A Comparison of Microchemical and Genetic Techniques for Evaluation of Stock Structure of the Jackass Morwong

Fishing Industry Research and
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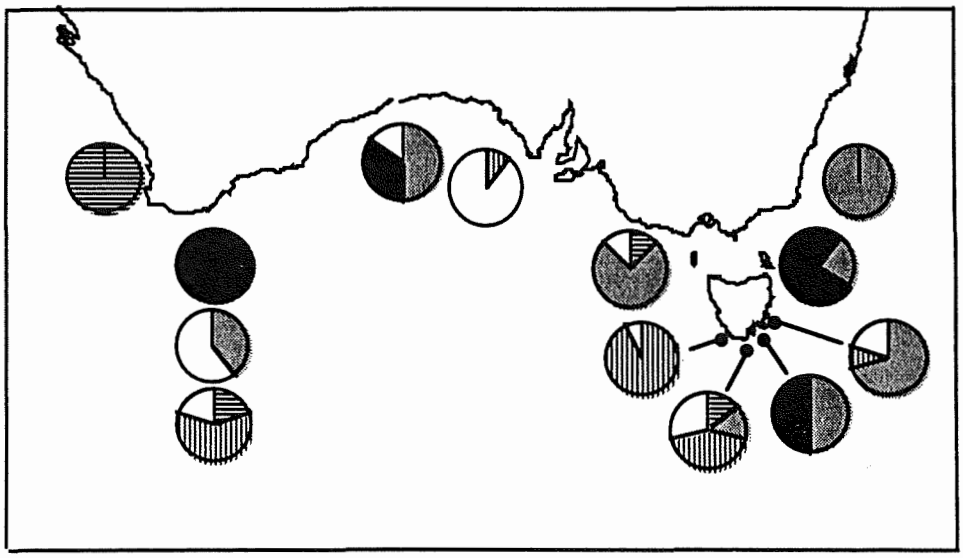
Final Report
Project 1991/32



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Contents

1. Non-technical Summary

Four different techniques for determining the stock structure of a marine fish species were compared, using as a subject the jackass morwong. The four techniques examined were allozyme analysis, mitochondrial DNA analysis and two techniques based on measuring the composition of otoliths ('ear stones') - electron probe microanalysis and proton probe microanalysis, each of which measures the concentrations of different sets of elements. The comparison was based on all four techniques being applied to the same samples, obtained from 14 sites distributed geographically from Perth to NZ. The principal result was that both genetic techniques distinguished between NZ and Australian samples, indicating strongly at least two genetically distinct stocks. The otolith analysis, however, suggested much greater structuring of the Australian samples, and suggests perhaps four stocks in Australian waters: off NSW and Victoria, off southern Tasmania, in the Great Australian Bight, and off Western Australia. There appears to be considerable mixing of individuals among sites, however, at least some of which appears to be the result of large scale seasonal movements of stocks. Otolith analysis could not separate the southern Tasmanian and NZ samples, which could be either because the environments in which the fish develop are similar in the two areas or because they are the same stock. Although the genetic analysis does not separate the southern Tasmanian sample (Maatsuyker) from other Australian sites, in practice sample size for genetic analysis of this site is too small for an effective comparison and, therefore, the genetic affinities of the SW Tasmanian and NZ stocks are not yet clear.

2. Background

Stock structure is widely held to be a key factor in the development of sustainable management practices for marine stocks. Yet, despite the emphasis routinely placed on the subject, little concrete is known about the stock structure of even the more intensively studied species. The principal difficulty is the lack of certainty associated with any specific means of delineating stocks. Tagging planktonic larvae, which are nearly ubiquitous among commercial species, is impractical given their small size and fragility, but nonetheless provides the only direct means of determining stock structure. Indirect approaches, such as analysis of larval distributions, tagging juveniles and adults, modelling larval advection from oceanographic features, analysing parasite loads, and locating and enumerating discrete spawning areas, all provide useful information, but are limited in scope and in the strength of the inferences that can be drawn from them. As a result of such problems, the current standard technique for analysis of the geographic structure of marine populations is genetic studies. But genetic techniques are far from ideal for this task: they will not detect differences at even low levels of larval or adult mixing among populations; they cannot measure directly rates of individual exchange among sites or, usually, specify the origin of individuals; and they have an asymmetric results field (i.e. a genetic difference between sites suggests little dispersal, but the lack of any difference is largely uninformative). As a result, there remains considerable uncertainty about the population structures of even the small number of marine species that have thus far been investigated. This has prompted continuing research into alternative, and perhaps more definitive, techniques for evaluating population structure.

One alternative is the analysis of the chemical composition of skeletal 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 are 1) that the calcified tissues of fish, with few exceptions, are not susceptible to dissolution or resorption and 2) that growth of these tissues continues throughout life. If these assumptions are correct, calcified tissues are permanent records of the influence of endogenous and exogenous factors on their calcium-protein matrices. The hypothesis is that genetic differences between populations or differences in the environments to which fish in each population are exposed affect the incorporation of elements in calcified tissues, 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 1984). The working hypothesis also appears reasonable, given an extensive literature on invertebrates that relates differences in the composition of, for example, mollusc shells

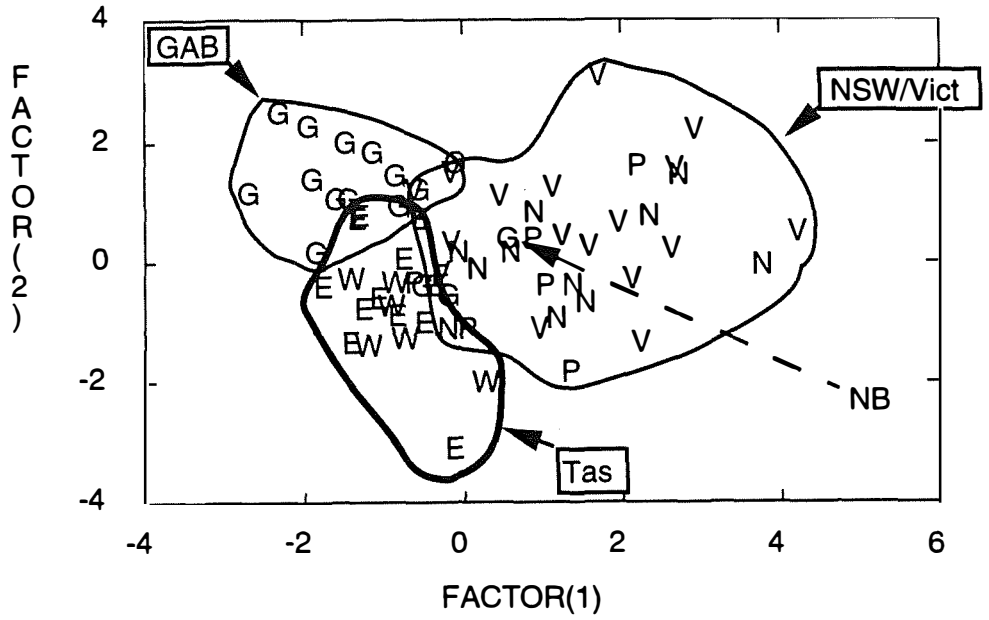
and coral skeletons to a range of environmental and physiological conditions (e.g., Schneider and Smith 1982).

In an previous FIRTA grant (1987/15), we evaluated this possibility by examining in detail regional and ontogenetic variability in the chemical composition of sagittae of juveniles and adults of the jackass morwong, *Nemadactylus macropterus*. Samples were collected at two points off Tasmania, two sites off Victoria, and one site each off NSW and South Australia. Six elements (in order of decreasing abundance, Ca, Na, Sr, K, S and Cl) were consistently detected in the otoliths 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 indicated 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 characterise groups of sites are manifest along most of, and perhaps all of, the growth axis of the otoliths. These results are consistent with the hypotheses that otolith composition reflects population structure, and that these populations are largely self-recruiting. Discriminant analysis suggested the six samples fell into three regional groups (Figure 1): one consisting of the Victorian and NSW samples, one consisting of the two Tasmanian samples, and the third consisting uniquely of the sample from the GAB. Hence, the results were overall consistent with the hypothesis that otolith chemical composition reflects population structure, although also suggesting that this composition is much less sensitive to environmental conditions than previously thought.

The current proposal was an extension of the earlier work, with two broad objectives.

First, as the results of the pilot study were encouraging, we proposed to extend the sampling and analysis of otoliths to cover the entire range of jackass morwong in Australia. Such an extension would, we hoped, fill two needs. It would allow us to test rigorously the hypothesis that otolith chemistry reflects population structure, by greatly expanding sample sizes and sites beyond that covered in the pilot study. And, based on this increased sampling effort, it would allow us to determine the number of and distribution of jackass morwong stocks.

The second proposed objective was a direct comparison of the results of three complementary techniques available to delineate marine fish stocks: otolith chemistry, allozyme distributions (electrophoresis), and analysis of mitochondrial DNA distributions. Preliminary genetic studies of *N. macropterus*, published by Richardson (1982), indicated a single genetic stock over much of SE Australia, the same region that the otolith analysis indicated at least two, very different stocks. There are two reasons why results from the microprobe and electrophoretic studies might differ: (1) the techniques provide complementary data and reflect different degrees of sensitivity to movement by individuals among populations, or (2) the probe and/or electrophoretic results are flawed, due to inadequate sample sizes, poor technique or incorrect implicit assumptions. The study we



Site Key: G = GAB, E = East coast of Tasmania, W = SW coast of Tasmania, V = Victoria, P = Phillip Island, N = Kiama, NSW.

Figure 1. Results of a discriminant function analysis in which adult morwong were asked to be separated into three stocks on the basis of the composition (six elements) of their otoliths. Two-stock discrimination pooled the GAB with Tasmania; a four-stock discrimination (attempting to further split off SW from E Tasmania or NSW from Victoria) was statistically not successful.

The sites are where the adults were caught. Length at age plots and examination of the number of strontium peaks in life history scans of a subset of the adults suggest the fish are a more-or-less uniform mixture of the 1981, 1982, 1983 and 1984 year-classes. The data for the discriminant function is based on the area of the otolith close to the primordium, i.e., early larval stage; analyses based on points farther out result in a virtually identical stock separations. We tentatively conclude therefore that the three stocks are separable from birth, suggesting either genetic differences or different spawning grounds.

Note the adult caught in the GAB (marked with the dasked arrow) that shows strong microchemical affinities with the NSW/Vict stock. We hypothesize that this individual moved between stocks, either actively or during larval drift.

proposed and report on here sought to resolve which of these two possibilities is correct and, not incidentally, determine the relative effectiveness of otolith chemical and genetic studies for stock identification. Microprobe studies for purposes of stock structure evaluation are time-consuming and expensive, though in some respects logistically more simple (e.g., do not require ultrafrozen material and often can use already extensive time-series collections of otoliths). They are warranted only if they provide information not available from less expensive, more conventional sources, such as analysis of allozymes and mitochondrial DNA. Hence the proposed comparison was seen as an essential first step before broadening studies of stock structure to other Australian species.

The model species chosen for study, jackass morwong, was also of particular interest given the uncertainty about its stock structure. In New Zealand, where it has been studied in some detail (e.g., Gauldie and Nathan 1977, Robertson 1978), research suggested this species has three geographically discrete populations, which jointly occupy an area similar in size to that inhabited by the species in Australia. However, the information on the Australian stocks, consisting of a small amount of tagging data for adults (Smith 1989) and the allozyme data for specimens collected in the south-east (Richardson 1982), suggested a single, broadly distributed population (Smith 1989, Tilzey et al. 1990). The early life history of the species also appears consistent with the hypothesis of a single, broadly distributed population. Spawning apparently occurs along the middle continental shelf (Vooren 1972). The planktonic eggs incubate for about 3 days (Robertson 1978) and hatch into larvae that live in the plankton for 9-12 months (Vooren 1972). Morphologically specialised late-stage larvae, known as 'paper fish', are neustonic and generally caught offshore of the continental shelf (Vooren 1972). The long planktonic duration and the off-shore distribution of the larvae suggest high rates of larval mixing among sites, and hence a single Australian population. In the light of the New Zealand data, however, it is not clear that the limited Australian data are being correctly interpreted. Nor is it clear how the observations above, and particularly those indicative of off-shore larval distributions and long planktonic durations, can be consistent with the results of the pilot study on morwong otolith chemistry.

3. Project Details

Objectives

1. To compare the stock structure of jackass morwong in Australian waters as indicated by four different techniques, in order to determine the most effective means of undertaking such studies;

2. To determine definitively the number of Australian stocks of jackass morwong and map their distribution; and

3. To determine if Jackass morwong in Australian and New Zealand coastal waters are parts of the same stock.

Personnel

R.E. Thresher	Ph.D.	(40)	Project Co-Supervisor
R. Ward	Ph.D.	(25)	Project Co-Supervisor
P. Grewe	Ph.D.	(40)	Mitochondrial Studies
C. Proctor	B. Sc. (Hons.)	.(50)	Microprobe Operations
N. Elliott	Ph.D.	(10)	Allozyme Studies
David Mills	B.Sc. (Hons.)	(100)	Otolith Specimen Preparation
Adam Smolenski	B.Sc. (Hons.)	(50)	Genetic Specimen Preparation

4. Technical Results

4.1. Stock structure as indicated by probe microanalysis of otoliths

Materials and Methods

Table 4.1.1 and Figure 4.1.1 provide catch location details for *N.macropterus* used in the otolith study. Note that sample sizes are generally smaller than those used in the genetic components of the work, but also that several additional sites were included (mainly because otoliths, but not whole fish,. were available for those sites). The samples of whole fish from different sites were obtained with the assistance of commercial fishermen, various State Departments of Fisheries, and fisheries research institutions. Samples were obtained from 11 sites distributed from Perth, WA, to NZ. Replication within sites was examined at two sites: three samples were collected at Albany (WA) and two from the Great Australian Bight (GAB). The Albany samples were all taken by the same vessel and all from approximately the same location, but span a period of 4-5 months (Albany #1 taken in September-October, 1993, versus Albany # 3 caught in mid-January, 1994). GAB # 2 was caught approximately 100 n. mi. east of GAB # 1, and approximately two months later.

All fish were frozen at -20°C following capture and remained frozen until otoliths and tissues for genetic analyses were extracted at CSIRO Marine Laboratories in Hobart (max. 30 days post capture). After extraction, sagittae and lapilli otoliths were cleaned of adhering tissue using fine forceps and a soft bristled brush in distilled water. Otoliths were then dried in an oven at 40-45°C for a minimum of 6 hrs, after which they were stored in polyurethane capsules within a desiccating cabinet.

Procedures for embedding, sectioning and preparing otoliths for probe microanalysis are detailed in Gunn et al (1992). The chemical analyses were conducted using lapilli in preference to sagittae in this study because of 1) the comparative ease of preparing a section of a lapillus at the primordial level, and 2) the higher level of standardisation of the position of the electron/proton beam (specimen to specimen) that can be achieved on the lapilli sections. Sections were prepared by fixing lapilli to the base of an embedding mould with a small droplet of 5-min. araldite. The mould was then filled with a clear, harder setting resin (Araldite D). A section containing the otolith was produced by cutting the resin block with a diamond saw blade (Struers Accutom), and sections were then fixed to Suprasil glass rounds. Grinding to the plane of the primordium was done by hand using 2400 grade silicon carbide wet/dry paper. The thickness of final section was 300-500μm. Final polishing was done using progressively finer grades of diamond paste (6, 3 μm) and aluminum oxide powder (Linde B, 0.5 μm) 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 coated

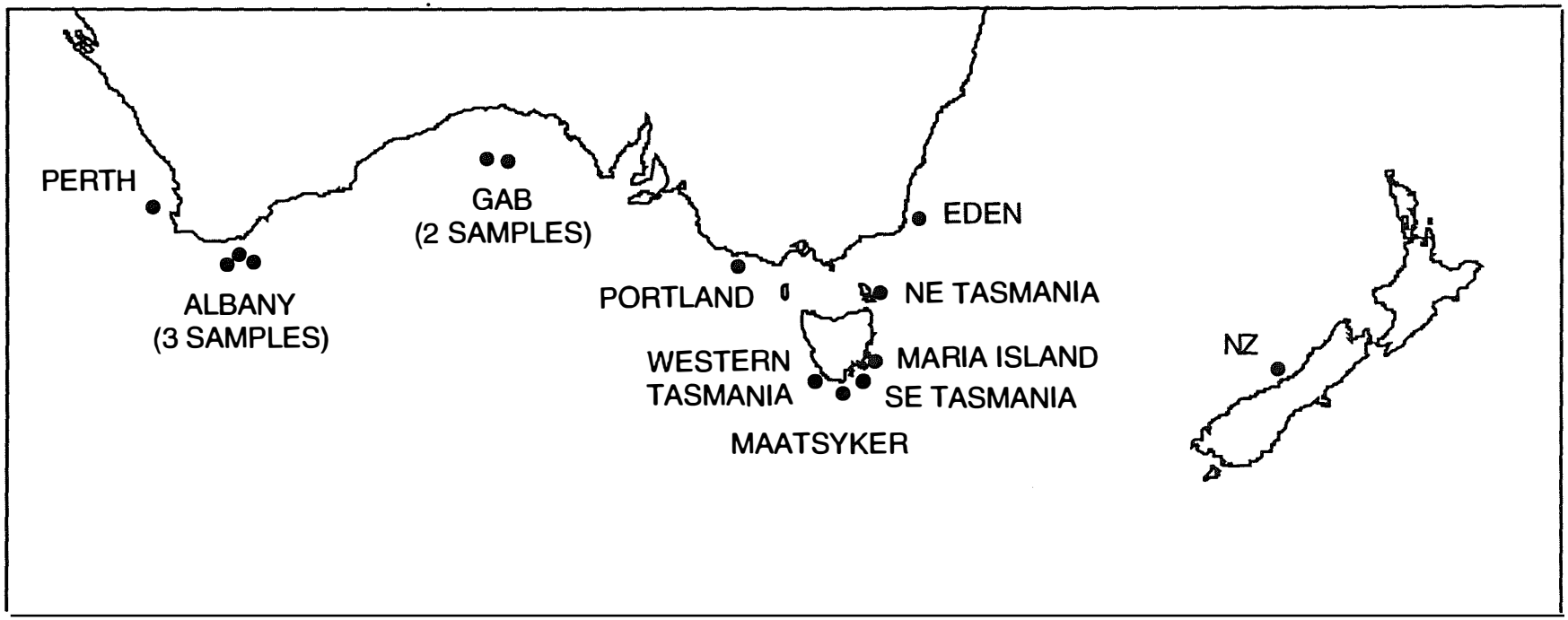


Figure 4.1.1. Sample locations for otolith probe microanalysis.

Table 4.2.1

Code	Location	Date Caught	Number of fish probed	Size range (F.L. cm)	Catch Depth (m)
PERTH	Lat.33°24'S Long.114°31'E	17 Feb 91	8	24.6 - 36.7	200
ALB1	Lat.35°11'S Long.114°28'E	late Sept- early Oct.91	18	31.2 - 41.4	
ALB2	Lat.35°11'S Long.114°48'E	13 - 16 Dec 91	20	29.9 - 38.6	110 - 140
ALB3	Lat.35°10'S Long.114°50'E	12 Jan 92	11	31.2 - 35.5	
GAB1	Lat.33°20'S Long.129°00'E	15 Nov 91	20	30.3 - 34.7	150
GAB2	Lat.33°19'S Long.127°35'E	24 Jan 92	20	29.8 - 41.5	
PORT	Lat.38°42'S Long.141°23'E	23 Sept 91	20	29.5 - 31.5	200 - 220
EDEN	Lat.37°20'S Long.150°11'E	3 Oct 91	23	28.9 - 31.5	110
NETAS	Lat.40°05'S Long.148°50'E	21 Oct.91	23	29.8 - 33.4	820??
MARIA IS.	Lat.42°43'S Long.148°20'E	17 Dec 91	20	29.9 - 37.7	110 - 223
SETAS	Lat.43°39'S Long.147°45'E	19 Sept.91	20	27.8 - 40.2	135 - 152
MAAT. IS.	Lat.43°52'S Long.146°24'E	4 Feb 92	20	25.2 - 35.0	160
WTAS	Lat.43°07'S Long.145°32'E	21 Mar 89	20	10.3 - 40.0	115
NZ	Lat.43°08'S Long.169°47'E	19 April 92	23	17.1 - 43.7	100

with a 250-300 Å coat of carbon, using a sputter coater, and then stored in vacuum until insertion into the probe.

Electron probe analysis

Details regarding the effects of operating conditions on determination of elemental concentrations by means of electron probe microanalysis are discussed in Gunn, et al. (1991) and FIRTA Final Report 1987/15. In the current study, concentrations of Na, Sr, K, S, Cl and Ca were determined using a Cameca Camebax electron probe microanalyzer fitted with three wave-length dispersive detectors. Weight-fractions of these elements were calculated based on count rates measured for the L_α line for Sr and the K_α lines for the other elements, on standard materials and the ratios of the intensities on standards and otoliths, computed using the "PAP" (Pouchou and Pichoir, 1984) matrix conversion software supplied by Cameca. Minimum detection limits and confidence intervals for the concentration estimates are based on equations provided by Ancey et al. (1978).

Beam conditions were as follows: a defocused beam of 50µm diameter, 15 kV accelerating voltage, 25nA beam current (a beam power density of 0.19 $\mu W\ \mu m^{-2)}$ and a total acquisition time of 3 min, 42 sec per point. The low beam power density ensured stability in measured count rate for all elements during the stated dwell time and negligible specimen damage. A single analysis was conducted on each lapillus section, adjacent to the primordium and on the line of longest growth axis. Standards ($CaSiO_3$, FeS_2 and $SrTiO_3$) were analysed at the beginning and end of each "run" of ten specimens to check for spectrometer drift. Our correction procedure for spectrometer drift is detailed in Gunn et al. (1992).

Proton probe analysis

Following electron probe analysis, the same position on the lapilli sections were analysed by proton probe analysis for Mn, Cu, Se, Br, Mo, Cd, Sn, Pb, Hg, Se, Ba, Rb, Zn, Fe, and Ni. Methodology employed in the analysis of otoliths with proton probe (micro-PIXE) is described in Sie and Thresher (1992). Operating conditions specific to this study were : 3MeV proton microbeam, 50µm beam diameter, and beam current of 7 to 10 nA. X-ray spectra, detected with a Si(Li) detector at 45° take-up angle were collected for 6 µC charge, resulting in ~3 ppm minimum detection limits for trace elements of interest (Cr to Rb) detected through their K lines, and 10-50 ppm for the heavy elements detected through L lines. Analyses were conducted with a 100µm Al filter, which attenuates the Ca lines and enhances the detection limits of all heavier elements.

Age determination

Sagittae, from the same fish for which lapillus chemistry was examined (ie. "sister" otoliths), were sent to the Central Ageing Facility, Marine Science Laboratories, Victoria for ageing. Age estimates, based on the reading of annuli, were made for whole otoliths immersed in water and viewed under reflected light.

Analytic Procedures

Analytic procedures for examining apparent stock structure in general follow those used in FIRTA grant (1987/15). The principal statistical method used to examine relationships among sites was linear discriminant function analysis (LDFA). Apparent stock structure was evaluated by three approaches: (1) LDFA of all fourteen sites, subsequently pooled on the basis of common factor scores, (2) a step-down procedure applied to LDFA, in which sites are sequentially pooled on the basis of highest levels of overlap in apparent source locations, and (3) identification of apparent 'source' locations based on unique compositions, and sorting of fish from all other sites into these 'sources' using LDFA. To assess the importance of each element to stock separation, the end-point of approach #2 was subsequently run using a step-down procedure in which elements were removed from the analysis on the basis of lowest significant difference among pooled sites.

