92/031 Genetic Study of the Spatial Structure of Southern Bluefin Tuna Population

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OBJECTIVES

The proposed project is intended as a pilot study to a subsequent larger research project dealing with the links between southern bluefin spawning grounds and recruitment to the fishery. The objectives of this project are as follows:

1. To assess the genetic variability in juvenile bluefin tuna collected from the south-west coast of Australia, adults collected in waters off south Africa, and adults collected from the east coast of Tasmania.
2. The genetic variation present among the sample locations will be used to test the hypothesis that the two length modes present in a single year-class of juvenile SBT (either one or two year old fish collected from the south-west coast of Australia) relate to at least two genetically different components of the population.
3. Genetic variation between the African and Tasmanian adult samples will help determine whether genetic differences observed in juvenile fish is related to their spatial distribution as adults.

NON TECHNICAL SUMMARY

Southern bluefin tuna (*Thunnus maccoyii* (Castelnau)) are large (up to 200 cm and 200 kg), long-lived (more than 40 years; see Gunn et al. 1995) and commercially valuable fish. They are fished in the southern oceans, from the south-east Atlantic across the southern Indian Ocean to east Tasmania and New Zealand. The majority of fishing is conducted by Japanese longline vessels who focus their effort off South Africa and Tasmania. They primarily target the medium to large-sized fish, which are destined for the lucrative Japanese sashimi market. In the past, Australian fishermen have concentrated on surface schools of juveniles along the coasts, but are increasingly targeting larger fish for the sashimi market.

Annual global catches have fallen from a peak of around 80 000 t in 1960 and 1961 (Caton, 1994) to 12 631 t in 1991 and 13 846 t in 1992 (FAO, 1994). The spawning stock is estimated to be around 6 to 11% of its 1960 size - a historically and critically low level (Polacheck, pers. com.). In response to declining stock levels, international management, through informal arrangements, had imposed increasingly restrictive global catch quotas. These arrangements were formalised in 1994 with the ratification of the Convention for the Conservation of Southern Bluefin Tuna by Japan, New Zealand, and Australia. The first meeting of the Convention agreed to a global catch quota for 1994-95 of 11,750 t, of which 5265 t was allocated to Australia, 6065 t to Japan, and 420 t to New Zealand.

Determining objective management strategies requires information on various population parameters including elucidation of stock structure. In many species, including SBT, stock structure has typically been examined by several non-genetic methods, chief among which is tagging. For SBT, these studies are on-going, but earlier data show extensive movements, with fish tagged off southern Western Australia, South Australia, and New South Wales being recaptured as far east as the east coast of New Zealand and as far west as southern Africa (Murphy and Majkowski, 1981). However, recent tagging results (Murphy 1977; Ishizuka 1987; Hearn and Polacheck 1991) suggest that there may be a differential east/west movement of two/three year old SBT from the coastal waters of Australia that persists in the spatial distribution as adults. These results indicate a complex spatial structuring of SBT stocks that may involve site fidelity of adults at different feeding areas that in turn correspond to major fishing grounds. This feeding ground fidelity could result in genetically distinct sub-stocks. Furthermore, juvenile SBT collected in western Australia demonstrate the presence of at least two length modes (Hynd 1965; Leigh 1991) suggesting two breeding pulses, further indicating the possibility of separate sub-stocks. Catch rates of sub-adults and adults from different locations within the major fishing areas show different temporal patterns of reductions which are also consistent with low exchange rates among areas. Considerable concern exists about the possibility of sequential depletion of local sub-stocks of SBT (e.g. some historically important SBT longline fishing areas now appear to be commercially extinct). Consequently, identification of the stock structure of SBT is critical for rational management.

This report examined data on genetic variability among and within four sampling areas (South Africa, Western and Southern Australia, and eastern Tasmania) to assess whether bluefin tuna recruited to domestic and foreign fisheries within the AFZ constitute more than a single genetically homogeneous population. Population structure was investigated by testing the null hypothesis that no genetic differentiation occurs among areas sampled. To test the null hypothesis, the frequencies of genetic markers (both allozyme and mitochondrial DNA) were studied in samples from different regions. In addition, samples of juveniles of different size modes were compared to each another and to larger fish.

Mitochondrial DNA (17 variants) and six polymorphic allozyme loci were screened in southern bluefin tuna samples taken from South Africa, Western Australia, South Australia, and eastern Tasmania. Frequencies of genetic markers were not statistically significant among the four sample locations. Comparison of the two samples representing extreme ends of the area sampled (Tasmania and South Africa, about 11 000 km apart) also failed to reveal any differences. Clearly, it is not possible from this data to reject the null hypothesis that southern bluefin tuna comprise a single genetic stock.

The null hypothesis was examined further by comparing genetic marker frequencies between various size classes. The daily growth rings on the otoliths of some of the small juveniles taken off Western Australia at the end of January/beginning of February 1993 suggest that the mode of 30-35 cm fish consists of fish 3 to 4 months old, while the 46 to 54 cm length mode consists of fish about 1 year old (Gunn et al., 1995).

