

# Stock structure across Northern Australia

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## **Abstract**

*A comprehensive analysis of allozyme frequency data for barramundi found significant stock structure throughout the species' range in Australian waters. Subsequent research refined some of these stock boundaries, in one case narrowing the boundary to a 50km stretch of coastline. In general, the results illustrate the genetic imprint left by momentous zoogeographic changes across northern Australia (the Top End), particularly changes in sea level over the past 135 000 years. The influence of these sea level changes on the genetic structure of marine populations has produced significant population subdivision across the Torres Straits for almost all species examined, including three small mackerel species, six estuarine species, several species of prawns, saucer scallops, turtles and mud crabs. Also, the sea level changes combined with the formation of Lake Carpentaria and a change in the course of the Fly River, explain the present day distribution of many freshwater species across the Top End and into New Guinea.*

*Recently, mtDNA sequencing techniques have been employed to re-examine stock structure of barramundi. These studies have reached basically the same conclusions, but highlight the extremely sensitive qualities of the technique, which may provide information superior to allozyme data, on current rates of migration between populations. To fully gain benefits from genetic stock structure analysis,*

*the models employed for the estimation of important stock characteristics such as migration rate and effective (breeding) population size need to be more fully developed. The dogma associated with the "island model" has impeded the development of more sophisticated models that may provide basic and important information, such as changes in breeding stock size and accurate estimates of migration rates between stocks, for fisheries management.*

## **Introduction**

The collection of information on the structure of fish stocks is an integral component in the implementation of successful management methods to maintain fisheries at sustainable levels. With little knowledge of the stock structure, overfishing can decimate isolated breeding populations to a level where recruitment cannot sustain the harvest, and the isolated populations which contribute to the total harvest can collapse, as occurred in the 1970s with the North Atlantic herring *Clupea harengus*. It is now generally agreed that allozyme electrophoresis is still the most appropriate method for looking at population structure, "... for reasons including cost effectiveness, ease of application, and necessary sample sizes, applying (other molecular) procedures should generally follow only when protein

electrophoresis cannot adequately resolve or identify differences among groups..." (Utter and Ryman 1993).

Allozyme electrophoretic-derived genetic information available on barramundi (*Lates calcarifer*) from the northern and east coasts of Australia has demonstrated that there is structuring of populations across the area that necessitates population-based management of the fishery (Shaklee and Salini 1985; Salini and Shaklee 1988; Shaklee *et al.* 1993; Keenan 1994). Following the research of Keenan (1994), new collections of barramundi were made to refine two stock boundaries, and molecular methods have been employed to examine his 1994 hypotheses (Chenoweth *et al.* in press a, b; Doupé and Lymbery submitted). Additional studies on barramundi have examined the possibility of marine only stocks that have not used freshwater habitat for part of their life cycle (Pender and Griffin 1996).

Apart from barramundi, the population structure of many other marine species from tropical Australia have been examined. There have been studies of nine fish species, including a recent study of three small mackerel species — school, spotted and grey mackerel (Fisheries Research Development Corporation (FRDC) 92/144: Fisheries biology and interaction in the Northern Australia Small Mackerel Fishery), Spanish mackerel (Shaklee *et al.* 1990), a recent study of four estuarine species (FRDC 92/145: Biology and harvest of tropical fishes in the Queensland Gulf of Carpentaria gillnet fishery), and a Lutjanid species (Elliot 1996); four crustacean species, including the mud crab *Scylla serrata* (Keenan *et al.* 1995), and three penaeid prawns — *P. esculentus* and *P. merguensis* (FRDC 94/165: DNA markers and genetic stock structure in commercial species of penaeid prawns in the east coast

fishery), and *P. monodon* (Benzie *et al.* 1992); several mollusc species: *Amusium japonicum balloti* — saucer scallops (Dredge *et al.* in prep.), and *Pinctada maxima* — pearl oysters (Johnson and Joll 1993); and a reptile species, the green turtle (Norman *et al.* 1994). Genetic differences between populations of all these species show distinct degrees of isolation between eastern (Pacific Ocean) and western (Arafura Sea) populations across the Torres Strait.

