. Fig. 12); the third function, separating the two Tasmanian samples, is not quite significant in the final step ($P=0.057$). Post-hoc analyses (Steffe's F-test) of the discriminant functions indicate that the samples from the Victorian and NSW sites do not differ significantly and consistently in any of the three functions, the GAB sample differs from all other sites (which do not differ significantly) in function 2, and none of the sites differs significantly in function 3, though the western Tasmanian sample nearly differs significantly from the other sites.

We draw three general conclusions from these analyses. First, there are significant differences among samples from different sites in terms of the composition of the primordial region of their otoliths. Second, analyses of the primordium itself, of a point 25 μm from the primordium, and of the mean value for the region between 25 and 125 μm from the primordium produce similar results, indicating that distributional differences of adults are manifest through at least the first 125 μm of otolith growth. And third, on the basis of common patterns of composition, the sites pool into three groups: one composed of the NSW and the two Victorian samples, a second consisting uniquely of the Bight sample, and a third consisting of the two Tasmanian samples. In both cases where sites are pooled, the pooled sites are geographically contiguous and nearest neighbors.

Site-specific differences and similarities in ontogenetic variation in composition

That differences in composition among sites can be discerned at points as far out as 125 μm from the primordium and to an apparent age of 45–55 days posthatching suggest that delineation among samples is not a function of conditions specific to the spawning sites. Any environmental differences must encompass at least several weeks, perhaps months, of larval development. To assess the ontogenetic patterning of these chemical differences, we compared the concentrations of apparent key elements for specimens from the three pooled areas (NSW/Victoria, the Bight, and both Tasmanian sites) at several points along their respective long growth axes. Five-point filtered data were assessed at four positions: points 2–6, 6–10, 36–40, and 80–84. The first position is immediately adjacent to the primordium; the second immediately exterior to the first (and presumably encompassing the second 2–3 months of planktonic larval development); the third we estimate to correspond approximately to the age when the prejuveniles recruit to the nursery areas; and the fourth, outermost position, is the farthest along the growth axis at which we had data for all specimens (the number of points depended upon the length of the axis) and, we estimate, corresponds to otolith deposition at an age of 2–3 years.

The results of the comparison (Fig. 14) lead to three conclusions. First, the mean pattern of ontogenetic change in composition is very similar for samples from all three pooled sets of sites, e.g. Na and Sr concentrations decline between points 2 and 6 and

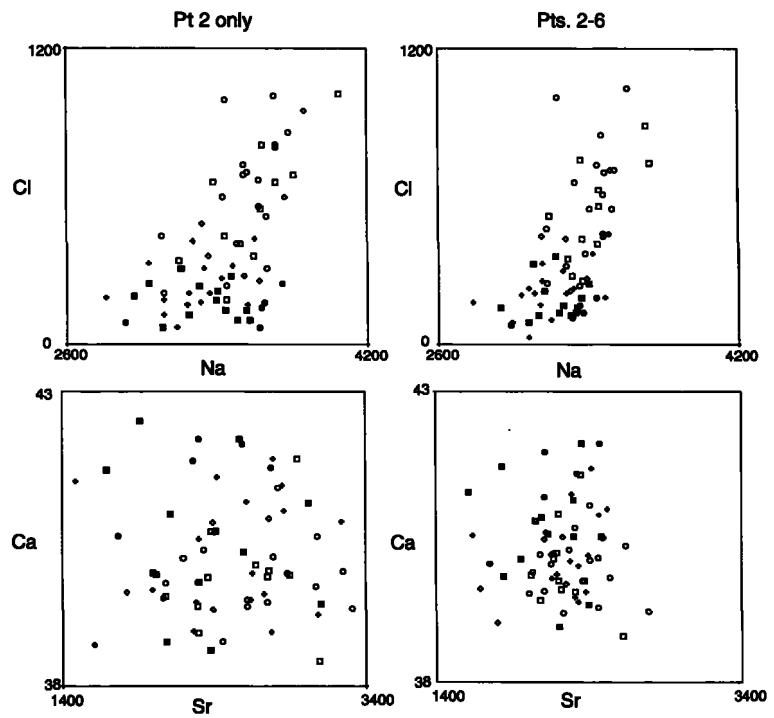


Figure 11

Scattergrams of the relationships between Na and Cl and between Sr and Ca concentrations among the 64 adult *Nemadactylus macropterus* examined, grouped by site, based on concentrations measured at point 2 only and the mean concentration for points 2–6, inclusive. Concentrations are reported in units of ppm by weight for all elements other than Ca, which is reported as percent by weight. Sites are described in Figure 1 and Table 1. Site key: E. Tas.=solid square; W. Tas.=solid circle; NSW=open square; E. Vict.=open circle; GAB= plus sign; and W. Vict.=open diamond.

then increase towards the otolith margin in samples from all three sites. Second, the pattern of ontogenetic variation in concentrations differs among elements. And third, similar mean differences are evident among sites irrespective of where the analysis was done in the otolith. NSW/Victorian specimens, for example, at all stages of their life histories to an apparent age of at least 2–3 years tend to have Cl levels higher than those of fish collected elsewhere. As a result, discriminant analyses based on mean concentrations at points 36 and 80 result in site delineations virtually identical to those derived from concentrations measured near the primordium.

