Stock Identity of Northern Fish Species

CSIRO Division of Fisheries Research
Cleveland Marine Laboratories

Final Report : FIRTA Project 83/48

CONTENTS

1. SUMMARY

The research program supported by this grant was directed at determining the number and distribution of subpopulations or genetic stocks which contribute to Australian fisheries for barramundi, northern sharks, and narrow-barred Spanish mackerel. Starch gel electrophoresis was used to reveal patterns of genetic variation in each species.

The analysis of approximately 4,500 barramundi collected at locations from the Ord River in Western Australia to the Rockhampton area of Queensland revealed the existence of at least 14 genetically discrete stocks. The significant amount of genetic subdivision characterizing Australian barramundi indicates that fisheries regulations and management policies must be focused on individual barramundi stocks in order to be maximally effective. The number and location of reserves, possible stocking of hatchery reared fish, and selective breeding of captive barramundi should all be based upon maintaining and exploiting the naturally occurring genetic heterogeneity of barramundi populations indicated by this study.

Our electrophoretic investigations of approximately 1,600 tropical sharks have revealed two important facts. First, the species dominating the fishery is not Carcharhinus limbatus as had been previously thought, but rather, Carcharhinus tilstoni. Second, there is no clear evidence of significant genetic subdivision in populations of either species in northern Australian waters. This latter observation suggests that management of the tropical shark fisheries can be conducted on a unit stock basis until evidence to the contrary is found.

Almost 2,000 narrow-barred Spanish mackerel from Australian localities were analyzed. The data indicate two major genetic groups in Australian waters: one along the eastern coast of Australia (Moreton Bay to Cape Grafton) and a second from the Torres Strait westward across northern Australia to the Monte Bello Islands of Western Australia. Spanish mackerel from southern Papua New Guinea (the port Moresby area) are not detectably different from those in northern and western Australia suggesting that a single, large stock may occur throughout this entire area.

2. IMPLICATIONS & RECOMMENDATIONS

2.1 Barramundi

The biochemical genetic investigations funded by this FIRTA grant have clearly demonstrated that barramundi exhibit considerable genetic heterogeneity throughout the range of the species in northern Australia. Indeed, the existence of at least 14 more-or-less distinct stocks has been documented. This relatively large amount of genetic differentiation among barramundi populations has several important implications for the management of the fisheries and conservation of the species:

1. Fisheries regulations and management policies must be focused on individual barramundi stocks, not on the species as a whole or even on major political units or geographic regions, in order to be maximally effective.

2. The establishment of "reserves" to provide protected areas for spawning and recruitment must be based upon the documented stock heterogeneity within this species. For all stocks to be protected in this way, it will be necessary to establish one or more such reserves within the geographic range of each stock.

3. Activities designed to enhance the production and/or promote the rehabilitation of exploited barramundi stocks, by way of transplantation or artificial propagation followed by intentional release into the wild, should be regulated to prevent the intermixing of genetically distinct stocks. Now that barramundi can be successfully spawned and reared in captivity, it is essential that policies and controls be instituted which will guarantee that the genetic integrity of native wild stocks is not compromised by intentional introductions of fish derived from other stocks.

4. At the same time, the information provided about genetic heterogeneity in barramundi may be used in selective breeding programs concerned with the production of fish for direct human consumption (not for release into the wild). The electrophoretic data furnish insight into the locations of genetically distinct

stocks. Artificial breeding programs should be designed to exploit these natural differences by utilizing broodstock from a diversity of source populations when selecting for desirable production traits. This is not to imply that the electrophoretic characteristics themselves are the basis of any desirable production traits, but rather, that they are indicators of where the genetic variation in the species is located geographically. Thus, the utilization of broodstock from populations found to be genetically differentiated from their electrophoretic characteristics should increase the likelihood of maximizing the genetic diversity of the broodstock, including those traits important in a hatchery context.

5. Although considerable insight into genetic aspects of stock structure in barramundi has resulted from this study, there is still much more work which should be done. We now have only a crude idea of the number and geographic distribution of barramundi stocks throughout northern Australia. It is important to continue and expand this research to refine our current knowledge of this species and provide needed information concerning several important questions such as:

(a) are there additional stocks of barramundi at locations which have not yet been characterized electrophoretically (e.g. Western Australia west of the Ord River; Northern Territory)?

(b) are there additional genetic markers (other polymorphic enzymes, etc.) that could be used to resolve more stocks and refine our present understanding of population structure in barramundi?

(c) what are the precise geographic locations of the boundaries between adjacent pairs of stocks, are the locations of these boundaries stable from year to year, and are these inter-stock boundaries characterized by abrupt discontinuities or gradual shifts in genetic traits?

(d) are the genetic characteristics of barramundi stocks stable over long time periods? and

(e) are there areas of stock intermixing (e.g. marine environments during spawning) and, if so, is it possible to identify the stock of origin of fish in such mixed-stock areas (cf. Milner et al 1985, "A genetic method of stock identification in mixed populations of Pacific salmon, Oncorhynchus spp." Mar. Fish. Rev. 47(1):1-8)?

2.2 Tropical Sharks

The electrophoretic investigations indicate that one of the two species which dominates the commercial shark fisheries in Northern Australia is Carcharhinus tilstoni, not Carcharhinus limbatus as previously thought. Because C. limbatus is also taken in the fishery, field identification of the catch must be improved to allow discrimination between these two morphologically similar species if harvest data and biological information collected for the fisheries are to be accurate. Furthermore, since the biology of these two species is likely to be different (e.g. they are known to exhibit different sizes at maturity in males) caution must be exercised in extrapolating data for the better studied C. limbatus from non-Australian areas and fisheries to C. tilstoni in Australia.

The electrophoretic data are entirely consistent with the existence of single, unit stocks of C. tilstoni and C. sorrah throughout the northern Australian waters studied. Although these results do not exclude the possible existence of multiple stocks, they provide no support for this. Thus, from a genetic perspective, there seems to be no justification for managing these fisheries on anything but a simple, unit stock basis.

2.3 Narrow-barred Spanish Mackerel

Our results suggest that only two major genetic stocks of narrow-barred Spanish mackerel occur in eastern and northern Australian waters. In spite of analyzing eight separate collections ranging from the Monte Bello Islands in Western Australia to Mornington Island in the Gulf of Carpentaria, little evidence of genetic subdivision among fish from northern Australian localities was obtained. Furthermore, these

fish were indistinguishable from fish collected in the Torres Strait and from the Port Moresby region of southern Papua New Guinea! However, the collections from eastern Australia (Cape Grafton and Moreton Bay) were distinct. These results suggest the existence of two major stocks: one restricted to eastern Australia and a second, wide-ranging stock occurring in southern Papua New Guinea, the Torres Strait, and across northern Australia. Regulation and conservation of this second, shared stock should involve joint efforts by biologists and managers in both Australia and Papua New Guinea. The existence of the Torres Strait Treaty provides a formal mechanism for implementing the coordinated, international management required by this shared resource.

3. OBJECTIVES

As outlined in the original 1983-84 grant proposal to FIRTA, this research program had, from its inception, three major goals:

1. To establish the optimal conditions for the electrophoretic analysis of stocks of barramundi (Lates calcarifer), tropical sharks (Carcharhinus tilstoni and Carcharhinus sorrah), and Spanish mackerel (Scomberomorus commerson).

2. To obtain frozen tissue samples of each species from various localities throughout the Australian range of each species and to analyze these samples for evidence of stock heterogeneity.

3. When the existence of two or more genetically differentiated stocks was detected, to determine the genetic characteristics and geographic boundaries of each stock.

4. METHODS

Specimens of each species under investigation were obtained from a variety of sources (capture by research vessel, purchase from commercial fishermen, gratis from interested commercial fishermen, etc.). The fish themselves had originally been captured using techniques common to each

species (e.g. barramundi - gillnets; tropical sharks - longlines; Spanish mackerel - trolling). Tissue samples were excised and frozen (at approx. -20°C) soon after capture of the fish. The samples were transported to the CSIRO Laboratory at Cleveland, Qld. (usually on dry ice) for analysis. Muscle, eye, and liver tissues were used in the case of the tropical sharks. In the laboratory, the tissue samples were thawed, homogenized in buffer, and centrifuged to yield a clear supernatant. The resulting tissue extracts were stored at -80°C in sealed glass vials until the electrophoretic analyses were performed.

Horizontal starch gel electrophoresis followed by specific histochemical staining was used to analyze genetic variation in enzymes in all three species. General proteins were analyzed using horizontal slab polyacrylamide gel electrophoresis followed by staining for non-specific proteins. The general experimental procedures utilized for all species are detailed in Shaklee and Keenan (1986 CSIRO Marine Laboratories Report 177) while the specific methods used in the analysis of barramundi have been described in Shaklee and Salini (1985).

The general approach used in interpreting observed patterns of presumed genetic variation has been described in some detail by Shaklee and Salini (1985). Similarly, the general methods of data analysis used for all species are outlined in Shaklee and Salini (1985).

5. RESULTS

5.1 Barramundi

Approximately 4,540 specimens from 30 major localities (ranging from the Ord River in Western Australia to the Rockhampton-Gladstone area of Queensland, and including two localities in Papua New Guinea) were analyzed (Table 1). Forty-six loci were surveyed for genetic variation in this species. Average heterozygosity in this species was approximately 0.03. Twelve loci were found to be polymorphic at the $P_{.99}$ level (in at least one population) and were screened in all populations (Table 2). All loci (except Ah, which exhibited several departures from expectation and was omitted from most analyses for that

reason) appeared to be in Hardy-Weinberg equilibrium since only 5 out of 228 tests involving the remaining 11 loci yielded significant departures from expectation. The Daly River (NT) was sampled five times over a 14-month period and allele frequencies did not differ significantly over this time interval. Several other localities were sampled more than once and allele frequencies remained stable at all of these localities as well. These observations suggest that the genetic characters used in this study are reasonably stable characteristics of populations of fish at particular localities and are not simply transient properties associated with individual collections. Collections taken in both fresh and saltwater habitats in the Gulf of Carpentaria (at both the Staaten and Nassau Rivers) revealed no significant differences in the genetic profiles of fish inhabiting these two different habitats. When all barramundi samples were tested simulataneously, an extremely significant chi-square value was obtained indicating significant stock heterogeneity among all collections. A series of tests involving geographically adjacent pairs of localities was then conducted to identify individual stocks and boundaries between adjacent stocks. These tests identified a total of 14 genetically distinct stocks in Australia (Table 3). These tests also demonstrated that Australian barramundi stocks along the Cape York Peninsula are genetically distinct from Papua New Guinea barramundi which spawn near the mouth of the Fly River in that country.

5.2 Tropical Sharks

Approximately 925 Carcharhinus tilstoni (14 collections) and 655 Carcharhinus sorrah (15 collections) were collected from ten major areas ranging from the North-west Shelf in Western Australia (C. sorrah only) to north-eastern Queensland (Table 1). A total of fifty-two loci were screened for genetic variation in each species. Average heterozygosity (H) was 0.037 for C. tilstoni and 0.035 for C. sorrah. There were 11 loci in C. tilstoni which were polymorphic at the $P_{.99}$ level (5 loci at the $P_{.95}$ level) and 9 loci in C. sorrah which were polymorphic at the $P_{.99}$ level (5 loci at the $P_{.95}$ level) (Table 2). The presumed genetic basis of the observed variation was verified for five loci by investigations of phenotypes in pregnant females and their pups. All loci in both species (except Est-1 in C. tilstoni which exhibited

numerous significant deficiencies of heterozygotes in numerous collections) were deemed to be in Hardy-Weinberg equilibrium as only four deviations from expectation were observed (out of a total of approximately 120 tests). No genetic heterogeneity was observed between males and females or between immature and mature individuals in either species. There was little or no evidence of significant genetic heterogeneity among all samples of either species (although the collections of C. tilstoni from the north-east Gulf of Carpentaria were slightly different from other collections of that species. Hierarchical analysis in C. tilstoni revealed that approximately 99.4% of the observed variation occurred within collections, 0.6% occurred among collections, and only approximately 0.1% occurred among areas. Similar analysis for C. sorrah indicated that approximately 99.7% of the observed variation occurred within collections, approximately 0.28% among collections, and only approximately 0.02% among areas and regions combined.

When this study was initiated, the tropical shark fishery in Northern Australia was thought to be dominated by two species, Charcharhinus sorrah and Carcharhinus limbatus. At about the same time, field investigations by J. Lyle (NT DPI Department of Fisheries) and J. Stevens (CSIRO Division of Fisheries Research) suggested that C. limbatus exhibited heterogeneity in several characteristics including size at maturity in males, pigmentation pattern, and number of vertebrae. More or less independently, our electrophoretic screening indicated that there were actually two distinct species lumped together under the name Carcharhinus limbatus in northern Australia. Integration of these various lines of investigation led to the discovery that C. limbatus was actually a relatively minor component of the commercial shark fisheries operating in northern Australia and that the second species, C. tilstoni (which had previously been presumed to be C. limbatus), was actually the major contributor to the fisheries.

5.3 Spanish Mackerel

A total of 2,719 Spanish mackerel from three major regions were analyzed (Australia, including the Torres Strait, N=1,928; Papua New Guinea, N=401; and other miscellaneous localities N=290) (Table 1). Over 40 loci were screened during this study, but only 30 were screened in all fish. Twenty loci were polymorphic at the 0.99 level in one or more collections, and at least ten of these loci were polymorphic at the 0.95 level. To date, only 15 of the most variable of these loci have been analyzed in detail. These 15 loci are indicated in underlined print in Table 2. All discussion and conclusions presented below are based on these 15 loci.

All loci except that encoding alcohol dehydrogenase (Adh) appeared to be in Hardy-Weinberg equilibrium. Adh exhibited a consistent excess of rare allele homozygotes in many individual collections and in pooled samples. For this reason, care should be exercised in interpreting Adh data in this species. Repeated annual samples were obtained from two Australian localities (Cape Grafton, N=2, and Moreton Bay, N=3) and one Papua New Guinea locality (New Ireland N=2) and these were used to test the temporal stability of allele frequencies. These tests failed to demonstrate any significant differences in allele frequency between annual samples at either Cape Grafton or in Moreton Bay, suggesting temporal stability of these genetic characters. In constract, the two samples from New Ireland did exhibit significant year-to-year genetic heterogeneity due to allele frequency differences at two loci: Ah and Ldh-B. Most of this heterogeneity was due to Ah and may be explained by possibly unreliable scoring of this locus in these collections due to poor sample quality. The repeat samples from both Cape Grafton and from Moreton Bay were pooled since no significant differences in allele frequencies were apparent. The two collections from New Ireland were maintained as separate entities for the tests of stock structure.

Tests of stock structure were initiated by conducting chi-square tests of pairs of geographically adjacent collections. When adjacent pairs of collections failed to exhibit significant genetic differences by this criterion, they were pooled together. Chi-square tests between adjacent pairs of such pooled samples were then conducted. This process

was continued until the pattern of stock differentiation was established and the inter-stock boundaries located. These tests indicated that at least seven genetically distinct stocks of narrow-barred Spanish mackerel exist throughout the localities surveyed in this study (Table 4). Two features concerning these Spanish mackerel stocks stand out and deserve comment. First, there is considerable genetic heterogeneity among Spanish mackerel stocks in some areas (e.g. Papua New Guinea). Second, in spite of the heterogeneity shown within Papua New Guinea, Spanish mackerel are quite homogeneous throughout the range from Port Moresby, PNG in the east, through the Torres Strait, and all across the north of Australia as far as the Monte Bello Islands of Western Australia. These two seemingly contradictory patterns are probably both explained, at least in part, by the same factor -- presence or absence of barriers to adult migration and/or larval dispersal.

6. TABLES

TABLE 1. Major collections analyzed for each species

BARRAMUNDI

Western Australia	Queensland
Ord River (N=69)	Norman River (N=407)
	Staaten River (N=238)
Northern Territory	Nassau River (N=114)
Daly River area (N=595)	Archer, Hey, & Embley R. (N=53)
Mary River (N=150)	Pennefather - Cotterell R. (N=151)
Glyde River (N=78)	Escape River (N=62)
Blue Mud Bay (N=100)	Orford - Weymouth Bay (N=155)
Roper River (N=116)	Lockhart River (N=157)
McArthur River (N=201)	Bobart Point - Friendly Point (N=177)
	Bizants & Normanby Rivers (N=159)
	Bathurst Bay & Ninian Bay (N=203)
	Bedford Bay - Cairns (N=84)
	Murray & Tully Rivers (N=65)
	Hinchenbrook Island (N=475)
	Repulse Bay - Mackay (N=338)
	Cape Palmerston - Broad Sound (N=51)
	Rockhampton - Baffle Creek (N=70)

TROPICAL SHARKS

Locality	Carcharhinus tilstoni	Carcharhinus sorrah
North-West Shelf	--	19
Timor Sea	51	57
Fog Bay	113	115
Melville Island	101	65
Croker Island	126	114
Wessel Island (offshore)	146	32
Wessel Island (innshore)	100	103
southern Gulf of Carpentaria	96	56
eastern Gulf of Carpentaria	54	39
northeastern Queensland	138	55

Table 1. (contd.)

NARROW-BARRED SPANISH MACKEREL

north central Western Australia
North West Cape -- Cape Leveque
5 localities N=716
northeastern Western Australia
Cape Bougainville N=102
Northern Territory
Cape Croker N=118*
Gulf of Carpentaria
Mornington Island N=162
northeastern Queensland
Cape Grafton N=290
southeastern Queensland
Moreton Bay N=354*
Torres Strait
2 localities N=262
southern Papua New Guinea
Duago Island area N=79
northeastern Papua New Guinea
Tufi N=109
northwestern Papua New Guinea
Wewak N=74
New Ireland -- New Britain
2 localities N=239
New Caledonia N=57
Fiji N=110
South Africa N=123

* = collection only partially analyzed

TABLE 2. Polymorphic ($P_{.99}$) enzyme loci. (Loci polymorphic at the 0.95 level in at least one Australian collection indicated by *).

BARRAMUNDI

Aconitate hydratase*
Creatine kinase-A
Esterase-2*
Esterase-D*
Fumarate hydratase
General protein*
Glucose-6-phosphate isomerase-A
L-iditol dehydrogenase
Isocitrate dehydrogenase-1*
Isocitrate dehydrogenase-2
Lactate dehydrogenase-C*
Malate dehydrogenase-2
Phosphogluconate dehydrogenase*
Phosphoglucomutase

TROPICAL SHARKS

	C. tilstoni	C. sorrah
Aconitate hydratase	yes*	yes
Creatine kinase	yes*	yes
Cytosolic aminopeptidase		yes*
Esterase-1	yes*	yes*
Esterase-D	yes	yes*
Fumarate hydratase	yes*	
Glucose-6-phosphate isomerase	yes	yes
Malate dehydrogenase-2		yes
Malate dehydrogenase-3	yes	
Malate dehydrogenase ($NADP^+$)-2	yes	yes*
Octanol dehydrogenase	yes	
Phosphoglucomutase	yes*	
Proline dipeptidase	yes	yes*

NARROW-BARRED SPANISH MACKEREL

Acid phosphatase*
Aconitate hydratase*
Adenosine deaminase-2*
Alcohol dehydrogenase*
Aspartate aminotransferase (mitochondrial)
Creatine kinase-A*
Esterase-4*
Glucose-6-phosphate isomerase-A*
Glycerol-3-phosphate dehydrogenase-2
Isocitrate dehydrogenase-2*
Lactate dehydrogenase-B
Malate dehydrogenase-1
Malate dehydrogenase ($NADP^+$)
Mannose-6-phosphate isomerase
Phosphoglucomutase
Phosphogluconate dehydrogenase*
Proline dipeptidase-1
Proline dipeptidase-2
Superoxide dismutase-1
Superoxide dismutase-2 (mitochondrial)

TABLE 3. Genetically distinct stocks of Australian barramundi.

Western Australia

1. Ord River

Northern Territory

2. Daly River area
3. Mary River
4. Glyde River
5. Blue Mud Bay
6. Roper River
7. McArthur River

Queensland

8. southeastern Gulf of Carpentaria
9. northeastern Gulf of Carpentaria
10. northeastern coast of Cape York
11. Shelburne Bay--Lockhart River area
12. Princess Charlotte Bay
13. central coastal Queensland (Cairns--Hinchenbrook Island)
14. southern coastal Queensland (Repulse Bay--Baffle Creek)

TABLE 4. Genetically distinct stocks of narrow-barred Spanish mackerel.

1. EASTERN AUSTRALIA
   (Moreton Bay - Cape Grafton)

2. NORTHERN & NORTHWESTERN AUSTRALIA, TORRES STRAIT, AND SOUTHEASTERN PAPUA NEW GUINEA
   (Gulf of Carpentaria to Monte Bello Islands; Torres Strait; Port Moresby area of PNG)

3. NORTHEASTERN PAPUA NEW GUINEA
   (Tufi - New Britain - Wewak)

4. NEW IRELAND

5. NEW CALEDONIA

6. FIJI

7. SOUTH AFRICA

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8. PUBLICATIONS

Studies suggest multiple stocks of Australian barramundi

RESEARCHERS in the CSIRO Division of Fisheries Research are utilizing electrophoresis, a biochemical technique for analysing proteins, to study barramundi fish stocks in northern Australia.

Their results, indicating the existence of several stocks, should allow fishery biologists to formulate future policies to protect and manage the existing fishery.

by James B. Shaklee and John P. Salini, Division of Fisheries Research, CSIRO (Cleveland, Qld).

Biology and fishery

Many important aspects about the basic biology of barramundi have recently come to light as the result of research projects carried out by the Fisheries Research Branch of the Queensland Department of Primary Industries, the Fisheries Division of the Northern Territory Department of Primary Production, and the Division of Fisheries Research of CSIRO (see *Australian Fisheries* July 1982, pp. 27-28).

These studies have revealed that barramundi change sex, first maturing as males at about age three and later becoming females in about their fourth or fifth year. The main spawning period is generally from September through February and individual females may produce as many as 10 million eggs. Because successful fertilization and embryonic development require at least 50 per cent seawater, adults living in freshwater streams and billabongs must migrate to the ocean during the spawning season. Tag-recapture studies in both the Northern Territory and Queensland have indicated that there is some movement of adult fish among neighbouring river systems.

Commercial barramundi fisheries generally are centred at river mouths along the coasts of Queensland and the Northern Territory. Current management policies restrict the length and mesh size of the gill nets used and a closed season during the peak of the spawning season further limits the commercial catch.

In recent years reports from fishermen have suggested that the composition of the commercial catch has changed, with smaller individuals comprising an ever-increasing percentage of the total catch.

This shift in the catch may have particularly profound effects on the future of the barramundi resource for two reasons.

First, as with any fishery, when the larger individuals have been removed, the reproductive potential of the species is decreased because large individuals usually produce the greatest number of eggs and are the most successful spawners.

Second, in the case of barramundi (where all large individuals are females), any shift in size and age composition brought about by the fishery can also result in a significant change in the sex ratio. In fact many catches of barramundi presently consist of 60 to 90 per cent males. This distorted sex ratio may further limit reproduction and thus recruitment of new generations to the fishery.

Given the ever-increasing demand for barramundi, the apparent shift in size and sexual composition of the catch, and the declining commercial catch (official figures indicate a decrease from about 3 200 tonnes in 1978-79 to slightly more than 1 000 t in 1980-81) there is a great deal of concern about the nature and viability of barramundi stocks in Australia and the future management of this fishery.

One important aspect of barramundi biology about which virtually nothing is known is the number of different stocks or sub-populations which can contribute to the barramundi fishery.

Fish stocks and management

Since a fish stock can be considered a self-reproducing unit within a fishery, the number and characteristics of such component stocks can be of critical importance to the management and long-term future of the entire fishery.

At one extreme, we can imagine that all barramundi in northern Australia could be members of a single large interbreeding stock. In this case, fishing harvest in any one location can actually affect the species throughout its range since all individuals contribute to the single large stock. In such a situation, one overall management program for the species would be appropriate.

At the other extreme, we can imagine that the barramundi fishery could be comprised of numerous smaller sub-populations, each associated with a major river system, and each more or less independent and reproductively isolated from the others. In this situation, the effects of the fishery on any one stock would be localized or semi-isolated so that the viability of other stocks should not be affected.

Given a situation where several barramundi stocks exist, the management of the entire fishery must be structured in such a way as to reflect the multiplicity of stocks

Australian Fisheries, February, 1983
42:36-38.

contributing to the overall harvest.

The barramundi fishery is presently managed on the assumption that it is composed of a single large stock. For this reason, certain streams are closed to all fishing to act as reserves — functioning as nursery grounds to stimulate the recruitment of juveniles into the fishery.

If only a single stock of barramundi actually exists, such a management policy would be expected to enhance the entire fishery.

On the other hand, if the barramundi fishery is, in reality, composed of many smaller stocks, this policy of setting aside small reserves may only strengthen certain isolated stocks and not benefit the overall fishery.

There are many ways to investigate the stock structure of a species. These include: tag-recapture programs; the study of biological features such as reproductive traits, life history characteristics, and behaviour; and the search for differences in parasites or anatomical characters.

However one of the most direct methods is to examine the genetic interrelationships of the fish in different areas.

We expect individuals within a stock to be genetically related to one another due to random interbreeding within the stock. Individuals from different stocks should be genetically different because of partial or complete reproductive isolation between stocks. Therefore genetic characteristics can provide considerable insight into the stock structure of the fishery.

In our laboratory at present we are studying the pattern of genetic variation of proteins in barramundi to investigate this problem of stock structure.

It first involves field sampling of tissues from a large number of individual fish from several localities. This aspect of the research is being conducted by biologists with the Fisheries Research Branch of the Queensland DPI and the Northern Territory Fisheries Division. The tissue samples are frozen and sent back to the CSIRO Division of Fisheries Research laboratory at Cleveland (Brisbane), where the actual biochemical tests are done. Extracts of each tissue (in this case muscle, liver and eyes) are prepared and subjected to electrophoretic analysis.

Electrophoresis

Electrophoresis is a procedure that depends upon the movement of charged molecules in an electric field. The rate of movement depends on the voltage applied and the overall electrical charge of each molecule. Protein molecules move in an electrical field because they are composed of amino acids, many of which are charged.

Each tissue of a fish contains thousands of different kinds of proteins which form the basis for both the structure and metabolism of the tissue. Electrophoresis provides a means of separating protein molecules that have different electrical charges. Therefore it can be used (in conjunction with a general protein stain which allows the separated proteins to be easily seen) to determine the pattern of proteins characteristic of a species of fish.