These findings are consistent with catch per unit effort (CPUE) studies of mature fish taken from the spawning area, which suggest that there are two peaks in CPUE: January to February, and October (Davis and Farley, 1995). These are likely to correspond to spawning peaks, as female fish in these catches have mature oocytes. The relative sizes of these two peaks, as judged from CPUE figures, varies from year to year, although in recent years the January to February peak appears to have been the larger (Davis and Farley, 1995). The larger (older) fish we sampled were probably spawned in the February to March 1992 period (although a few may have been spawned in September to October 1991) and the smaller (younger) fish were spawned September to October 1992. The lack of intermediate-sized fish

reflects the likely paucity of spawning between May and July, or possibly poor survival of larvae spawned in this period. The null hypothesis that these two groups of fish were drawn from a single gene pool could not be rejected for either the allozyme or the mtDNA data. However, sample sizes were small, and the strength of this inference is correspondingly weak: the extent of gene flow between fish spawning at these two peaks may range from very limited to complete. Because of subsequent differences in growth rates, these two groups of fish cannot be distinguished in larger-size categories and therefore cannot then be compared with one another.

Another technique for defining stock structure examines the microchemistry of otoliths. Using this non-genetic approach, examination of both the primordial region deposited in the first few days of larval life and the margin deposited shortly before the individual is caught, were consistent with a single southern bluefin tuna stock (Proctor et al., 1995). However, no clear differences in the compositions of the otolith margins were apparent in individuals collected from widely separated localities, suggesting the approach will not be useful for studying southern bluefin tuna movements.

The conclusion from these various lines of evidence must be that the null hypothesis of a single stock of southern bluefin tuna cannot be rejected. However, failure to reject this hypothesis does not necessarily mean that it is correct. In particular, the biological basis of the two spawning peaks needs further investigation. If offspring from one peak preferentially return to spawn at that time, then there will be some restriction on gene flow between these two components of the population, and the nature and extent of these stocks would have to be determined. Our attempt to resolve this question did not provide a robust test, as only small numbers of individuals (n=56) were examined from the two spawning peaks. Further investigations should attempt to analyse larger numbers of fish, if possible by sampling spawners or larvae rather than their putative juvenile offspring. Future research should also incorporate analysis of hypervariable microsatellite DNA markers, which may detect levels of population structuring not apparent from allozyme or mtDNA surveys.

KEYWORDS: southern bluefin tuna, stock discrimination, analysis of population structure, allozymes, mitochondrial DNA (mtDNA)

December 1996

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Grewe, Peter, 1961-
Genetic study of the spatial structure of the
southern bluefin tuna population

ISBN 0 646 05958 X

1. Bluefin tuna – Genetics
   I. Grewe, Peter. 1961-
   II. CSIRO. Division of Fisheries
   III. Fisheries Research and Development Corporation
   (Australia)
   IV. Title

597.78313

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1. BACKGROUND

This project was a pilot study intended to provide the information needed to assess the feasibility of a larger research project to a) determine the source of bluefin recruits to domestic and foreign commercial fisheries in Australian waters and b) determine whether bluefin tuna sub-populations are present within the AFZ. This information will be reported to Australian Fisheries Management Authority, SBTMAC and relevant Granting Agencies. Results will be presented to the Demersal and Pelagic Fisheries Research Group, the Australian Marine Science Association and the Australian Society for Fish Biology. This pilot study will determine whether a larger research project is practical and if so, it will provide the basic information needed to carry out such a project. Data provided from this pilot study will also result in publications in international journals

Data on genetic variability among and within three sampling areas (south western Australia, South Africa, and eastern Tasmania) will provide a means of assessing whether bluefin tuna recruited to domestic and foreign fisheries within the AFZ constitute more than a single genetically homogeneous population. One-year-old, juvenile southern bluefin tuna SBT collected in western Australia demonstrate the presence of at least two length modes (Hynd 1965; Leigh 1991) suggesting 2 breeding pulses and the possibility of separate sub-stocks. Early reports by Hynd (1965) suggested the existence of three separate size modes, but the third mode appears to have disappeared. In addition, recent tagging results (Murphy 1977; Ishizuka 1987; Hearn and Polacheck 1991) suggest that there may be a differential east/west movement of two/three-year-old SBT from the coastal waters of Australia that persists in the spatial distribution as adults. Preliminary data indicate that approximately equal proportions of SBT tagged as juveniles off western Australia are recovered as adults in fishing grounds located both east and west of 70°E. However, fish tagged at more easterly locations are less likely to be recruited to the fisheries west of 70°E (*i.e.* only one-third of juveniles tagged off South Australia are recovered west of 70°E and juveniles tagged off NSW were only recovered in fishing grounds east of 70°E). Tagging results indicate a complex spatial structuring of SBT stocks which may involve site fidelity of adults that exist as genetically distinct sub-stocks.

2. NEED

Catch rates of sub-adults and adult southern bluefin tuna from different locations within the major fishing areas show different temporal patterns of reductions which are also consistent with low exchange rates among areas. Considerable concern exists about the possibility of sequential depletion of local sub-stocks of SBT (*e.g.* some historically important SBT longline fishing areas now appear to be commercially extinct). Consequently, identification of the stock structure of SBT is critical for rational management.

This project will test the hypothesis that the two length modes relate to at least two genetically different components of the population, and that this genetic difference is related to the spatial distribution of these fish as adults. Observer programs on longline vessels are expanding as a result of the real time monitoring program so that for the first time it will now be possible to collect biological samples from all fishing areas (particularly the high seas).

3. OBJECTIVES

The proposed project is intended as a pilot study to a subsequent larger research project dealing with the links between southern bluefin spawning grounds and recruitment to the fishery. The objectives of this project are as follows:

1. To assess the genetic variability in juvenile bluefin tuna collected from the south-west coast of Australia, adults collected in waters off south Africa, and adults collected from the east coast of Tasmania.

2. The genetic variation present among the sample locations will be used to test the hypothesis that the two length modes present in a single year-class of juvenile SBT (either 1- or 2-year-old fish collected from the south-west coast of Australia) relate to at least two genetically different components of the population.