Evaluation of signatures specific to nursery areas and the links between nursery areas and adult groups

The links between nursery areas and spatial components of the adult populations can be assessed in two complementary ways: 1) by determining the source affinities (e.g. spawning site) of juveniles collected in each nursery area and 2) by developing a specific

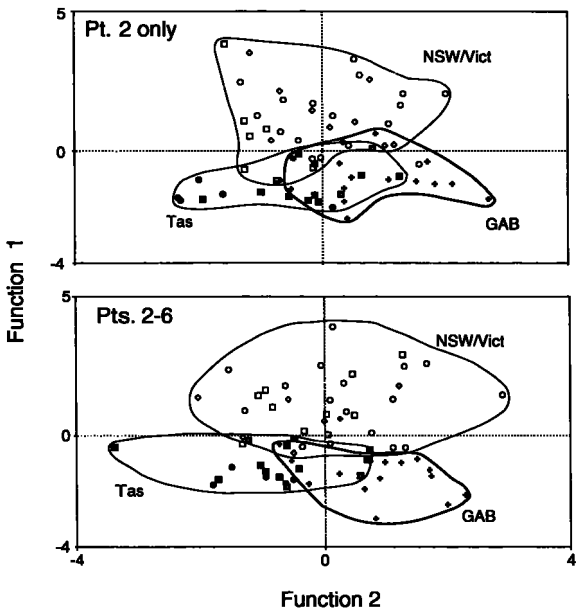


Figure 12

Distribution of adults from each of the six sampled sites (see Fig. 11 for key) in two-function space as determined by a three-group linear discriminant function analysis based on point 2 data and the mean concentrations for points 2–6 inclusive of the six detected elements for each individual. Outlines around each group were drawn by eye. Differences among groups are significant at $P<0.001$ in both analyses (for point 2 only, $F_{12,120}=6.21$, Wilk's lambda=0.35; for filtered data, $F_{12,120}=8.72$, Wilk's lambda=0.29).

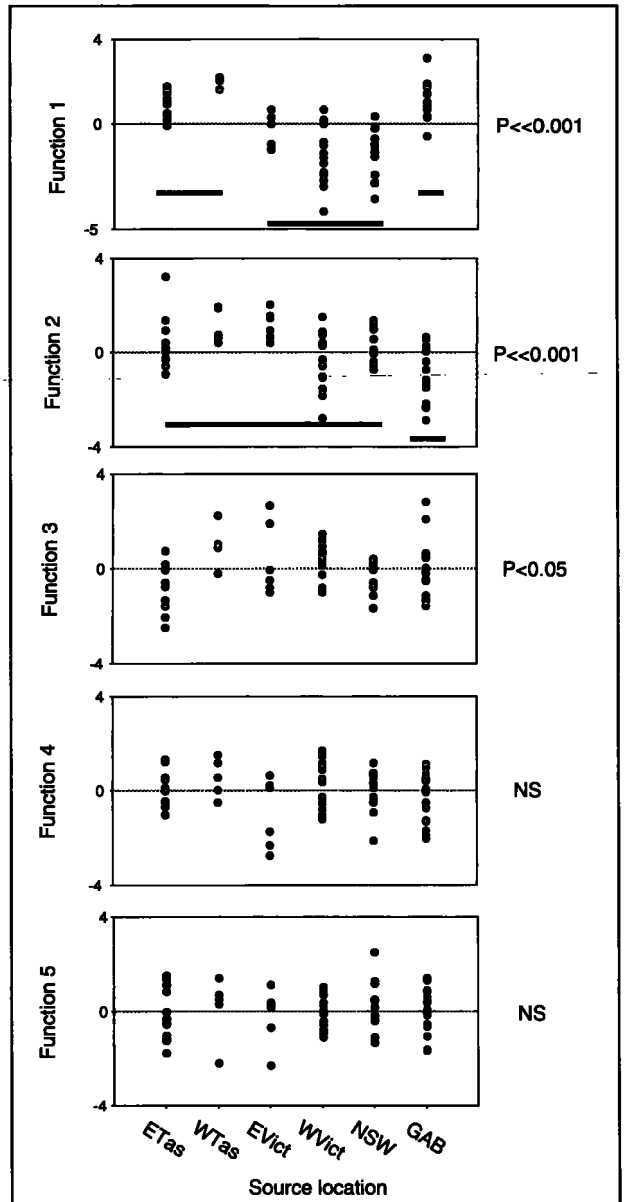
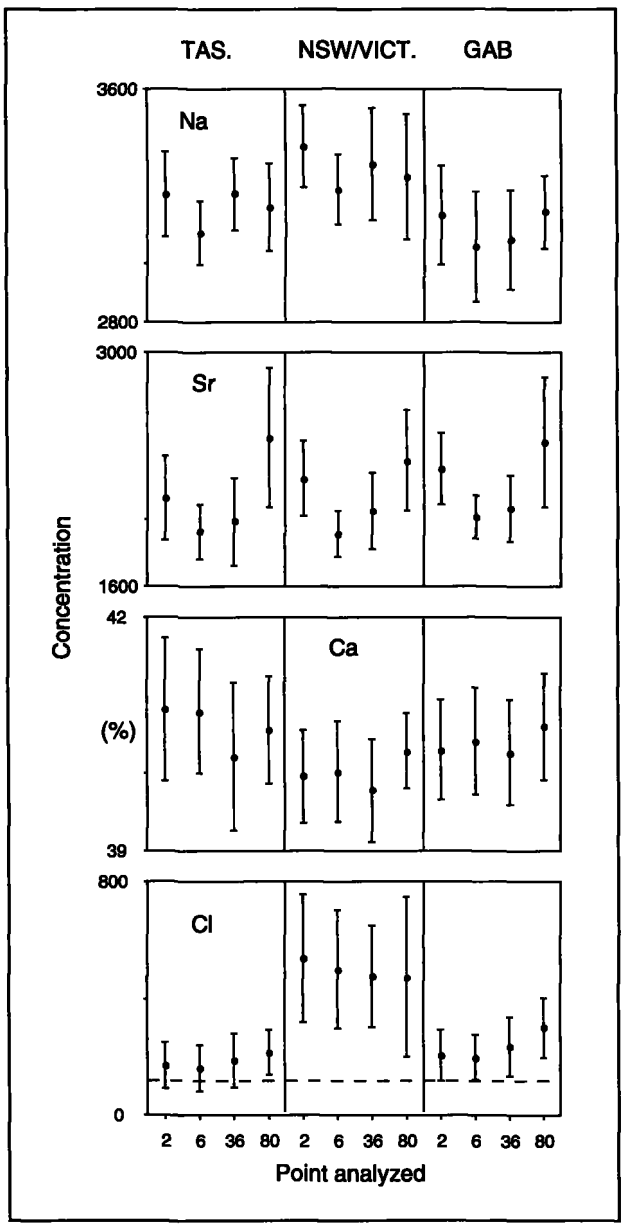