Results

Composition of *N. macropterus* lapilli

Lapilli from 261 *N. macropterus* were analyzed using the electron and proton probe microanalyzers. Nominally, twenty-two elements were detected, but several of these were either of dubious presence (mis-reported due to pulse pile-ups in the X-ray spectra, e.g., rubidium closely associated with the dominant Sr peak) or artefacts of specimen preparation, e.g., molybdenum (see Sie and Thresher, 1992). Ultimately, 17 elements were recorded as reliably detected. These consisted of the six macro- and micro-constituents previously detected in *N.macropterus* sagittae using wavelength-dispersive electron probe microanalysis (calcium, sodium, strontium, potassium, sulphur and chlorine, in order of decreasing mean concentration) and eleven trace elements, detectable only using the proton probe microanalyzer. Means, ranges and minimum detection limits (MDL's) for the elements detected at our standard operating conditions are indicated in Figure 4.1.2. Among the trace elements, mean concentrations exceeded the respective MDL for only three

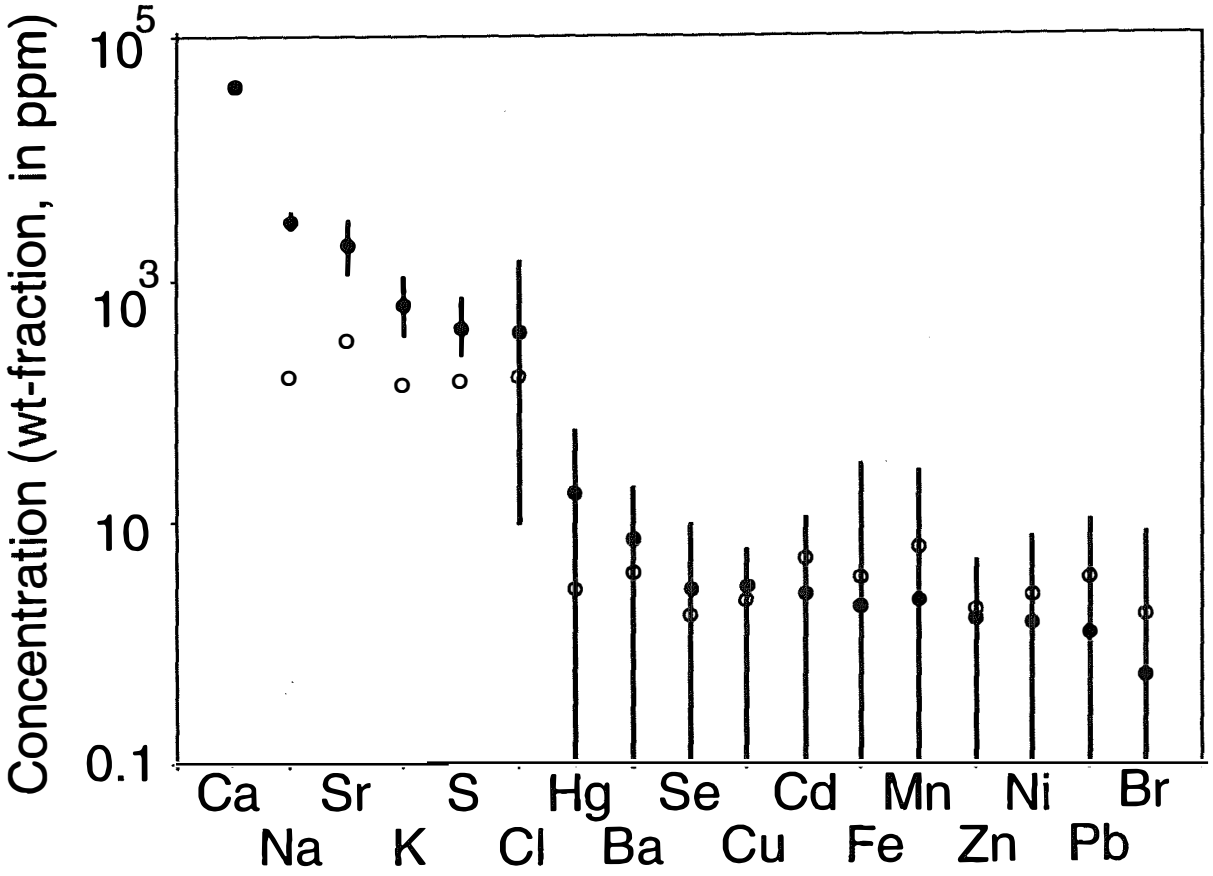


Figure 4.1.2. Mean (solid circles), ranges (vertical lines) and minimum detection limits (open circles) for elements detected in the lapillae of 261 N. macropterus.

elements (Cu, Hg and Se). For all other trace elements, presence was the result of a relatively few individuals with high concentrations. Among the macro- and micro-constituents, only Cl was occasionally present at less than its MDL.

Of the seventeen elements, mean concentrations differed among sites for only nine (Figure 4.1.3).

When all specimens were pooled, mean concentrations did not differ among ages for any element. Differences among ages were nearly significant for Cl concentrations ($F_{8, 219} = 1.92$, $P=0.06$), but this is driven mainly by the relatively low Cl values for one sight (Western Tasmania) which consisted mainly of young fish (figure 4.1.4). Within sites, there were nine significant ($p<0.05$) correlations between age and the concentration of some element. As there are 238 combinations of sites X elements, nine significant correlations is less than would be expected on the basis of chance alone. Nonetheless, of the nine, three correlations involve Na which could perhaps indicate a weak interaction between year-class and Na concentrations.

Among the seventeen elements, mean concentrations differed between sexes for only one (Fe, at $P=0.04$). As means for both sexes were below the minimum detection limit for Fe, we attribute the apparent difference to chance, rather than any effect of sex per se. Within sites, only two of the 34 pair-wise comparisons were significant at $P < 0.05$, which again, we attribute to the effects of chance.

Evaluation of stock structure

Linear discriminant analysis, applied to all 14 sites and using as input all 17 elements, resulted in four significant discriminant factors and a scatter of sites in factor space (Figure 4.1.5a). Within the scatter, however, a number of sites separated out as relatively discrete clusters of points (Figure 4.1.5b). The distinctiveness of several sites and the overall pattern of grouping among all sites is indicated in a post hoc analysis (Scheffe's F-test) of the four significant underlying factors (Figure 4.1.6). Factor 1, which was loaded onto mainly by Cl ($r = 0.78$) and Br ($r=0.42$), divided the sites into two, roughly equal sized groups. Factor 2, predominantly the influences of K ($r=0.52$) and Pb ($r=0.43$), separated Perth from all other sites. Factors 3 and 4, which loaded predominantly on to Sr (0.62) and Pb (0.42) and Hg (0.46) and Na (0.43), respectively, did not unambiguously define any site or groups of sites; rather, factor 3 tended to separate Perth and Gab #2 from all remaining sites and factor 4 weakly distinguished between Albany #1 and other sites.

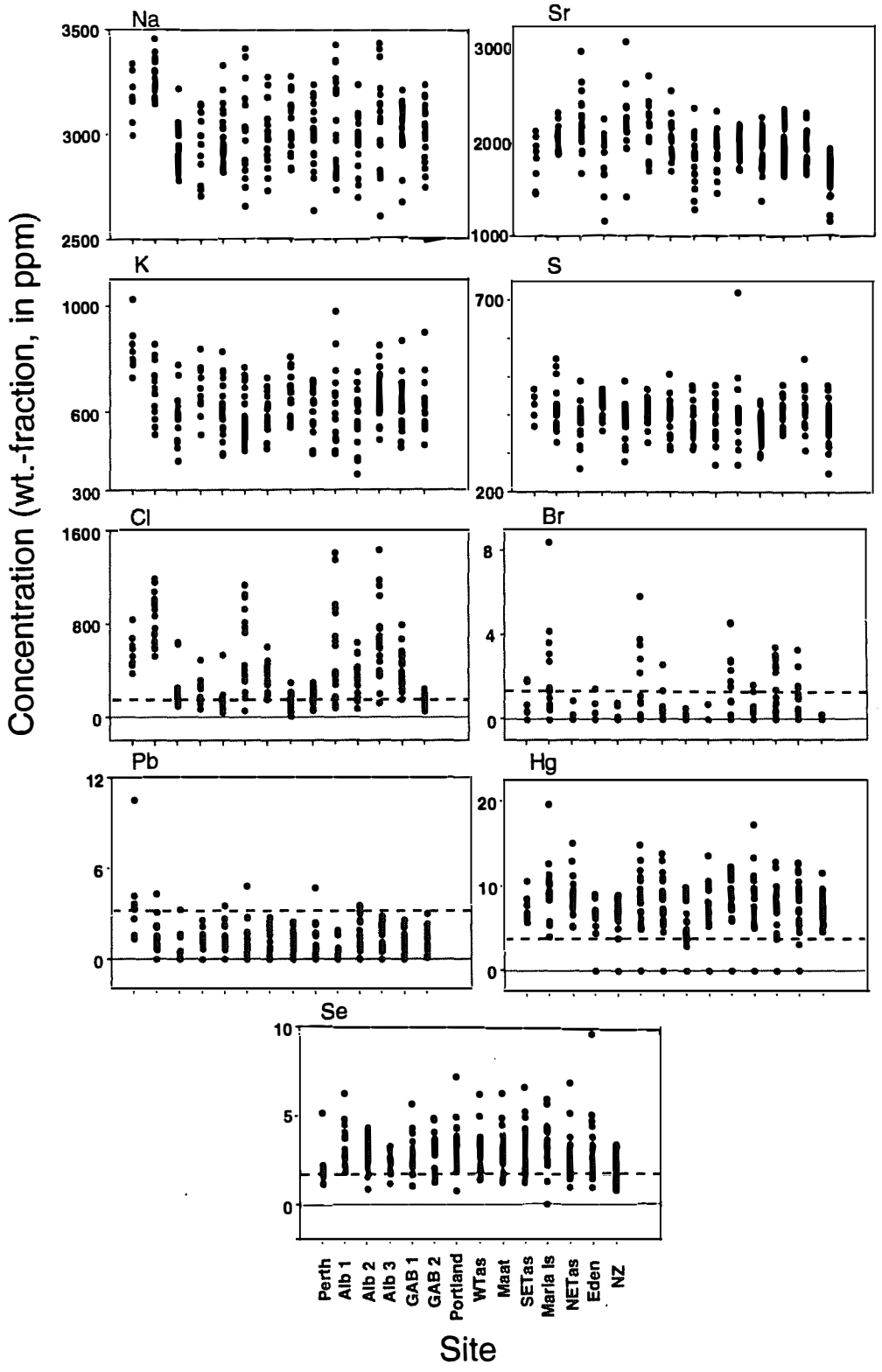


Figure 4.1.3. Distribution of the concentrations of the seven elements which differed significantly among sites. Horizontal dashed line indicates minimum detection limit..

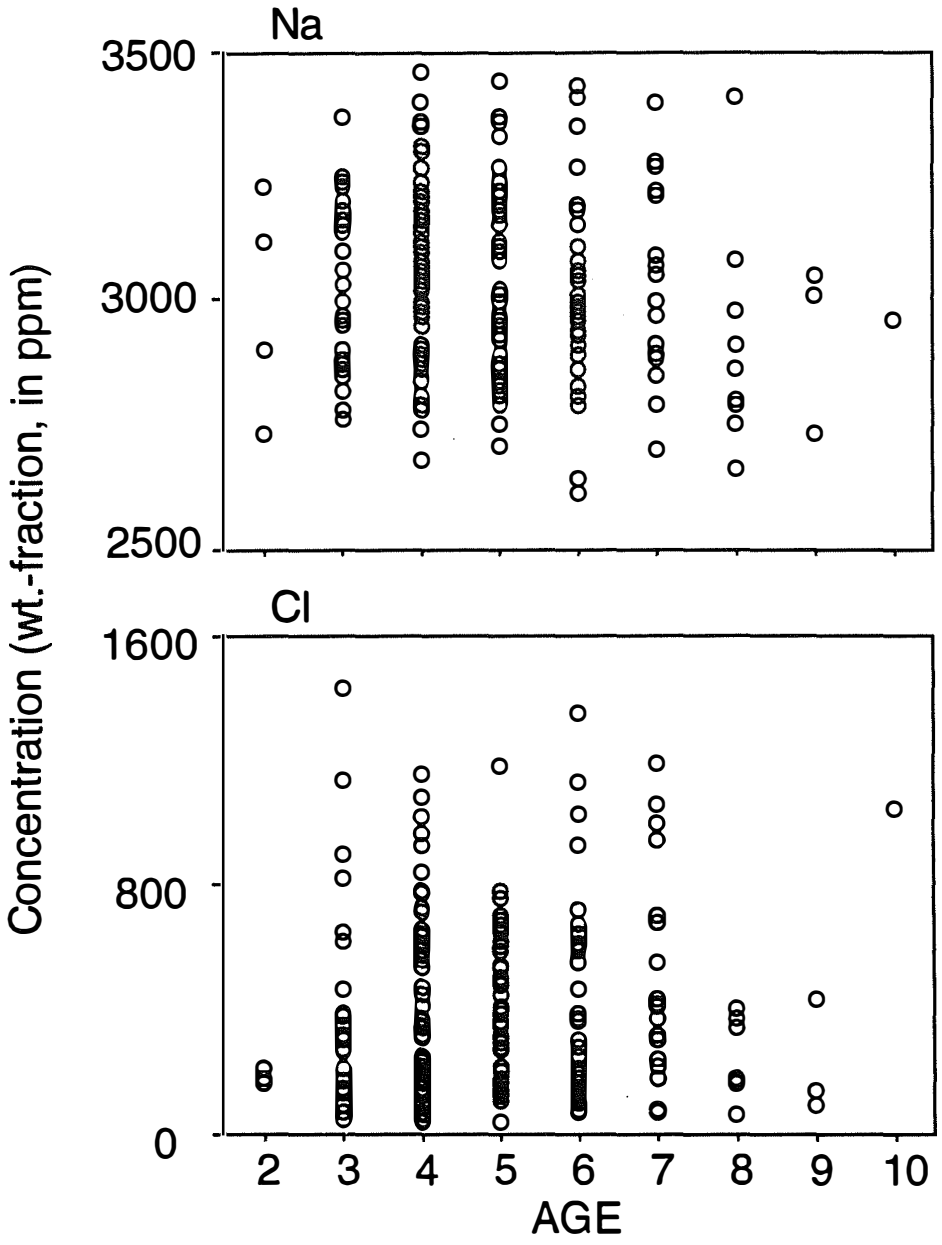


Figure 4.1.4. Effects of estimated specimen age on the concentrations of Na and Cl measured at the otolith primordium.

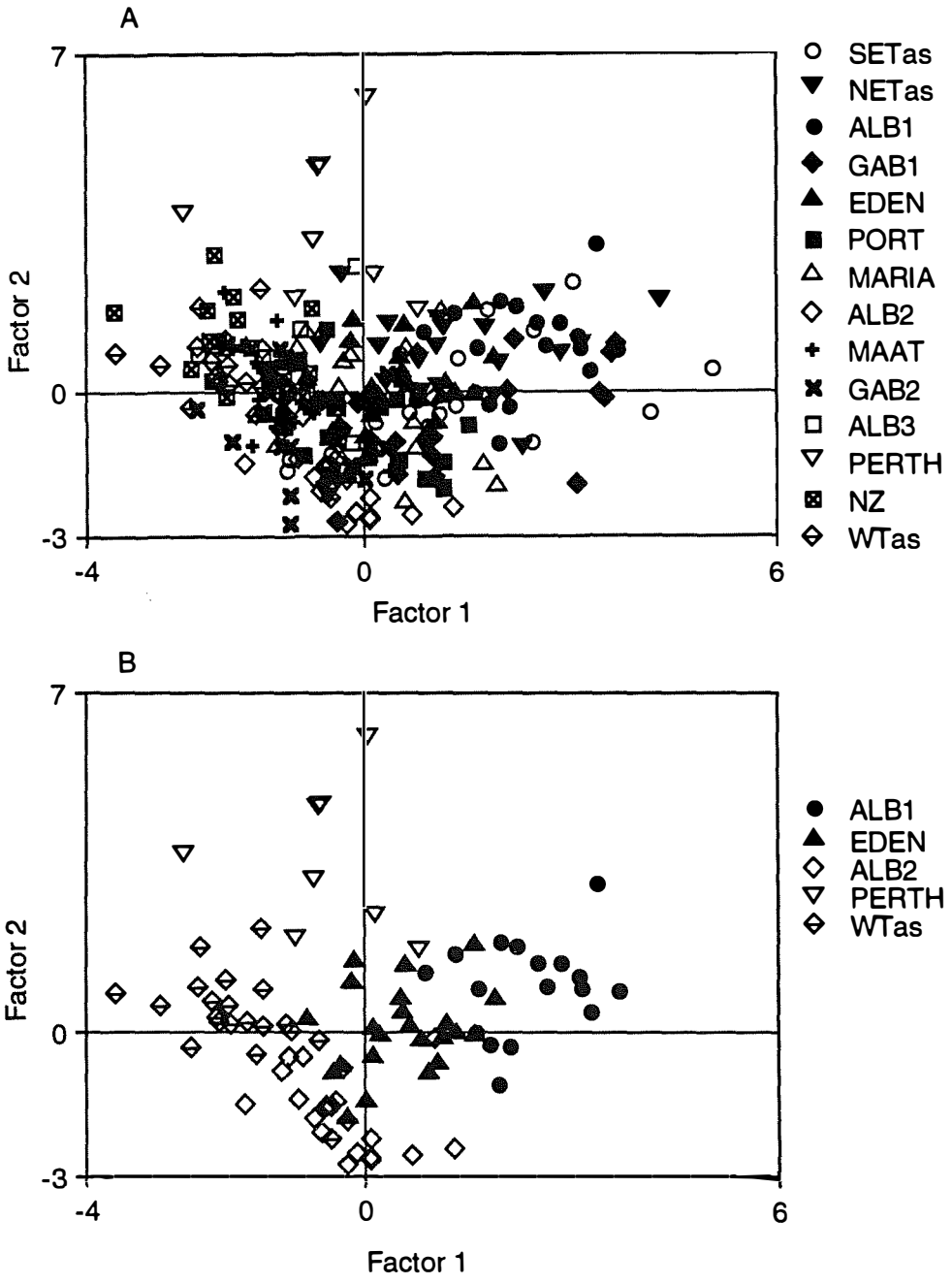


Figure 4.1.5. Distribution of specimens in factor space defined by a linear discriminant analysis separating all fourteen sites. A. All sites depicted. B. Selected sites.

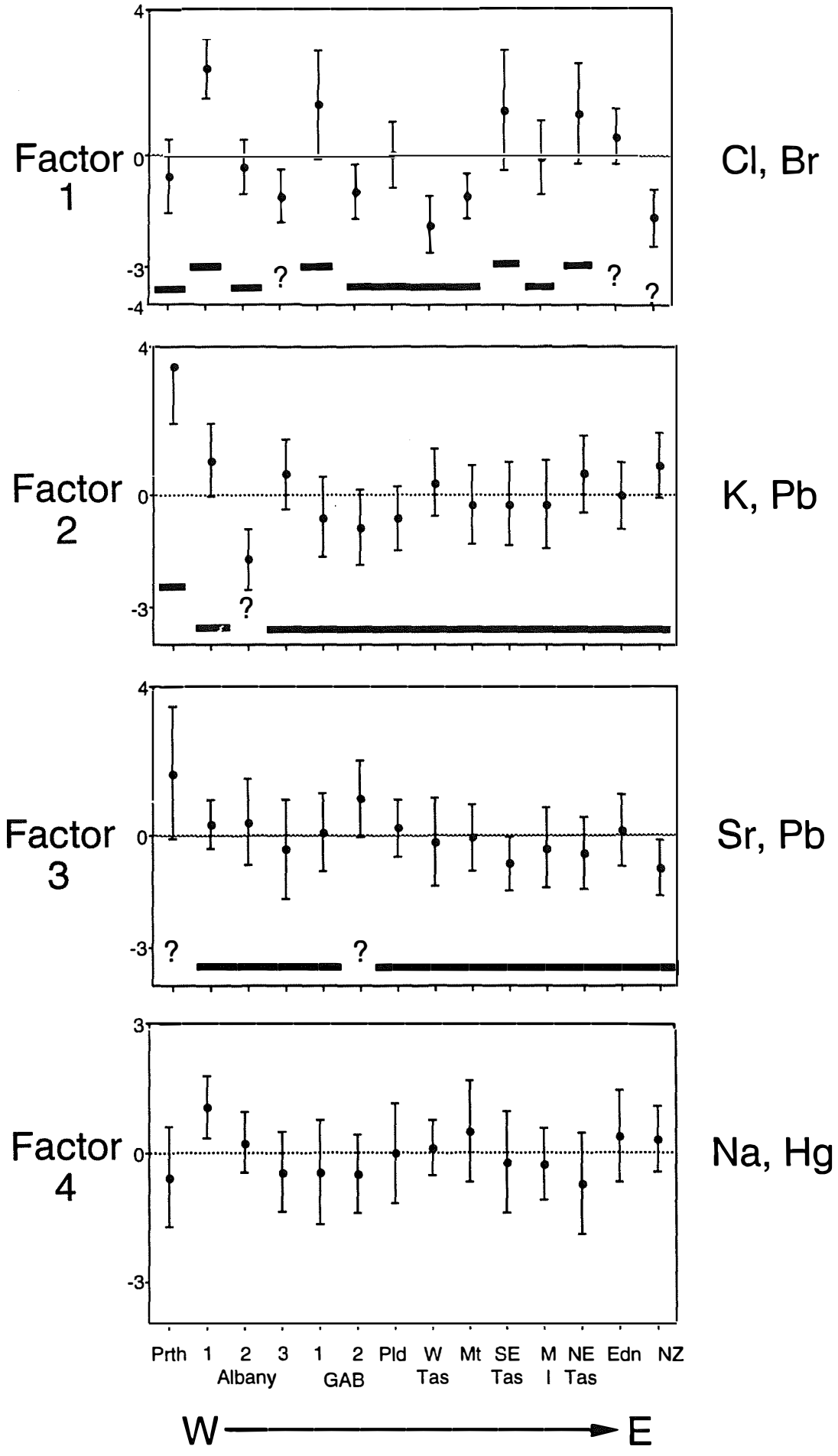


Figure 4.1.6 Distribution of discriminant factors across sites. Horizontal lines link sites not significantly different.

Inspection of the raw data generally confirms these groupings. There are well marked differences among sites in Cl concentrations, suggestive of perhaps three groups: one group consisting of Albany #2, GAB #2, Western Tasmania, Maatsuyker and New Zealand, with mean Cl concentrations close to or below the Cl minimum detection limit, a second group (Albany #3, Portland and Eden) with intermediate concentrations of Cl (in the range of 200-500 ppm), and a third group (Perth, Albany #1, GAB #1, NE Tasmania and SE Tasmania) with mean Cl concentrations > 500 ppm. Perth and Albany #1 group, and are split off from other sites, on the basis of higher than average Na values, whereas Perth separates from Albany #1 on the basis of K and, to a lesser extent, Pb concentrations.

A step-down procedure applied to the LDFA, in which sites were sequentially pooled based on degree of overlap in predicted 'origin', produces a final stock structure based on four probable groups (Figure 4.1.7). The stopping rule used was when at least 70% of the individuals in each group were correctly assigned; in practice, this stopping rule coincided with another possible stopping rule, which is the point at which all remaining discriminant factors are significant. Both criteria were achieved at the four-group stage. The groups consisted of (1) Perth, (2) Albany #2 and GAB #2, (3) Western Tasmania, Maatsuyker, New Zealand and Albany #3, and (4) all remaining sites (Albany #1, GAB #1, Portland, Eden and three sites off eastern Tasmania).

In part, the overlap of sites in factor space could result from individuals of the same putative population mixing to various extents, i.e., the Albany #3 sample could consist of 5 fish 'from' Eden, 7 'from' Tasmania, and the remainder 'from' Perth. To evaluate this possibility, five sites were selected as most likely to represent 'pure' samples, based on their distribution in two-factor space in the LDFA, and samples from all sites classified against these putative source populations. The five samples chosen were Eden, Perth, Western Tasmania, GAB #2, and Albany #1, all of which were well separated in factor-space when run alone (Figure 4.1.8). The affiliations of individuals from all fourteen sites, when classified against the five 'source' sites, is depicted in Figure 4.1.9. Individuals which classified ambiguously are excluded from the analysis.

Four points emerge from this and the previous analyses. First, the New Zealand sample did not differ significantly from those off Western Tasmania or Maatsuyker for any element or in any discriminant analysis. Second, the geographic structuring of the groups is not clear-cut, that is, fish which classify together are broadly distributed across much of southern Australia. In the most extreme example, fish which group together as 'Albany #1' are caught off Albany and off eastern Tasmania, with relatively few caught in the GAB or off western Tasmania. Third, despite this, there is a general patterning in the distributions, seen most clearly in Figure 4.1.9. 'Eden' fish tend to dominate in the southeast; 'western

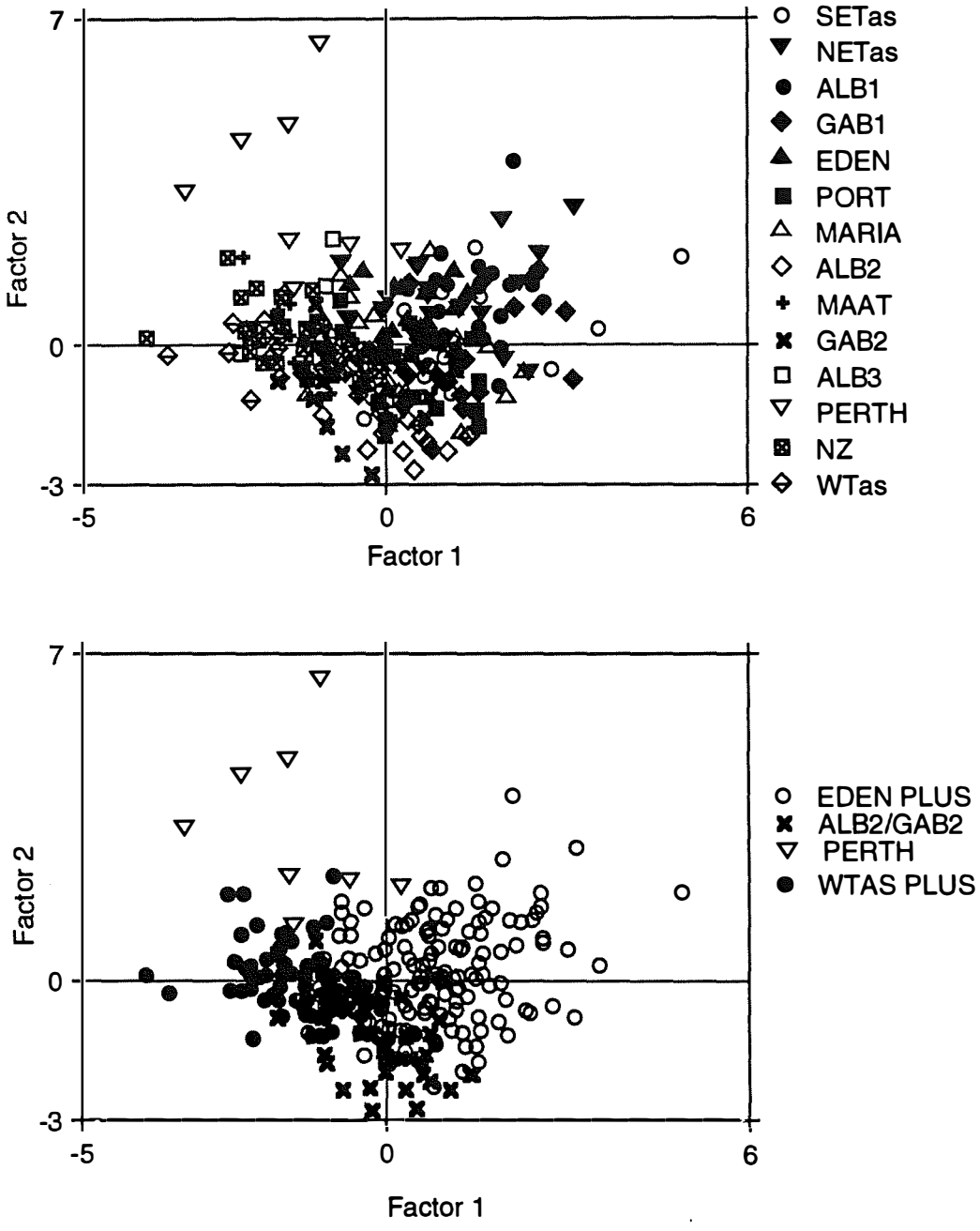


Figure 4.1.7. 4-group end-point of a step-down LDFA of the 14 sites sampled for Jackass Morwong. a. All sites. B. Sites labled by group.