3. Genetic variation between the African and Tasmanian adult samples will help determine whether genetic differences observed in juvenile fish is related to their spatial distribution as adults.

4. METHODS

Genetic Analysis

Four areas were extensively sampled: Tasmania, Western Australia, South Australia, and South Africa (Fig. 1 and Table 1). The samples from Tasmania and South Africa were taken by observers on Japanese longline vessels, and the samples from Western Australia and South Australia were taken by scientists on research cruises.

Table 1. Southern bluefin tuna. Approximate sites and sampling dates. *n* = number of fish.

Location	Date	*n*	Lat., Long.
South Africa	April 1992	32	42°S, 3°W
	April-June 1992	86	38-42°S, 20-26°W
	April-June 1992	16	42°S, 6-7°W
	June-July 1993	96	39-41°S, 27°W
Western Australia	January 1992	14	35°S, 112-118°W
	January 1993	68	34°S, 121-124°W
	January-March 1994	35	34-35°S, 117-122°W
(*Shoyo Maru*)	January-February 1993	101	27-33°S, 113-115°W
South Australia	December 1992	9	35°S, 136°W
	January 1993	19	32-43°S, 133°W
	January-February 1994	62	32-35°S, 132-135°W
Tasmania	May-June 1992	152	39-44°S, 148-150°W
	November-December 1993	23	42-44°S, 142-145°W
	October 1994	45	44°S, 144-148°W

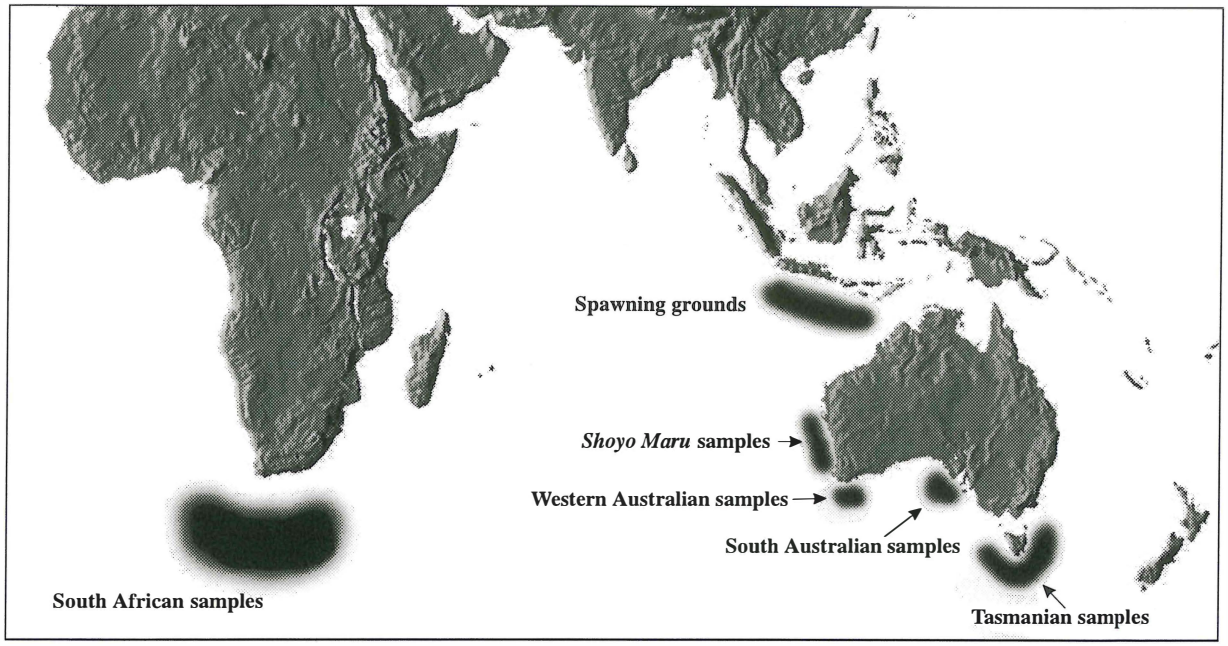

Figure 1. Southern bluefin tuna sampling sites and spawning ground.

Muscle and liver tissues were dissected shortly after capture and immediately frozen. Lengths (fork lengths) and sexes were noted. The tissue samples were transported frozen to the laboratory, where they were stored at -80°C. For electrophoresis, tissue samples were placed in 1.5 mL microcentrifuge tubes, homogenised manually with a few drops of distilled water, and spun at 11 000 g in a microcentrifuge for 2 min. The supernatant was used.

In an initial survey of 35 allozyme loci (Elliott and Ward, 1995), six showed sufficient polymorphism to be useful for stock-structure analysis: *ADA** (adenosine deaminase, EC 3.5.4.4), *GDA** (guanine deaminase, EC 3.5.4.3), *GPI-A** (glucose phosphate isomerase, fast allozyme, EC 5.3.1.9), *MPI** (mannose phosphate isomerase, EC 5.3.1.8), *PGDH** (phosphogluconate dehydrogenase, EC 1.1.1.44) and *PGM-1** (phosphoglucomutase, fast allozyme, EC 5.4.2.2). *ADA**, *GPI-A**, *MPI**, and *PGDH** were examined in white muscle tissue, *GDA** and *PGM-1** in liver tissue. All loci, except *PGDH**, were scored from Helena Titan III cellulose acetate plates run with a tris-glycine buffer system (0.02 M tris, 0.192 M glycine, see Hebert and Beaton, 1989, for details). *PGDH** was scored from 8% Connaught starch gels with a histidine/citrate buffer system (gel buffer: 0.005 M histidine HCl, adjusted to pH 7.0 with 0.1 M sodium hydroxide; electrode buffer: 0.41 M trisodium citrate, adjusted to pH 7.0 with 0.5 M citric acid). Alleles were numbered according to the mobility of their product relative to that of the most common allele observed in yellowfin tuna (*Thunnus albacares*), which was designated '100' (Elliott and Ward, 1995)