## Results

### *Barramundi stock structure in Australia*

Figure 1 presents a summary of the present knowledge of the stock structure of barramundi in Australian waters. Since the publication of Keenan (1994), additional samples of barramundi were collected from Cooktown on the east coast and an unsampled region of western Cape York. In the western Cape York area between the Staaten River and Weipa, more than 50% of the total genetic diversity of the species in Australia was attributed to the difference between two extensive populations, (i) SE Gulf and (ii) NW Cape York (Keenan 1994). Analysis of the new, closely-spaced samples allowed the extent of this major stock boundary to be reduced and the significant difference between adjacent stocks was clearly mapped to just a 50 km wide geographic feature, Pera Head, just south of Weipa. This prominent stock restriction, basically between the Coral Sea and Arafura Sea populations had significant differences at six of thirteen loci examined and must be attributed to a continued discontinuity in migration (of larvae, juveniles and/or adults that survive to contribute to the breeding population) around this barrier. Statistical details of these results are given in Table 1.

The general stock structure within the Gulf of Carpentaria was formed recently during major migrations of estuarine species into the Gulf of Carpentaria which started only 12 000 years ago (Keenan 1994), after the end of the last ice age during the seawater flooding of the area. Keenan also proposed the recent formation of an hybrid zone of barramundi between east coast and western-derived populations in this region, caused by migrations following the most recent opening of the Torres Strait.

### Other fish species

Apart from several published studies of marine fish population structure across northern Australia (Shaklee *et al.* 1990; Elliot 1996) there have been two FRDC-funded studies that in total have examined seven additional fish species. Detailed results from these studies will eventually be published in full as FRDC reports, and aspects are being prepared for publication elsewhere. Basically, all seven species examined show significant differences between populations separated by the Torres Strait. Unweighted pair-group [clustering] method using arithmetic averages (UPGMA — Sneath and Sokal 1973) of Rogers' genetic distance for the three small mackerel species examined by Cameron *et al.* (1997) shows significant differences between populations separated by the Torres Strait for; school (Figure 2), spotted (Figure 3) and grey mackerel (Figure 4). The apparent clustering of the Darwin and Hervey Bay samples in the dendrogram for the grey mackerel (Figure 4) is an anomaly caused by low sample sizes and is not statistically significant. The comparison between the east coast (Mackay, Townsville) and Arafura Sea (Gulf, Gove) samples is statistically significant ( $G=19.14$ ,  $df=8$ ,  $p<0.05$ ).

Similarly a genetic study of four estuarine species (Garrett *et al.* 1997) from the eastern

and western coasts of Cape York showed significant genetic differences across the Torres Strait; black jew, *Protonibea diacanthus* (Figure 5), blue salmon, *Eleutheronema tetradactylum* (Figure 6), golden grunter, *Pomadasys kaakan* (Figure 7) and jewel fish, *Nibea squamosa* (Figure 8). There are some anomalies in the relationships between populations of blue salmon, which may result from stream capture events, that deserve further investigation.

### Crustacean species

There is evidence of population subdivision across the Torres Strait in several crustacean species. Benzie *et al.* (1992; 1993) found that a Western Australian population of *P. monodon* was significantly different from populations from the east coast. Similarly, *P. esculentus* and *P. merguensis* show distinct genetic differences, based on mitochondrial DNA haplotypes (Lavery and Keenan 1995; Lavery 1998). Preliminary work on the mud crab, *Scylla serrata*, shows evidence of distinct populations in the Arafura Sea and from the Pacific Ocean (Keenan *et al.* 1995). There is approximately 2% sequence difference between the samples examined to date (Figure 9). Additional research has substantiated this difference for a much larger sample size (D. Gopurenko pers. comm.)

### Mollusc species

The genetic structure of two mollusc species has been examined across the Top End. For the subtropical saucer scallop, *Amusium japonicum balloti*, Dredge *et al.* (in prep.) found that the Western Australian population was so differentiated from its east coast congeners that the two populations presumably represented different species. Johnson and Joll (1993) found significant population structure across the Top End between a north-west Australian, two Northern Territory and a

Torres Strait population of the tropical pearl oyster, *Pinctada maxima*.

