Refinement of Barramundi Stock Definition; Particularly in Relation to Access and Allocation to Competing User Groups.

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NON-TECHNICAL SUMMARY

This study addressed two separate but related aspects of the biology of barramundi. The first extended the knowledge of the distribution of genetic stocks of barramundi. The second examined the possibility that populations of barramundi exist which do not have a freshwater phase as the current understanding of the life cycle dictates. This aspect of the work was conducted on barramundi from the Mary River and Chambers Bay (Figure 1).

Section (i) - Genetics stocks

Previous barramundi genetic studies have shown the existence of several distinct stocks in Northern Territory waters. With one exception, each sample has proved to be distinct from all others. Thus it was considered likely that the actual number of discrete stocks in the Northern Territory was much greater than indicated by the original sampling undertaken. The accurate definition of unit stocks enhances the opportunities for applying different management regimes to stocks in separate areas. In addition, because of the debate on the pros and cons of transferring barramundi of different genetic stocks between areas for reasons of stocking or aquaculture, clarification of the population structure would benefit these discussions.

In the Northern Territory, this study examined six samples, five from areas not previously sampled and a repeated sampling of the Daly River stock for a temporal comparison, seven years after the original sample was taken. Another 12 new Queensland samples were included in the overall study to provide a thorough re-examination and re-interpretation of the nature of barramundi genetics in tropical Australia. Three new barramundi stocks were identified from the six additional Northern Territory samples (P#; III, V and VII in Table 1). The ten stocks of barramundi that have been identified in the Northern Territory are essentially reproductively isolated and hence are self-sustaining populations that require independent management (in the short term).

Table 1. Ten identified genetic stocks of barramundi from the Northern Territory:

P#	STOCK	GEOGRAPHIC AREA
I.	Joseph Bonaparte Gulf	Ord River, W.A. - Moyle River
II.	Anson & Fog Bays	Daly River - Finniss River
III.	Darwin	Darwin Harbour - Shoal Bay
IV.	Chambers Bay	Mary River
V.	Bathurst Island	Port Hurd
VI.	Castlereagh Bay	Goyder River
VII.	Buckingham Bay	Buckingham River
VIII.	Blue Mud Bay	Walker River
IX.	Limmen Bight	Roper River
X.	Sir Edward Pellew Group	McArthur River

This study went beyond describing stock structure to develop innovative statistical analysis of the extensive data (6000 fish). Through the use of the (theoretically correct) one-dimensional stepping model, migration between populations was estimated to be two orders of magnitude higher than previously thought, at approximately 1% between adjacent stocks every generation.

Five major conclusions were derived from this study.

- The study confirmed the subdivided nature of barramundi stocks around the coast of Australia. New stocks were found and the geographic extent of other stocks were refined. The importance of management of population levels within each stock so that recruitment overfishing does not occur was emphasised.

- The level of inter-stock migration was far higher than first predicted. The level of migration observed could result from either infrequent migrations of juveniles, primarily driven by flood plumes that travel along the coast, or from the movement of

a 'marine' stock of fish for which tagging data has never been obtained (see study (ii). Tagging studies on barramundi in Australia have only been conducted on riverine fish.

- The overall study is responsible for a reappraisal of the management of translocations between barramundi stocks. The results indicate that the stock structure within Australian populations has developed recently. Natural hybridisation has been proposed, and there appears to be little evidence of decreased viability usually associated with hybridisation between distantly related populations.

- An explanation of the evolution of population structure has been provided, with reference to the biogeography of the habitat. Because of the recent and rapid nature of the recolonisation of the Arafura Sea and Torres Straits, this zoogeographic hypothesis is applicable to many of the other marine species inhabiting this region and assists with understanding the current distribution of tropical freshwater species.

- While studies have often suggested that the one-dimensional stepping stone model may be used to explain population differentiation, this study is the first that examines the application of this model to observed data. On both theoretical and practical grounds the one-dimensional model accurately explains the data. Application of this model to other studies, particularly within river systems, is recommended.

The results of this study were combined with the results of a parallel study funded through an ARC Postdoctoral Fellowship and were published in full in the following paper:

Keenan, C.P. (1994) Recent evolution of population structure in Australian barramundi, *Lates calcarifer* (Bloch): An example of isolation by distance in one dimension. *Australian Journal of Marine and Freshwater Research* **45**:1123-1148

Section (ii) - Scale chemistry and marine stocks

Commercial fishermen, particularly in the Mary River region (Figure 1) of the Northern Territory, have suggested that there are possibly populations of barramundi which live in areas relatively remote from rivers, have no fresh water residence phase, and therefore are possibly never readily accessible to the recreational sector which operates largely in freshwaters.

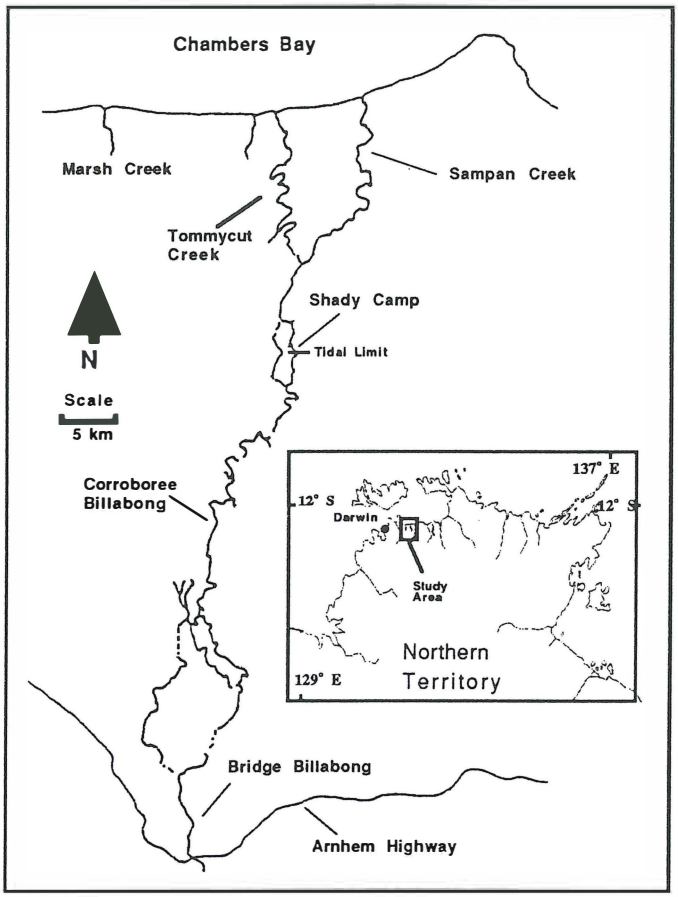
If such stocks are found to exist, it may be that some degree of commercial utilisation of them, together with the other major target of the coastal gillnet fishery, threadfin salmon, could be permitted with minimal effects on stocks available to the recreational sector.

The chemical composition of scales, otoliths or bones of fish have been found to reflect the chemical composition of the waters in which the fish has been resident. Using this method it has been possible to reliably identify fish from different regions of similar environment and from different environments (such as fresh, brackish or salt water) within a region. By examining parts of scales or otoliths laid down in particular years (ie. between specific growth checks or rings) it is possible that residence environment in particular years of life could be determined. In this study the concentrations of various elements (primarily barium and strontium which indicate freshwater and saltwater respectively) in scales of barramundi from different habitats were determined. Scales of barramundi raised for a year in fresh and saltwater aquaculture ponds were used to determine the levels of barium and strontium accumulated by barramundi in those habitats. Then the scales of 197 barramundi (Table 2) taken from the freshwater parts of the Mary River (Corroboree and Bridge Billabongs), estuarine parts (Shady Camp and Sampan Creek) and marine areas of Chambers Bay near Marsh Creek were analysed.

Table 2. Details of barramundi scale sample collections.

Sample Set	Sample Location	Habitat Type	No. of Fish
"Wildstock" Set	Marsh Creek	Marine	105
	Sampan Creek/Shady Camp	Estuarine	12
	Corroboree Billabong	Fresh	61
	Bridge Billabong	Fresh	19
"Growout" Set	Howard River	Estuarine	34
	Berry Springs	Fresh	22

Figure 1. Scale chemistry study area showing sampling locations in and adjacent to the Mary River. Discontinuous streams represent dry season conditions. Inset shows the study area in relation to the northern part of the Northern Territory.



It was clearly shown that the barramundi raised in freshwater had high levels of barium and those raised in saltwater had high levels of strontium, reflecting the concentrations in the water.

Two different statistical methods, discriminant function analysis and cluster analysis, were used to classify the fish taken from the different areas of the Mary River and Chambers Bay. The first method classified the wild caught fish based on how similar their scale chemistry was to either the fresh or salt water pond raised fish. The cluster analysis grouped the fish into clusters according to the similarity of their barium and strontium levels. The discriminant analysis classified all the Marsh Creek fish as saltwater fish and 100% of the Bridge Billabong and 80% of the Corroboree Billabong fish as fresh water fish (Table 3). Twenty percent of the fish from Corroboree Billabong had levels of strontium high enough for them to be classified as saltwater fish, indicating that they perhaps had spent one year in saltwater before migrating upstream to the billabong.

The cluster analysis essentially placed the fish in to three groups (Table 3); a marine cluster with high strontium and low barium, a billabong cluster with high barium and low strontium and a mixed cluster with intermediate levels of both elements. The marine cluster contained 92% of the fish from Marsh Creek and 17% of the Sampan Creek fish. The low level of barium in these fish suggests that they have not had a period of residence in freshwater as is believed to be typical of barramundi. The billabong cluster contained 72% of the Bridge Billabong fish 33% of the Corroboree Billabong fish. The mixed group consisted of 67% of the Corroboree Billabong fish, 28% of those from Bridge billabong, all of those from Shady Camp and only 8% of those from the Marsh Creek area. The intermediate levels of both the freshwater and saltwater indicator elements suggests that these fish have all had a mixed residence history, partly in fresh water and partly in saltwater. Fish in the mixed cluster which were found in the billabong tended to be younger than those in the billabong which were placed in the billabong cluster. Those fish, mostly two year olds, probably spent one year in the sea and then moved into freshwater, hence the saltwater component of the scales is more influential than in those fish which migrated upstream in their first year.

Table 3 Composition of clusters and discriminant groups showing the number in each group and the mean concentration of barium and strontium (parts per million) for the group. SE = standard error of the mean.

Group/Cluster Name	Group/Cluster Composition	No. Fish	Ba (ppm) Mean	Ba (ppm) SE	Sr (ppm) Mean	Sr (ppm) SE
Clusters						
'Marine' Cluster	100% of Howard River 92% of Marsh Creek 17% of Sampan Creek	132	13.7	1.4	552.0	9.7
'Mixed' Cluster	67% of Corroboree B'bong 28% of Bridge B'bong 100% of Shady Camp 8% of Marsh Creek 17% of Sampan Creek	61	111.2	5.2	251.8	6.7
'Billabong' Cluster	100% of Berry Springs 72% of Bridge Billabong 33% of Corroboree B'bong	56	199.1	5.5	289.9	4.6
Discriminant Groups						
'Saltwater' Group	100% of Marsh Creek 100% of Shady Camp 50% of Sampan Creek 20% of Corroboree B'bong	126	28.9	3.4	489.8	13.8
'Freshwater' Group	100% of Bridge Billabong 80% of Corroboree B'bong 50% of Sampan Creek	71	180.7	8.7	291.5	13.3

These results indicate quite strongly that most barramundi which live in areas relatively remote from the main river channels such as the Marsh Creek area, probably do not migrate to fresh water but live their whole lives in saltwater. While this is the most likely explanation for the observed result it is not the only one possible. Further work examining the chemical composition of otoliths, using recently developed microprobe techniques, could however be used to describe more conclusively the lifetime habitat usage of individual barramundi. Some exploratory work of this nature conducted in the course of this study provided strong indications that this is the case. The results of this project are to be published in Transactions of the American Fisheries Society in a paper entitled "Habitat History of Barramundi *(Lates calcarifer)* in a North Australian River System Based on Barium and Strontium Levels in Scales".

BACKGROUND

As management of the barramundi fishery in the Northern Territory is refined, and questions of resource allocation are being addressed, it is becoming necessary to more accurately determine the distribution of distinct fishery 'unit stocks'. Previous studies in northern Australia (Shaklee and Salini, 1985; Salini and Shaklee, 1988) using electrophoretic genetic techniques had shown the existence of several distinct stocks in Northern Territory waters. With one exception, each sample taken (usually representing the population of a major river system) proved to be distinct from all others. Thus it was considered likely that the actual number of distinct stocks was much larger than indicated by these studies. The accurate definition of the Northern Territory barramundi stocks would enhance the opportunities for applying different management regimes to stocks under different fishing pressures.

There was also significant debate on the pros and cons of transferring barramundi of different genetic stocks between areas for reasons of stocking or aquaculture (Stoddart and Trendall, 1990; Keenan and Salini, 1990). A lack of knowledge about the adaptive significance of stock structure led Keenan and Salini (1990) to conclude that a cautious approach to translocations between stocks should be used. They recognised that knowledge of the evolutionary history of these stocks would benefit these discussions through an understanding of the time taken for the stocks to develop and therefore the adaptive changes that may have occurred in each stock.

Section (i) of the project addresses this question.

Studies of catch distribution, irregularities in observed growth patterns and observations by commercial fishermen suggest that there are possibly populations of barramundi which live in areas relatively remote from rivers, have no fresh water residence phase, and therefore are possibly never readily accessible to the recreational sector.

