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The origin of recruits to the east coast yellowfin tuna fishery and the delineation of the structure of yellowfin stocks in the western Pacific

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1 Non-technical Summary

1994/022 The origin of recruits to the east coast yellowfin tuna fishery and the delineation of the structure of yellowfin stocks in the western Pacific

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Objectives

1. To develop the use of microsatellites for the genetic analysis of yellowfin tuna.
2. To determine the genetic relationships of yellowfin tuna from the Coral Sea to those from the east coast of Australia (mainly NSW), and to those from other Western Pacific fisheries (the Philippines, Solomons, Papua New Guinea and Indonesia) and thereby test the null hypothesis that there is a single panmictic WP stock.
3. To develop techniques for estimation of oxygen 18/16 isotopic ratios (currently used in bivalve research) in otoliths using a SIMS microprobe.
4. Using analysis of otolith chemistry and isotopic ratios, examine the hypothesis that all recruits to the ECTUNA fishery are derived from spawning grounds in the Coral Sea.
5. In the event of rejection of Ho, use otolith chemical data collected from yellowfin collected throughout the WP to estimate the proportion of immigrant recruits and determine their most likely origin.

Outcomes Achieved

The results of this project have had significant impacts on the assessment of yellowfin tuna in the eastern AFZ. In 1998, 1999 and 2000, the Eastern Tuna and Billfish Fishery (ET&BF) Fishery Assessment Group (FAG) used the information provided by this study on linkages between the Coral Sea and the ET&BF in its interpretation of catch rates and recruitment patterns of yellowfin. The project data were also provided at an important effort setting workshop in 1999 at which representatives from industry, science and management met to discuss the optimal levels of effort within the ET&BF. The likelihood that yellowfin recruitment into the ET&BF was strongly linked to the Coral Sea, rather than the alternative scenario that all recruits were sourced from the greater Western Pacific, was a significant contributing factor to a majority decision within the meeting to adopt a low risk position in setting an effort cap for the ET&BF.

The outputs of the project have also been used within a major AFMA report on the trends in abundance of yellowfin on the east coast of Australia and in the SW Pacific Ocean (Campbell 1998). In this report an understanding of the probable sources of recruits to the ET&BF was used in the interpretation of observed inter-annual variation in abundance, and the development of hypotheses explaining these.

In 2002, AFMA and the ET&BF FAG will conduct a second effort-setting workshop to develop a total allowable effort (TAE) for the ET&BF under its new management plan. As previously, it is expected that the outputs of this project will provide critical inputs into the development of the TAE, and in ensuring the sustainable use of the east coast yellowfin tuna resource.

This study of the population structure of yellowfin tuna (*Thunnus albacares* (Bonnaterre)) in the south-west Pacific Ocean was initiated in the mid-1990's in response to calls from the Australian Fisheries Management Authority's Eastern Tuna Management Advisory Committee (ECTUNAMAC) for more information on the structure of yellowfin stocks in our region. To provide advice on how to manage yellowfin resources – at that time exploited by Japanese and domestic longliners in the eastern Australian Fishing Zone (AFZ) – ECTUNAMAC required an answer to a very simple question - where did the recruits to the lucrative yellowfin fishery on the NSW coast come from? If we could show that yellowfin recruiting into the fishery came primarily from the very large populations of yellowfin known to inhabit equatorial waters to Australia's north and north east, then restricting catches of yellowfin in the AFZ may have little impact on the status of stocks, or future recruitment to the fishery. If, however, the fish caught in the Coral and Tasman Seas recruited primarily from more localised spawning (perhaps in the Coral Sea), then a more precautionary view on the control of exploitation rates in our fishery would likely be necessary.

From ECTUNAMAC's simple question grew a complex and detailed study focussed on a number of aspects of yellowfin genetics, biology and movement. Our study has used cutting edge techniques to provide new data on geographical variation in yellowfin genetics and the chemistry of their otoliths (ear bones). In seeking an answer to the ECTUNAMAC's question we've synthesised these new data with other available information collected over the last two decades. That we found the answer to the simple question somewhat complex is not surprising—delineating stocks of highly migratory fish, and in particular tuna, has presented a major challenge to all who have tried.

Genetics

Microsatellite techniques for yellowfin were successfully developed and applied (objective 1). Five polymorphic microsatellite loci (i.e. loci on the yellowfin genome that show two or more different states) were examined in 1391 yellowfin tuna samples collected in eight regions of the western (Coral Sea, east Australia, Fiji, Indonesia, Philippines and Solomon Islands) and eastern (California and Mexico) Pacific Ocean. All loci showed high levels of genetic variation, but little of this could be attributed to among-sample variation. Samples that were collected in two different years for three areas allowed us to examine the stability of the genetic patterns we were observing. Statistical tests detected no significant differences between repeated collections from the three areas, except at one locus between two Philippines samples. Four loci showed no evidence of population differentiation following statistical tests and the fifth locus showed small but significant differentiation.

No significant differences in allele frequencies at any locus between yellowfin tuna from the Coral Sea, East Australia (eastern AFZ) and the Solomon Islands were detected. This is consistent with the proposal that yellowfin tuna spawning in the north west Coral Sea contribute to recruitment in the eastern AFZ.

On a wider Pacific scale, microsatellite data provided only weak support for population heterogeneity; no consistent genetic differences were detected across the entire Pacific Ocean. Only one locus of the five microsatellite loci showed significant heterogeneity of allele frequencies, largely attributable to small differences between the temporal Philippines samples.

These Philippines differences may reflect sampling artefacts rather than real differences. It is possible that there is some very weak population structuring within the Pacific that we did not detect, perhaps because the present sample sizes were too small to detect very small differences. Genetic differentiation between samples from the east coast fishery and other western Pacific areas was minimal, and the null hypothesis could not be unequivocally rejected (objective 2).

It appears therefore that there is sufficient gene flow throughout the Pacific to prevent the formation of genetically discrete populations, at least as can be ascertained with the markers and sample sizes that have been used so far. The finding of very limited population heterogeneity accords with most of the earlier allozyme and mitochondrial DNA studies of yellowfin tuna in the Pacific Ocean.

Otolith chemistry

We collected otoliths from young-of-the-year yellowfin at five locations in the western Pacific where yellowfin spawning is known to occur—the Banda Sea in Indonesia, the southern Philippines, northeast Solomon Islands, Fiji, and the northwest Coral Sea—and from two and three year old fish caught by the domestic fishery off the east coast of NSW and southern Qld. Our sampling design included collecting samples for two or three years at each site, to allow us to examine inter-annual variation in the chemical signal or natal area fingerprint. This wasn't possible in all areas due in some cases to inter-annual variation in recruitment of young fish and in other cases logistical problems prevented collection of samples. Despite these difficulties, we collected enough samples from each of the areas to examine the extent of variation in otolith chemistry in at least one year.

The variation in otolith chemistry was examined by measuring stable isotopes, using a method we adapted for yellowfin tuna otoliths (objective 3), trace elements and microconstituents in the otoliths. There was significant variation in the concentrations of many of the microconstituents among the different sampling locations from which we derived a 'natal fingerprint'. Perhaps not surprisingly, given the extent of inter-annual variability in the environment of sampling sites, we also found significant variation between years at sites we sampled on more than one occasion – i.e. Philippines, Indonesia and Solomon Islands. That finding meant that when looking at natal fingerprints we needed to take into account the cohort (the year in which a fish was born) as well as the area from which it came. Multivariate analyses – taking into account all of the variation within a site/cohort–allowed us to determine whether we could use the otolith chemistry data to characterize the different locations for any given year. Our analyses showed that we could correctly classify where fish from each of the natal sites had come from in 78% of cases for the 1994 cohort and 68% for the 1995 cohort – a very good result given the degree of overlap between sites in the concentrations of most elements.

We then used the otolith fingerprint data for each of the natal sites to classify fish from the same cohorts caught on the NSW coast as 2-3 year olds. The lack of replicate data for the Coral Sea and other natal sites limited these analyses to some extent. However, the chemical fingerprints of fish from the 1994 cohort caught on the NSW coast were predominantly classified (74%) into the Coral Sea group, with alternate classifications being to Fiji (11%) and the Philippines (15%). When the 1995 cohort was classified using data from the Solomon

Islands, Indonesia and the Coral Sea 1994 data set, a similar classification resulted – the majority of fish (63%) were classified to the Coral Sea, 27% to Indonesia and 10% to the Solomons. Without having sampled every possible yellowfin spawning site in the Pacific, it is not possible to say conclusively that the similarities in otolith chemistry between NSW and Coral Sea yellowfin indicate the latter serves as the primary source of recruits to the east coast fishery. However, having sampled a number of sites, over a broad geographic range, the strong similarity between these two, provides compelling evidence that the majority of recruits to the ECTUNA fishery come from the Coral Sea in the two years we sampled. That we saw a proportion of the fish with chemistry significantly different to that exhibited by Coral Sea fish, suggests that recruitment is also derived from immigrants spawned outside the Coral Sea. Thus, our original hypothesis that all recruits are derived from the Coral Sea is not supported (objective 4). The classification levels provide a crude indication of the proportions of immigrants to the fishery during the two years studied (objective 5).

Synthesis of all available data

We synthesized the new data on otolith microchemistry and genetics with existing data from a wide range of sources (scientific and recreational tagging studies; catch, effort and size data collected from throughout the region; information on spawning distribution in our zone and the possible influence of ocean currents in this region on the patterns of movement of larvae and recruits to the fishery) in an attempt to draw conclusions regarding the recruitment dynamics of yellowfin in the eastern AFZ.

Our syntheses of these data lead us to the conclusion that in some years the majority of the yellowfin caught on the NSW coast each year derive from localised spawning in the Coral Sea/northern Tasman Sea. In other years however, very high recruitment of 0-1 year-old fish along the Australian east coast indicate either a very strong year class of locally-spawned fish, or a large influx of fish into the Coral Sea from equatorial regions to the north (or perhaps both).