Figure 13

Distribution among sites (see Fig. 1) of values of each of the five discriminant functions defined by the initial (six-site) discriminant function analysis for adult *Nemadactylus macropterus*, based on mean concentrations for points 2–6, inclusive. Horizontal bars indicate sites that pool together based on post-hoc analysis (Steffe's *F*-test). In the case of Function 3, the overall ANOVA is just significant at $P<0.05$, but pairwise comparisons among sites indicate no single or set of sites that differs consistently and significantly from the others. NS=not significant.

signature for each nursery area (based on otolith material deposited during residence in the nursery areas) and using these to classify adults collected in different regions. In essence, the former assesses how juveniles from each of the putative populations are distributed among nursery areas, whereas the latter assesses the contribution of each nursery area to adults collected at each site. With specific regard to *N. macropterus*, if SE Tasmania is the sole nursery area for the species in Australia, then we would expect that 1) the complete range of chemical patterns documented in the adults, at all sites sampled, would be seen in juveniles collected in the single, common nursery area, and 2) adults collected at all sample sites would have a nursery area 'fingerprint' similar to that of the juveniles collected.



For the first analysis, the three-site discriminant functions developed from the adults were used as a training set to classify each of 116 recently settled *N. macropterus*. The data for the juveniles were acquired in the same way as for the adults. Analysis is based on the mean values for points 2–6 from the primordium. Most juveniles were collected in SE Tasmania; a small number were also collected at Phillip Island, Victoria (Table 1).

Most juveniles examined fell within or close to the areas in discriminant function space defined by the adult groups (Fig. 15); only one, with an exceptionally high value on the function 2 axis, did not match the characteristics of at least one of the three adult groups. Moreover, most juveniles classified with the adults collected in the same area. Of the 106 juveniles caught in Tasmania, all but 25 classified with the Tasmanian adult samples, and of these, 13 classified ambiguously, with a probability >25% of being Tasmanian. Overall, only 7% of the Tasmanian-collected juveniles had a probability of <10% of classifying with the Tasmanian-caught adults (Fig. 16). Samples from the five Tasmanian sites were distributed similarly in two-function space (Fig. 15), though the variance was conspicuously higher at one site (Cygnet).

The pattern was similar for juveniles collected off Victoria (Phillip Island), although sample sizes were too small to draw strong inferences. Of the 10 individuals examined, six classified with the NSW/Victorian adults, three classified with the Tasmanian-caught adults (at probabilities ranging from 72 to 85%), and one classified with the Bight-caught adults (at P=63%). The probability that the Victorian juveniles classify with the NSW/Victorian adults is markedly bimodal (Fig. 16) with peaks at >95% and between 5–10%. That is, most individuals had either a very high or very low probability of classifying with the local adults. A similar pattern may also be the

Figure 14

Comparisons of mean concentrations of four elements (Ca, Na, Sr, and Cl) at four points along the life history scan of each adult *Nemadactylus macropterus* pooled by groups identified by linear discriminant function analysis (LDFA). For each individual and point, data were calculated as the mean of the 5-point moving average, beginning at the point indicated (i.e. 2=mean of points 2–6, inclusive; 6=mean of points 6–10, inclusive, etc.). K and S are not depicted as the former generally varies similarly to Na, whereas the latter did not differ significantly among groups. Horizontal dashed line in Cl plot indicates minimum detection limit. Vertical lines about each mean indicate one standard error of the mean. Concentrations are reported in units of ppm by weight for all elements other than Ca, which is reported as percent by weight.