If such stocks are found to exist, it may be that some degree of commercial utilisation of them, together with the other major target of the coastal gillnet fishery, threadfin salmon, could be permitted with minimal effects on stocks available to the recreational sector.

In many cases the chemical composition of scales, otoliths or bones of fish have been found to reflect, to varying degrees, the chemical composition of the waters in which the fish has been resident (Bagenal et al, 1973; Lapi and Mulligan, 1981; Mulligan et al, 1983, 1987; Belanger and Cherry, 1987; Edmonds et al, 1988). Using this method it has been possible to reliably identify fish from different regions of similar environment and from different environments (such as fresh, brackish or salt water) within a region. By examining parts of scales or otoliths laid down in particular years (ie. between specific growth checks or rings) it is possible that residence environment in particular years of life could be determined.

In the course of ageing fish from those areas, a significant proportion of the scales could not be reliably aged because of the confused and atypical nature of the growth ring pattern (Griffin, unpub. data). It is possible that this atypical pattern arises because they do not undergo the same life cycle patterns as those which utilise or are influenced by the fresh water. This question is addressed by sections (ii) and (iii) of the project.

The general life cycle of barramundi, as defined by studies so far, (Moore and Reynolds, 1982; Moore, 1982; Davis, 1985, 1986; Griffin, 1987) is such that spawning occurs in marine/estuarine areas at river mouths and, after a period of growth in coastal nursery swamps, juvenile fish (3-6 months) migrate to upper fresh waters. After some 3-4 years, maturing fish move to estuarine areas to take part in spawning. It is likely that those mature fish remain in the estuarine or upper tidal sections of rivers, however some may return to upper fresh water or billabong areas. Traditional tagging methods have not been able to satisfactorily resolve this question.

The question is of relevance because there is a degree of separation of the commercial and recreational sectors of the fishery, with commercial fishing being confined by law to lower estuarine and coastal areas, while recreational fishing occurs mainly in the more accessible inland waters.

If the movement of fish back to recreational areas is not significant, then commercial fishing at levels not affecting recruitment, could be considered to have little effect on catches by anglers in upstream areas. Section (iii) addresses this question.

OBJECTIVES

To investigate aspects of the behaviour and biology of barramundi in the Northern Territory which impinge on resource access and allocation with particular reference to :

(i) further definition of genetically distinct stocks of barramundi by electrophoretic analysis of gene frequency;

(ii) the possible existence of entirely marine stocks of barramundi which do not depend on a period of residence in estuarine or fresh water;

(iii) the movement of barramundi within river systems and exchange between the river and adjacent coastal waters.

METHODS: Section (i) - Genetics

Sample Collection and Tissue Preparation

Of the 50 collections analysed for evidence of population subdivision, 18 were new and 32 were derived from published data (Shaklee and Salini 1985; Salini and Shaklee 1988; Shaklee et al. 1993;). Some collections that were pooled in these original publications have been split for the purposes of geographic data analysis. The new collection sites were selected to provide information; (i) on fine scale variation between nearby populations, and (ii) for new locations between distant populations. Duplicate collections from two locations (Daly River, Northern Territory and Hinchinbrook Channel, Queensland) were made four to seven years after the original CSIRO collections; (i) to check that the data being collected by this project was comparable to that of the earlier studies, and (ii) to assess population stability over longer time periods.

New collections of juvenile and adult barramundi were made from 18 locations. The collection number (C#, Table 1), location, date (month/year) and sample size (n) were; C#2 Moyle River, 6/90 & 3/91, 176; C#4 Daly River, 2/90 - 8/90, 132; C#6 Darwin Harbour, 2/90 - 3/91, 159; C#7 Shoal Bay, 7/90 - 3/91, 41; C#8 Port Hurd, Bathurst Island, 3/90 - 6/91, 148; C#11 Buckingham Bay, 8/90, 150; C#15 Leichhardt River, 9/90 - 4/91, 105; C#37 Hinchinbrook Channel, 3/88, 112; C#38 Townsville, 5/88, 41; C#39 Burdekin River & Bowling Green Bay (BGB), 8/90 - 11/90, 207; C#42 Cape Palmerston, 8/88 - 10/88, 40; C#44 Broad Sound, 8/88 - 10/88, 81; C#45 Shoalwater Bay, 6/88, 40; C#46 Fitzroy River, 4/88 & 4/90, 163; C#47 Gladstone, 5/88, 58; C#48 Burnett River, 11/88, 30; C#49 Burrum River, 1/88 - 2/90, 56; and C#50 Mary River, Qld, 1/89 - 5/90, 38.

Fish were caught using monofilament gill nets. Many samples were shipped to the laboratory, frozen at -20°C, as `head and guts'. White muscle, liver and eye (retinal) tissues were collected when available. Liver samples could not be taken from the commercially supplied samples from Bowling Green Bay because the guts had been removed. Samples taken prior to 2/90 were homogenised and prepared as described in Keenan and Shaklee (1985). To simplify sample preparation, subsequent samples were often dissected in the field immediately after capture and frozen in liquid nitrogen. These samples were not homogenised but were placed whole into microcentrifuge tubes with a few drops of homogenising buffer (Selander et al. 1971), centrifuged in a microcentrifuge (@ 13000

rpm) for 15 minutes and stored in an ultrafreezer. These 'tissue drip' samples were more concentrated and exhibited no detectable change in isozyme banding pattern, apart from the advantage of more intense staining (Keenan and Shaklee 1985).

Electrophoretic Techniques

Starch and polyacrylamide (PAGE) gel electrophoretic techniques were used to collect data for thirteen known polymorphic loci (Shaklee and Salini 1985). However, changes were required to techniques used by previous studies because one ingredient of the CAEA buffer described in Shaklee and Keenan (1986) was not available. Details of polymorphic loci used for data collection, source tissues and the modified buffer systems are given in Appendix 1. Three loci were scored using PAGE (EST, IDDH and LDH-C). Only one allele (PGDH*92 on TC-1) could not be reliably resolved by the modified techniques. For the purposes of analysis, this allele was pooled with the *100 allele for all collections. Otherwise, the nomenclature of this study is comparable to that of previous studies of barramundi (Shaklee and Salini 1985; Salini and Shaklee 1988; Shaklee et al. 1993).

Statistical Analyses

The log likelihood ratio, or G-test, was used in preference to the chi-square test (as used in the previous studies) because the G statistic and the degrees of freedom for individual loci are completely additive (Sokal and Rohlf, 1981). "Genes in Populations", a microcomputer program designed by B. May and C.C. Krueger and written in C by W. Eng, was used for these statistical tests (May and Krueger, 1990). F_{ST} statistics were calculated using unweighted means by the method of Wright (1965) and also by the method of Weir and Cockerham (1984) using BIOSYS (Swofford and Selander 1989).

Tests of conformance of genotypic counts to Hardy-Weinberg equilibrium were made using G-tests for individual collections as well as for pooled samples. In cases where one or more genotypes were uncommon, they were pooled with other uncommon genotypes to yield expected cells of five or more wherever feasible. Contingency G tests were used to evaluate the homogeneity of allele frequencies of temporally separated collections, pairwise between all collections from one locality.

To determine the presence of population boundaries, a progressive pattern of statistical analysis was used. All pairs of adjacent collections were compared using contingency G-tests. When not significantly different ($P > 0.05$ over all loci) the adjacent collections were pooled and another round of tests conducted, until all tests resulted in significant differences. In cases where sample sizes were inadequate to resolve differences ($n<50$) e.g. C#30, adjacent samples were pooled to perform these tests. To account for multiple tests of the same hypothesis, differences between collections were considered significant only if the total G statistic (summed over all loci) was significant at a level $a_{0.05}$, adjusted by the method of Cooper (1968), where $a_{0.05} = (0.05/n)$ and n was the number of polymorphic loci (i.e. individual tests) contributing to the total G statistic.

For each collection the mean sample size, proportion of polymorphism, number of alleles, mean observed and expected heterozygosity (genetic diversity) were calculated for the same 13 variable loci. In addition, the most direct coastal geographical distance between adjacent collections was estimated from maps. The interrelationships of these variables were examined statistically and graphically using NTSYS-pc (Rohlf 1992). Relationships between collections, including data from Papua New Guinea collections (Shaklee et al. 1993), were summarised by calculation of Rogers' genetic distance and Cavalli-Sforza and Edward's chord distance (CS&E distance) and UPGMA clustering of these distance matrices (Swofford and Selander 1989). The ordination procedure, nonmetric multidimensional scaling (MDS) (Kruskal 1964 in Rohlf 1992), was used to represent the CS&E distance matrix in 2 dimensions. A minimum spanning tree, which links nearest neighbours, was superimposed over the MDS plot to facilitate interpretation. The CS&E distance matrix was compared to a geographical distance matrix using Mantel's test (Rohlf 1992).

Migration rate was calculated by substituting estimated values into the one-dimensional stepping stone model of Kimura and Weiss (1964) (Hartl 1980):

$$F_{ST} \approx 1/(4N_e(2m\mu)+1) \qquad (1)$$

when solved for the number of migrants:

$$N_e m = 0.03125 \times N_e\mu^{-1} \times (1/F_{ST} - 1)^2 \qquad (2)$$

An average estimate of effective population size, N_e, was derived to use in (2) from the proportional loss in heterozygosity observed between selected collections. Allendorf (1986) presents an equation (from Wright, 1931 in Leberg, 1992) to calculate the proportional loss of heterozygosity as a function of effective population size (which is assumed to be constant) and time (t generations). The proportional loss of heterozygosity:

$$H_t/H_o = (1-1/(2N_e))^t \quad (3)$$

where H_t is the heterozygosity after t generations and H_o is the original heterozygosity. Values can be substituted into this equation and solved for:

$$N_e = 1/(2(1-\sqrt[t]{(H_t/H_o)})) \qquad (4)$$

H_t and H_o were estimated after consideration of the loss in heterozygosity found to occur across certain collections (see Discussion). N_e is a theoretical concept and is extremely difficult to estimate from population census data. The effective population size under natural conditions is usually considerably smaller than the actual population size (Crow and Kimura 1970). Because *L. calcarifer* is a protandrous hermaphrodite, changing sex from male to female between 3 to 8 years of age (Davis 1987) the effective population size would be expected to be much lower than in a bisexual species. Sex-changed females would have a relatively higher chance of mating with their offspring, than in a (normal) bisexual population thus reducing the comparable effective population size through increased levels of inbreeding.

DETAILED RESULTS Section (i) - Genetics

Allele frequencies

Details of allele frequencies and their geographic variation can be found in the publication by Keenan (1994). Table 1 identifies loci where the frequency change between adjacent populations was large enough to show statistical significance and the significance level associated with this difference (see below).

Across the 50 Australian collections, total sample heterozygosity (H_t) was 0.089 (±0.04 SE) when including data for the 13 variable loci examined by the present study. Another polymorphic locus, aconitate hydratase (which is susceptible to enzyme degradation), and 32 monomorphic loci have been identified for Australian samples of the species (Salini and Shaklee 1988). When these 32 monomorphic loci are included in the sample, the actual total heterozygosity (H_T) was reduced to 0.026. This level of heterozygosity is less than half the average heterozygosity levels reported by Ward et al. (1994) for 57 marine and 49 freshwater fish species (0.064 and 0.062 respectively). Subpopulation heterozygosity (H_S) was found to be only slightly less (0.024) than total heterozygosity, resulting in an average F_{ST} value of 0.064 (Table 3). This level of genetic subpopulation differentiation is slightly greater than the average G_{ST} of 0.062 found for 57 wholly marine species (Ward et al. 1994).

Variation between collections

Interesting patterns were revealed during examination of summary statistics. Plots of mean sample heterozygosities (from the 13 polymorphic loci) and total number of alleles against coastal distance revealed unexpected patterns. Far from being random, the relationship between genetic diversity and distance followed a pattern of peaks and troughs that appeared to be related to geography. Collections with the highest heterozygosity, from Joseph Bonaparte Gulf, W.A. and the NE Coast of Queensland, had almost three times the mean sample heterozygosity of collections from the south-west Gulf of Carpentaria, the lowest level of all collections. The regression of heterozygosity against sample size was not significant ($P>>0.05$, $r^2=0.003$). Further, the collections with high heterozygosity did not have the greatest number of alleles, but collections with lower heterozygosity

commonly had fewer alleles, with no significant correlation between these variables ($P>>0.05$, $r^2= 0.004$). The SE Gulf of Carpentaria collections revealed the highest number of alleles (31), almost twice that observed in one collection (16), and an intermediate level of heterozygosity.

Not all the variation in number of alleles could be explained by sample size, although the regression was significant ($P<0.001$, $r^2= 0.395$). Only rare alleles with a frequency of <0.005 were augmenting this relationship ($P<0.001$, $r^2=0.533$). Regression of the number of alleles with a frequency of >0.95 ($P>>0.05$, $r^2=0.008$) or between 0.005 and 0.95 ($P>0.05$, $r^2=0.065$) against sample size were not significant. While the SE Gulf of Carpentaria collections were large (n = 238 & 406) they were not as large as the Daly River 1983 collection (n = 452) which had 5 fewer alleles. The duplicate collections from Hinchinbrook Channel, which varied in size from n = 63 - 275, consistently displayed the same 22 alleles, with no significant variation in gene frequencies.