Tagging studies conducted in the equatorial Pacific and in areas adjacent to the Coral Sea rim in the 1970's and late 1980's/early 1990's released large numbers of tags into young yellowfin. Only two of these have ever been reported recaptured in the Coral Sea or Tasman Sea. Negative evidence is always difficult to interpret. However, the lack of recaptures provides no basis for accepting the hypothesis that equatorial fish move freely into our region. Tagging in the eastern AFZ suggest a high degree of residency of fish along the entire east coast, a finding consistent with the otolith chemistry data. Local and international tagging studies have shown that while yellowfin are capable of swimming long distances, the majority in fact travel relatively small distances while at liberty. Limited movement, combined with the regional oceanography, and observations of recruitment of yellowfin in the eastern AFZ in the form of discrete pulses of fish moving along the East Australian Current, all suggest the strong possibility of a semi-discrete population of yellowfin tuna in the Coral Sea/Tasman Sea.

To further examine the linkage between the local population and those of the neighbouring waters, and importantly to determine the levels of exploitation of the domestic fishery on this population, we recommend a conventional tagging study be initiated over the next few years, preferably in collaboration with the Secretariat for the Pacific Community Oceanic Fisheries Program and the Central and Western Pacific Tuna Commission. Ideally this study would

release tags over a number of consecutive years, allowing us to examine the hypothesis developed in this study that in some years there is an increased likelihood of movement into the eastern AFZ from equatorial regions and from outside the Coral Sea rim.

Finally, to provide a long term view of the patterns of recruitment to the east coast fishery, we recommend that the size monitoring program currently run on a year-to-year basis by AFMA is developed into a routine part of the monitoring and catch data collection for the fishery.

Keywords

Yellowfin tuna, stock structure, microsatellite, mitochondrial DNA, heterozygosity, genetic variability, population differentiation, otolith chemistry, stable isotope, tagging.

2 Background

The following was provided as background to this study in the 1994 FRDC application.

The yellowfin tuna, Thunnus albacares, is a pan-tropical species and the subject of important fisheries in the Indian, Pacific and Atlantic Oceans. In 1992, an estimated 431 000 metric tons were taken from the western, central and southern Pacific region monitored by the South Pacific Commission (Anon. 1993).

The Australian domestic yellowfin fishery was developed following the demise of the NSW SBT fishery in the early 1980's and the "discovery" (recreational fishermen had been enjoying the annual yellowfin runs for many years) that large and good quality yellowfin could be caught by trolling and small-scale longlining along the south coast of NSW. When Australian, "cold-water" yellowfin, began to command hitherto-unheard-of prices on the lucrative Japanese fresh sashimi market, the boom was on. In 1987, the AFS placed a moratorium on new endorsements in the ECTUNA fishery and today there are 292 endorsement holders, of which only 50-60 fish regularly (R. Miller AFMA pers. comm.). Throughout a broad cross-section of industry and management, there are now serious concerns about latent effort in the fishery.

Following the good catches of the 1980's, many operators, and in particular the longline fleet that take the bulk of the catch, invested heavily in the fishery. Bigger boats, more hooks, monofilament technology, satellite imagery and electronic aids all served to rapidly increase the effective effort in the fishery. At the same time, limits on the maximum size of boats permitted to fish in the historical fishing zone off southern NSW, an area in which catch rates have been high throughout the fishery, effectively restricted the domestic operations to inshore waters.

In offshore waters, a Japanese fleet, setting in the order of 7 million hooks per year (approximately 5 times more than the domestic fleet), takes 1500-3000 tonnes annually; 2-4 times the domestic catch.

The Australian domestic yellowfin tuna fishery extends from Cairns in north Queensland down the east coast to St Helens in Tasmania. With the exception of operations in Cairns, where yellowfin are taken throughout most of the year, the fishery is highly seasonal. In the 1990's it has concentrated on a "run" of small to medium-sized fish. These have been associated with southward incursion of the East Australian Current during spring and summer, and subsequent northwards retraction of the current during autumn and winter.

Anecdotal evidence (length records are not routinely kept in the fishery) suggests that since its beginnings in the mid-1980's, the average size of fish caught by domestic longliners has decreased markedly, to the point today where it would appear that the bulk of returns come from recruitment of one or two year classes - probably the 1 + and 2+ cohorts. With this history, and recent claims of localised depletion of east coast stocks, the identification of the source of recruits to the fishery and the broader delineation of stock structure in the region has become a high priority to scientists and managers alike (Ward 1990).

This requirement for information on the stock structure of yellowfin tuna in the AFZ led to FIRDC funding a one year pilot study in 1991/2 (FIRDC 91/27) aimed at determining the extent of genetic and otolith microchemical variability in yellowfin populations from different parts of the Pacific Ocean.

The genetic component of this pilot study developed appropriate allozyme and mitochondrial DNA (mtDNA) protocols for yellowfin, and then examined around 400 fish from a wide range of locations: Coral Sea, Philippines, Kiribati, Hawaii, California and Mexico (Ward et al. 1994). Major differences were detected for one locus (GPI-F) between central/western and eastern samples, implying strong reproductive isolation between the stocks of these two regions. No statistically significant differences were noted for the polymorphic allozymes and mitochondrial DNA variants between fish from the Coral Sea, Philippines and Kiribati. However, sample sizes per site were relatively small, ranging from 35 to nearly 100 per site, and small but biologically meaningful differences would not have been detected.*

The otolith microchemistry component of the pilot study examined the potential for the discrimination of yellowfin tuna spawning grounds using this new technique. For this purpose, the primordia (corresponding to the first few days of life) of otoliths from young-of-the-year yellowfin collected at sites throughout the Western Pacific were analysed for chemical variation. The objective was to determine whether fish from different spawning sites showed site-specific chemistry - i.e. were signals from Philippine fish different from those in the Coral Sea, Hawaii, or Indonesian fish? If so, this chemical "signature" for each site may be useful in determining the origin of fish at any time later in their lives.

Seven of 15 elements present in the otolith matrices exhibited significant variation among sites. The multivariate data were analysed using linear discriminant function analysis, kernal analysis and canonical variate analysis to examine the extent and nature of intra-and inter-site variation. These analyses showed clear separation of the sites with correct classification rates of approximately 80% and overall misclassification rates approximately 20%. These were excellent results bearing in mind the limited sample sizes. Perhaps the most interesting result was not the differences between sites, but the similarity between Hawaiian and Coral Sea samples. These two sites, both with semi-tropical locations, showed 30-40% misclassification rates, but varied very significantly from samples collected from all tropical sites. Our hypothesis for these differences between tropical and sub-tropical yellowfin is that they are most likely due to the temperature at which larvae were developing. If this is the case, observed differences may be indicative of a generalised latitudinal variation in chemistry. Such a signal would be extremely valuable in examining whether fish recruiting to the ECTUNA fishery are derived from the tropical spawning grounds of the Western Pacific, or from the more southerly Coral Sea.

The limited nature of the pilot study precluded examination of the temporal stability of the otolith chemical signals in yellowfin. This analysis would be a priority in the proposed follow-up study.

An additional source of information on the structure of Australian yellowfin stocks and their relationship to those of the broader Western Pacific is provided by the results of tagging

studies conducted by the South Pacific Commission's Regional Tuna Tagging Project (RTTP). Since 1990, over 40,000 yellowfin have been tagged by the RTTP throughout the tropical Western Pacific, including approximately 3,000 within the AFZ in the NW Coral Sea. Of 37,000+ yellowfin tagged outside the Coral Sea, one has been recaptured within the AFZ; a 135 cm fish tagged off New Britain and recaptured off Ulladulla. This contrasts with an average return rate of around 10% from throughout the region. Undoubtedly the low return rate within the AFZ reflects the low effort when compared with that placed in tropical regions. However, of the 3,000 yellowfin tagged in the Coral Sea, 42 have been recaptured, 80% of these after 30 days. 56% have been caught close to the points of release. However, of the 44% that have moved significant distances (>100 miles), none has moved outside of the Coral Sea, and four had moved south along the east coast of Australia.

The present application seeks to build on the pilot project in order to examine in depth the links between the Coral Sea spawning ground and other spawning grounds around the Coral Sea rim, and to determine the source of recruits to the Australian east coast tuna fishery. Again, both genetic and otolith microchemical techniques will be used.

The genetic techniques will use not only the allozyme and mtDNA methods developed in the pilot project, but will also examine a class of genetic variation, microsatellites, that has only recently been discovered. Microsatellites are non-coding stretches of DNA with high levels of variation and very high mutation rates. This latter feature makes them especially useful for population structure examination, as populations will diverge not only be genetic drift but also by mutation. The development of methods for microsatellite analysis is technically demanding and will be carried out in year one.

Similarly, the analysis of otolith chemistry will be extended to include the estimation of oxygen isotope ratios. During the first year of the study, this will involve adaptation of techniques used for marine invertebrates for use on otoliths.

Since the proposal and project were developed there have been a few changes in the yellowfin fishery off the eastern coast of Australia. The total catch of yellowfin has remained in the order of 2000 tonnes per annum. The domestic fishery has expanded very rapidly: in 1994 it was setting a little over one million hooks per year, by 2000 this had grown to approximately 10 million hooks. The number of vessels operating in the fishery has also more than doubled in this time, and the average number of days each vessel sets has increased. In 1998 Japanese vessels were excluded from the Australian Fishing Zone, and since that time all of the tropical tuna and billfish catch has been taken by domestic longliners.