### Reptiles

Four green turtle populations, two from the Arafura Sea and two from the east coast were examined for mtDNA haplotype variation (Norman *et al.* 1994). Pairwise percentage sequence variation was higher than for mud crabs from the same region, up to 6.98%. Three common haplotypes and several additional variants were observed. Haplotypes A and B were found in different frequencies from the southern and northern Great Barrier Reef (east coast) and haplotype C was found in the Gulf of Carpentaria and Western Australian populations (Arafura Sea and Indian Ocean). This strong regional differentiation, primarily across the Torres Straits, parallels the variation found in the other species discussed above.

### Discussion

Molecular genetic techniques have developed rapidly over the past two decades. The study of natural genetic variation in almost any species can now find markers that can easily identify individuals and their offspring and therefore can show very significant, fixed differences between families, demes and farther up the scale, populations. This is apparent in barramundi, comparing the results of Keenan (1994) and Chenoweth *et al.* (in press a), where statistically significant population boundaries were more apparent from smaller sample sizes using mtDNA-based data. Such sensitivity results from the discovery of highly variable regions of the genome, both in the female inherited mitochondria and within the cytoplasmic diploid genome. We are therefore in a strong position to use a wide variety of genetic tools to

illustrate the population structure of our fisheries resources.

In terms of understanding population structure, it is necessary to relate the genetic data that can be collected, with a model that we can use to interpret the data. Genetic population models are, at first glance, deceptively simple being based on three basic parameters:  $N_e$  — the effective population size;  $m$  — the migration rate between populations; and  $\mu$  — the mutation rate. Two of these parameters,  $N_e$  and  $m$  are fundamental to understanding very important characteristics of wild fished populations and are basically tools for the management of these fisheries.  $N_e$  — the effective population size (i.e. the number of spawners successfully contributing to the next generation), is a crucial parameter in understanding stock-recruitment relationships (Lavery and Keenan 1995) and  $m$  — the migration rate, can be used to estimate the average, long-term rates of migration between stocks.

### *The significance of stock structure across the Top End*

The stock structure apparent between the Coral and Arafura Seas, for a large number of marine species, is a result of the accumulation of genetic changes or mutations, over time. The mutation rate is different for different types of genetic data, and is quite difficult to estimate accurately. Mutations record changes over time in a variety of ways. They can be detected as gene frequency changes, levels of heterozygosity (genetic variation), and changes in gene sequences through which family history can be derived using parsimony analysis. These genetic changes reflect changes in zoogeography, with the level of mutations and genetic differentiation increasing in proportion to the period of isolation between populations.

There are several examples of major zoogeographic changes from which molecular evolutionary rates can be inferred. One of the most studied is the population differentiation caused by the rise of the Panamanian Isthmus between 2.9 and 3.5 million years ago (reviewed by Bermingham *et al.* 1997). Other, more recent, examples derive from the zoogeographic change in population structure resulting from the glaciation events of the Tertiary: between the Gulf of Mexico and Atlantic Oceans (Avice 1992); and between the Black and Mediterranean Seas (Magoulas *et al.* 1996). The isolation between the Coral and Arafura Seas, across the Torres Strait, discussed above, provides an even more recent, and possibly better documented, model to study. Because of the recent history of the isolation and rejoining of waters across the Torres Strait it is more relevant to the understanding of population (stock) structure than the example of the Panamanian Isthmus, which is more relevant to allopatric speciation models.

These examples can be viewed as natural experiments in genetics, in that they provide an opportunity to investigate the processes of population and molecular evolution across a variety of species. Accurate timing of the isolation events can be used to determine rates of molecular evolution, which is an important parameter in appropriate population models. Given accurate estimates of molecular evolution  $\mu$ , the other two important parameters of genetic models (see above) can be calculated.

### Genetic models

The most widely used genetic model to estimate migration rate is the "island model" of Wright (1943). It is mathematically simple but not biologically realistic (Wright 1943; Keenan 1994). It has become acceptable

through familiarity but when applied to genetics data, provides unrealistically low estimates of migration. These estimates are often questioned by non-geneticists because they are easily refutable. The dogma associated with the "island model" has invaded text books and even the definition of 'sub-populations' in IUCN guidelines (Mace and Stuart 1994). More realistic, one and two dimensional migration models are mathematically "intractable" but have been derived independently by many authors, including Wright himself. Additional understanding and development of better population genetic models will strengthen the role of genetics research in stock-based fisheries management.