Hardy-Weinberg equilibrium

Very few tests (10 of a total 340, 2.94%) showed significant levels of deviation from the expected values and further, they were not consistent across loci or sampling sites. The ten significant tests ($P<0.05$) were: PROT in C#1; sIDHP in C#4; EST in C#8; GPI-A in C#8; EST in C#13; ESTD in C#16; PGDH in C#20; sIDHP in C#24; PGDH in C#31; and PGDH in C#34. At the 5% level, just by chance at least 17 of the tests could have been significant and further, nine of these ten tests were not statistically valid because of low expected values, despite pooling. It is concluded that the samples conformed to Hardy-Weinberg expectations.

Temporal variation

No significant differences were observed between the duplicate collections taken by this project and those from the original data for two widely separated locations, the Daly River (C#3, 4) and Hinchinbrook Channel (C#34-37). This project's sample from the Daly River was made 7 years after the previous samples from this location (Salini and Shaklee 1988). Likewise, this project's sample from Hinchinbrook Channel (3/'88) was compared to each of three samples taken from this area by the earlier studies ('82, '83, '84; Shaklee et al. 1993). No significant differences were found by contingency log-likelihood ratio (G) tests between these collections. This provided further confirmation of the stability of gene frequencies within populations and has extended the interval to longer than a generation span (>> 5 years).

Population structure

The broad conclusions from the earlier work were supported by the present study. When all populations were tested together, G-tests among the 50 collection locations across northern Australia were highly significant. However, not all loci showed significant heterogeneity across all collections. While most loci showed highly significant heterogeneity ($P<0.001$), loci with low levels of variation were either just significant (PGM, $0.025>P>0.01$) or were not significant (CK-A and IDDH).

Sixteen barramundi populations (I to XVI) were identified by this study (Table 1). From the eighteen new collections taken for this study, four previously unrecognised populations were identified; III (based on C#6, 7); V (C#8); VII (C#11); and XVI (C#46-50). The other new collections were not significantly different from existing defined populations. Within an identified population (Table 1) no statistically significant differences could be found between adjacent collections. In the case of the Port Hurd collection (C#8), from Bathurst Island approximately 50 kms off the coast, comparisons were made with the two closest populations from the mainland, Darwin Harbour (C#6+7) and the Mary River (C#9) (Table 1). In this case the three collections were significantly different from each other, with the comparison between the mainland collections having the least number of significant loci (Table 1).

An important difference between this and earlier studies was in the interpretation of the results of statistical tests. Shaklee et al. (1993), using the chi-square test, found significant differences at one or more loci between populations from Cape York, comprising C#19+20, C#21+22, C#23-26 and C#27-29. However, the results using the G-test, summed over all polymorphic loci, suggest a different population structure for this region. The G-test value, summed over loci, between combinations of collections was maximised if C#21 was pooled with C#19+20. No overall heterogeneity was observed for the pooled collections C#22-29, using the G-test, however some individual loci did show significant differences between these collections. The discrepancy between the chi-square and G-test results can be attributed to using corrected probability levels (Cooper 1964) when summing the results across loci. The validity of this approach is verified by the congruence between the population structure seen in the UPGMA dendrogram and that derived by the statistical tests. For almost all populations the UPGMA clustering reflected the statistical difference (Figure 1), particularly in the region where the discrepancy discussed above was realised.

The UPGMA dendrogram of the CS&E distance (Figure 1) provides the clearest representation for this method of determining relationships between the collections and their amalgamation into populations. This dendrogram had a cophenetic correlation of 0.816, much higher than the cophenetic correlation of 0.722 for Rogers' genetic distance. Mantel's correlation between the CS&E distance and cumulative coastal distance matrices was highly significant ($P<0.001$, $Z = 0.855$) and not linear confirming an "isolation by distance" type of population structure (Richardson et al. 1986). Upon examination of the output dendrograms from both CS&E (Fig. 1) and Rogers' (unpublished) distances three distinct groups of populations can be recognised. There is a western group consisting of populations I-XI, a central group of populations XII-XIII and an eastern group consisting of populations XIV-XVI. However there is a distinctive difference between these two dendrograms, concerning the relationship of the central Cape York populations (XII & XIII) with the eastern and western groups. The CS&E distance revealed that these populations were more closely related to the Queensland east coast populations (XIV, XV & XVI), whereas Rogers' genetic distance had them clustered with the western group (I-IX). The cause for this discrepancy and the poor cophenetic correlations can be explained in terms of the evolutionary relationships of the populations (discussed later).

Results produced by 2-dimensional multidimensional scaling of the CS&E distance matrix clarify this discrepancy (Figure 2). The first axis has separated collections within each of the large eastern and western groups, which correspond to areas from the east (C#22-50) and west (C#1-18) of Cape York Peninsula. The second axis has clearly separated between these distinct east and west groupings. A minimum-length spanning tree, which links nearest neighbours, joins the two large groups together through the collections (C#19, 20, 21) which constitute the population (XII) present at the tip of Cape York Peninsula.

The geographic spread of the identified barramundi populations was generally smaller in the west and greater in east. For the western populations from the Northern Territory, bounds of identifiable populations were spread over distances less than 300 kms and there were 2-5 significantly differentiated loci between populations (Table 1). In contrast, Queensland populations were spread over larger distances (>500 kms) with 6 - 10 loci exhibiting significant differences, with an exception between the Central and Southern Qld populations (XV & XVI) where only 3 loci showed significant levels of heterogeneity (Table 1). To explain these differences in population range from the west to the east, environmental parameters that may affect larval and/or juvenile migration, including tide height, prevailing winds, river discharges and oceanic currents were examined.

Table 1 Contingency log likelihood ratio (G) tests between pooled collections from identified populations of barramundi. The boundary comparison indicates the significance level between collections (C#) adjacent to population boundaries, which in some cases is equivalent to the population comparison. Population V (C#8) from Port Hurd, Bathurst Island, was compared with the two closest mainland populations, Darwin Harbour III (C#6+7) and Mary River, Northern Territory, IV (C#9).

Population Comparison	G	d.f.	Significant loci [1]	n/N[2]	Boundary Comparison	P[3]
I / II	99.18	29	GPI-A**, IDHP-1***, sMDH-2***	3/12	C#2 / C#3+4	***
II / III	105.53	27	EST***, IDHP-1***, LDH-C*, sMDH-2**, PROT*	5/11	C#5 / C#6	n.s.
III / V	53.48	15	EST***, GPI-A*, PROT**	3/7	C#6+7 / C#8	***
III / IV	44.53	16	EST***, FH**	2/8	C#6+7 / C#9	**
IV / V	61.01	13	EST***, GPI-A**, IDHP-1*, PROT**	4/7	C#8 / C#9	**
IV / VI	54.87	18	EST***, FH*, PGM**, ESTD*	4/9	C#9 / C#10	**
VI / VII	54.17	16	EST**, IDHP-1***, ESTD*	3/8	C#10 / C#11	**
VII / VIII	104.60	15	EST***, IDHP-1***, LDH-C*, PROT***	4/8	C#11 / C#12	**
VIII / IX	24.20	6	IDHP-1***, PROT*	2/4	C#12 / C#13	**
IX / X	45.75	15	EST**, sMDH-2*, ESTD***	3/8	C#13 / C#14	**
X / XI	116.14	28	EST*, IDHP-1***, LDH-C***, PGDH*	4/12	C#14 / C#15	**
XI / XII	257.01	28	EST***, FH**, IDHP-1**, IDHP-2*, LDH-C***, sMDH-2**, PROT***, PGDH***, PGM*, ESTD***	10/13	C#18 / C#19	**
XII / XIII	150.62	22	EST***, IDHP-1***, IDHP-2***, sMDH-2* PROT***, PGDH**, PGM**,	7/11	C#21 / C#22+23	ns
XIII / XIV	583.69	26	EST***, FH***, IDDH*, IDHP-1***, IDHP-2***, LDH-C***, PGDH***, ESTD***	8/12	C#29 / C#30+31	***
XIV / XV	138.80	24	EST**, IDHP-1*, LDH-C***, PROT***, PGDH***, ESTD***	6/11	C#39 / C#40	**
XV / XVI	78.59	25	FH**, LDH-C***, PROT**	3/10	C#45 / C#46	ns
OVER ALL POPULATIONS						
	5684.8	510				

All population level tests were significant at the P<0.01 level using adjusted probabilities. See text for details.

[1]- The asterisks indicate the level of significance for each locus - *** P<0.001, ** 0.001<P<0.01, * 0.01<P<0.05, ns not significant.

[2] n, number of loci exhibiting significant differences between populations, N, total number of loci polymorphic for the populations tested.

[3] - represents the probability of G tests between collections adjacent to the boundary between populations (see above).

Figure 1. UPGMA cluster analysis of Cavalli-Sforza and Edwards chord distance. Population numbers refer to populations identified from statistical testing. Two additional collections from Papua New Guinea (Shaklee et al. 1993) are included.

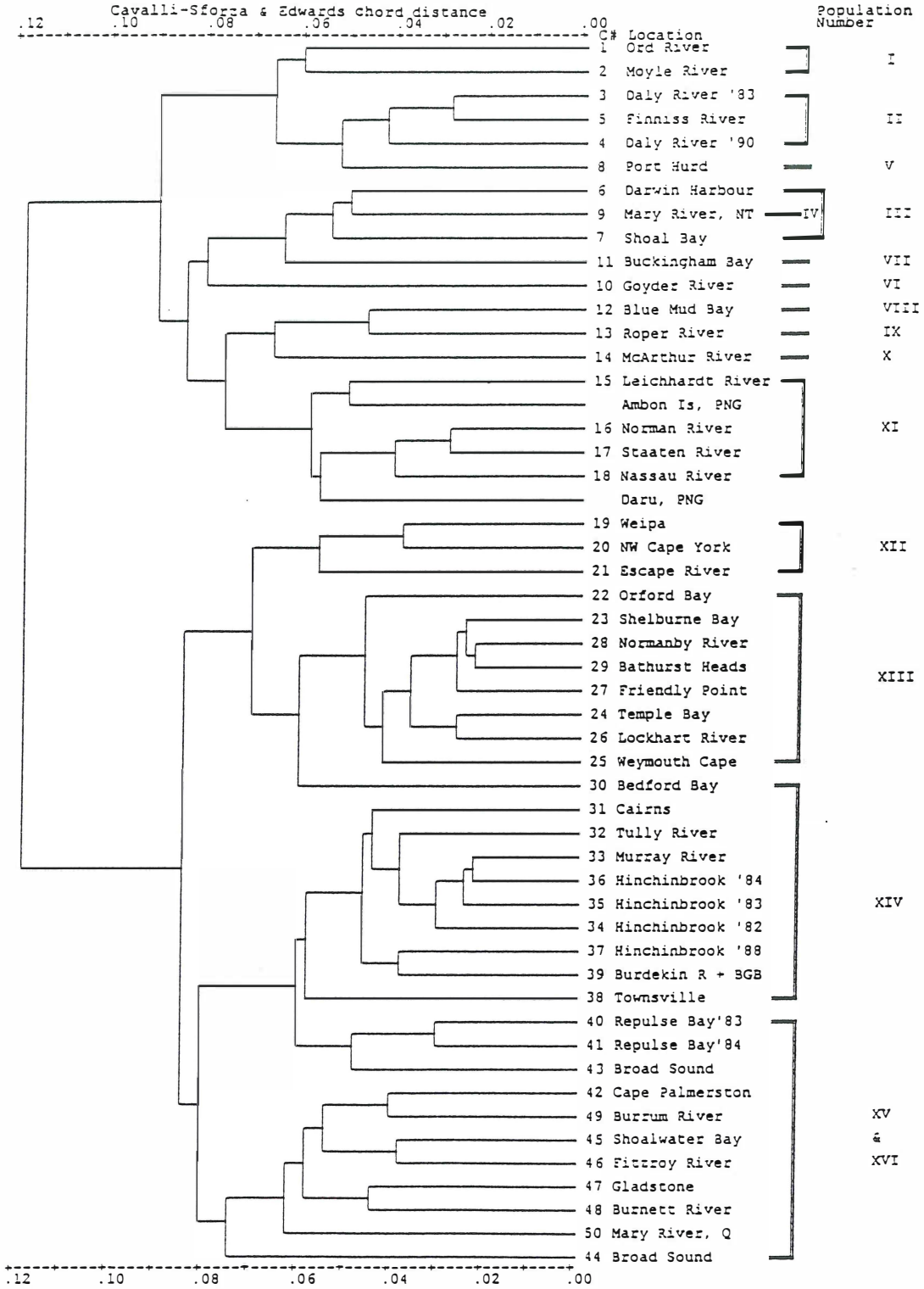
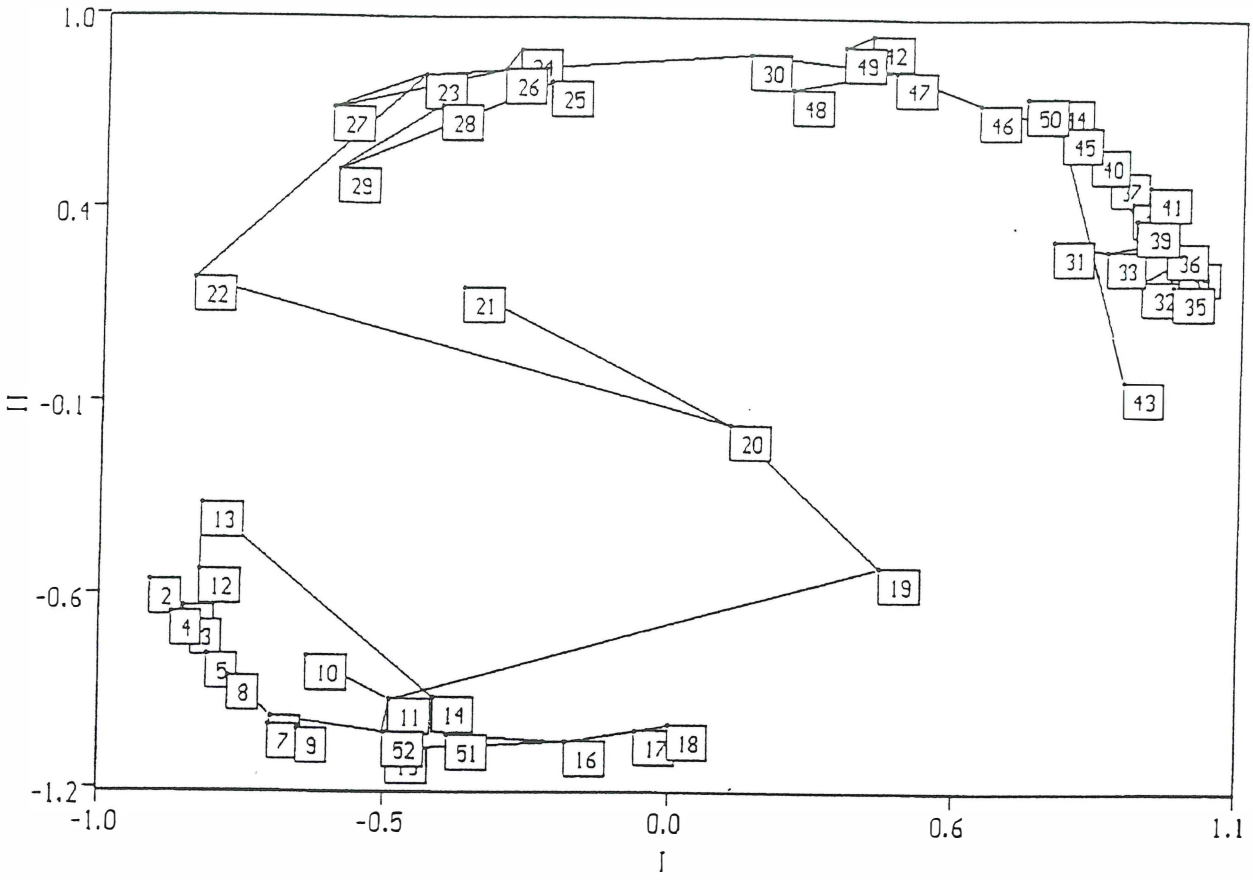