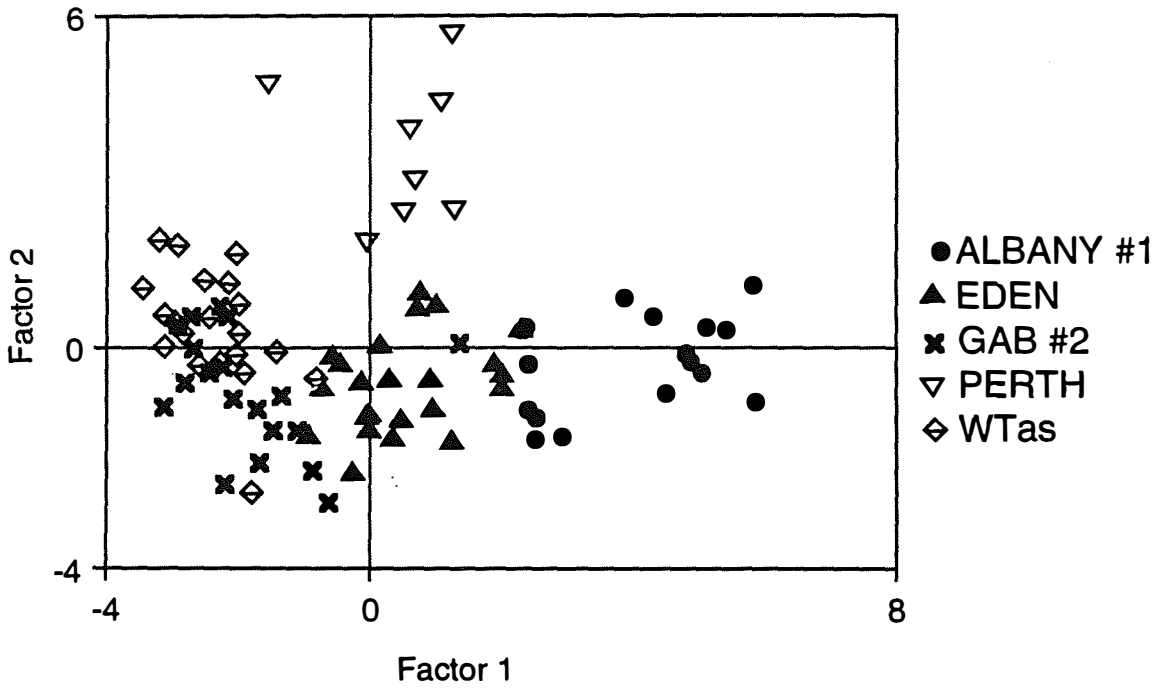


Figure 4.1.8. The distribution of five putative 'pure' source populations in LDFA two-factor space

Figure 4.1.9. The afilliations of specimens collected at fourteen sites across Southern Australia and NZ, as determined by comparisons against five putative 'pure' source populations (Perth, Eden, Western Tasmania, Albany #1 and GAB #2). Only individuals which classified with a putative source group at P>0.9 are included.

Tasmanian' fish dominate off SW Tasmania (and New Zealand); 'GAB #2' fish are common in the Bight and, to a lesser extent, east and west of the Bight; 'Albany #1' fish dominate off Albany (and off eastern Tasmania, as noted above); and 'Perth' fish are found predominantly off the western Australian coast. And fourth, 'replicate' samples from approximately the same location, but separated in time, consist of fish with wholly different affiliations.

The last point could be the result of sampling errors, artefacts in specimen preparation, or changes in population structure due to seasonal (for example) movements. The possibility of seasonal migrations was not specifically tested for in this study, but it can be approached by examining composition over the period for which samples were collected, in the case of Albany, over a five-month period. The results of the analysis for three sites are shown in Figure 4.1.10. For the purpose of the comparison, we assume that the Tasmanian samples are drawn from approximately the same area, though there is a clear confounding of spatial and temporal effects. Comparisons involving samples from Albany and the GAB are more direct, since samples were in all cases drawn from approximately the same location at each.

Although sample sizes are small and cover only part of a year, the Tasmanian, Albany and GAB samples are suggest a seasonal change in otolith composition, from fish with relatively high chlorine values in late winter/spring to fish with relatively low chlorine concentrations in summer/early autumn.

Evaluation of the Effect of Different Elements on Stock Delineation

Of the seventeen elements, nine differed significantly among samples (Figure 4.1.4). The nine included all five of the micro-constituents (but not Ca), as well as four of the eleven trace elements. In general, overlap among sites was extensive for all elements. Differences among sites were largest for Cl and Br (which varied among sites in parallel); several sites (such as GAB #2, western Tasmania and NZ) had mean Cl concentrations below the MDL, whereas others (Perth and Albany #1) had no individual fish with Cl levels that low. Although there in no indication of an east-west cline for any element, there is a general tendency (with the noteworthy exception of Albany #3) for Sr concentrations to peak around the center of the sampled distributions, with relatively low values at both the western and eastern extremes of the sampled geographic range. As noted above, the elements also showed a range of correlated relationships, the strongest of which were between Cl and Br ($r = 0.62$, $p \ll 0.001$) and among Na, K and Cl ($r = 0.44$ to 0.47, all at $p \ll 0.001$).

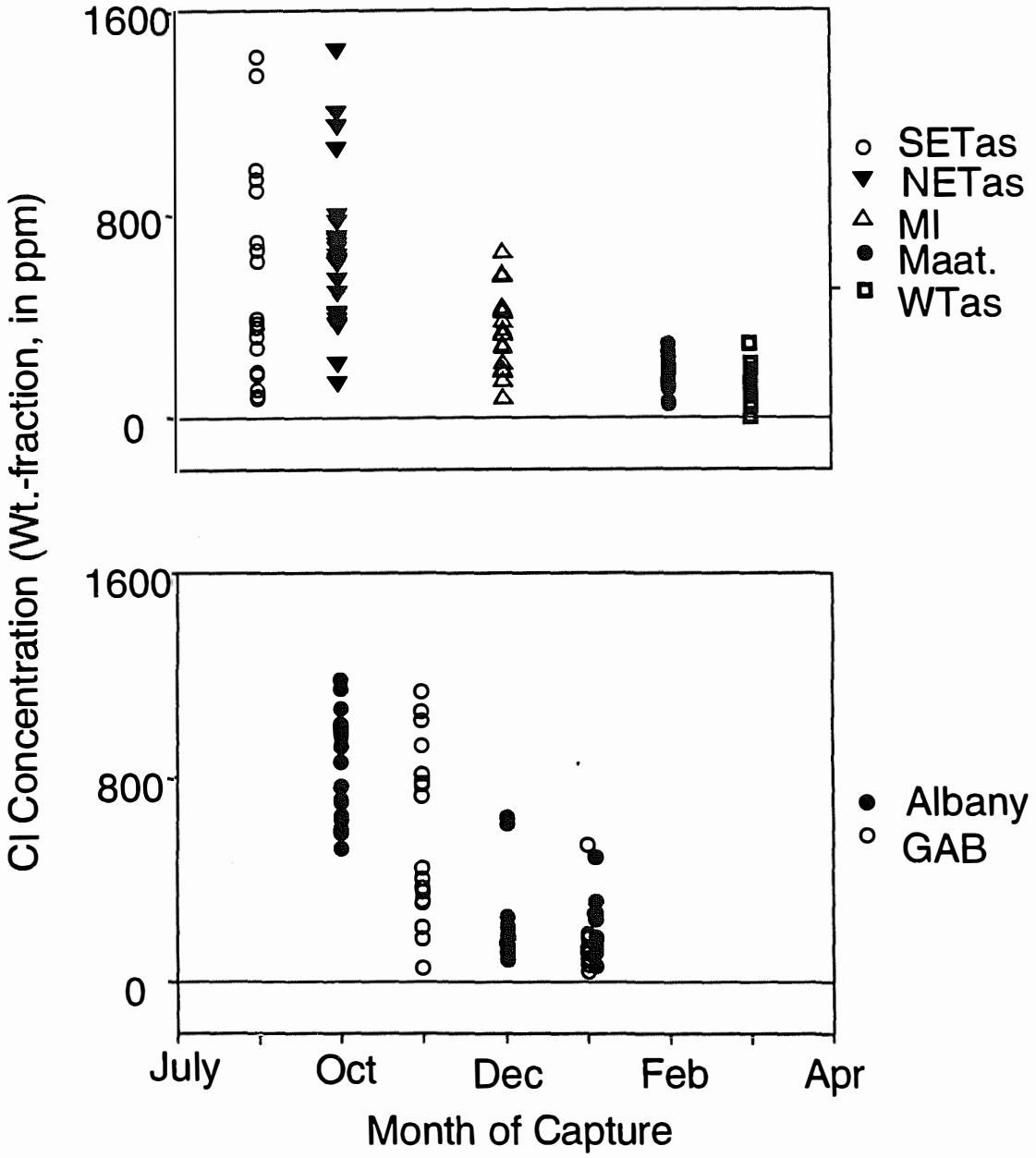


Figure 4.1.10. Relationship between month of capture and Cl concentrations in the otoliths of specimens caught off Albany, the GAB and Tasmania. In both scattergrams, the correlation between capture date and Cl concentration is significant at $p < 0.01$.

To evaluate the contribution of the various elements to the discrimination among apparent stocks, a step-down procedure was applied to the seventeen elements at the four-group end-point of the LDFA (e.g., Figure 4.1.7). Elements were sequentially removed from the analysis, in the order of lowest to highest F-value, and the impact of the removal on the discrimination assessed by examining Wilk's lambda, a measure of group separation. Of the seventeen elements, eleven could be removed with only a slight affect on lambda (Figure 4.1.11). However, lambda abruptly increased (i.e., the discrimination abruptly worsened) when K, Pb, Sr or Cl are removed from the analysis (Figure 4.1.11), indicating that all four contributed significantly to group identification. Of the four, the role of Pb is perhaps questionable. Although contributing significantly in delineating Perth from all other sites, the mean value of Pb concentrations measured was well below an admittedly conservative Minimum Detection Limit (set at 3 sigma above the mean level of the background signal). The mean concentration at Perth was only just above the MDL (3.86 ppm versus an MDL of 3.6 ppm), which suggest caution in its involvement in the analysis, particularly given the small sample size for Perth (8 specimens).

Discussion

The results of the current study, in general, are consistent with those of the pilot study, reported in FIRTA Final Report Number 1987/15. Figure 4.1.12 depicts the end-points of the linear discriminant analysis for the two studies, plotted on the equivalent factor scores (the Sr-dominated factor score versus the Cl-dominated factor score). The pilot study, using sagittae and individuals from six sites in SE Australia and the GAB, found evidence of a clear separation between the NSW/Victorian sites and those from Tasmania, and an indication of a weaker separation between Tasmanian and GAB-derived fishes. The principal element involved in this stock separation was Cl, which was consistently much higher for SE mainland fishes than those from either Tasmania or the GAB. Results of the current study, overall, parallel these results closely, though obviously with much larger sample sizes and more extensive overlap among sites. Nonetheless, the principal separation among sites still distinguishes between a SE mainland set of samples, characterized by relatively high Cl concentrations, and a SW Tasmanian sample, characterized by very low CL concentrations. As in the pilot study, there is also an indication of a weak separation between a GAB population (in the current study, the combination of GAB #2 and Albany #2) and the Tasmanian fishes.

The results of the current study differ from those of the pilot study in a number of key outcomes, however, at least some of which are likely to be due to the much wider range of sites sampled. The principal differences are 1) identification of a distinct fourth phenotype, off Western Australia, 2) a much broader overlap among sites in terms of the apparent source

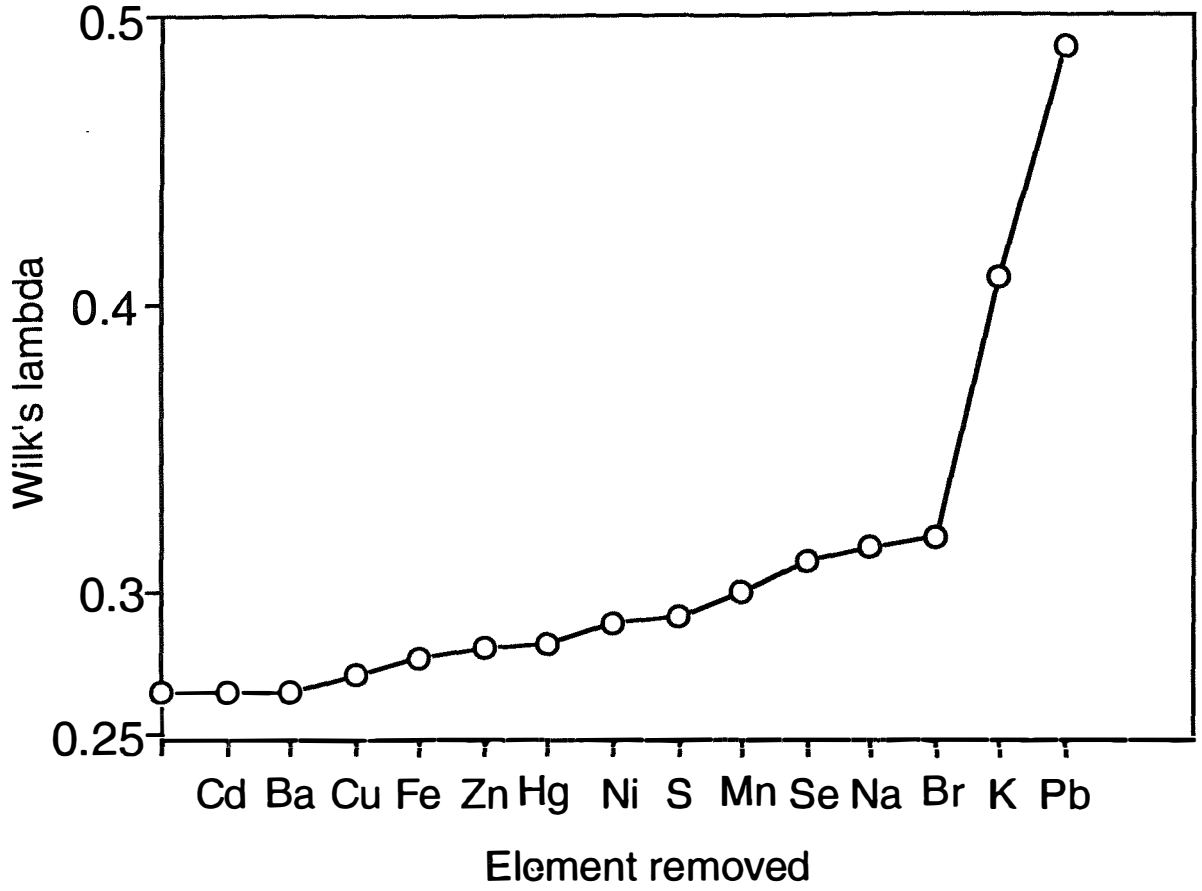


Figure 4.1.11. The effect of sequentially removing elements from the LDFA, in the order of the lowest to highest significant difference among sites. Wilk's lambda is a measure of the separation among populations, increasing as the separation among stocks worsens.

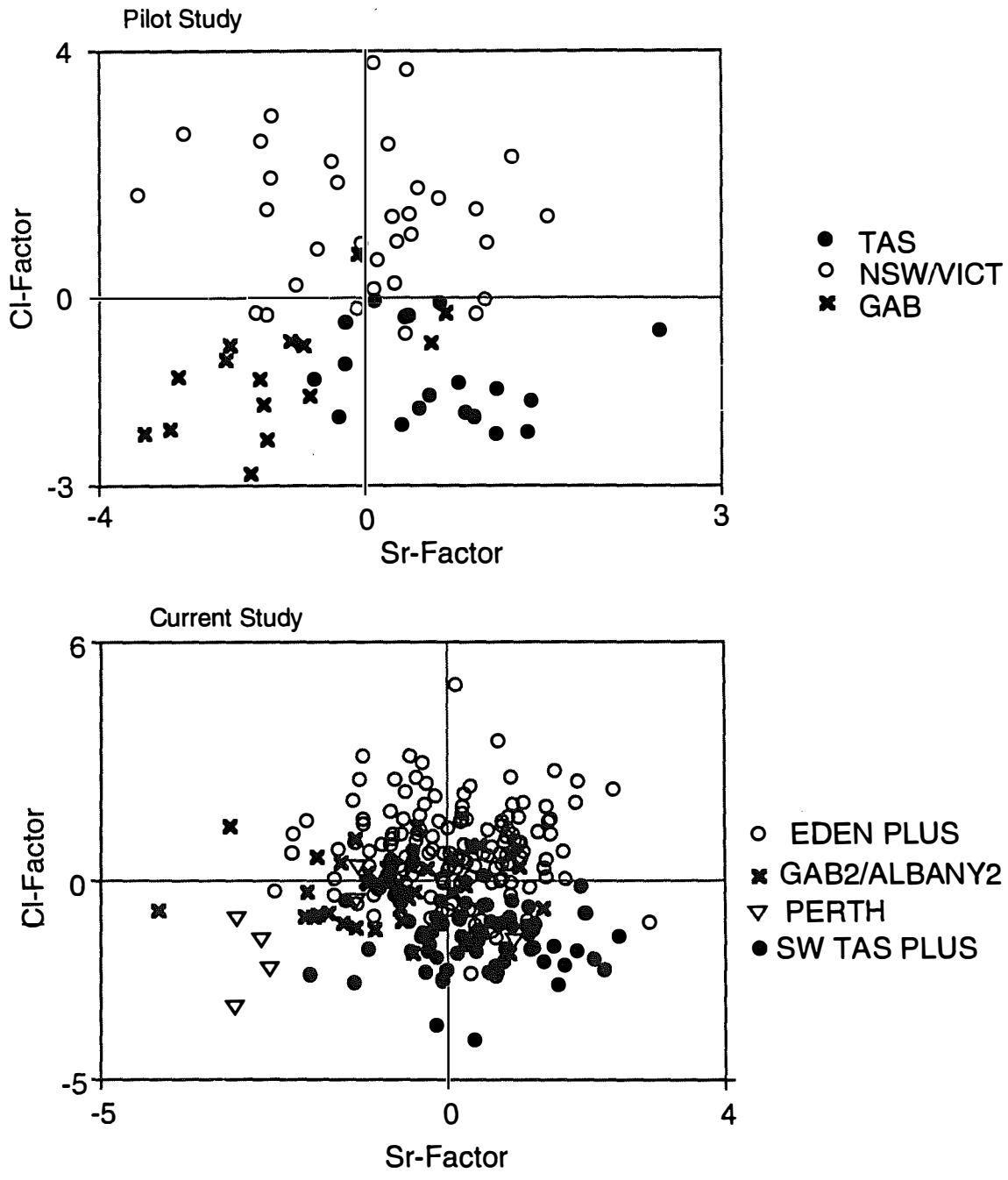


Figure 4.1.12. Comparsion of end-points for LDFA for pilot study of SE Australian Jackass Morwong stock structure, based on analysis of sagittae, with results of the current study, based on analysis of lapillae. For basis of comparison, both results are plotted against equivalent factors for the two studies.

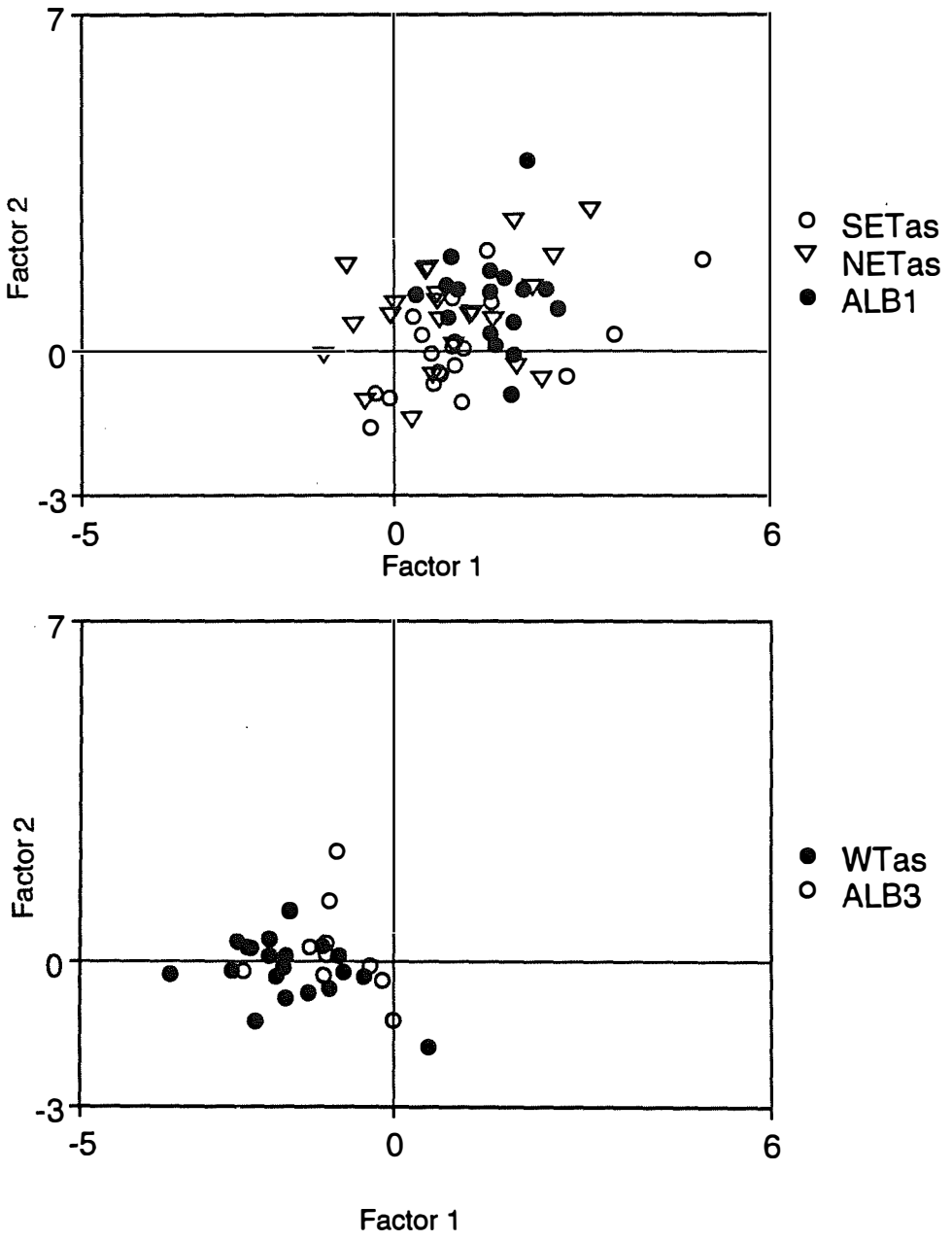


Figure 4.1.13. Distribution in two-factor space, as defined by the end-point four-group LDFA, of specimens from disjunct sites that pooled together.

affiliations of the fish, and 3) a shift in the affiliations of the eastern Tasmanian samples, from Tasmanian, in the pilot study, to 'Eden plus', in the current study.

The distinctiveness of the Perth sample, although based on a very small sample size (8 specimens) is evident in a number of elements: higher than average Na, K and Pb concentrations, and lower than average Se concentrations. Among other samples, the closest affiliation with Perth appears to be Albany #1, which uniquely shares with the Perth sample both high Na and high Cl concentrations (Figure 4.1.3). Statistically, however, Albany #1 and Perth are discernible at the $P<0.01$ level, on the basis of, principally, the differences between them in mean K and Pb concentrations (note that by comparison, Perth differs from all other sites at $P<0.001$). The reliability of these differences is not entirely clear, however, and we suspect that the Perth and Albany #1 samples may both represent a previously unidentified west coast population of jackass morwong. The difficulty of relying on Pb and K as discriminators are two. First, as noted above, the Pb levels are overall extremely low; the main difference between Perth and Albany #1 (and other sites) is that Perth is the only site at which mean Pb concentrations were not below the detection limit for the element. Even so, Pb concentrations in Perth fish were only just above the MDL. The second problem concerns K. In general, concentrations of K correlate highly with those of Na, both among individuals within sites and among sites. On that basis, we would expect that Perth and Albany #1, both with high Na levels, would also both have equivalently high K levels. That this is not the case could reflect a real difference in Na and K metabolism between the two samples, or could be an artefact of the sampling. In that regard, we again note the very small sample size from Perth, and feel that drawing a firm conclusion that the Perth and Albany #1 fish differ is premature until we can increase sample sizes and draw a statistically robust comparison between the two samples. Nonetheless, the point is a relatively slight one. The principal conclusion is still that there appears to be a distinctly different stock of jackass morwong off the west coast; the question remaining is whether that stock extends around to Albany (perhaps seasonally, see below) or is confined to the west coast.