An initial survey of mitochondrial DNA using 15 restriction enzymes (Ward *et al.*, 1995) showed that the bulk of the variation could be detected by three enzymes: *Bam* HI, *Bcl* I and *Eco* RI. Total DNA was extracted from white muscle tissue by a modified CTAB (hexadecyltrimethylammoniumbromide) protocol, digested and electrophoresed as described by Grewe *et al.* (1993). Total DNA was transferred onto a nylon membrane filter (Hybond N+, Amersham Ltd.) by southern transfer (Sambrook *et al.*, 1989). The filters were probed either with trevalla (*Hyperoglyphe antarctica*, Teleostei: Stromateoidei) mitochondrial DNA (50 ng used per ten 20 cm x 20 cm blots) purified by caesium chloride (CsCl) ultracentrifugation or with a mtDNA probe produced by the PCR amplification of southern bluefin tuna mtDNA with conserved primers. The probe was labelled with 32P dCTP (Bresatec Pty Ltd) using a GIGAprime DNA labelling kit (Bresatec Pty Ltd). The membrane filters were then exposed to Kodak XAR-5 X-ray film for 12 to 48 h.

Statistical analysis

Allele and haplotype frequency homogeneity across samples was tested by the randomised Monte Carlo chi-square procedure of Roff and Bentzen (1989). This procedure obviates the need to pool rare alleles. For each test, 2000 randomisations of the data were carried out, each giving a randomised chi-square value (χ^2null). The probability that the null hypothesis of genetic homogeneity was correct was given by $P = n/2000$, where n was the number of randomisations that generate $\chi^2\text{null} \geq \chi^2$, where χ^2 is the chi-square value given by the observations.

The extent of genetic differentiation among samples was quantified by the gene diversity statistic G_{ST} (Nei, 1987), which reflects the proportion of total genetic variation attributable to differentiation between populations. For each locus it was estimated as $(H_T - H_S)/ H_T$, where H_T represents total heterozygosity and H_S is average (Hardy-Weinberg expected) population heterozygosity (or, in the case of mtDNA, haplotype diversity). The proportion or magnitude of G_{ST} generated by sampling error, which we have termed $G_{ST.null}$, was estimated with a bootstrapping program (Ward and Grewe, unpublished), given the observed allele or haplotype frequencies and sample sizes. Simulations were run 1000 times to provide a mean value of $G_{ST.null}$ and a standard deviation. The probability of obtaining a value of $G_{ST.null}$ as large or larger than that obtained from the actual observations, *GST*, was given by $P = n/1000$, where n is the number of randomisations that generate $G_{ST.null} \geq G_{ST}$. Values of $P < 0.05$ indicated significant differentiation between areas that could not be explained by sampling error alone.

Probability corrections for multiple tests were carried out by the sequential Bonferroni procedure of Hochberg (1988; see also Lessios, 1992), using $\alpha = 0.05$ as the predetermined significance level.

5. DETAILED RESULTS

Unlike the Tasmanian and South African samples, the Western Australia and South Australia samples comprised juvenile fish less than 100 cm fork length (Fig. 2). There were no significant differences in allozyme allele or mitochondrial DNA haplotype frequencies among the Western Australia and South Australia samples and they were combined in the analyses of spatial differentiation.

The six polymorphic allozyme loci screened (Table 2) had average heterozygosities per locus ranging from 0.049 (*MPI**), through 0.290 (*ADA**), 0.435 (*PGDH**), 0.507 (*GPI-A**) and 0.511 (*PGM-1**) to 0.612 (*GDA**), and numbers of alleles per locus ranged from four (*MPI**, *ADA**) through five (*GPI-A**, *PGM-1**) to six (*PGD**, *GDA**).

The average size of the mtDNA genome was 16779 bp (from data in Ward *et al.*, 1995). Seventeen different haplotypes were found (Table 2), nine in Tasmania, 10 in Western and South Australia, and 12 in South Africa.

Spatial differentiation

Gene and haplotype frequencies were estimated from observed genotype and haplotype numbers (Table 2). The sample sizes for the two liver-specific enzymes (*GDA** and *PGM-1**) were smaller than the four muscle-specific enzymes, especially in the Western Australia and South Australia samples, because livers were not collected from all fish.

The six polymorphic allozyme loci screened (Table 2) had average heterozygosities per sample ranging from 0.394 (Tasmania) through 0.399 (South Africa) to 0.406 (Western and South Australia), and mean numbers of alleles per locus ranging from 4.0 (Tasmania) through 4.17 (South Africa) to 5.17 (Western and South Australia). The increased number of alleles in the latter sample reflects its increased sample size.

Chi-square tests for goodness-of-fit of genotype numbers to Hardy-Weinberg expectations were carried out for all loci and sampling areas, except for *MPI**, whose gene frequencies were too skewed to permit valid tests. Rare alleles were pooled as necessary. No significant deviations were observed in any of the 15 tests (three areas x five loci). Samples were then pooled across areas, and the tests repeated. None of these five tests showed any significant deviations from expectations.