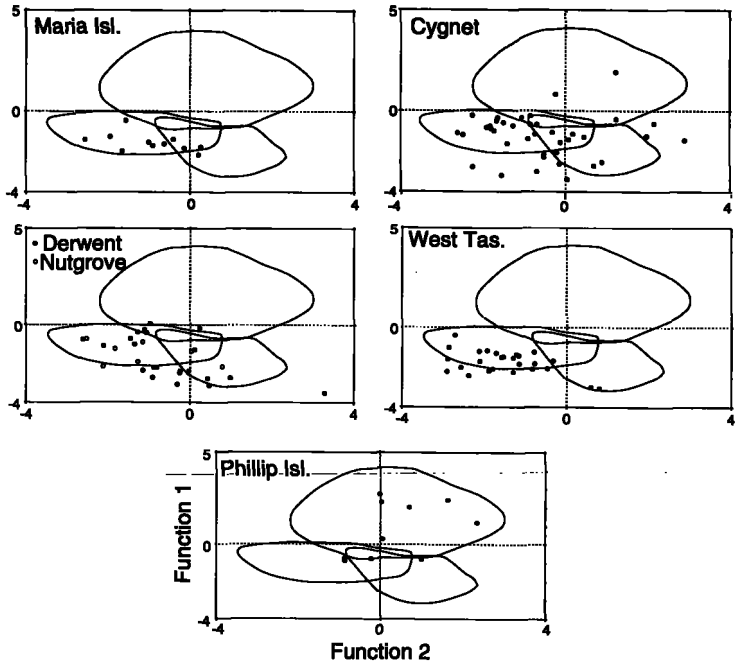


Figure 15

Distribution of individual juvenile *Nemadactylus macropterus* from each of the six sampling sites (see Fig. 1 and Table 1) in two-function space as defined by the three-group LDFA of the adults, based on mean concentrations of the six detected elements at points 2–6, inclusive. Circles enclose areas in two-function space defined by the three adult groups (see Fig. 12).

case among the Tasmanian-caught juveniles. There is an indication of modes at either end of the probability spectrum and possibly a third mode centered near 35%.

The second analysis of the link between nursery areas and the adult population requires analysis of that portion of the otolith deposited while the individuals were in the nursery areas. The otolith of the smallest juvenile we found had a longest growth axis (posterior to primordium) 500 μm in length; several other small fish had similar growth axes in the range of 570–650 μm. Therefore, we examined a standard region approximately 600–800 μm posterior to the primordium along the main growth axis as otolith deposited early in the nursery area stage of development. Specifically, we used as the datum of interest for

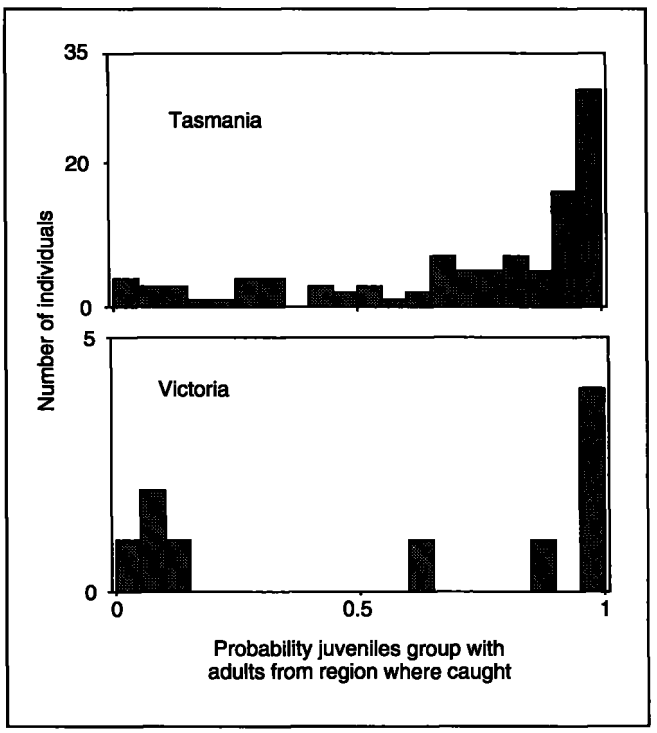


Figure 16

Distribution of probabilities that juvenile *Nemadactylus macropterus* caught in each of the two regions sampled (Tasmania and Victoria) group with adult samples collected in the same regions as defined by the three group LDFA of the adults.

each specimen the mean composition of points 35–39, inclusive, in a standard life history scan (680 to 780 μm from the primordium). All juveniles used in these analyses had otoliths at least this large.

Discriminant analysis of the juveniles indicated highly significant differences among all six areas sampled. The weakest discriminator (the fifth root of the discriminant analysis) was significant at $P<0.01$. The preliminary conclusion then is that there are signatures specific to each nursery-area that could be sought in the adult population.

Further analyses of the data, however, indicated this conclusion was premature. Specifically, if there are nursery-area-specific environmental signals in the otolith, then we would expect them to be manifest ontogenetically in either or both of two ways. First, we would expect that at the end of the larval period (approximately points 25–30), the mean concentrations of various elements would diverge among sites, reflecting the specific environment at each (i.e. the nursery area 'fingerprint'). Second, we would also expect that among individuals, concentrations of these same elements would converge within sites, reflecting recruitment into a common environment. Again, this convergence should occur at approximately points 25–30. For the second prediction, we analyzed in detail one site (Cygnet) for which the sample size of juveniles was large enough that we could reduce possible variability due to differences in date of recruitment. This was done by examining juveniles caught on the same day and falling within a narrow size range (7–11 cm SL).