Because different species have different general protein patterns in, for example, muscle tissue, this procedure can be used to identify fish fillets and detect substitution. This simple application of the technique for identifying barramundi fillets has recently been described in *Australian Fisheries* (July 1982, pp. 19-20— and is being used by several agencies (including the CSIRO Tasmanian Food Research Laboratory, The Queensland Government Chemical Laboratory and the Australian Government Analytical Laboratory) to monitor the barramundi substitution problem.

We are utilizing this same basic technique — electrophoresis — together with biochemical and genetic studies, to investigate the stock structure of barramundi.

In our case, we take advantage of the fact that the amino acid composition of each protein is determined by the gene in the DNA specifying that protein. Any change in protein structure detected by a different rate of movement during electrophoresis indicates a change in the gene encoding that protein. Such different forms of a gene are called alleles.

By monitoring many proteins by electrophoresis, we can determine the genetic characteristics of individual fish. However, since each tissue contains several thousand different kinds of protein molecules, we restrict our analysis to one class of protein — enzymes.

Because each enzyme catalyses one and only one biochemical reaction, we employ specific enzyme staining following electrophoresis to show up and identify one type of enzyme at a time. This makes the approach more sensitive and allows us to make genetic interpretations of the resulting patterns seen after electrophoresis.

When the analysis is done repeatedly for many different enzymes and many individual fish from several localities, we can describe the genetic characteristics of the fish populations sampled in each area and determine whether or not different samples come from different stocks or breeding groups.

Genetic differences

To date we have examined the genetic characteristics of barramundi from three general localities: eastern Queensland (Princess Charlotte Bay), the Gulf of Carpentaria (Staaten and Nassau Rivers in the south-eastern Gulf), and the Northern Territory (Daly River).

Samples of fish from each locality have been electrophoretically analysed on starch gels for at least 15 different enzymes. Figure 1 schematically illustrates the pattern of variation seen for the enzyme esterase (EST) in fish from the Northern Territory (NT) and the east coast of Queensland (PCB).

Although we have detected four different esterase types (alleles) in our study of barramundi, only three were common in these samples and these three (labelled **+**, **m**, **s**) are shown in the figure.

The proportions of all four types (**f**, **+**, **m** and **s**) in the samples are shown in the 'pie' diagrams in Figure 2.

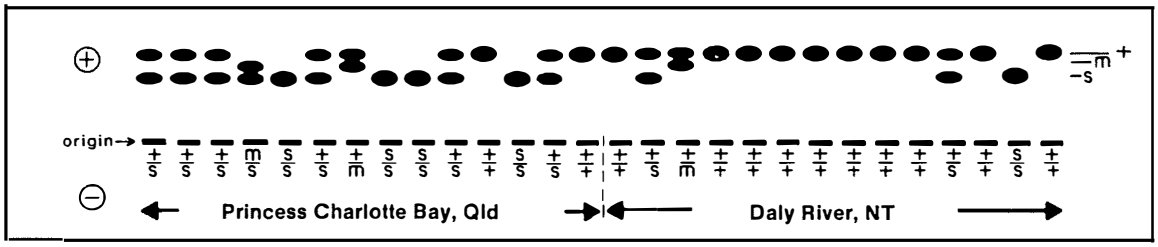

Figure 1. *Electrophoretic analysis of barramundi esterases (schematic drawing). Tissue extracts from 28 individual fish (14 from Queensland and 14 from the Northern Territory) were loaded onto a starch gel at the sample origin and electrophoresed for six hours. A slice of the gel was then stained for esterase. Three esterase types — **+, m** and **s** —each due to a different allele of the esterase gene were observed. The allelic composition of each fish is indicated at the bottom of the figure. Note that approximately equal numbers of + and **s** alleles occur in the eastern Queensland sample while the + allele predominates in the Northern Territory sample. The Gulf sample is somewhat intermediate.*

In the sample of barramundi from the Northern Territory, only three types were observed (the **f** allele was absent). The + type made up 84 per cent of the total while the **s** allele contributed only 11 per cent.

In contrast, the barramundi samples from the south-eastern Gulf and from Princess Charlotte Bay each had all four alleles present and the contribution of the **s** type was much greater (making up 34 per cent of the total in the Gulf sample and 43 per cent in the PCB sample) than in the NT sample.

Statistical tests of these data for esterase indicate that the three samples are significantly different from each other, implying that each barramundi sample was derived from a different stock. Preliminary studies of a barramundi esterase by Dr Barry J. Richardson (formerly of the Australian National University) also suggested such stock differences.

We have completed similar electrophoretic analyses for an additional 14 enzymes. Genetic variation has been observed for at least five of these (isocitrate dehydrogenase-IDH in both liver and muscle, lactate dehydrogenase-LDH, phosphogluconate dehydrogenase-PGDH and umbelliferase-UMB). Four of these enzymes also show evidence of genetic differences among the three areas.

Taken together, these data indicate that at least three separate stocks of barramundi exist in northern Australia. How many additional stocks, and what their boundaries may be, can be determined only by additional investigations (which are planned and in progress).

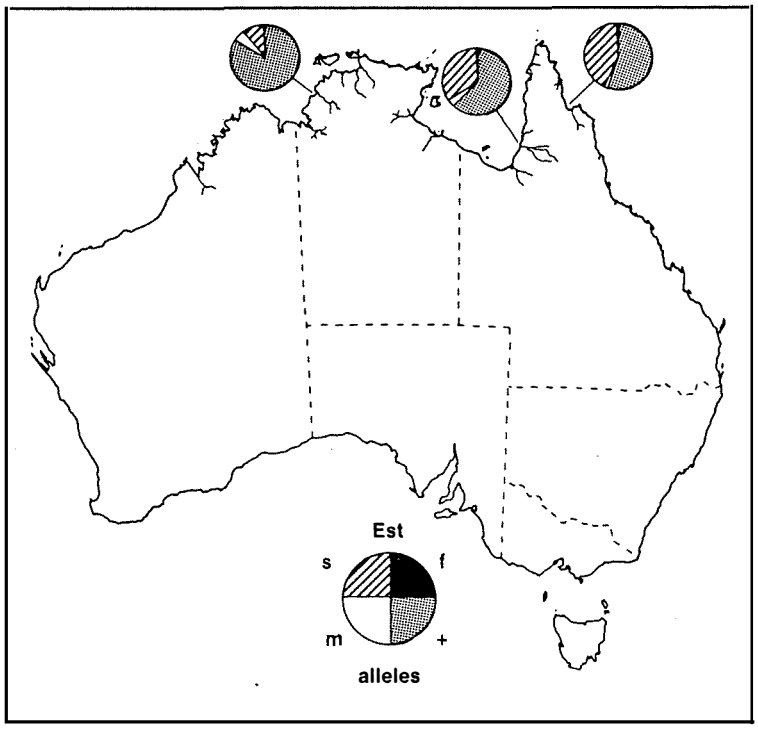

Figure 2. *Esterase allele proportions in barramundi from three localities: Princess Charlotte Bay, Queensland (58 fish); south-eastern Gulf of Carpentaria, Queensland (84 fish); Daly River, Northern Territory (48 fish). The proportions of each allele (**f, +, m** and **s**) in each sample are represented by the size of the wedge in each pie diagram.*

These results emphasize two major points.

The first, which is specific to barramundi, is that several stocks exist. This means that a single uniform management policy for the entire species is undoubtedly non-ideal and possibly quite inappropriate.

The second point is that biochemical genetic studies of fishes utilizing electrophoresis can provide valuable insight regarding the number, characteristics, and boundaries of fish stocks and thus make a significant contribution to fisheries management in Australia.

Commonwealth Scientific and Industrial Research Organisation
Marine Laboratories Reprint No. 1398
Reprinted from *Australian Fisheries* Vol. 42 No. 2 February 1983
Australian Fisheries Reprint No. 108

Reprinted from *Australian Fisheries*
Vol. 42 No. 2 February 1983
Australian Fisheries Reprint No. 107

Aust. J. Mar. Freshw. Res., 1985, **36**, 203–18

Genetic Variation and Population Subdivision in Australian Barramundi, *Lates calcarifer* (Bloch)

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Abstract

Starch gel electrophoresis of enzymes and polyacrylamide gel electrophoresis of muscle proteins were used to study genetic variation in 589 barramundi from three widely separated regions in northern Australia. Presumed genetic variation was observed at 16 loci (out of 46 screened). However, only 11 of these were polymorphic at the $p_{0.99}$ level. The average heterozygosity in this species was 0·032. In nearly all cases, genotypic proportions agreed closely with Hardy–Weinberg expectations. Repeated sampling in each region revealed little or no change in allele frequencies over a period of several months. Similarly, comparisons of allele frequencies for fish from marine and from freshwater localities in the south-eastern Gulf of Carpentaria failed to reveal significant genetic differences between habitats. Between-region heterogeneity χ^2 tests indicated substantial genetic differentiation at 10 of the 11 polymorphic loci. These data indicate the existence of at least three distinct stocks or subpopulations of barramundi in Australia.

Introduction

The barramundi, *Lates calcarifer* (Bloch) (Centropomidae), is a tropical, Indo-west Pacific species ranging from the Persian Gulf to Australia and Papua New Guinea (Greenwood 1976). In Australia, the species ranges from the Ashburton River (east of Exmouth Gulf) in Western Australia, across the north of the continent, and southward along the east coast of Queensland to the Mary River at Maryborough (Fig. 1). It is euryhaline and is known to inhabit freshwater ponds and rivers, tidal swamps, estuaries, and coastal reefs. Adults are long-lived (>10 years) and grow to a large size (>100 cm total length, >20 kg weight) (Reynolds and Moore 1982). There is evidence that barramundi in both Papua New Guinea and Australia are protandrous hermaphrodites—individuals first becoming sexually mature as males and subsequently becoming functional females (Moore 1979; Davis 1982, 1984). Although adults are known to prosper in entirely freshwater habitats, rearing studies in Thailand have indicated that successful embryonic development and hatching in this species require brackish waters (salinity >10) and that optimal salinity for larvae is 20–25 (Wongsomnuk and Manevonk 1973). These characteristics of barramundi mean that adults living in freshwater habitats must move downstream to brackish waters to reproduce successfully. It is generally thought that barramundi spawn in coastal waters. In Papua New Guinea, such coastal spawning often occurs after extensive downstream or alongshore migrations (Moore 1982; Moore and Reynolds 1982).

Throughout much of its range, *L. calcarifer* supports substantial fisheries. In Australia, it supports a major commercial gillnet fishery. Total commercial barramundi landings in Australia have varied from 181 t liveweight in 1969–1970 to 1686 t in 1977–1978 (Cameron 1982). In addition, the species supports a considerable angling sport fishery.

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Accurate catch statistics for this amateur fishery are not available, but estimates indicate that its total yield is high (Bandaranaike and Hampton 1979; Griffin 1979, 1982). Indeed, it seems likely that the overall harvest of the recreational fishery may equal or exceed that reported for the commercial fishery.

The decline in total commercial landings of barramundi since 1978, the recognition of the monetary and recreational values of the barramundi fisheries, and a desire to ensure the long-term viability of these fisheries have focused considerable attention on management programs for this species in Australia (Grey and Griffin 1979; Rohan 1981). Both Queensland and the Northern Territory have recently instituted numerous specific management initiatives.

Effective, long-term management of Australia's barramundi resource will also be dependent upon an understanding of the genetic basis of population structure in the species. That is, it is necessary to know whether or not multiple breeding groups or stocks contribute to the total resource and what the qualitative and quantitative contributions of each stock are. Furthermore, since management plans are usually administered on a geographic basis, information regarding the actual boundaries separating such barramundi stocks is essential to the formulation of an optimal management scheme.

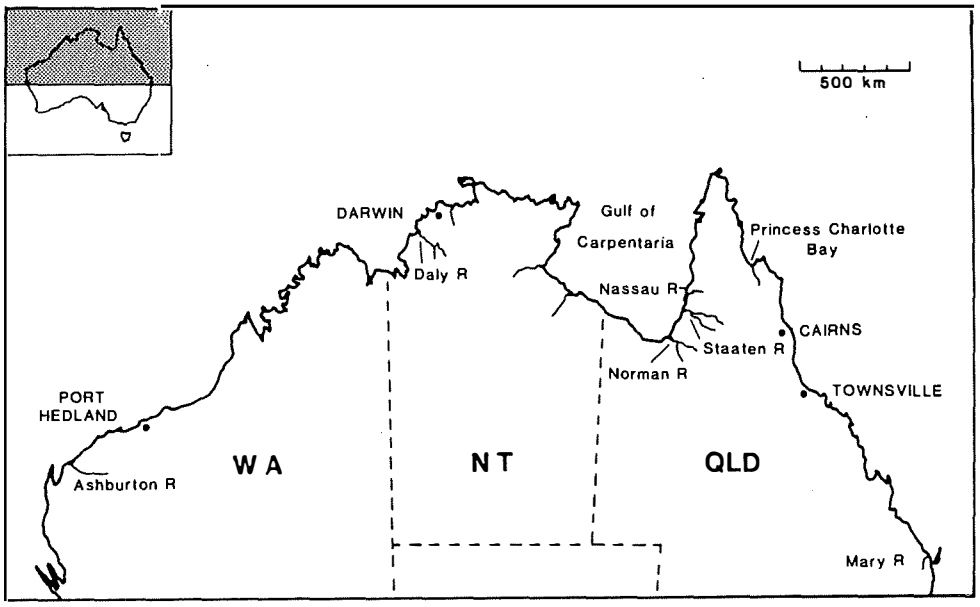

Fig. 1. Map showing the three localities in northern Australia sampled for barramundi in the present study.

The present investigation was undertaken to determine the nature and level of electrophoretically detectable genetic variation in *L. calcarifer* and to assess whether or not there was evidence for the existence of multiple barramundi stocks in Australian waters. The ultimate goal of this research program is to provide a stock basis for management of the fisheries.

Materials and Methods

Field Collection and Sample Preparation

Adult barramundi were collected from three widely separated geographic regions: the Princess Charlotte Bay area in north-eastern Queensland (from Cape Melville north-west to Lloyd Bay), the south-eastern Gulf of Carpentaria in northern Queensland (from the Norman River north to the Nassau River), and the Daly River in the north-western Northern Territory (adjacent to Anson Bay). They

were caught using commercial-size gill nets (stretch mesh size, 130–200 mm for the Queensland samples, 175 mm for the Northern Territory sample). See Fig. 1 for collection locations and Table 1 for sample details.

Animals from the two locations in Queensland were measured, sexed, and dissected immediately. Isolated samples of muscle, liver, and eye were individually frozen at −20°C or colder until homogenates were prepared in the laboratory. Some of the fish from the Northern Territory were treated in the same way, and others were frozen as 'frames' (carcass minus fillets) and shipped in this form to the laboratory where the frames were thawed, measured, and sexed. Individual tissue samples were then dissected from the thawed frames and homogenized. Pieces of white skeletal muscle were homogenized directly. Where necessary, livers were dissected to avoid the inclusion of macroscopic parasites (larval shark tapeworms—Trypanorhyncha) and to minimize the content of lipid in the samples. The eye samples consisted of the entire eyeball(s) minus the sclera and lens. Homogenates were prepared with a loose-fitting, motorized, stainless steel pestle in a polycarbonate centrifuge tube using approximately 1 volume of homogenizing buffer (1×10^{-1} M Tris, 1×10^{-3} M EDTA, 5×10^{-5} M $NADP^+$, pH 7·0) to 1 volume of tissue. All homogenates were centrifuged for at least 40 min at 20 000 × *g* (at 5°C). The resulting liver supernatants were centrifuged a second time under these conditions to minimize lipid and particulate content. Final supernatants were placed in sealed glass vials and stored at −70°C until the electrophoretic analyses were completed. Samples could be stored for many months in this way without qualitative changes in the isozyme patterns.

Table 1. Locality information and collection details for samples of *Lates calcarifer*

Locality	Collection No.	Date of collection	No. of specimens	Ratio of ♂ : ♀[A]	Average size (±s.d.) (mm)
Princess	1	Nov. 1981	51	n.d.	706·1 (±154)
Charlotte	2	Mar. 1982	53	n.d.	626·5 (±130)
Bay	3	June–July 1982	58	44 : 6	572·4 (±140)
South-eastern	4	May 1982	55	51 : 4	592·9 (±127)
Gulf of	5	May 1982	84	74 : 9	667·0 (±117)
Carpentaria	6	July 1982	49	37 : 12	721·4 (±99)
	7	July 1982	58	51 : 6	649·1 (±101)
Daly River	8	Aug.–Sept. 1982	48	39 : 2	729·7 (±112)
	9	Feb.–Mar. 1983	133	108 : 18	815·7 (±113)

[A] Sex was not determined for all individuals. n.d., no data.

Electrophoresis, Histochemical Staining, and Data Analysis

Horizontal starch gel electrophoresis (Selander *et al.* 1971) was used to investigate genetic variation in specific enzymes. Horizontal, thin slab, polyacrylamide gel electrophoresis (modified after Gahne *et al.* 1977) was used to monitor variation in general muscle proteins. Specific details of both techniques have been recently described (Shaklee and Keenan, unpublished manuscript). Thirty-six different enzyme systems were initially screened for variation using two or more different buffers for each enzyme. Enzyme-specific histochemical staining followed the recipes of Shaw and Prasad (1970), Siciliano and Shaw (1976), Harris and Hopkinson (1976), and Richardson (1983) with minor modifications. General proteins in muscle were stained with either Coomassie blue or the silver technique [see Keenan and Shaklee (1985) for details]. Population genetic data for all variable enzymes were obtained from gels loaded with one or more known variants at each locus.

Patterns of enzyme variation that were consistent with the subunit structure of the enzyme (when known) and simple models of Mendelian inheritance were scored and recorded as genotypes. Names of enzymes and Enzyme Commission numbers follow the recommendations of the Commission on Biochemical Nomenclature (Anon. 1978). For multilocus enzyme systems, loci were given alphabetic designations to indicate homology with loci of other species when appropriate (e.g. *Gpi-A*) or were simply assigned a number, beginning with 1, for the most anodally migrating isozyme. For each genetic system, alleles were designated according to the relative electrophoretic mobilities of the homomeric isozymes they encode. The most common homomeric isozyme of each system was assigned a mobility of '100' and the allele encoding the polypeptide was similarly designated '100'. All other alleles at

that locus were numbered according to the electrophoretic mobilities of their products relative to the mobility of the product of the 100 allele. Negative numbers refer to alleles encoding isozymes with cathodal migration. The putative genotype data were summarized as genotype and allele frequency distributions. The genotype distributions were examined for internal consistency with the Mendelian inheritance model by χ^2 testing for goodness-of-fit of observed genotype ratios with those expected for a single randomly mating population in the absence of differential selection among the alleles. The expected ratios were computed from 'observed' allele numbers using Levene's (1949) unbiased method for small samples. Heterozygosity for each locus (h) was calculated as $h = 1-x_i^2$, where x_i is the frequency of the ith allele. Average heterozygosity (H) was calculated as the mean of h over all loci examined. A locus was considered to be polymorphic (p) if the frequency of the most common allele was less than 0·99 at one or more localities. Heterogeneity χ^2 analyses for all polymorphic loci were conducted at three levels to investigate patterns of genetic differentiation among the samples. Temporal variability within a region was investigated by comparing individual collections taken at different times. Habitat-related variability within a region was examined by comparing saltwater and freshwater collections within river systems in the south-eastern Gulf of Carpentaria. Finally, large-scale geographic differentiation was analysed by tests of aggregate sample data pooled by region (i.e. Princess Charlotte Bay *v.* south-eastern Gulf of Carpentaria *v.* Daly River). Among-region differentiation was also characterized using Wright's F_{ST} statistic (Wright 1965, 1978) calculated for aggregate sample data pooled by region. The BIOSYS-1 computer program of Swofford and Selander (1981) was used throughout.

Table 2. Characteristics and conditions for analysis of polymorphic proteins in *Lates calcarifer*

Protein (EC number)	Locus	Subunit structure[A]	Tissue	Buffer[B]
Aconitate hydratase (4.2.1.3)	*Ah*	Monomer	Liver	CAEA
Esterase (3.1.1.–)	*Est-2*	Monomer	Eye	LiOH
Glucosephosphate isomerase-A (5.3.1.9)	*Gpi-A*	Dimer	Liver	Poulik
Isocitrate dehydrogenase ($NADP^+$)-1 (1.1.1.42)	*Idh-1*	Dimer	Liver	TRIC
Isocitrate dehydrogenase ($NADP^+$)-2 (1.1.1.42)	*Idh-2*	Dimer	Muscle	TC-1
Lactate dehydrogenase-C (1.1.1.27)	*Ldh-C*	Tetramer[C]	Eye	LiOH
Malate dehydrogenase-2 (1.1.1.37)	*Mdh-2*	Dimer	Liver	CAEA
Muscle protein	*Mp*	Monomer	Muscle	LiOH[D]
Phosphogluconate dehydrogenase (decarboxylating) (1.1.1.44)	*Pgdh*	Dimer	Liver	TRIC
Phosphoglucomutase (2.7.5.1)	*Pgm*	Monomer	Muscle	Poulik
Umbelliferyl esterase (3.1.1.–)	*Est-D*	Dimer	Liver	EBT

[A] Presumed structure based on isozyme banding pattern in heterozygotes.
[B] CAEA, citric acid–aminopropylmorpholine, pH 6·0 buffer of Clayton and Tretiak (1972). LiOH, lithium hydroxide–boric acid buffer; modified buffer 2 of Selander *et al.* (1971). Poulik, sodium hydroxide–boric acid, pH 8·7 buffer; buffer 3 of Selander *et al.* (1971). TRIC, triethanolamine–citric acid, pH 7·2 buffer of Clayton and Tretiak (1972). TC-1, Tris–citric acid, pH 7·0 buffer; buffer 1 of Shaw and Prasad (1970). EBT, EDTA–boric acid–Tris, pH 8·6 buffer of Boyer *et al.* (1963).
[C] Resolution not adequate to separate five expected isozymes.
[D] Electrophoresis in polyacrylamide gel.

Results

Fifty-three presumed enzyme loci were initially surveyed for genetic variation. Six enzyme systems (guanine deaminase, octanol dehydrogenase, phosphoglycerate kinase, pyruvate kinase, purinenucleoside phosphorylase, and triosephosphate isomerase) were not scorable either because of inadequate staining or because the resulting patterns were uninterpretable. These enzymes (treated conservatively as a single locus each) were not included in the calculations of polymorphism (p) or average heterozygosity (H). Thirty-one presumed loci were monomorphic in the first 150 animals (50 from each region).