Since 1994, the primary target and catch of the Eastern Tuna and Billfish fishery (ET&BF) has changed from yellowfin tuna, to a combination of swordfish, yellowfin and bigeye. Fishermen targeting bigeye and swordfish set at night using squid baits and light sticks and as a result tend to catch fewer yellowfin. The termination of the Japanese fleet catch and effort data series that began in the 1950's, and the major change in targeting within the domestic fishery, has made the interpretation of catch per unit effort trends difficult. In the absence of a full stock assessment for yellowfin there remain significant uncertainties over the status of stocks. Campbell (1998) in a major review of yellowfin catch and effort data for the SW Pacific

highlighted the need for information on the stock structure of yellowfin in our region. He pointed out that indicators of abundance on the Australian east coast appeared not to reflect patterns of recruitment and CPUE series in the greater Western Pacific, and hypothesized that the recruitment into the east coast fishery may rely on a combination of both localised and more equatorial spawning, with the latter taking place only intermittently.

Along the central and south coast of NSW recreational fishermen have found 30+kg yellowfin — their principal target — to have been extremely scarce for a number of years, prompting claims that the commercial fishery has fished these year classes out. The last strong recruitment of one-year-old yellowfin along the central and south-east coast was in a strong la Nina year — 1996.

Management of the ET&BF continues to be based on input controls, and to date there has been no cap placed on the catch or effort in the fishery. However, the extent to which the resource of yellowfin on the east coast of Australia is linked to the large populations in the Western Pacific remains a core management issue.

References:

Anon. (1 993), Status of tuna fisheries in the SPC area during 1992, with annual catches for 1922-1938 and 1952-1992. Working paper no. 2, Standing Committee on Tuna and Billfish. South Pacific Commission, Noumea, New Caledonia. 76pp

Ward, P. J. 1990. East Coast Tuna and Billfish Research and Monitoring Workshop. 15-16 March, 1990. Papers and summary of discussions. Bureau of Rural Resources.

Ward, R.D., Elliott, N.G., Grewe, P.M. and Smolenski, A.J. (1994). Allozyme and mitochondrial DNA variation in yellowfin tuna (Thunnus albacares) from the Pacific Ocean. Marine Biology, in press.

3 Need

This proposal was developed in response to a call from ECTUNAMAC for research into the structure of yellowfin stocks exploited in the east coast tuna fishery. ECTUNAMAC have assigned this topic their top priority. Briefly, the reasons for this are as follows:

Detailed assessments of the east Australian yellowfin tuna resource have not been conducted, and there are serious questions concerning the accuracy of log books used by AFS, and subsequently AFMA, to monitor catches in the fishery. However, the ECTUNA industry has for some years claimed that the resource that appeared so promising in the 1980's is showing signs of serious decline. A rapid decrease in the number of large, adult yellowfin taken along the east coast over the last five years, coupled with what fishermen have termed localised depletion of stocks, but is more likely to be a fish-down of the annual "runs" or recruitment pulses of juvenile fish, have caused concern. In response to these concerns, in 1990 the AFS reduced effort in the offshore Japanese longline fishery.

For many years, the generally accepted dogma regarding the east coast yellowfin resource was that it originated from the enormous tropical Western Pacific (WP) yellowfin stocks. These stocks support fisheries taking over 400,000 tonnes per year and recent assessments by the South Pacific Commission conclude that catches of one million tonnes annually across the region could be biologically sustainable (Hampton 1993).

Clearly the short history of the ECTUNA fishery, characterised by apparent fish-downs of adults and an annual exhaustion of recruitment pulses of juveniles associated with the East Australian Current, does not fit well with SPC assessments of an under-exploited resource in the tropical WP. These anomalies and the preliminary results of large-scale tagging programs by the SPC in the WP and the Coral Sea (discussed in section 8.3), pose some doubt that the model of a free flow of recruits from the WP into the AFZ is appropriate.

From the ECTUNAMAC/AFMA perspective, determination of the origin of recruits and the stock structure of yellowfin in the AFZ is a critical prerequisite for development of rational exploitation strategies. If, for example, the bulk of recruits to the ECTUNA fishery originate from spawning in the Coral Sea rather than from the broader WP, a very different management scenario would arise to that for a situation where the Australian fishery was based simply on an overflow of the “inexhaustible” tropical yellowfin stocks. Given the generally complex structure of many pelagic fishes, it seems unlikely that the ECTUNA situation will be simple. However, to clarify the issue, Appendix 1 provides a series of alternative stock scenarios for the east coast yellowfin, each of which will provide differing results in the proposed study, and necessitate specific management strategies if catches in the domestic fishery are to be maximised on a sustainable basis.

References:

Hampton, J. 1993. Status of tuna stocks in the SPC area: A summary report for 1993. SPC WP3, 6th Standing Committee on Tuna and Billfish, 53 pp.

4 Objectives

1. To develop the use of microsatellites for the genetic analysis of yellowfin tuna.

2. To determine the genetic relationships of yellowfin tuna from the Coral Sea to those from the east coast of Australia (mainly NSW), and to those from other Western Pacific fisheries (the Philippines, Solomons, Papua New Guinea and Indonesia) and thereby test the null hypothesis that there is a single panmictic WP stock.

3. To develop techniques for estimation of oxygen 18/16 isotopic ratios (currently used in bivalve research) in otoliths using a SIMS microprobe.

4. Using analysis of otolith chemistry and isotopic ratios, examine the hypothesis that all recruits to the ECTUNA fishery are derived from spawning grounds in the Coral Sea.

5. In the event of rejection of Ho, use otolith chemical data collected from yellowfin collected throughout the WP to estimate the proportion of immigrant recruits and determine their most likely origin.

5 Sample collection and handling

The total number of yellowfin sampled between 1994 and 1999 was 2032 (figure 5.1 and table 5.1.1). Although CSIRO scientists undertook some sampling, we were given valuable assistance by AFMA observers, fish processors, recreational and commercial fishers and scientists from international agencies. We gratefully acknowledge the cooperation and interest from these groups.

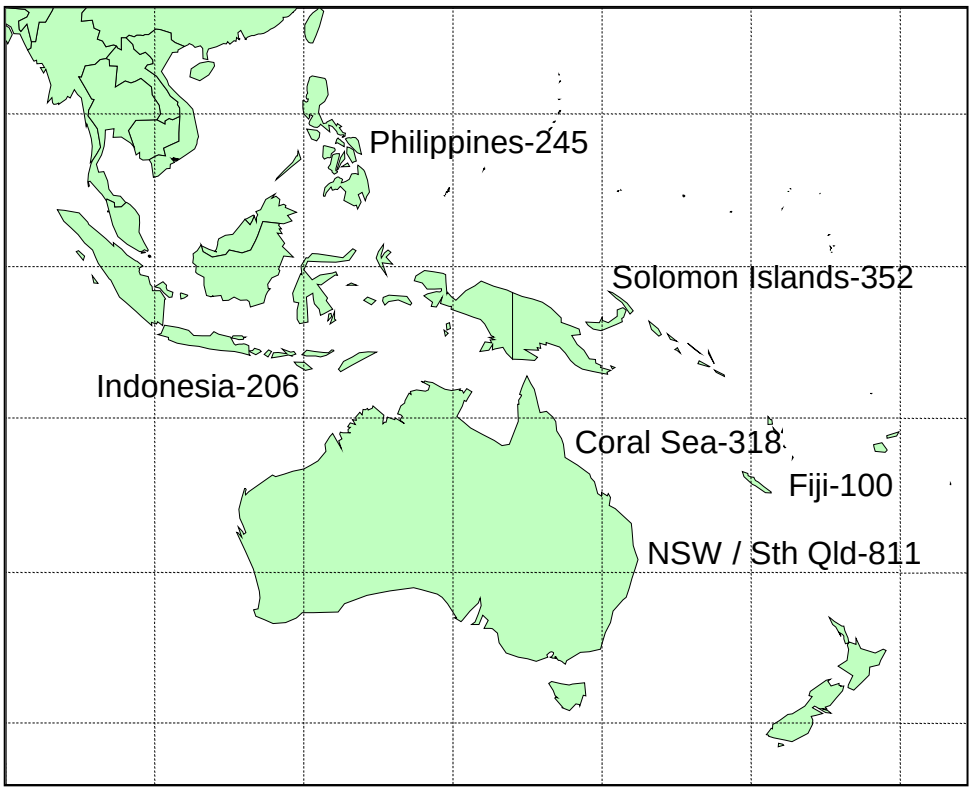

Figure 5.1. The number of fish sampled at each location

5.1 Sample Collection

Samples were collected from a range of tropical sites around the western Pacific: Fiji, Indonesia, Philippines and Solomon Islands, and from the Coral Sea. These areas were known yellowfin tuna spawning areas and at each location we aimed to collect 100 samples from fish estimated to be 0+ during the first year of the project. Older fish (estimated to be at least 1+) were collected from the eastern tuna and billfish fishery along the NSW/southern Queensland coast (which we will refer to as 'NSW samples'). We planned to repeat the collection in the second year to provide samples with which to assess temporal stability of genetic and otolith chemistry signals. In order to compare the otolith chemistry and genetics of cohorts — a group of fish spawned during the same season — we needed samples from fish spawned in the same years from every location. As we could only sample larger fish from the east coast we planned to sample at this location for a third year.

Because samples were to be collected in five countries over such a wide area a considerable

amount of time was spent organising the sampling logistics: who was to carry out the sampling and what remuneration they would receive; what equipment and training would be provided for sampling; and how samples would be sent to CSIRO. Sampling protocols were established and equipment, information sheets and data recording sheets were produced and a reward T-shirt designed for payment where money was not appropriate.

Overall, the sampling was a success (table 5.1.1). However, despite careful planning of the sampling program, we did not receive samples from some locations in the first year. During this 1994/95 season, samples from 4 of the 6 locations were collected successfully but we didn't receive samples from Fiji and Indonesia and the number of samples from the Coral Sea and the Solomon Islands was lower than we had anticipated. During the second year samples were collected from 5 sites; we didn't receive samples from the Philippines. In an attempt to acquire samples from at least 2 years from each location we extended the sampling beyond the originally planned period. Despite this, from one location, Fiji, we received samples from only one season. (Tables 5.1.1 and 7.2.1 give details of the number of otolith and genetics collected in each year of the project from each location. Note that genetics analyses included samples that had been collected in the early 1990s, prior to the commencement of this project). Sampling continued until the 1998/98 season in 2 locations, the Coral Sea and off the Australian east coast, from where the largest (oldest) fish were sampled.