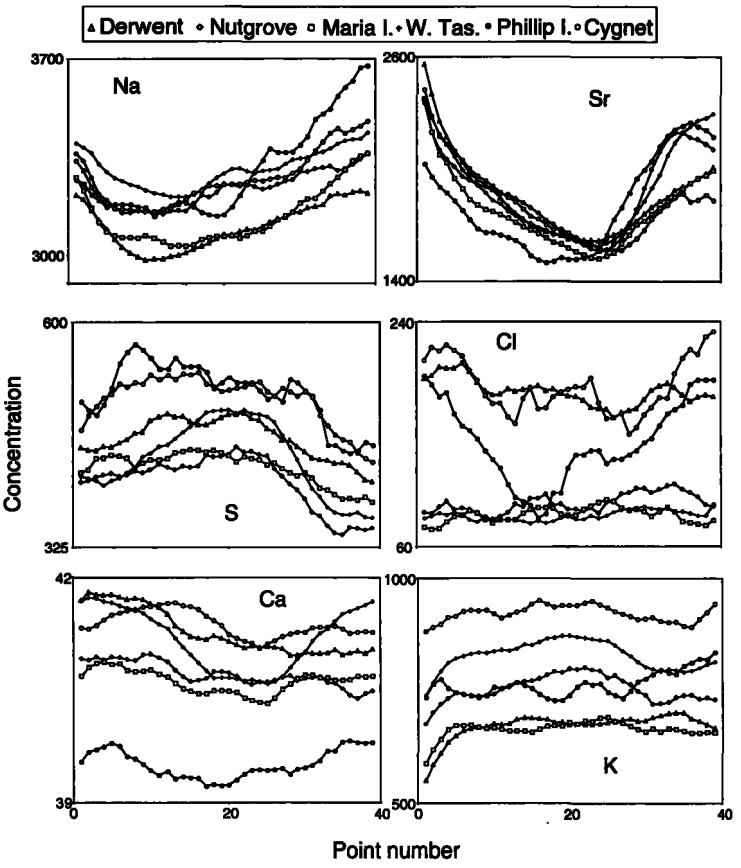


Figure 17

Group mean concentrations of each of the six detected elements (Ca, Na, Sr, K, S, and Cl) for the interior-most 40 points analyzed along the long growth axis of sagittae of juvenile *Nemadactylus macropterus* caught at each of the six nursery areas sampled (see Fig. 1 and Table 1). Point 1 is on the primordium. Data for each individual were filtered through a five-point moving average before the group mean was calculated.

For most elements (all but Sr), neither prediction is supported by the data (Figs. 17 and 18). Although variance is high at all points, there is little or no indication of either divergence among sites (in the case of mean concentrations) or convergence among individuals (in the case of variation within the single site) at or near points 25–30 for any element other than Sr. For Sr, however, both predictions appear to be borne out. Mean concentrations overlap broadly among juveniles from all sites during the larval stage but diverge significantly among sites at about point 25. Among individuals, juveniles at Cygnet appear to converge on two different postrecruitment Sr trajectories, also beginning at about point 25. The available evidence suggests that concentrations of elements other than Sr are largely unaffected by the transition from the larval to the juvenile stages and that the apparent discrimination among nursery-areas is the manifestation of differences among individuals already evident in their larval stages.

We tested this conclusion by reanalyzing for "nursery-area-specific signals" using data for points 2–6 (early larval life) rather than for points 35–39 (early juvenile stage). In general, the results were similar to those obtained with points 35–39, with good discrimination among most nursery grounds and a comparable level of overall site separation (Wilk's lambda=0.20 for points 2–6 vs. 0.22 for points 35–39, $P<0.001$ in both cases). However, the accuracy of correctly assigning juveniles to nursery areas was less in the point 2–6 analysis (51% vs. 82%), which reflects the divergence of Sr concentrations in the nursery areas and its increased importance as a discrimi-