These enzyme loci, which were not studied further, were aspartate aminotransferase-1 and -3, alcohol dehydrogenase, adenylate kinase, alanine aminotransferase, creatine kinase-B and -C, enolase, liver esterases (several presumed loci; only esterase-1 included here for

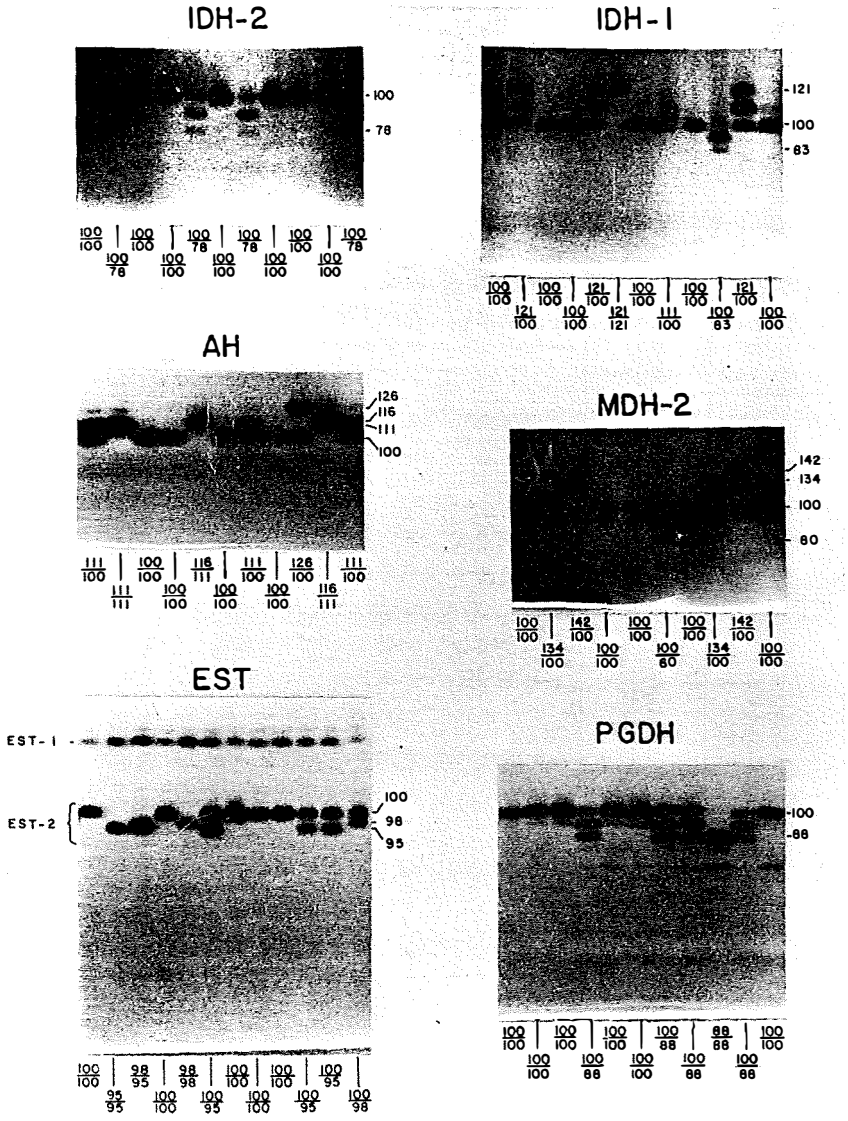

Fig. 2. Isozyme patterns for six variable enzyme systems in barramundi. The enzymes are: AH, aconitate hydratase; EST, esterase; IDH-1, isocitrate dehydrogenase-1 (liver); IDH-2, isocitrate dehydrogenase-2 (muscle); MDH-2, malate dehydrogenase-2; PGDH, phosphogluconate dehydrogenase. The anode is toward the top of each gel and the sample origin is at the bottom of each gel. The allelic classes for each enzyme are indicated to the right of each gel by their relative mobilities. The presumed genotype of each individual is indicated at the bottom of each gel.

calculation of H and p), glyceraldehyde-phosphate dehydrogenase (two loci), glutamate dehydrogenase, glycerol-3-phosphate dehydrogenase (two loci), glucose-6-phosphate dehydrogenase, glucosephosphate isomerase-B, fructose-bisphosphatase, hexokinase,

Table 3. Allele frequencies (and number of genes scored) for eleven variable loci in *Lates calcarifer* from nine collections in three regions (Princess Charlotte Bay, PCB; south-eastern Gulf of Carpentaria, SE. Gulf; Daly River) in northern Australia

— No data

Locus	Allele	Allele frequency											
		PCB			SE. Gulf				Daly River		PCB	SE. Gulf	Daly River
		1	2	3	4	5	6	7	8	9	1+2+3	4+5+6+7	8+9
Ah	126	—	—	0·018	—	—	0·047	0·056	0·000	0·008	0·018	0·052	0·006
	116	—	—	0·232	—	—	0·116	0·120	0·045	0·094	0·232	0·119	0 082
	111	—	—	0·027	—	—	0·151	0·213	0·318	0·165	0·027	0·186	0·203
	100	—	—	0·723	—	—	0·686	0·611	0·636	0·733	0·723	0·643	0·709
				(112)			(86)	(108)	(88)	(266)	(112)	(194)	(354)
Est-2	102	0·000	0·000	0·009	0·000	0·000	0·000	0·009	0·000	0·000	0·003	0·002	0·000
	100	0·578	0·708	0·543	0·618	0·667	0·633	0·596	0·844	0·789	0·608	0·633	0·804
	98	0·010	0·000	0·017	0·027	0·042	0·031	0·061	0·052	0·064	0·009	0·041	0·061
	95	0·412	0·292	0·431	0·355	0·292	0·337	0·333	0·104	0·147	0·380	0·324	0·135
		(102)	(106)	(116)	(110)	(168)	(98)	(114)	(96)	(266)	(324)	(490)	(362)
Est-D	114	0·000	0·000	0·000	0·027	0·048	0·051	0·017	0·000	0·000	0·000	0·037	0·000
	100	1·000	1·000	1·000	0·973	0·952	0·949	0·983	1·000	1·000	1·000	0·963	1·000
		(102)	(106)	(116)	(110)	(168)	(98)	(116)	(96)	(266)	(324)	(492)	(362)
Gpi-A	106	0·000	0·000	0·000	0·000	0·000	0·010	0·000	0·000	0·000	0·000	0·002	0·000
	100	1·000	1·000	1·000	1·000	1·000	0·980	1·000	0·979	0·992	1·000	0·996	0·989
	95	0·000	0·000	0·000	0·000	0·000	0·010	0·000	0·021	0·008	0·000	0·002	0·011
		(102)	(106)	(116)	(110)	(168)	(98)	(116)	(96)	(266)	(324)	(492)	(362)
Idh-1	121	0·186	0·170	0·216	0·056	0·116	0·061	0·138	0·250	0·222	0·191	0·097	0·229
	109	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·010	0·000	0·000	0·000	0·003
	100	0·814	0·830	0·784	0·907	0·878	0·918	0·845	0·740	0·778	0·809	0·884	0·768
	83	0·000	0·000	0·000	0·037	0·006	0·020	0·017	0·000	0·000	0·000	0·019	0·000
		(102)	(106)	(116)	(108)	(164)	(98)	(116)	(96)	(266)	(324)	(486)	(362)

Idh-2	100	0·961	1·000	0·940	1·000	1·000	1·000	1·000	1·000	1·000	0·966	1·000	1·000
	78	0·039	0·000	0·060	0·000	0·000	0·000	0·000	0·000	0·000	0·034	0·000	0·000
		(102)	(106)	(116)	(108)	(168)	(98)	(116)	(96)	(266)	(324)	(490)	(362)
Ldh-C	100	1·000	0·971	0·991	0·909	0·952	0·990	0·947	1·000	0·992	0·987	0·949	0·994
	90	0·000	0·029	0·009	0·091	0·048	0·010	0·053	0·000	0·008	0·013	0·051	0·006
		(102)	(102)	(116)	(110)	(168)	(98)	(114)	(96)	(264)	(320)	(490)	(360)
Mdh-2	142	0·000	0·000	0·000	0·000	0·006	0·000	0·009	0·000	0·000	0·000	0·004	0·000
	134	0·000	0·000	0·000	0·000	0·030	0·010	0·000	0·000	0·004	0·000	0·012	0·003
	100	1·000	1·000	1·000	1·000	0·964	0·990	0·991	0·958	0·974	1·000	0·984	0·969
	60	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·042	0·023	0·000	0·000	0·028
		(102)	(106)	(116)	(110)	(168)	(98)	(116)	(96)	(266)	(324)	(492)	(362)
Mp	100	0·933	0·915	0·948	—	—	0·980	0·986	0·969	—	0·933	0·982	0·969
	97	0·067	0·085	0·052	—	—	0·020	0·014	0·031	—	0·067	0·018	0·031
		(30)	(106)	(116)	—	—	(98)	(70)	(96)	—	(252)	(168)	(96)
Pgdh	100	0·931	0·887	0·897	0·973	0·988	0·990	0·983	1·000	0·996	0·904	0·984	0·997
	88	0·069	0·113	0·103	0·027	0·012	0·010	0·017	0·000	0·004	0·096	0·016	0·003
		(102)	(106)	(116)	(110)	(168)	(98)	(116)	(96)	(266)	(324)	(492)	(362)
Pgm	110	0·000	0·000	0·000	0·018	0·012	0·000	0·000	0·000	0·000	0·000	0·008	0·000
	100	1·000	1·000	1·000	0·982	0·988	0·969	0·991	1·000	1·000	1·000	0·984	1·000
	90	0·000	0·000	0·000	0·000	0·000	0·031	0·009	0·000	0·000	0·000	0·008	0·000
		(102)	(106)	(116)	(110)	(168)	(98)	(116)	(96)	(264)	(324)	(492)	(360)

dihydrolipoamide reductase (NAD^+) (=diaphorase), lactate dehydrogenase-A and -B, malate dehydrogenase-1 (cytoplasmic) and -3 (mitochondrial), malate dehydrogenase ($NADP^+$) (=malic enzyme), peptidases (four loci), superoxide dismutase (cytoplasmic), and xanthine dehydrogenase. Five loci (aspartate aminotransferase-2, adenosine deaminase, creatine kinase-A, L-iditol dehydrogenase [=sorbitol dehydrogenase], and mannosephosphate isomerase) exhibited only one or two variants each (out of the 589 animals screened) and, thus, contributed no information regarding patterns of genetic differentiation. Ten enzyme-coding loci were polymorphic. Details of these enzymes and the conditions for their analysis are summarized in Table 2. Since 10 out of the 47 enzyme-coding loci successfully screened were polymorphic, $p_{0.99} = 0.21$ for *Lates calcarifer*. If the more stringent 95% criterion for polymorphism is applied, $p_{0.95} = 0.11$, since only aconitate hydratase, esterase-2, isocitrate dehydrogenase-1, lactate dehydrogenase-C, and phosphogluconate dehydrogenase are polymorphic at this level. Zymogram patterns for six of the polymorphic enzymes are shown in Fig. 2.

Table 4. Results of χ^2 contingency tests

χ^2 value, degrees of freedom and probability are given. n.s., not significant; *$P < 0.05$; **$P < 0.025$; ***$P < 0.01$; ****$P < 0.0001$

(*a*) Temporal comparisons within areas

Locus	Princess Charlotte Bay			South-eastern Gulf: Collection 4 *v.* 6			South-eastern Gulf: Collection 5 *v.* 7			Daly River		
	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Ah	1·51	6	n.s.	0·91	3	n.s.	1·26	3	n.s.	11·02	3	**
Est-2	9·46	6	n.s.	0·08	2	n.s.	2·91	3	n.s.	1·36	2	n.s.
Est-D	No variation			0·79	1	n.s.	1·86	1	n.s.	No variation		
Gpi-A	No variation			2·27	2	n.s.	No variation			1·14	1	n.s.
Idh-1	0·77	2	n.s.	0·52	2	n.s.	1·14	2	n.s.	3·15	2	n.s.
Idh-2	6·28	2	*	No variation			No variation			No variation		
Ldh-C	3·80	2	n.s.	6·74	1	***	0·04	1	n.s.	0·73	1	n.s.
Mdh-2	No variation			1·13	1	n.s.	3·57	2	n.s.	1·31	2	n.s.
Mp	0·97	2	n.s.	0·04	1	n.s.	0·03	1	n.s.	Partial data		
Pgdh	1·32	2	n.s.	0·80	1	n.s.	0·14	1	n.s.	0·36	1	n.s.
Pgm	No variation			5·16	2	n.s.	2·83	2	n.s.	No variation		

(*b*) Comparisons of saltwater and freshwater habitats in the south-eastern Gulf

Locus	Collection 6 *v.* 7			Collection 4 *v.* 5		
	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Ah	1·48	3	n.s.	No data		
Est-2	2·03	3	n.s.	1·46	2	n.s.
Est-D	1·92	1	n.s.	0·72	1	n.s.
Gpi-A	2·39	2	n.s.	No variation		
Idh-1	3·40	2	n.s.	6·03	2	*
Ldh-C	2·97	1	n.s.	2·06	1	n.s.
Mdh-2	2·03	2	n.s.	4·02	2	n.s.
Mp	0·09	1	n.s.	No data		
Pgdh	0·19	1	n.s.	0·89	1	n.s.
Pgm	1·40	1	n.s.	0·19	1	n.s.

(*c*) Geographic comparison of all three areas

Locus	χ^2	d.f.	*P*
Ah	45·98	6	****
Est-2	67·06	6	****
Est-D	25·49	2	****
Gpi-A	7·31	4	n.s.
Idh-1	42·89	6	****
Idh-2	29·20	2	****
Ldh-C	19·98	2	****
Mdh-2	31·27	6	****
Mp	6·32	2	*
Pgdh	53·06	2	****
Pgm	11·20	4	**
Total	349·57	50	****

Polyacrylamide gels of muscle homogenates stained for general proteins yielded approximately 10 bands when stained with Coomassie blue and approximately 25 bands when stained with the more sensitive silver technique (see Materials and Methods). Genetic variation in general muscle proteins was monitored in only 258 fish. Consistent variation in banding patterns that could be interpreted using a simple genetic model was observed

for only two of these general protein bands. Specific enzyme staining revealed one of the two variable general protein bands to be creatine kinase-A. The other variable general protein band behaved as a monomer, exhibiting single- and double-banded phenotypes (only two alleles were detected). [A detailed discussion of barramundi general muscle proteins, including photographs of representative banding patterns, is included in Keenan and Shaklee (1985).] The polymorphic muscle protein band was also detected in liver and eye extracts. For any given individual fish, the banding pattern for this protein was

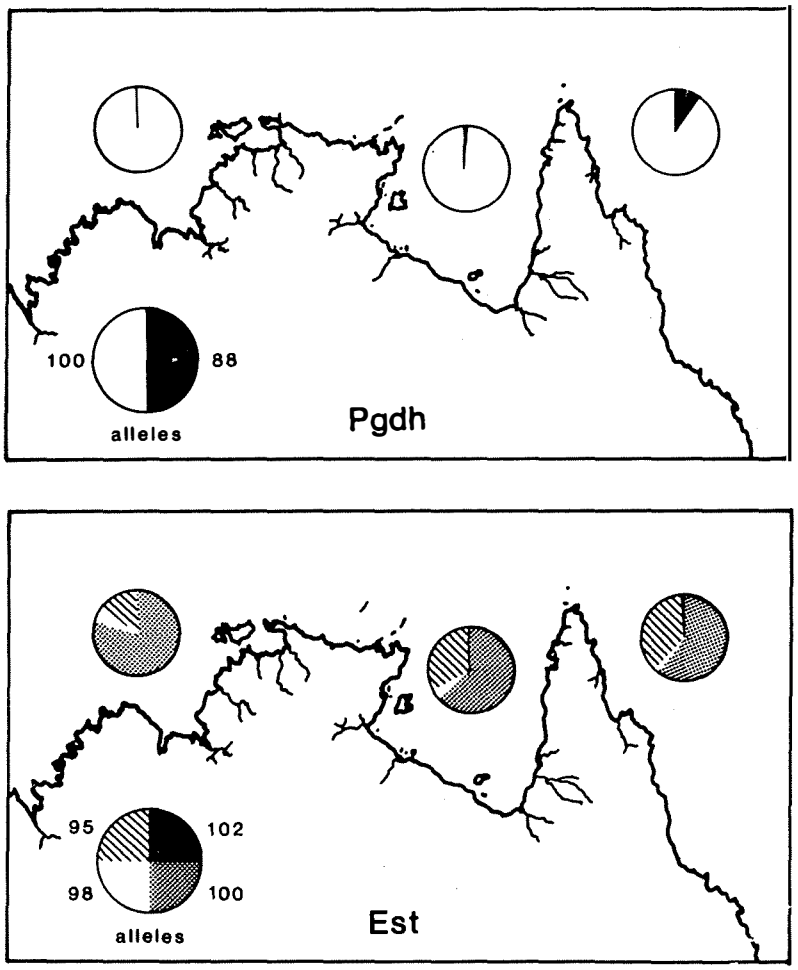

Fig. 3. Maps showing allele frequency distributions for *Pgdh* and *Est-2* at the three localities sampled. The frequency of each allele is represented by the area of the section of the pie diagram devoted to it. See Table 3 for sample sizes and exact allele frequencies.

qualitatively identical in all three tissues. That is, animals scored as heterozygotes based on the protein pattern in muscle homogenates also exhibited the expected two-banded pattern for this protein in liver and in eye homogenates. Similarly, presumed homozygotes exhibited single-banded phenotypes in all three tissues. Because it was not possible to determine how many gene loci were represented by the 20–30 general protein bands observed in muscle homogenates, the data for general proteins have not been used to calculate the estimates of H and p for *L. calcarifer*.

The allele frequencies at each polymorphic locus for the nine individual collections are presented in Table 3. χ^2 tests for goodness-of-fit of genotype proportions to Hardy–Weinberg expectations for each variable locus in each collection (total of 61 tests) yielded only two significant outcomes—both due to expected values in one class of less than 0·5. These were for *Ldh-C* in collection 4 and *Est-D* in collection 5. From this analysis we can conclude that the observed gene frequency data are consistent with the simple genetic models applied to each polymorphic system (cf. Table 2). Contingency χ^2 tests were used to examine the temporal stability of allele frequencies in each of the three collecting areas. For the Princess Charlotte Bay region, collections 1–3 (covering an 8-month period) were compared. One locus, *Idh-2*, exhibited a significant difference ($P<0·05$) among collections (Table 4). For the south-eastern Gulf of Carpentaria, collections 4 and 6 and collections 5 and 7 (each pair covering a 4-month period) were compared. Only *Ldh-C*, in the comparison of collections 4 and 6, exhibited a significant difference ($P < 0·01$) between collections. Finally, for the two Daly River collections, 8 and 9 (representing a 7-month period), only *Ah* exhibited a significant difference ($P < 0·025$) between collections. Overall, these analyses suggest minimal temporal variation in allele frequencies in the three regions over the sampling period since only three significant outcomes were observed in a total

Table 5. Per locus heterozygosity and F_{ST} values for *Lates calcarifer* from three regions (Princess Charlotte Bay, PCB; south-eastern Gulf of Carpentaria; Daly River) in northern Australia

Locus	Heterozygosity at			F_{ST}
	PCB	SE. Gulf	Daly River	
Ah	0·422	0·533	0·449	0·025
Est-2	0·486	0·492	0·332	0·042
Est-D	0·000	0·071	0·000	0·025
Gpi-A	0·000	0·008	0·022	0·004
Idh-1	0·309	0·207	0·358	0·019
Idh-2	0·066	0·000	0·000	0·023
Ldh-C	0·024	0·097	0·012	0·018
Mdh-2	0·000	0·032	0·058	0·012
Mp	0·125	0·035	0·060	0·012
Pgdh	0·174	0·031	0·006	0·046
Pgm	0·000	0·032	0·000	0·008
H^A	0·035	0·033	0·028	
Mean F_{ST}				0·028

[A] Calculated for all 46 loci surveyed.

of 33 tests. Collections of barramundi from both marine and freshwater habitats in the south-eastern Gulf of Carpentaria allowed an assessment of the amount of genetic difference between fish in the two habitats. Comparisons were made between collections 4 and 5 and between collections 6 and 7. Of the 19 χ^2 tests, only one was significant (*Mdh-2*, collection 4 *v.* 5, $P < 0·05$). Since one significant outcome in 20 tests is expected by chance (given an $\alpha = 0·05$) this analysis provides no evidence that fish collected from the two habitats were genetically different.

The statistical analyses presented above provide evidence of, at most, only minimal genetic differentiation among barramundi based upon temporal or habitat (seawater *v.* freshwater) considerations. Given these results, it was possible to pool the individual collections by region to test for genetic differentiation on a geographical basis. The pooled allele frequencies characteristic of the three areas are also presented in Table 3. χ^2 tests (total of 25) of agreement with Hardy–Weinberg expectations for all variable loci within each of the three regions (pooled collections) yielded only one statistically significant outcome—*Est-D* in the south-eastern Gulf of Carpentaria, $P < 0·01$. However, the validity

of this result is doubtful since this particular calculation involved an expected value for one class of less than 0·5. Thus, we feel that there is no evidence of a Wahlund effect (excess of homozygotes) within any of the pooled samples. This result is consistent with the conclusion reached earlier that barramundi exhibit little genetic heterogeneity due to either seasonal or habitat (saltwater *v.* freshwater) variation. On the other hand, contingency χ^2 analysis among regions indicated that 10 of the 11 polymorphic loci exhibited significant differences in allele frequency (Table 4, Fig. 3). Indeed, the cumulative χ^2 of 349·57 across all 11 loci had a probability of less than 0·00001. These results clearly indicate that barramundi in northern Australia are not behaving as a single, large, Mendelian population. The values of F_{ST} calculated for barramundi (cf. Table 5) also suggest population subdivision.

Discussion

The combined use of biochemical techniques (such as electrophoresis and specific enzyme histochemical staining), genetic interpretations, and population genetics theory provide a robust and direct means of testing the subpopulation or stock structure characteristics of a given species. Because of the theoretical power of this approach, numerous investigations of genetic aspects of stock structure of commercial fishes have been accomplished (see reviews by Allendorf and Utter 1979; Berst and Simon 1981; Shaklee 1983). In such studies, validity of the results and conclusions is dependent upon the correct interpretation of observed banding patterns. For several fish species, direct inheritance tests establishing the genetic basis of the observed patterns of enzyme variation have been accomplished (Allendorf and Utter 1973; Dando 1974; Allendorf et al. 1975; Purdom *et al.* 1976; Whitt *et al.* 1976; Leslie and Vrijenhoek 1977; Place and Powers 1978; May *et al.* 1979, 1980, 1982; Kornfield *et al.* 1981; Van Beneden *et al.* 1981; Morizot and Siciliano 1983; Wright *et al.* 1983). In most cases, however, it is impractical or impossible to carry out such direct tests. In these circumstances it is necessary to rely on indirect evidence to verify the assumed genetic basis of the observed variation. Lacking direct genetic evidence for barramundi, we have relied upon three indirect criteria to establish the genetic nature of the observed variation in enzyme banding patterns. First, the isozyme banding patterns of presumed heterozygotes (see Fig. 2 and Table 2) for MDH, LDH, GPI, and PGM agreed with those predicted from the known subunit structures of these enzymes in other fishes (Hashimoto and Handler 1966; Bailey *et al.* 1970; Whitt 1970; Mo *et al.* 1975). Similarly, for AH, EST, EST-D, GPI, IDH, LDH, MDH, PGDH, and PGM, the observed banding patterns of presumed heterozygotes agreed in all cases with those predicted from the known subunit structures of these same enzymes in other vertebrates (Darnall and Klotz 1975; Harris and Hopkinson 1976). Second, the genetic basis for similar electrophoretic variation has been directly demonstrated in other fish species for AH, EST, GPI, IDH, LDH, MDH, PGDH, and PGM. Third, χ^2 tests of observed genotypic proportions for agreement with Hardy–Weinberg expectations failed to reveal significant deviations. In total, these criteria support the assumption that the observed electrophoretic variation has a genetic basis.

The overall values for polymorphism ($p_{0.99} = 0·21$) and average heterozygosity ($H = 0·032$) estimated for Australian barramundi are within the range of values observed for these statistics with other fish species (Nevo 1978; Winans 1980). The among-locality χ^2 tests (cf. Table 4*c*) reveal a substantial amount of genetic differentiation within barramundi and indicate the existence of at least three major subpopulations or stocks—one on the east coast of Queensland (Princess Charlotte Bay region), one in the south-eastern Gulf of Carpentaria, and one in the western part of the Northern Territory (Daly River). That barramundi stocks in these three areas are genetically (and, therefore, reproductively) semi-isolated from each other seems clear given the results of the χ^2 tests. The values of Wright's F_{ST} statistic summarized in Table 5 are small but do suggest a

modest amount of among-population differentiation. The average F_{ST} value for barramundi in the present study is significantly greater than the values reported for North Sea plaice and for pink salmon but slightly less than that for milkfish (Winans 1980).

The genetic differentiation of Australian barramundi stocks indicated by the results of this study may at first seem surprising given the fact that the species' range is essentially uninterrupted throughout coastal northern Australia. Furthermore, spawning of barramundi (which is reported to be preceded by long-range adult migrations in Papua New Guinea—Moore and Reynolds 1982) occurs only in estuarine or nearshore marine habitats and results in planktonic embryos and larvae, which theoretically could be dispersed over considerable distances by longshore and/or tidal currents. However, two lines of evidence suggest that the substantial alongshore prespawning migrations documented for barramundi in Papua New Guinea by Moore and Reynolds (1982) may not be characteristic of fish in Australia. First, based on collections of ripe prespawning adults, and on observed distributions of eggs and small larvae (<5 mm total length), only one major barramundi spawning area is recognized in western Papua (Moore 1982). In contrast, Garrett and Russell (unpublished data) report the presence of running ripe male and female barramundi, of small larvae, and of juvenile nursery grounds at several locations in north-eastern Queensland, including the Princess Charlotte Bay, Cairns, and Tully regions. Second, the tag–recapture studies of Moore and Reynolds (1982) have established a pattern of substantial prespawning migrations of adult barramundi in Papua New Guinea. These migrations include both riverine (up to 350 km) and coastal (up to 622 km) movements. In Australia, however, a pattern of much more restricted adult movements may characterize barramundi. Thus, unpublished studies in Queensland involving over 4200 releases and 517 recaptures (R. Garrett, personal communication) and in the Northern Territory involving over 2500 releases and 180 recaptures (R. Griffin and G. White, personal communication) and approximately 4000 releases and 275 recaptures (T. L. O. Davis, personal communication) suggest that net coastal movements of less than 50 km are typical of *Lates calcarifer* in Australia and movements exceeding 100 km are very rare. These data for movements of Australian barramundi should be interpreted with some caution, however. Most of the tagging and nearly all of the returns of tagged fish in the Australian studies occurred outside the spawning season. This means that movements during the reproductive period were not directly measured by these studies. This seems particularly important since Moore and Reynolds (1982) suggested that adult barramundi in Papua New Guinea, tagged during nonspawning periods, tended to be recaptured in the same general location in which they had been tagged, even if they participated in spawning migrations. If barramundi initiate reproductive migrations shortly before spawning, and exhibit some degree of homing migration directly after spawning, recaptures of tagged fish at times of the year other than during the reproductive period would be expected to underestimate movements significantly. In addition, much of the tagging in the Australian studies has been undertaken at upstream localities. Given that the commercial fisheries in Australia are located at river mouths and surrounding areas, migrating tagged fish in many areas must first successfully run the gauntlet of commercial gill nets (during the fishing season) at the mouth of the river in which they were tagged before they have any chance of being recaptured at another location. This bias means that both the frequency and extent of coastal migration will be underestimated by such tagging studies.

A second characteristic of barramundi contributing to the genetic differentiation of stocks is the apparently limited dispersal abilities of the embryonic and larval stages. The embryonic stage is so short-lived (approx. 20 h) as to effectively preclude significant tidal or current-borne dispersal. Furthermore, the fact that very small larvae—1·5–10 mm total length—are found in 'inland' nursery swamps (Moore 1982; Russell and Garrett 1983; T. L. O. Davis, personal communication) would mean that the potentially planktonic larvae are in open waters for only a few days. This too would severely limit passive dispersal. In situations where there are numerous, localized spawning grounds and the spawning

grounds and nursery areas are adjacent or close to adult barramundi habitat, one would expect this type of life-history strategy to favor regional genetic differentiation.

The among-locality allele frequency differences observed in the present study clearly indicate genetic differences among barramundi from Princess Charlotte Bay, the south-eastern Gulf of Carpentaria, and the Daly River. Such genetic differentiation can be explained either by restricted gene flow among localities (the existence of distinct stocks) or by strong, differential selection in the three regions. The latter explanation seems unlikely for two reasons. First, significant differences in allele frequency have been observed for 10 of the 11 polymorphic systems studied. If the differences were due to selection, this would require selection to be acting in a similar way at many, presumably unlinked, loci simultaneously to generate the same basic pattern of genetic heterogeneity. Secondly, the three localities sampled are not expected to exhibit major differences in general physical attributes or in the biological communities they support so that a possible basis for such strong selection is unclear. In most studies where natural selection has been implicated in determining geographic patterns of genetic variation in populations of fishes, a major environmental variable such as temperature is thought to be responsible (Johnson 1971; Powers and Place 1978; Smith 1979). Water-temperature differences do not seem a likely candidate in the present case since all three localities are at approximately the same latitude (13–18°S.) and would be expected to exhibit similar temperature regimes. Indeed, limited data suggest that all three areas experience a maximum water temperature (at river mouths) of approximately 33°C, while minimum water temperatures are approximately 25°C at the Northern Territory site, 22°C in the Princess Charlotte Bay region, and 17°C in the south-eastern Gulf of Carpentaria (T. L. O. Davis, R. Garrett and D. Vance, personal communication).