This question also bears on the pattern of apparent overlap among sites, which is much broader than suggested by the pilot study. In the earlier study, not only was the chemical signature of the apparent stocks much more pronounced than in the current study, but also the apparent mixing of phenotypes much less, resulting in separations among sites that were much more clear-cut. That there would be some mixing of individuals is biologically inevitable, given a prolonged planktonic larval stage (6-9 months) and mobile adults. That 'Eden plus' fish are identified from NSW and Victoria, and also in the GAB and as far west as Albany and as far south as SE Tasmania, is perhaps a reasonable expectation given the continuous nature of the continental shelf linking these areas. What is more difficult to understand, however, is evidence of apparently discontinuous distributions of

various phenotypes. As indicated in Figure 4.1.9, eastern Tasmania and Albany #1 share a common diagnostic signature, with very few fish of this type occurring in the GAB or off western Tasmania, and western Tasmanian phenotypes occur both there and, in a single sample, off Albany (Albany #3), where they constitute more than half the sample.

There are three ways these data can be interpreted. First, the apparent overlap is an artefact of the statistical procedures used to classify individuals. Second, fish derived from a number of sites across southern Australia could have virtually identical chemical signatures, despite different source populations. Hence, the apparent equivalence of the NE Tasmanian and Albany #1 samples could indicate two separate spawning populations, which are coincidentally similar in chemical signatures. Third, there is large scale movement/exchange at the larval stage among widely separated sites.

The first possibility — a statistical artefact— appears to explain the irregular collection of a few 'Perth' fish off Portland and Maatsuyker. As indicated in Figure 4.1.5, there is virtually no overlap in factor space between Maatsuyker and Perth, and very little between Portland and Perth. The identification of individuals from the two eastern sites as 'Perth' fish is likely to be due to the linear component of the discriminant analysis, which draws straight lines in factor space among defined populations, in order to classify individuals. Given the relative distinctness of the samples involved, we strongly suspect that a more sophisticated statistical analysis (basically a non-linear discriminant analysis) would quickly resolve this apparent disjunction in the Perth population.

Statistical problems do not so easily account for other disjunctions, however. In that regard, some evidence supports the possibility of 'coincidentally' identical chemical signatures for fish from different populations. Comparison of the Albany #1 and, combined, the SE and NE Tasmanian samples (Figure 4.1.13) suggests that the two sets of samples, in fact, are not distributed identically in factor space, despite classifying in the same group. By comparison with the tightly clustered Albany #1 sample, both of the Tasmanian samples are much more heterogeneous, a point also evident in scatterplots of the raw element data; Na/Cl ratios in the NE Tasmanian sample, in particular, range much more widely than those from any other sample. One interpretation of Figure 4.1.13 is that SE and NE Tasmania represent samples of unknown origin, which are distributed in factor space in part overlapping the Albany sample and identified as such in the absence of any other possibility. If this is the case, where these highly variable Na/Cl fish originate is not clear, though high Cl levels, at least, also characterize fish from NSW/Victoria.

Much the same can be said for the western Tasmanian versus Albany #3 comparison. The samples certainly overlap in factor space (Figure 4.1.13, but are not identical. Whether

the difference is a sampling artefact, or represents subtle differences due to different, but unidentified source populations is very unclear.

The possibility of extensive migration also cannot be ruled out as a source of the mixed affiliations. An explicit assumption underlying this study from its onset was that jackass morwong, as adults, were relatively sedentary. The principal evidence to support this assumption was a tagging study carried out off NSW by Smith (1989), who found evidence of localized movement only near the Eden tagging site over a four year period. These results were basically similar to those of a previous tagging study off Victoria, by Wankowski & Hobday (1984). Two observations suggest that this assumption of a relatively sedentary population may be incorrect. First, tagging studies in New Zealand indicate at least some long-distance movement by adult jackass morwong; Annala (1993) reports that most individuals tagged off Kaikoura (South Island) were re-captured near the release point, but also that 40% of recaptures were caught as far away as several hundred kilometres from that point. Second, industry sources tell us that they can reliably follow seasonal movements of schools of adult jackass morwong down the shelf from NSW to NE Tasmania, and in fact express considerable scepticism when presented with the idea that the stocks are sedentary.

Our data are fully consistent with the possibility of seasonally migratory stocks. In particular we note that the fish caught off the east coast of Tasmania classify very differently between the pilot study, carried out in 1987, and the current study, carried out in 1991. In the initial study, the sample off Maria Island classified with the west coast Tasmanian material, and was clearly distinct from the high Cl-NSW/Victorian specimens; the sample in question was taken in late spring (November). In the later study, the samples classified primarily with the Eden plus group, with typically high Cl levels; these samples were taken in winter and spring (July through November). One interpretation of the data was that in the later study, fish previously identified from only the NSW/Victorian region dominated the east coast of Tasmania, and the fish previously evident on both the east and west coast of Tasmania were now found only on the west and southwest coast, i.e., there had been a seasonal shift in the distributions of the two populations.

Such a seasonal shift is suggested for both Tasmanian and GAB/Albany samples. As noted earlier, comparisons of replicate samples from the same sites (Albany and the GAB) proved unambiguously that the samples were not identical. In the most extreme example, the three samples from Albany showed almost no overlap in terms of their putative source populations: Albany #1 consisted wholly of Albany/Perth fish; Albany #2 consisted of GAB and Eden-type fish; and Albany #3 consisted primarily of fish with a western Tasmanian signature. Again, there are several possibilities to account for this variability: sampling errors, artefacts in specimen preparation or analysis, and seasonal migration and change of

populations are three most likely prospects. We cannot at this stage rule out a sampling problem, that is, that three independent samples taken within a few days of each other might also have revealed this level of variability, and that the differences among samples perhaps reflect some schooling behaviour of fish derived from the same stock. We think it less likely that the results are due to an analytical artefact, given that all the samples were taken by the same vessel, fishing with the same gear, preserved in much the same way each time, and prepared and analysed in random order. The possibility of a seasonal change in population structure, while difficult to prove directly, is fully consistent with the apparent change in the characteristics of the fishes over the period we did sample. At both Albany and in the GAB, we see evidence of high Cl fish present in late winter/early spring, and low Cl fish over the summer, a pattern very similar to that suggested by the samples off Tasmania. Presumably the common direction of this Cl-shift is coincidental, in that if the migration hypothesis is correct there must also be sites where high Cl-fish migrate into previously unoccupied areas or replace low Cl-specimens. Irrespective of their affiliations, the samples collected at each of these sites have clearly different chemical signatures, depending upon when they were caught.

We conclude, therefore, that our assumption of relatively sedentary adult populations is not likely to be valid for much of the Australian coastline, and hence that our current analysis confounds location and season in trying to sort out and identify the stock structure of jackass morwong. To determine accurately the stock structure of the species will require at least summer and winter sampling at each location. The basic picture emerging, however, is that there appear to be relatively discrete populations of NSW/Victoria, off southern and western Tasmania, perhaps in the GAB and off western Australia. These populations appear to be mobile, however, with suggestions of northward movement of low Cl populations north along the coast and into the GAB during late spring and early summer.

4.2 Stock structure as indicated by analysis of allozyme distributions

Materials and Methods

Samples

Between January 1991 and April 1992, twelve samples of Nemadactylus macropterus were obtained from eight different areas around southern Australia and two samples from one area off the west coast of the South Island of New Zealand (Table 4.2.1, Figure 4.2.1). All samples consisted of whole fish that were frozen after capture and transported frozen to the laboratory. Each frozen fish was dissected for samples of muscle, liver and eye tissue for the genetic studies, and otoliths were removed for micro-chemistry analysis. All fish were measured (standard length), sexed (except the Maria Island sample), and meristic counts taken of the dorsal, anal and pectoral fins. Tissue samples were stored in sealed polyethylene bags at -70°C until electrophoretic analysis.

Electrophoresis

Small pieces of liver, muscle or eye tissue were placed in 1.5 ml microcentrifuge tubes, homogenized manually with a few drops of distilled water (no water added to eye homogenates), and spun at 10 000 rpm in a micro-centrifuge for 2 minutes. The supernatant was used for electrophoresis, which was carried out using two gel systems. Gel system A used Helena Titan III cellulose acetate plates run with a tris-glycine buffer system (0.02M tris, 0.192M glycine; see Hebert and Beaton 1989 for details), and gel system B used 12% Sigma starch gels and a histidine/citrate buffer system (gel buffer: 0.005M histidine HCL, adjusted to pH 7.0 with 0.1M sodium hydroxide; electrode buffer: 0.41M trisodium citrate, adjusted to pH 7.0 with 0.5M citric acid).

Loci and alleles were designated by the nomenclature system outlined by Shaklee et al. (1990), except peptidase loci were identified by position of migrating system (PEP-1*, PEP-2* and PEP-3*). Multiple loci encoding the same enzyme were designated by consecutive numbers, with"1" denoting the fastest migrating system. Alleles within each locus were numbered according to the anodal mobility of their product relative to that of the most common allele which was designated as "100" (cathodal migration was designated "-"). The cellulose acetate mobilities were used in all cases except ACP*, ESTD*, IDH-2*, sMDH-1*, sMDH-2*, ME-1* and PGDH*, where starch mobilities were used.

Every specimen was screened for the loci with the most common allele having a frequency of less than 0.90, and a minimum of 20 fish from each of the nine areas screened

Figure 4.2.1. Map of locations. WA= West Australia, SA=South Australia, Maats=Maatsuyker, NZ= New Zealand.

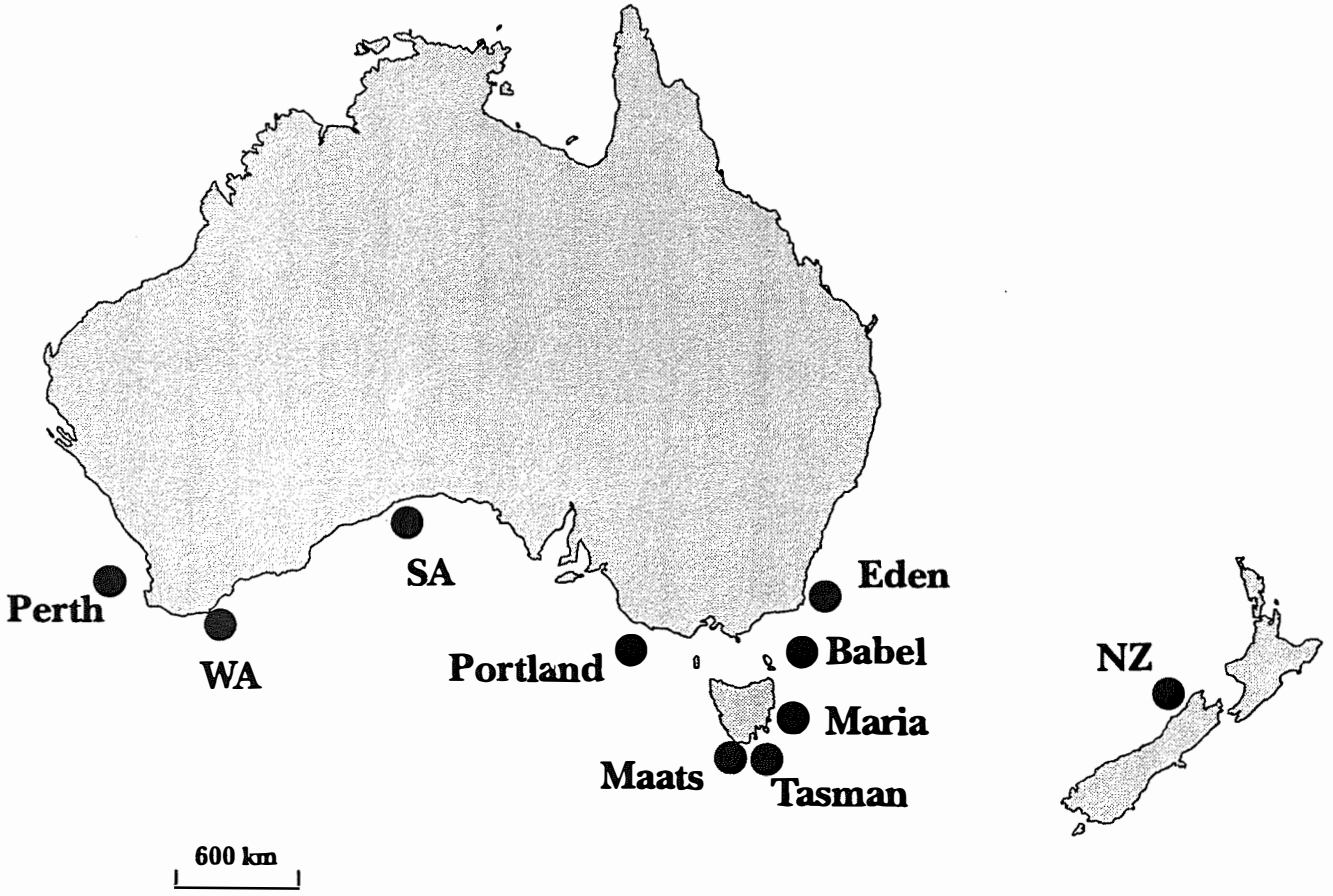


Table 4.2.1. Collection details of samples of *Nemadactylus macropterus*, including numbers of fish.

Sample	No. Fish	Collection Date	Location Details
Perth	9	February 1991	33° 24'S 114° 31'E
West Australia #1	20	October 1991	South-east of Albany, W.A
West Australia #2	32	December 1991	South-east of Albany, W.A
West Australia #3	40	February 1992	South-east of Albany, W.A
South Australia #1	71	April 1991	GAB 129°E
South Australia #2	49	January 1992	33° 19'S 127° 35'E
Portland	106	September 1991	38° 42'S 141° 23'E
Maatsuyker	25	February 1992	43° 52'S 146° 24'E
Tasman Island	138	September 1991	43° 39'S 147 45'E
Maria Island	92	December 1991	42° 43'S 148° 20'E
Babel Island	97	October 1991	East of Babel Isl - 40°S
Eden	108	October 1991	37° 20'S 150° 11'E
New Zealand #1	23	April 1992	42° 32'S 170° 43'E
New Zealand #2	76	April 1992	43° 08'S 169° 47'E

for less variable loci. On a few occasions, notably the (invariant) eye-specific CK-1* locus, sample sizes were smaller than 20 owing to enzyme degradation.

Analyses

Calculations of gene frequencies, conformance to Hardy-Weinberg equilibrium, and cluster analysis of the data were performed using BIOSYS-1 (Swofford and Selander 1981, using version 1.7). Deviations from expected Hardy-Weinberg genotype proportions were tested using chi-squared tests for goodness-of-fit, with pooling of rare alleles so that expected numbers of genotypes in a given class were always greater than one. Pooling was required for all eight polymorphic loci; six were subsequently tested as di-allelic polymorphisms (sAAT-1*, ADH*, GPI-1*, IDDH*, PGM-1*, and PGM-2*) and two (ADA* and PGDH*) as tri-allelic polymorphisms in some samples and di-allelic in others.

Nei's (1973) gene diversity (GST) statistics were used to apportion variation among areas. GST is equal to (HT-HS)/HT, where HT (total genetic diversity) is the average of the Hardy-Weinberg expected heterozygosity in the total population and HS (mean genetic diversity per area) is the average Hardy-Weinberg expected heterozygosity within areas. Across all loci, GST was estimated from the mean of the HT and HS values. The GST value represents the proportion of genetic diversity that can be attributed to differences between areas. A bootstrapping procedure (Elliott and Ward 1992) was used to estimate the magnitude of GST that could be attributed to sampling error alone. This quantity is termed GST-null and a mean value of GST-null was estimated for each locus from 1000 replications. The number of times each of the 1000 estimates of GST-null was equal to or greater than the observed GST was determined, and this divided by 2000 gave the probability of obtaining the observed GST by chance.

Heterogeneity among areas was tested using chi-square contingency tests of allele numbers. Two techniques were used, a standard procedure necessitating pooling of rare alleles as implemented in BIOSYS-1 (Swofford and Selander 1981), and a Monte Carlo procedure (Roff and Bentzen 1989) which obviated the need to pool rare alleles. This second technique involved testing the significance of the observed value (c2o) at each locus based on 1000 randomizations of the data. The number of times each of the randomized replicates (chi-square) was greater than or equal to the observed value, divided by 1000, was a measure of the probability of obtaining the observed value by chance; again a significance level of 0.05 was used to test the hypothesis of no geographic variation. Since chi-square tests are not completely additive, a procedure outlined by Sokal and Rohlf (1981) was used to test the significance across all eight loci. Here, chi-square = $-2 \sum \ln P$, where P is the probability obtained from the chi-square test at an individual locus. The across loci chi-square is distributed with 2k degrees of freedom (k = number of separate tests).

When multiple tests were carried out, the standard Bonferroni technique was used to adjust significance levels. The predetermined significance level of 0.05 was divided by the number of tests to obtain a corrected significance level.

An estimate of the number of migrants per generation (Nem; where Ne is the effective population size and m is the rate of gene flow per generation) was calculated from Wright's (1943) equation:

Nem = 0.25 (1/FST - 1),

by setting FST equal to the relative gene diversity (GST). The relationship between Nem and FST is approximately true if m<<1. Mutation is ignored, the populations are assumed to be at demographic equilibrium, and the various genotypes are assumed to be selectively neutral. Under these circumstances, population differentiation is solely a consequence of genetic drift and migration.

Cluster analysis used the UPGMA (unweighted pair-group method with averaging) algorithm with Nei's (1978) unbiased genetic distance measure.

Results

Enzyme loci

Twenty-two enzymes representing thirty-seven gene loci were initially examined, and of these, twenty-one enzymes and thirty-three loci showed sufficient activity and definition to be reliably scored (Table 4.2.2). The four unscored and poorly resolved loci were the liver loci IDH-1*, G3PDH-1*, G6PDH* (glucose-6-phosphate dehydrogenase; E.C. No. 1.1.1.49) and EST* (esterase, using a-naphthyl acetate as substrate).

The frequency of the most common allele was less than 0.90 for eight loci: sAAT-1*, ADA*, ADH*, GPI-1*, IDDH*, PGDH*, PGM-1* and PGM-2*(Table 4.2.3). Every specimen was screened for these eight loci, and these results were primarily used in tests of genetic differentiation among areas. Fourteen loci showed more limited variation (mAAT*, sAAT-2*, ACP*, ESTD*, GPI-2*, G3PDH-2*, IDH-2*, sMDH-1*, sMDH-2*, ME-1*, MPI*, PEP-1*, PEP-2* and PEP-3*). Eleven loci were monomorphic within all areas examined (AK*, CK-1*, CK-2*, FH*, GAPDH-1*, GAPDH-2*, LDH-1*, LDH-2*, LDH-3*, mMDH*, and sSOD*). All heterozygote banding patterns showed the appropriate banding patterns based on known subunit numbers (e.g. Ward et al. 1992).

A ratio of 1:1.15 between males and females was observed from the 783 individuals that were sexed. There was no evidence (contingency tests) of any variation in genotype

frequencies between the sexes at any of the eight polymorphic loci, therefore sexes were combined in the following analyses.

The three West Australia samples came from the same area, although temporally spaced. No significant difference at the eight polymorphic loci were observed between these samples, and therefore they were pooled in subsequent analyses. Similarly the two South Australia samples were pooled, as were the two New Zealand samples. The Perth sample was small (only nine fish) and was not included in the following analyses. However, heterogeneity chi-squared tests of allele frequencies revealed no significant differentiation between this sample and the West Australia sample off Albany. Following pooling, and elimination of the Perth sample, fish from nine areas were analysed (Table 4.2.3).

Analysis of the Nine Areas

The average heterozygosity (HT) for the 33 loci was 10.1% ranging from 0% to 62.3% per locus; the most heterozygous locus was ADA*. Heterozygosity per area ranged between 8.4% for the relatively small sample from Maatsuyker to 11.1% for the Tasman Island sample. Polymorphism per sample ranged from 24.2% to 27.3% (mean 25.23%) using the $P_{0.95}$ criterion, and from 27.3% to 48.5% (mean 36.7%) using the $P_{0.99}$ criterion.

Tests of conformance to Hardy-Weinberg genotypic proportions within areas indicated disequilibria ($P < 0.05$) in 5 out of 66 valid (expected frequencies ≥ 1.0 per cell) tests. Four of these had probabilities exceeding 0.04 and the fifth had a probability of 0.013. All these probabilities were considerably greater than the Bonferroni adjusted significance level of 0.0008 (0.05/66).

Heterogeneity chi-squared tests on allele frequency variation at the eight polymorphic loci revealed some genetic stock structure amongst the nine areas sampled (Table 4.2.4). There was significant ($P=0.003$) differentiation across all eight loci, which was attributable predominantly to variation at the sAAT-1* locus. Heterogeneity among the areas at this locus was highly significant ($P< 0.001$ with pooled rare alleles, $p=0.003$ without pooling).

The suggestion of differentiation at the ADH*, IDDH* and PGM-1* loci following analysis without pooling of rare alleles (although not significant following Bonferroni adjustment) was not supported by the pooled allele analyses. The differences in probabilities between the two procedures reflect the sensitivity of the Monte Carlo technique of Roff and Bentzen (1989) to the presence of rare alleles.

Differentiation among the nine areas sampled was supported by the gene diversity analyses. As Table 4.2.4 shows, there is again significant differentiation at the sAAT-1* locus. GST at this locus was equal to 0.0214, which was greater than the GST-null value in 997 out of 1000 replicates (i.e. probability of achieving such a GST value by chance alone was P= 0.003). However, the GST across all eight polymorphic loci was equal to 0.0064, indicating that 0.64% of the total genetic variation was attributable to differentiation between samples. Even this low figure is an overestimate, as the mean GST-null (0.0058) was not significantly (P = 0.269) less than 0.0064, and thus the value of 0.0064 could be attributable to sampling error alone.

In order to locate the geographic source of the genetic differentiation revealed by the contingency chi-square tests, further chi-squared tests were carried out by comparing each area in turn with the remaining eight areas combined into one pooled sample. Each of these area tests yielded a single significance value, as for each area test the probabilities from the eight independent locus tests were combined into a single figure (Sokal and Rohlf, 1981). Significant differentiation was only observed when the New Zealand sample was compared to the remaining eight areas (P<0.001, considerably less than the Bonferroni adjusted significance level of 0.05/9=0.0056). Other comparisons were: West Australia, P=0.483; South Australia, 0.964; Portland, 0.020; Maatsuyker, 0.718; Tasman Island, 0.502; Maria Island, 0.761; Babel Island, 0.438; Eden 0.178.

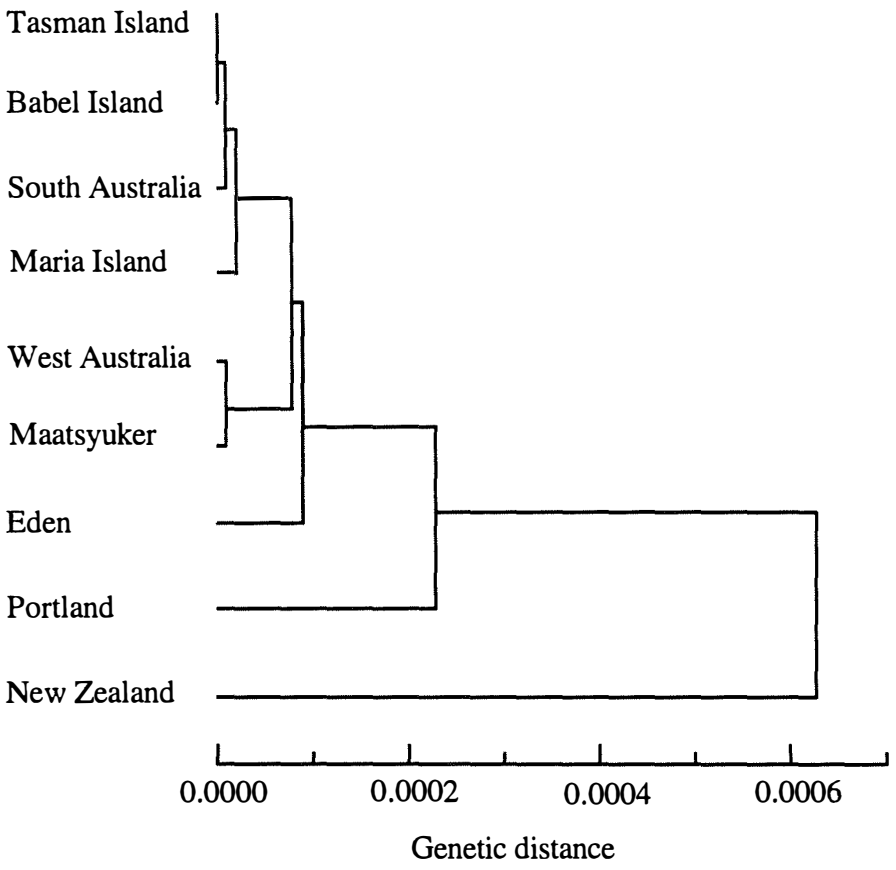
Likewise, chi-squared tests (Roff and Bentzen 1989) on allele numbers at the sAAT-1* locus between pairs of areas revealed only seven cases of highly significant (P < 0.001) variation out of the total 36 tests (Table 4.2.5). All seven cases involved the New Zealand sample. The only sample not significantly differentiated from the New Zealand sample was Maatsuyker, which was a relatively small sample of 25 fish and which showed no differentiation from any other sample.