Chi-square analyses of allele frequency differentiation across samples (Table 3) showed evidence of differentiation at only one of the six allozyme loci, *MPI** ($P = 0.026$). This proved not to be significant after Bonferroni probability correction for multiple tests. The observed degree of population division estimated from G_{ST} analysis was, for five of the allozyme loci, around 0.001-0.002 (Table 3). For these cases, sampling error alone would have generated similar values ($G_{ST.null}$). For the sixth locus, *PGM-1**, the observed value of G_{ST} was marginally significant ($P = 0.042$). With Bonferroni correction, this result became non-significant.

With respect to the mtDNA haplotype data, neither chi-square nor G_{ST} analyses revealed any significant spatial differentiation (Table 3). Nucleon diversities (Nei and Tajima 1981) assessed from the three restriction enzymes ranged from 0.399 for the Western Australia and South Australia sample, through 0.467 for the sample from Tasmania, to 0.532 for the South African sample. Pooling all fish produced a nucleon diversity of 0.468. This is about 64% of the nucleon diversity judged from 15 restriction enzymes, estimated at 0.729 (from the data in Ward *et al.* 1995).

A comparison of fish (all of which were 2-year-old plus) from the two extremities of the sampled distribution – Tasmania and South Africa – did not reveal any significant differentiation (probability values: *ADA**, $P = 0.376$; *GDA**, $P = 0.765$; *GPI-A**, $P = 0.704$; *MPI**, $P = 0.065$; *PGDH**, $P = 0.100$; *PGM-1**, $P = 0.592$; mtDNA, $P = 0.383$).

Sex differentiation

A comparison of male and female fish did not reveal any significant sex-related differentiation in either allele or mtDNA haplotype frequencies (probability values: *ADA**, $P = 0.093$; *GDA**, $P = 0.983$; *GPI-A**, $P = 0.288$; *MPI**, $P = 1.000$; *PGDH**, $P = 0.890$; *PGM-1**, $P = 0.678$; mtDNA, $P = 0.766$).

Size differentiation

The overall size distribution shows several distinct modes, with the smallest fish taken from Western Australia and South Australia (Fig. 2). Many of the smallest fish were taken in the space of one week (30 January 1993 to 5 February 1993) by the research vessel *Shoyo Maru*, operating off Western Australia (Fig. 1). The lengths of these fish ($n = 102$) were strikingly bimodal (Fig. 3): all fish except two fell into either the mode 30 to 35 cm or the mode 46 to 54 cm. The two exceptional fish had lengths of 62.5 and 64.5 cm. The gene and haplotype frequencies of fish of these two modes were compared by chi-square analyses (Table 4). Only one of the six polymorphic loci, *PGM-1**, showed evidence of differentiation ($P = 0.023$), and this proved not to be significant after Bonferroni probability correction for multiple tests. The mtDNA haplotype frequencies showed no significant differences.

The second set of size tests compared all juvenile fish (taken to be those < 70 cm) with all non-juvenile fish (≥ 70 cm). These tests, too, showed no significant differentiation in allele or haplotype frequencies (uncorrected probability values: *ADA**, $P = 0.411$; *GDA**, $P = 0.051$; *GPI-A**, $P = 0.123$; *MPI**, $P = 0.683$; *PGDH**, $P = 0.355$; *PGM-1**, $P = 0.083$; mtDNA, $P = 0.895$).