The most reasonable explanation for the data presented above is that there are several semi-isolated reproductive groups (=populations) of barramundi in northern Australia. Each of these populations can be considered to be a separate stock. Since barramundi from only three localities have so far been analysed, several questions regarding the nature of stock structure in this fish remain to be answered. Our future research will focus on four of these questions, which are central to the formulation of a comprehensive management plan for the species: 1. How many stocks of barramundi occur in Australia? 2. Do the different barramundi stocks intergrade into one another, or do they exhibit discrete boundaries? 3. Where are the geographic boundaries of the individual barramundi stocks? 4. Are the various stocks and interstock boundaries stable through time?

For a single-species fishery in which the species behaves as a unit stock (a single panmictic population) throughout its range, a management plan treating the fishery as a single unit should be appropriate and adequate. This is because the entire fishery should respond to perturbations as a unit (e.g. fishing mortality in one area amounting to 10% of the total species abundance should, in the short term, reduce abundance throughout the species range by 10%, and in the long term should have no effect on abundance because successful reproduction within the unit stock would eventually lead to replacement of the 10% harvested). However, for a fishery composed of numerous component stocks, management of the overall fishery must incorporate this biological heterogeneity (multiple stocks) if it is to be successful. This is because each stock behaves as a semi-independent unit (e.g. fishing mortality in one area amounting to 10% of the total species abundance could completely eliminate one component stock that initially contributed 10% to the total fishery; the long-term effect in this case would be a 10% reduction in the fishery due to the complete loss of one stock). In this way, each stock must be managed and protected in order to ensure its continued viability and future contribution to the total fishery. It will not be possible to formulate specific management plans to ensure the long-term viability of the barramundi fishery without detailed knowledge of the number and distribution of component stocks involved. Additional electrophoretic analyses are therefore needed to provide a more complete understanding of subpopulation structure in this species.

Acknowledgments

This study was funded, in part, by a research grant from the Fisheries Industry Research Trust Account (FIRTA).

We wish to thank R. Garrett (Queensland Fisheries Service, Cairns, Qld) and R. Griffin, G. White and N. Gill (Northern Territories Fisheries, Darwin, N.T.) for collecting and/or arranging for all of the barramundi samples used in this study. Without their enthusiastic support and cooperation the present investigation would not have been possible. We also thank these same investigators and T. L. O. Davis (Division of Fisheries Research, CSIRO, Cronulla, N.S.W.) for sharing unpublished results and observations with us. Finally, we thank M. Shaklee for typing the manuscript.

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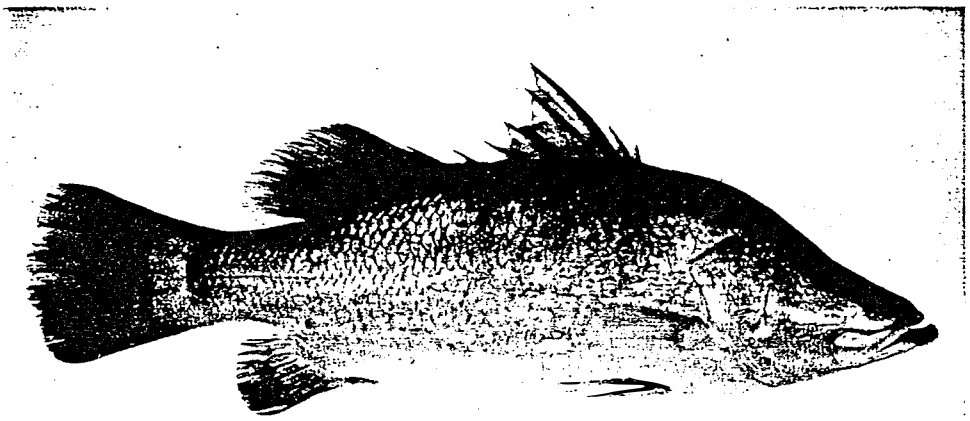
Manuscript received 23 July 1984, accepted 5 October 1984

MANAGEMENT OF WILD AND CULTURED

SEA BASS / BARRAMUNDI

(Lates calcarifer)



No. 20

Management of Wild and Cultured Sea Bass/Barramundi *(Lates calcarifer)*

Proceedings of an international workshop held at Darwin, N.T. Australia, 24–30 September 1986

Editors:
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Australian Centre for International Agricultural Research
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Northern Territory Department of Ports and Fisheries

Stock Structure of Australian and Papua New Guinean Barramundi *(Lates calcarifer)*

John Salini* and James Shaklee**

INTEREST in the population stucture of barramundi was stimulated by the perceived decline in both landings and catch per unit effort (CPUE) after the peak 1977–78 catch of over 1600 t liveweight. A broad-based biological study of barramundi was initiated in 1978 on the part of the CSIRO and the state fisheries departments in both Queensland and the Northern Territory. The Fish Population Genetics Group in Cleveland began assessing the extent of electrophoretically detectable genetic variation in barramundi in late 1981. Although levels of variation were low relative to other fishes, sufficient polymorphic loci for population studies were detected. Subsequently, large-scale collecting of tissue was established through both Queensland and NT state fisheries departments and by direct contact with cooperative commercial fishermen.

Over 4500 samples have been collected since our program commenced, covering 32 locations and about 7000 km of coastline. In this report we present the results and their implications for WA and NT collections, the tip of Cape York and two PNG locations. Details of the nature of the genetic variation have been reported elsewhere (Shaklee and Salini 1985). The Cape York samples form part of a separate paper on barramundi stock structure in Queensland (Shaklee and Salini, in prep.), but have been included here to compare the closest Australian location to the PNG samples.

Genetically discrete stocks are ones where there is no normal mixing between them by way of either migrating adults which interbreed or by exchange of juveniles and/or long-lived larvae; that is a self-reproducing unit. This stock concept is the cornerstone of sound management policies for the maintenance of a fishery resource. In the case of Australian barramundi, the question of concern should be: Do we manage the fishery on the basis of a single unit stock across its entire geographic range or as a resource composed of multiple stocks? At present, the first strategy has been the basis for fishery management with only minor differences between the NT and Queensland approaches.

The essence of the existing controls concerns protecting the spawning adults from gill-netting and in addition there is a series of controls on fishing gear to minimise the removal of females (size of capture) and on numbers of commercial fishermen operating. This last control has been pursued with some success by both states to reduce the fishing effort. The other scenario of multiple barramundi stocks would obviously require a different emphasis in that each individual stock would have to be protected from overfishing because of the lack of substantial recruitment from other populations. In this case, recovery from a severely overfished situation would be a protracted event.

Tagging studies on barramundi in Van Diemen Gulf revealed considerable within-river movement of barramundi (Davis 1986). There was some evidence of limited movements between nearby rivers, less than 50 km apart, but essentially little or no coastal migrations were observed. Similar results have been obtained from tagged barramundi in Queensland waters (Garrett, pers. comm.) and in the Daly River (White, pers. comm.). Given these tagging results and the lack of definitive information on barramundi stocks, we planned to sample as many locations as possible over most of their geographic range. The aim of our population genetic approach was to determine: (1) if there are genetically discrete stocks of barramundi; and if so (2) how many stocks can be identified; (3) where are the boundaries of such stocks; and (4) if the genetic data (allele frequencies) are stable temporally.

Materials and Methods

Samples used for this part of the study were collected from Western Australia, the Northern Territory, Queensland (Cape York) and Papua New Guinea. These locations were: WA, the Ord River; NT, the Daly, Finniss, Mary, Glyde, Roper and McArthur

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rivers and Blue Mud Bay, Cape York, Pennefather, Ducie, Dulhunty, Jackson, MacDonald, Doughboy, and Cotterell rivers and Janie Creek on the west or Gulf side of the Cape, and Escape and Lockhart rivers and Orford, Shelburne and Temple bays on the eastern cape; PNG, Toro Passage at the south western mouth of the Fly River and an unnamed stream 100 km northwest of the Fly River. For the sake of statistically valid sample sizes in the genetic analyses, several small collections had to be pooled on a geographic basis. This pooling was only necessary for the Cape York collections and consisted of two separate locations on the Gulf side, Cotterell River area, Port Musgrave area, and five locations on the eastern Cape. From north to south these eastern cape locations were, 12 (Escape River), 14 (Orford Bay), (Shelbourne Bay), 16 (Temple Bay) and 13 (Lockhart River). Electrophoretic techniques have been described in detail together with explanations of the statistical analyses (Shaklee and Salini 1985, Salini and Shaklee in prep.). Validation of the electrophoretic interpretation has been discussed at length for our results (Shaklee and Salini 1985). For the purposes of this paper, results of the contingency chi square tables testing the homogeneity of alleles is the principal concern and these analyses were all computed using the BIOSYS-1 genetics program of Swofford and Selander (1981).

The null hypothesis for this analysis is that the samples being tested are drawn from the one gene pool, that is, from the one stock. A significant difference implies that the samples are from different gene pools or stocks. This analysis is carried out for each polymorphic locus and over all polymorphic loci.

Results

WA–NT Samples

The Daly and Finniss rivers were sampled five times and twice respectively to test for genetic stability over time and to test geographically close streams for stock discreteness. The samples within the Daly and from within the Finniss rivers were not genetically different and the pooled Daly River and pooled Finniss River collections were not significantly different at any of the polymorphic loci either. This implies that for geographically close rivers, in this case separated by about 50 km, the barramundi populations can be considered to be uniform or of the same stock. In addition, these samples were obtained over 14 months (Table 1) which demonstrates the stability of the genetic data over time. Most of the other locations sampled were barramundi fishing centres with at least one or more streams (capable of sustaining barramundi populations) between them. In general, the size and hence age of the barramundi used reflected the commercial sampling

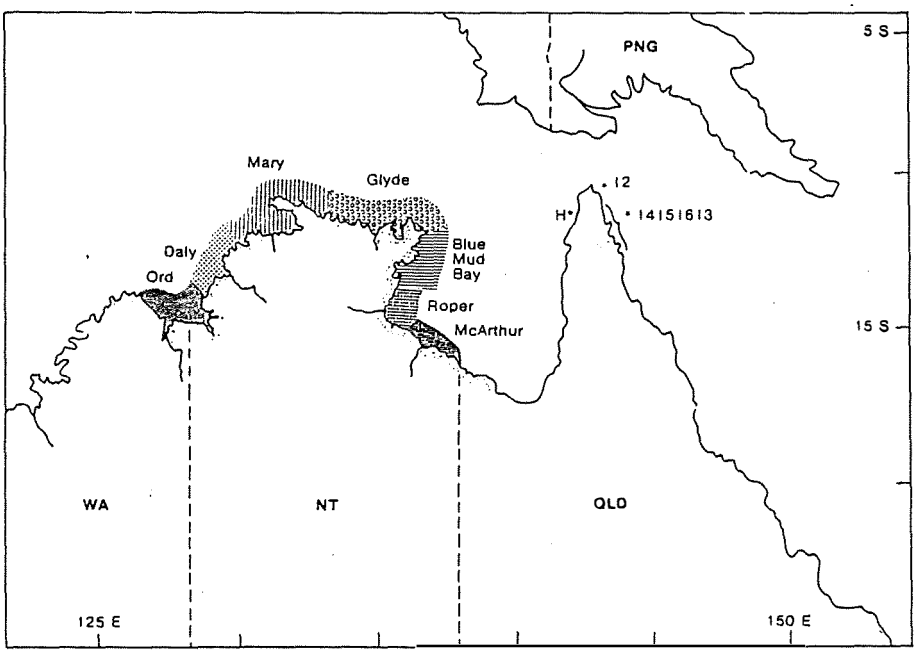

Fig. 1. Genetically discrete stocks in northern Australia and PNG. Other collections from SE Gulf of Carpentaria and the east coast of Queensland are not included.

with most fish conforming to the legal size for netting and with males predominating in the commercial catch samples.

When all seven localities were compared as if they were one population, they were strongly heterogeneous with $X^2 = 612$ with 54 degrees of freedom and seven loci out of the 11 polymorphic loci showing significant deviations from expectations for a single homogeneous population. To determine where the genetically discrete stocks occurred within our sample locations, each adjacent pair of rivers was statistically tested for genetic homogeneity by contingency chi square tables. The results were highly significant for each of the comparisons, that is Ord vs Daly area (Daly plus Finniss samples), Daly area vs Mary, Mary vs Glyde, Glyde vs Blue Mud Bay, Blue Mud Bay vs Roper and Roper vs McArthur indicating strong barriers to gene flow between each of the seven locations. Thus, for the WA–NT samples, there are genetically discrete stocks represented by the Ord, Daly (including Finniss), Mary and Glyde Rivers, Blue Mud Bay, the Roper and MacArthur Rivers represented by the different shading in Fig. 1.

Cape York and PNG samples

The western Cape samples comparison of area H1H2H3 (Port Musgrave) and "Cotterell River" area revealed no significant differences for all loci. This means all these collections can be considered one stock. On the eastern Cape, all five locations represented by Escape River (I2), Orford Bay (I4), Shelburne Bay (I5), Temple Bay (I6) and Lockhart River (I3) revealed genetically discrete stocks for the Escape River and a pooled area incorporating locations I4, I5, I6 and I3.

Similarly, two PNG samples represented a single population such that our samples over northern Cape York represented three genetic stocks each of which was distinct from the PNG stock. That is, there is little chance of significant genetic exchange between Australian and PNG barramundi.

If geographic separation between populations of barramundi is the major determinant of whether there are discrete stocks or not, then the Escape River and PNG barramundi populations appear to be within the range for uniform populations, given the distance between NT stocks of at least 150 km. However, despite their apparent geographic proximity at their nearest points, the Torres Straits constitute a major geographic barrier for what is essentially a shallow-water, coastal species. The WA–NT samples contained individual river collections, unlike the Cape York samples which consisted of several small collections from different streams which required grouping for analysis. Hence, the WA–NT samples provide a better indicator of the approximate coastal distances involved in the formation of genetically discrete stocks. There is no genetic distinction between the Daly and Finniss rivers separated by about 50 km, but the nearest genetically distinguishable stocks are separated by 150 km (approximately), e.g. Blue Mud Bay, Roper and MacArthur rivers.

Discussion

The primary goal of our biochemical genetics approach to studying barramundi stock structure was to attempt to provide the relevant fishery managers with previously unobtainable information. Until now, the main source of stock structure information on barramundi was provided by tagging programs, which revealed the nature and extent of fish movements within and outside their natal rivers. Davis (1986) carried out a comprehensive tagging program on Australian barramundi in Van Diemen Gulf, Northern Territory. His results confirmed the downstream spawning movement prior to and during the wet season, together with a small amount of movement between streams less than 50 km apart. Similar movements have been observed for barramundi tagged in the Daly River (White, pers. comm.) and on the east coast of Queensland (Garrett, pers. comm.). The genetic data we have gathered complements all the available tagging data and is especially useful as a research technique when covering such a wide geographic range. To attempt a tagging program on such a scale would have been impossible with the same resources. It should be noted that, given sufficient levels of detectable genetic variations, the life history of the barramundi lends itself to the biochemical genetics approach. Lack of long range migrations, a short planktonic larval stage and a dependence on coastal nursery habitats adjacent to spawning sites for larval and juvenile barramundi (Davis 1985; Russell and Garrett 1983, 1985) provide the basis for establishing barriers to gene flow. This life history results in genetically distinct populations through random genetic drift. In contrast to the observed Australian tendency to form identifiable localised populations, Moore and Reynolds (1982) and Moore (1982) were able to document significant coastal migrations by Papua New Guinea barramundi originating from within the Gulf of Papua and its streams.

This behaviour enhances the mixing of genes and so there is less chance of localised populations forming and becoming genetically distinct from one another. More important than the description of these stocks is their implications for the management of the commercial (and amateur) fishery and, in the near future, the control of hatchery brood stock and fingerlings. As far as the commercial fishery is concerned, it should be clear that it would be biologically more appropriate to manage the fishery as a multiple stock fishery rather than the present modus operandi which acknowledges the unit or single-stock strategy. Because we have a

multiple stock situation with documented short distance movements, then this biological information must be taken into account with regard to the location of habitat reserves. The obvious diversity of stocks throughout their range, evident from our work, requires barramundi reserves to be located in such a way that they can feasibly contribute to and, in times of resource depletion, replenish commercially fished stocks.

With the very real prospect of barramundi hatcheries supplying fingerlings for restocking purposes, the consequences of uncontrolled mixing of different genetic stocks must be addressed. Biologically, the danger in releasing fingerlings derived from brood stock which were not indigenous to the release site is the loss of genetic discreteness for the mixed stocks. The genetic data we have collected indicates that only a few fish moving between stocks per generation is sufficient to eliminate their genetic discreteness. It follows from hatchery experience in North America and Europe with salmon and trout species that it is essential to regularly obtain new genetic stock from the wild to minimise the loss of desirable genetic traits as a result of inbreeding depression (Aulstad and Kittelsen 1972; Shaklee 1983; Vuorinen 1984; Hynes et al. 1981). At the 1980 Stock Concept Symposium in Ontario, Maclean and Evans (1981) cite examples of reduced viability attributed to direct mixing of stocks or via hatchery releases. To minimise such risks Krueger et al. (1981) recommended that, where there is a likelihood of gene flow between stocked fish (hatchery releases) and the local wild population, the broodstock should be taken from environmentally similar locations. Again the intention is to maintain genetically discrete stocks. It is generally recognised that genetically discrete stocks represent the best adapted genotypes for that particular area and as such constitute a valuable component of the species' genetic variability (Shaklee 1983; Thorpe et al. 1981; MacLean and Evans 1981). Because of the high public profile and economic value of the barramundi fishery, commercial and recreational, it would be prudent to choose the least risk of resource deterioration in considering future developments. In conclusion, we endorse the three recommendations to fishery management from the 1980 Stock Concept Symposium (Spangler et al. 1981):

1. "That research be intensified to resolve further the existence of genetic and environmental stocks and mechanisms underlying their maintenance or persistence in wild populations. This research should include intensive studies of gene flow within and between stocks to determine the risks associated with the extirpation of discrete stocks.
2. "That fishery agencies concerned with the rehabilitation of self-producing stocks re-examine fish culture practices in the light of the stock concept according to the specific concerns raised by Hynes et al. (1981), and that hatchery stocks designated for release in wild environments be carefully evaluated with respect to their potential genetic impact on extant populations.
3. "That fishery regulatory mechanisms be developed so as to protect exploited species from selective exploitation of stocks exhibiting the extremes of vital life history characteristics, such as sexual dimorphisms, early or late spawning, size, and growth."

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Australian Fisheries

January
1987

Barramundi discoveries may lead to management changes

Barramundi discoveries may change management

by John Salini* and James B. Shaklee**

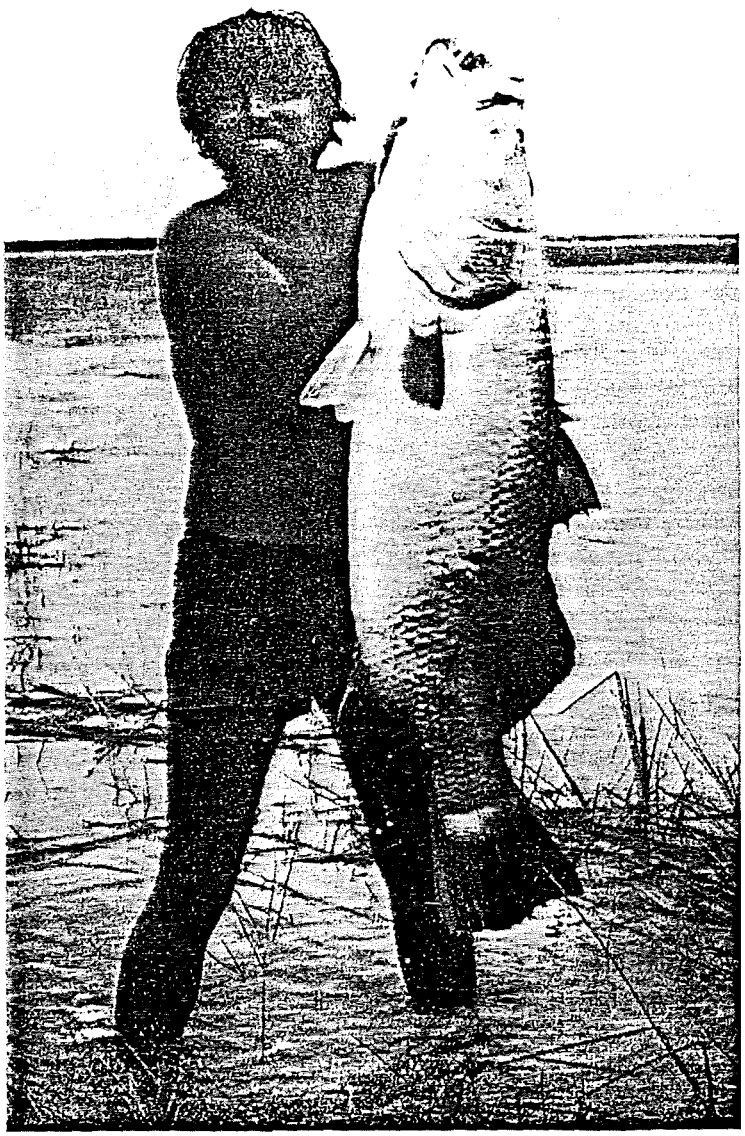
AUSTRALIA's barramundi population consists of numerous genetically different stocks — a discovery which has important ramifications for management and breeding programs.

Research begun in 1982 has identified seven reproductively isolated sub-populations, and yet we use a uniform management strategy across northern Australia. Uncontrolled hatchery releases could destroy the genetic variability of wild stocks, and there is now new scope for selective breeding for aquaculture.

Data analysis of existing samples will certainly show other stocks, although complete definition of barramundi stocks throughout Australia may never be complete. Nevertheless, we should be able to provide enough information to allow management on a true stock basis for most of this important resource.

The biochemical genetics group of the CSIRO Marine Laboratories at Cleveland has been studying the genetic structure of barramundi stocks, *Lates calcarifer*, since 1982. The major goal is to determine the number of different spawning populations which contribute to various barramundi fisheries and the locations of boundaries between adjacent populations.

We have employed electrophoretic techniques in conjunction with specific enzyme (=protein) staining to examine genetically determined patterns of protein variation. A simple



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explanation of the technique was presented in an earlier report in *Australian Fisheries* February 1983, pp. 36-38.[1] The same electrophoretic technique has also been used as a powerful 'fingerprinting' tool for fish species identification in the policing of fish substitution regulations.[2]

Our first investigations were restricted to barramundi from three general regions: Princess Charlotte Bay on the east coast of Queensland, the south-eastern Gulf of Carpentaria (including fish from both the Staaten and Nassau Rivers, and the Daly River in the western Northern Territory.[3] On the basis of observed patterns of genetic variation at 12 enzymes, barramundi from these three areas were considered to represent genetically distinct populations.

This report presents information obtained from more than 1400 barramundi representing seven further locations and some replicate samples. The present study shows that numerous localised populations of barramundi occur throughout the species' range in northern Australia.

The samples

To make the laboratory experiments and data analysis, samples of muscle, liver and eye were obtained with the assistance of fisheries officers of the Queensland Department of Primary Industries and the Northern Territory Fisheries Division, and the co-operation of interested commercial fishermen. These samples were sent frozen to our laboratory in Cleveland as head and guts, dissected tissues or whole fish frames.

Sampling locations illustrated in Figure 1 were the Daly River (replicate samples about eight months apart), Finniss, Mary, McArthur Rivers, south-eastern Gulf of Carpentaria, north-eastern Gulf of Carpentaria, Princess Charlotte Bay and Tully-Hinchinbrook Channel. In several

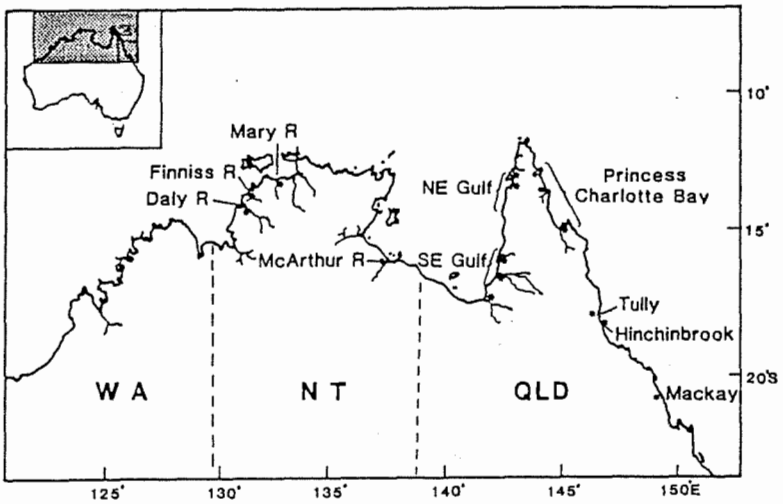

Figure 1. Barramundi-sampling locations across northern Australia. SE Gulf represents the Nassau, Staaten and Norman Rivers; NE Gulf represents the Hay and Embley Rivers of Albatross Bay and Port Musgrave to the north, while Princess Charlotte Bay represents collections from Lockhart River through to Bathurst Bay.

cases, individual collections were grouped together by geographic areas for statistical analysis:

- The south-eastern Gulf of Carpentaria covering the Norman, Staaten and Nassau Rivers;
- North-eastern Gulf of Carpentaria, including streams from Albatross Bay and Port Musgrave; and
- Princess Charlotte Bay which encompassed collections from Lloyd Bay to Bathurst Bay. This Princess Charlotte Bay accumulation of 208 fish represents about 250 km of coastline. In addition, a small sample (41 fish) from Mackay was included with the Tully collection for statistical purposes.

Laboratory procedures and results

At the laboratory, extracts containing the water soluble proteins were prepared on ice and then stored at −70°C until electrophoresis to minimise loss of enzyme activity.

The enzymes we screen from barramundi tissues are the proteins represented by the genetic code at a locus on the barramundi DNA. If there is detectable genetic variation, then the same enzyme may appear in different forms (enzyme 'polymorphism') due to slight difference in the genetic code. The different forms of the same enzyme are referred to as 'alleles'. It is a difference in frequency of these alleles from separate locations that allows us to detect non-interbreeding populations (stocks) in the barramundi.

We have found useful enzyme polymorphisms at 12 genetic loci.[3] Not all of them showed dramatic differences between locations, but two of the more pronounced differences are illustrated as pie diagrams in Figures 2(a) and 2(b) for two gene loci, Est-2 and Pgdh. Only locations with significant differences between them are shown.