A dendogram derived from cluster analysis of pairwise genetic distance indices (Nei 1978) among the nine areas over all 33 loci is given in Figure 4.2.2. Whilst these distances are small and little significance can be placed on the branch order, the phenogram is consistent with the identification of the New Zealand morwong as a distinct assemblage.

Analysis of Eight Australian Areas

With the removal of the New Zealand sample from the analyses there was no differentiation found among the eight Australian areas following gene diversity analysis (GST) (Table 4.2.4.II). GST values were not significantly greater than the GST-null at any of the eight loci. Considering all eight polymorphic loci, GST for the eight Australian areas was

Figure 4.2.2. *Nemadactylus macropterus*. Dendogram based on UPGMA clustering of unbiased genetic distance indices (Nei 1978) utilizing all 33 loci examined at the nine areas sampled.



0.0052 which was not significantly different to the mean GST-null value of 0.0059. The low observed GST value could be attributed solely to sampling error.

The low degree of differentiation among the Australian areas was confirmed by the chi-squared analyses at each of the eight polymorphic loci (Table 4.2.4.II). However the overall significance test across all eight loci without pooling of rare alleles suggested some differentiation (P = 0.033), but this was not supported when rare alleles were pooled. Again, this result may be a consequence of the sensitivity of the Monte Carlo technique. The sAAT-1* and ADH* loci contribute most to the significant value yet pairwise comparisons failed to reveal any significant differentiation between pairs of samples.

Pooling the eight Australian areas into a single sample gave no significant deviations to Hardy-Weinberg expectations at seven of the eight loci (Table 4.2.5). The lack of any Wahlund effect for these loci supports the conclusion of little genetic differentiation among samples. The PGM-1* locus was marginally significant following Bonferroni adjustment, and showed a small heterozygote deficiency. For this locus, the probability of equilibrium was 0.005, marginally less than the Bonferroni adjusted significance level of 0.05/8 = 0.006.

New Zealand versus Australia

Evidence of differentiation between the New Zealand sample and the pooled Australian sample over the eight polymorphic loci was shown by both the allele frequency chi-square analyses and the gene diversity analyses (Table 4.2.4.III). Across all eight loci the heterogeneity chi-squared analysis was significant (P = 0.011), a result which was primarily attributable to the sAAT-1* locus (P = 0.001). At this locus, New Zealand fish had a higher frequency (0.939) of the common allele sAAT-1*100 compared to the average (0.792) for fish from the eight Australian areas (Table 4.2.3).

The GST value across all eight polymorphic loci and at the sAAT-1* locus confirm the genetic differentiation between the one New Zealand area sampled and the pooled Australian sample (Table 4.2.4.II). Across all loci, GST was equal to 0.0046 which was significantly greater (P=0.002) than the mean GST-null of 0.0014, and at the sAAT-1* locus the GST of 0.0446 was likewise significantly greater (P<0.001) than the mean GST-null value of 0.0014.

Gene frequencies shown in Table 4.2.3 reveal that the MPI* locus also shows differentiation between New Zealand and Australia (chi-square = 16.408, d.f. = 2, P = 0.0003). This was a consequence of the presence of two rare alleles in the New Zealand fish while all other fish examined were homozygous at this locus.

Level of migration

Whilst an overall GST value of 0.0064 was recorded for the eight polymorphic loci across all nine areas examined, the 'true' estimate of GST among the nine areas is likely to be in the range of 0 to 0.001, as the mean GST-null value was 0.0058 (Table 4.2.4.I). Assuming demographic equilibrium, and using the range of 'true' values in Wright's (1943) equation above, a minimum estimate of the number of migrants per area per generation (Nem) is approximately 250, with a maximum number equivalent to unlimited exchange. Among the eight Australian areas examined there is apparently extensive exchange, as the GST value obtained was not greater than the mean GST-null value.

The 'true' estimate of GST between the Australian and New Zealand samples is likely to be around 0.003 (estimated from GST - GST-null) when considering all eight polymorphic loci. This value equates to an estimate of the number of migrants per generation (Nem) of approximately 80. When all 33 loci are considered, the GST value was 0.0047, which with a mean GST-null of 0.0017 gives the same approximate estimate for Nem of around 80 migrants per generation between Australian and New Zealand populations.

Discussion

Compared to the average total heterozygosity for marine species (around 5%, Ward et al. 1992 and Smith and Fujio 1982), the jackass morwong has a relatively high amount of genetic variation with an average heterozygosity of 10.1% for 33 loci. However, despite this high level of genetic variability and sample sizes averaging around 97 fish, there was no convincing evidence of any genetic structuring in the morwong population within southern Australian waters. Eight polymorphic allozyme loci were screened in 787 morwong from localities ranging from West Australia to New South Wales, a distance exceeding 3 000 km, yet the null hypothesis that these morwong form a single panmictic population cannot be rejected.

This conclusion supports the earlier and somewhat more limited work of Richardson (1982). He examined five polymorphic loci (ADA*, ADH*,GPI-1*, PGDH* and PGM-2*) in between 374 and 510 morwong from the south eastern region of Australia (New South Wales, east and west Victoria, and Tasmania), and found no evidence for population structuring. Despite the use of different electrophoretic techniques, allele frequencies obtained in 1981 for these five loci were very similar to ours (Table 4.2.6), arguing for temporal stability over time and further supporting the lack of spatial genetic differentiation in this region. The only locus showing significant differences in allele frequencies was ADH*. Richardson found, for this locus alone, significant between school (although not between area) variation in allele frequencies and deviations from Hardy-Weinberg

equilibrium (with variable direction) in some schools. This could have resulted either from selection, typing errors or non-Mendelian genetics (Richardson, 1982; Richardson et al., 1986, page 292). Since we did not observe similar deviations from Hardy-Weinberg proportions, some typing errors at this locus in Richardson's study may be the cause of the small differences in allele frequencies between the studies.

The two samples from New Zealand were from adjacent collections off the west coast of the South Island, and showed no significant genetic differentiation for any locus. Gauldie and Johnston (1980) examined 3200 morwong from localities around the coast of New Zealand for variation at a single locus, PGM-2*. Significant differentiation was detected, with the frequency of the common allele ranging from 0.69 to 0.87, with a mean of 0.78. Gauldie and Johnston attribute this variation to selective factors rather than genetically isolated stocks. Their samples taken from the same general area as ours had frequencies of around 0.05, 0.76 and 0.19 for the three major alleles (F, M and S respectively in their notation), frequencies very close to those we found (0.06, 0.78 and 0.16).

While we found no differences among the ten Australian nor among the two New Zealand samples, there were differences between Australian and New Zealand collections. These two sets of samples were differentiated at the polymorphic sAAT-1* locus. There is also some evidence of differentiation at the MPI* locus, where one common and two rare alleles were present in the New Zealand samples, whilst the two rare alleles were not observed in any Australian samples. Richardson (1982) had earlier proposed a marked genetic separation of fish from these two zones. This conclusion was based on the recording by Gauldie and Smith (1978) of ADH* and GPI* as monomorphic in New Zealand fish whilst Richardson found these same loci to be polymorphic in Australian fish (liver GPI* reported in these studies is equivalent to GPI-1* of our study). Gauldie and Johnston (1980) also report GPI* to be monomorphic in samples of 30 fish from two widely separated locations. In this study we found these two loci to be polymorphic and at similar frequencies in both Australian and New Zealand jackass morwong, and the reports of monomorphism of ADH* and GPI* by Gauldie and Smith (1978) and GPI* by Gauldie and Johnston (1980) appear to be erroneous. Thus while our analysis does support the conclusion of Richardson (1982), it does so on completely different evidence. These findings of genetic homogeneity among Australian samples of jackass morwong and differentiation from New Zealand samples are also supported by mitochondrial DNA analysis (Grewe, Smolenski and Ward, unpublished).

Data from New Zealand suggest that the morwong has a 8-10 month off-shore pelagic post-larval stage, followed by metamorphosis to bottom living juveniles and growth in identified nursery areas, distinct from known spawning grounds (Tong and Vooren, 1972;

Vooren, 1972 and 1975). The location of the nursery grounds in Australian waters remains unclear, although there is speculation that at least some of these are in Tasmania waters (Tilzey et al., 1990). Recruitment of juveniles to the adult population has not been recorded, although it appears that the species shows a continuous distribution throughout southern Australian waters. Data on 69 tagged and recaptured morwong off the New South Wales coast showed that the greatest distance moved, after one year, was 74 km (Smith, 1989). Most fish showed little movement, with no net migrations north or south. If this species does have a continuous distribution throughout southern Australia, then even localized adult movement would retard genetic differentiation. However, the major factor promoting genetic homogeneity is likely to be larval drift. The long pelagic post-larval period and the availability of the Leeuwin Current in the west and south and the East Australian Current in the east means that gene flow over long distances in Australian waters could be considerable. The Tasman Sea would appear to be only a partial barrier to gene flow, with the level of differentiation between New Zealand and southern Australia corresponding to a ball-park estimate of about 80 migrants per generation.

Our GST value of 0.0064 (uncorrected for sampling error) for the eight polymorphic loci and the nine areas sampled is an order of magnitude lower than the average (similarly uncorrected) value reported for 57 species of marine teleosts (0.062; Ward et al. 1993). Samples of jackass morwong from different areas are clearly relatively more homogeneous than most other marine fishes thus far studied.

The hypothesis of a single panmictic population of Australian jackass morwong is compatible with the possible scenario of unique nursery grounds, little adult migration and relatively discrete spawning groups. There may be enough gene flow existing between the spawning groups via (most likely) larval drift to prevent the morwong from evolving into genetically distinct stocks. Alternatively, morwong may have begun this evolutionary process in recent geographic time, so that genetic differences have not had time to evolve, or are too small to have been detected by the current genetic studies.

A number of other species have been compared from New Zealand and south east Australian waters. Allozyme data from blue grenadier (*Macruronus novaezelandiae*) parallel the morwong results closely. No significant genetic differences were observed among blue grenadier from three localities off south-eastern Australia, while there were small but significant differences between these fish and ones sampled from the other side of the Tasman Sea (Milton and Shaklee, 1987). The snapper, *Chrysophrys auratus*, also showed allozymic differentiation across the Tasman (MacDonald, 1980). Limited mitochondrial DNA data from the rock lobster *Jasus verreauxi* were also suggestive of trans-Tasman differences,

although more extensive data from the related species Jasus edwardsii failed to reveal such differentiation (Ovenden et al., 1992; Brasher et al., 1992).

Our results have certain implications to the management of the morwong population. They suggest that the demise of a single stock (fished area), be it a spawning stock or not, would not adversely affect the underlying genetic structure of the Australian morwong population. However, such individual stocks may be a consequence of particular spawning grounds, spawning behaviour, appropriate larval conditions, or oceanographic features. Gene flow between areas could be sufficient to maintain genetic homogeneity while being low enough to mean that overfished stocks would take a long time to recover. Therefore until an understanding is available on the actual spawning and nursery grounds, and their relationship to the population size and condition, management policy should be to maintain all existing stocks.

Table 4.2.2. Enzyme systems employed in this study. Tissue: l - liver, m - muscle - e - eye. Gel system: A - cellulose acetate, B - starch (see text).

Enzyme	Locus		EC Number	Tissue	Gel System
Alcohol dehydrogenase	*ADH**	dimer	1.1.1.1	l	A
Glycerol-3-phosphate dehydrogenase	*G3PDH-2**	dimer	1.1.1.8	m	A/B
L-Iditol dehydrogenase	*IDDH**	dimer	1.1.1.14	l	A
Lactate dehydrogenase	*LDH-1**	tetramer	1.1.1.27	e	A/B
	*LDH-2**			m	A/B
	*LDH-3**			m	A
Malate dehydrogenase	*mMDH**	dimer	1.1.1.37	m	A/B
	*sMDH-1**			m	A/B
	*sMDH-2**			m	A/B
Malic enzyme	*ME-1**	tetramer	1.1.1.40	m	A/B
Isocitrate dehydrogenase	*IDH-2**	dimer	1.1.1.42	m	B
Phosphogluconate dehydrogenase	*PGDH**	dimer	1.1.1.44	m	B
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH-1**	tetramer	1.2.1.12	e/m	A/B
	*GAPDH-2**			m	A
Superoxide dismutase	*sSOD**	dimer	1.15.1.1	l	A/B
Aspartate aminotransferase	*mAAT**	dimer	2.6.1.1	l/m	A
	*sAAT-1**			m	A
	*sAAT-2**			l	A
Creatine kinase	*CK-1**	monomer	2.7.3.2	e	B
	*CK-2**			m	A/B
Adenylate kinase	*AK**	monomer	2.7.4.3	m	A/B
Esterase-D (UV, umb acetate)	*ESTD**	dimer	3.1.-.-	m/l	A/B
Acid phosphatase	*ACP**	monomer	3.1.3.2	l	B
Peptidase - L-Leucyl-L-Tyrosine	*PEP-1**	dimer	3.4.-.-	m	A
L-Leucylglycyl-Glycine	*PEP-2**			m	A
L-Leucyl-L-Tyrosine	*PEP-3**			m	A
Adenosine deaminase	*ADA**	monomer	3.5.4.4	l	A
Fumarate hydratase	*FH**	tetramer	4.2.1.2	m	A
Glucose-6-phosphate isomerase	*GPI-1**	dimer	5.3.1.9	m	A
	*GPI-2**			m	A
Mannose-6-phosphate isomerase	*MPI**	monomer	5.3.1.8	l	A
Phosphoglucomutase	*PGM-1**	monomer	5.4.2.2	l	A
	*PGM-2**			l/m	A

Table 4.2.3. *Nemadactylus macropterus*. Allele frequencies at 33 loci in samples from eight Australian and one New Zealand region. (n): number of individuals scored for a given locus; negative mobility of an allele indicates cathodal migration

Locus, allele	West Australia	South Australia	Portland	Maatsu-yker	Tasman Island	Maria Island	Babel Island	Eden	New Zealand
variable loci:									
*mAAT**									
(n)	(92)	(100)	(106)	(25)	(32)	(92)	(73)	(108)	(99)
-50	0.011	0.010	0.009	-	0.016	-	-	-	-
-100	0.984	0.990	0.991	1.000	0.984	1.000	1.000	1.000	1.000
-150	0.005	-	0.005	-	-	-	-	-	-
*sAAT-1**									
(n)	(92)	(120)	(106)	(25)	(138)	(92)	(97)	(108)	(99)
140	-	-	-	-	0.007	-	-	-	0.005
120	0.185	0.179	0.198	0.100	0.225	0.185	0.206	0.222	0.056
100	0.810	0.813	0.764	0.900	0.761	0.810	0.789	0.773	0.939
80	0.005	0.008	0.038	-	0.007	0.005	0.005	0.005	-
*sAAT-2**									
(n)	(6)	(24)	(24)	(24)	(32)	(24)	(73)	(24)	(24)
100	1.000	1.000	1.000	0.979	1.000	1.000	1.000	1.000	1.000
80	-	-	-	0.021	-	-	-	-	-
*ACP**									
(n)	(92)	(27)	(24)	(25)	(23)	(87)	(25)	(24)	(24)
130	0.022	0.007	0.042	0.020	0.022	0.046	0.020	-	0.042
100	0.978	0.993	0.958	0.980	0.978	0.954	0.980	1.000	0.958
*ADA**									
(n)	(92)	(120)	(106)	(25)	(138)	(91)	(97)	(108)	(99)
125	0.011	-	0.005	-	0.004	0.011	-	0.009	0.010
115	0.261	0.329	0.358	0.300	0.348	0.352	0.304	0.296	0.303
105	0.005	-	-	-	-	-	-	-	-
100	0.592	0.517	0.538	0.560	0.533	0.484	0.562	0.532	0.545
95	-	0.004	-	-	-	-	-	-	-
90	0.130	0.138	0.090	0.140	0.116	0.137	0.129	0.153	0.136
75	-	0.008	0.005	-	-	0.016	0.005	0.009	0.005
60	-	0.004	0.005	-	-	-	-	-	-
*ADH**									
(n)	(92)	(120)	(106)	(25)	(138)	(91)	(97)	(108)	(99)
400	-	-	-	-	0.007	-	-	-	-
300	-	-	-	-	0.004	-	-	-	0.005
180	0.022	-	0.005	-	-	-	0.005	0.005	-
40	0.348	0.400	0.363	0.320	0.395	0.335	0.351	0.292	0.419
-60	0.005	-	0.009	-	0.004	-	-	-	-
-100	0.609	0.571	0.623	0.660	0.580	0.626	0.619	0.671	0.561
-200	0.016	0.029	-	0.020	0.007	0.033	0.026	0.019	0.015
-250	-	-	-	-	0.004	0.005	-	0.014	-

Table 4.2.3 cont.

Locus, allele	West Australia	South Australia	Portland	Maatsu-yker	Tasman Island	Maria Island	Babel Island	Eden	New Zealand
*ME1**									
(n)	(20)	(4)	(24)	(24)	(128)	(24)	(25)	(2)	(24)
130	-	0.125	-	0.021	0.016	0.021	0.020	-	-
100	1.000	0.875	1.000	0.979	0.980	0.979	0.980	1.000	1.000
80	-	-	-	-	0.004	-	-	-	-
*MPI**									
(n)	(20)	(24)	(24)	(24)	(32)	(24)	(24)	(24)	(24)
125	-	-	-	-	-	-	-	-	0.021
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.958
90	-	-	-	-	-	-	-	-	0.021
*PEP-1**									
(n)	(20)	(27)	(24)	(24)	(22)	(24)	(25)	(24)	(24)
100	1.000	1.000	0.958	1.000	1.000	1.000	1.000	1.000	0.958
90	-	-	0.042	-	-	-	-	-	0.042
*PEP-2**									
(n)	(20)	(27)	(24)	(24)	(22)	(24)	(25)	(24)	(24)
100	1.000	1.000	1.000	1.000	0.977	1.000	1.000	1.000	1.000
90	-	-	-	-	0.023	-	-	-	-
*PEP-3**									
(n)	(20)	(27)	(24)	(24)	(22)	(24)	(25)	(24)	(24)
190	0.025	-	-	-	0.023	-	0.020	-	-
100	0.975	1.000	1.000	1.000	0.977	1.000	0.980	1.000	1.000
*PGDH**									
(n)	(92)	(120)	(106)	(25)	(138)	(92)	(97)	(108)	(99)
150	0.005	-	-	-	-	-	-	-	-
135	0.016	0.008	0.005	-	0.004	0.022	0.021	0.005	-
125	0.005	-	-	-	-	-	-	-	-
120	0.288	0.300	0.250	0.280	0.301	0.261	0.325	0.273	0.303
110	0.011	0.004	0.014	-	0.014	0.005	-	0.009	0.005
100	0.598	0.592	0.637	0.580	0.605	0.609	0.598	0.644	0.601
90	-	0.017	-	-	0.004	0.005	-	-	0.015
85	0.076	0.079	0.094	0.140	0.072	0.098	0.052	0.069	0.071
70	-	-	-	-	-	-	0.005	-	0.005
*PGM-1**									
(n)	(86)	(120)	(104)	(25)	(138)	(91)	(97)	(108)	(99)
120	0.006	0.004	0.029	-	0.014	0.005	0.021	0.009	-
115	0.023	0.013	0.063	-	0.040	0.038	0.041	0.046	0.068
100	0.773	0.696	0.731	0.740	0.681	0.681	0.696	0.690	0.708
95	-	-	-	-	-	-	-	0.005	-
90	0.198	0.262	0.173	0.240	0.232	0.247	0.237	0.245	0.224
75	-	0.021	0.005	0.020	0.033	0.027	-	0.005	-
65	-	0.004	-	-	-	-	0.005	-	-

Table 4.2.3 cont.

Locus, allele	West Aust	South Aust	Portland	Maatsu-yker	Tasman Island	Maria Island	Babel Island	Eden	New Zealand
*ESTD**									
(n)	(101)	(120)	(106)	(25)	(32)	(92)	(25)	(108)	(99)
110	0.010	-	-	-	0.016	0.005	-	-	-
100	0.960	0.979	0.981	1.000	0.984	0.978	0.940	0.991	0.970
80	0.020	0.013	0.014	-	-	0.011	0.040	0.009	0.020
65	0.010	0.008	0.005	-	-	0.005	0.020	-	0.010
*G3PDH-2**									
(n)	(20)	(27)	(24)	(24)	(32)	(24)	(24)	(24)	(24)
150	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	0.984	1.000	1.000	1.000	1.000
*GPI-1**									
(n)	(87)	(120)	(104)	(25)	(138)	(92)	(95)	(108)	(99)
115	0.086	0.100	0.120	0.080	0.091	0.087	0.089	0.116	0.089
105	0.006	-	0.005	0.020	-	-	-	-	-
100	0.891	0.888	0.851	0.900	0.888	0.891	0.911	0.870	0.900
90	-	-	-	-	0.004	-	-	-	-
85	0.017	0.013	0.024	-	0.018	0.022	-	0.014	0.005
75	-	-	-	-	-	-	-	-	0.005
*GPI-2**									
(n)	(101)	(120)	(106)	(25)	(138)	(92)	(97)	(108)	(99)
400	-	-	-	-	0.004	-	0.010	0.005	-
100	1.000	1.000	1.000	1.000	0.996	0.995	0.990	0.991	1.000
-200	-	-	-	-	-	0.005	-	0.005	-
*IDDH**									
(n)	(90)	(120)	(106)	(25)	(138)	(90)	(97)	(108)	(99)
160	0.122	0.108	0.142	0.080	0.112	0.089	0.113	0.083	0.172
135	-	0.004	-	-	-	0.011	-	-	-
100	0.850	0.888	0.844	0.860	0.862	0.883	0.861	0.884	0.823
70	0.022	-	0.014	0.060	0.025	0.017	0.021	0.032	0.005
50	0.006	-	-	-	-	-	0.103	-	-
*IDH2**									
(n)	(20)	(27)	(23)	(24)	(32)	(24)	(9)	(23)	(24)
100	1.000	1.000	0.978	1.000	1.000	1.000	1.000	1.000	1.000
65	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000
*sMDH-1**									
(n)	(20)	(27)	(24)	(24)	(42)	(24)	(25)	(24)	(24)
115	-	-	-	-	-	-	-	0.021	-
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.979	1.000
*sMDH-2**									
(n)	(20)	(27)	(24)	(24)	(42)	(24)	(25)	(24)	(24)
100	1.000	0.981	1.000	1.000	1.000	1.000	1.000	1.000	0.979
70	-	0.019	-	-	-	-	-	-	0.021

Table 4.2.3 cont.

Locus, allele	West Australia	South Australia	Portland	Maatsu-yker	Tasman Island	Maria Island	Babel Island	Eden	New Zealand
*PGM-2**									
(n)	(92)	(120)	(106)	(25)	(138)	(91)	(97)	(108)	(99)
430	-	-	0.005	-	-	-	-	-	-
300	0.060	0.071	0.080	-	0.047	0.077	0.052	0.069	0.061
130	-	-	0.005	-	-	-	-	-	-
100	0.793	0.779	0.708	0.860	0.786	0.775	0.845	0.782	0.778
-300	0.147	0.150	0.203	0.140	0.167	0.148	0.103	0.148	0.162
monomorphic loci (only sample sizes given)									
*AK**	(20)	(27)	(16)	(24)	(32)	(24)	(25)	(19)	(24)
*CK-1**	(10)	(14)	(7)	(24)	(32)	(5)	(11)	(6)	(17)
*CK-2**	(20)	(27)	(24)	(24)	(31)	(24)	(25)	(24)	(24)
*FH**	(20)	(27)	(24)	(24)	(32)	(24)	(25)	(24)	(24)
*GAPDH-1**	(20)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)
*GAPDH-2**	(20)	(27)	(24)	(24)	(24)	(24)	(25)	(24)	(24)
*LDH-1**	(20)	(27)	(24)	(24)	(32)	(24)	(24)	(24)	(24)
*LDH-2**	(20)	(27)	(40)	(24)	(32)	(24)	(25)	(24)	(24)
*LDH-3**	(20)	(27)	(40)	(24)	(32)	(24)	(25)	(24)	(24)
*mMDH**	(20)	(27)	(24)	(24)	(42)	(24)	(25)	(24)	(24)
*sSOD**	(101)	(100)	(106)	(25)	(80)	(91)	(97)	(106)	(76)

Table 4.2.4. *Nemadactylus macropterus*. Summary of heterogeneity chi-square tests for differences in gene frequencies (without and with pooling of rare alleles, see text) and gene diversity statistics for the eight polymorphic loci among (I) the nine areas sampled, (II) the eight Australian areas, and (III) the pooled Australian samples versus New Zealand.