Table 5.1.1. The samples collected for this project from 1994 to 1999. Additional samples for genetics analyses included samples that had been collected in the early 1990s (see table 7.2.1).

	Number of Samples Collected							
Sampling Location	**94/95**	**95/96**	**96/97**	**97/98**	**98/99**	**All Years**	**LCF (cm)**	**Approximate sampling positions**
Coral Sea	69	200	27	22	-	217	46-132	15°S 146°E
Fiji	-	100	-		-	100	51-61	18°S 178°E
Indonesia	-	100	106	-	-	206	20-45	3°S 128°E
Philippines	134	-	111	-	-	246	22-48	12°N 125°E
Solomon Islands	42	160	150	-	-	353	29-55	8°S 159°E
NSW/ Sth Qld Coast	141	79	212	338	41	813	49-165	30-36°S 150-153°S
Total	386	639	606	360	41	2032		

Two main reasons accounted for the failure to acquire the samples to complete the experimental design. Firstly fish were absent or in very low numbers at some locations during some years. When dealing with such a highly migratory species such as yellowfin, it is impossible to guarantee that sampling will always be possible; variable movements and recruitment can affect local availability. Such was the case in Fiji during 1996/97 —no 0+ yellowfin were caught

during that year. A low availability of 0+ in the Coral Sea meant that we could not follow the original experimental design; instead we sampled older fish from this area. Secondly the lack of samples was due to collectors being unable to sample at the time when fish were available or, if the samples were collected, not being able to transport them. This occurred in the Philippines during 1995/96; samples were collected from 100 fish but were lost in transit to CSIRO.

We recommend that any similar research planned in the future, in which large numbers of juveniles are required in a multi-year and multi-site study, take these factors into account. To ensure adequate numbers of samples are collected it would prudent to conduct sampling over several years, rather than restricting it to two consecutive years.

5.2 Handling of samples

The material required for genetic and otolith chemistry analysis posed challenges for the collectors. Firstly the white muscle tissue that was required for the genetics component of this study breaks down quickly if it is not preserved by freezing or preserved in alcohol solutions. A delay in preservation of the tissue decreases the power of the genetic techniques and, if the delay is extended, the tissue can't be used. Hence it was imperative that muscle samples were collected quickly and handled appropriately before they were dispatched to CSIRO for analysis (see section 7.2.1 for more details about the samples used in the genetic component of this study). The other samples required were sagittal otoliths — small bony structures that lie posterior to the brain. To successfully extract otoliths from the heads of fish takes some experience so we firstly sought staff experienced in extracting otoliths or we gave training in these extraction techniques. In one location, the Philippines, it was not possible to give the required training so whole fish heads were sent to CSIRO before the otoliths were sampled.

Figure 5.2.1. Otoliths sampled from yellowfin tuna heads.

Samples were sorted, catalogued and prepared for analysis at the CSIRO Marine Laboratories in Hobart. Each sample for inclusion in this project was assigned a unique identification number. This allowed linking of results from the two components of the study: genetics and otolith chemistry. At the size at which 0+ fish were sampled (as small as 22 cm), it was sometimes difficult to differentiate bigeye tuna and yellowfin tuna in the field. To ensure that

only yellowfin tuna were included for analysis a genetic test using mitochondrial DNA was performed on each sample to identify any non-yellowfin samples.

5.3 Data collection and storage

In addition to collecting the sample material, collectors were asked to record other information for each fish: details about the fishing operations and biological data — sex, length-to-caudal-fork (LCF) to the nearest cm and weight to the nearest kg. This information was entered into our relational database along with details about the condition of the samples, how they were prepared for analysis and the data acquired from analyses (see Appendix B for a display of the database table structure). The original Helix Express database has been superseded by an Oracle database so that data derived from this project is now linked to the CSIRO Hard Parts Archives. Regular access to this data is restricted to CSIRO staff working in the Pelagic Ecosystems group, and is protected by the requirement of a user code and password. The information is archived using a centralised daily back-up system ensuring long-term secure storage of the data.

The raw genetics data (microsatellite scans and genotype information), statistical analysis of all data and report preparation for the genetics component of this report are stored on both zip disks and CD's located within the genetics laboratory at CSIRO Marine Research (CMR). The information technology group at CMR has also backed up externally all information.

5.4 Assigning individual fish to a cohort

In order to compare the otolith chemistry and genetics of cohorts we needed to assign individual fish to their cohort using an approximate birth date, i.e. when the fish had been spawned. To determine the birth date we needed to estimate the age of individual fish when they were caught. This age-at-capture was estimated by converting length measurements to age using growth equations derived from studies of western Pacific yellowfin tuna (tables 5.4.1 and 5.4.2). Yamanaka's (1990) study estimated ages from counts of microincrements on sagittal otoliths. Yabuta *et al.* (1960) used the von Bertalanffy equation with parameters derived from the results of an age determination study using scales.

Table 5.4.1. The growth equations used to calculate age from length.

LCF (cm)	Growth equation	Source	Method
up to 35 cm	$LCF = 0.25 \times \text{age}(\text{days}) + 5.87$	Yamanaka (1990)	otoliths
35 – 80 cm	$LCF = 0.096 \times \text{age}(\text{days}) + 22.42$	Yamanaka (1990)	otoliths
greater than 80 cm	$L_t = L_\infty(1 - \exp[-k(t - t_0])$, where t = age (years), k = 0.33, L_∞ = 190, t_0 = 0	Yabuta *et al.* (1960)	scales

The length-at-age calculated from these equations is shown in table 5.4.2 and compared with results from studies of eastern Pacific yellowfin tuna (Wild 1986).

Table 5.4.2. Length-at-age calculated from Yamanaka (1990) and Yabuta *et al.* (1960), compared with Wild (1986) lengths-at-age for studies of eastern Pacific yellowfin tuna.

Age (years)	Yamanaka and Yabuta *et al.*, 1960	Wild, 1986 (eastern Pacific)
1	57	49
2	89	89
3	119	127
4	139	154

An estimate of birth date was back-calculated using the catch date and calculated age. From this birth date, fish were assigned to a cohort. Samples from some locations fell into obvious cohorts (Figure 5.4.1) however cohorts from NSW and the Coral Sea were not as evident.
There were some uncertainties in using this method of assigning individuals to cohorts because of inaccuracies introduced when calculating age and birth dates. These were due possibly to:

- Estimating the catch date. Mostly, sampling was carried out at the landing ports and when catch date wasn't known we used the date of sampling as an estimate of catch date. However, the discrepancy between catch date and landing date was estimated to be less than a week in all cases.
- Estimating LCF. In some cases (13 fish from the Coral Sea), fish were weighed but not measured, so an estimate of length was made using a length-weight conversion (Morita 1973). In some other cases (30 fish from NSW and 22 fish from the Coral Sea), neither lengths nor weights were measured. Heads were removed from the body and kept aside for later sampling, so we estimated LCF from measurements of snout to operculum, a standard measurement used regularly in taxonomic studies.

Further inaccuracies in assigning individuals to a cohort were possibly introduced from individual variation in growth rates, which are not accounted for in growth equations. The likelihood and extent of individual variation in length-at-age increases as fish get older hence we would expect the greatest differences in individual growth in the largest (oldest) fish in our sample set, i.e. fish from NSW/southern Qld and Coral Sea. In the other locations, from where we had sampled relatively small fish, the distribution of birth dates fell in discrete peaks providing evidence for spawning periods lasting several months. Conversely, there was more spread in the distributions of birth dates of fish from NSW/southern Qld and Coral Sea. This may be explained by a difference in growth rates — a group of fish spawned at the same time would not be expected to have identical growth rates and hence would reach different lengths at the time they were caught. Applying these lengths to growth equations results in a variety of ages spread around the true birth date.