Statistical analysis over the entire sample range from Daly River to Tully clearly showed that the samples as a whole were not taken from a single genetically homogeneous population but that numerous subpopulations existed.

To define the different subpopulations, the computer analysis of the genetic data was re-

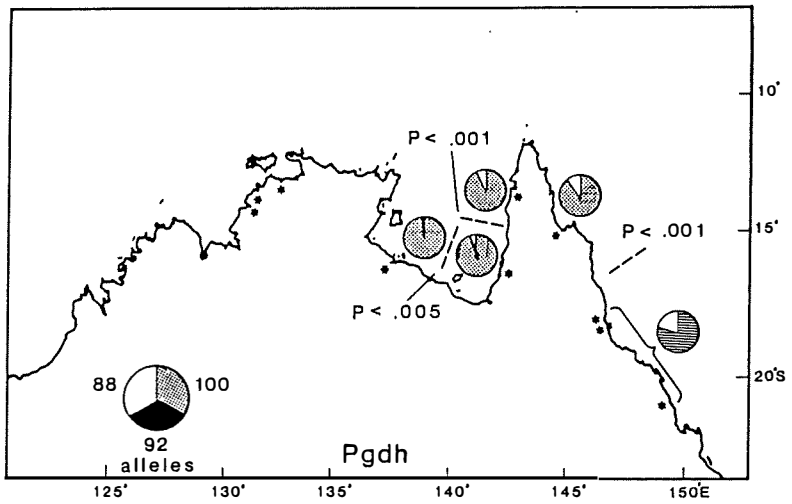

Figure 2a. Pie diagram representation of changes in allele frequencies at the esterase-2 locus between locations. Only those adjacent locations that were statistically different are shown. 'P' is the probability that the difference observed is due to chance.

run to compare each location with its adjacent location. As noted, one limitation to this procedure was the pooling of individual collections from separate river systems in four of the areas (south-eastern Gulf of Carpentaria, north-eastern Gulf of Carpentaria, Princess Charlotte Bay and Tully-Hinchinbrook Channel-Mackay) which was necessary to overcome the small sample sizes available. Continued sampling from these areas should allow analyses based on individual rivers within these large areas in the future.

Genetically detectable stocks

There was no evidence of heterogeneity either among the Daly River replicate samples or between the Daly and Finniss Rivers, so these two locations were treated as one area (Daly River area). Each of the six other area groupings and river samples exhibited highly significant differences when tested against adjacent localities.

These results mean that at least seven reproductively isolated populations or stocks of barramundi are recognisable from the samples so far examined. These distinct barramundi stocks are found in:

- the Daly-Finniss Rivers in western Northern Territory,
- the Mary River in Van Diemen Gulf,
- the McArthur River in the western Gulf of Carpentaria,
- the south-eastern Gulf,
- the north-eastern Gulf,
- the Princess Charlotte Bay area, and
- the Tully-Hinchinbrook Channel-Mackay area, the latter two situated on the east coast of Queensland (Fig. 3).

One of the advantages of the population genetics approach is that we can assume that none of the genetic loci we have examined in the barrmundi is strongly affected by natural selection caused by the environment. The mechanism for the maintenance of genetically discrete populations or stocks is therefore a balance between the isolating effects of both geographic distance barriers and the homogenising effect of passive dispersal and active migration of the species during all of its life history stages — larval, juvenile and adult. The results of our investigations indicate that in general the dispersal ability of Australian barramundi populations is overshadowed by geographic isolating effects, thus providing the conditions which lead to genetic differentiation through random genetic drift.

In the barramundi, dispersal ability is limited. The embryonic stage lasts only about 24 hours and small larvae probably spend only a few days in open coastal waters before being found inshore in nursery habitats.[3,4] There is therefore little scope for dispersal at the larval stage.

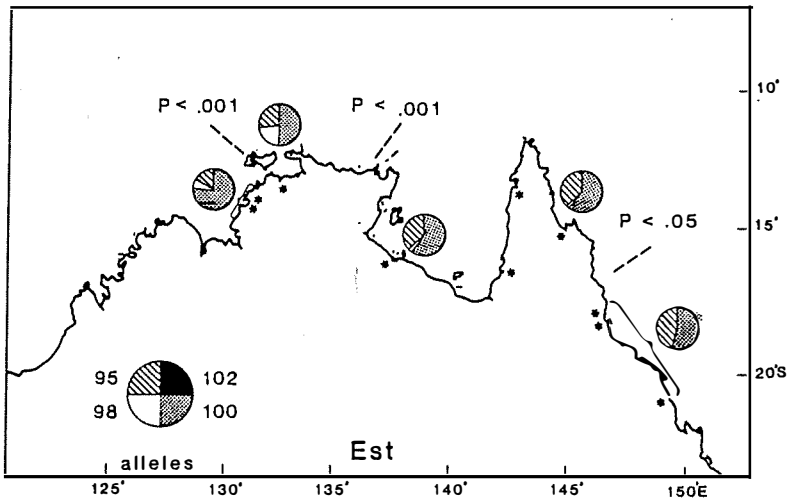

Figure 2b. Pie diagram representation of changes in allele frequencies at the phosphogluconate dehydrogenase (Pgdh) locus. Only adjacent locations that are statistically different are shown.

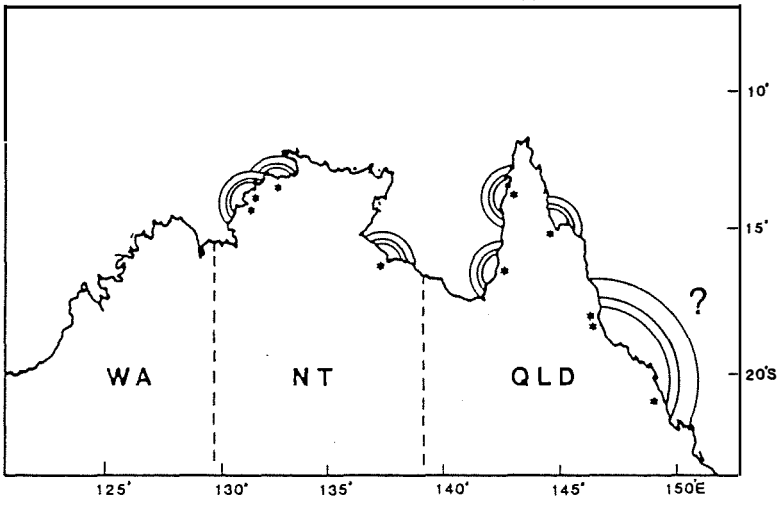

Figure 3. Diagrammatic representation of the seven genetically detectable barramundi stocks.

Studies by Dr Tim Davis,[5] of the CSIRO Division of Fisheries Research, and by John Russell and Rod Garrett,[6] of the Queensland Department of Primary Industries, have established that very young juveniles reappear in inshore waterways and eventually colonise the nearby river systems, extending up into freshwater until they are adults ready to participate in the spawning run (see *Australian Fisheries*, July 1982).

As adults, barramundi migrate downstream in the spawning season (spawning requires salinities of at least 20 ppt). The bulk of the commercial catch is obtained towards the end of this stage of their life cycle. Closures from October 1 to January 31 in the Northern Territory and from November 1 to January 31 in Queensland are in force to protect spawning fish.

Findings supported by tagging

Tagging studies by Davis[7] in Van Diemen Gulf revealed little evidence that tagged fish travel far outside their river. Only a few moved between adjacent river mouths less than 50 km apart. The four rivers studied were the Mary, West Alligator, South Alligator and East Alligator, which cover roughly 100 km of the Van Diemen Gulf coastline. Of 278 fish whose recaptures were reported, only nine had moved between rivers. One fish had travelled about 120 km along the coast before being recaptured 213 days later. However, the remainder were recaptured within or just outside their resident rivers up to two wet seasons after tagging, with no evidence of coastal migrations.[5,7] Similar short-range movements were recorded for barramundi in Queensland waters by John Russell and Rod Garrett.

How do the patterns of stock structure suggested by the population genetics research compare with these tagging results? To maintain the discrete populations revealed by our electrophoretic studies, only a few fish can move between genetic stocks per generation without affecting their discreteness.

The tagging results clearly complement our results and illustrate that most Australian barramundi probably do not participate in synchronised, long-distance, coastal spawning migrations, as was reported for barramundi in the Gulf of Papua by Moore and Reynolds.[8] Instead, Australian barramundi tend to form localised populations with little or no exchange between populations more than 100 km apart.

The Daly and Finniss Rivers are about 50 km apart yet there was no evidence of genetic heterogeneity. The Daly River area population (Daly plus Finniss River samples) was significantly different from the Mary River population about 250 km away. Whether there are several more separate stocks, between say the Mary River and the McArthur River for example (Fig. 3), can only be answered by the collection and analysis of further samples from rivers between those locations.

If the tagging data had shown that barramundi from a wide geographic range make long-range spawning migrations, with the larvae and juveniles returning at random to all the rivers in the region, then we would have expected little or no genetic differentiation among the samples. In fishes with a natal river homing instinct (trout), genetic differentiation can still occur because the isolating mechanism necessary is a behavioural characteristic.

Management implications

The management of the commercial barramundi fisheries in northern Australia at present involves complete closure to netting between October 1 and January 31 in the Northern Territory and between November 1 and January 31 in Queensland. The fishing effort of each fisherman is restricted during the barramundi season and some nursery streams are completely closed to fishing. Thus, the management strategy is uniform across northern Australia.

This strategy would be appropriate if the fishery were composed of a unit stock. In such a situation, overfishing in any one location would affect the whole resource because each location contributes fish to the one overall stock and is similarly replenished by reproduction throughout the overall stock.

However, since our data clearly indicate that the fisheries are supported by many independently reproducing stocks, prolonged overfishing in any one stock will almost certainly cause long-term depletion of that particular stock because of the absence of recruitment from outside populations.

The picture emerging from our biochemical genetics approach, together with information from earlier tagging programs, suggests that Australian barramundi fisheries are exploiting at least seven (and probably many more) reproductively separate stocks between the Daly River and Mackay.

Consequently, we recommend that future management policies take account of the existence of these multiple stocks. Optimally, such a management strategy will entail assessing the barramundi resource of each identifiable stock and then determining the level of effort that each stock can tolerate and still produce a sustainable yield.

Production models developed for the Northern Territory barramundi fishery have progressed towards this strategy by treating catch per unit effort (CPUE) data on the basis of eight fishery areas: the Victoria River, Daly River, Finniss River (which includes Bynoe Harbour, Darwin and the Adelaide River), Mary River, Alligator Rivers, Arnhem coast, Roper River and McArthur River.

In Queensland, the commercial barramundi fishery is segregated into east coast and Gulf of Carpentaria endorsements. Extending this policy to the recognition of several sub-areas within these two large areas would greatly enhance the possibility of stock-by-stock management in Queensland.

One of the first fisheries biologists to study barramundi in Australia, D. J. Dunstan,[9] evaluated the suitability of streams in various catchment areas for barramundi. This type of approach, together with commercial (and amateur) catch data obtained on a regional basis, would help in assessing the allowable fishing effort for any particular stock.

Barramundi nursery steams closed to fishing should obviously be spread throughout the fishery so that they are located within the geographic boundaries of each recognised barramundi stock. In this way, movement of fish from the protected nursery into the fished streams would result in the natural restocking of commercially fished rivers. This is necessary because, as the genetic and tagging evidence suggests, these barramundi do not move far from their spawning grounds.

Hatchery implications

In the light of current research on barramundi spawning and rearing experiments by the Queensland Department of Primary Industries and Sea Hatcheries Pty Ltd of Queensland, the genetic implications of stock mixing are well worth considering.

Salmon and trout hatcheries in North America and the United Kingdom have reduced the genetic variability of their stock. This can, and has, lead to significant inbreeding depression which has reduced traits like growth rate, longevity and fecundity.

In addition to the risk of inbreeding depression, our data suggest that genetically discrete stocks on the east coast could easily be eliminated by successful release of hatchery-reared barramundi spawned from a few brood stock.

The danger in uncontrolled hatchery releases lies in the risk of losing the naturally occurring genetic variability of wild barramundi populations. If hatchery rearing of barramundi is to expand, then it is in that industry's best interest to maintain this primary biological resource. This could be best achieved by using only brood stock from sites where fingerlings are to be released.

If any type of selective breeding program is initiated in support of a barramundi aquaculture program (not involving releases), the genetic heterogeneity among wild stocks should be exploited. This would provide maximal genetic variation within the culture environment and help select particular production traits.

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9. PAPERS IN PREPARATION

The Population Genetic Structure of Barramundi (Lates calcarifer) from the Northern Territory, Australia

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ABSTRACT

Barramundi were collected from eight locations, including the Daly, Finniss, Mary, Glyde, Roper and McArthur Rivers and Blue Mud Bay in the Northern Territory, and the Ord River in Western Australia. The Daly River area (Daly and Finniss Rivers) was sampled seven times over a fourteen-month period. Eleven loci, polymorphic at the $p_{0.99}$ level, were analysed, using both polyacrylamide and starch gel electrophoresis. One polymorphic general protein was resolved on polyacrylamide slab gels, while eleven polymorphic enzymes (aconitate hydratase, creatine kinase-A, esterase-2, esterase-D, fumarate hydratase, glucose-6-phosphate isomerase-A, isocitrate dehydrogenase-2, lactate dehydrogenase-C, malate dehydrogenase-2, phosphoglucomutase, and phosphogluconate dehydrogenase) were analysed on starch gels.

All loci were deemed to be in Hardy-Weinberg equilibrium, as only 2 out of 86 tests yielded significant deviations from expectations. A contingency χ^2 analysis for homogeneity of alleles over all loci and all seven locations was highly significant ($P << 0.001$). Comparisons of adjacent pairs of localities revealed that the overall heterogeneity was attributable to heterogeneity among seven of the eight localities (the Daly and Finniss River areas were not significantly different from one another). No evidence of heterogeneity over time was found among the seven replicated Daly River area collections. The considerable amount of inter-locality heterogeneity observed suggests that each of these seven localities supports a genetically discrete stock of barramundi. This conclusion is consistent with the documented life history of

Australian barramundi and indicates that management of the barramundi fisheries should include considerations of this genetic heterogeneity of stocks.

INTRODUCTION

The barramundi, Lates calcarifer, is one of the premier table and sport fishes native to Australia. A consequence of strong consumer demand and high prices in the early 1980s was the large-scale substitution in the retail market of other fish fillets (Anon. 1982a and 1982b, Seymour 1983, Keenan and Shaklee 1985). Since the peak catches of the late 1970s and early 1980s, escalating fishing pressure (Rohan et al. 1981, Anon. 1982c, Seymour 1983, Quinn 1984) has resulted in a steady decline in both the commercial catch in northern Australia and the catch per unit effort (CPUE). The barramundi is not only of commercial importance to professional fishermen: the catch of the amateur fishery is estimated to be about 25% by weight of the commercial catch in the Northern Territory (and an undetermined amount in Queensland) (Griffin 1982). This high public profile of barramundi, together with the marked decline in CPUE, has prompted considerable demand for biological information to assist in formulating management policies for this species.

An essential requirement for the successful management of any fishery is an understanding of the major components of that fishery. We define stocks as self-sustaining panmictic breeding units within a species (Shaklee 1983). Our research has been directed at examining the stock structure of the barramundi, using established biochemical genetic techniques.

Barramundi living in the freshwater and estuarine reaches of rivers migrate downstream to spawn near or outside the river mouths. The developing embryos are thought to hatch within 24 h of fertilisation (Wongsomnuk and Manevonk 1973). Very small larvae are often found in coastal swamps and estuaries (Davis 1985, Russell and Garrett 1985, Morrissey 1985). As the fish grow, many juveniles and subadults enter estuaries and rivers and eventually move upstream a considerable distance, often remaining in freshwater until mature. Some of the barramundi living in a river system may remain in that river and not participate in the general spawning movement downstream in the wet season, while others may not return upstream once they have spawned, but remain near the mouths of their natal rivers (Davis 1986, R. Garrett, Qld. Fisheries Service, Cairns, Qld., personal communication). The commercial barramundi fishery is centred on the coastal prespawning migration during the monsoon or wet season; the fish are gillnetted in the tidal reaches of rivers or close to the river mouths. Historically, electrophoresis has been used to investigate the stock structure of a fish resource when tagging was either unsuccessful or impractical. In the present application, it offers the opportunity to compare the results of population genetics and tagging in one area. Over 4,000 barramundi

had previously been tagged in the Van Diemen Gulf area by the CSIRO in a wide-ranging study of the movements of barramundi within and outside several different river systems (Davis 1982, Davis 1986). Electrophoresis made it possible to assess the barramundi stock structure over a much wider geographic area, at lower cost, and without the problems of tag recovery.

The heterogeneity of the barramundi populations in three widely spaced localities in northern Australia (Daly River, Northern Territory; south-eastearn Gulf of Carpenaria, Queensland; and Princess Charlotte Bay, north-eastearn coast of Queensland) has been reported in Shaklee and Salini (1985). The present work focusses on a narrower geographic sampling range to provide fisheries management officers with previously unavailable data on the stock structure of barramundi in the Northern Territory. The aims of the present study were: to determine the extent of stock heterogeneity in the Northern Territory; to test the stability of allele frequencies over time; and to define the boundaries of individual stocks.

MATERIALS AND METHODS

Collections and Sample Preparation

Collections of adult barramundi were obtained from eight river systems (see Fig. 1): 1) the Ord River (Western Australia), 2) the Daly River (five replicate collections), 3) the Finniss River (two replicate collections), 4) the Mary River, 5) the Glyde River, 6) Blue Mud Bay, 7) the Roper River, and 8) the McArthur River. The distance from the Ord

to the McArthur estuaries was approximately 1600 km. All samples, except for three of the Daly River replicates, were shipped frozen to the laboratory as "head and guts" samples or as "frames". The remaining samples consisted of tissues dissected and frozen in the field and shipped frozen to the laboratory. Details of these individual collections are summarised in Tables 1 and 2.

Three tissues were collected wherever possible; muscle, eye and liver. The muscle sample was usually taken from the cheek (when heads were provided) or from the belly flap, if the sample was dissected in the field. Muscle samples taken from these two different locations generally exhibit indistinguishable electrophoretic profiles (Shaklee and Keenan 1986). The frozen samples were maintained at -20°C until processed and analysed. No detectable changes in isozyme banding patterns resulted from this storage treatment. The tissue extracts were prepared as described in Shaklee and Salini (1985).

Electrophoretic Analysis

Starch and polyacrylamide gel electrophoresis and histochemical staining for most enzymes followed the methods described in Shaklee and Salini (1985) and in Shaklee and Keenan (1986). Although the starch gels were made with two different lots of Connaught starch (lots 380-1 and 396-3), they yielded virtually identical results. Four enzymes (N-acetyl-beta-glucosaminidase, fumarate hydratase, beta-galactosidase and alpha-mannosidase) not included in the earlier study of Shaklee and Salini (1985), were surveyed in the present investigation. These were stained according to the methods of Harris and Hopkinson (1976).

The so-called 'muscle protein' described in Shaklee and Salini (1985) is present in all three tissues, but as it stains most intensely in eye supernatant, it was screened in eye extracts for all fish in the present study. As silver-stained gels produce a second, weak, zone of staining at the same position on the gel as the product of the slow allele, we routinely visualised this locus with Coomassie blue staining (4 ml of 1% Coomassie blue R or Serva violett, 15 ml glacial acetic acid, 70 ml methanol, 70 ml distilled water). To identify the protein more precisely (Coomassie blue is a non-specific general protein stain), we used the specific transferrin staining technique of Chung (1985). This techique resulted in weak, but definite, staining of the polymorphic protein revealed by Coomassie blue staining. Furthermore, the phenotypes of both homozygotes and heterozygotes were consistent among individuals using both staining techniques. These results suggest that "muscle protein" may actually be transferrin, although many other protein bands also stained weakly with the so-called specific transferrin staining technique, we refer to this protein as "general protein" throughout the present report.

Statistical Tests

Isozyme banding patterns were interpreted as genotypes when the observed enzyme variation was consistent with the known subunit structure of the enzyme (see Shaklee and Salini 1985 for discussion). Genotype frequencies were tested for agreement with Hardy-Weinberg expectations for a randomly mating population with no differential selection acting among the alleles. Levene's (1949) correction for small sample sizes

was used in the calculation of expected genotype frequencies. Homogeneity of alleles was analysed using contingency χ^2 tests. Analyses for temporal changes in allele frequency were carried out on the seven replicate Daly River area samples collected between August 1982 and September 1983. Individual locus heterozygosities (h) were calculated for all polymorphic loci using the standard formula $h = 1-x_i^2$, where x_i is the frequency of the ith allele. Average heterozygosity (H) was calculated as the mean of all individual locus heterozygosities (including monomorphic and variable loci). All statistical tests and allele frequencies were calculated using the BIOSYS-1 computer program of Swofford and Selander (1981).

Geographic differentiation was tested by comparing all localities and all adjacent pairs in the seven major collections: Ord River v. Daly River area (Daly River collections + Finniss River collections); Daly River area v. Mary River; Mary River v. Glyde River; Glyde River v. Blue Mud Bay; Blue Mud Bay v. Roper River; and Roper River v. McArthur River.

RESULTS

The results presented in this paper represent an extension of the data from our preliminary investigation of genetic variation in Australian barramundi (Shaklee and Salini 1985). Those enzymes which had exhibited genetic variation in the preliminary study were screened in the present investigation. In addition, four enzymes not included in the earlier study were also examined. Two of these, N-acetyl-beta-glucosaminidase (EC 3.2.1.30) and beta-galactosidase (EC 3.2.1.23), did not exhibit

adequate resolution and could not be used in the population screening. In addition to the 31 monomorphic loci reported earlier (Shaklee and Salini 1985), one additional locus, alpha-mannosidase (EC 3.2.1.24), was screened in 100 fish from both the east coast of Australia and from the Daly River and showed no genetic variation. Although this enzyme was not screened further, it was considered to be monomorphic. Thus, there are 32 known monomorphic loci in Australian barramundi.

The other enzyme not surveyed before, fumarate hydratase, proved to be polymorphic in barramundi from the Northern Territory. When electrophoretic resolution was adequate, heterozygotes at this locus exhibited the five-banded phenotypes expected for this tetrameric enzyme. This enzyme was routinely screened for all samples. Particulars of the polymorphic loci (locus abbreviations, EC numbers, subunit structures, source tissues, and optimum buffers) are given in Table 3. The muscle-predominant form of isocitrate dehydrogenase (Idh-2), which has been shown to be polymorphic in barramundi from the Princess Charlotte Bay area of the east coast of Australia (Shaklee and Salini 1985), did not exhibit variation in any of the collections from the Northern Territory or Western Australia. This increased the number of monomorphic loci in this region to 33. Similarly, although L-iditol dehydrogenase (Iddh) exhibited low levels of variation in one or more populations in the Northern Territory, it did not meet the $p_{0.99}$ criterion for polymorphism, thus resulting in a total of 34 monomorphic loci for these collections. As there were 12 polymorphic protein-coding loci (out of 46 screened), $p_{0.99} = 0.26$ for Northern Territory barramundi. However, in only five loci (aconitate hydratase, esterase-2, general protein, isocitrate dehydrogenase-1, and malate

dehydrogenase-2) was the frequency of their most common allele less than 0.95 so that $p_{0.95} = 0.11$ for barramundi from the Northern Territory. These values are almost identical to the corresponding figures of 0.21 and 0.11 calculated for for a smaller sample of barramundi from a much wider geographic range (Daly River, southeastern Gulf of Carpentaria, and Princess Charlotte Bay) as reported by Shaklee and Salini (1985). Mean heterozygosity (H) for all Northern Territory and the Western Australian collections was 0.029. Allele frequencies for all the polymorphic loci are presented for the individual Daly River area collections (Table 4) and for the seven major locations (Table 5).

For the individual Daly River area collections, none of the 38 possible tests for deviation from Hardy-Weinberg expectations was significant. In the analysis of collections from the seven different localities, only 2 out of 48 possible χ^2 tests (Ah from the Mary River and Est-2 from the Roper River) differed significantly (at the 5% significance level) from expectations, which could be attributed to chance alone. Therefore, these samples can be considered to be in agreement with Hardy-Weinberg expectations and the genetic models used for interpreting the gel banding patterns are deemed consistent with the observed patterns of variation.

The temporal stability of allele frequencies at the Daly River area was tested in three ways. First, the five Daly River replicates were compared by a five-way contingency χ^2 analysis, while the two Finniss River collections were compared by a two-way contingency χ^2 analysis. Neither test detected any overall or individual locus heterogeneity. Given the apparent homogeneity at each site, a pooled Daly River sample

(consisting of the five individual collections combined) was then tested by two-way contingency χ^2 analysis with the pooled Finniss River sample. Again, no overall or individual locus heterogeneity could be detected, which suggests that all these samples were taken from the same interbreeding population. These two pooled collections were then combined into a "Daly River area" collection.

Second, a contingency χ^2 test among the seven individual collections from the Daly River area (representing a 14-month period) was conducted. This overall test using the seven loci polymorphic ($p_{0.99}$) in these collections (Ah, Ck-A, Est-2, Gpi-A, Idh-1, Mdh-2 and Gp) revealed no significant differences among collections (see Table 4).

Third, χ^2 goodness-of-fit tests of genotypic proportions to Hardy-Weinberg expectations were conducted at all seven polymorphic loci in the pooled Daly River area collection. These tests did not yield any significant deviations (out of a total of 38 tests) from the expected genotypic proportions. The results of all three tests indicated that all the alleles tested were drawn from a homogeneous population with no detectable change in allele frequencies over the 14 months of sampling. To the extent that this result can be generalized to barramundi in other areas, it would seem that allele frequencies are stable for at least one year.

The Daly River and Finniss River collections were therefore combined and treated as a single, homogeneous collection (= "Daly River area") for the remainder of the contingency χ^2 tests for homogeneity of alleles between locations.

The overall χ^2 test for all seven locations and 12 possible polymorphic loci was reduced to a comparison using 7 polymorphic loci. Four (*Fh, Ldh-C, Pgdh* and *Pgm*) were unreliable, as in the χ^2 analysis, each had one or more cells with an expected value less than 1.0, and further pooling to remove these low expected values was not possible. The locus *Ah* was also omitted from the comparison because only three collections were satisfactorily scored for this locus. Despite this reduction from twelve to seven polymorphic loci, the overall χ^2 test for all seven localities was highly significant: $\chi^2 = 612$ (54 degrees of freedom), $P << 0.001$, with all seven polymorphic loci (*Ck-A, Est-2, Est-D, Gp, Gpi-A, Idh-1* and *Mdh-2*) exhibiting significant heterogeneity (Table 6).