	Heterogeneity χ^2 tests					Gene diversity tests		
	No pooling		Pooled			Nei	Mean	
Locus	χ^2	P^a	χ^2	d.f.	P	G_{ST}	$G_{ST\text{-null}}$	P^a
I. Comparison of 9 areas sampled								
*sAAT-1**	62.533	0.003[s]	33.654	8	<0.001[s]	0.0214	0.0058	0.003[s]
*ADA**	45.771	0.851	11.513	16	0.777	0.0035	0.0057	0.796
*ADH**	76.943	0.037	11.966	8	0.153	0.0060	0.0056	0.349
*GPI-1**	38.575	0.501	4.912	8	0.767	0.0028	0.0058	0.891
*IDDH**	46.776	0.055	6.102	8	0.636	0.0060	0.0058	0.367
*PGDH**	65.618	0.373	9.708	16	0.881	0.0029	0.0060	0.923
*PGM-1**	69.730	0.029	8.344	8	0.401	0.0050	0.0059	0.551
*PGM-2**	31.318	0.445	13.668	8	0.091	0.0081	0.0057	0.154
Across all eight loci	36.390[b]	0.003[s]	32.386[b]	16[b]	0.009[s]	0.0064	0.0058	0.269
II. Comparison of 8 Australian areas								
*sAAT-1**	34.203	0.032	8.350	7	0.303	0.0100	0.0059	0.119
*ADA**	42.335	0.726	11.315	14	0.661	0.0039	0.0058	0.733
*ADH**	69.177	0.048	9.463	7	0.221	0.0049	0.0058	0.489
*GPI-1**	26.075	0.544	4.475	7	0.724	0.0029	0.0057	0.812
*IDDH**	34.505	0.168	3.250	7	0.861	0.0038	0.0059	0.711
*PGDH**	58.600	0.353	9.423	14	0.803	0.0031	0.0059	0.864
*PGM-1**	56.245	0.080	8.343	7	0.303	0.0053	0.0059	0.477
*PGM-2**	29.372	0.316	13.662	7	0.058	0.0091	0.0058	0.128
Across all eight loci	27.820[b]	0.033[s]	15.717[b]	16[b]	0.473	0.0052	0.0059	0.683
III. Comparison of Australian (pooled) and New Zealand fish								
*sAAT-1**	27.630	0.001[s]	24.771	1	<0.001[s]	0.0446	0.0014	<0.001[s]
*ADA**	2.041	0.959	0.211	2	0.900	0.0003	0.0014	0.859
*ADH**	6.502	0.413	2.572	1	0.109	0.0033	0.0015	0.109
*GPI-1**	10.274	0.162	0.381	1	0.537	0.0005	0.0014	0.613
*IDDH**	9.056	0.077	2.952	1	0.086	0.0058	0.0014	0.033
*PGDH**	10.297	0.297	0.317	2	0.853	0.0003	0.0014	0.817
*PGM-1**	9.932	0.132	0.000	1	0.983	0.0008	0.0015	0.550
*PGM-2**	0.654	0.954	0.025	1	0.874	0.0001	0.0014	0.936
Across all eight loci	31.008[b]	0.011[s]	40.068[b]	16[b]	<0.001[s]	0.0046	0.0014	0.002[s]

a - P value equals proportion of 1 000 bootstrap replicates where randomized value ≥ observed value;

b - overall significance test ($\chi^2 = -2\Sigma \ln P$ with d.f. = 2 x no. separate tests) from Sokal and Rohlf (1981);
s - significant at P=0.05, which with Bonferroni adjustment for 8 loci becomes P=0.006.

Table 4.2.5. *Nemadactylus macropterus*. Probabilities of no significant differentiation among pairs of areas following chi-square analysis (Roff and Bentzen, 1989) of allele numbers at the *sAAT-1** locus.

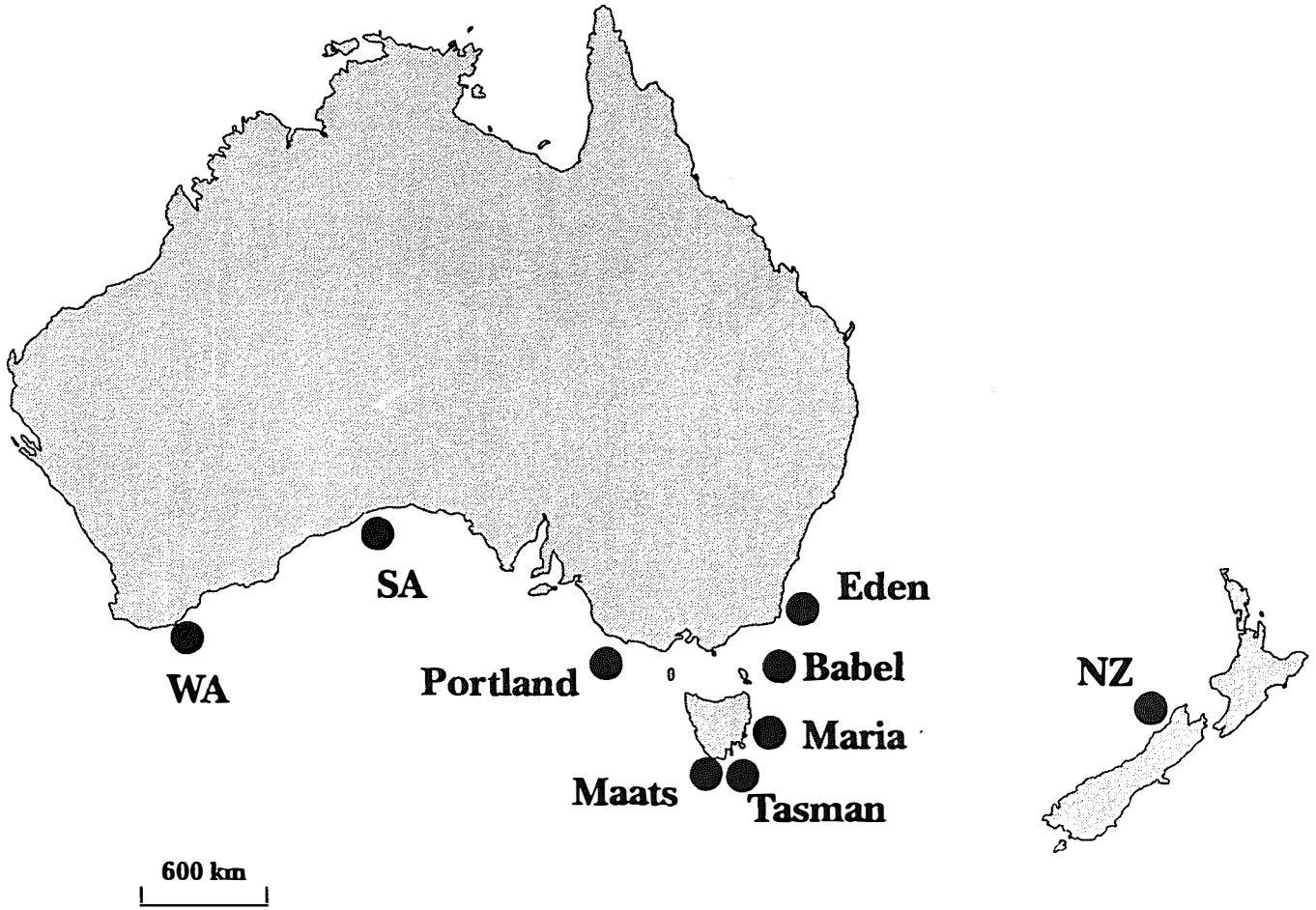
Area	South Australia	Portland	Maatsuyker	Tasman Island	Maria Island	Babel Island	Eden	New Zealand
West Australia	0.964	0.072	0.362	0.494	1.000	0.801	0.685	<0.001^{s}
South Australia		0.084	0.257	0.341	0.969	0.767	0.491	<0.001^{s}
Portland			0.083	0.048	0.110	0.097	0.049	<0.001^{s}
Maatsuyker				0.143	0.373	0.284	0.245	0.461
Tasman Island					0.526	0.664	0.717	<0.001^{s}
Maria Island						0.813	0.702	<0.001^{s}
Babel Island							0.867	<0.001^{s}
Eden								<0.001^{s}

s - significant at P=0.05, which with Bonferroni adjustment for 36 tests becomes P=0.0014.

Table 4.2.6. *Nemadactylus macropterus.* Comparison of overall gene frequencies between 1981 (Richardson 1982) and 1991 (this study, Australian fish only) for the five loci common to each study. Alleles are numbered in order of increasing mobility; n: number of individuals examined; - not detected. Chi-square tests follow pooling to three alleles for each locus, giving 2df. Values of 0.00 in Richardson's data indicate rare alleles with (unspecified) frequencies of less than 0.005.

Allele	*ADA**		*ADH**		*GPI-1**		*PGM-2**		*PGDH**	
	1981	1991	1981	1991	1981	1991	1981	1991	1981	1991
n	486	786	414	786	374	778	507	786	510	787
1	-	0.002	-	0.003	0.01	-	0.00	-	0.00	0.001
2	0.00	0.006	0.00	0.018	0.02	0.015	0.13	0.153	0.09	0.079
3	0.14	0.127	0.54	0.613	-	0.001	0.80	0.783	-	0.004
4	-	0.001	-	0.003	0.88	0.885	-	0.001	0.58	0.607
5	0.58	0.536	0.43	0.357	-	0.002	0.08	0.062	-	0.008
6	-	0.001	0.03	0.004	0.09	0.096	-	0.001	0.32	0.288
7	0.28	0.322	-	0.001	-	0.001			-	0.001
8	-	0.005	-	0.001					0.01	0.010
9									-	0.001
$\chi 2$	5.590		12.549		2.520		4.645		2.945	
P	0.061		0.002		0.284		0.098		0.229	

Fig 4.3.1. Map of locations. WA= West Australia, SA=South Australia, Maats=Maatsuyker, NZ= New Zealand. Further information about localities and sampling dates is in Elliot and Ward, 1993.



4.3 Stock structure as indicated by analysis of MtDNA haplotype distributions

Materials and Methods

Between January 1991 and April 1992, samples of Nemadactylus macropterus were obtained from eight areas around southern Australia and one area off the west coast of the South Island of New Zealand (Figure 4.3.1). Samples consisted of whole fish frozen after capture and transported to the laboratory. All fish were measured (standard length) and sexed (except the Maria Island sample), and meristic counts were taken of the dorsal, anal and pectoral fins. Muscle, liver and eye tissues were sampled for genetic studies, and the otoliths removed for chemical analysis. The tissue samples were stored at -70°C.

Total DNA was extracted from approximately 100 mg of white muscle tissue per fish by a modified CTAB (hexadecyltrimethylammoniumbromide) protocol described by Grewe et al. (1993). Nine restriction enzymes (Bam HI, Ban I, Bcl I, Bst BI, Eco RI, Hind III, Pvu II, Sal I and Xho I) were used to examine 166 individuals representing seven sampling locations. As three of these enzymes (Bam HI, Ban I and Bcl I) revealed more genetic variation than the remaining six, these were used to analyse 334 additional fish representing nine locations (the original seven plus two more).

Restriction fragments were separated in horizontal 1.0% agarose gels submerged in a tris-borate-EDTA (TBE) buffer system (Sambrook et al., 1989). DNA was transferred to a nylon membrane filter (Hybond N+, Amersham Ltd) by southern transfer (Sambrook et al. 1989). The nylon membrane filters were probed with blue eye trevalla (Hyperoglyphe antarctica, Teleostei: Stromateoidei) mitochondrial DNA (50 ng used per ten 20 cm x 20 cm blots) purified by caesium chloride ultracentrifugation. The trevalla probe was labelled with 32P dCTP (Bresatec Pty Ltd) by a GIGAprime DNA labelling kit (Bresatec Pty Ltd). The membrane filters were then exposed to Kodak XAR-5 X-ray film for 12-48 hours.

Haplotype (nucleon) and nucleotide diversities within samples, and nucleotide diversities and divergences between samples, were computed with the REAP package (McElroy et al., 1992), using the formulations of Nei and Tajima (1981) and Nei (1987).

All chi-square analyses used the Monte Carlo randomisation approach of Roff and Bentzen (1989), which eliminates the need to pool rare haplotypes. Two thousand randomisations were carried out for each test. The number of times each of the randomised

replicates was greater than or equal to the observed value divided by 2000 was an estimate of the probability of obtaining the observed value by chance.

The proportions of total genetic diversity attributable to variation among localities (estimated both by treating haplotypes as alleles, GST-Nei (Nei, 1973), and by considering restriction site data, GST-TP (Takahata and Palumbi, 1985)) were estimated. The significance of these statistics was evaluated by bootstrapping (Ovenden and White, 1990; Palumbi and Wilson, 1990; Elliott and Ward, 1992). Two thousand bootstrapping runs were performed for each test. The number of times each of the bootstrapped replicates was greater than or equal to the observed value was determined. This value divided by 2000 was an estimate of the probability of obtaining the observed value by sampling error alone.

Matrices of genetic distances among localities (estimated both by treating haplotypes as alleles, DNei, using Nei's (1978) distance, and by considering restriction site data, DTP, using Takahata and Palumbi's (1985) distance) were compared with a matrix of geographic distance. The significance of correlations were tested by Mantel's t-test method, as outlined by Douglas and Endler (1982).

Results

Nine-enzyme data

The nine restriction enzymes (Bam HI, Ban I, Bcl I, Bst BI, Eco RI, Hind III, Pvu II, Sal I and Xho I) revealed 28 haplotypes among the 166 fish from seven Australian localities (Table 4.3.1). Fragment sizes are given in Table 4.3.2. Two of these enzymes (Hind III and Pvu II) produced fragments that permitted the resolution of a 300 nucleotide base pair insertion in the mitochondrial genomes of five individuals. Given the resolution limitations of the southern blotting used to visualise restriction profiles (i.e. fragments < 400 base pairs are usually not observed), the loss or gain of a 300 base pair fragment in the restriction profiles of either Hind III or Pvu II could easily explain the observed differences. However, the parallel size increase of 300 base pairs in both the 5,300 base pair Hind III and 4,600 base pair Pvu II restriction fragments is strong evidence for a mtDNA size polymorphism in those individuals. The mean size of the mtDNA molecule, excluding the size insertion, is 16,639 bp. No heteroplasmic individuals were observed.

Nucleon diversity was 0.671 overall (Table 4.3.1). Chi square analysis showed no significant differentiation of haplotype numbers among these localities (P=0.35). Mean percent nucleotide diversity per sample was 0.458, with a mean (corrected) percent nucleotide divergence between pairs of samples of 0.001 (Table 4.3.3).

Three-enzyme data

Five hundred fish from eight Australian localities and one New Zealand locality were analysed with each of the three restriction enzymes Bam HI, Ban I and Bcl I that detected polymorphic cut sites.

These three enzymes differentiated 33 haplotypes (Table 4.3.4). Haplotype AAA predominated (59% over all samples), followed by AAC (12%), BCB (9.6%), ABA (4.6%), AAB (3.6%), BAB (1.8%) and ACA and BCC (1%). The 25 remaining haplotypes each had frequencies of less than 1%. Nucleon diversity ranged from 0.529 (New Zealand) to 0.793 (Maatsuyker). Mean percent nucleotide diversity per sample was 1.247, and mean (corrected) percent nucleotide divergence between pairs of samples was 0.002 (Table 4.3.3).

The first set of analyses considered haplotypes as alleles. Chi-square analysis showed that these haplotypes were not distributed randomly with respect to locality (P=0.0135). In order to identify the source of this heterogeneity, all possible pair-wise comparisons of localities were carried out (Table 4.3.5). Eleven of the 36 tests were significant at the 5% level. Although none of these was significant after applying the (conservative) Bonferroni correction procedure for multiple tests (alpha=0.05, adjusted alpha=alpha/36=0.0014), seven of the eleven comparisons with P<0.05 were comparisons of New Zealand with Australian localities. The eighth New Zealand comparison yielded P=0.064. Furthermore, the five comparisons with probabilities less than 0.01 were five of the eight comparisons between New Zealand and Australian localities. There was also some suggestion that the Maatsuyker sample might be different from other samples, with three pairwise comparisons having probabilities less than 0.05 (but greater than 0.01) and the New Zealand comparison having a P value of 0.064. This suggestion was not supported by the results of the next tests, which compared each sample in turn against all other samples pooled. Only the New Zealand sample was shown to be distinguishable at the 5% level (West Australia, P=0.810; South Australia, 0.811; Portland, 0.272; Babel Island, 0.104; Eden, 0.188; Maria Island, 0.075; Tasman Island, 0.536; Maatsuyker, 0.339; New Zealand, 0.023).

This suggests that the significant heterogeneity among localities detected in the first test was primarily attributable to differentiation of the New Zealand sample. Removing this sample and testing for haplotype heterogeneity among the eight Australian samples alone gave a non-significant result (P=0.104). These analyses therefore indicated that the New Zealand sample was significantly different from the Australian samples, while the Australian samples did not differ significantly among themselves. Much of this heterogeneity was attributable to the AAB haplotype, which had a frequency of 0.113 in the New Zealand

sample but only 0.023 in the pooled Australian sample. Eliminating this haplotype from the comparison of the pooled Australian sample versus the New Zealand sample removed the significant heterogeneity (P=0.132).

About 2 percent of the total haplotype variation was attributable to variation among the nine localities (GST-Nei=0.023, Table 4.3.6), but this was not significantly greater than that attributable to sampling error alone (P=0.13). Pooling the Australian localities and comparing this pooled sample to the New Zealand sample reduced the proportion of variation arising from locality differences to about 1 per cent (GST-Nei=0.013), but this is significantly greater than that arising from sampling error alone (P=0.024).

Nei's unbiased genetic distances (Nei, 1978) were estimated for all pair-wise sample comparisons by treating the mtDNA haplotypes as alleles. A UPGMA-derived dendrogram separated the New Zealand sample from the others (Fig. 4.3.2), although there was no significant relationship between DNei and geographic distance between pairs of localities (r=0.342, t=1.41, P>0.05).

A second set of analyses considered the patterns of restriction site variation among haplotypes across all 9 localities, using the formulae of Takahata and Palumbi (1985). Table 4.3.7 gives the within (I) and between-locality (J) identity probabilities, and between-locality distances (DTP). GST-TP was estimated at 0.130, which implies that about 13% of the restriction site variation was attributable to geographic differentiation. However, of 2000 bootstrapped values, this value was equalled or exceeded on 1664 occasions (P=0.832). Thus the observed GST-TP value, although apparently quite large, is not significantly greater than that assignable to sampling error alone. Comparing the pooled Australian samples with the New Zealand sample gives a similarly non-significant result (GST-TP = 0.124, P = 0.404). These analyses therefore fail to confirm the significant heterogeneity shown by the chi-square haplotype analysis. There was no significant relationship between genetic distance among restriction sites (DTP) and geographic distance between pairs of localities (r=-0.184, t=-0.684, P>0.05).

Discussion

Nucleon diversity (h) of the jackass morwong at around 0.67 for the nine-enzyme analysis, is similar to that of the bluefish (h=0.696, Graves et al., 1992b) and yellowfin tuna (h=0.724, Ward et al., 1993a). Some marine fishes have nucleon diversities approaching the maximum attainable of 1 (e.g. red drum, h=0.94, Gold and Richardson, 1991; Atlantic menhaden, h=0.99, Bowen and Avise, 1990), others are around 0.90 (e.g. haddock, h=0.87, Zwanenburg et al. 1992; herring, h=0.91, Kornfield and Bogdanowicz, 1987), while some

are well below those of morwong (e.g. cod, h= 0.36, Carr and Marshall, 1991, h=0.10, Smith et al 1989; American plaice, h=0.35, Stott et al., 1992; weakfish, h=0.16, Graves et al., 1992a). However, nucleon diversity is a crude measure of variability. It is based on haplotype frequencies alone, and as more restriction enzymes are employed in a study and additional variable cut sites detected, the number of haplotypes and therefore the value of h increases. The mean percent nucleotide diversity per morwong sample is 0.458 for the nine-enzyme study and 1.247 for three-enzyme study (which selected those restriction enzymes that revealed the most genetic variation).

This study focussed on the mtDNA haplotype distribution of the 500 fish characterised by the restriction enzymes Bam HI, Ban I and Bcl I. A chi-square analysis based on haplotype numbers showed that fish from New Zealand were significantly different from those in Australian waters (P=0.024), while Australian samples, despite spanning a coast line of about 3000 km, were not significantly different from each other (P=0.104). However, the degree of difference between these two sets of samples was small, and primarily attributable to frequency differences in a single haplotype (AAB). The overall similarity of fish from the Austral-New Zealand region was supported by a GST-based analysis of restriction site diversity, which failed to demonstrate significant differentiation among samples. The mean percent nucleotide divergence between pairs of samples, having corrected for diversity within samples, was 0.002 for the three-enzyme study.

It therefore seems probable that there is gene flow throughout this region, with the Tasman Sea between Australia and New Zealand acting as a partial barrier. This conclusion is supported by the lack of significant correlations between genetic and geographic distance and the observation that 14 of the 15 haplotypes recorded more than once were found in two or more localities. The exceptional haplotype was AAG, found in only two fish, both from off Babel Island. While this might represent a remnant of population structuring, it is more likely to be a chance occurrence. There is thus very little evidence of haplotypes being restricted to single localities, although much larger sample sizes would be required to evaluate this hypothesis for the many rare haplotypes.

How do these findings accord with those of the allozyme analysis ? While the mitochondrial DNA analysis showed the morwong to have levels of mtDNA diversity, h, around the mean for marine fishes, the average allozyme heterozygosity per locus (0.101) was estimated to be about twice the mean of other marine fishes (around 0.05, Smith and Fujio, 1982; Ward et al., 1993b). However, both analyses concluded there was a lack of genetic differentiation among the Australian localities, but there was significant, if minor, differentiation of the New Zealand sample. The allozyme differentiation was primarily attributable to variation at one of eight polymorphic loci, sAAT-1*, where the common allele

are well below those of morwong (e.g. cod, h= 0.36, Carr and Marshall, 1991, h=0.10, Smith et al 1989; American plaice, h=0.35, Stott et al., 1992; weakfish, h=0.16, Graves et al., 1992a). However, nucleon diversity is a crude measure of variability. It is based on haplotype frequencies alone, and as more restriction enzymes are employed in a study and additional variable cut sites detected, the number of haplotypes and therefore the value of h increases. The mean percent nucleotide diversity per morwong sample is 0.458 for the nine-enzyme study and 1.247 for three-enzyme study (which selected those restriction enzymes that revealed the most genetic variation).

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had a frequency of around 0.80 in the Australian samples and 0.95 in the New Zealand sample.

The haploid, maternally inherited nature of mitochondrial DNA should make it a more powerful tool for examining population subdivision than the allozymes coded by nuclear DNA (see, for example, Avise et al., 1979; Lansman et al., 1983; Saunders et al., 1986; Ward et al., 1989, Reeb and Avise, 1990), but in the morwong study the allozyme approach arguably provided more evidence for population subdivision than did the mtDNA analysis. For example, with respect to chi-square analysis, the probabilities of no significant differentiation between the New Zealand sample and the pooled Australian sample were 0.001 for alleles at the sAAT-1* locus, 0.011 for a combined probability across all eight polymorphic allozymes, and 0.023 for mtDNA haplotypes. Furthermore, while the mtDNA GST-Nei values are about three times greater than for the allozymes (Table 4.3.6), much of this is attributable to the increased sampling error associated with the mtDNA analysis: the probability that the allozyme GST-Nei comparison between the pooled Australian samples and the New Zealand samples arises by chance is in fact an order of magnitude smaller than that derived from the mtDNA comparison (P=0.002 and 0.024, respectively). The greater variance of the haplotype than allozyme GST-Nei values to a large extent reflects the substantially greater sample size used in the latter analysis. Nearly 1800 nuclear alleles (880 fish) were examined for each of eight independent allozyme loci, with heterozygosities or genetic diversities ranging from 0.237 to 0.592 per allozyme. This compares with 500 mitochondrial DNA alleles with a genetic diversity of 0.635. However, we are not arguing that one approach should be preferred over the other: both have advantages and disadvantages, and their joint application to studies of population structure will always be more powerful than the application of a single approach. In our case, the congruence of the two data sets gives added confidence that there was indeed weak but significant differentiation of the New Zealand sample from the Australian samples.

Other genetic studies have compared marine species from New Zealand and south east Australian waters, and several of these have suggested that the Tasman Sea is a partial barrier to gene flow. Allozyme data from blue grenadier (*Macruronus novaezelandiae*) parallel the morwong results closely. No significant genetic differences were observed among samples from three localities off south-eastern Australia, while there were small but significant differences across the Tasman Sea (Milton and Shaklee, 1987). The snapper, *Chrysophrys auratus*, similarly showed allozymic differentiation across the Tasman (MacDonald, 1980). Limited mitochondrial DNA data from the rock lobster *Jasus verreauxi* were also suggestive of trans-Tasman differences, although more extensive data from Jasus edwardsii failed to show such differentiation (Ovenden et al., 1992; Brasher et al., 1992).

While our data indicate mixing among Australian localities, the extent of this mixing is uncertain. A tagging study off the New South Wales coast, based on 69 recaptured fish, indicated little movement of adults over one year (Smith, 1989). However, the main factor promoting genetic homogeneity in the jackass morwong may well be larval and pre-juvenile drift. New Zealand studies found that the morwong has an 8-10 month off-shore pelagic post-larval period, followed by metamorphosis to a bottom-living juvenile resembling the adult in form (Tong and Vooren, 1972; Vooren, 1972; Vooren, 1975). In Australian waters, the availability of the Leeuwin Current in the west and south and the East Australian Current (with associated eddies) in the east mean that gene flow resulting from larval drift could be considerably greater than that resulting from adult migrations.