Figure 2. Multidimensional scaling (MDS) in 2 dimensions using Cavalli-Sforza and Edwards chord distance, with a minimum spanning tree superimposed. Numbers refer to collections (Fig. 1) with the addition of two collections from Papua New Guinea (Shaklee et al. 1993); 51 - Daru, 52 - Ambon Island.

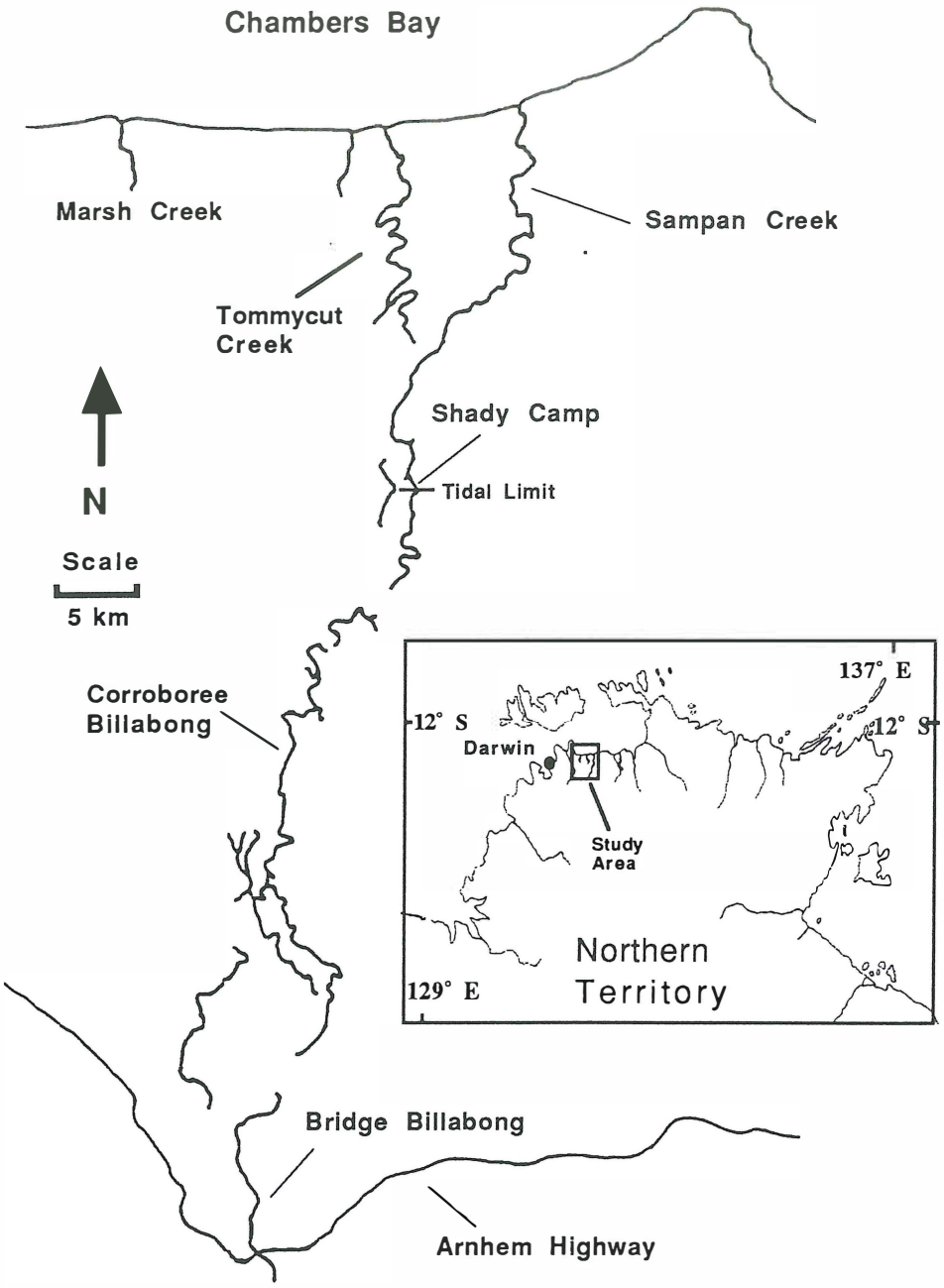


METHODS: Section (ii) - Scale Chemistry

Study site

The Mary River system is a complex floodplain river system with two main entrances to the sea, Sampan Creek and Tommycut Creek (Figure 3). The non-tidal section of the river flows only during the 'wet' (north west monsoon) season from December - May and becomes a series of separate waterholes or 'billabongs' during the 'dry' season from June - November. The tidal limit of the system is 30 km upstream at Shady Camp. Corroboree Billabong, 5 km upstream from Shady Camp and Bridge Billabong, a further 10 km upstream, are isolated during the dry season. Marsh Creek is a short tidal creek 24 km west of Sampan Creek. It is not directly connected to the seasonal freshwater channels of the Mary River but during the peak of the monsoon season receives considerable freshwater input. The geology of the catchment and estuarine sections of the study area is relatively homogeneous with Marsh Creek, Tommycut Creek and Sampan Creek all situated on an estuarine plain which has existed in its present form for approximately 2000 years (Woodroffe and Mulrennan 1993).

Figure 3. Study area showing sampling locations in and adjacent to the Mary River. Discontinuous streams represent dry season conditions. Inset shows the study area in relation to the northern part of the Northern Territory.



Analytical trials were based on a "trial" set of scales of fish (Table 2) taken from coastal waters of Chambers Bay and from freshwater at Corroboree Billabong during 1986 and 1987. Scales ("wildstock" set) were also collected during February - June 1991 from 197 barramundi caught from coastal marine waters near Marsh Creek, estuarine waters of Sampan Creek and Shady Camp) and fresh waters of Corroboree Billabong and Bridge Billabong. A "grow-out" set of scales was taken during October 1991 from 56 farm reared barramundi aged about 12 months; 22 from a freshwater grow-out pond at Berry Springs (42 km south of Darwin) and 34 from a saltwater pond at the mouth of Howard River (17 km east of Darwin). All fish were aged from scales according to methods described by Davis and Kirkwood (1984) with the exception of ten to which an age could not be confidently assigned.

Table 2. Details of barramundi scale sample collections.

Sample Set	Sample Location	Habitat Type	No. of Fish
"Trial" Set	Chambers Bay	Marine	30
	Corroboree Billabong	Fresh	30
"Wildstock" Set	Marsh Creek	Marine	105
	Sampan Creek/Shady Camp	Estuarine	12
	Corroboree Billabong	Fresh	61
	Bridge Billabong	Fresh	19
"Growout" Set	Howard River	Estuarine	34
	Berry Springs	Fresh	22

Water samples were taken from Berry Springs and Howard River ponds during October - November 1991 and from Marsh Creek, Corroboree Billabong and Bridge Billabong during October - November 1991 and February - March 1992, and stored in plastic containers under refrigeration. Salinity of the Howard River grow-out pond was recorded on a weekly basis by the farm operator.

Chemical analysis

At least ten original scales (those not replaced during the lifetime of the fish) were taken from behind the pectoral fin of each fish to allow for potential variability between scales and to ensure sufficient quantities for exploratory analysis as well as final analysis. Depending on the size of the fish one or two grams of cleaned and dried scales were weighed and then digested with 5 to 15 ml of 70% HNO_3 (depending on the weight of scales) at 100°C until the digests reduced to 5 ml. Cooled digests were made up to volume with Hi Pure deionised water, stored in polypropylene containers and refrigerated prior to analysis. Standards and blank digests were prepared in a similar manner.

Exploratory analyses on the "trial" set of scales was carried out at the State Chemistry Laboratories, Adelaide, using inductively coupled plasma mass spectroscopy (ICP-MS) and atomic absorption flame spectroscopy (Flame-AA) (precision and accuracy ± 5-10%). These indicated that 12 elements, with readily detectable concentrations and with discernible differences in sample concentrations from fish of freshwater and saltwater origin, could be further examined as possible residence indicators (Table 3). Subsequent trials found that Ba, Ca, Mg, Na, Sr, Mn, and Zn levels in scales could be reliably analysed at the Northern Territory Department of Primary Industry and Fisheries Chemistry Section, Darwin, by ICP-AES, a less expensive technique.

Table 3. Element concentrations (as dry weight, ppm) in scales of barramundi from Chambers Bay and Corroboree Billabong. Note: Multiply Ca values by 100 to obtain actual values.

Element	**Corroboree Billabong**	**Chambers Bay**
Al	80	24
Ba	190	4.4
Ca	1050	1000
Mg	1850	1700
Na	4000	5500
Sr	420	670
Fe	74.6	23.6
Mn	23.2	8.1
Zn	60	50
La	0.100	0.0165
Ce	0.170	0.036
Nd	0.064	0.0109

A single scale from each of seven fish of the "wildstock" set (three from Marsh Creek, two from Bridge Billabong and two from Sampan Creek) was examined to investigate element

concentration in the marginal growth ring. The last annual increment ring was cut from the whole scale using a scalpel and scissors. Using the same methods as for whole scales the rings were cleaned, weighed, digested and analysed by ICP-AES.

Data analysis

Identification of residence history was based on discriminant function analysis of element concentrations in scales using fish of known habitat history from grow out ponds. The stepwise selection method was applied to the "grow-out" set for Ba, Ca, Mg, Na, Sr, Mn and Zn. Wilk's lambda likelihood ratio criterion was used to measure discriminatory power at 0.05 and 0.10 probability levels. The discriminant function was then applied to the "wildstock" set.

Groupings based on scale element concentrations were also examined by hierarchical cluster analysis using the complete linkage method. Euclidean distance measure was used because element concentrations are continuous (Wilkinson, 1989). Element concentrations were standardised (range standardisation) to account for dominance of elements with very high values which would otherwise almost entirely determine the dissimilarity between cases (Belbin, 1989).

METHODS: Section (iii) - Otolith Chemistry

Eight larger barramundi were sampled from salt and freshwater locations of the Mary River system (Marsh Creek, Sampan Creek and Bridge and Corroboree Billabongs). Their scales and otoliths were aged and were tested for indicator element concentration.

The otoliths were embedded in araldite, sectioned and aged from band counts. To enhance age detection, some otolith sections were also charred over a bunsen burner and others etched with 10% HCl. The sections were marked at each age band. The chemical composition within each age band was then determined by energy dispersive electron microprobe and proton microprobe.

DETAILED RESULTS: Section (ii) Scale chemistry and marine stocks

Concentrations of elements in scales of fish from fresh and salt grow-out ponds were significantly different for all elements measured except Zn. Strontium, Na and Mg concentrations were significantly higher in Howard River and Ba, Ca and Mn concentrations were significantly higher in Berry Springs. Barium and Sr concentrations were more variable in scales from Howard River fish than in scales from Berry Springs fish (Table 4). Examination of concentrations in scales of the "wildstock" set indicated considerable differences in most elements between scales from fish taken from fresh water and those from fish taken from marine waters (Table 5, Figure 4).