To determine where inter-stock boundaries occurred, all the adjacent localities were compared over all possible loci. The six comparisons revealed heterogeneity between adjacent localities at between two and five loci, with the number of polymorphic loci available for testing in each pairwise comparison varying between three and ten (Table 6). These results indicate that each of the locations sampled supports a discrete breeding unit or stock, with apparently limited gene flow between adjacent sites. Indices of fixation, F_{st}, although low for each locus over all locations, revealed greatest fixation of alleles at the *Ah* locus (Table 7), which also exhibited the highest overall levels of per-locus heterozygosity (Table 5). However, it should be noted that reliable data for *Ah* were only available for three of the seven areas. Even so, over the seven areas, per-locus F_{st} values were all significantly different from zero according to the test statistic: $\chi^2 = 2NF_{st}$ with $s-1$ degrees of freedom where N = the number sampled and s =

the number of sites (Workman and Niswander 1970).

DISCUSSION

Our genetic interpretations of the observed polymorphic isozyme banding patterns in barramundi have already been discussed in a previous report (Shaklee and Salini 1985). The levels of polymorphism for the three widely separated locations surveyed in this earlier study (Princess Charlotte Bay on the northeastern coast of Australia, the southeastern Gulf of Carpentaria and Daly River in Northern Territory) are similar to those observed in the Northern Territory and Western Australia in the present investigation ($\underline{p}_{0.99}$ = 0.21 and $\underline{p}_{0.95}$ = 0.11 previously: $\underline{p}_{0.99}$ = 0.26 and $\underline{p}_{0.95}$ = 0.11 in this report). The slight differences between these studies are a result of the Idh-2 locus not exhibiting any variation in the samples from the seven Northern Territory and one Western Australian sites, the fact that Ck-A is polymorphic in Western Australia and the Northern Territory, and the addition of the extra polymorphic locus, Fh, in the present study. The similarity in the levels of variation is also reflected in the values for per-locus heterozygosities and overall heterozygosity (see Tables 5 and 7).

One aim of this study was to test the stability of allele frequencies over time. Stability over a 14-month period was demonstrated in the Daly River area. Temporal stability of allele frequencies supports our use of allele frequency data as characteristics of localities rather

than simply as characteristics of collections.

Another goal of this investigation was to determine the extent of stock heterogeneity in barramundi throughout the Northern Territory. Contingency χ^2 analyses of all polymorphic loci at all locations revealed strong evidence of genetic heterogeneity within our samples. Indeed, pairwise tests of adjacent localities indicated genetically discrete populations at seven of the sites sampled. The exception was the comparison of the Finniss and Daly Rivers; 60 km between their mouths is certainly within the movement range of adult barramundi reported from tagging studies in the East and West Alligator Rivers (Davis 1986). All of the remaining pairwise comparisons, after pooling the Daly and Finniss River collections, revealed significant genetic heterogeneity between adjacent pairs of localities. This result is interpreted as a clear demonstration of the existence of at least seven discrete stocks of barramundi in this region of Australia. These stocks are associated with the following riverine localities: 1) Ord River, 2) Daly River area, 3) Mary River, 4) Glyde River, 5) Blue Mud Bay, 6) Roper River and 7) McArthur River (Fig. 2).

A third aim of this work was to attempt to define the boundaries of all detectable stocks. This was most effectively tested with the sampling of 5 Daly River replicates and 2 Finniss River replicates. Since no heterogeneity was detected within either river or between the two rivers over a period of 14 months, the barramundi from the Daly and Finniss Rivers can be presumed to have sufficient gene flow between localities to prevent the establishment of significant allele frequency differences. In contrast, the next closest pairs of sampling locations

(the McArthur River and Roper River, and the Roper River and Blue Mud Bay;- each pair is separated by about 150 km) showed significant genetic differences. Because of the highly discontinuous distribution of collection localities in the present study, it is entirely possible that additional, discrete stocks of barramundi may occur within the overall region studied. Indeed, on geographic grounds, it seems likely that additional stocks may occur in, for example, southeastern Joseph Bonaparte Gulf (associated with the Victoria River), eastern Van Diemen Gulf, and Arnhem Bay about 150 km east of the Glyde River. The seven discrete stocks recognised in the present study should therefore be considered to represent the minimum number of barramundi stocks in this region of Australia.

The evidence for the existence of distinct stocks of barramundi at the locations compared implies that either the geographic separation of such localities or the behaviour of the barramundi in these areas, or both, is sufficient to restrict gene flow to a level incapable of overriding the effects of random genetic drift. The possibility of selection maintaining the observed genetic discreteness is remote in light of, firstly, the lack of any noticeable change in major environmental variables (for example, temperature) between sites that would be capable of driving such selection and secondly, by the "patchwork" pattern of the observed variation in allele frequencies throughout the region (cf. Shaklee and Salini 1985). Table 5 shows no evidence of a consistent clinal change in allele frequencies across the collection sites from west to east or from north to south.

The most important factor modulating the presence or absence of

detectable stocks is the number of individuals exchanged between populations. Given the difficulty of obtaining such information for many fisheries, various estimates have, nevertheless, been made. Menzies (1981) calculated that between 1 in 10,000 and 1 in 100,000 immigrants per year would prevent the establishment of genetic discreteness in spiny lobster, Panulirus argus, populations in Florida and the Caribbean. Allendorf and Phelps (1981) estimated that exchanges between populations of less than 50 individuals per generation would not swamp genetic discreteness under conditions of neutrality. They also concluded from population genetics theory and from computer simulations that, for salmonids at least, it is the absolute numbers, and not the proportion of individuals exchanged, that are of primary importance in preventing allelic divergence between populations. However, in a comparison of the genetic structure of marine, anadromous and freshwater fishes, Gyllensten (1985) estimated that the number of migrants per generation that were adequate to "homogenize" allele frequencies and prevent population differentiation ranged from about 40 in marine species to less than 1 for freshwater species such as brown trout. The above calculations all require estimates of the effective population size, N_e, and approximations of F_{st} in the formula $F_{st}=(4N_em+1)^{-1}$, with m = immigration rate (Wright 1978). Although estimates, the number of barramundi moving between river systems can only be tentative, given the limited data on tagged barramundi, and the limitations of the data (see Shaklee and Salini 1985 for discussion), Wright's formula can be usefully applied. The one-dimensional stepping-stone model is probably the best model for barramundi as fish movements are most likely to be between adjacent populations, as the tagging data suggests. Substitiuting F_{st} = 0.087 from Table 7 and $\mu = 10^{-6}$ in the formula N_em =

$\frac{1}{4}(1/F_{st} - 1)^{-1} - N_e\mu$, where μ = mutation rate, then the number of exchanges, $N_e m = 2.62 - N_e 10^{-6}$. In this case, the number of movements between populations, $N_e m$, is less than 2.6 per year, depending on the value of N_e. Similarly, $N_e m$ becomes 2.6 and is independent of N if the more simple, island model of migration is used. In this model, N_e becomes N, the population size (Hartl 1980). Given the assumptions inherent in these models, this can only be treated as a very approximate figure, but it is consistent with the reported movements of tagged barramundi among the Alligator Rivers and the Mary River in Van Diemen Gulf reported by Davis (1986): only nine of 278 tags returned (of the 4,000 barramundi tagged) indicated that the fish had moved between these rivers in three years. Furthermore, eight of these nine fish had moved between river mouths less than 100 km apart, with only one moving about 120 km along the coast.

In Australian barramundi, the critical characteristics for the establishment of genetically discrete populations would seem to be:
1) the limited dispersal potential of the short-lived, pelagic eggs and larvae (Wongsomnuk and Manevonk 1973),
2) the rapid movement of juveniles into the river systems (Moore 1982, Davis 1984, Russell and Garrett 1985),
3) the relatively short migrations of tagged adults (Davis 1986, R. Garrett, Qld. Fisheries Service, Cairns, Qld., unpublished data) and,
4) the more-or-less disjunct distribution of suitable riverine and estuarine adult habitat.
Management of the barramundi fishing resource should incorporate such basic knowledge of stock structure. Since these stocks represent self-reproducing units with little or no exchange between them, then the

total resource should be managed accordingly. A depleted stock (from overfishing or natural causes) will not be replenished, in the short term, from adjacent stocks. Hence, our results suggest that the barramundi should be managed as separate stocks rather than one single unit stock (Shaklee and Salini 1983, Salini and Shaklee 1987). Another repercussion of the extent of barramundi stock structure is in aquaculture. The danger lies in the unmonitored mixing of barramundi from different stocks during hatchery releases for restocking purposes. The consequence of mixing stocks is the loss of naturally occurring genetic diversity, which is an essential resource for any viable breeding program (Salini and Shaklee 1987).

ACKNOWLEDGMENTS

All the barramundi samples in this report were obtained through the assistance of R. Griffin, G. White and N. Gill (Northern Territory Fisheries, Darwin, NT) and the cooperation of various interested commercial fishermen (W. Barrett, B. Carter, R. Connelly, D. Fazio, M. Fraser, C. Noar, M. Sargent, R. Timms, G. Tybell and L. Will). T. L. O. Davis (CSIRO Division of Fisheries Research, Hobart) and R. Garrett (Queensland Department of Primary Industries, Cairns) kindly shared unpublished results and other observations. We thank Shane Lavery for his assistance with the computer programs used to organise and analyse the data in this study. Part of this study was funded by a grant from the Fisheries Research Trust Account Grant Number 1983/48.

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TABLE 1. Daly River area replicate collections. Not all fish were sexed in all collections, hence the totals discrepancies in collections 2, 3, 6 and 7. $\bar{x}$ = mean; sd = standard deviation; nd = no data;

COLLECTION NUMBER	LOCALITY	DATE	NUMBERS EXAMINED			STANDARD LENGTH(mm)	
			males	females	totals	$\bar{x}$	sd
1	Daly River	Aug/82	39	2	48	730	112
2	Daly River	Feb/83	108	18	133	816	113
3	Finniss River	Apr/83	97	7	105	nd	nd
4	Daly River	Sep/83	48	12	60	744	122
5	Daly River	Sep/83	78	3	81	685	109
6	Daly River	Aug/83	115	13	130	727	122
7	Finniss River	Feb/83	34	3	38	677	80

TABLE 2. Sample details for the seven major collections of barramundi. Not all fish were sexed in all collections. Daly River and Finniss River collections were pooled as "Daly River Area" locality.
$\bar{x}$ = mean; sd = standard deviation; nd = no data.

LOCALITY	DATE	NUMBERS EXAMINED			STANDARD LENGTH(mm)	
		males	females	totals	$\bar{x}$	sd
Ord River	Aug/84	nd	nd	69	768	143
Daly River Area	Feb/83	519	58	595	771	134
Mary River	Mar/83	nd	nd	150	810	108
Glyde River	Sep/84	63	14	78	772	102
Blue Mud Bay	Aug/85	56	42	100	708	93
Roper River	Jul/83	nd	nd	116	798	141
McArthur River	Apr/83	107	88	201	nd	nd

TABLE 3. Characteristics of the polymorphic proteins in Lates calcarifer in the Northern Territory and Western Australia and conditions for electrophoresis.

EC = Enzyme Commission

PROTEIN	EC NUMBER	LOCUS	SUBUNIT STRUCTURE[a]	TISSUE	BUFFER[b]
Aconitate hydratase	4.2.1.3	Ah	Monomer	Liver	CAEA
Creatine kinase-A	2.7.3.2	Ck-A	Dimer[c]	Muscle	TC-1
Esterase-2	3.1.1.-	Est-2	Monomer	Eye	LiOH
Esterase-D	3.1.1.-	Est-D	Dimer	Liver	EBT
Fumarate hydratase	4.2.1.2	Fh	Tetramer[d]	Liver	TRIC
Glucose-6-phosphate isomerase-A	5.3.1.9	Gpi-A	Dimer	Eye	LiOH
Isocitrate dehydrogenase($NADP^+$)-1	1.1.1.42	Idh-1	Dimer	Liver	TRIC
Lactate dehydrogenase-C	1.1.1.27	Ldh-C	Tetramer[d]	Eye	LiOH
Malate dehydrogenase-2	1.1.1.37	Mdh-2	Dimer	Liver	CAEA
General protein		Gp	Monomer	Muscle	LiOH[e]
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh	Dimer	Liver	TRIC
Phosphoglucomutase	5.4.2.2	Pgm	Monomer	Muscle	TC-1

[a] - Presumed structure based on isozyme banding pattern in heterozygotes

[b] - CAEA = Citric acid-aminopropylmorpholine, pH 6.0 buffer of Clayton and Tretiak (1972)

LiOH = Lithium hydroxide-boric acid buffer; modified buffer 2 of Selander et al. (1971)

TABLE 3. Footnote continued.

POULIK = Sodium hydroxide-boric acid, pH 8.7; buffer 3 of Selander et al. (1971)

TRIC = Triethanolamine-citric acid, pH 7.2; buffer of Clayton and Tretiak (1972)

TC-1 = Tris-citric acid, pH 7.0; buffer 1 of Shaw and Prasad (1970)

EBT = EDTA-boric acid-Tris, pH 8.6; buffer of Boyer et al. (1963)

(see Shaklee and Keenan 1986 for detailed buffer recipes)

[c] - although this enzyme is a dimer, presumed heterozygotes exhibited two-banded patterns as reported for other species (cf. Ferris and Whitt 1978)

[d] - resolution not always adequate to separate the five expected isozymes

[e] - electrophoresis in polyacrylamide gels

TABLE 4. Allele frequencies and individual locus heterozygosities in replicate collections from the Daly River area (Daly River and Finniss River as described in Table 1).

The number of fish successfully scored at each location and for each locus is given in ( ); nd = no data.

LOCUS	ALLELES	LOCATION						
		1	2	3	4	5	6	7
Ah		(44)	(133)	nd	nd	nd	nd	(38)
h=0.448	100	0.636	0.733					0.711
	126	0.000	0.008					0.000
	116	0.045	0.094					0.066
	111	0.318	0.165					0.224
Ck-A		(48)	(133)	(105)	(60)	(81)	(129)	(37)
h=0.022	100	1.000	0.996	0.990	0.992	0.975	0.992	0.959
	106	0.000	0.004	0.010	0.008	0.025	0.008	0.041
Est-2		(48)	(133)	(103)	(60)	(77)	(103)	(38)
h=0.387	100	0.844	0.789	0.718	0.742	0.760	0.806	0.697
	102	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	98	0.052	0.064	0.087	0.092	0.058	0.049	0.132
	95	0.104	0.147	0.194	0.167	0.182	0.146	0.171
Est-D		(48)	(133)	(105)	(60)	(81)	(128)	(38)
h=0.002	100	1.000	1.000	0.995	1.000	1.000	1.000	1.000

	114	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	85	0.000	0.000	0.005	0.000	0.000	0.000	0.000
Fh		(47)	(133)	(104)	(53)	(78)	(118)	(38)
h=0.004	100	1.000	0.996	1.000	1.000	0.994	1.000	1.000
	40	0.000	0.004	0.000	0.000	0.006	0.000	0.000
Gpi-A		(48)	(133)	(104)	(60)	(81)	(118)	(38)
h=0.031	100	0.979	0.992	0.986	0.983	0.981	0.979	0.987
	95	0.021	0.008	0.014	0.017	0.019	0.021	0.013
Idh-1		(48)	(133)	nd	(50)	nd	nd	(38)
h=0.338	100	0.740	0.778		0.840			0.829
	121	0.250	0.222		0.160			0.171
	60	0.000	0.000		0.000			0.000
Ldh-C		(48)	(132)	(105)	(52)	(79)	(127)	(38)
h=0.011	100	1.000	0.992	0.990	1.000	1.000	0.992	1.000
	90	0.000	0.008	0.010	0.000	0.000	0.008	0.000
Mdh-2		(48)	(133)	(104)	(60)	(81)	(130)	(38)
h=0.061	100	0.958	0.974	0.966	0.983	0.963	0.962	0.974
	134	0.000	0.004	0.014	0.000	0.019	0.004	0.000
	60	0.042	0.023	0.019	0.017	0.019	0.035	0.026
Pgdh		(48)	(133)	(101)	(58)	(73)	nd	(37)
h=0.002	100	1.000	0.996	1.000	1.000	1.000		1.000
	92	0.000	0.000	0.000	0.000	0.000		0.000

	88	0.000	0.004	0.000	0.000	0.000		0.000
Pgm		(48)	(132)	(105)	(59)	(81)	(130)	(37)
h=0.000	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	90	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gp		(48)	(133)	(105)	(60)	(81)	(129)	(38)
h=0.148	100	0.969	0.929	0.900	0.892	0.907	0.938	0.895
	97	0.031	0.071	0.100	0.108	0.093	0.062	0.105

TABLE 5. Allele frequencies and individual locus heterozygosities at seven major locations for all polymorphic loci.

The number of fish successfully scored at each location and for each locus is given in ( ); nd = no data.

LOCUS	ALLELES	LOCATION						
		Ord	Daly	Mary	Glyde	BMBay	Roper	McAth
Ah		nd	(217)	(144)	nd	(99)	nd	nd
h=0.508	100		0.712	0.563		0.727		
	126		0.005	0.059		0.000		
	116		0.078	0.122		0.020		
	111		0.205	0.240		0.253		
	89		0.000	0.017		0.000		
Ck-A		(69)	(578)	(150)	(77)	(100)	(115)	(202)
h=0.011	100	0.993	0.989	1.000	1.000	1.000	1.000	1.000
	106	0.007	0.011	0.000	0.000	0.000	0.000	0.000
Est-2		(70)	(566)	(149)	(75)	(98)	(116)	(199)
h=0.469	100	0.771	0.762	0.503	0.427	0.781	0.724	0.608
	102	0.007	0.000	0.007	0.013	0.000	0.000	0.000
	98	0.071	0.074	0.248	0.153	0.015	0.000	0.025
	95	0.150	0.163	0.242	0.407	0.204	0.276	0.367
Est-D		(70)	(578)	(150)	(78)	(101)	(115)	(202)
h=0.015	100	0.986	0.999	1.000	0.974	0.995	1.000	0.970

	114	0.000	0.000	0.000	0.000	0.005	0.000	0.030
	85	0.014	0.001	0.000	0.026	0.000	0.000	0.000
Fh		(66)	(557)	(150)	(70)	(100)	(112)	(195)
h=0.007	100	1.000	0.998	0.980	1.000	1.000	1.000	0.997
	40	0.000	0.002	0.020	0.000	0.000	0.000	0.003
Gpi-A		(70)	(567)	(150)	(78)	(101)	(116)	(202)
h=0.018	100	1.000	0.984	0.983	1.000	1.000	1.000	1.000
	95	0.000	0.016	0.017	0.000	0.000	0.000	0.000
Idh-1		(67)	(270)	(150)	(74)	(101)	(114)	(201)
h=0.233	100	0.672	0.791	0.937	0.899	0.827	0.930	0.958
	121	0.328	0.207	0.063	0.101	0.173	0.070	0.040
	60	0.000	0.002	0.000	0.000	0.000	0.000	0.002
Ldh-C		(70)	(567)	(150)	(78)	(100)	(112)	(202)
h=0.009	100	0.993	0.995	0.983	1.000	1.000	1.000	1.000
	90	0.007	0.005	0.017	0.000	0.000	0.000	0.000
Mdh-2		(70)	(579)	(150)	(78)	(101)	(116)	(202)
h=0.044	100	0.943	0.969	0.987	1.000	1.000	1.000	0.975
	134	0.036	0.006	0.003	0.000	0.000	0.000	0.025
	60	0.021	0.025	0.010	0.000	0.000	0.000	0.000
Pgdh		(69)	(520)	(150)	(69)	(100)	(113)	(202)
h=0.004	100	1.000	0.999	1.000	1.000	1.000	1.000	0.990
	92	0.000	0.000	0.000	0.000	0.000	0.000	0.010

	88	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Pgm		(70)	(577)	(149)	(78)	(100)	(115)	(202)
h=0.007	100	1.000	1.000	1.000	0.968	1.000	1.000	0.990
	90	0.000	0.000	0.000	0.032	0.000	0.000	0.010
Gp		(70)	(579)	(150)	(78)	(99)	(116)	(202)
h=0.113	100	0.821	0.920	0.937	0.968	1.000	0.983	0.975
	97	0.179	0.080	0.063	0.032	0.000	0.017	0.025

TABLE 6. Contingency χ^2 analyses for all polymorphic loci at the seven locations examined.

df = degrees of freedom, P = probability, n = number of loci exhibiting significant differences between localities, N = total number of loci polymorphic at the locations tested.

COMPARISON	χ^2	df	P	SIGNIFICANT LOCI	n/N
7-way (all locations)	612	54	<0.001	Ck-A, Est-2, Est-D, Gp, Gpi-A, Idh-1, Mdh-2	7/11[a]
Ord v. Daly area	50	10	<0.001	Est-2, Gp, Idh-1, Mdh-2	4/6
Daly area v. Mary	182	15	<<0.001	Ah, Est-2, Fh, Idh-1, Ldh-C	5/9
Mary v. Glyde	51	16	<0.001	Est-2, Est-D, Pgm	3/10
Glyde v. B.M.Bay	109	11	<<0.001	Ah, Est-2, Pgm, Gp	4/6
B.M.Bay v. Roper	20	4	<0.001	Est-2, Idh-1	2/3
Roper v. McArthur	33	9	<0.001	Est-2, Est-D, Mdh-2	3/7

[a] = Fh, Ldh-C, Pgdh and Pgm were ignored (even though they had "significant" χ^2 values) as the tests for these loci contained cells with expected values <1.0 and further allele pooling was not possible.

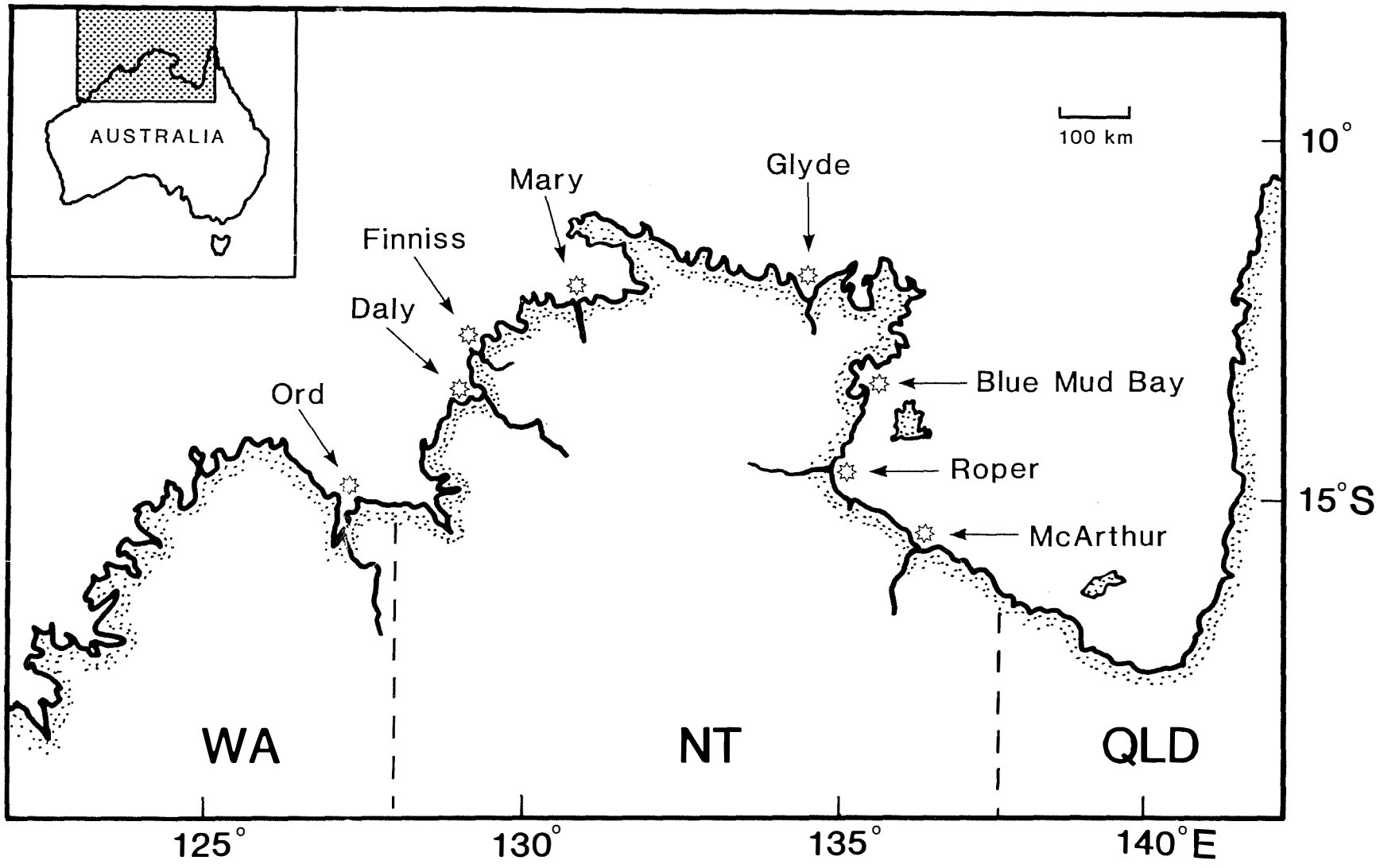
Table 7. Indices of fixation (F_{st}) for all polymorphic loci in barramundi in the Northern Territory and Western Australia. s.e.= standard error, Total = across all locations, N = number of fish successfully scored, H = mean heterozygosity, nd = no data.