The failure of the data to reject the null hypothesis that Australian morwong comprise a single stock does not mean that the null hypothesis is correct. It remains possible that several genetic stocks are present in Australian waters, but that our techniques failed to uncover their existence. However, we examined eight polymorphic allozymes in 780 fish, and 33 mtDNA haplotypes in 429 fish (numbers which exclude the New Zealand sample), from samples covering a 3000 k coastline. The power of these tests suggests that any genetic differentiation is likely to be very limited, although the observed levels of homogeneity could nevertheless be maintained by levels of gene flow ranging from high to quite low. Managers may manage two stocks separately if there is 5% gene flow between them, but jointly if there is 45% gene flow. The present genetic data do not allow us to distinguish between these possibilities.

Table 4.3.1. Numbers of nine-enzyme haplotypes of morwong, in the order *Bam* HI, *Ban* I, *Bcl* I, *Bst* BI, *Eco* RI, *Hind* III, *Pvu* II, *Sal* I and *Xho* I, from seven localities. *h* refers to nucleon diversity (Nei and Tajima, 1981).

	West Australia	South Australia	Portland	Babel Island	Eden	Maria Island	Tasman Island	Total
AAAAAAAAA	9	18	12	19	16	9	10	93
AAABAAAAA	1	0	0	1	0	0	1	3
AAAAABAAA	0	1	0	0	1	1	1	4
AAAAAAABA	0	1	0	0	0	1	1	3
AAAAAAAAB	0	0	0	0	0	0	1	1
AAAAAAAAC	0	1	0	0	0	0	0	1
AACAAAAAA	1	1	0	3	4	1	1	11
AACAAABAA	0	2	1	0	2	1	1	7
AACAAAABA	1	0	0	0	0	0	0	1
AACAAAAAB	0	0	0	1	0	0	0	1
AACAAACAA	0	0	1	0	0	0	0	1
AACAAAACA	0	0	1	0	0	0	0	1
AADAAAAAA	0	0	1	0	0	0	0	1
AAEAAAAAA	0	0	0	0	0	1	0	1
ABAAAAAAA	0	1	2	0	1	0	3	7
ABBAAABAA	1	0	0	0	0	0	0	1
ACAAAAAAD	0	1	0	0	0	0	0	1
BABAAAAAA	0	0	2	0	0	3	0	5
BABAAAACA	0	1	0	0	0	0	0	1
BCBAAAAAA	1	1	3	5	1	0	0	11
BCBAAAAAB	0	0	1	0	0	0	0	1
BCCAAAAAA	0	0	1	0	1	0	0	2
CCAAAAAAA	0	0	0	0	1	0	0	1
CCCAAAAAA	0	0	0	0	1	0	0	1
DACAAAAAA	0	1	0	0	0	0	0	1
AAAAAA$^+$A$^+$AA	0	1	0	0	0	1	1	3
AAABAA$^+$A$^+$AA	0	0	0	0	1	0	0	1
BABAAA$^+$A$^+$BA	0	0	1	0	0	0	0	1
n	14	30	26	29	29	18	20	166
h	0.604	0.646	0.782	0.547	0.687	0.745	0.747	0.671

The A$^+$ haplotypes represent a size polymorphism - see text.

Table 4.3.2. Restriction fragment lengths of the different haplotypes.

Bam HI

A	B	C	D	E	F
-	-	16,575.	-	-	-
-	-	-	-	11,085.	-
-	13,850	-	-	-	-
8,360	-	-	-	-	-
-	-	-	7,780.	-	-
5,490	-	-	5,490.	5,490.	5,490
-	-	-	-	-	4,450
-	-	-	-	-	4,000
2,725	2,725	-	2,725.	-	2,725
-	-	-	580.	-	-
16,575	16,575	16,575	16,575	16,575	16,665

Ban I

A	B	C	D	E	F
9,371	9,371	9,371	-	-	9,371
-	-	-	-	7,620	-
-	-	-	5,171	-	-
-	-	-	4,200	-	-
3,500	-	3,500	3500.	3,500	-
-	2,806	-	-	-	2,806
-	-	2,250	-	-	2,220
2,063	2,087	-	2,087	2,063	-
1,856	1,856	1,856	1,856	1,856	1,856
-	-	-	-	1,750	-
-	700	-	-	-	700
220	220	-	220	220	-
17,010	17,040	16,977	17,034	17,009	16,953

Bcl I

A	B	C	D	E	F	G	H	I	J
-	-	-	-	-	-	-	14,482	-	-
-	-	-	-	-	-	-	-	-	14,281
12,249	-	-	-	12,249	-	-	-	-	-
-	-	-	10,649	-	-	10,649	-	-	-
-	-	-	-	-	-	-	-	10,386	-
-	8,153	8,153	-	-	8,153	-	-	-	-
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
-	4,096	4,096	-	-	4,096	-	-	4,096	-
-	-	-	-	-	3,282	-	-	-	-
2,233	2,233	-	2,233	-	-	-	-	-	2,233
-	-	-	-	-	-	-	-	-	-

Table 4.3.2 continued.

2,032	2,032	2,032	2,032	2,032	-	2,032	2,032	2,032	-
-	-	-	1,600	-	-	1,600	-	-	-
-	-	1,250	-	1,250	-	1,250	-	-	-
-	-	983	-	983	983	983	-	-	-
16,514	16,514	16,514	16,514	16,514	16,514	16,514	16,514	16,514	16,514

Bst BI

A	B
14,500	-
-	7,950
-	6,550
2,115	2,115
16,615	16,615

Eco RI

A	B
16,560	-
-	9,550
-	7,010
16,560	16,560

Hind III

A	A+	B
-	5,600	-
-	-	5560
5300	-	5300
4,467	4,467	-
3,832	3,832	3,832
1,145	1,145	1,145
1,092	1,092	-
564	564	564
16,400	16,700	16,401

Table 4.3.2 continued.

Pvu II

A	A+	B	C
5,820	5,820	5,820	-
-	4,900	-	-
4,600	-	4,600	4,600
3,400	3,400	3,400	3,400
-	-	-	3,390
-	-	-	2,430
1,680	1,680	-	-
1,390	1,390	1,390	1,390
-	-	950	950
-	-	730	730
16,890	17,190	16,890	16,890

Xho I

A	B	C	D
11,434	11,434	11,434	-
-	-	-	6,520
-	-	-	4,914
-	2,680	-	-
2,035	-	2035	2,035
-	-	1700	-
1,300	1,300	-	1,300
650	650	650	650
645	-	645	645
-	-	-	-
400	400	-	400
16,464	16,464	16,464	16,464

Table 4.3.3. Percent nucleotide diversity within and between samples, after Nei and Tajima (1981), Nei (1987).

	9 enzymes	3 enzymes
percent nucleotide diversity per sample		
mean	0.458	1.247
minimum	0.360	0.703
maximum	0.645	1.499
percent nucleotide diversity between pairs of samples		
mean	0.459	1.249
minimum	0.382	0.927
maximum	0.588	1.438
percent nucleotide divergence between pairs of samples*		
mean	0.001	0.002
minimum	-0.017	-0.026
maximum	0.050	0.054

*This is percent nucleotide diversity between pairs of samples corrected for the percent nucleotide diversity within samples.

Table 4.3.4. Numbers of three-enzyme haplotypes of morwong, in the order *Bam* HI, *Ban* I and *Bcl* I, from nine localities. *h* refers to nucleon diversity (Nei and Tajima, 1981).

	West Australia	South Australia	Portland	Babel Island	Eden	Maria Island	Tasman Island	Maatsuyker	New Zealand	Total
AAA	31	38	30	37	32	34	35	10	48	295
AAB	2	0	1	0	1	2	1	3	7	18
AAC	7	6	7	8	12	3	9	3	5	60
AAD	0	0	1	0	0	0	0	0	0	1
AAE	0	0	0	0	0	1	0	0	2	3
AAF	0	0	0	0	0	0	1	0	0	1
AAG	0	0	0	2	0	0	0	0	0	2
AAH	0	0	0	0	1	1	0	1	0	3
AAI	0	0	0	1	0	0	0	0	0	1
AAJ	0	0	0	0	0	0	0	0	1	1
ABA	0	2	3	2	4	1	6	3	2	23
ABB	1	0	0	0	0	0	0	0	0	1
ACA	0	2	1	0	0	2	0	0	0	5
ACB	0	0	1	0	0	0	0	0	2	3
ADA	0	0	0	0	1	0	0	0	0	1
AEA	0	0	0	0	0	1	0	0	0	1
AFA	0	0	1	0	0	0	0	0	0	1
BAA	0	0	0	0	1	0	0	0	0	1
BAB	1	1	3	0	0	4	0	0	0	9
BAC	0	0	0	0	1	0	1	0	0	2
BAE	0	0	0	0	0	0	1	0	0	1
BCB	9	8	6	7	3	5	5	3	2	48
BCC	0	0	3	0	1	1	0	0	0	5
BCF	0	0	0	1	0	0	0	0	0	1
CCA	0	0	0	0	1	0	0	0	0	1
CCB	0	0	0	0	0	1	0	0	0	1
CCC	0	0	0	0	1	1	0	0	0	2
DAC	0	1	0	0	0	0	0	0	0	1
EAA	0	0	1	1	1	1	0	0	0	4
EAB	0	0	0	0	0	0	1	0	0	1
EAC	0	0	0	0	0	1	0	0	0	1
FAA	0	0	0	1	0	0	0	0	0	1
BFC	0	0	0	0	0	0	0	1	0	1
n	51	58	58	60	60	59	59	24	71	500
h	0.578	0.547	0.710	0.595	0.677	0.660	0.616	0.793	0.529	0.635

Table 4.3.5. Probabilities from chi-square analysis (Roff and Bentzen, 1989) of pairwise sample comparisons of haplotype numbers based on three restriction enzymes and 2000 Monte Carlo iterations. Probabilities less than 5% are given; probabilities greater than 5% are given as n.s. (not significant).

	South Australia	Portland	Babel Island	Eden	Maria Island	Tasman Island	Maatsuyker	New Zealand
W. Australia	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.025	0.012
S. Australia	-	n.s.	n.s.	n.s.	n.s.	n.s.	0.014	0.004
Portland	-	-	n.s.	n.s.	n.s.	n.s.	n.s.	0.006
Babel Island	-	-	-	n.s.	n.s.	n.s.	0.037	0.003
Eden	-	-	-	-	n.s.	n.s.	n.s.	0.005
Maria Island	-	-	-	-	-	0.026	n.s.	0.034
Tasman Island	-	-	-	-	-	-	n.s.	0.009
Maatsuyker	-	-	-	-	-	-	-	n.s.

Table 4.3.6. Comparison of G_{ST}-Nei analyses between the allozyme and mitochondrial DNA data, treating the mtDNA haplotypes as alleles. G_{ST}-Nei (null) values, which are those derived from 2000 bootstrapped replications, represent the level of G_{ST}-Nei that can be attributed to sampling error alone.

	All nine sites			Australian (pooled) vs New Zealand		
	G_{ST}-Nei	G_{ST}-Nei (null)	*P*	G_{ST}-Nei	G_{ST}-Nei (null)	*P*
mtDNA	0.0230	0.0178	0.130	0.0126	0.0043	0.024
*sAAT-1**	0.0214	0.0058	0.003	0.0446	0.0014	<0.001
Eight polymorphic allozymes*	0.0064	0.0058	0.269	0.0046	0.0014	0.002

* from Elliott and Ward (1994)

Table 4.3.7. Pairwise sample comparisons of restriction site data, after Takahata and Palumbi (1985). Identity probabilities within localities (I) on diagonal (bold), identity probabilities between localities (J) above diagonal, distances (D_{TP}) below diagonal. Mean I = 0.680, mean J = 0.626, mean D_{TP}=0.083.

	West Australia	South Australia	Portland	Babel Island	Eden	Maria Island	Tasman Island	Maatsuyker	New Zealand
W. Australia	**0.749**	0.699	0.653	0.583	0.701	0.648	0.706	0.703	0.665
S. Australia	0.035	**0.699**	0.614	0.552	0.657	0.610	0.662	0.658	0.627
Portland	0.066	0.093	**0.650**	0.522	0.616	0.574	0.621	0.656	0.589
Babel Island	0.125	0.145	0.165	**0.583**	0.554	0.546	0.589	0.554	0.561
Eden	0.035	0.065	0.093	0.145	**0.703**	0.612	0.664	0.661	0.628
Maria Island	0.067	0.093	0.117	0.113	0.093	**0.641**	0.654	0.612	0.620
Tasman Island	0.035	0.064	0.092	0.090	0.064	0.033	**0.713**	0.665	0.673
Maatsuyker	0.033	0.065	0.031	0.146	0.063	0.094	0.064	**0.705**	0.629
New Zealand	0.069	0.094	0.120	0.114	0.095	0.061	0.033	0.095	**0.678**

4.4 Re-analysis of genetic data based on microprobe results

The distributions of eight polymorphic allozyme loci and the mtDNA haplotypes were re-examined, based on a classification of fish into one of five putative source populations (see Figure 4.1.8), based on the otolith probe microanalysis. Results are given in Table 4.4.1. One of these loci, sAAT-1*, showed just significant differentiation at the 5% level, but this test became non-significant following Bonferroni adjustment for multiple tests. The remaining seven allozyme tests all showed probability levels greater than 5%. Similarly, there was no significant mtDNA differentiation among these five assemblages, although the overall level of significance for the comparison was P = 0.08.

The genetic data do not, therefore, indicate any significant differentiation among the five putative source populations. However, it should be stressed that in this subset of data the sample sizes are small, ranging from 7 to 37, and therefore the statistical tests lack power. Even if these populations were reproductively semi-isolated, only small differences in gene frequency would be expected, and these would go undetected given the sample sizes.

As an attempt to overcome the sample size problem, we re-examined in detail two samples which, based on the sub-sample used for probe analysis, constituted relatively 'pure' source populations. The two samples were Eden and Albany #1. Genetic comparison of these two samples alone are summarized in Table 4.4.2. Overall, there is no evidence of genetic divergence between the two samples.

Table 4.4.1. Analysis of allele and mtDNA haplotype frequencies of the five putative source populations identified by otolith microchemistry results.

Sample sizes were, for the allozymes, 8, 39, 21, 26 and 17 respectively, and for the mtDNA, 7, 37, 13, 24 and 16, respectively.

Locus	allele/ haplotype	Perth	Eden	W. Tas.	Albany	GAB	χ^2	P
*sAAT-1**	120	0.125	0.115	0.167	0.096	0.324	9.927	0.039
	100	0.875	0.885	0.883	0.904	0.676		
*ADA**	125	-	0.026	-	-	0.029	17.812	0.884
	115	0.313	.372	.262	0.269	0.324		
	100	0.563	0.500	0.595	0.519	0.500		
	95	-	-	-	0.019	-		
	90	0.063	0.090	0.143	0.173	0.118		
	75	-	0.013	-	-	-		
	60	0.063	-	-	0.019	0.029		
*ADH**	180	-	-	0.024	-	0.029	9.617	0.688
	40	0.250	0.462	0.405	0.346	0.353		
	-100	0.750	0.526	0.571	0.654	0.618		
	-200	-	0.013	-	-	-		
*GPI-1**	115	-	0.051	0.119	0.087	0.088	15.723	0.053
	100	0.938	0.949	0.881	0.913	0.912		
	85	0.063	-	-	-	-		
*IDDH**	160	0.125	0.077	0.190	0.120	0.088	11.541	0.174
	100	0.813	0.923	0.786	0.880	0.912		
	70	0.063	-	0.024	-	-		
*PGDH**	150	-	-	-	0.019	-	26.105	0.326
	135	-	0.013	-	0.038	-		
	120	0.500	0.346	0.381	0.327	0.206		
	110	-	-	-	0.019	0.029		
	100	0.375	0.590	0.524	0.558	0.735		
	90	-	-	-	0.019	0.029		
	85	0.125	0.051	0.095	0.019	-		

*PGM-1**	120	-	0.013	-	-	-	19.177	0.245
	115	-	0.064	0.048	0.022	0.029		
	100	0.875	0.705	0.643	0.891	0.618		
	90	0.125	0.205	0.310	0.065	0.353		
	75	-	0.013	-	0.022	-		
*PGM-2**	300	0.125	0.077	0.048	0.038	0.029	2.898	0.950
	100	0.750	0.769	0.786	0.808	0.794		
	-300	0.125	0.154	0.167	0.154	0.176		
mtDNA	AAA	0.714	0.622	0.308	0.583	0.563	67.220	0.082
	AAB	0.143	-	0.154	-	-		
	AAC	-	0.081	0.231	0.167	0.188		
	AAE	-	0.027	-	-	-		
	ABA	-	0.054	0.077	-	0.125		
	ABB	-	-	-	0.042	-		
	ACA	-	-	-	-	0.063		
	AEC	0.143	-	-	-	-		
	BAB	-	0.081	-	-	-		
	BCB	-	0.108	0.077	0.167	0.063		
	BCC	-	-	0.077	-	-		
	CCA	-	0.027	-	-	-		
	DAC	-	-	-	0.042	-		
	EAB	-	-	0.077	-	-		

Table 4.4.2. Analysis of allele and mtDNA haplotype frequencies of the Albany-1 (*n*=20) and Eden (*n*=108) samples.

Locus	allele/ haplotype	Alb-1	Eden	χ^2	P
*sAAT-1**	120	0.075	0.222	4.834	0.214
	100	0.875	0.773		
	80	-	0.005		
*ADA**	125	-	0.009	6.674	0.278
	115	0.350	0.296		
	105	0.025	-		
	100	0.475	0.532		
	90	0.150	0.153		
	75	-	0.009		
*ADH**	180	0.025	0.005	3.238	0.490
	40	0.325	0.292		
	-100	0.650	0.671		
	-200	-	0.019		
	-250	-	0.014		
*GPI-1**	115	0.063	0.116	1.313	0.436
	100	0.937	0.870		
	85	-	0.014		
*IDDH**	160	0.158	0.083	3.198	0.201
	100	0.842	0.884		
	70	-	0.032		
*PGDH**	150	0.025	-	8.234	0.159
	135	0.025	0.005		
	120	0.225	0.273		
	110	0.025	0.009		
	100	0.625	0.644		
	85	0.075	0.069		
*PGM-1**	120	-	0.009	0.919	0.914
	115	0.029	0.046		
	100	0.735	0.690		
	95		0.005		

	90	0.235	0.245		
	75	-	0.005		
*PGM-2**	300	0.075	0.069	1.557	0.524
	100	0.700	0.782		
	-300	0.225	0.148		
mtDNA	AAA	0.500	0.533	11.400	0.649
	ABA	-	0.067		
	ADA	-	0.017		
	AAB	-	0.017		
	ABB	0.050	-		
	AAC	0.250	0.200		
	AAH	-	0.017		
	BAA	-	0.017		
	BCB	0.200	0.050		
	BAC	-	0.017		
	BCC	-	0.017		
	CCA	-	0.017		
	CCC	-	0.017		
	EAA	-	0.017		

5. General Discussion

Genetic versus Probe-based Approaches to Stock Delineation

The basic premise underlying the current study was that there is a discernible stock structure in Australian jackass morwong, which might be variously detectable depending upon the technique used to assess it. In this study, we attempted resolving stock structure using four techniques: two genetic techniques (allozyme analysis and analysis of mtDNA haplotypes) and two techniques based on probe microanalysis of otoliths (electron probe analysis and proton probe analysis).

The principal conclusion drawn from the genetic techniques was that there is no indication of significant differentiation among the Australian samples, but that, pooled, the Australian samples differ significantly from that collected in New Zealand. In this regard, both the allozyme and mtDNA techniques produced virtually identical conclusions. The otolith-based techniques, however, resulted in just the opposite conclusion: otolith chemistry suggests considerable, if perhaps still confusing structure within the Australian samples, but no detectable difference between samples collected off SW Tasmania and NZ. There are fundamentally two different reasons for these 'contradictory' results.

First, sample sizes or locations may not have been adequate for the analysis. Although sample sizes for all sites were chosen to satisfy the statistical requirements of the various techniques, sample sizes were nonetheless too small at Perth (8 specimens) for effective analysis using either genetic or probe techniques and too small at Maatsuyker (25 specimens) for genetic analysis (neither site was in the original list of sites to be sampled, but were run opportunistically when samples became available). Unfortunately, in retrospect both of these samples turn out to be critical to an interpretation of the stock structure of morwong or a comparison of the various techniques.

In the case of Perth, the otolith techniques consistently identified the Perth sample as distinctive, suggesting strongly a population specific to the west coast. Sample sizes are too small, however, to determine if this population also includes Albany #1, with which it shares several, otherwise distinct chemical characteristics, or to include it in the genetic comparison. Hence, although we infer a Western Australian stock of jackass morwong, we cannot at this stage identify what, if any links it has with the Albany or GAB fishes, or determine whether or not it is also genetically identifiable.

The small sample sizes for Maatsuyker are also unfortunate, in that, again in retrospect, this site could provide a critical test of the relative power of the genetic and microprobe-based techniques for stock delineation. Both the allozyme and mtDNA approaches pool all Australian samples, but distinguish between fish from Australia and NZ. The principal conclusion drawn is that the Australian sites constitute a single genetic population (or more precisely, there is no evidence of regional differentiation among the Australian samples), but that there are clearly separate populations in Australia and NZ. The probe techniques, however, distinguish between, essentially, the Australian mainland sites and, as a group, western Tasmania, Maatsuyker and New Zealand. The separation between these samples is the strongest signal derived from the probe analysis, and confirms the results of the pilot study, which also separated NSW/Victoria from Tasmania. The critical feature here is that the probe data link this Tasmanian population to that in NZ. Such a link, while not inevitable, is a reasonable hypothesis given the off-shore, Tasman Sea distribution of the pelagic post-larvae of jackass morwong (B. Bruce, pers. comm.), previously documented cross-Tasman transport of passive drifters, and a water mass (Sub-Antarctic Water) common to the two sites

The prediction tested, therefore, is that genetic analysis of the Maatsuyker (and western Tasmanian) sample will show a close affinity with the NZ sample, if the probe and genetic techniques reflect similar structuring of the population. In the current study, we were unable to run genetic analyses on fish from western Tasmania (due to lack of samples). The sample for Maatsuyker is too small for a statistically valid comparison of genetic data. Nonetheless, both the allozyme and the mtDNA analyses suggest an affinity between the NZ and Maatsuyker sample, although, again, this affinity at this stage has to be discounted due to the small sample size.

The second reason why the genetic and probe-based techniques produce different scenarios regarding the stock structure of jackass morwong is that they are sensitive to different levels of migration among stocks, and hence provide complementary information. There are grounds to support this argument. As noted above, the probe-based techniques consistently distinguish between a SE Australian mainland population of jackass morwong and one from Tasmania, and provide evidence for further sb-structuring of the population in the GAB and western Australia. Within the constraints of small sample sizes noted above (e.g., Perth, Maatsuyker and western Tasmania), the genetic techniques generally do not support this structuring within the Australian population. Again, there are two reasons why this might be the case.

First, given the relatively long planktonic duration of the larval stage of *N. macropterus* (6-9 months) and the off-shore distribution of the larvae, some exchange of

individuals among stocks is likely. Indeed in the pilot study, we found evidence suggesting a minimum exchange rate between the NSW/Victorian and Tasmanian stocks of about 7%/year, which is high enough to minimize genetic divergence among the stocks. Consequently, it is perhaps reasonable that there is little divergence across southern Australia, but that some separation would be evident across the Tasman Sea, which is perhaps a more effective barrier to individual movement. In this scenario, the probe techniques, which are hypothesised to derive at least some of their resolving power from environmentally induced changes in otolith composition, are less sensitive to this mixing, and hence provide a level of stock resolution finer than that achievable by currently available genetic techniques. Also in this scenario, the lack of a separation between the NZ and SW Tasmanian samples in the otolith results reflects the common environment (e.g., the Sub-Antarctic water mass) linking the two, such that in this case the genetic techniques provide a better means of discriminating among stocks that, although separate, share a common environment. The principal conclusion, then, is that the two sets of techniques resolve different sets of problems: the genetic techniques can resolve better geographically separate stocks that share similar environments, whereas the probe-based techniques resolve better geographically contiguous stocks that, over their range, inhabit somewhat different environments.

The second possibility is that both the genetic and probe-based techniques can resolve the same basic stock structure, but that the former are insensitive to individual variability and these differences are obscured due to pooling of individuals derived from different spawning grounds. We hypothesize that movement of individuals away from their spawning areas results in small percentages of individuals from several source populations being present at each site, such that when all individuals from a site are pooled the genetic features that distinguish among source populations are lost. In this regard, because the probe-based techniques produce a classification for each individual, rather than for the site as a whole, the probe-based techniques can presumably provide a better resolution of migration, etc.

We tested this hypothesis, in part, by re-examining the genetic data in the light of the probe results. Specifically, we used the classification of individuals derived from the probe-data to re-sort fish for the genetic analysis, ignoring where the individuals were caught. Results, shown in Table 4.4.1, are suggestive, but, unfortunately, not definitive. Unlike the larger scale genetic analysis of the Australian specimens, which indicated no differences among the sites, re-pooling based on probe-based classification results in one allozyme being different among sites (at $p = 0.04$), another not quite significant ($p = 0.053$), and the mtDNA distribution approaching significance (at $p = 0.08$). Correction for multiple comparisons renders none of these statistically conclusive, but the shift in overall significance from broadly non-significant to several samples approaching significance appears suggestive. The principal reason for ambiguity in the conclusions that can be drawn is, again, sample size.