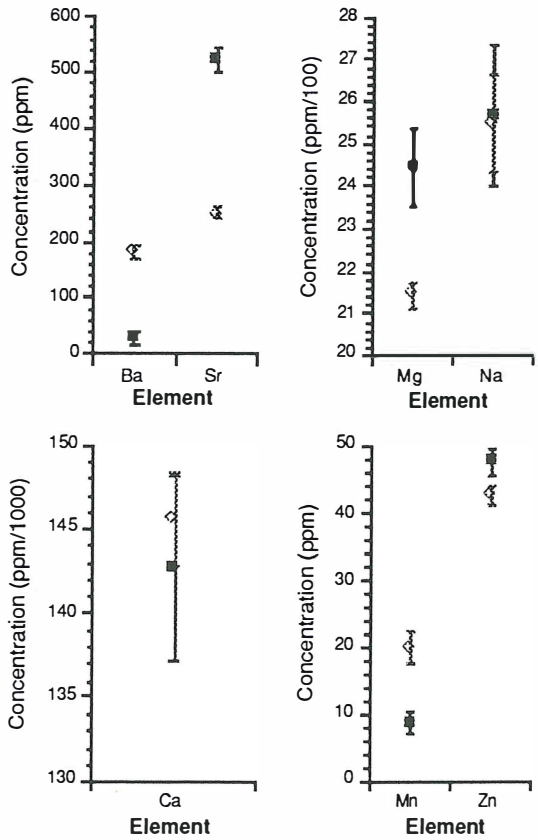
Table 4. Mean, standard error (SE) and coefficient of variation (CV) of element concentrations (ppm) in scales of barramundi taken from Howard River (n=34) and Berry Springs (n=22) grow out ponds. Note 1: Multiply Ca values by 100 to obtain actual values. Note 2: * indicates significant difference (t-test, P≤0.05).

Element	Howard Springs		Berry Springs	
	mean ± SE	CV(%)	mean ± SE	CV(%)
Ba *	7.5 ± 0.2	22	276 ± 7	11
Mn *	2.1 ± 0.2	56	18.5 ± 0.7	18
Zn	46.6 ± 0.6	7	46.7 ± 1.2	12
Ca *	1402 ± 11	5	1553 ± 14	4
Mg *	2434 ± 18	4	2315 ± 25	5
Na *	1960 ± 24	7	1856 ± 62	16
Sr *	512 ± 10	11	191 ± 2	5

Table 5 Element concentrations (as dry weight, ppm) in scales of barramundi from Chambers Bay and Corroboree Billabong. Note: Multiply Ca values by 100 to obtain actual values.

Element	Corroboree Billabong	Chambers Bay
Al	80	24
Ba	190	4.4
Ca	1050	1000
Mg	1850	1700
Na	4000	5500
Sr	420	670
Fe	74.6	23.6
Mn	23.2	8.1
Zn	60	50
La	0.100	0.0165
Ce	0.170	0.036
Nd	0.064	0.0109

Figure 4. Element concentration (ppm, mean ± 95% CI) in scales of barramundi taken from marine (■) and freshwater (◇) environments in or adjacent to the Mary River.



Discriminant function analysis using stepwise selection at the 0.05 level chose Ba and Sr as important predictor variables (average squared canonical correlation = 0.985). At the 0.1 level, Ba, Sr, Ca, Mg, Mn and Na were chosen (average squared canonical correlation = 0.989). At the 0.05 level, 100% of the "grow-out" set were correctly grouped by the discriminant function. Thus Ba and Sr alone were adequate predictor variables. On the basis of canonical coefficients, Ba was the more important predictor element from scale analysis.

The discriminant function based on Ba and Sr was applied to the "wildstock" set, producing two groups. The larger 'saltwater' group contained 126 fish and consisted of all those from Marsh Creek and Shady Camp, three of the six fish from Sampan Creek and 12 of the 61 fish from Corroboree Billabong. The second 'freshwater' group contained 71 fish and consisted of the remaining three fish from Sampan Creek, all 19 from Bridge Billabong and 49 from Corroboree Billabong (Table 4). Fish from Corroboree Billabong which were placed in the 'saltwater' group were mostly young fish with only 10% greater than 2 years and none older than 3 years. However 22% of fish from Corroboree Billabong placed in the 'freshwater' group were older than 3 years.

Table 6. Composition of clusters and discriminant groups.

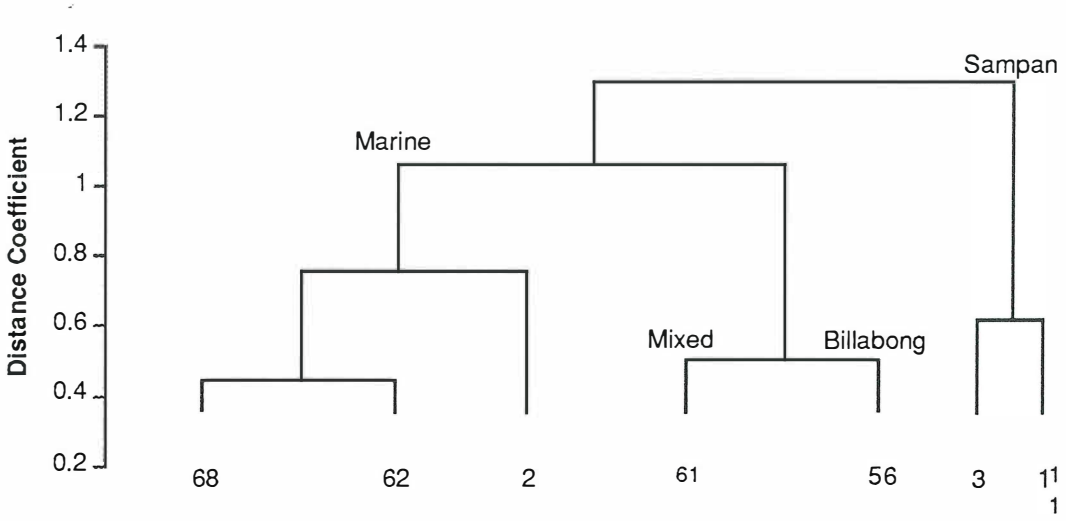
Group/Cluster Name	Group/Cluster Composition	No. Fish	Ba (ppm) Mean	Ba (ppm) SE	Sr (ppm) Mean	Sr (ppm) SE
Clusters						
'Sampan' Cluster	67% of Sampan Creek	4	395.3	84.8	776.0	60.9
'Marine' Cluster	100% of Howard River 92% of Marsh Creek 17% of Sampan Creek	132	13.7	1.4	552.0	9.7
'Mixed' Cluster	67% of Corroboree B'bong 28% of Bridge B'bong 100% of Shady Camp 8% of Marsh Creek 17% of Sampan Creek	61	111.2	5.2	251.8	6.7
'Billabong' Cluster	100% of Berry Springs 72% of Bridge Billabong 33% of Corroboree B'bong	56	199.1	5.5	289.9	4.6
Discriminant Groups						
'Saltwater' Group	100% of Marsh Creek 100% of Shady Camp 50% of Sampan Creek 20% of Corroboree B'bong	126	28.9	3.4	489.8	13.8
'Freshwater' Group	100% of Bridge Billabong 80% of Corroboree B'bong 50% of Sampan Creek	71	180.7	8.7	291.5	13.3

Cluster Analysis

All fish were clustered on the basis of scale concentrations of Ba and Sr (Figure 5, Table 6). Interpretation becomes significant at the seven cluster solution (above the 0.35 squared Euclidean distance coefficient), but groups were most clearly defined at the three cluster solution. Four older fish from Sampan Creek ('Sampan' cluster) grouped independently from the remainder of the population. Two larger clusters possibly reflecting residence habitat were apparent. The larger 'marine' cluster contained only saltwater fish; all those from the Howard River ponds, 97 fish (92%) from Marsh Creek and one fish from

Sampan Creek. The other cluster, with mainly freshwater affiliations contained all the Berry Springs grow-out fish, all fish caught from Bridge Billabong and Corroboree Billabong, all fish from Shady Camp, eight fish caught from Marsh Creek and one fish from Sampan Creek. That cluster could be logically sub-divided into two smaller clusters designated as 'billabong' and 'mixed'. Subdivision of the 'marine' cluster was also indicated but because Howard River fish were represented in both its subgroups there was no obvious basis for subdivision.

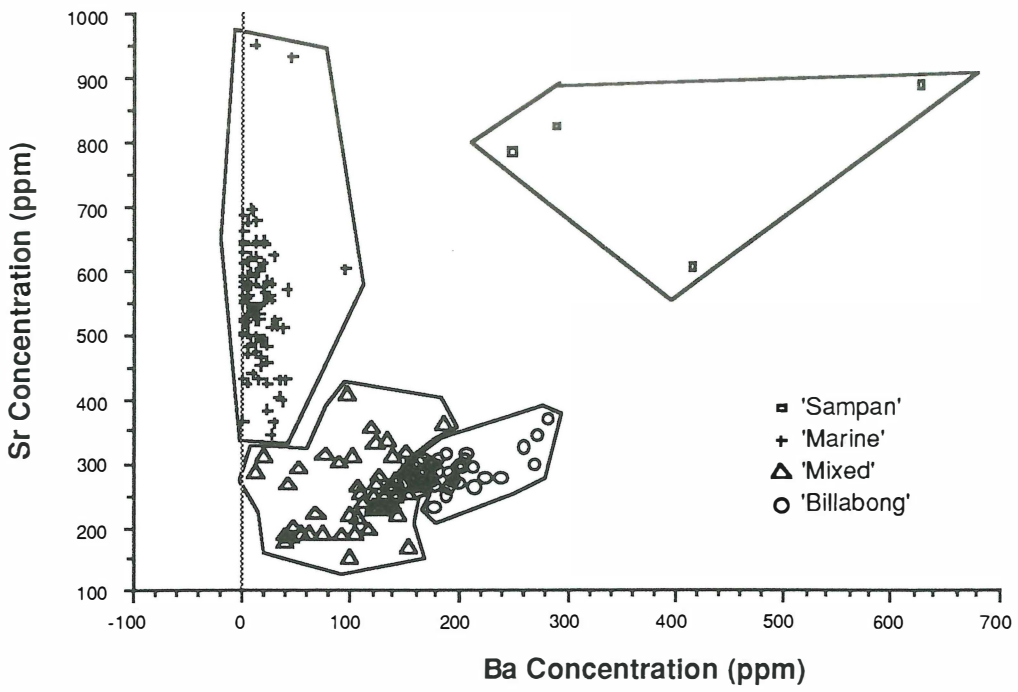
Figure 5. Cluster diagram of 'wildstock' and 'growout' barramundi based on concentrations of Ba and Sr in scales. Group names and numbers are indicated.



The 'billabong' cluster contained all fish from Berry Springs grow-out ponds, 72% of Bridge Billabong fish and 33% of Corroboree Billabong fish. The 'mixed' cluster was made up of 28% of the Bridge Billabong fish, 67% of the Corroboree Billabong fish, all 6 fish from Shady Camp, 8% of fish from Marsh Creek and one older (6 years) fish from Sampan Creek. There was a significant difference in age distribution between Bridge Billabong and Corroboree Billabong fish from the 'mixed' cluster and those from the 'billabong' cluster (Kolmogorov-Smirnov test P=0.0176). This indicated that there was a higher proportion of older (greater than 2 years) Corroboree Billabong and Bridge Billabong fish in the 'billabong' cluster compared with in the 'mixed' cluster. All fish from Shady Camp were 2 years old and were grouped with the 'mixed' cluster.

Examination of the chemistry of fish of unknown history via a plot of Sr concentration versus Ba concentration of wildstock fish only (Figure 6) shows the four cluster groups clearly. Analysis of variance showed that mean Ba and Sr concentrations of wildstock fish in the 'marine', 'mixed' and 'billabong' clusters were significantly different ($P<0.0001$). Barium concentrations were 13.7 (SE 1.4) ppm, 111.2 (5.2) ppm and 199.1 (4.5) ppm in the 'marine', 'mixed' and 'billabong' clusters respectively . Multiple range tests (Scheffé $P\leq0.05$) showed significant differences in Ba levels between all three clusters ($P<0.0001$). Strontium concentrations were 552.0 (SE 9.7) ppm, 251.8 (6.7) ppm and 289.9 (4.6) ppm in the 'marine', 'mixed' and 'billabong' clusters respectively. Strontium concentrations differed significantly between 'marine' and 'billabong' clusters ($P<0.0001$) and between 'marine' and 'mixed' clusters ($P<0.0001$), but there was no significant difference between 'mixed' and 'billabong' clusters ($P=0.9522$). Very high concentrations of both Ba (395.3 ppm) and Sr (776.0 ppm) were found in the 'Sampan' cluster but sample size was too small for further analysis.

Figure 6. Plot of Sr versus Ba concentration for all wildstock fish showing the 'marine', 'billabong', 'mixed' and 'sampan' clusters.



Barium and Sr concentrations in the marginal zone (representing the most recent growth) of scales of the two fish from freshwater were 237 ppm and 159 ppm and 366 ppm and 278 ppm respectively. In the five fish of marine origin Ba ranged from 13 ppm to 46 ppm (mean 25.6, SE 6.4) and Sr from 473 ppm to 663 ppm (mean 551, SE 37.1). These values are comparable with those of the 'billabong' and 'marine' cluster groups.