### Acknowledgements

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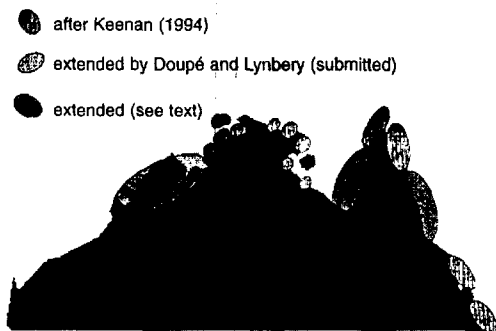
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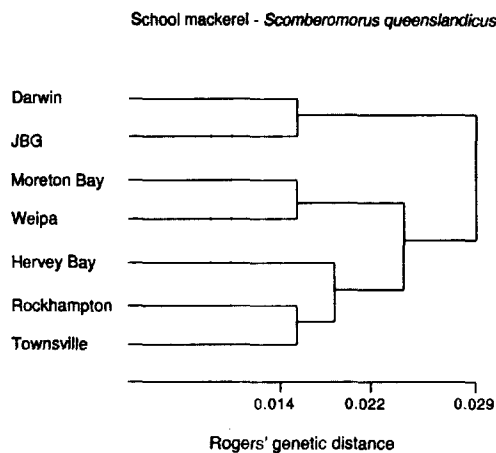
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**Table 1.** Summary of statistical tests for barramundi samples from the western Cape York region of the Gulf of Carpentaria.

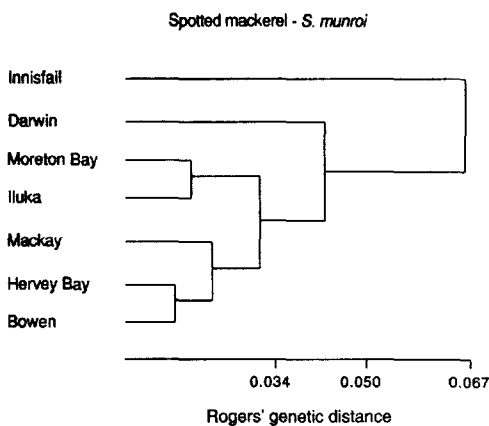
<b>Overall heterogeneity</b>	<b>N</b>	<b>Location</b>	<b>Partitioned heterogeneity within populations</b>
Total G = 353.365 df 234	406	Norman River	<u>SE GULF</u> G = 138.089, df 150, n.s. 1 of 13 loci significant
	238	Staaten River	
	114	Nassau River	
	84	Mitchell + Coleman Rivers	
	18	Edward River	
	67	Holyroyd River	
	90	Archer Bay	
<b>P &lt; 0.01</b>	<b>heterogeneity between populations:</b> <b>G = 189.097, df 26, P &lt; 0.001,</b>		<b>6 of 13 loci significant</b>
3 of 13 loci significant	58	Weipa	<u>NW CAPE</u> G = 26.180, df 36, n.s. 0 of 13 loci significant
	58	Port Musgrave	
	86	Mac. + Cott. + Janie Rivers	



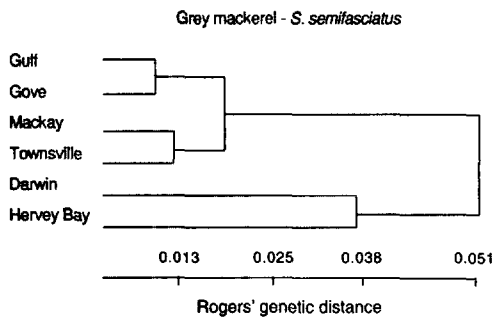
**Figure 1.** Map of northern Australia illustrating population subdivision as identified by allozyme and mtDNA genetic analysis. After Keenan (1994) and Doupé and Lynbery (submitted).