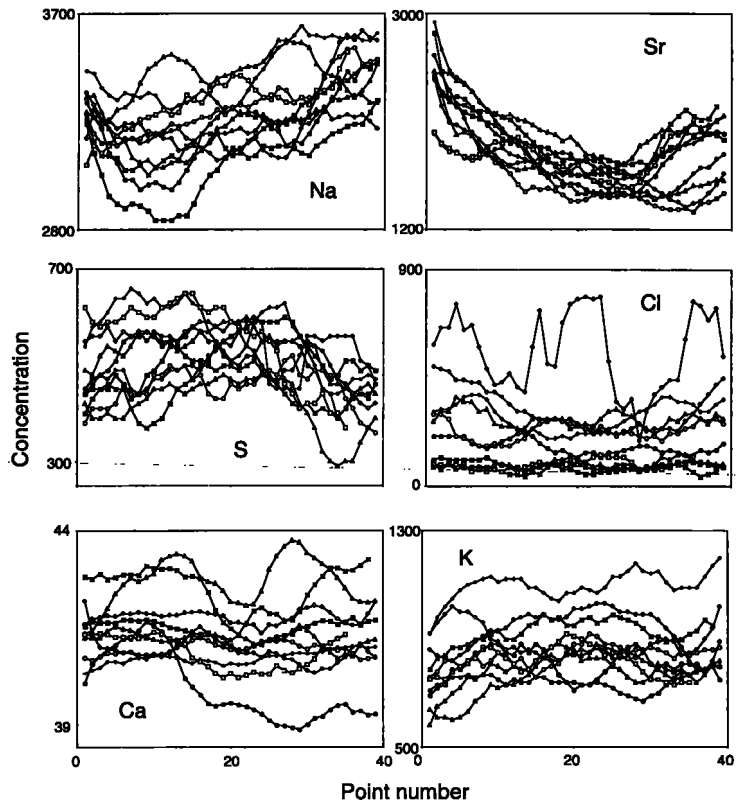


Figure 18

Individual life history trajectories (interior-most 40 points) for each of the six elements (Ca, Na, Sr, K, S, and Cl) detected in the sagittae of juvenile *Nemadactylus macropterus* collected at Cygnet (SE Tasmania). To minimize potentially confounding effects of variation in date of settlement, the comparison was restricted to juveniles collected on the same day (19 December 1987) and in the same size range (7–11 cm standard length). The data for each individual have been filtered through a five-point moving average.

nator. Two discriminant functions (2 and 3) load onto Sr at $r>0.5$ in the original analysis, whereas Sr does not achieve this load level for any function in the point 2–6 analysis. Reflecting this, mean Sr concentrations differ among nursery areas at $P<0.001$ ($F_{5,110}=6.21$) in the original analysis, but at only $P<0.02$ ($F_{5,110}=2.82$) for the point 2–6 analysis. By comparison, differences among nursery areas for the other five elements are significant at similar levels for the two analyses.

Discussion

Effects of data quality on stock delineation

Electron-probe microanalysis with WD-spectrometers revealed extensive variability in the concentrations of six elements in *N. macropterus* otoliths. Some of this variability is induced by the inherent, small-scale compositional heterogeneity of otoliths and some is noise that reflects the limits of detectability and precision of the electron probe. However, comparisons of life history scans along similar growth axes of left and right otolith pairs indicate significant ontogenetic variability for all elements. For most elements, there is also evidence of geographic variability in composition.

The extent to which this ontogenetic and geographic variability can be used to detect differences in either life histories or population structure critically depends on the scale of the life history or population 'signal' relative to analytical 'noise.' In that regard, data quality varies widely among elements. Two identifiable sources of this 'error' are the effects

of beam conditions, which differ among elements (e.g. Na analysis was more sensitive to effects of pitting than was analysis of Sr) (see Gunn et al., 1992), and the low precision of estimates for elements at low mean concentrations (e.g. Cl and S). The effects of these factors can be estimated from standard formulae and empirically by comparing 'replicate' analyses on the same otolith. In practice, true replication is impossible, owing to the effects of beam damage and small-scale heterogeneity in composition, but it can be approximated by comparing points in two parallel life history scans. Our comparison (Fig. 6) is also a worst-case scenario in that it also includes the effects of different beam-power densities and point spacing in the two scans, which can be expected to have a marked effect on the estimated concentrations of some elements, such as Ca. Nonetheless, for the five elements other than Ca, differences between 'replicate points' are still on the order of the theoretical analytical precision (Table 2) and suggest a conservative difference criterion between point analyses that ranges from 331 ppm for Sr to 73 ppm for Cl (Table 2).

Ontogenetic comparisons also require that otolith sections be accurately duplicated among individuals. Our test of this accuracy—a comparison of left and right otoliths from single individuals—leads to three conclusions. First, despite our best efforts we could not guarantee duplication of the life history track between otoliths. Pairwise comparisons suggest variable compression and expansion of the ontogenetic signal between pairs, which presumably reflects slight differences between otoliths in the beam path relative to the main growth axis. The accuracy of duplication was generally high, but also differed among individuals and declined as distance from the primordium increased. Second, nonetheless, the overall pattern of peaks and troughs in the pairs of otoliths compared was generally quite similar. As a result, the principal ontogenetic patterns in, for example, Sr concentrations would be reflected in both otoliths, but examination of one alone could lead to erroneous conclusions about the life history stage at which a particular change in concentration occurred. The variability in life history scans induced by differential compression renders statistical comparisons of ontogenetic patterns extremely difficult and liable to subjective interpretation. In theory, these difficulties could be overcome by calibrating ontogenetic changes in composition against real age—as opposed to distance along the growth axis—but difficulties in resolving the ages of larger individuals are likely to make this approach problematic for most species.

Third, for at least some elements, mean concentrations and ontogenetic patterns appear to differ between otoliths even within the same individual. In two of the three pairs examined, mean Cl and S concentrations differed significantly between otoliths over relatively large sections of the main growth axis and at levels well above measurement error. This asymmetry was so surprising that we repolished and reanalyzed one pair of otoliths (specimen #312) to confirm the results; the second series of data were virtually identical to the first. The implication is that otolith pairs do not encode life history information in the same way. As yet, sample sizes for this comparison are much too small to assess the generality of mismatches and the scale of the problem, but the available information suggests treating with caution data obtained from single point analyses in otoliths.

Another potential source of methodological errors is specimen contamination. J. Calaprice (in press), for example, discounted Cl as a stock discriminator in his studies on Atlantic bluefin tuna, *Thunnus thynnus,* because the element is widely present in the laboratory environment and easily transferred during specimen preparation and handling. Given this, the dependence of our site separation on variation in Cl concentrations is of concern. Although contamination is a critical issue (particularly at the sub-ppm level), several of our observations are not immediately consistent with the contamination hypothesis. First, Cl does not vary independently; its concentrations in otoliths covaries among specimens with Na and K. If Cl concentrations are principally contaminants, the same contamination must affect Na and K concentrations, which is unlikely. Second, samples collected in the same region but at different times, different places, and with different gear types (e.g. juveniles and adults from the Tasmanian sites collected by hook-and-line and trawling) exhibit similar concentrations of Cl, suggesting that observed variability is not a consequence of the way individuals are caught and handled. And third, the order in which the specimens were prepared and analyzed was randomized to check for systematic error; none was detected.