This study has provided strong indications that purely marine populations of barramundi exist in the Marsh Creek area which in turn suggests that the established "typical" life cycle is not obligatory. While this study provides no direct insight into the mechanisms underlying this departure from the established life cycle two mechanisms are suggested. A marine population may be simply a result of spawning in coastal areas remote from the river. Alternatively recruits resulting from late spawning may not mature sufficiently to migrate upstream before river levels subside and as a consequence they remain in coastal waters. If the latter is the case the relative importance of the marine population might vary according to seasonal conditions. Current stock assessments, which are strongly influenced by studies in fresh water, may need to be reappraised as a consequence of this information because a poor year class in fresh water may be offset to some extent by recruitment to the marine population. The presence of a small proportion of fish at Marsh Creek which have had a freshwater phase suggests that these largely marine populations do not constitute separate genetic stocks.

The results of this project are to be published in Transactions of the American Fisheries Society in a paper entitled "Habitat history of barramundi (Lates calcarifer) in a north Australian river system based on Ba and strontium levels in scales".

DETAILED RESULTS: Section (iii) Otolith chemistry.

A suitable method of preparing barramundi otolith samples for aging and for chemical testing was developed. Neither charring nor etching techniques enhanced the clarity of banding. In the otolith sections a small central origin layer was observed representing the first months of growth. This was surrounded by alternating transparent and opaque bands within which are numerous thinner rings (10-100). Literature suggests that for most fish a

set of transparent and opaque bands may correspond to a single year of growth while the smaller rings may be daily, weekly or monthly patterns.

Generally, aging from otoliths produced similar results to the established method of counting of scale annuli and correlates with fish length (Table 7).

Table 7. Barramundi age estimates by otolith and scale methods, fish length and location.

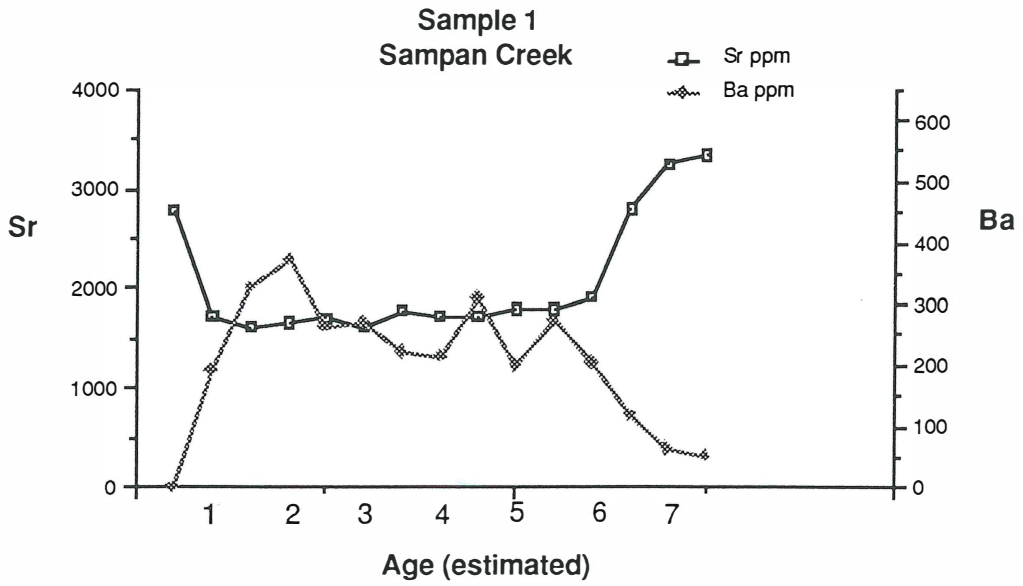
Otolith	Age (otolith)	Age(scales)	Length (cm)	Residence
1	7	6-7+	102	Sampan Cr
2	9-14	9+	106	Marsh Cr
3	6.5	6+	94	Marsh Cr
4	> 7.5	4+	87	Bridge Bb
5	5.5-8.5	4-6+	92	Sampan Cr
6	1.5	2+	54	Corrob Bb
7	7-9.5	7+	86	Bridge Bb
8	2.5	3-4+	74	Bridge Bb

The otoliths were found to comprise about 1% organic material. They are composed of almost pure calcium carbonate ($CaCO_3$) in a protein matrix of otolin.

Electron microprobe analysis of otolith sections could not reliably detect environmental indicator elements at the levels present. Proton microprobe analysis did provide reliable estimates of concentrations of several elements including the two strongest indicators of fresh and saltwater residence, barium and strontium.

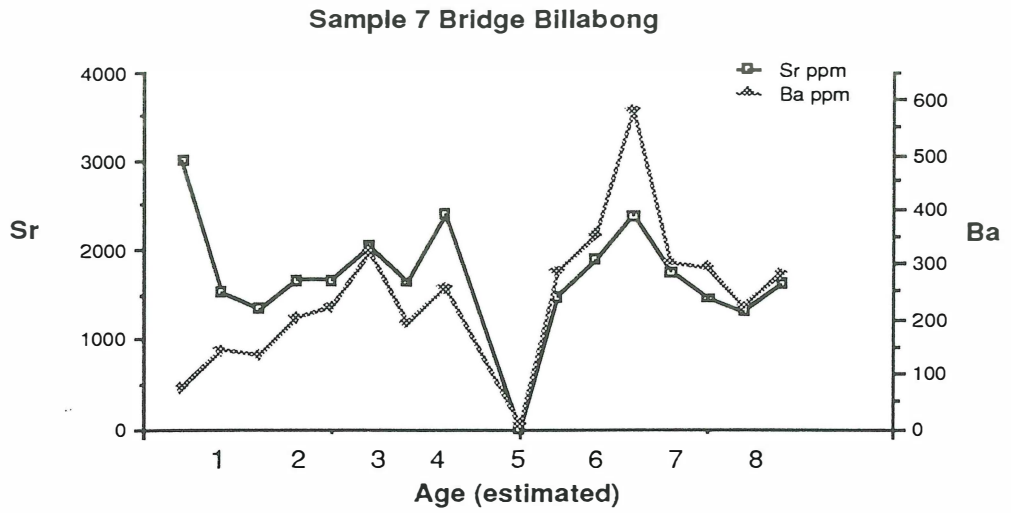
Of the barramundi tested, two appear to have hatched in saltwater followed by freshwater and saltwater resident phases, eg Sample 1 (Figure 7a). In one of these fish during the freshwater phase there seems to be a short period in salt water, represented by high strontium and low barium levels.

Figure 7a Barium and Strontium concentration at age bands in an otolith of a barramundi from Sampan Creek (Mary River) NT, determined by proton microprobe.



The remainder of the barramundi seemed to have hatched in salt water and spent the remainder of time in freshwater, eg Sample 7 (Figure 7b). In sample 7 there are two data points difficult to interpret, occurring when both barium and strontium levels fall to zero (with a corresponding increase in zinc level) and when both barium and strontium levels increase simultaneously (with a corresponding increase in copper concentration).

Figure 7b Barium and Strontium concentration at age bands in an otolith of a barramundi from Bridge Billabong (Mary River) NT, determined by proton microprobe.



While no real conclusions can be drawn from this small sample these preliminary results of otolith band analysis suggest that the technique could successfully be used to examine the residence histories of individual barramundi. Recent work in this field in Australia and overseas indicates that wavelength dispersive electron microprobe analysis could probably replace the more expensive proton microprobe analysis for this work. New developments in methodology in this area of research were detailed at an international conference in South Carolina, USA in January 1993. That conference was attended by the supervisor of this project, Mr Roland Griffin, with support from the NT Department of Primary Industry and Fisheries. Preliminary electron microprobe scans across barramundi otoliths conducted at the University of Maryland in January 1995 indicate that that method is suitable for determination of residence habitat based on the ratio of strontium to calcium.

BENEFITS: Section (i) Genetics

Discussion of Results including analysis of research outcomes compared with the objectives.

The objective to further define the stock structure of barramundi in the Northern Territory by electrophoretic analysis of gene frequency was successfully completed. Three new barramundi stocks were identified from the six additional Northern Territory samples (P#; III, V and VII in Table 1). The ten stocks of barramundi that have now been identified in the Northern Territory are essentially reproductively isolated and hence are self-sustaining populations that require independent management (in the short term). This knowledge of the stock structure is being applied to the fishery through independent management of isolated river systems.

In addition to this singular objective, the collection of additional data from new locations allowed a re-interpretation of the development of population structure in Australian barramundi. This required an understanding of the breeding cycle of the species and the biogeographical history of the fishes' habitat. With this knowledge it was possible to interpret the evolutionary significance of the observed variation in allele frequencies, heterozygosity and the number of alleles between collections to address the issue of translocations that may result from restocking or aquaculture. The remainder of this discussion deals primarily with this issue.

Constraints inherent in the life cycle can influence dispersal patterns of larvae and juvenile fish. For barramundi under experimental conditions, larval survival is optimal at salinities of 20-25 ppt and temperatures of 27°-28°C (Wongsomnuk and Manevonk 1973 in Grey 1987). Eggs hatch rapidly under these conditions in about 18 hrs and the larvae are

actively feeding by the 3rd day. By the 15th day larvae can tolerate completely fresh water and exploit flooded wetlands adjacent to river mouths that are abundant with food resources (Russell and Garrett 1983). Juvenile fish can tolerate greater fluctuations in environmental parameters than the larvae (Russell and Garrett 1983, 1985), and adults can tolerate temperatures as low as 15.5°C, which occur near the species' southern limit in the Mary River, south-east Queensland. Such rapid larval and juvenile development, which includes a move from almost marine to freshwater conditions within two weeks, is unusual for a fish species. This developmental behaviour, which appears to be identical for SE Asian and Australian sea bass/barramundi based on observations at fish hatcheries, obviously places limits on the potential for oceanic distribution of juvenile fish and associated gene flow.

Historical zoogeography

In addition to these life history constraints, there are well documented historical zoogeographic constraints to the distribution of marine populations across tropical northern Australia, resulting from ice-age sea-levels changes. Data on sea-level changes over the last 300 kyr for the Huon Peninsula in nearby north-eastern New Guinea have been deciphered from uplifted coral reefs by Chappell (1983) and refined by Chappell and Shackleton (1986) to estimate global changes. Recently, Lambeck and Nakada (1990) examined the variation in highstands around the Australian continent, to interpret sea-level changes for the last 18 kyr. At the peak of the two most recent ice-age glaciations, approximately 18 kyr and 135 kyr BP, the sea-level dropped to such an extent (at least -130m globally and possibly to -155m in this region (Chappell 1983)) that the entire area between New Guinea and Australia formed a land bridge. Obviously no barramundi populations, nor in fact any other marine species, could have inhabited the area. Further, only during the peaks of the warmest periods, 7,000 years BP to present and also approximately 130 kyr - 115 kyr BP, were sea-levels of a height sufficient to open the Torres Strait, because the present depth of the Strait is only 7.5 metres. During these warm periods, sea-levels are recorded as being 6m above present levels in some areas (Chappell 1983) although Lambeck and Nakada (1990) suggested a maximum of only 2m above present levels for the most recent rise. Some care must be exercised in the interpretation of past isobaths from existing levels because significant levels of marine sedimentation have been documented for the Arafura Sill [Jones and Torgersen (1988) reported at least 22m of sediments infilling a 36 kyr old river valley] and areas of erosion where no sediments are currently accumulating (i.e. the Torres Strait, D.E. Searle pers. comm.). In addition hydro-isostatic warping of up to 3m has been reported for the region (Chappell et al. 1982, Lambeck and Nakada 1990).

An interesting feature of the region during the period 70 - 10 kyr BP, when sea-level was often below -50m, was the formation of a substantial lake (Lake Carpentaria) in a depression in the present Gulf of Carpentaria (Torgersen et al. 1983, 1985). Of importance to this study is the salinity of Lake Carpentaria during the glacial maximum. If salinity in Lake Carpentaria was similar to estuarine conditions a refuge population of barramundi could have bred in the lake and survived prior to reinundation. Jones and Torgersen (1988) have determined that from 28 - 18 kyr BP the Lake was, at most, brackish and the Arafura Sill at 53m below present water level was not overtopped by marine water until 11,000 years ago. Fully marine conditions may not have been established until 9500 years BP. De Deckker et al. (1988) confirmed that the lake's water was almost fresh from 40 kyr to » 11 kyr BP by examining Mg/Ca and Sr/Ca ratios of ostracod shells from sediment cores.

These historical observations imply that a refuge population of barramundi could not have established in Lake Carpentaria, for the salinity would have been too low for breeding and larval survival. However, juvenile and adult barramundi most probably used the lake's food resources as the lake was connected to the sea via a western flowing river (Jones and Torgersen 1988). Therefore the most easterly population from the western coastline, until approximately 11000 years ago, was based to the west of the Arafura sill 500 kms to the north-east of Darwin, at the outflow of Lake Carpentaria.

Evolutionary history

Considering the constraints discussed above, an hypothesis for the evolution of present population structure of Australian barramundi can be developed. The most recent development in population structure of barramundi across northern Australia began when the sea-level dropped below -12m at about 115 kyr BP and for the ensuing 108 kyrs a land bridge reconnected Australia and New Guinea. Throughout this period the primary east-west component of genetic isolation and population subdivision developed. It is possible that migration through the Torres Strait occurred prior to 130 kyr BP at 340-320 kyr BP and/or earlier during another period of high sea level. However, any earlier migrations are of no significance to the latest re-colonisation, but only of significance to the historical development of heterozygosity levels and allelic variation between these divided populations. Evidence of east-west isolation can be clearly seen in the allozyme data. The UPGMA dendrogram (Figure 1) and multidimensional scaling (Figure 2) separate collections into two major groups as well as a smaller central group. The major "western" group consists of populations positioned near the Joseph Bonaparte Gulf (I-II) and populations from the Northern Territory and Gulf of Carpentaria coast (III-XI). The major "eastern" division consists of east coast populations south of Princess Charlotte Bay (XIV-XVI). Central between these major groups are the populations of Cape York (XI-XIII), particularly that from the tip (XII), which display variable relationships according to the method of analysis. Cavalli-Sforza and Edwards chord distance (Fig. 1) reveals the derivation of XII from the "eastern" group (XIV-XVI), while MDS (Fig. 2) places this population centrally between the east-west dichotomy.