LOCUS	ORD	DALY	MARY	GLYDE	BMBAY	ROPER	McATH	TOTAL	χ^2	df	N	P
Ah	nd	0.448	0.608	nd	0.407	nd	nd	0.155	157.5	3	508	<0.001
Ck-A	0.014	0.022	0.000	0.000	0.000	0.000	0.000	0.007	18.1	6	1291	<0.01
Est-2	0.377	0.387	0.627	0.629	0.358	0.400	0.495	0.065	141.6	6	1089	<0.001
Est-D	0.028	0.002	0.000	0.050	0.010	0.000	0.058	0.015	33.0	6	1100	<0.001
Fh	0.000	0.004	0.039	0.000	0.000	0.000	0.005	0.013	29.9	6	1068	<0.001
Gp	0.293	0.148	0.119	0.062	0.000	0.034	0.048	0.058	127.5	6	1099	<0.001
Gpi-A	0.000	0.031	0.033	0.000	0.000	0.000	0.000	0.012	24.2	6	1100	<0.001
Idh-1	0.441	0.338	0.119	0.182	0.286	0.131	0.081	0.073	146.5	6	977	<0.001
Ldh-C	0.014	0.011	0.033	0.000	0.000	0.000	0.000	0.008	17.4	6	1087	<0.01
Mdh-2	0.109	0.061	0.026	0.000	0.000	0.000	0.048	0.019	41.8	6	1100	<0.001
Pgdh	0.000	0.002	0.000	0.000	0.000	0.000	0.020	0.008	15.1	6	1080	<0.025
Pgm	0.000	0.000	0.000	0.062	0.000	0.000	0.020	0.021	46.0	6	1095	<0.001
H	0.085	0.095	0.107	0.106	0.071	0.038	0.052					
(±s.e.)	0.039	0.040	0.054	0.055	0.038	0.027	0.032					
Mean F_{st}								0.087	178.8	63	1028	<0.001

FIGURE LEGENDS

Figure 1. Place names and locations of collecting sites for barramundi in Western Australia and the Northern Territory.

Figure 2. Diagrammatic representation of genetically discreet barramundi stocks in central northern Australia between the Ord and the McArthur Rivers.


AUSTRALIA
100 km
Mary
Glyde
Finniss
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Ord
Blue Mud Bay
Roper
McArthur
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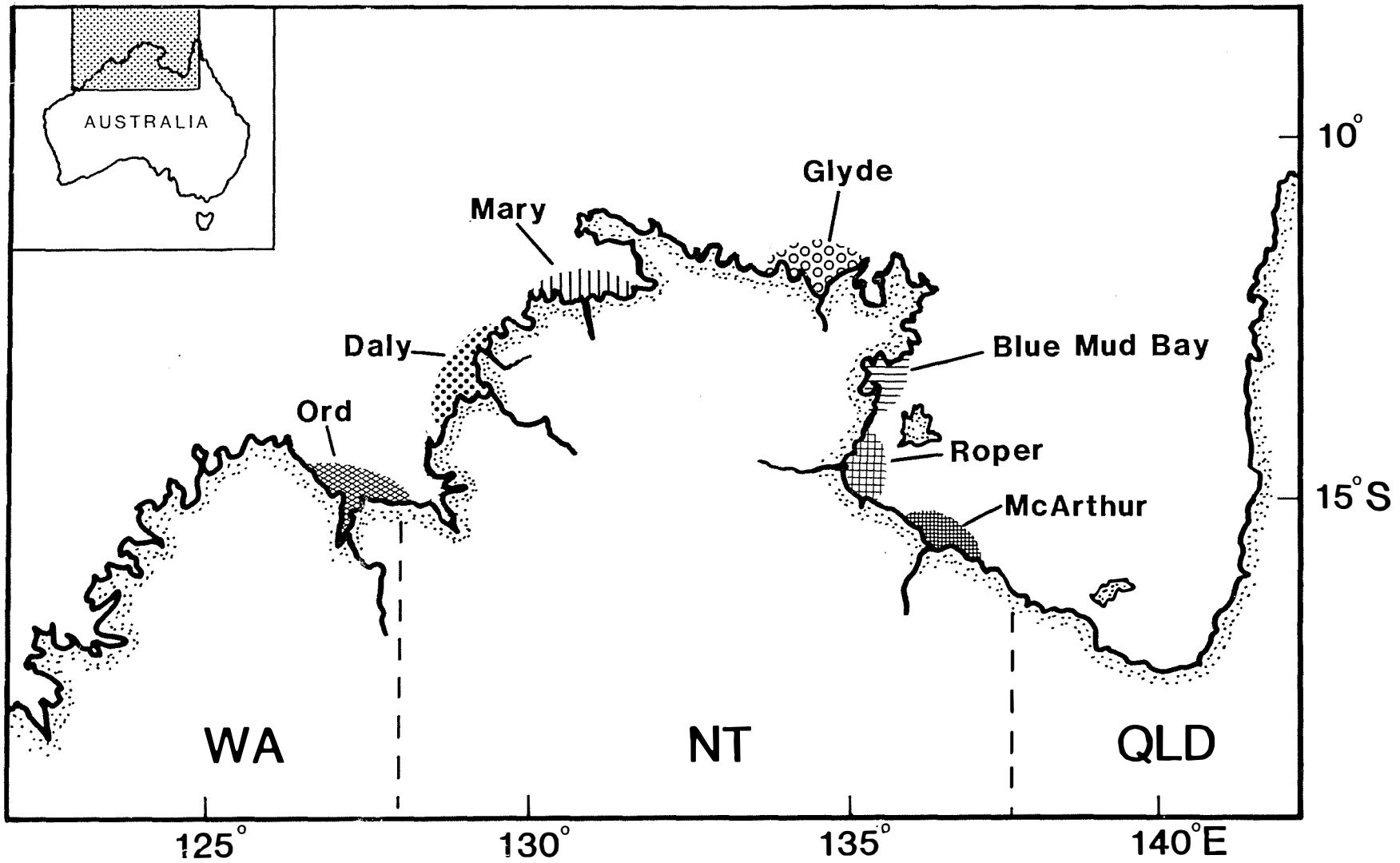
QLD
125°
130°
135°
140°E


AUSTRALIA
Glyde
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Daly
Blue Mud Bay
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Roper
McArthur
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QLD
125°
130°
135°
140°E

Fig 2

Population Genetics of Two Tropical Sharks, *Carcharhinus tilstoni* and *C. sorrah*, in Northern Australia

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ABSTRACT

The technique of starch gel electrophoresis was used to study possible stock heterogeneity in the two species of shark (Carcharhinus tilstoni and C. sorrah) which comprise the bulk of the northern Australian shark fishery. Tissue samples were taken from a total of over 1700 sharks, from locations ranging from the North-West Shelf (in Western Australia) to the north-east coast of Queensland, both inshore and offshore. From a total of 47 enzyme loci screened in each species, 13 proved to be polymorphic ($P_{.99}$) for at least one species, with only 5 loci for each species showing sufficient variation ($P_{.95}$) to be of use in our analysis of stock structure. Mean heterozygosity values were relatively low, being 0.037 and 0.035 respectively for C. tilstoni and C. sorrah. A low level of population subdivision was found within each species, with F_{ST} values of 0.0094 for C. tilstoni and 0.0076 for C. sorrah. There was insufficient evidence to suggest that any more than one stock exists within either species of shark in Australian waters.

INTRODUCTION

A Taiwanese gill-net fishery has been making significant use of the pelagic fish resources off the coast of northern Australia since the early 1970's. Although now restricted to offshore areas (since the declaration of the Australian Fishing Zone in 1979), the Taiwanese had previously caught over 17 000 tonnes in one year in this fishery (Walter 1981). Approximately 80% of this catch is composed of sharks. The two species examined in this study, Carcharhinus tilstoni and C. sorrah, comprise about 58% and 25%, respectively, of the shark catch (Stevens and Wiley 1986). In recent years, Australian fishermen have become increasingly interested in the tropical shark resource and there are indications that tropical sharks could become the object of a major Australian fishery (Lyle and Timms 1984).

In attempting to manage such a fishery, it is important to know of the possible existence of distinct stocks or isolated subpopulations (MacLean and Evans 1981). For example, in this shark fishery it would be of considerable interest to determine whether or not the sharks taken by the Taiwanese in the offshore regions are from the same stock or stocks found close inshore by Australian fishermen. As the range of these sharks includes waters controlled by three Australian State or Territory fisheries bodies, it would also be of interest to know whether these bodies need to manage separate stocks within their boundaries independently, or if the fishery should be managed co-operatively as one stock.

Particular problems are encountered in trying to understand the effects of exploitation on a shark fishery as compared to other species of fishes. Firstly, sharks generally appear to have slow growth rates and are long-lived

(reference?), therefore, heavy fishing pressure may have severe effects on a population. Secondly, very little research has been undertaken on the stock structure of sharks anywhere so that little is known about population subdivision in these primitive fishes.

Data from the electrophoretic analysis of protein variation has proven to be of considerable use in defining the population structure of many commercial fish species (for reviews see Berst and Simon 1981, Shaklee 1983).

There is some indication from the biology of the two species involved in this study that stock differentiation would be possible within each of them. Unlike most other marine fishes, both species of shark exhibit placental viviparity (Stevens and Wiley 1986). Thus, there is no planktonic stage of the life cycle which would facilitate substantial dispersal and mixing of the entire population. On the other hand, both species are relatively large, strong-swimming fishes which could conceivably move large distances as adults.

For some time, C. tilstoni has been regarded as a synonym for the world-wide species C. limbatus. However, our electrophoretic analysis has revealed that "C. limbatus" in Australian waters is actually comprised of two very similar, but distinct, sympatric species (Lavery and Shaklee, unpublished data). The species dominating the Australian catch (by approximately 300 to 1) has proved to be a currently unrecognised, although previously named, species -- C. tilstoni (Lyle 1986, Stevens and Wiley 1986). It is not known if the distribution of this species extends beyond Australian waters, however, C. sorrah is distributed throughout the Indian and western Pacific Oceans.

This study used electrophoretic protein variation to examine possible stock differentiation within both C. tilstoni and C. sorrah in Australian waters. Because this study represents the first major electrophoretic study on elasmobranch population structure, the electrophoretic results are described in some detail.

MATERIALS AND METHODS

Sample Collection

Tissue samples for electrophoresis were taken from sharks caught between 1982 and 1985. The bulk of the samples came from catches of the fishing vessel 'Rachel' during cruises under the Northern Pelagic Fish Stock Research Programme conducted by the CSIRO Division of Fisheries Research and the Northern Territory Fisheries Service. Additional samples came from Northern Territory Fisheries Service cruises, the Commonwealth Department of Primary Industry 'Observer' programme, and from the private vessel 'Kiama'. Samples were collected from 925 C. tilstoni (comprising 14 collections) and 655 C. sorrah (15 collections), details of which appear in Table 1. The collections were grouped into logical geographic areas shown in Figure 1.

Electrophoresis

Tissue samples were frozen at -20° C immediately after dissection and transported and stored at this temperature until extracts were prepared in the laboratory. The extracts were stored at -70° C until electrophoresis. Tissue preparation, horizontal starch gel electrophoretic techniques and histochemical enzyme staining procedures largely followed those of Shaklee and Salini (1985) and Shaklee and Keenan (1986).

Difficulties in obtaining adequate resolution were encountered for many enzymes. The high frequency of smeary staining or considerable sub-banding observed for many of the shark enzymes was in sharp contrast to the clear isozyme patterns obtained from several species of teleostean fishes examined

in our laboratory. Numerous variations of the standard techniques referred to above were employed in an attempt to overcome these problems. Variations included the use of: different tissue homogenising buffers containing urea concentrations of up to 0.05M (as suggested by Peterson 1970); a wide range of tissues, including muscle, heart, eye, liver, kidney and blood; a large number of electrophoretic buffer systems (up to 14); a range of starch lots; different electrophoretic media (both starch gel and polyacrylamide gel); variation of the quantity of sample applied to the gel (by using 3 different thicknesses of filter paper wicks); and a wide range of enzyme stains (76), as well as two general protein stains (Coomassie blue, and the very sensitive silver stain technique (Shaklee and Keenan 1986)). Although some of these alternative methods did improve the clarity of banding patterns for some enzymes, many enzymes still exhibited uninterpretable patterns.

Only those patterns of enzyme variation that were consistent with the subunit structure of the enzyme (where known) and simple models of Mendelian inheritance were scored and recorded as genotypes. Names of enzymes and Enzyme Commission numbers follow the recommendations of the Commission on Biochemical Nomenclature (1984), and enzyme abbreviations were derived from these recommended names. Locus designations for multi-locus enzyme systems and allelic designations follow Shaklee and Salini (1985).

Statistical Analyses

Genotype frequencies were tested for conformity to Hardy-Weinberg expectations by chi-square goodness-of-fit tests. Contingency table analyses by the likelihood-ratio (G) statistic were employed to test for independence of allele frequencies and collections or areas. For both these analyses, alleles of closest relative electrophoretic mobility were pooled into

classes, when necessary, to avoid excessively low expected frequencies. A test for linear trend of allele frequencies across areas was performed. This is a chi-square test with 1 degree of freedom, equivalent to testing the slope of a simple linear regression, where the dependent variable is the allele frequency (Cochran 1954).

The degree of genetic heterogeneity was described by use of Wright's F-statistics (Wright 1965, 1978) at both a hierarchical and non-hierarchical level. F_{ST} values were tested for significance by the method of Brown (1985) and Workman and Niswinder (1970) i.e., $X^2 = 2NF_{ST}$, with appropriate degrees of freedom. Rogers' measure of genetic distance (modified by Wright, 1978), calculated from all polymorphic ($P_{.95}$) loci, was used to compare sharks from different areas, and Sneath and Sokal's (1973) UPGMA clustering method was used to construct dendrograms.

For age class comparisons, sharks were considered to be mature if their fork length was greater than the average minimum fork length of mature sharks, as determined by Stevens and Wiley (1986) (i.e., 84cm for C. tilstoni and 71cm for C. sorrah).

Statistical significance throughout is indicated by * for $P<.05$, ** for $P<.01$ and *** for $P<.001$.

RESULTS

Electrophoresis

Electrophoretic analysis of soluble enzymes in each species of shark began with the search for variable loci exhibiting sufficient genetic variation to be of use in detecting possible population differentiation. Initially, 76 enzyme stains were used on extracts from six tissues using up to 14 electrophoretic buffers. Those enzymes showing uninterpretable, weak or no staining for animals from a variety of collections were dropped from further analysis, together with those tissues (eye, kidney and blood) which did not contribute additional information. The optimum tissue/enzyme/buffer combinations were then used to score the genotypes of individuals. All monomorphic enzymes (Table 2) were scored for approximately 50 or more fish from each area (A-I) of Figure 1. Attempts were made to score all polymorphic enzyme loci (Table 3) for all individuals of a species. Examples of banding patterns and genotype scoring appear in Figure 2. Two enzymes, AH and EST-1, exhibited greatly reduced activity or partial breakdown in some collections, resulting in a relatively high proportion of unscorable individuals in these collections. When this occurred, there was a high risk of there being a differential probability of successfully scoring different phenotypes, thus biasing any allele frequencies calculated (see Shaklee 1983). In such cases in this study, all data for that locus for that particular collection were omitted from the statistical analyses.

Additional evidence of a genetic basis for the observed electrophoretic variation became available for some loci. Inheritance data from family groups (pregnant females plus litters of pups) gave clear support to the genetic

interpretation of the observed variation in the enzymes: creatine kinase (CK), phosphoglucomutase (PGM), umbelliferyl esterase (EST-D), $NADP^+$-dependent malate dehydrogenase (MDHp) and cytosolic aminopeptidase (CAPEP) (Lavery, unpublished data). Sufficient data were not available for the less polymorphic loci.

Polyacrylamide gel electrophoresis was used to search for polymorphic general proteins in all tissues. All proteins visualised with the non-specific protein stains used appeared as monomorphic bands, except for two proteins in heart tissue of C. sorrah. One slowly-migrating (anodal) protein exhibited a low level of tetrameric variation ($H < 0.01$) with 3 alleles, while a fast-migrating (anodal) protein exhibited a much higher level of di-allelic, monomeric variation ($H \sim 0.22$). Unfortunately the latter locus could not be resolved adequately in the majority of collections and had to be omitted from the calculations. No general protein loci were included in any statistical analyses.

The overall levels of genetic variation in each species are summarised in Table 4. For each collection and each area, allele frequencies were calculated for all polymorphic loci. Due to relatively small sample sizes for some collections, only those loci polymorphic at the 0.95 level (frequency of the most common allele less than 0.95) were included in the statistical analyses, and the allele frequencies for these loci appear in Tables 5 and 6.

Population Structure

C. tilstoni

Chi-square tests of conformity of observed genotype frequencies to those expected for Hardy-Weinberg equilibria were carried out for each locus in

each collection (Table 5). Significant results occurred for both Ah and Est-1 in two collections and for Ck-A in one collection. The 'significant' outcome for Ck-A was primarily due to a small expected frequency (<1). These significant results represented 7.5% of the 67 comparisons made, and were also reflected in the tests for each area.

Likelihood ratio (G) tests of allele frequencies were conducted to examine the pattern of variation within and among collections (Table 7). Comparisons of allele frequencies among collections were significant for Ck, Ah and over all loci. When this variation was partitioned into among-area and within-area components, only Ck showed overall significant heterogeneity among areas, while only Ah exhibited overall significant differences between collections within areas. Standardised statistics (X^2 divided by degrees of freedom) were calculated for these two components of genetic variation. Comparison of the values for among-area variation (1.51) and within-area variation (1.47) suggested that allele frequency differences among areas were not significantly greater. By studying the pattern of within-area heterogeneity it was seen that the Ah differences were restricted to the two collections from the Wessel Islands (area F), one inshore (#?) and one offshore (#?). One of these collections (#14) also had a significant deviation from Hardy-Weinberg equilibrium for Ah. No other tests, either overall or for individual loci, gave a significant result for this comparison between inshore and offshore locations. The two collections from Fog Bay (#4 and #5) had significant allele frequency differences for two loci, Ck and Pgm.

Heterogeneity among areas was examined in more detail by comparing adjacent, paired areas. This was done to identify the location of possible discontinuities in allele frequencies. Seven of these paired comparisons, from a total of 39 tests (18%), proved significant. The area contributing most to the allele frequency differences was the north-east Gulf of

Carpentaria (area H). This area was different from all adjacent areas (F, G and I) for Ck, as well as over all loci, and different from the Wessel Is collections for Fh. Other significant differences occurred between Fog Bay (area C) and Melville I.(area D) for Pgm, and Croker I. (area E) and Wessel Is (area F) for Ck. Comparison of Wessel Is (area F) and the southern Gulf (area G) proved significant for Ah, however this is again associated with collection #14 which was out of Hardy-Weinberg equilibrium. For all other loci in this comparison, chi-square values were very small and non-significant.

None of the five loci exhibited a significant linear trend (cline) of allele frequencies across areas (Table 7). There was also no significant correlation between the genetic distance and geographic distance separating areas.

In an attempt to isolate the observed frequency differences between areas into differences between larger regions, adjacent areas not exhibiting significant heterogeneity were pooled. This resulted in the following five regional groups: (B + C), (D + E), (F + G), (H) and (I) (refer to Figure 1). For this purpose, the difference at Ah between areas F and G was not considered. This grouping was supported by the clustering of areas based on their genetic distances averaged over all loci (Figure 3). The dendrogram also indicated that the north-east Gulf of Carpentaria (area H) was the most dissimilar area. Overall heterogeneity among the five regions was significant for Ck ($G=13.44^{**}$, 4df) and Pgm ($G=10.87^{*}$, 4df), and over all loci ($G= 40.35^{*}$, 24df), and was greatest between regions (B + C) and (D + E) (Pgm: $G=7.69^{**}$ 1df), and between (F + G) and (H) (Ck: $G=12.23^{***}$ 1df) (Figure 4).

Another method of analysing the variation in allele frequencies between collections is to employ Wright's F-statistics (Wright 1978, Nei 1977). The usual simplified formula for the fixation index is

$$F_{ST} = s^2 / p(1-p),$$

where s^2 is the estimated variance of allelic frequencies among areas and p is the frequency of the most common allele averaged over areas. This statistic estimates the degree of genetic differentiation between areas.

F_{ST} values across the eight areas were calculated for all loci (Table 7). Chi-square tests of their difference from zero give significant results for Ck and over all loci. These results are comparable with the results from the heterogeneity chi-square tests. However, this simple F_{ST} formula takes no account of either the sample sizes or the variation between collections within areas. It is inappropriate to allow for sample sizes simply by weighting means and variances, as such weighting is intended for variation in population sizes, which are rarely known for fish species (Nei 1977, Swofford and Selander 1981), as opposed to sample sizes. Both sampling variances and within-area variation can be taken into consideration by Wright's application of hierarchical F-statistics (Wright, 1978). This method gives a series of F-ratios which measure the proportion of the total genetic variation attributable to differentiation among the subpopulation units comprising each level of the hierarchy. The consideration of additional variance components in this analysis results in more conservative estimates of heterogeneity.

For the present analysis, collections were grouped into areas and then into regions in the same manner as in the contingency table analyses. The results indicated that very little of the genetic variance between collections in the total population ($F_{CT} = 0.006$) is due to between-area or between-region differences ($F_{AT} = 0.001$, $F_{RT} = 0.000$). Most of the variance between collections was due to differences within areas or regions ($F_{CA} = 0.005$, $F_{CR} = 0.006$).

These results can also be expressed in terms of Nei's gene diversity (Nei 1977, Swofford and Selander 1981). Using his terminology, approximately 99.4% of all genetic variation exists within collections, while only 0.6%

exists among collections, 0.1% among areas and a negligible proportion exists among regions.

Genetic variation was also examined for any effects of sex or age class. Tests were carried out for all collections which had sufficient data on individual lengths and sexes. Of 34 G-tests for allele frequency differences between sexes, only one was significant (Est-1 in collection #19, G=12.20***, 1df). A test between all males and all females (all collections pooled together) was not significant. Due to small sample sizes of measured individuals, the sharks were divided into only two age classes: mature and immature fish. Only two of 29 tests comparing these classes were significant. These were for Ah in collection #14 (G=7.08*, 2df) and Fh in collection #19 (G=6.25*, 1df). There was no difference between all immature and mature C. tilstoni.

C. sorrah

Tests of Hardy-Weinberg equilibrium were again calculated for each locus for each collection. Eleven tests (15%) gave significant deviations (Table 6). Genotype frequencies for two loci, Pdpep and Est-D, differed from the expected in only one collection each. However these tests had low expected frequencies (<1) or a low total number of individuals (N < 20). Capep gave significant results in the two collections (#4 and #5) within Fog Bay (area C). These departures from expectations were in opposite directions, however,indicating that they may simply be sampling artifacts. All other significant results occurred at Est-1, which had a deficiency of heterozygote genotypes for all but one of the 15 collections, with seven of these being significant. In all the Est-1 comparisons however, the expected frequency of the alternate homozygote class was less than one, which disproportionately

increased the chi-square values. Hardy-Weinberg tests were also performed on the pooled data for each area, where all genotype classes were sufficiently large. The only significant results here were for Est-1, in which there was a very highly significant heterozygote deficiency in areas B, D, and F.

Results of likelihood ratio tests of allele frequencies appear in Table 8. Among all collections there was no overall significant heterogeneity in frequencies. No significant differences were found between collections within each area. Included in this was a comparison between an offshore collection and two inshore collections in the Wessel Is area (area F).

When collections within areas were pooled, there was again no significant genetic heterogeneity observed among areas. Considering all adjacent pairwise comparisons of areas for all polymorphic loci, only one test out of 45 was significant. This was the comparison between Croker I. (area E) and Wessel Is (area F) for Pdpep. The North-West Shelf collection (area A) was relatively small (19 fish), and this may have substantially reduced the level of significance of any possible differences with adjacent areas. When this collection was pooled with the adjacent ones from the Timor Sea (area B), a significant difference was found between this combined area and Fog Bay (area C) for Est-D ($G = 5.15^{*}$ 1df). All tests for a cline (linear trend) in allele frequencies across areas proved non-significant. There was also no significant correlation between the genetic distance and geographic distance separating areas.

Pairwise genetic distances were used to cluster all areas into groups based on overall genetic similarity. The dendrogram (Figure 5) indicates that all but the most geographically remote areas (areas C to H) are genetically very similar to one another. Using these results, and the significant differences found between pairs of adjacent areas, the areas could be grouped into larger regions of genetic similarity (i.e., (A + B), (C + D + E), (F + G + H) and (I)) to determine if a large-scale pattern of heterogeneity exists.

However, contingency table analysis of these pooled frequencies revealed no significant differences, either overall or for individual loci.

F_{ST} values (using the usual simplified formula) were calculated for all loci and appear in Table 8. The values for Est-1 and Est-D were significantly different from zero. This is not in agreement with the contingency chi-square values, but these F_{ST} values take no account of sample size. As with C. tilstoni, a hierarchical analysis was performed to take into account sampling error and within-area variation. In the three level hierarchical analysis, collections were grouped into areas and then into regions, in the same manner as in the contingency table analyses. Similarly to those for C. tilstoni, the results for C. sorrah showed that most of the the genetic variance among collections (F_{CT} = 0.0030) occurred within areas (F_{CA} = 0.0028), rather than between areas or regions (F_{AT} = 0.0002, F_{RT} = 0.0001). Expressed in terms of Nei's genetic diversity, approximately 99.70% of all genetic variation existed within collections, and 0.28% among collections, while only 0.02% was accounted for by both among-area and among-region genetic diversity.

There were no differences in allele frequencies between male and female C. sorrah within any individual collection, or over the total population. Comparisons between age classes (immature and mature) showed a similar lack of distinction, with only one test significant (Capep in collection #10 : G = 6.55** 1df) out of a total of ?? tests.

DISCUSSION

Population Structure

Before dealing with the question of genetic heterogeneity among collections, the question of conformity of genotypes to Hardy-Weinberg

expectations must be addressed, for the validity of any interpretations of genetic variation are totally dependent on the appropriateness and accuracy of the genetic models. There must be confidence both in the assignment of a genetic basis to the observed phenotypic variation, and also in the assumption that the subpopulations under consideration are each behaving as randomly-mating groups with all alleles encountered following true Mendelian inheritance laws. These assumptions have traditionally been statistically analysed by chi-square goodness-of-fit tests of observed genotype frequencies to those expected under Hardy-Weinberg expectations. Apart from ensuring that the correct method of calculation is used (Pamilo and Varvio-Aho 1984, Crisp et al. 1978), it is necessary to remember that this chi-square test has a low power to detect departures from the expected, particularly when, as is the case in most studies, sample sizes are less than 200 (Fairbairn and Roff 1980, Valenzuela 1985). As suggested by Fairbairn and Roff, it is important to try to reduce the β error (the probability of accepting the null hypothesis of equilibrium, when in fact it is false). This has been attempted in this study by (1) using well-characterised protein systems with *a priori* genetic models, (2) independently testing Mendelian inheritance for the most polymorphic loci and (3) eliminating alternative hypotheses for phenotypic variation (e.g. relationships between isozyme phenotypes and age or sex, or secondary isozyme formation due to storage). Despite these precautions, and because of the low power of the test, it is unwise to ignore significant deviations which clearly require some explanation. Heterozygote deficiencies are often explained by hypothesising a Wahlund effect, where more than one genetically distinct stock may have been sampled in the one collection (e.g. Richardson 1982). However this could only account for a small degree of deviation from the expected, equal to F_{ST} (Johnson et al. 1986). The alternative possibility that such departures are associated with non-genetic variation is often not even considered.