Evidence for regional variation in otolith composition

Several previous studies, using probe microanalysis (e.g. Radtke, 1989; Kalish, 1990) and whole otolith analysis (e.g. Gaudie et al., 1986; Edmonds et al., 1991), have demonstrated that otoliths vary in composition ontogenetically and regionally. Our data permit a detailed evaluation of the interaction between these components of variability. However, because we could not collect and analyze otoliths of larvae from known spawning areas our results only test

indirectly the potential of the technique to resolve spawning stock structure. Specifically, we sought evidence of regionally different patterns in otolith composition that might reflect stock structure.

In that regard, concentrations measured near the primordium differed significantly for four of six elements among adults from the six sites. In all four cases, the range of mean values among sites exceeded an empirically derived 'minimum significant difference' by at least 50% (Table 2). Furthermore, the pattern of differences among sites appeared to be regionally based: the two Tasmanian samples pooled together in the discriminant analysis, as did the geographically contiguous NSW and Victorian samples. Such a grouping of sites could imply any of four different mechanisms: 1) all sites differ, and the grouping is a statistical artefact of the small number of sites and individuals sampled; 2) regional differences result from retrospective changes in otolith chemistry in response to the latest conditions encountered by each adult, and adjacent sites pool because their environmental characteristics are more similar than those of widely separated sites; 3) the sites pool because each regional set derives uniquely from a common spawning ground or spawning population; and 4) each set is derived from a number of spawning grounds or populations that have similar chemical fingerprints, within which individuals mix widely and the boundaries of which are set by constraints on adult or larval mixing.

The possibility that the regional groupings are an artefact is difficult to evaluate without knowing the range of chemical fingerprints possible and their likelihood of occurrence. Assuming three chemical phenotypes randomly distributed among six individuals (=sites), then the probability that at least two adjacent sites will have identical characteristics is extremely high. However, given the number of possible permutations, the probability that all pooling of sites will be only among nearest neighbors is less than 0.01. Therefore, we reject the hypothesis that the apparent regional groupings are a statistical artefact.

We also think it unlikely that the groupings (and similarity of fish within sites) are the result of retrospective modification of otolith chemistry. It is a consistent assumption of otolith-based aging studies that otolith structure is not modified after deposition. A similar assumption underlies chemical studies, although there are no experimental data to verify the point (as opposed to studies on scales, the chemical compositions of which are modifiable retrospectively, e.g. Sauer and Watabe, 1989). In fact, it is likely that at least some water- and alcohol-soluble compounds are transported into or out of otoliths during preservation. However, our data are not consistent with such retrospective modification of the micro-constituents. *Nemadactylus macropterus* collected at the same site and time show little evidence of convergence on a common marginal composition. This implies that recent environmental history has little or no effect on the composition of the otolith margin and presumably even less on the interior. Where a common marginal composition was evident, as in Sr levels among juveniles collected in the same area, it appears to be related to an environmental effect during deposition rather than to retrospective modification.

Distinguishing between the other two hypotheses—a single spawning ground for each regional phenotype or multiple spawning grounds with regionally restricted mixing—is not possible without additional information. As noted, information on the reproductive biology of Australian *N. macropterus* is sparse. Smith (1989) found running-ripe individuals in autumn (February–March) off NSW; we found large numbers of relatively young larvae present along the east, but not the west, coast of Tasmania (for sampling sites and protocol, see Thresher et al., 1989), and several unpublished reports indicate similar larvae off Victoria and South Australia (in the Bight). These scattered observations suggest that *N. macropterus* spawn at a number of sites along the southeastern Australian coast and certainly spawn in each of the three regional groupings of sites identified by otolith chemical analysis. But sampling is not yet detailed enough to determine whether there are discrete spawning areas, or whether spawning occurs in a continuous band of activity all along the coast. Genetic data provide little additional information. Richardson's (1982) samples were drawn from Tasmanian and NSW/Victorian sites and hence appear to bracket two otolith-based regional groupings but indicate no significant genetic differences across this range. This result has recently been confirmed by Elliott and Ward (1994) for allozymes and Grewe et al. (1994) for mitochondrial DNA.

The lack of genetic differentiation in southeastern Australian *N. macropterus* populations is consistent with our observations of apparent examples of larval mixing. Probe microanalysis of otoliths of juveniles from Victorian and Tasmanian coastal habitats indicated that most are similar in composition to those of adults collected at the same sites, which suggests regional, self-recruiting populations. However, the distribution of the probabilities that each juvenile originated in the region where it was collected was conspicuously bimodal. Four out of ten juveniles caught off Victoria had chemical phenotypes more typical of Tasmanian (3) or Bight (1) origin, whereas 8 of 106 Tasmanian-caught juveniles classified mainly with the NSW/Victorian adult sample.