The heterozygosity data for "ancestral" populations provide further evidence for the "western" and "eastern" isolation hypothesis. For the purposes of this discussion, "ancestral" populations are defined as the source populations prior to recolonisation after sea-level rises, i.e. they would have been based on major river systems which would have been capable of supporting large numbers of fish, much larger than surrounding regions, similar to the Fly River of Papua New Guinea today. Of the "western" populations, the population based in Joseph Bonaparte Gulf (I) has an "ancestral" nature because the major rivers that drain into this Gulf once combined to form a large river basin. The remnant contours of this basin can still be seen extending from the coast. With rising sea-levels this "ancestral" population would have extended back into the river valley and separated as river valleys were drowned and new river mouths became exposed to marine influences. Unfortunately, data from other areas along the north-west coast of Western Australia that maintain populations of barramundi were not available for comparisons of heterozygosity. Another western "ancestral" population which cannot be assigned to any one present population would have existed at the western mouth of the Arafura Sea, where rivers from southern New Guinea and northern Australia, as well as Lake Carpentaria and the Fly-Strickland Basin (Torgersen et al. 1983) must have once joined to form a massive river.

On the east coast, the Mid NE Coast population (XIV) extending from Cairns to the Burdekin River (C#31-39) would have been "ancestral" to more southerly populations after the initial colonisation of the east coast. This population was also, most likely, the source of fish for the more northerly populations of eastern Cape York. There are two pieces of evidence which indicate that NE Cape York populations of barramundi did not exist until after the latest rise in sea-level. Firstly, the geographically closest collections to Cape York from Daru and Ambon Island (on the Papua New Guinea side to the east of the Torres Strait) cluster with collections from the "western" derived population (Figs 1 & 2). Secondly, the Fly River has only recently, 24 kyrs ago, been diverted from a westerly course across the Arafura Sill to its present mouth east of the Torres Strait (Blake and Ollier 1969). During low sea levels the coastline in this area would have been close to the fringing coral reefs of the outer Barrier Reef. At present no populations of barramundi are found to the north and east of Papua New Guinea where the continental shelf is close to the coast and fringed by coral reef.

Both of the "ancestral" populations exhibit considerably higher heterozygosity levels than adjacent populations. Similar large changes in heterozygosity have been reported for two dissimilar fish species, *Fundulus heteroclitus* and *Oncorhynchus kisutch* , whose

distribution has been altered by the retreat of glaciers on the east and west coast of the USA respectively. When Ropson et al. (1990) graphed the geographic variation in gene diversity of *F. heteroclitus* from 15 sites along the Atlantic coastline, they found comparatively low gene diversity in the most northern population (H_{exp} = 0.06). Gene diversity increased steadily to the very high level of H_{exp} = 0.36 at a site from northern New Jersey and then remained at a plateau level of approximately H_{exp} = 0.33 in sites further south. Further, Ropson et al. (1990) reported that the marsh habitat of *F. heteroclitus* to the north of the New Jersey site would have been buried under an ice sheet during the last glacial maxima until 15000 years ago. Therefore, the "ancestral" population, most likely the source of migrants to the north, has maintained a relatively high level of heterozygosity throughout its undisrupted range, whereas the heterozygosity was reduced in "founder" populations as the fish spread north following the retreating ice sheet. It should be noted that this interpretation is the alternate hypothesis to the secondary intergradation hypothesis promoted by González-Villaseñor and Powers (1990) based on mitochondrial DNA data. While the data for *O. kisutch* is not so well developed the same pattern can be seen. Wehrhahn and Powell (1987) reported that the gene diversity of populations from areas of southern British Columbia, which were affected by glaciation, was only 22% that of fish from Oregon, which was not glaciated during the last ice-age. They reported a further reduction in heterozygosity, of 46% (of 22%), between fish from the Upper Fraser River and Lower Coastal Mainland fish.

With respect to the barramundi of northern Australia, the rapid rise in sea-level between 18000 and 6000 years BP would have had spectacular effects on the marine/land interface and the barramundi's habitat (Galloway and Löffler 1974). The low gradients present on the 1,500 km wide continental shelf between Australia and New Guinea (1:7500) meant that marine inundation was encroaching the land at an average rate of approximately 125 metres per year for these 12000 years. Therefore, in order to survive, the animals and plants of the coastal fringe areas must have been capable of quick colonisation and utilisation of this new habitat. In the Gulf of Carpentaria, where this colonisation was the most rapid and also the most recent, these changes in population structure can be seen in the genetic data. As discussed above, the freshwater Lake Carpentaria was not overtopped by marine water until 11000 years ago and fully marine conditions, suitable for barramundi breeding may not have been established until 9500 years BP. Consequently, the timing of migration and colonisation of barramundi from the west, as well as the east, into the Gulf of Carpentaria can be established with a degree of certainty. The summary data, mean heterozygosity and number of alleles, reveals the impact these migrations had on the genetic structure of new populations. The heterozygosity levels present in the colonising populations were almost halved as the migration front extended from the "ancestral" populations, and migrated with the encroaching sea towards the SE Gulf of Carpentaria. Likewise, the number of alleles possibly decreased in conjunction with this migration, except where the two migration fronts met and hybridised and an increase in the number of alleles is observed (see below). These events can be explained by the escalating effects of genetic drift at the periphery of the colonising population.

Estimation of population size from heterozygosity data

Allendorf (1986) modelled the influence of bottlenecks in population size on heterozygosity and retention of alleles and found that the these two factors did not respond equally to genetic drift, particularly when there was a large reduction in effective population size for a few generations. His simulations of the effect of population size on heterozygosity corresponded closely to the theoretical equation when population sizes were stable. Recent experimental work by Leberg (1992) basically confirmed the results from these simulations. As a logical extension of these results, it could be concluded that large effective population sizes are necessary for the maintenance of high stable levels of heterozygosity, like those seen in *F. heteroclitus*.

Assuming that the level of heterozygosity has been stable in the "ancestral" barramundi populations over time, values can be substituted into equation (4) above, to calculate the average effective population size (N_e) of the fish that have migrated and colonised new habitat in the Gulf of Carpentaria, from both the west and the east. Setting H_o» 0.1 (i.e. the

western "ancestral" populations original heterozygosity) and H_t » 0.05 which is approximately the present observed sample heterozygosity after t generations (from IX, Fig. 3a) and t » 3600 (i.e. a generation time of 5 years for barramundi, for the last 18000 years), an N_e of 2597 fish is calculated. If the total heterozygosity value is used, which includes 32 monomorphic loci, the ratio H_t/H_o » 0.016/0.027 and an N_e of 3440 fish is calculated. A similar calculation can be made for the reduction in heterozygosity observed between populations XIV and XI, which most likely occurred in the last 7000 years after the opening of the Torres Strait. In this case where the proportional loss H_t/H_o » 0.08/0.11 » 0.73, and t » 1400, N_e is calculated to be 2198 fish. Similarly, if the total heterozygosity value is used, the ratio H_t/H_o » 0.023/0.031 and an N_e of 2345 fish is calculated. The gross similarity of these estimates of effective population size, that range from 2198 to 3440, are of particular interest because of the accuracy that can be placed on the timing of these recent migrations. These estimates will be used later to calculate migration rates using the one-dimensional stepping stone model and the island model.

Hybridisation

Information on the composition of these migrating populations can also be derived from the number and type of alleles present in collections. According to Allendorf (1986), loss of alleles can most likely be attributed to reductions in population size for a few generations and complete loss of allelic variation can result from severe bottlenecks. While complete loss of allelic variation is not apparent in the data, there is an example of reduction in the number of alleles, between populations V and IX. This could be related to the smaller sample sizes of populations VIII and IX. However, at population XI there is a substantial increase in the number of alleles that is not associated with an increase in heterozygosity or can be attributed to sample size variation.

The data suggest that hybridisation has occurred within the last 7000 years, after the Torres Strait opened, between the east and west coast derived populations. Collections within population XI, from the south-east Gulf of Carpentaria, have a level of heterozygosity intermediate between adjacent populations that can be simply explained by colonisation of new habitat by a limited number of migrants (above). However, the number of alleles for collections within this population are notably more (26-31) than those observed from adjacent populations (21-23). Examination of the data reveal that not only are rare alleles present in population XI at twice the frequency than are found in either one or the other adjacent populations, but three new alleles, GPI-A*95, sMDH*142 and PGM*110, not observed in any other population are present. Other studies have often reported finding new alleles in interspecies hybrid zones (Barton et al. 1983 and references therein). However, the increase in the number of alleles observed in population XI is not only produced by the presence of new alleles. Population XI also contains five alleles that are derived from the western populations that have not spread into the eastern populations, as well as four alleles from the eastern populations that have not spread into the western populations.

The five alleles found predominantly to the west and including XI are CK-A*106, CK-A*95, ESTD*82, IDDH*350 and PGM*90. The four alleles found predominantly to the east and including XI are ESTD*114, sIDHP*83, sIDHP*111 and PGDH*88. Another three alleles sIDHP*116, mIDHP*78 and LDH-C*104 are only present in some populations along the east coast and presumably arose via mutation after the (most recent) isolation of the "eastern" group 115 kyr BP. It is of interest to note that the dichotomous nature of UPGMA dendrograms cannot resolve the convergence and hybridisation of populations as discussed above. Perhaps this explains the large difference seen in the relationships of the three major groups for the dendrograms derived from Rogers' and CS&E distances, as well as their modest cophenetic correlations. Multidimensional scaling (Fig. 2) and a minimum-length spanning tree on the other hand clearly resolved the relationships between collections.

Estimation of migration rate

As discussed in the introduction, the one-dimensional stepping stone model rather than the island model should best represent the observed population structure. Compared with the equation derived from the island model (Wright 1943):

$$N_e m = 0.25(1/F_{ST} - 1) - N_e m \qquad (5)$$

where mutation rate (m) has little effect on the estimate of $N_e m$ and is usually ignored, the factor $N_e m^{-1}$ can have a large effect on the estimate derived from the one-dimensional model (2). Kimura and Weiss (1964) used the rate of long range dispersal per generation to develop their model, the effect of which is formally equivalent to mutation. For the purposes of estimation of migration rate, a range of values for the mutation rate per generation (m), has been used from $m = 1x10^{-5}$ to $1x10^{-6}$, which is within the range of values found for allelic mobility variants (Nei 1987).

Apart from the mutation rate, two other constants are required to calculate migration rates; (i) the observed fixation index $F_{ST} = 0.064$, and (ii) an estimate of effective population size, N_e. When Weir and Cockerham's method was employed to provide an average measure of subpopulation differentiation, $F_{ST}= 0.0636$ ($\pm$ 0.0108 S.D.), which is effectively the same as Wright's F_{ST} value for this data. A range of values for effective population size based on the previous calculation, from $N_e = 2000$ to 3500, was employed to estimate gene flow as measured by migration rates between populations. In the case of the one-dimensional model, the migration estimate m is the probability of migration between adjacent demes of an average size N_e. The assumption that $m<<1$, required by the island model, is not an assumption of the one-dimensional stepping stone model which is an exact solution.

It is evident from these results that estimates of both the number of migrants contributing genes to other populations ($N_e m$) and the migration rate are two orders of magnitude higher using the one-dimensional stepping stone model than the island model for the population sizes used. Variation in population size has very little effect on the Nem estimates derived from the island model (3.621 - 3.654) regardless of mutation rate, whereas the estimates derived from the 1-D model for the same population sizes range from 191 - 334, for a mutation rate = 10^{-5}. As mentioned earlier, because mutation rate (or long range dispersal per generation) is a factor in the 1-D model, a decrease of this factor (10^{-5} - 10^{-6}) increases migration rate by the same order of magnitude. Assuming a mutation rate of 5×10^{-6} and an effective population size of 3000 the estimate of migration rate derived from the 1-D model is substantially higher than previously attributed for this species, from genetic data (Salini and Shaklee 1988, $N_e m \pm 2.6$) or movements of adults (Davis 1986) i.e. if N_e = 3000, $N_e m = 446$, $m = 0.148$, or 14.8% of the population per generation. Surprisingly, to preserve the observed degree of population subdivision if the effective population size is halved to 1500, the number of migrants required is doubled ($N_e m = 891$) and therefore the migration rate is quadrupled to $m = 0.594$. In barramundi it is more probable that if the average population size were halved (to 1500), the migration rate would remain the same (0.148), and the degree of population subdivision i.e. F_{ST} value would increase substantially (from 0.064 to 0.120). Thus, the extremely high migration rates of over 100% calculated are not likely to be experienced by barramundi populations but are theoretically possible if mutation rates are as low as $1x10^{-6}$.
This comparison highlights the dynamic interaction between these fundamental variables in the 1-D stepping stone model compared to their inconsequential interaction in the island model. The mechanism that produces an increase in population subdivision through isolation by distance is simple to explain; mutation and genetic drift operate independently in different parts of the population (Goldstein and Holsinger 1992).