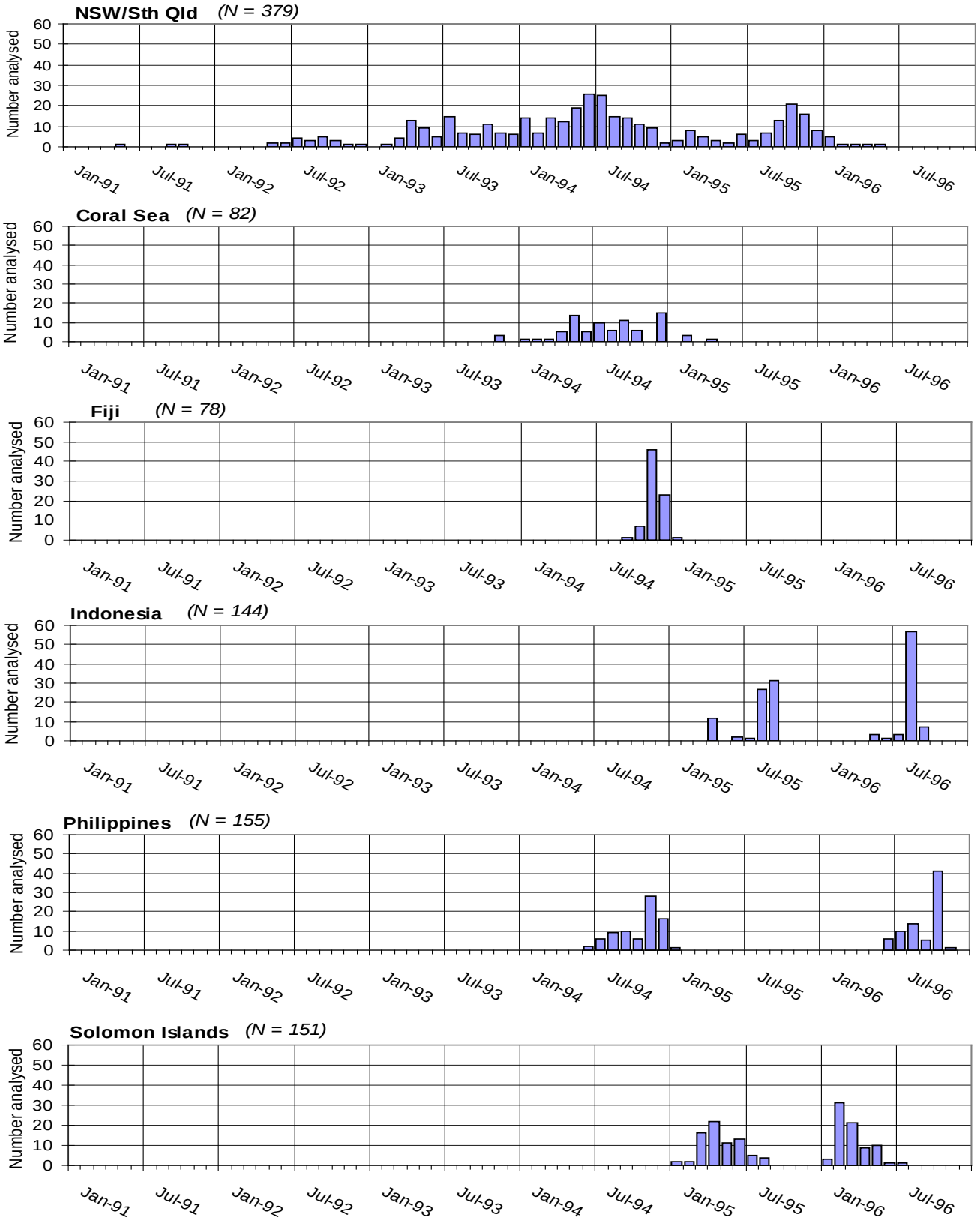

Figure 5.4.1. Distribution of calculated birth dates of yellowfin tuna sampled.

6 Genetics

6.1 Background

Morphometric studies have demonstrated variation in yellowfin stocks from Japan, Australia, Hawaii, Eucador and Mexico (Schaefer 1991). Size and catch composition of longline and purse-seine landings of yellowfin tuna within the Pacific also suggest semi-independent populations of equatorial (Kamimura and Honma 1963) and eastern, central and western Pacific (Suzuki *et al.* 1978) sub-populations of yellowfin. In addition, otolith microchemical variability analysis detected significant geographic variations in chemical signatures of the primordial region of 111 yellowfin tuna from four western and central Pacific Ocean localities (Gunn and Ward 1994).

While the above evidence suggests that yellowfin tuna does demonstrate population structure within the Pacific Ocean, various genetic analyses have given inconsistent results. Transferrin and esterase variation indicated no spatial heterogeneity in yellowfin stocks (Barrett and Tsuyuki 1967; Fujino and Kang 1968). Sharp (1978) demonstrated that GPI polymorphisms revealed significant differences between two eastern Pacific and one western Pacific yellowfin stock. This was confirmed by Ward *et al.* (1994), with the most common *GPI-A** allele in western and central region differing from that in the eastern region. However, three other polymorphic allozyme loci (*ADA**, *FH**, and *GPI-B** *)* showed no differentiation. Another allozyme, *GDA** , was only examined in Hawaii, Coral Sea and Philippines samples; it showed very weak evidence of differentiation following chi-square analysis but not following a boot-strapped analysis of G_{ST} values.

As these studies demonstrate, variation in the yellowfin tuna nuclear genome has up to now been assessed through allozyme electrophoresis. MtDNA variation has also been examined in two studies (Scoles and Graves 1993; Ward *et al.* 1994); neither revealed significant spatial differentiation in the Pacific Ocean.

Since these studies, new DNA analysis techniques have been described, and some of these are potentially more powerful than allozyme and mtDNA methods for studying levels of genetic variation within and among populations. Foremost among these are microsatellites.

Microsatellites are tandem repeats of short sequence motifs which are distributed randomly and believed to be abundant in all eukaroytic genomes (Tautz 1989). Polymorphisms at microsatellite loci were first demonstrated by Tautz (1989) and Weber and May (1989). Microsatellite allelic variation is assessed from the different electrophoretic mobility of PCR products which display length polymorphisms (Weber and May 1989; Brooker *et al.* 1994).

Microsatellite loci typically show high levels of variation in fish, and in recent years have become popular in studies of fish stock structure (see O'Connell and Wright 1997; DeWoody and Avise 2000 and references within). They show high levels of genetic variation and high mutation rates; meaning that populations are likely to diverge not only by genetic drift but by mutation as well.

Objectives for the current genetic research included developing microsatellites for the genetic analysis of yellowfin tuna and determining the genetic relationships of yellowfin tuna from the Coral Sea to those from the eastern coast of Australia and other Western Pacific fisheries (e.g., Indonesia, Philippines, Fiji). These genetic results would allow the testing of the null hypothesis of a single panmictic yellowfin tuna stock in the western Pacific.

6.2 Methods

6.2.1 Tuna Population Sampling

Samples from identified cohorts were collected around the western Pacific Ocean from 1991 to 1998. Samples from fish estimated to be 0+ were collected from the Coral Sea, Fiji, Indonesia, Philippines and Solomon Islands. Fish estimated to be 1+ were collected from southern Queensland and New South Wales (the Australian east coast fishery, subsequently referred to as East Australia) throughout the project. Two smaller samples of fish from the eastern Pacific, California and Mexico, were used as outlying populations; they were collected in 1992. Samples that formed part of the genetic analysis are outlined in table 6.2.1 and locations given in Figure 6.2.1. Samples consisted of pieces of white muscle dissected from whole fish and stored either frozen at -20^0C or in alcohol preserving solutions (and also stored at -20^0C) until DNA extraction.

Table 6.2.1. Populations, sampling locations (approximate longitude and latitude) and sample sizes of yellowfin tuna examined for five microsatellite loci.

Population	Sample Sizes					
	1991-1992	1994-1995	1995-1996	1996-1997	1997-1998	Total
California	34 33^0N, 117^0W	–	–	–	–	34
Coral Sea	96 15^0S, 146^0E	–	192 14-19^0S, 145-154^0E	–	–	288
East Australia	–	118 30-36^0S, 150-153^0E	50 30-33^0S, 151-153^0E	126 30^0S, 151^0E	266 30^0S, 151^0E	560
Fiji	–	–	96 18^0S, 178^0E	–	–	96
Indonesia	–	–	–	94 3^0S, 128^0E	–	94
Mexico	40 29^0N, 117^0W	–	–	–	–	40
Philippines	–	95 12^0N, 125^0E	–	96 no details	–	191
Solomon Is.	–	–	95 8^0S, 159^0E	–	–	95
Total Fish	170	213	433	316	266	1398

Genetic analysis involved the use of mitochondrial DNA for species identification (identifying individuals as yellowfin/non-yellowfin tuna) and nuclear DNA microsatellites for population discrimination. For both approaches, total genomic DNA was extracted from approximately 50mg of frozen or alcohol stored tissue from each individual using a modified CTAB (hexadecyltrimethylammonium bromide) extraction protocol described in Grewe *et al.* (1993). After precipitation with isopropanol and ethanol, genomic DNA pellets were resuspended in 150-200μl of deonized H_2O and stored at 4^0C. Stock DNA was diluted to a 1:10 ratio for future working applications for both mtDNA and microsatellite analyses