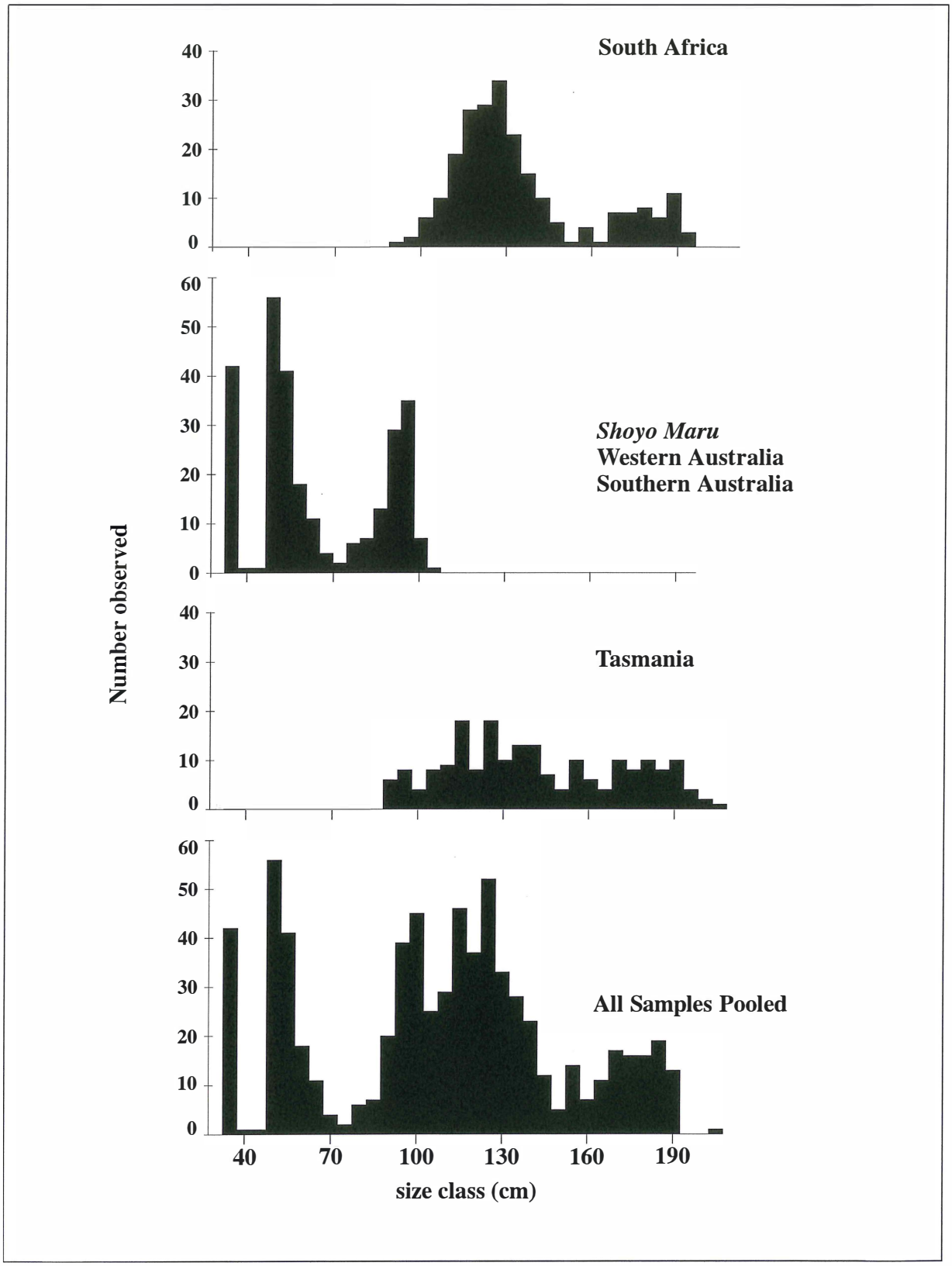

Figure 2. Size distributions of southern bluefin tuna examined in this study (numbers of fish measured per 5 cm size category).

Table 2. Southern bluefin tuna. Allozyme allele and mtDNA haplotype frequencies. MtDNA letters reflect, in order, *Bam* HI, *Bcl* I and *Eco* RI fragment patterns. *n* = number of fish analysed. Dashes indicate alleles that were not observed in a sample.

Locus	Allele / Haplotype	Tasmania	W & S Australia	South Africa
*ADA**	115	0.112	0.113	0.101
	100	0.829	0.824	0.849
	85	0.053	0.057	0.051
	70	0.007	0.005	-
	n	*219*	*296*	*218*
*GDA**	120	0.002	-	-
	110	0.460	0.465	0.432
	100	0.049	0.030	0.048
	90	0.421	0.395	0.444
	80	0.061	0.091	0.061
	70	0.007	0.019	0.015
	n	*214*	*186*	*198*
*GPI-A**	130	-	0.002	-
	100	0.507	0.478	0.514
	90	-	0.002	-
	75	0.489	0.515	0.477
	40	0.005	0.003	0.009
	n	*218*	*295*	*216*
*MPI**	120	0.0141	0.024	0.014
	100	0.9859	0.970	0.970
	85	-	0.005	0.009
	65	-	0.002	0.007
	n	*213*	*296*	*218*
*PGDH**	125	0.005	-	0.002
	100	0.716	0.707	0.677
	85	-	0.002	-
	75	0.262	0.263	0.297
	50	0.005	0.019	0.018
	30	0.014	0.011	0.005
	n	*218*	*295*	*217*
*PGM-1**	110	0.002	0.008	0.005
	100	0.416	0.465	0.376
	90	0.563	0.516	0.601
	80	0.019	0.011	0.016
	70	-	-	0.003
	n	214	188	193
mtDNA	BFA	0.711	0.765	0.661
	AFA	0.155	0.123	0.169
	AFB	0.059	0.028	0.037
	BFB	0.043	0.022	0.048
	BAA	0.011	0.028	0.026
	BQA	-	0.006	0.016
	BVA	-	0.011	0.005
	DFA	-	-	0.016
	BTA	0.005	-	0.005
	BUA	0.005	-	0.005
	AFN	-	0.006	-
	ARA	0.005	-	-
	AVA	-	0.006	-
	BFH	-	-	0.005
	BFI	-	-	0.005
	BSB	0.005	-	-
	EFA	-	0.006	-
	n	187	179	189

Table 3. Southern bluefin tuna. Analyses of genetic differentiation among the three areas.

Loci	Number of alleles	Number of individuals	Chi-square analysis $\chi 2$	Chi-square analysis P	Genetic diversity analysis G_{ST}	Genetic diversity analysis $G_{ST.null} \pm SD$	Genetic diversity analysis P
*ADA**	4	733	3.563	0.741	0.001	0.001±0.001	0.782
*GDA**	6	598	11.290	0.317	0.002	0.002±0.001	0.421
*GPI-A**	5	729	6.173	0.699	0.001	0.002±0.002	0.516
*MPI**	4	727	13.345	0.026	0.002	0.001±0.001	0.205
*PGDH**	6	730	12.025	0.271	0.001	0.001±0.001	0.388
*PGM-1**	5	595	10.361	0.209	0.005	0.002±0.002	0.042
mtDNA	17	555	35.639	0.235	0.006	0.004±0.002	0.138