In the present study, the number of significant Hardy-Weinberg tests warrants examination. Some of the significant results can undoubtedly be counted as statistical artifacts, compounded by a problem of relatively small expected frequencies for some genotypes. However, many of the significant deviations cannot be attribute to such obvious factors. Unfortunately, as a result, allele frequencies for those collections concerned should be treated cautiously as subpopulation estimates. In C. tilstoni, significant deviations occurred only in those enzymes subject to relatively rapid breakdown and loss of activity (AH and EST-1). Although all reasonable precautions were taken to prevent this from biasing allele frequency estimates, it is not at all inconceivable that an undetected degradation of particular phenotypes took place in some collections. For example, heterozygotes in general have weaker staining of each isozyme due to the fact that the enzyme activity is dispersed over a larger number of isozymes (allozymes). Thus, heterozygotes are more likely to be unscorable in samples having weak activity. In C. sorrah, the consistent trend of a deficit of heterozygotes at Est-1 needs to be seriously considered. As there were no apparent relationships between phenotype and obvious factors such as sex or age, the genetic interpretation of the isozyme banding patterns at this locus could be questioned. In any case, the above interpretations of significant Hardy-Weinberg deviations are at least as likely as explanations based on non-random mixing of totally unknown groups. Ultimately, this means that allele frequencies may be unreliable for the loci such as Est-1 in the particular collections concerned.

The potential to identify discrete populations or stocks within each of the two species was hampered by the small size of some collections. These smaller samples had the effect of increasing the inherent sampling error in allele frequencies and also, as a consequence, reducing the number of polymorphic loci that could be confidently used to only those polymorphic at

the 0.95 level. Relatively low levels of genetic variation exist in both species (*C. tilstoni*: H = 0.037; *C. sorrah*: H = 0.035), which ultimately means that only a few enzyme loci could be used to study population heterogeneity.

The degree of genetic differentiation among all collections was relatively small in both species. The results of comparisons of collections demonstrated a number of factors. Firstly, there was no obvious difference between the genetic composition of inshore and offshore sharks, at least in the Wessel Is area where this possibility was tested most rigorously. Secondly, there was no clear difference in allele frequencies between collections within any one area (except Fog Bay - see below). That is, within each area considered in this study, allele frequencies were relatively stable over time or exact location of sampling. Thirdly, there was some degree of genetic heterogeneity between areas or regions in *C. tilstoni*.

The locations of the most significant geographic discontinuities are shown in Figure 4. These could be interpreted as indicating the existence of genetically differentiated populations in the western and eastern sections of this species' range in Australian waters. The biological significance of these results is reduced, however, by other factors. It is unwise to infer overall genetic differentiation between areas based on only one or two loci from the species' entire genome. In this study, support from three or more of the polymorphic loci for any proposed stock boundaries would be far more conclusive. Further, when the level of genetic variance between individual collections *within* areas or regions is considered, it is found to be comparable to the level of variance *between* areas or regions. That is, the differences between areas are not greater than those within areas. The statistical significance of among-area comparisons is primarily due to the increased sample sizes. Furthermore, although the genetic distances calculated between areas give some indication of relative similarity, even

the greatest distances are small (D_{ROGERS} < 0.07), particularly considering that only polymorphic loci were included in these calculations.

From the statistical results, the genetic variance between collections appears to have only a small component due to geographic isolation. Another biological factor may be involved. These sharks have a tendency to be found in age- and sex-specific aggregations (Lyle and Timms 1984). This is perhaps reflected in a number of collections in this study which show a bias towards one sex or age class (Table 1). Although the observed genetic heterogeneity among collections might be attributed to genetic differences between sexes or age classes, the results of statistical tests of this hypothesis indicate that these are not explanatory factors.

An inconsistency in the results was the apparent difference between the two C. tilstoni collections from Fog Bay. Although not highly significant, this significant outcome was due to differences at two of the most reliable enzyme loci. The vast majority of both of these samples were collected from the same exact location, but the collections were separated in time (Sept., 1984 vs. June, 1985).

It may be possible that these collections sampled two different stocks utilising the same area at different times as has been reported for other fish species such as skipjack tuna (Richardson 1983). There is some evidence that the Fog Bay area may act as a nursery ground for C. tilstoni (J. Lyle, Northern Territory Fishery Service, Darwin, Northern Territory and J. Stevens, CSIRO Division of Fisheries Research, Hobart, Tasmania: personal communication). Although pups may be found throughout the species' range at the appropriate time of year (January onwards), there is a distinct concentration of young sharks in Fog Bay. This is reflected by the relatively small average size of C. tilstoni in the two collections from Fog Bay (Table 1). However, no purely age-class effect on allele frequencies was shown to exist although we tested for this as explained above. In the vicinity of Fog

Bay some degree of geographic discontinuity of allele frequencies was indicated by Pgm (Figure 4). It is possible that if such a boundary truly exists in this vicinity, then pregnant sharks from both sides may be migrating to Fog Bay at the time of parturition, attracted by its apparent suitability as a nursery ground. The sharks could maintain any possible genetic isolation either by temporal segregation (there is some evidence that Western Australian C. tilstoni have a breeding time slightly earlier in the year than more easterly individuals - J. Stevens, personal communication) or simply by a movement back to their parents' waters prior to the attainment of sexual maturity. The possibility of such an occurrence could be taken into consideration in the analyses by not pooling the two Fog Bay collections. When this is done, there is an increased level of overall differentiation between areas and regions as expected, particularly for Ck and Pgm. Collection 5 is more similar to the 'western region' of Figure 4, while collection 4 is more similar to the 'central region'. These results could be summarised by the F-statistics where for Ck, F_{RT} = 0.0059 (compared to 0.0029 in the previous analysis) and for Pgm, F_{RT} = 0.0035 (compared to 0.0001). However it is clear that although the above proposal may explain a greater proportion of the genetic variation between collections, the overall heterogeneity is still low.

In general, we found no compelling reason, based on our electrophoretic studies, to conclude that more than one stock exists within Australian waters for either species of shark. Of course, a lack of detectable genetic differences between areas can not prove that population subdivision does not occur, however the rigorousness of the present analysis suggests that significant stock differentiation is unlikely.

Evidently, sufficient interbreeding and/or mixing among the populations seems to be occurring to provide gene flow between relatively widely separated areas. Estimates of migration can be calculated from the values of

F_{ST}, the degree of subpopulation differentiation. The distribution of sharks along the northern Australian coast is expected to resemble the linear stepping-stone model of migration (Kimura and Weiss 1964) most closely. This model states that

$$F_{ST} = 1/(4N(2mu)^{1/2}+1)$$

where N is the effective subpopulation size, m is the proportion of fish migrating into each subpopulation each generation and u is the electrophoretic mutation rate (10^{-7}). Assumptions of the model include those of equal subpopulation size, the restriction of migration to adjacent areas and the equilibrium of migration and genetic drift (Hartl 1980). The subpopulation sizes in each area can be estimated at $1*10^6$ to $1*10^7$ for C. tilstoni and, proportionally, $7*10^5$ to $7*10^6$ for C. sorrah (based on previous total catch weights, average individual weights and estimates of exploitation rates). Using the previously determined values of F_{ST} gives estimates of migrants per generation of between 3500 and 350 for C. tilstoni and between 7500 and 750 for C. sorrah.

Although many assumptions and estimates are involved, it is clear that the observed level of genetic heterogeneity does allow for considerable movement of individuals between areas. This is in contrast with many other marine species in which low levels of heterogeneity can be accounted for by the movement of only a few individuals (e.g. Grant 1985, Milton and Shaklee in prep.). The difference is primarily due to the much smaller effective population sizes of the sharks relative to the other species of fishes studied.

Preliminary results of tag-recapture data lend support to the findings of this study (J. Stevens, personal communication). The results indicate that many individuals of both species move hundreds of kilometres (up to 1000 km) between captures. The fact that C. sorrah appear to move longer distances, on average, than C. tilstoni may explain the lower level of genetic

heterogeneity found in C. sorrah and is consistent with the larger migration estimates obtained above. Although evidence for such large movements may appear to preclude any stock differentiation, this need not necessarily be the case. Despite evidence of long distance movements and substantial exchange among subpopulations, studies of skipjack tuna have indicated the presence of significant stock heterogeneity (Richardson 1983 and references cited therein).

It is of considerable interest to compare the presumed population structure found in these sharks with the patterns of population differentiation that have been reported for various species of teleost fishes. The degree of population subdivision in any one species appears to be strongly related to its habitat and breeding patterns (Gyllensten 1985) with considerable variation occurring even within taxonomically related groups (e.g. salmonids, Ryman 1983). Many freshwater fishes exhibit highly differentiated populations (e.g. brown trout - Crozier and Ferguson 1986), most anadromous species studied have many distinct stocks (e.g. salmonids - Beacham et al. 1985) and catadromous species may also possess considerable heterogeneity (e.g. barramundi - Shaklee and Salini 1985). However many marine species have been shown to have very low levels of differentiation, even over great oceanic distances, with relatively indistinct genetic subpopulations (e.g. blue grenadier - Milton and Shaklee 1986, cod - Mork et al. 1985, orange roughy - Smith 1986, anchovy - Grant 1985, halibut - Haug and Fevolden 1986) or a confusing pattern of genetic relationships (e.g. herring - Anderson et al. 1981, Isles and Sinclair 1982, Grant 1984). In marine species in general, therefore, population subdivision is less distinct. This is perhaps as expected considering the relative lack of physical barriers to movement, and hence gene flow in the marine environment.

With this perspective, the lack of distinct population subdivision of the two tropical sharks observed in this study is not too surprising,

especially considering that the geographic range under consideration is not great. Although there has been no previous large-scale study of the population genetics of sharks, their possible population structuring has been hypothesized to be similar to that of tuna, on the basis of body size and mobility (Kirpichnikov 1981). Pacific skipjack tuna have been the most intensively studied species of tuna (see Shaklee 1983 for a summary). A degree of population heterogeneity exists in this highly mobile species, but a variety of models have been used to explain the pattern of genetic heterogeneity observed. Perhaps the most successful to date is that of Richardson (1983) who proposed an isolation-by-distance model, with the entire population comprising a number of genetically distinct 'neighbourhoods' up to 2000 km in diameter. There is, however, little evidence to support such a population structure in the two species of sharks under study. Richardson's model was based on an obvious cline in allele frequencies from west to east across the Pacific. No such cline is apparent for either species of shark, and no significant correlation exists between genetic distance and geographic distance. It would be interesting to estimate the possible size of a 'genetic neighbourhood' for the sharks, but sufficient movement data are not available. C. sorrah is dispersed over a wide range in the Indian and western Pacific Oceans, and it is likely that C. tilstoni could be as well. It may, therefore, prove valuable to look at the population genetics of these species over a much wider geographic area.

The genetic evidence we have collected leads us to conclude that C. tilstoni and C. sorrah could each be managed as single stocks within Australian waters. Our data show no compelling need for distinct management policies within each State or Territory. Furthermore, the data suggest that any impact on the fishery in offshore grounds would also be felt in inshore areas and vice-versa. However, as the unit stocks have such a large geographic size, they may be well buffered to disturbance or perturbation.

If, in the future, this tropical shark fishery exerts a level of fishing pressure similar to that on the southern Australian shark fishery, the total population size rather than the local population size is likely to be the limiting factor.

Table 1. Collection Details

Area	Collection number	Date	*C. tilstoni* Number of individuals	*C. tilstoni* Sex ratio[A] (m:f)	*C. tilstoni* Mean fork length[A] (cm)	*C. sorrah* Number of individuals	*C. sorrah* Sex ratio[A] (m:f)	*C. sorrah* Mean fork length[A] (cm)
A. North-West Shelf	1	Oct 83	0	-	-	19	2:17	91.2
B. Timor Sea	2	May 84	51	41:15	85.1	16	15: 1	72.3
	3	Sep 84	0	-	-	41	22:17	72.9
C. Fog Bay	4	Sep 84	41	20:16	64.6	43	22:18	72.5
	5	Jun 85	72	12:14	57.6	72	12: 4	65.2
D. Melville Is	6	Oct 83	0	-	-	26	10:15	77.5
	7	Apr 84	101	62:34	81.8	39	21:18	78.2
E. Croker Is	8	Aug 83	19	4:15	95.0	0	-	-
	9	Jan 84	107	29:28	90.7	66	22:26	77.7
	10	Mar 84	0	-	-	48	27:20	72.1
F. Wessel Is (offshore)	11	Nov 82	76	35:29	109.6	32	14:15	73.0
	12	Feb 85	70	58:12	78.0	0	-	-
(inshore)	13	Feb 84	0	-	-	50	5: 8	79.8
	14	Mar 84	100	59:40	86.5	53	33:14	75.0
G. Southern Gulf	15	Jun 84	32	18:14	87.6	0	-	-
	16	Jul 84	64	31:25	82.9	56	11:10	76.7
H. Eastern Gulf	17	Jul 84	54	37:11	81.0	39	19:16	80.0
I. North-eastern Queensland	18	Apr 85	22	11:11	90.6	0	-	-
	19	Apr 85	116	55:59	84.9	55	21:16	80.3

[A] Data on sex and length were not available for all individuals.

Table 2. Monomorphic[A] Loci

Enzyme (EC number)	Enzyme (EC number)
Aspartate aminotransferase-2 (2.6.1.1)	
N-Acetyl-β-glucosaminidase (3.2.1.30)	Hydroxyacylglutathione hydrolase (3.1.2.6)
Acid phosphatase (3.1.3.2)	L-Iditol dehydrogenase (1.1.1.14)
Alcohol dehydrogenase (1.1.1.1)	Isocitrate dehydrogenase (1.1.1.42)
Adenylate kinase (2.7.4.3)	L-Lactate dehydrogenase (1.1.1.27) (1. 1.1.37)
Alanine aminotransferase (2.6.1.2)	Lactoylglutathione lyase (4.4.1.5)
Creatine kinase - C (2.7.3.2)	Malate dehydrogenase -2[T] and -3[S] (1.1.1.37)
Cytosolic aminopeptidase (3.4.11.1) [T]	Mannose-6-phosphate isomerase (5.3.1.8)
"Diaphorase" (1.6.-.-)	α-Mannosidase (3.2.1.24)
Enolase (4.2.1.11)	Octanol dehydrogenase (1.1.1.73) [S]
Esterase-2 and -4 (3.1.1.-)	Peptidase-C (substrate?) (3.4.-.-)
Fructose-bisphosphate aldolase (4.1.2.13)	Peptidase-E (substrate?) (3.4.-.-)
Fumarate hydratase (4.2.1.2) [S]	Phosphoglycerate kinase (2.7.2.3)
β-Galactosidase (3.2.1.23)	Phosphoglucomutase (5.4.2.2) [S]
Glutamate dehydrogenase (1.4.1.-)	Pyruvate kinase (2.7.1.40)
Glyceraldehyde-3-phosphate dehydrogenase -1 and -2 (1.2.1.12)	Purine-nucleoside phosphorylase (2.4.2.1)
	Superoxide dismutase (1.15.1.1)
Glycerol-3-phosphate dehydrogenase (1.1.1.8)	Tripeptide aminopeptidase (3.4.11.4)
	Xanthine dehydrogenase (1.2.1.37)

[A] Only one allele or frequency of most common allele > 0.99.
[T] Monomorphic only in *C. tilstoni*.
[S] Monomorphic only in *C. sorrah*.

Table 3. Characteristics and conditions for analysis of polymorphic[A] enzymes

Enzyme (EC number)	Locus	Subunit structure	Tissue	Buffer[B]
Polymorphic in both species:				
Aconitate hydratase (4.2.1.3)	Ah	monomer	liver	CAAPM
Creatine kinase (2.7.3.2)	Ck-A[D]	dimer	muscle	CAAPM
Esterase (3.1.1.-)	Est-1	monomer	liver	LiOH
Esterase-D (3.1.1.-)	Est-D	dimer	muscle	EBT
Glucose-6-phosphate isomerase (5.3.1.9)	Gpi	dimer	muscle	CAAPM
Malate dehydrogenase ($NADP^+$) (1.1.1.40)	MdhP-2	tetramer	muscle	TRIC
Proline dipeptidase (3.4.13.9)	Pdpep[C]	dimer	muscle	TRIC
Polymorphic in *C. tilstoni* only:				
Fumarate hydratase (4.2.1.2)	Fh	tetramer	liver	TRIC
Malate dehydrogenase-3 (1.1.1.37)	Mdh-3	dimer	muscle	TRIC
Octanol dehydrogenase (1.1.1.73)	Odh	dimer	liver	Poulik
Phosphoglucomutase (5.4.2.2)	Pgm	monomer	muscle	CAAPM
Polymorphic in *C. sorrah* only:				
Cytosolic aminopeptidase (3.4.11.1)	Capep[C]	hexamer	liver	LiOH
Malate dehydrogenase-2 (1.1.1.37)	Mdh-2	dimer	muscle	TRIC

[A] Frequency of most common allele < 0.99.

[B] CAAPM: citric acid - aminopropylmorpholine pH 6.0 (Clayton and Tretiak 1972)
LiOH: lithium hydroxide - boric acid: modified buffer 2 of Selander et al. (1971)
EBT: EDTA - boric acid - Tris pH 8.6 (Boyer et al. 1963)
TRIC: triethanolamine - citric acid pH 7.2 (Clayton and Tretiak 1972)
Poulik: sodium hydroxide - boric acid pH 8.7: buffer 3 of Selander et al. (1971)
(detailed buffer recipes can be found in Shaklee and Keenan 1986)

[C] Presumed homology with peptidase loci of other fishes and vertebrates based on substrate specificity (Frick 1983). Substrates employed: PDPEP - leucyl-proline. Capep - leucyl-tyrosine.

[D] Presumed homology with Ck-A locus of other vertebrates based on relative mobility and tissue specificity (Fisher and Whitt 1978).

Table 4 Summary of observed genetic variation

	C. tilstoni	C. sorrah
Number of Polymorphic Loci (0.95 level)	5	5
Number of Polymorphic Loci (0.99 level)	11	9
Number of Monomorphic Loci	36	38
Total number of loci scored	76	76
Mean heterozygosity (H)	.037	.035

Table 5. C. tilstoni allele frequencies for five polymorphic loci.

Area/ Collection		N	Locus and alleles[A] Ah 100	Ah 120+	Ah 79+	Ck-A 100	Est-1 100	Fh 100	Pgm 100
B	2	51	-	-	-	.957	.915	.692	.897
C		113	.505	.240	.255	.916	.886	.641	.907
	4	41	.536	.214	.250	.866	.882	.632	.963
	5	72	.493	.250	.257	.944	.889	.646	.875
D	7	101	.458	.239	.303	.922(*-)[B]	.851(*-)	.628	.960
E		126	.504	.230	.266	.921	.872	.665	.948
	8	19	.500	.263	.237	.921	.789	.735	.895
	9	107	.505	.223	.272	.921	.887	.654	.958
F		246	.477	.182	.341(*-)	.957	.856(*-)	.645	.919
	11	76	.526	.026	.447	.941	.950	.625	.936
	12	70	-	-	-	.964	.850	.643	.921
	14	100	.456	.228	.316(*-)	.965	.843(***-)	.649	.908
G		96	.570	.273	.156	.947	.856	.633	.926
	15	32	-	-	-	.906	.828	.556	.922
	16	64	.570	.273	.156	.968	.871	.667	.929
H	17	54	.510	.240	.250	.870	.806	.532	.926
I		138	.465	.213	.322(**+)	.942	.873	.622	.894
	18	22	.333	.310	.357	.955	.955	.591	.909
	19	116	.491	.194	.315(**+)	.939	.858	.628	.891

[A] Alleles are identified by the relative electrophoretic mobilities of the homomeric isozymes they encode compared to that of the most common allele (100) at each locus. Rare alleles were pooled with alleles of closest relative mobility. For all loci except Ah, there were two allele classes. Only the frequency of most common allele class (100) is given for these loci. The following are the allelic compositions of the allele classes for each locus.

Ah: 100; 120+ = 120,142,168; 79+ = 79,47
Ck-A: 100; other = 180,400
Est-1: 100; other = 105
Fh: 100; other = 80
Pgm: 100; other = 180

[B] Asterisks indicate the degree of deviation of genotype frequencies from Hardy-Weinberg expectations within that collection (chi-square probability: * = P < .05; ** = P < .01; *** = P < .001). A '+' indicates a heterozygote excess, a '-', a heterozygote deficiency.

Table 6. C. sorrah allele frequencies

Area/ Collection	N	Locus and alleles[A] Capep 100+	Est-1 100	Est-D 100+	MdhP-2 100+	Pdpep 100+
A 1	19	.711	.789	.474(*+)[B]	.868	.895
B	57	.705	.892(***-)	.509	.851	.918(*-)
2	16	.733	1.000	.594	.781	.967
3	41	.695	.859(**-)	.476	.878	.900(*-)
C	115	.670	.915	.617	.826	.861
4	43	.663(*-)	.927(*-)	.616	.837	.884
5	72	.674(*+)	.908	.618	.820	.847
D	65	.715	.897(***-)	.600	.846	.885
6	26	.769	.942(***-)	.635	.884	.846
7	39	.679	.865(*-)	.577	.821	.910
E	114	.693	.923	.544	.864	.889
9	66	.705	.923	.568	.849	.908
10	48	.677	-	.510	.885	.865
F	135	.692	.891(***-)	.559	.860	.940
11	32	.617	.941	.594	.891	.969
13	50	.700	.870(**-)	.540	.850	.959
14	53	.726	.896(***-)	.557	.849	.906
G 16	56	.727	.861(***-)	.618	.873	.900
H 17	39	.713	.863	.610	.914	.878
I 19	55	.657	.843	.491	.855	.882

[A] Refer to note [A] in Table 5. Allele classes:
Capep: 100+ = 100.112: other = 67
Est-1: 100: other = 108
Est-D: 100+ = 100.72: other = 125.115
MdhP-2: 100+ = 100.83: other = 141.118
Pdpep: 100+ = 100.80: other = 117.157.145.122.108

[B] Refer to note [B] in Table 5.

Table 7. C. tilstoni: Tests of allele frequency heterogeneity.

	Ah		Ck-A		Est-1		Fh		Pgm		Total	
	df	G	df	G	df	G	df	G	df	G	df	G
A. G-statistics:												
Among areas	12	19.76	7	14.69*	7	7.02	7	6.67	7	12.06	40	60.20*
B-C	-	-	1	2.13	1	0.59	1	0.68	1	0.10	4	3.50
C-D	2	1.07	1	0.05	1	1.13	1	0.07	1	5.00*	6	7.32
D-E	2	0.86	1	0.00	1	0.39	1	0.64	1	0.39	6	2.28
E-F	2	3.16	1	4.05*	1	0.30	1	0.27	1	2.14	6	9.92
F-G	2	14.64**	1	0.29	1	0.00	1	0.06	1	0.11	6	14.70*
F-H	2	3.03	1	9.90**	1	1.58	1	3.91*	1	0.06	6	18.48*
G-H	2	3.36	1	5.37*	1	1.28	1	2.63	1	0.00	6	12.64*
H-I	2	1.86	1	4.98*	1	2.72	1	2.35	1	0.89	6	12.80*
Within Areas	8	15.63*	6	8.72	6	10.88	6	3.22	6	8.58	32	47.03*
C	2	0.37	1	4.01*	1	0.03	1	0.04	1	5.53*	6	9.98
E	2	0.37	1	0.00	1	2.44	1	0.90	1	2.13	6	5.84
F	2	9.10*	2	1.39	2	3.97	2	0.07	2	0.78	10	17.33
G	-	-	1	3.06	1	0.62	1	1.98	1	0.03	4	5.69
I	2	4.20	1	0.17	1	3.80	1	0.22	1	0.13	6	8.35
Total (among all collections)	20	35.39*	13	23.41*	13	17.90	13	9.89	13	20.64	72	107.23**
B. Test for linear trend among areas:	1	0.07	1	0.07	1	1.55	1	1.89	1	0.80	5	4.38
C. F_{ST} among areas:	6	0.008	7	0.011**	7	0.007	7	0.008	7	0.007	34	.0094**

Table 8. C. sorrah: Tests of allele frequency heterogeneity

	df	Capep G	Est-1 G	Est-D G	MdhP-2 G	Pdpep G	Total df	Total G
A. G statistics:								
Among areas	8	2.43	10.17	10.81	6.01	10.46	40	39.88
A-B	1	0.00	2.31	0.14	0.07	0.19	5	2.71
B-C	1	0.44	0.43	3.68	0.34	2.45	5	7.34
C-D	1	0.81	0.32	0.11	0.24	0.41	5	1.89
D-E	1	0.20	0.60	1.06	0.22	0.02	5	2.10
E-F	1	0.00	1.08	0.12	0.02	4.17*	5	5.39
F-G	1	0.47	0.63	1.12	0.12	1.80	5	4.14
F-H	1	0.13	0.46	0.66	1.87	1.44	5	4.56
G-H	1	0.05	0.00	0.01	0.85	0.23	5	1.14
H-I	1	0.64	0.14	2.69	1.66	0.01	5	5.14
Within areas	6	3.80	9.98	2.94	3.41	8.14	30	28.27
B	1	0.15	6.30*	1.29	1.60	1.52	5	10.86
C	1	0.03	0.23	0.00	0.12	0.61	5	0.99
D	1	1.25	2.11	0.43	1.01	1.23	5	6.03
E	1	0.20	0.32	0.75	0.66	1.03	5	2.96
F	2	2.17	1.49	0.46	0.36	3.75	10	8.23
Total (among all collections)	14	6.23	20.15	13.75	9.42	18.60	70	68.15
B. Test for linear trend among areas:								
	1	0.01	1.63	0.04	2.90	0.59	5	5.17
C. F_{ST} among areas:								
	8	0.002	0.014*	0.012*	0.005	0.006	40	0.0076