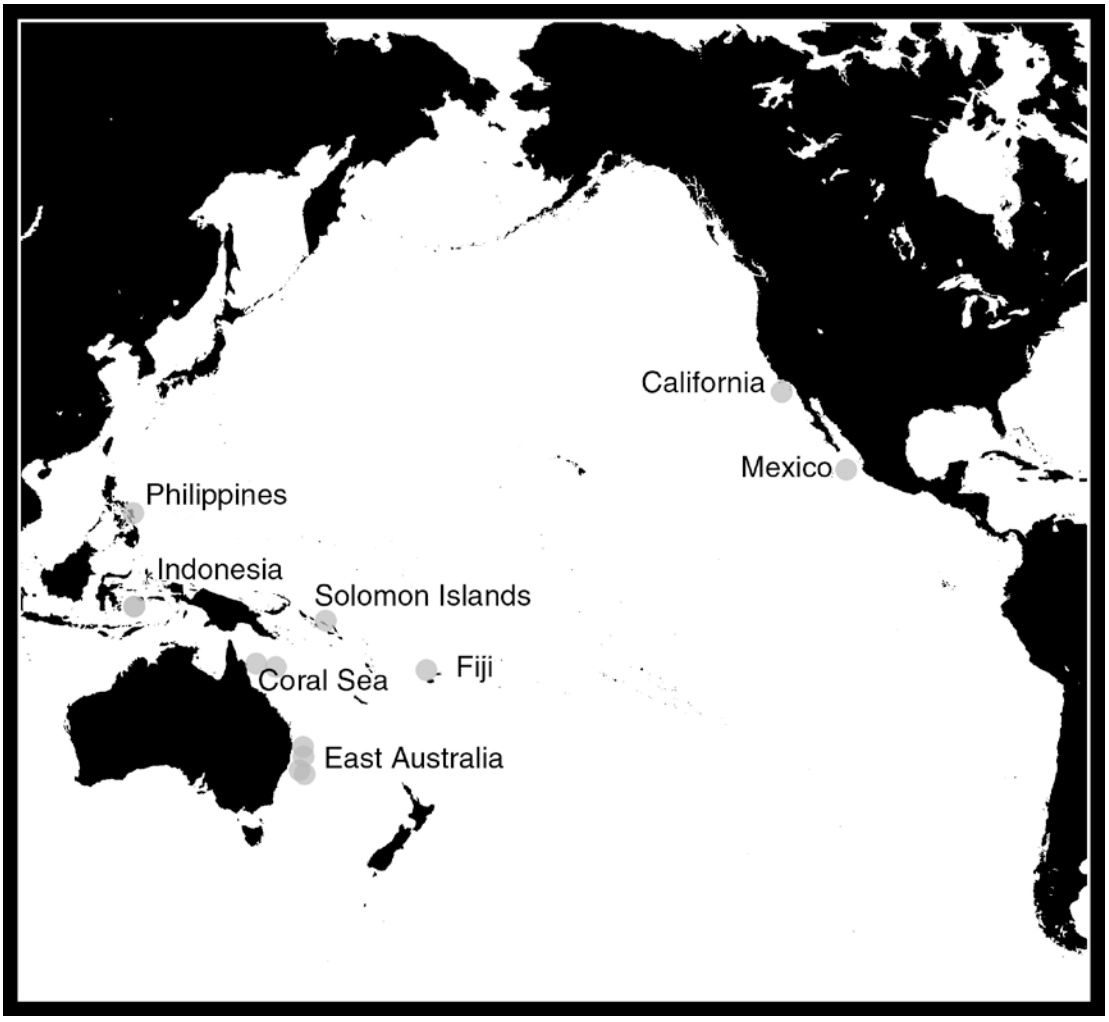

Figure 6.2.1. Yellowfin tuna populations within the Pacific Ocean sampled for genetic analysis.

6.2.2 MtDNA Haplotype Analysis

MtDNA variation for the identification of yellowfin tuna was examined through restriction digestion of the amplified ATCO fragment. This fragment contains the flanking region between the ATPase-6 and cytochrome oxidase subunit III genes and was amplified using primers (LAT6 (L8562) 5'- CTTCGACCAATTTATGAGCCC-3' and HCOIII (H9432) 5'-GCCATATCGTAGCCCTTTTTG-3') described by Chow and Inoue (1993). PCR amplifications were performed in a PE-Applied Biosystems 9600 thermocycler in a total volume of 50μl. Individual amplifications consisted of 200μM dNTP's, 10mM TrisHCL pH 8.3, 50mM KCL, 1.5mM $MgCl_2$, 0.2μM HCOIII & LAT6, 0.025μ/μl Amplitaq Gold (Perkin Elmer) and 10μl of the 1/10 dilution of genomic DNA.

After an initial cycle of 93^0C × 10 minutes, 60^0C × 45 seconds and 72^0C × 2 minutes, samples were subjected to 93^0C × 30 seconds, 55^0C × 45 seconds and 72^0C × 2 minutes for 40 cycles with a final extension step of 72^0C × 10 minutes. Resulting PCR products were directly subjected to restriction endonuclease digestion with the enzyme *MseI* (New England Biolabs, 4000U/μl) to confirm species identity. 10μl of PCR product was added to buffer, BSA (bovine serum albumin) and 0.4μl of the enzyme and incubated at 37^0C for 90 minutes. Fragments were then run on a 2% 1X TBE agarose gel (containing ethidium bromide) at 120 volts for 80 minutes. A Promega 100 base pair ladder was loaded on the gel to enable sizing of various fragments. Resulting fragments were visualised under UV light and photographed using a digital camera.

Individuals that were identified as yellowfin tuna were used in the microsatellite analysis; others were excluded from further analysis.

6.2.3 Microsatellite DNA Analysis

Six DNA microsatellite loci examined in this study were isolated from yellowfin tuna (Grewe, unpublished data). PCR primers were designed for flanking regions around the microsatellite repeats (table 6.2.2). Oligonucleotides were synthesised by Bresatec Pty Ltd (Adelaide, South Australia) with one of the primer pairs end-labelled with a fluorescent tag; FAM, TET or HEX. The six loci are designated *cmrTa-113*, *cmrTa-117*, *cmrTa-125*, *cmrTa-144*, *cmrTa-161* and *cmrTa-208* (*cmr*=CSIRO Marine Research, *Ta=Thunnus albacares*), but are referred to hereafter as *113*, *117*, *125*, *144*, *166* and *208*. Due to the large number of alleles present and a difficulty in consistently scoring samples collected later in the study, *117* was not used in the final analyses.

In the development phase, individual microsatellite loci were amplified separately in a subsample of individuals. These were then run on an ABI377 DNA autosequencer (PE Applied Biosystems, England) for three hours to check for overlapping allele sizes and colours. The five loci (excluding *117*) were then optimised for use in a single multiplex reaction where all five pairs of primers were added to a single PCR reaction. PCR amplifications were performed in a PE-Applied Biosystems 9600 thermocycler (PE Applied Biosystems, England) in a total volume of 25μl. Individual amplifications for samples identified as yellowfin tuna consisted of 100μM dNTP's, 10mM TrisHCL pH 8.3, 50mM KCL, 2.5mM $MgCl_2$, 0.8μM for each forward

and reverse primer, 0.05U/μl Amplitaq Gold (Perkin Elmer) and 10μl of the 1/10 dilution of genomic DNA. After an initial cycle of 93^0C × 10 minutes, 55^0C × 15 seconds and 72^0C × 2 minutes, samples were subjected to 93^0C × 15 seconds, 54^0C × 15 seconds and 72^0C × 2 minutes for 35 cycles with a final extension step of 72^0C × 10 minutes.

Table 6.2.2. Microsatellite motif, primer sequences, number of alleles observed and allele size of yellowfin microsatellite loci. The motif listed is that obtained from the original yellowfin sequence used to generate amplification primers.

Locus	Motif	Primer sequences	Number of alleles	Allele size range[a]
cmrTa -113	$(CA)_{12}$	5'- CAT ATT GTC TGC ATC TGA AAA CTG –3'	25	107-155
		5'- CAT CCT CCT GCT TGA ACT GA –3'		
cmrTa-117	$(CA)_{12}$	5'-TCA CAG CAT GGG ACA AC–3'	36	150-220
		5'-ATA GTG AAA TGA TTA GAA CAG TGC–3'		
cmrTa-125	$(CA)_{10}$	5'- TTG GGC TGC CTG ATG AAG –3'	13	148-176
		5'-GTG TCT CTG AAA TGA TGG AAA CA –3'		
cmrTa-144	$(CA)_6$	5'- TCC TCA TTT AGA AAG CCA CTG TA –3'	7	166-178
		5'- ACC TGT TGA TTA TTG CTT TTA TGT –3'		
cmrTa-161	$(CA)_{19}$	5'- CAG TAT TTT CTC ATG GAT ACC AGC AC –3'	30	180-238
		5'- GAT TTC GTG CAG CCT TGT GCA G –3'		
cmrTa-208	$(CA)_{10}$	5'- CAC AAA CTT CCT CTT AAA CCG ATC ATG –3'	10	135-153
		5'- GAT GTA TGG AAA GCA GGG GAC TG –3'		

[a]sizing in base pairs

Amplified products were used undiluted and mixed with a formamide loading dye containing ABI Prism GeneScan350 Tamra internal lane size standards (PE Applied Biosystems, England) and blue dextran loading dye, denatured at 94^0C × 2 minutes, and immediately placed on ice. 1.3μl of sample was stagger loaded into a 4.8% 6M urea denaturing polyacrylamide gel and run using 1X TBE buffer on the ABI Prism 377 DNA autosequencer for three hours. PCR products for each locus were analysed using GENESCAN™ 3.1 collection software (PE Applied Biosystems) and local southern size calling method. Genotyping at each locus was completed using GENOTYPER™ 1.1.1 software (PE Applied Biosystems) which enabled the formation of approximately two base pair bins for each locus. Bin widths generally corresponded to a repeat unit.

6.2.4 Statistical Analysis

Levels of genetic variation for each yellowfin tuna population were assessed as the mean number of alleles per locus and the number of polymorphic loci. Allele frequencies were compiled using BIOSYS-1 (Swofford and Selander 1981).

Tests of microsatellite allele frequency homogeneity between temporal populations and among spatial populations were assessed using the Monte-Carlo chi-square program CHIRXC (Zaykin and Pudovkin 1993). Such methods (Roff and Bentzen 1989) obviate the need to pool rare alleles. The null hypothesis was that samples belonged to the same gene pool and thus did not differ significantly in allele frequencies. The first tests involved temporal samples from the

same spatial area; if these did not differ significantly in allele frequency they were pooled. Subsequently different spatial areas were compared together. If significant heterogeneity was demonstrated, populations were compared pairwise using CHIRXC to locate the source of the heterogeneity.

Observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}) and tests for deviations from Hardy Weinberg Equilibrium (HWE) within samples were estimated using the Monte-Carlo based program CHIHW (Zaykin and Pudovkin 1993). As well as testing goodness-of-fit, this program also computed an index of heterozygote deficiency or excess D, where $D=[H_0-H_e]/H_e$ (H_0=observed heterozygosity and H_e=expected heterozygosity). HWE was tested on temporally pooled samples if no significant variation in allele frequency at any of the five loci was detected.

The CHIHW and CHIRXC programs used 5000 randomisations of the data to give 5000 resultant randomised χ^2_{null} values. The total number of times these randomised replicates was greater than or equal to the observed χ^2_{null} value, divided by 5000, gave an estimate of the probability of obtaining the observed χ^2 value by chance.

The AMOVA (Analysis of Molecular Variance) procedure developed by Excoffier *et al.* (1992) was also used to measure population differentiation. The proportion of gene diversity within and among yellowfin populations was estimated using ϕ_{ST}, an analogue of F_{ST}. ϕ_{ST} is obtained as the ratio of the estimated variance components due to differences among populations, calculated over the estimated total variance (Michalakis and Excoffier 1996). For the current study, ϕ_{ST} values are equivalent to F_{ST} values, when equal distances among haplotypes are assumed. The significance of each F_{ST} value was tested by comparison with a null distribution of random sampling from the global population and was based on 16000 re-sampling trials.