Although the data are obviously preliminary, these mismatched individuals could be direct evidence of an exchange of individuals among populations during the larval stage. The apparent exchange rate varies depending upon the criterion selected and may well differ with site. Even a conservative estimate for the Tasmanian samples (i.e. defining migrants as individuals with a probability >90% of not being derived from the Tasmanian adults) nonetheless suggests an exchange rate of about 7–8%, which is high enough to prevent genetic divergence among samples from the NSW, Victorian, and Tasmanian sites (Elliott and Ward, 1994).

Determinants of otolith composition

The working hypothesis underlying our approach is that otolith composition is largely determined by environmental factors that presumably differ at relatively fine space scales. The data to support this environmental sensitivity, however, are not abundant and to a large extent are drawn from the invertebrate literature (e.g. Rosenberg, 1980; Schneider and Smith, 1982). Studies on teleosts are ambiguous. To date, all reported effects have involved Sr, which has been reported as sensitive to changes in salinity (Radtke et al., 1988; Kalish, 1990; Secor, 1992) and temperature (Radtke et al., 1990; Townsend et al., 1992; however, see Kalish, 1989; and Gallahar and Kingsford, 1992).

There are two reasons to suspect that most of the elements detected in our study are less responsive to the environment than is widely assumed. First, most are physiologically important and their concentrations tightly regulated in plasma and hence presumably in endolymph (Kalish, 1991). For example, an expectation that relatively slight changes in salinity significantly affect the incorporation of Na and Cl in otoliths is unrealistic in an animal with well-developed osmoregulatory mechanisms. Of the six elements detected, only Sr is likely to be relatively unaffected by such physiological controls, though it is presumably affected by many of the same factors that constrain variation in Ca concentrations and may well be subject to a suite of other physiological constraints (see Kalish, 1991).

Second, our data are not consistent with a strong and direct effect of the environment on composition. Two observations are particularly relevant: 1) settlement into nursery areas had no apparent effect on otolith chemistry, other than a slight effect on Sr, and 2) differences among regional groupings are manifest from the primordium to nearly the otolith margin and hence were apparently unaffected by life history stage, irrespective of habitat occupied. Regarding the transition to the nursery areas, for elements other than Sr there was no indication of convergence on a common chemical phenotype by individuals in a given nursery area, nor evidence of divergence among nursery areas in response to local conditions. This suggests that the concentrations of 5 of the 6 elements we measured do not vary in response to environmental conditions in the nursery areas in any direct way. The nursery areas sampled ranged from mid-shelf to shallow coastal embayments and differed markedly in temperature and salinity histories, water-column chemistry, depth, substratum, turbidity, and in invertebrate composition (and hence presumably in the diets of the juveniles). The apparent lack of an impact of any of these on otolith composition suggests their effects at the >100 ppm level are weak or indirect (or both), except possibly for effects on Sr. Similarly, the consistency of regional differences in concentrations through life suggests these differences are largely unaffected by changes in habitats that range from high seas nekton to coastal embayments. Although the concentrations of several elements (Sr, Na, K, and S) clearly vary ontogenetically in otoliths, this variation is superimposed on, and apparently separate from, whatever determines regional differences in composition.

The causes of the regional 'base' differences in composition are not clear. There are several broad possibilities:

- The chemical phenotype is modified retrospectively, based on the adult habitat or sample preparation; for reasons discussed above, we think this mechanism unlikely;
- Life cycles for each region are closed within areas of a uniquely diagnostic environment. This seems unlikely given the diversity of habitats occupied by the species during its life history, but cannot be rejected until the factors that affect otolith composition are determined;
- The base composition is ontogenetically set by environmental influences early in the larva's life, and then maintained, although overlayed by ontogenetic modification, throughout its subsequent life and environmental history;
- The base composition is determined genetically.

The information currently available is not sufficient to discriminate between a 'locked phenotypic effect' (#3) and a genetic hypothesis (#4). A key datum that would permit such discrimination is a measure of year-class effects on otolith composition. Regional differences in otolith composition that vary among year classes argue against a genetic basis and for an environmental influence early in larval devel-

opment. Our current data are much too sparse for any statistically powerful test of year-class differences, but preliminary results suggest only small differences among years for most sites. This is consistent with the similar classifications of adults and juveniles for both the NSW/Victorian and Tasmanian regional groupings; although the adult and juvenile samples differ in mean birth date by five years (adults from the 1980–84 year classes, juveniles from the 1987 and 1988 year classes), the samples overlap broadly in the concentrations of the regionally diagnostic elements.

We tentatively conclude that the regionally diagnostic 'base' concentrations of most measured elements probably have a genetic basis. This conclusion conflicts with both genetic analyses of the species in Australia (Richardson, 1982; Elliott and Ward, 1994; Grewe et al., 1994), which indicate no regional differences and with our preliminary, conservative estimate of larval mixing among regional groupings. At this point, the data are not adequate to resolve this contradiction. Its resolution, however, critically affects the way compositional data obtained from electron-probe microanalysis are used for stock delineation. If the regional differences are primarily genetically determined, then year-class effects are likely to be relatively unimportant. This simplifies analysis but also implies that the usefulness of the approach depends on the extent and pattern of genetic differentiation among populations. However, if regional differences in 'base' concentrations are primarily determined by environmental effects, perhaps via a 'locked phenotype' mechanism of some kind, then variability among year classes could be a critical covariate in an analysis of population structure. In that case, electron-probe microanalysis is likely to be useful wherever significant environmental differences between spawning grounds are known or suspected.

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