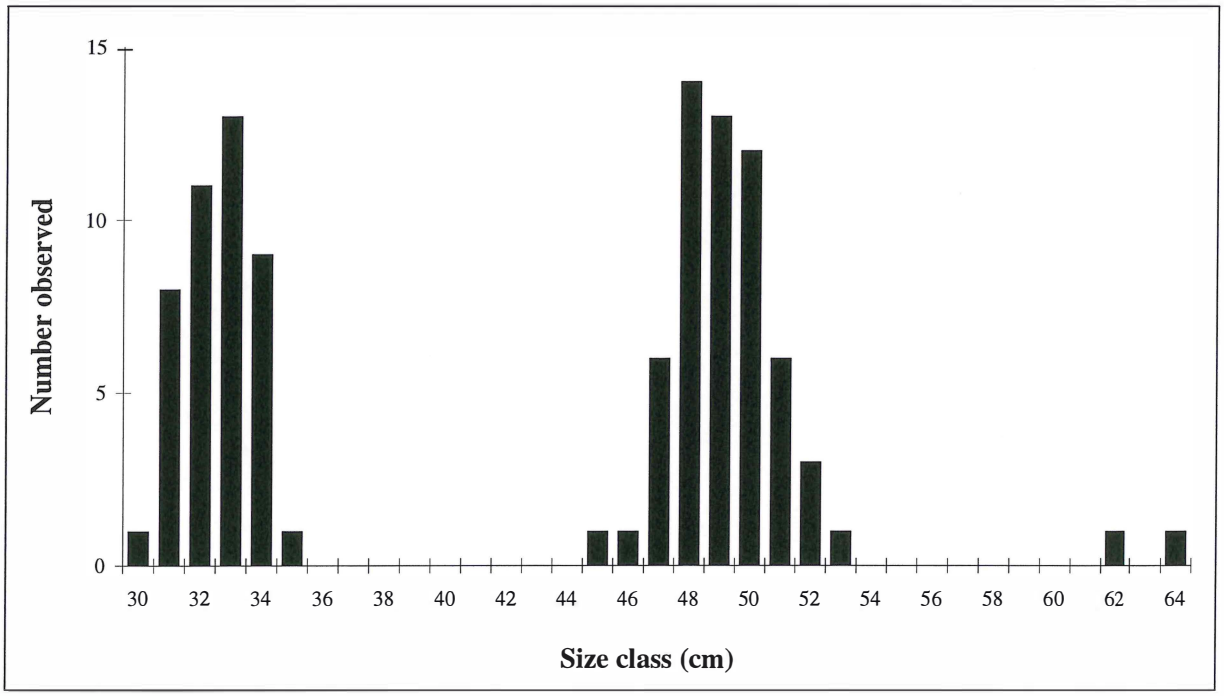

Figure 3. Size distribution of juvenile southern bluefin tuna collected onboard the *Shoyo Maru*.

Discussion

Six polymorphic allozyme loci and mtDNA variants (17 haplotypes) were screened in southern bluefin tuna samples taken from South Africa, Western Australia, South Australia, and eastern Tasmania, but no statistically significant genetic differentiation was observed. Samples from two extremities of this distribution (Tasmania and South Africa, about 11 000 km apart) also failed to reveal any differences. No significant deviations from Hardy-Weinberg expectations were found. Clearly, it is not possible from this data to reject the null hypothesis that southern bluefin tuna comprise a single genetic stock.

This hypothesis was examined further by comparing various size classes. The daily growth rings on the otoliths of some of the small juveniles taken off Western Australia at the end of January/beginning of February 1993 suggest that the mode of 30-35 cm fish consists of fish 3 to 4 months old, while the 46 to 54 cm length mode consists of fish about 1-year-old (Gunn *et al.*, 1995).

These findings are consistent with catch per unit effort (CPUE) studies of mature fish taken from the spawning area, which suggest that there are two peaks in CPUE: January to February, and October (Davis and Farley, 1995). These are likely to correspond to spawning peaks, as female fish in these catches have mature oocytes. The relative sizes of these two peaks, as judged from CPUE figures, varies from year to year, although in recent years the January to February peak appears to have been the larger (Davis and Farley, 1995). The larger (older) fish we sampled were probably spawned in the February to March 1992 period (although a few may have been spawned in September to October 1991) and the smaller (younger) fish were spawned September to October 1992. The lack of intermediate-sized fish reflects the likely paucity of spawning between May and July, or possibly poor survival of larvae spawned in this period. The null hypothesis that these two groups of fish were drawn from a single gene pool could not be rejected for either the allozyme or the mtDNA data. However, sample sizes were small, and the strength of this inference is correspondingly weak: the extent of gene flow between fish spawning at these two peaks may range from very limited to complete. Because of subsequent differences in growth rates, these two groups of fish cannot be distinguished in larger-size categories and therefore cannot then be compared with one another.

There were also no significant genetic differences between fish <70 cm (less than 2-years-old) and those >70 cm (mostly 3- to 6-year-old fish, although with a few 2-years-old and some 7-years and older; Gunn *et al.* 1995). There is therefore no evidence of selection operating to change gene or mtDNA haplotype frequencies as fish age.

The only other published population genetic study of southern bluefin is Fujino and Kang (1968), who found no significant differences in allele frequencies at the transferrin locus in 302 fish from different Australian sites. This is also consistent with the hypothesis of a single genetic stock of southern bluefin tuna.

Other tuna species have been the focus of population genetic analysis. Many thousands of skipjack tuna (*Katsuwonus pelamis*) from the Atlantic, Indian and (especially) Pacific Oceans have been examined for variation at the *EST** locus. Inter-ocean differences in gene frequencies were described, as was heterogeneity within the Pacific Ocean (*e.g.* Fujino *et al.* 1981; Richardson, 1983). Whether the heterogeneity reported in the Pacific Ocean reflects multiple stocks or clinal variation (isolation by distance) remains uncertain (IATTC, 1984). With respect to yellowfin tuna (*Thunnus albacares*), striking genetic differentiation was found for one of four polymorphic allozyme loci, *GPI-A**; mtDNA differentiation was also studied but was less pronounced (Scoles and Graves 1993, Ward *et al.*, 1996). Four genetic stocks of yellowfin tuna were proposed: Atlantic Ocean, Indian Ocean, west-central Pacific Ocean, and east Pacific Ocean (Ward *et al.*, 1996). MtDNA analyses of albacore tuna (*Thunnus alalunga*) found differences between Atlantic and Pacific Ocean samples, but no intra-ocean heterogeneity (Chow and Ushiama, 1995); there have as yet been no allozyme studies of this species.