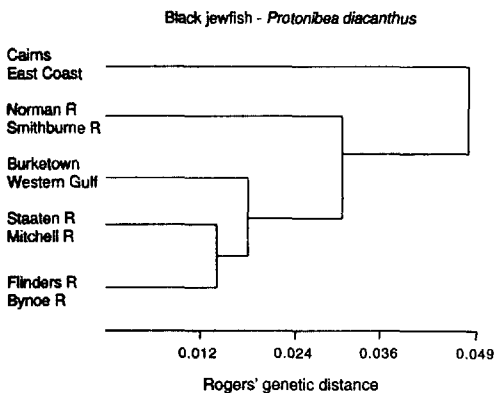
**Figure 2.** Unweighted Pair Groups using Mathematical Averages (UPGMA) dendrogram school mackerel samples based on Rogers' genetic distance. After Begg *et al.* (submitted).



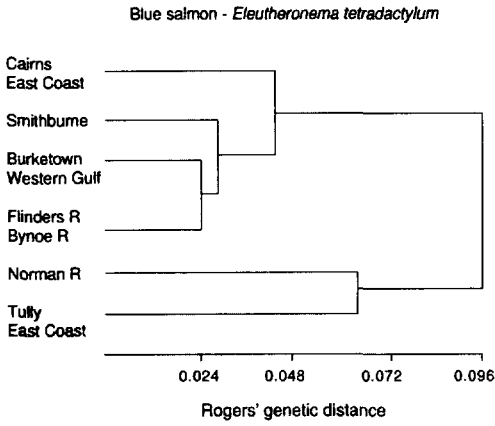
**Figure 3.** UPGMA dendrogram spotted mackerel samples based on Rogers' genetic distance. After Begg *et al.* (submitted).



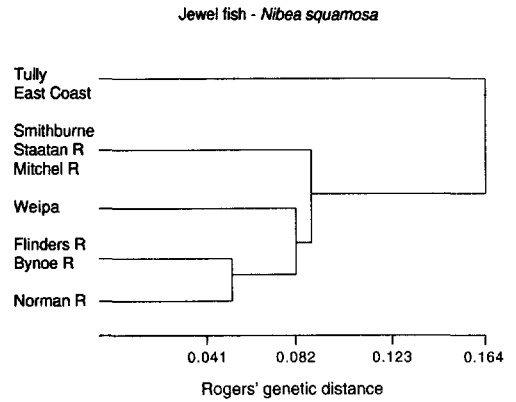
**Figure 4.** UPGMA dendrogram of grey mackerel samples based on Rogers' genetic distance.



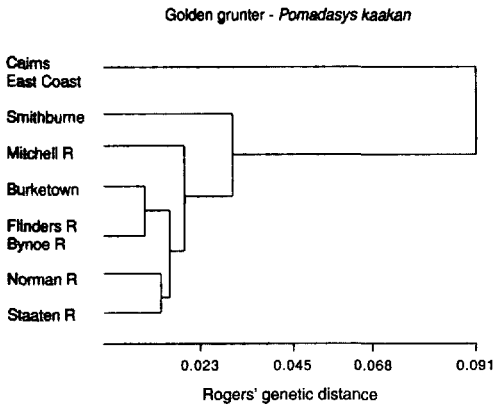
**Figure 5.** UPGMA dendrogram of black jewfish — *Protonibea diacanthus* samples based on Rogers' genetic distance.



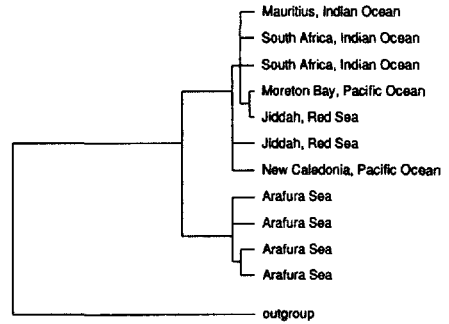
**Figure 6.** UPGMA dendrogram of blue salmon — *Eleutheronema tetradactylum* samples based on Rogers' genetic distance.



**Figure 8.** UPGMA dendrogram of jewel fish — *Nibea squamosa* samples based on Rogers' genetic distance.



**Figure 7.** UPGMA dendrogram of golden grunter — *Pomadasys kaakan* samples based on Rogers' genetic distance.



**Figure 9.** UPGMA dendrogram of mud crab, *S. serrata*, based on a number of nucleotide differences of cytochrome oxidase I (COI) mtDNA sequence between individuals.