Migration via flood plumes

From the above interpretation, barramundi are clearly capable of considerable levels of migration along a coastline and there is more migration occurring than is seen in the movements of tagged fish. Within Australia there are only isolated reports of coastal migrations of tagged fish over 100 kms. Of 17135 tagged barramundi only 11 of the reported 2046 recaptures (0.54%) have moved outside of their native river and more than

100kms along the coast (Sawynok, pers. com.). However, this included a single fish, at liberty for 1401 days that migrated 650 km north, through two population boundaries, along the east coast from the Fitzroy River (C#46) to Bowling Green Bay (C#39) following the massive Fitzroy River flood of 1991.

If larger tagged fish are not the basis of the predicted high migration rates between adjacent rivers, then larval or juvenile migration could be an important factor. Evidence for this hypothesis can be found through the examination of variation in environmental parameters for populations of differing geographic ranges. Where the range of populations is small, often less than 100 kms (i.e. in the Northern Territory), the tidal range during summer spring tides can be over 7 metres and tidal mixing of fresh and salt waters is therefore substantial. Further, onshore NW monsoon winds would keep any fresh water flood plume close to the mouths of rivers. On the other hand, along the east Queensland coast, where populations extend for 500-600 kms, summer spring tides are less than 2.5 metres (except for XV) and the main rain bearing SE winds can drive extensive flood plumes along the coast. These flood plumes, which can exist long enough for larval development (2 weeks), contain huge amounts of nutrients and are responsible for massive algal blooms (O'Neill et al. 1992, Brodie and Mitchell 1992) similar to those developed in fertilised larval rearing ponds used for culture of this species (Rutledge and Rimmer 1991). The transport of larvae via the leading edge of freshwater flood plumes is the most likely explanation for the high level of migration deduced from the genetic data, particularly within populations. Rather than larval migration occurring regularly, the stochastic nature of extensive flooding in Australia, with major floods occurring approximately every 25 years, could produce a massive, pulsed transfer of juvenile fish from one location to another.

If this hypothesis is correct it could also explain the introduction of L. calcarifer into Australian waters. During periods of extremely low sea-level, the channel between north-western Australia and Timor would have been less than 200 kms across. Flood plumes from the extension of the Ord/Victoria River valley and/or the central river valley between New Guinea and Australia would have been able to reach the island of Timor and attract juvenile or adult fish into their freshwaters. Under similar conditions it is possible that estuarine fish such as the flathead *Platycephalus indicus*, which was originally endemic to Australia, migrated in the opposite direction (Keenan 1988, 1991). During the Quaternary, such migrations could have occurred from 16 -20, 150 - 155 or 250 - 260 kyrs ago, or earlier, when sea-levels were close to their lowest levels (Chappell 1983). Because the founding populations of barramundi would have represented a small offshoot of the south-east Asian population, the comparatively low level of gene diversity found in Australian barramundi is not unexpected. The extent and timing of the migration of barramundi into Australia could be determined by a genetic comparison of Australian and south-east Asian populations.

To summarise the results of this study, much of the population structure observed in Australian barramundi has developed in the last 18000 years and is primarily a function of isolation-by-distance. Not only are population differences very small, although statistically significant, but also there is evidence of natural hybridisation between two "ancestral" populations of barramundi that had been isolated for 110 kyrs. Biological differences that have been observed between natural populations of barramundi, including sexuality, growth, predation, breeding and migration patterns, can vary between sites within populations and from season to season. In addition, laboratory studies have shown little difference in growth rate and temperature response between tropical and temperate derived populations (J. Burke, L. Rodgers pers. comm.). These biological differences are therefore, most likely, a phenotypic response to specific environments. An experiment designed to examine genotype by environment interaction would clarify the relative importance of genotype on phenotype. Because of the recent and rapid nature of the recolonisation of the Arafura Sea and Torres Strait, the zoogeographic hypothesis proposed by this study would be applicable to many of the other marine species inhabiting this region. Recent studies have found significant levels of population differentiation between the east and west coasts of Australia; in the prawn, *Penaeus monodon* by Benzie et al. (1992), green turtles *Chelonia mydas* (Norman, pers. comm.), and the saucer scallop, *Amusium japonicum balloti* (unpublished data).

While studies have often suggested that the one-dimensional stepping stone model should be used to explain population differentiation, an extensive literature search has revealed few applications of this model to observed data. A comparison of levels of subpopulation differentiation within and among vertebrate classes reveals large differences in FST values (Ward et al. 1994). For example, the mean G_{ST} level of 57 species of marine fish (0.062) is similar to that of birds (0.078, 27 species), whereas freshwater fish (0.222, 49 species), mammals (0.207, 83 species), reptiles (0.258, 33 species) and amphibia (0.308, 33 species) have a much higher degree of subpopulation differentiation (Ward et al. 1994). This study has highlighted the fundamental importance of dimensionality in the development of population structure and can provide a simple explanation for these intriguing differences. It would appear that the level of subpopulation differentiation is inversely related to the dimensionality of migration. The groups with two dimensional access to their environment, marine fish and birds, have much lower levels of subpopulation differentiation than species limited to essentially a single dimension. Even within marine species, Ward et al. (1994) were surprised to find that of the five fish species with G_{ST} levels greater than 0.20, four were limited to movement along a coast. They attributed these large differences in G_{ST} to different rates of migration between subpopulations for marine and freshwater environments. However, the migration rate necessary to maintain a certain level of subpopulation differentiation in one dimension is two orders of magnitude higher than that required in two dimensions. Therefore, while the migration rate may be approximately equal for both one and two dimensional environments, the large increase in G_{ST} in one-dimensional (e.g. freshwater) environments results from the limitation of gene flow to only two adjacent populations (in a linear array) in contrast to four or more populations in a two-dimensional space. Of course there are many other variables that effect the flow of genes between populations, including effective population size, migratory ability and the evolutionary age of populations, however the most significant factor would appear to be the degree of dimensionality. The importance of one-dimensional population structure was illustrated in recent work by Goldstein and Holsinger (1992), who demonstrated that it allowed the maintenance of far higher levels of polygenic variation in the overall population than a two-dimensional model.

On both theoretical and practical grounds, the one-dimensional model accurately explains the barramundi allozyme data. This is not the case for the island model. The extremely low migration rate predicted by the island model does not concur with the substantial levels of migration that must have occurred during the Recent re-colonisation. Application of the one-dimensional model to similar studies, particularly studies of populations distributed within river systems, may provide new insights into population evolution. One can predict that the results of such studies on salmonid species will produce much higher levels of gene flow between populations within rivers than has previously been accepted.

BENEFITS: Section (i) Genetics

Implications and Recommendations

This study confirmed the subdivided nature of barramundi stocks around the coast of Australia. Three new stocks were determined in the Northern Territory and the geographic extent of other stocks in Queensland were refined. Management based on the 'stock structure concept' requires that the first step in successful management is the definition of the stocks. "Mis-management is inevitable in the absence of adequate knowledge of population structure" (Utter, 1995). The definition of stock structure has now been completed for almost all of the range of barramundi in Australia and fisheries managers are now able to confidently use this information when making management decisions.

In addition to the definition of unit stocks, the study provided an insight into the fundamental factors that have influenced the present day structure of the tropical Australian marine environment. Several implications arise from this insight.

Firstly, the level of inter-stock migration was far higher than predicted by the earlier genetic and tagging studies. Within Australia there are only isolated reports of coastal

migrations of tagged fish over 100 kms. Of 17135 tagged barramundi only 11 of the reported 2046 recaptures (0.54%) have moved outside of their native river and more than 100kms along the coast (Sawynok, pers. com.). The level of migration predicted by this study could result from either infrequent migrations of juveniles, primarily driven by flood plumes that travel along the coast (as suggested in the paper), or alternatively, from the movement of a 'marine' stock of fish from which tagging data has never been obtained (see part (ii) of this project).

Secondly, the overall study is responsible for a reappraisal of the management of translocations between barramundi stocks, primarily for aquaculture. The results indicate that the stock structure of Australian populations has developed relatively recently. Natural hybridisation has been proposed, and there appears to be little evidence of decreased viability usually associated with hybridisation between distantly related populations. These results have been adopted by several State Fisheries Departments issuing permits for the transfer of fingerlings. The tough approach originally adopted has been softened by the arguments developed by this study.

Thirdly, an explanation of the evolution of population structure has been provided, with reference to the biogeography of the habitat. Because of the recent and rapid nature of the recolonisation of the Arafura Sea and Torres Straits, this zoogeographic hypothesis is applicable to many of the other marine species inhabiting this region and assists with understanding the current distribution of tropical freshwater species.

Finally, this study has made a significant contribution towards the continuing development of genetic methods of analysis. While studies have often suggested that the one-dimensional stepping stone model may be used to explain population differentiation, this study is the first that examines the application of this model to observed data. On both theoretical and practical grounds the one-dimensional model accurately explains the data. Application of this model to other studies, particularly within river systems, is recommended.

BENEFITS: Sections (ii) & (iii) Scale chemistry and marine stocks; Otolith chemistry.

Discussion of Results including analysis of research outcomes compared with the objectives.

The objectives of this section of the project have been achieved in that:

- A method to determine fresh water and saltwater habitat history by examination of scale chemistry has been developed; and

- application of that method to barramundi from the Mary River and Chambers Bay has shown that there is a very high likelihood that many of the barramundi in Chambers Bay do not conform to the established life cycle of barramundi in that they have not had a fresh water residence period.

Unfortunately the methods used do not allow absolute proof of this. Examination of the chemistry of otoliths undertaken during this project (section iii) indicates that that method would be able to more conclusively describe the residence history of individual barramundi.

Implications

The fact that a substantial proportion of the barramundi population residing in coastal waters apparently does not undergo a freshwater phase represents a significant change to the understanding of barramundi biology and is of importance to the question of resource allocation. Sustainable commercial utilisation of that part of the population might not have a significant impact on availability of barramundi to the recreational sector which operates mainly in fresh waters and upper estuarine waters. Threadfin salmon, the other major target species of the coastal gillnet fishery in the NT, is possibly under-utilised to some extent in

some areas largely because of management measures in place to protect barramundi and ensure the quality of recreational barramundi fishing.

Given the findings of this study it is possible that some of the commercial value of barramundi and threadfin resources might be currently foregone without any real benefit accruing to the recreational sector. The current management of barramundi resources in the NT, particularly in the Mary River region, reflects these findings in that commercial fishing is allowed in areas remote from the river, apparently without any substantial negative impact on the quality of recreational fishing in the river.

While these findings are quite clear for the Mary River region they should not be applied to other areas without verification that a similar situation exists, particularly in river systems with different hydrological and geographic circumstances. It should also be made clear that the 'marine stock' barramundi should not be regarded as a separate, independent population but only as a part of the population which by circumstance has not had a freshwater phase. The progeny of those fish may well undergo the 'normal' upstream migration and spend their first 3-4 years in freshwater. While this is a most intriguing question there does not appear to be any real way to answer it at present although developments in mitochondrial DNA techniques in the future may be useful.

It is likely that the relative importance of the 'marine' part of the populations will vary according to seasonal conditions, mainly according to whether the wet season is early or late. Current stock assessments, which are strongly influenced by studies in fresh water, may need to be reappraised as a consequence of this information because a poor year class in fresh water may be offset to some extent by good recruitment to the marine part of the population.

INTELLECTUAL PROPERTY

No significant intellectual property has been generated by either section of this project.

FURTHER DEVELOPMENT - Section (i) - Genetics

Recent developments in genetic studies have shown that allozyme electrophoresis, as conducted by this study, is still a valuable and cost effective tool for the analysis of population structure (Carvalho and Hauser, 1995). Rapid new developments have occurred in the examination of DNA (both mitochondrial and cytoplasmic) but the present high cost of these techniques limits their application in many areas where large sample sizes are required for statistical confidence (Utter, 1995).

Several studies have been spawned from the results of this study. A PhD student at Griffith University, Stephen Chenoweth, is testing the hypothesis proposed by examination of the highly variable control region of barramundi mitochondrial DNA. Initial findings support the conclusions of this study. Because of the high level of genetic variability at the DNA sequence level, this PhD study also provides a valuable look into the potential application of genetic markers in restocking studies on barramundi and other species. A study at AGAL is examining the presence of RAPD markers in barramundi DNA using samples provided through this study.

As our knowledge of the genetics of barramundi increases, there are many potential applications that could be considered, primarily in the field of aquaculture and stocking. McPhee (1995) has suggested the development of a selection program for aquacultured barramundi which could increase growth rate by approximately 50% in a ten year period. As mentioned above, there is also considerable potential for the monitoring of the genetic impacts of restocking programs, and also identification of stocked fish, through the use of genetic markers. There is certainly a need for the monitoring of restocking programs to ensure that genetic variability in the stocked fish and the natural gene pool is not being lost through the use of inadequate number of parental fish (Keenan, 1995).

FURTHER DEVELOPMENT - Section (ii) Scale chemistry and marine stocks

While this study has shown that residence habitat of barramundi can be determined by the concentration of certain elements in the scales it has also shown that examination of otoliths using microprobe techniques is probably a more reliable method. Microprobe scans are being increasingly used to examine the lifetime residence environment of fishes and there is scope for use of those techniques to confirm and extend the results of this study. There is also scope for experimental rearing of barramundi in controlled environments to validate the conclusions of this study.

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FINAL COST

The final financial statement has already been submitted.

DISTRIBUTION

Northern Territory Fishing Industry Council

Amateur Fishermen's Association NT

Australian Nature Conservation Agency

Queensland Dept of Primary Industry

Queensland Commercial Fishermen's Organisation

Queensland Fisheries Management Authority

WA Fisheries Department

Barramundi Farmers Association

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