These three species of tuna are much more widely distributed than southern bluefin, and all have multiple spawning areas. In all three cases there is evidence of genetic stock differentiation, although (and commensurate with their migratory abilities) this tends to be on a large scale (intra- or inter-oceanic) rather than more local scales (Ward, 1995).

The stock structure of southern bluefin tuna has been examined by several non-genetic methods, chief among which is tagging. These studies are on-going, but earlier data show extensive movements, with fish tagged off southern Western Australia, South Australia, and New South Wales being recaptured as far east as the east coast of New Zealand and as far west as southern Africa (Murphy and Majkowski, 1981).

Another non-genetic approach examines the microchemistry of otoliths. Such studies, of both the primordial region deposited in the first few days of larval life and the margin deposited shortly before the individual is caught, were consistent with a single southern bluefin tuna stock (Proctor *et al.*, 1995). However, no clear differences in the compositions of the otolith margins were apparent in individuals collected from widely separated localities, suggesting the approach will not be useful for studying southern bluefin tuna movements.

The conclusion from these various lines of evidence must be that the null hypothesis of a single stock of southern bluefin tuna cannot be rejected. However, failure to reject this hypothesis does not necessarily mean that it is correct. In particular, the biological basis of the two spawning peaks needs further investigation. If offspring from one peak preferentially return to spawn at that time, then there will be some restriction on gene flow between these two components of the population, and the nature and extent of these stocks would have to be determined. Our attempt to resolve this question did not provide a robust test, as only small numbers of individuals ($n \leq 56$) were examined from the two spawning peaks. Further investigations should attempt to analyse larger numbers of fish, if possible by sampling spawners or larvae rather than their putative juvenile offspring. Future research should also incorporate analysis of hypervariable microsatellite DNA markers, which may detect levels of population structuring not apparent from allozyme or mtDNA surveys.

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6. BENEFITS

This project was designed as a pilot study intended to provide the information needed to assess the feasibility of a larger research project to a) determine the source of bluefin recruits to domestic and foreign commercial fisheries in Australian waters and b) to determine whether bluefin tuna sub-populations are present within the AFZ. The data gathered has promoted a better understanding of population dynamics of southern bluefin tuna. Southern bluefin tuna appear to be a single genetically homogeneous population or stock. This information provides an objective criterion from which to base sound management decisions on bluefin tuna quotas which are designed to provide long term sustainability of the resource. Thus, our results have benefited parties involved in the southern bluefin tuna fishery at both the commercial and recreational level.

7. INTELLECTUAL PROPERTY AND VALUABLE INFORMATION

No commercial intellectual property arose from this work.

8. FURTHER DEVELOPMENT

Failure to reject the null hypothesis (Ho: "there exists only a single genetic stock of SBT") does not prove that the null hypothesis is true. In other words, the null hypothesis is not the only alternative explanation or conclusion which can be drawn from the data. For example, separate genetic stocks of SBT may exist, however, our methods may have been unable to detect this structure at the level of resolution required. In particular, the biological basis of the two spawning peaks needs further investigation. If offspring from one peak preferentially return to spawn at that time, then there will be some restriction on gene flow between these two components of the population, and the nature and extent of these stocks would have to be determined. Our attempt to resolve this question did not provide a robust test, as only small numbers of individuals ($n \leq 56$) were examined from the two spawning peaks. Further investigations should attempt to analyse larger numbers of fish, if possible by sampling spawners or larvae rather than their putative juvenile offspring. Future research should also incorporate analysis of hypervariable microsatellite DNA markers, which may detect levels of population structuring not apparent from allozyme or mtDNA surveys.

9. STAFF

Dr P. Grewe	Research Scientist (CSOF4)	40%
Dr. T. Polacheck	Research Scientist (CSOF6)	5%
A. Smolenski	Experimental Scientist (CSOF3)	50%
B. Innes	Experimental Scientist (CSOF3)	50%
Dr. R. Ward	Principal Research Scientist (CSOF7)	10%
W. Whitelaw	Research Scientist (CSOF5)	5%

Acknowledgements

We thank S. Meyer and P. Lowry for assisting with some of the allozyme analyses.
B. Evans assisted with mtDNA extraction and analysis. We thank all those who assisted with sample collections, especially N. Clear, T. Carter, C. Stanley, the crew of the *Shoyo Maru*, and Real Time Monitoring Program and Australian Fishing Zone observers.

10. FINAL COST

PROJECT BUDGET SUMMARY

	1993-94	TOTAL
FRDC Contribution		
Salaries and On-costs	**$43,881**	**$43,881**
Travel	$3,640	$3,640
Operating	$23,900	$23,900
Capital	$0	$0
Total FRDC	**$71,421**	**$71,421**
Research Organisation Contribution		
Salaries and On-costs	$61,433	$61,433
Travel	$3,640	$3,640
Operating	$	$
Capital	$255,000	$255,000
Total Research Organisation	**$320,073**	**$320,073**
Contribution by other sources		
Cash	$	$
Other (include 'in-kind')	$	$
Total Contribution by other sources	**$**	**$**
TOTAL BUDGET	**$391,494**	**$391,494**