In addition, genetic distances among populations were assessed with Nei's (1978) unbiased genetic distance (a value of 0 = total similarity, value of 1 = total dissimilarity) and Cavalli-Sforza & Edwards (1967) chord distance. Genetic distance matrices were converted to a dendogram of cluster analysis by UPGMA (unweighted pair-group arithmetic averaging) using BIOSYS-1 (Swofford and Selander 1981). This analysis assumes a constant rate of evolution.

In all cases with multiple tests, a conservative but standard Bonferroni procedure was adopted (Miller 1980, Lessios 1992). The critical α value of 0.05 was divided by the number of tests performed to give an adjusted significance level of α_b. *P* values had to be equal to or less than this adjusted value to be deemed significant.

6.3 Results

All tuna sampled for this study were examined for variation of the ATCO fragment to determine species identity. Examination of the ATCO fragment revealed patterns diagnostic for yellowfin and bigeye tuna. The yellowfin haplotype consisted of bands of approximately 270, 230, 195 & 120 base pairs in size, whereas the bigeye haplotype had bands of 300, 230, 195 & <100 bp. Eight fish identified as bigeye tuna (one, Coral Sea 91-92; one, East Australia 94-95; five, Philippines 94-95; one, Indonesia 96-97) were excluded from further analysis.

Allele frequencies at the five microsatellite loci for confirmed yellowfin tuna are given in Appendix 3. Seven (locus *144*) to thirty (locus *161*) alleles were detected at the five loci. Loci *125, 144* and *208* produced relatively "clean" banding patterns, generally free of subbanding or "stuttering". Locus *113* and in particular locus *161*, were however prone to stuttering. Dinucleotide repeats, as used in the current study, are often characterised by stuttering. Stuttering is thought to be caused by slipped strand mispairing during PCR (Tautz 1989). This laddering of bands results in difficulties in allele scoring and while minimised using fluorescent labelled primers and analysis on the ABI377 DNA sequencer, was still a problem in the current study. Of the five loci, locus *161* produced the largest number of band stutters which could have led to inaccurate allele scoring in some instances.

Tests of microsatellite allele frequency homogeneity at the five loci between temporal populations (i.e., populations sampled more than once over time) formed the first statistical tests. As table 6.3.1 demonstrates, only Philippines 94-95 and Philippines 96-97 showed significant heterogeneity at locus 161 ($P<0.001$), even after Bonferroni correction of α levels. They continued to be treated as separate populations. Allele frequencies in all other temporally-separated population samples (Coral Sea 91-92 & Coral Sea 95-96; East Australia 94-95, 95-96, 96-97 & 97-98) were homogenous; these populations were combined to form a pooled Coral Sea population (91-96) and a pooled East Australia population (94-98).

The two small and spatially closely-linked samples from California and Mexico were tested for heterogeneity in a similar fashion (table 6.3.1); no significant differences were found and they were pooled for subsequent analyses (91-92).

Genetic diversity for each population (after pooling as above) was estimated by the numbers of alleles per loci and observed and expected heterozygosity per locus and per population (table 6.3.2). All five microsatellite loci examined were highly polymorphic in all populations. The less variable loci, *125* and *144*, had more common allele of higher frequencies, while the more variable loci, *113* and *161*, did not have any alleles with frequencies greater than 0.300 (Appendix 3). Locus *161* had the highest number of alleles present in all populations amongst the loci (mean of 26 alleles) while locus *144* showed the lowest number of alleles (mean of 3.37 alleles). Not unexpectedly, locus *161* also had the highest observed heterozygosity in all populations (0.833-1.000) while locus *144* was characterised by the lowest observed heterozygosity (0.168-0.316). Most alleles at all loci were present in all populations. There were no fixed or diagnostic loci (i.e., relative frequency of >0.100) among the tuna populations. Observed and Hardy-Weinberg expected heterozygosities per locus ranged from 0.230 to 0.936 and 0.246 to 0.915 respectively (table 6.3.2).

Overall, total numbers of alleles per locus ranged from 7 to 30 with an average of 17 alleles/locus across the populations (Appendix 3). The mean number of alleles per locus ranged from 11.2 (Fiji 95-96) to 15.8 (East Australia 94-98) (table 6.3.3). Mean number of alleles per locus was highly correlated with sample size ($r=0.926$, $P<0.01$). This is unsurprising given that many alleles are rare and the larger the sample size the more rare alleles are expected to be detected.

Nei's (1978) unbiased estimate of total expected heterozygosity per locus under Hardy-

Weinberg expectations for each of the populations ranged from 0.607 (Solomon Islands 96) to 0.649 (Philippines 94-95) (table 6.3.3) with an average Hardy-Weinberg expected heterozygosity level of 0.619 across the eight populations.

Table 6.3.1. Homogeneity χ^2 analysis for comparisons of microsatellite allele frequencies in temporal yellowfin tuna populations, bolded *P* values are significant after probability of H_0 calculated from 5000 Monte Carlo runs, critical *P*=0.01[b]

Locus	Total fish	Population[a]	Population	Population	Population	χ^2	*P*
113	287	Coral Sea 91-92	Coral Sea 95-96			26.284	0.190
113	553	East Aust 94-95	East Aust 95-96	East Aust 96-97	East Aust 97-98	82.277	0.085
113	187	Philippines 94-95	Philippines 96			18.500	0.591
113	74	California 91-92	Mexico 91-92			10.954	0.819
125	288	Coral Sea 91-92	Coral Sea 95-96			15.158	0.038
125	554	East Aust 94-95	East Aust 95-96	East Aust 96-97	East Aust 97-98	29.040	0.657
125	187	Philippines 94-95	Philippines 96			12.356	0.123
125	74	California 91-92	Mexico 91-92			7.932	0.197
144	287	Coral Sea 91-92	Coral Sea 95-96			2.412	0.484
144	191	Philippines 94-95	Philippines 96			3.236	0.346
144	74	California 91-92	Mexico 91-92			0.262	0.771
161	283	Coral Sea 91-92	Coral Sea 95-96			34.964	0.123
161	553	East Aust 94-95	East Aust 95-96	East Aust 96-97	East Aust 97-98	90.947	0.199
161	187	Philippines 94-95	Philippines 96			50.780	**0.003**
161	74	California 91-92	Mexico 91-92			30.092	0.181
208	287	Coral Sea 91-92	Coral Sea 95-96			5.415	0.618
208	558	East Aust 94-95	East Aust 95-96	East Aust 96-97	East Aust 97-98	37.058	0.100
208	191	Philippines 94-95	Philippines 96			12.788	0.041
208	74	California 91-92	Mexico 91-92			4.446	0.676

[a]East Aust = East Australia
[b]after Bonferroni correction for multiple tests

Genotype proportions in each population for each locus were tested for goodness-of-fit to Hardy Weinberg expectations (table 6.3.2). There were eight tests (eight populations) of goodness-of-fit for each locus. If the number of tests was taken as eight for each locus, then all genotype frequencies at loci *113*, *125*, and *161* conformed to Hardy Weinberg expectations, while genotype frequencies in locus *144* in Philippines 96, Coral Sea 91-96 and East Australia 94-98, and in locus *208* in Coral Sea 91-96 and East Australia 94-98, were significantly different from that expected (corrected α_b significance value of 0.05/8=0.006). If however a more conservative approach is taken and the significance level is adjusted by the total number of comparisons undertaken (corrected α_b significance value of 0.05/40=0.001), only locus *208* in Coral Sea 91-96 remains significant. There was a small but significant excess of homozygotes in this sample (D=-0.052). There was some evidence of a generalised homozygote excess. Of the total of 40 tests, 30 showed a homozygote excess, 10 a homozygote deficiency. This is statistically significant (χ^2=10, df=1, *P*=0.002). The overall heterozygote deficiency was about 2.5%. This may have a variety of causes including the presence of (rare) non-amplifying alleles, typing errors, and mixing of genetically discrete populations (the Wahlund effect).

Table 6.3.2. Summary of genetic variability data per locus in each yellowfin tuna population

Population		Loci *113*	*125*	*144*	*161*	*208*
Cal/Mex 91-92	N[a]	74	74	73	73	73
	Nallele	16	7	2	26	6
	H_{obs}[b]	0.743	0.581	0.178	1.000	0.466
	H_{exp}[b]	0.878	0.501	0.162	0.927	0.549
	$D_{Selander}$	-0.153	0.161	0.098	0.079	-0.152
Coral Sea 91-96	N[a]	287	288	287	283	287
	Nallele	22	10	4	28	8
	H_{obs}[b]	0.814	0.451	0.214	0.855	0.516[*]
	H_{exp}[b]	0.882	0.494	0.278	0.926	0.544
	$D_{Selander}$	-0.077	-0.087	-0.231	-0.078	-0.052
East Aust 94-98	N[a]	553	554	559	553	558
	Nallele	23	12	5	29	10
	H_{obs}[b]	0.829	0.481	0.223	0.853	0.486
	H_{exp}[b]	0.876	0.489	0.258	0.933	0.517
	$D_{Selander}$	-0.053	-0.017	-0.134	-0.086	-0.060
Fiji 95-96	N[a]	90	88	89	87	92
	Nallele	15	7	4	23	7
	H_{obs}[b]	0.789	0.466	0.269	0.833	0.467
	H_{exp}[b]	0.881	0.541	0.275	0.912	0.472
	$D_{Selander}$	-0.104	-0.138	-0.019	-0.086	-0.011
Indonesia 96-97	N[a]	92	68	94	96	92
	Nallele	17	6	3	25	8
	H_{obs}[b]	0.844	0.412	0.202	1.000	0.532
	H_{exp}[b]	0.858	0.506	0.203	0.875	0.571
	$D_{Selander}$	-0.017	-0.186	-0.001	0.143	-0.067
Philippines 94-95	N[a]	91	94	95	93	95
	Nallele	20	9	4	25	7
	H_{obs}[b]	0.725	0.543	0.316	1.000	0.568
	H_{exp}[b]	0.868	0.578	0.282	0.903	0.589
	$D_{Selander}$	-0.164	-0.061	0.121	0.108	-0.035
Philippines 96-97	N[a]	96	96	96	96	96
	Nallele	19	6	3	27	6
	H_{obs}[b]	0.896	0.489	0.271	0.947	0.458
	H_{exp}[b]	0.881	0.515	0.323	0.907	0.474
	$D_{Selander}$	0.017	-0.049	-0.162	0.044	-0.033
Solomon Is 95-96	N[a]	95	95	95	96	95
	Nallele	18	6	2	25	6
	H_{obs}[b]	0.800	0.305	0.168	1.000	0.568
	H_{exp}[b]	0.881	0.475	0.188	0.918	0.552
	$D_{Selander}$	-0.092	-0.358	-0.106	0.090	0.030
Mean	N[a]	172.25	169.65	173.50	172.12	173.50
	Nallele	18.75	7.87	3.37	26.00	7.25
	H_{obs}[b]	0.805	0.466	0.230	0.936	0.508
	H_{exp}[b]	0.875	0.512	0.246	0.913	0.533