Because only 261 specimens were run on the probes and hence classifiable, the total sample sizes of 'classified' individuals available for genetic re-analysis is too small for statistically robust analysis of allozyme or haplotype frequency distributions. For genetic analysis, samples sizes on the order of 100 individuals per group are preferable, a sample size not approached for any of the groups based on re-classification (which range in size from 8 to 39 fish).

Hence, at this stage we think it likely that the genetic and probe-based techniques provide different and complementary levels of resolution of population structure in *N. macropterus*, but cannot yet rule out the possibility that results from the two techniques may well be similar if fish can be analyzed on an individual, rather than a site-pooled basis.

Comparative Power of the Genetic and Probe-based Techniques

One objective of the current study was to compare the resolving power of the two genetic techniques to each other, and to assess the relative value of two different probe-based techniques (electron probe microanalysis and proton probe microanalysis) for stock delineation.

With regard to the genetic techniques, the haploid, maternally inherited nature of mitochondrial DNA should make it a more powerful tool for examining population subdivision than the allozymes coded by nuclear DNA. In this study, however, the allozyme approach arguably provided more evidence for population subdivision than did the mtDNA analysis. For example, with respect to chi-square analysis, the probabilities of no significant differentiation between the New Zealand sample and the pooled Australian sample were 0.001 for alleles at the sAAT-1* locus, and 0.011 for a combined probability across all eight polymorphic allozymes, but only 0.023 for mtDNA haplotypes. Furthermore, while the mtDNA GST-Nei values are about three times greater than for the allozymes, much of this is attributable to the increased sampling error associated with the mtDNA analysis: the probability that the allozyme GST-Nei comparison between the pooled Australian samples and the New Zealand samples arises by chance is in fact an order of magnitude smaller than that derived from the mtDNA comparison (P=0.002 and 0.024, respectively).

In this instance, we suspect that the greater variance of the haplotype than allozyme GST-Nei values to a large extent reflects the substantially greater sample size used in the latter analysis. Nearly 1800 nuclear alleles (880 fish) were examined for each of eight independent allozyme loci, with heterozygosities or genetic diversities ranging from 0.237 to 0.592 per allozyme. This compares with 500 mitochondrial DNA alleles with a genetic diversity of 0.635.

The results of this study, therefore, do not argue that one approach should be preferred over the other: both clearly have advantages and disadvantages, and their joint application to studies of population structure will always be more powerful than the application of a single approach. .In the current study, the congruence of the two data sets gives added confidence that there is indeed weak but significant differentiation of the New Zealand sample from the Australian samples.

Regarding the probe-based techniques, nine elements differed significantly across the sites sampled: the five micro-constituents detectable using electron probe microanalysis and 4 of the 11 elements present in lower concentration (<100 ppm) and detectable using the proton probe micro-analyzer. Ultimately, most of these elements proved of little or no significance in terms of apparent stock delineation in jackass morwong: most of the information on which stock structure was deduced was conveyed by four elements - Cl, Sr, K and Pb. Of these, Pb is of questionable value for two reasons: first, its mean concentration at all sites but one (Perth) was less than the minimum detection limit for the element, and second, the Perth sample, while significantly different from other sites, is based on the smallest sample size of any of the sites examined and hence the difference could well derive from a sampling problem. In practice, much the same separation of sites develops from an analysis of only the electron probe data as from the full, 17-element data set, which implies that, in this instance, involving proton probe microanalysis in the study contributed relatively little to the outcome.

Nonetheless, that four of the eleven trace elements differed significantly among sites suggest it is premature to discount proton probe analysis as a tool for stock delineation. Of the four elements that differed among sites, two (Hg and Se) were consistently present at concentrations well above their respective MDL's. Until there are more data available about geographic variability in the concentrations of these two elements, at least, in fishes, it is probably unwise to discount either as the basis for stock identification. Hg, in particular, may be of value in delineating among local populations of deep-water fishes, which are subject to high, and highly variable sources of natural Hg accumulation, due to their apparent great age (maximum > 50 years) and localised sources of natural seepage in the SE Australian region.

Stock Structure of Jackass morwong

The totality of the available data suggest at least two genetic stocks of jackass morwong: one stock appears confined to Australia, the second consists of at least NZ, but may also include southern and western Tasmania. As noted above, sample sizes for the latter are too small for an effective genetic analysis, such that the affinities of the Tasmanian and NZ stocks of morwong remain uncertain. The probe-based data suggests at least one

additional stock, off western Australia; we have inadequate samples for this site for a genetic analysis. Finally, the probe-based techniques also suggest a weakly definable stock in the GAB, with affinities primarily for the Tasmanian stock.

As noted above, there are several possible explanations for the lack of genetic differentiation among sites off Australia. The most parsimonious explanation, however, is that there is enough mixing at the larval and adult stages along a continuous continental shelf to prevent local genetic differentiation.

The probe-based results are much more difficult to interpret. Concentrations of nine elements differ significantly among sites in Australia, which appears to imply considerable local structuring of the morwong population. Exactly what this structure is, however, is difficult to specify. Both the pilot study and the current one strongly suggest a 'SE Australian mainland population', characterized best by very high Cl concentrations, and a 'Tasmanian' population easily diagnosed based on extremely low Cl concentrations. The distribution of these two stocks and the degree of overlap between them is uncertain, however. In the pilot study, the mainland population included samples of NSW and off eastern Bass Strait, and were easily distinguishable from Tasmanian samples taken off both the east and west coasts of the island. The more recent, and more detailed study, however, linked the east coast Tasmanian samples with that of the mainland, and indicated the 'Tasmanian' fish were present only on the SW and west coast of the island. There are similar problems in interpreting other data sets: the marked differences among three samples taken off Albany, over a five month period, and the differences between two GAB samples, taken two months apart, have been previously noted.

We strongly suspect that these apparent difficulties are the result of stock movements. The explicit assumption that underlied our choice of jackass morwong for this developmental study was that adults were relatively sedentary. This assumption was based primarily on tagging studies carried out off NSW and Victoria, which reported the large majority of recaptures, even after several years, near the tagging sites. This observation, however, conflicts with both reports from industry (that the NSW/Victorian fish migrate to NE Tasmania seasonally) and with tagging studies off NZ, where high level of long distance movement has been reported. The apparent mixing of individuals from a number of putative source populations (e.g., Figure 4.1.9) is certainly suggestive of this sort of long distance movement along the Australian continental shelf, and evidence of a change in population characteristics seasonally (e.g., Figure 4.1.10) further suggest large scale seasonal changes in stock distributions.

If such movements are the case, how then to explain the results of the NSW and Victorian tagging studies? If the NSW/Victorian morwong stock migrates to Tasmania seasonally, then at least some tag returns should have come from that region; in fact, none apparently did. We suspect the answer lies in the relative sampling effort. As indicated by Tilzey et al. (1990)(figures 3 and 4), total annual fishing effort off southern NSW is ten to thirty times greater than fishing effort off the NE Tasmanian coast. Targeted fishing for morwong off Tasmania, until recently at least, was also likely to be confined mainly to the immediate vicinity of Hobart, to supply the local fresh fish market. The overall effort devoted to this species farther up the coast may well have been many times less than even the summary statistics suggest. Smith (1989) indicates a total recapture of 120 of the approximately 2500 fish he tagged off southern NSW (4.78%). Given a much lower sampling effort, returns off the Tasmanian coast were likely to never be more than 10 tags, and even this would depend on the nature of any seasonal movements, how long the fish spent in Tasmanian waters and the extent to which this coincides with the seasonality of fishing effort of that coast. Hence, while we cannot be certain we suspect that the absence of tag returns off Tasmania from this study are not definitive, particularly in the light of industry comments of a well defined migration down the coast.

Unfortunately, if such migrations are the norm, they vastly complicate both defining the structure of the jackass morwong stocks and increase substantially the data needed to do so. At the minimum, we would need samples from all sites at least 6 months apart (winter versus summer, or spawning period versus non-spawning period). The current study was never designed with that level of sampling effort in mind. Hence although we feel we can block out the main stock structure, i.e., at least three stocks focussed on SE Australia, western Australia and NZ/possibly southern Tasmania, we cannot do more than that without additional sampling effort to resolve the question of seasonal shifts in stock distributions.

6. Implications and Recommendations

The implications and recommendations derived from this study fall into two groups - those related to the comparative power of the various techniques for documenting stock structure, and those related specifically to stock structure of jackass morwong.

1. Comparative power of techniques

- All four techniques trialed in this study contribute to resolving the structure of the jackass morwong stocks. Of the four, proton probe microanalysis was relatively unimportant, with regard to the probe-based stock structure, and analysis of allozyme and mtDNA distributions revealed essentially the same stock structure. To the extent that redundancy strengthens the interpretations drawn, application of both genetic techniques to the problem appears justified.
- Although the analysis is weakened by small sample sizes for key sites, it appears that the probe-based techniques reveal a finer level of stock structure than either of the genetic techniques.
- However, the techniques appear to be complementary, to the extent that they resolve different sets of problems: the genetic techniques appear to resolve better geographically separate stocks that share similar environments, whereas the probe-based techniques resolve better geographically contiguous stocks that, over their range, inhabit somewhat different environments.
- Due to the typically ambiguous nature of stock structure in marine fishes, which has prevented such information being widely incorporated into management plans, we recommend that at least one genetic technique and a probe-based technique be routinely applied to questions of stock structure.
- The strength of the current comparison of techniques would be increased greatly by a small amount of additional data. Sample sizes for several sites, sampled opportunistically but ultimately critical to the analysis, are much too small for effective analysis. In particular, genetic analysis of samples off western Tasmania and Western Australia, and probe analysis of additional specimens from Perth, would permit resolution of the status of those sites with respect to the remaining sites off Australia and NZ..

2. Stock Structure of Jackass morwong

- There are at least two, and possibly five stocks of jackass morwong in Australian and NZ waters. Despite small sample sizes and lack of genetic data for Perth, there is almost certainly different stocks off Western Australia and the SE Australian mainland. Probe analysis also indicates a separation between a southwestern Tasmanian stock and the mainland stocks. Genetic analysis indicates a separation between the Australian and NZ

stocks, but the question of whether or not the southern Tasmanian stocks are linked to NZ, rather than (genetically) to the Australian mainland, is unresolved.

- The available evidence suggests much greater mobility of the jackass morwong stocks than has previously been thought likely. As a result, management based on static stock boundaries are likely to be ineffective unless they are so large as to encompass the entire ranges of several seasonally overlapping stocks. We note, in particular, current problems regarding SEF stocks, where jackass morwong off the SE mainland coast are considered fully or over-exploited, whereas those off Tasmania are suggested to be lightly exploited. Drawing an unambiguous boundary between the two stocks is likely to be difficult, given their possible overlap seasonally off the east coast of Tasmania. If any part of the SEF can be managed separately, it is likely to be only the far southwest region.
- Although data are still preliminary, it is likely that jackass morwong in Western Australia should be managed as a stock separate from the SEF and GAB fisheries.

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Appendicces

Appendix 1. Original Application

Fishing Industry Research and Development Trust Fund
Application for New Grant
1991/92

1. Project Title

A comparison of otolith microchemistry and genetic techniques for evaluation of stock structure of the jackass morwong, Nemadactylus macropterus.

2. Keywords

Jackass morwong, Biochemical genetics, Microcomposition, Otoliths, Stock structure.

3. Objectives

1. To compare the stock structure of Jackass morwong in Australian waters as indicated by four different techniques, in order to determine the most effective means of undertaking such studies;
2. To determine definitively the number of Australian stocks of Jackass morwong and map their distribution; and
3. To determine if Jackass morwong in Australian and New Zealand coastal waters are parts of the same stock.

4. Justification

Over the last three years, we have been working on a new technique to determining stock structure and measuring rates of movement by fishes. This technique involves analysis of otolith composition. Results to date on one of the prime study species, jackass morwong, indicate strongly three stocks in southern Australian waters: one stock that encompasses NSW and Victoria, a second that encompasses both eastern and western Tasmania, and a

third stock in the Great Australian Bight (Figure 1). The results of this work have been presented in summary form to FIRDC and the Demersal and Pelagic Fisheries Research Group and will be detailed in a final report to FIRDC nearing completion. These results are important for two reasons. First, they appear to conflict with available genetic data regarding stock structure of morwong in Australian waters. Richardson (1982) analyzed allozymes of fishes from Tasmanian, Victorian and NSW waters, and found no evidence of structuring of the stock over this area. The microprobe data, however, indicates strongly that Victorian/NSW and Tasmanian stocks are quite distinct. Second, the results bear on management strategies for the species. Currently, DPFRG and SETMAC manage jackass morwong on the basis that it is a single stock (see Tilzey, et al., 1990), based largely on the allozyme analysis of Richardson (1982). The microprobe results suggest that this is inappropriate.

There are two reasons why results from the microprobe and electrophoretic studies might differ: (1) the techniques provide complementary data and reflect different degrees of sensitivity to movement by individuals among populations, or (2) the probe and/or electrophoretic results are flawed, due to inadequate sample sizes, poor technique or incorrect implicit assumptions. We need to resolve which of these two possibilities is correct. In the near future we anticipate evaluating definitively the geographic structure of all of the major species in the SET. To do this efficiently, we need first to determine the relative effectiveness of probe and genetic studies for stock identification. Microprobe studies for purposes of stock structure evaluation are time-consuming and expensive, though in some respects logistically more simple (e.g., do not require ultrafrozen material and often can use already extensive time-series collections of otoliths). They are warranted only if they provide information not available from less expensive, more conventional sources, such as analysis of allozymes and mitochondrial DNA. At this stage, it is not clear that this is the case.

Therefore, we propose to conduct parallel analyses of the stock structure of jackass morwong using two microprobe techniques (electron and proton probe microanalysis) and two genetic techniques (mitochondrial DNA and allozyme analysis). Mitochondrial DNA analysis is likely to have greater resolving power than allozyme analysis for sub-population discrimination (Avise 1987; Ward et al. 1989). This is because restriction enzyme analysis of DNA allows variability at synonymous and non-coding DNA sites to be detected, and because mtDNA evolved at 5-10 times the rate of nuclear DNA.

Samples previously collected for the probe work were not preserved suitably for genetic studies, so the work proposed will involve, first, collections of suitable representative samples from throughout the Australian (and NZ) range of the species, and second, analysis of the material using now well developed procedures for each of the techniques. The proposed study will have four benefits: first, it will resolve the question of the sensitivity of the different techniques in evaluation of stock structure; second, it will confirm (or refute),

with more extensive samples, Richardson's (1982) conclusions based on allozyme distributions; third, it will determine whether Australian and New Zealand stocks are different (Richardson suggested this was the case, but apparently did not directly compare samples from the two populations); and fourth, it will produce a more detailed analysis of stock structure in the Australian morwong population, including identification of overlap zones between adjacent stocks and extension of the microprobe and genetic results to the full Australian geographic range of the species.

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5. Proposal in Detail

(a) Plan of Operation

Collection of samples

Adult jackass morwong will be obtained from the commercial catch and, opportunistically, from research cruises in southern Australian waters that involve demersal trawling. With regard to the latter, we will solicit the assistance of state fisheries organizations throughout the southern Australian region and, for New Zealand, Dr. Peter Smith (MAF). Dr. Smith has previously examined allozyme distributions in NZ morwong (Gauldie & Smith, 1978) and has indicated his willingness to supply specimens. As it is critical for allozyme and mitochondrial studies that specimens be either fresh or frozen, we will ensure that in research

and commercial fishing operations the appropriate level of preservation is carried out. The spatial resolution of the study will depend on availability of specimens, though clearly we will make special efforts to obtain material from apparent areas of overlap between adjacent stocks (Bass Strait and western Victoria). We expect to examine at least twenty individuals (up to 100 specimens for genetic studies) from at least ten sites in Australian waters, plus material from NZ waters. From each individual, we will extract tissues for genetic analysis and both sagittae, to be used in probe studies.

Genetic Analysis

Allozymes: Richardson (1982) concluded his search for suitable genetic markers after he had found five polymorphic enzymes. We will continue the search for polymorphic proteins to enhance the power of such tests of stock structure, and will use both starch and cellulose acetate gel electrophoresis. All fish will be analysed electrophoretically for all loci identified as polymorphic. Gene frequencies from different areas will be compared statistically and use will be made of the BIOSYS-1 software package to estimate the genetic distances between subpopulations. Repeat samples will be taken from one or two locations to assess the extent, if any, of inter-seasonal genetic variability.

Mitochondrial DNA: Mitochondrial DNA will be extracted from liver and/or ovary samples, purified, and digested with a range of restriction enzymes recognizing different six base nucleotide sequences. Fragments will be separated using both agarose and acrylamide gels and visualized using ethidium bromide staining or through the use of end-labelling radioactive techniques. Haplotype frequencies will be determined and samples from different areas compared. Statistical analysis will include the use of software packages such as PHYLIP and PAUP.

Analysis of Otolith Composition

Procedures for sectioning and preparing morwong otoliths for probe analysis have been well established as a result of our previous work on the species. In brief, sagittae are sectioned along the major growth axis, at a plane approximately 50 μm dorsal to the primordium. The primordial plane is then exposed by successive polishing with increasing fine compounds, ultimately to produce the "mirror smooth" surface required for electron probe microanalysis. We have also developed an appropriate strategy for the fine scale chemical analysis of morwong otoliths (beam conditions, dwell times, order of analysis) that provides an acceptable trade-off between sensitivity and (expensive) probe time (Gunn, et al., ms.). These now standard procedures will be used in the proposed work.

Stock structure in morwong will be determined using two complementary probe techniques: electron probe microanalysis and proton probe microanalysis. There are two reasons to compare results of the two techniques. First, the two analytical machines provide data on concentrations of almost entirely different set of elements; the proton probe provides high quality data on, for example, trace metals, but cannot effectively measure concentrations of the light elements, such as sodium and chlorine, that are measured using the electron probe. It is not clear that the different sets of elements will indicate the same stock structure, or be equally sensitive to movements between areas. Second, pilot studies with the proton probe suggest it may enhance stock separation based on data from the electron probe. The stock separation depicted in figure 1 is based on the six elements we can reliably measure using the electron probe; this would presumably be our primary means of continued analysis of stock structure. In this analysis, separation of the NSW/Victorian stock from those in the GAB and off Tasmania is clear-cut; separation of the GAB and Tasmanian stocks, while statistically robust is more problematical. First results from work on the proton probe indicate that morwong otoliths from the GAB have much higher trace metal concentrations than those from fish collected elsewhere. If this is confirmed, then use of trace metal data along with the electron probe data could result in 100% separation of all three apparent Australian morwong stocks, i.e. each individual could be unambiguously identified as to its source location and stock identity.

Literature Cited

Gauldie, R.W. & Smith, P.J. 1978. The adaptation of cellulose acetate electrophoresis to fish enzymes. Comp. Biochem. Physiol., 61B: 421-425.

Gunn, J.S., I. Harrowfield, C. Proctor & R. E. Thresher. Ms. in review. Electron probe microanalysis of calcified tissues in fishes - evaluation of techniques appropriate to studies of age and stock discrimination.

(ii) Facilities Available

CSIRO Marine Laboratories, Hobart provide office and general laboratory facilities and a full back-up of computing, technical and library services. Biochemical genetic studies will be conducted at the Marine Laboratories, which has a fully equipped facility for undertaking such work. Preparation of otoliths for probe microanalysis will also be done at the Marine Laboratories. Electron probe microanalysis will be done on the Cameca Wavelength Dispersive Probe, at the CSIRO Division of Mineral Products, Port Melbourne,

and the proton probe work at the Heavy Ion Analytical Facility, CSIRO Division of Exploration Geoscience, Sydney. We have established good working relationships with staff at both facilities.

(b) Supporting Data:

Biochemical genetic studies will be supervised by Dr. Robert Ward.
Dr Ward joined CSIRO Fisheries in January 1990 from Loughborough University, U.K., and is currently carrying out an appropriation and GITLC funded study into morphological and genetic variation in Australian samples of the orange roughy. He is an experienced biochemical geneticist who for the last twenty years or so has used allozyme-based techniques in analyses of the population structure of a variety of invertebrate and vertebrate species, and has published about 60 papers in this area, including a general review. Recent work includes studies of stock structure, hybridization and phylogeny in several groups of freshwater fishes and studies of the phylogenetic relationships of a number of European species of the intertidal molluscan genus Littorina.

Dr Grewe has six years experience of mitochondrial DNA analysis in fishes, having previously worked on mtDNA variation in lake trout populations in Canada and the US. He is experienced with all relevant techniques including recent approaches such as DNA amplification using the polymerase chain reaction.

Dr Thresher's group has been engaged in a detailed study of the use of otolith microchemistry as a means of stock discrimination in jackass morwong (and southern bluefin tuna) for the last three years. The techniques developed for analysis of fish otoliths are state-of-the-art, and have been discussed extensively with the few other scientists engaged in similar studies elsewhere in the world. In part as a result of such studies, Dr. Thresher has received funds (from DITAC and, in the USA, the National Science Foundation) to host an international workshop in Hobart on application of microprobe techniques to fisheries science. The workshop will be held in March, 1991 (immediately prior to the start of this grant) and will involve in round-table discussions approximately 20 physicists, chemists, statisticians and biologists from Australia, New Zealand and North America.

6. Research Priority

The proposed study is directly relevant to FIRDC priorities regarding innovative methods of stock assessment, and will establish a firm basis for future studies of stock structure in

Australian finfish species. The proposed study will also have direct relevance to current management strategies for a major component of the SET fishery.

7. Transfer of Results to Industry

Results of this work will be communicated directly to DPFRG and SETMAC. A summary of the work will be published in Australian Fisheries, and technical publications will be submitted to major international scientific journals.

8. Predicted Commencement and Completion Date

Commencement Date 1 July 1991 Completion Date 30 June 1992

9. Requested Budget

Requested Budget	$
Salaries	58,139
Operating Expenses	39,000
Travel	11,421
Capital Items	0
Total	108,560

10. Funds Sought from Other Sources

None

11. Financial Contribution of Applicant

(a) Salaries of Existing CSIRO Staff
(including Overhead)

1 CSOF7 (Thresher 40%) 23171

1 CSOF7 (Ward 25%)	15364
1 CSOF4 (Grewe 40%)	14556
1 CSOF4 (Proctor 50%)	17434
1 CSOF5 (Elliott 10%)	4474
Total Salaries(including on costs)	112,577

(b) Operating Expenses Nil

(c) Capital Cost of Equipment Available

Genetics Laboratory	130000
Leitz Orthoplan Microscope and Associated Image Analysis Equipment	55000
Leitz Laborlux D Transmitted/ Incident Light Compound Microscope	10000
Electron Microscope Unit	275000
Proton Probe Microanalysis Unit (Division of Exploration Geoscience)	1500000
Electron Probe Microanalysis Unit (Division of Mineral Products)	750000
Total Capital	2,720,000

TOTAL CONTRIBUTION OF APPLICANT 2,848,270

12. Detailed Budget

Item	Requested Budget $
SALARIES	
Technical Officer (2 Max, to be appointed, to assist in preparation of otoliths for microanalysis)	32319
Super-annuation	5947
Comcare	807
Leave-loading	510
Sub-total	40583
Technical Officer (1 Max, 50%, to be appointed, to assist in preparation of material for biochemical genetic studies)	15149
Super-annuation	2787
Comcare	380
Leave-loading	240
Sub-total	19056
Total Salaries and Wages	58139
OPERATING EXPENSES	
Cost of Operating Electron Probe (based on estimate provided by Division of Mineral Products)	10000
Cost of Operating Proton Microprobe (based on estimate provided by Division of Exploration and Geoscience)	10000

Consumable supplies for biochemical genetic studies

1. Allozyme electrophoresis 5500
2. Mitochondrial DNA 6000

Total 11500

Consumable supplies for preparation of material for probe analysis (largest single item - 200 ultrapure glass rounds required for proton probe analysis, at $22 @) 6000

Cost of collecting and shipping specimens 1500

Total Operating Expenses 39000

TRAVEL EXPENSES

Three trips to Melbourne, each of 7 days duration (per diem based on Melbourne rate of $127.50/day). 3877

Three trips to Sydney, each of 7 days duration (per diem based on $154.50/day) 5044

Two trips to assist in collecting suitably preserved specimens for genetic studies (unspecified locations, as required to fill gaps in sampling coverage) 2500

Total Travel expenses 11421

CAPITAL ITEMS Nil

TOTAL REQUESTED BUDGET 108,560

ESTIMATED INCOME Nil

13: Organization:

Division of Fisheries,
CSIRO Marine Laboratory, G.P.O. Box 1538,
Hobart Tasmania 7001
Telephone: (002) 206 222 Telex: 57182 Fax: (002) 240 530
P.C. Young, Ph.D., Chief

14: Project Supervisors:

Ronald Thresher and Robert Ward
Principal Research Scientists
Division of Fisheries,
CSIRO Marine Laboratory, G.P.O. Box 1538,
Hobart Tasmania 7001
Telephone: (002) 206 222 Telex: 57182 Fax: (002) 240 530

15. Staff:

		% of Time on Project		Role
		CSIRO	FIRDC	
R.E. Thresher	Ph.D.		(40)	Project Co-Supervisor
R. Ward	Ph.D.		(25)	Project Co-Supervisor
P. Grewe	Ph.D.		(40)	Mitochondrial Studies
C. Proctor	B. Sc. (Hons.)		(50)	Microprobe Operations
N. Elliott	Ph.D.		(10)	Allozyme Studies
Technical Officer	(to be appointed)		(100)	Otolith Specimen Preparation
Technical Officer	(to be appointed)		(50)	Genetic Specimen Preparation

16. Administrative Contact

P. Green
Divisional Finances Officer,
Division of Fisheries,
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