[a]N=total number of fish, [b]H_{obs}=observed heterozygosity, H_{exp}=expected heterozygosity under Hardy-Weinberg expectations
[*]significant after Bonferroni correction for total number of comparisons.

Table 6.3.3. Genetic variability at five loci in all yellowfin tuna populations (standard errors in parenthesis)

Population[c]	Mean sample size per locus	Mean number alleles per locus	% of poly loci[a]	Hardy-Weinberg observed	Hardy-Weinberg expected[b]
Cal/Mex 91-92	74.0 (0.5)	11.4 (4.3)	100	0.593	0.609
Coral Sea 91-96	286.4 (0.9)	14.4 (4.5)	100	0.570	0.619
East Aust 94-98	555.4 (1.3)	15.8 (4.4)	100	0.574	0.609
Fiji 95-96	89.2 (0.9)	11.2 (3.5)	100	0.565	0.619
Indonesia 96-97	88.4 (5.2)	11.8 (4.0)	100	0.598	0.616
Phil 94-95	93.6 (0.7)	13.0 (4.0)	100	0.630	0.649
Phil 96-97	96.0 (0.0)	12.2 (4.6)	100	0.612	0.627
Sol Is 95-96	95.2 (0.2)	11.4 (4.3)	100	0.568	0.607

[a] locus is considered polymorphic if more than one allele is detected

[b] unbiased Nei (1978) estimate of Hardy Weinberg expectations

[c]East Aust = East Australia; Phil = Philippines; Sol Is = Solomon Islands

Chi-square analysis of homogeneity of allele frequencies at each of the five loci produced significant results for one locus only (table 6.3.4) after corrections for multiple tests (corrected α_b significance value of 0.05/5=0.01). This was locus *161*, for which the probability of population homogeneity was <0.001. There was no evidence of spatial heterogeneity of allele frequencies at the other four microsatellite loci.

Table 6.3.4. χ^2 analysis for differentiation of five microsatellite loci in eight yellowfin tuna populations, bolded *P* values are significant after probability of H_0 calculated from 5000 Monte Carlo runs, critical *P*=0.01.

Locus	Total[a]	Cal/Mex 91-92	Coral Sea 91-96	East Aust 94-98	Fiji 95-96	Indo 96-97	Phil 94-95	Phil 96-97	Sol Is 95-96	χ^2	*P*[b]
113	1374	74	287	553	90	92	91	92	95	206.844	0.034
125	1357	74	288	554	88	68	94	96	95	120.028	0.020
144	1391	76	287	559	89	94	95	96	95	57.313	0.113
161	1378	73	283	553	87	96	93	96	97	314.179	**0.0002**
208	1388	73	287	558	92	92	95	96	95	83.795	0.055

[a]sample sizes given

[b] after Bonferroni correction for multiple tests

The eight samples were then compared pairwise to determine the source of the heterogeneity at locus *161* (table 6.3.5). Significant heterogeneity was mainly a result of the Philippines 94-95 and Philippines 96-97 populations. These two populations were significantly different (*P*<0.001) from each other and also from Indonesia 96-97, Coral Sea 91-96, and Fiji 95-96. In Philippines 94-95, the 200 allele had a frequency of 0.231 while in Philippines 96-97 this allele was only 0.141 and the 214 allele had a frequency of 0.016 in the former and 0.063 in the later population. In addition, alleles characterised by relative frequencies less than 0.095 differed between the two Philippine populations. However, even after removal of the two Philippines populations from the chi-square analysis for locus *161*, significant residual heterogeneity was

still observed among the six remaining populations (χ^2=201.137, *P*<0.001). The significant pairwise comparisons did not appear to be correlated with geographic distance and there were no differences between Coral Sea and East Australia populations.

Table 6.3.5. Locus *161* pairwise comparisons of allele frequencies, χ^2 values above the diagonal & *P* values below. *P* values in bold are significant after probability of H_0 calculated from 5000 Monte Carlo runs

Populations	Indo 96-97	Cal/Mex 91-92	Coral Sea 91-96	East Aust 94-98	Fiji 95-96	Phil 94-95	Phil 96-97	Sol Is 95-96
Indo 96-97	*****	44.406	34.822	46.076	53.673	55.277	40.959	26.054
Cal/Mex 91-92	0.009	*****	41.129	38.240	37.253	33.531	30.353	28.959
Coral Sea 91-96	0.147	0.046	*****	35.609	52.383	74.625	43.744	14.295
Est Aust 94-98	0.030	0.103	0.183	*****	46.275	55.579	41.627	22.418
Fiji 95-96	**0.0002**	0.049	0.003	0.027	*****	45.508	50.575	35.640
Phil 94-95	**0.0004**	0.194	**0.0002**	0.004	0.006	*****	50.779	47.166
Phil 96-97	0.025	0.348	0.031	0.089	**0.0006**	**0.0003**	*****	26.767
Sol Is 95-96	0.410	0.311	0.980	0.757	0.047	0.007	0.421	*****

F_{ST} values (average = 0.002) indicated very low levels of genetic differentiation across the five microsatellite loci over different geographic locations (table 6.3.6). An F_{ST} value of 0.002 means that only 0.2% of all the detected genetic variation is attributable to differences among populations; the remaining 99.8% arising from within population variation. F_{ST} values per locus ranged from zero to 0.004, with an average of 0.002 (table 6.3.6). There is clearly very little genetic differentiation for any locus over the eight collections. The only significant F_{ST} value, after Bonferroni correction, was that for locus *161* (F_{ST}=0.002, *P*<0.001).

Table 6.3.6. F_{ST} values obtained from AMOVA analysis of five microsatellite loci in eight yellowfin tuna populations, bolded *P* values are significant after Bonferroni correction.

Locus	Total[a]	F_{ST}	*P*
113	1374	-0.000	0.558
125	1357	0.003	0.031
144	1391	0.004	0.015
161	1378	0.002	**0.0002**
208	1388	0.001	0.140

[a]sample sizes given

While the allele frequencies at four of the five loci do not show statistically significant evidence of spatial heterogeneity, probabilities from each individual locus test (both χ^2 and F_{ST} estimates) are all quite low (tables 6.3.4 & 6.3.6). A combined probability test from these independent tests of significance across all five loci (Fishers test, in Sokal and Rohlf 1995) gave significant overall values (chi-square tests table 6.3.4, $P<0.001$; F_{ST} tests table 6.3.6, $P<0.001$). Even if locus *161* is dropped, there is still significant overall heterogeneity (chi-square tests, $P=0.002$; F_{ST} tests, $P=0.009$). There is therefore evidence of weak but statistically significant differentiation among the populations.

Genetic distances estimated using both Nei (1978) unbiased genetic distance and Cavalli-Sforza and Edwards (1967) chord distance among the eight populations were all very small (table 6.3.7); the average Nei pairwise distance was 0.0037.

Table 6.3.7. Matrix of genetic distance coefficients between yellowfin populations, below diagonal = Nei (1978) unbiased genetic distance, above diagonal = Cavalli-Sforza and Edwards (1967) chord distance

Population	Indo 96-97	Coral Sea 91-96	Cal/Mex 91-92	East Aust 94-98	Fiji 95-96	Phil 94-95	Phil 96-97	Sol Is 95-96
Indo 96-97	*****	0.128	0.166	0.131	0.172	0.185	0.171	0.158
Coral Sea 91-96	0.000	*****	0.143	0.083	0.145	0.157	0.140	0.138
Cal/Mex 91-92	0.001	0.000	*****	0.128	0.161	0.164	0.160	0.153
East Aust 94-98	0.002	0.001	0.001	*****	0.130	0.132	0.115	0.130
Fiji 95-96	0.007	0.003	0.004	0.002	*****	0.182	0.170	0.169
Phil 94-95	0.004	0.008	0.011	0.009	0.008	*****	0.174	0.182
Phil 96-97	0.005	0.004	0.003	0.000	0.004	0.008	*****	0.146
Sol Is 95-96	0.000	0.000	0.000	0.001	0.003	0.008	0.002	*****

A derived dendogram of yellowfin tuna population relationships was produced from Nei's (1978) unbiased genetic distance estimates using cluster analysis and the unweighted pair-group method with arithmetic averaging (UPGMA). Very close relationships among all yellowfin tuna populations were observed as can be seen in Figure 6.3.1. The standard deviations of the branch points are high; for example, the mean genetic distance value of 0.0037, based on five loci, has a standard deviation of approximately 0.028. Thus Figure 6.3.1 simply shows that all populations are closely related, the pattern of clusters is not meaningful.

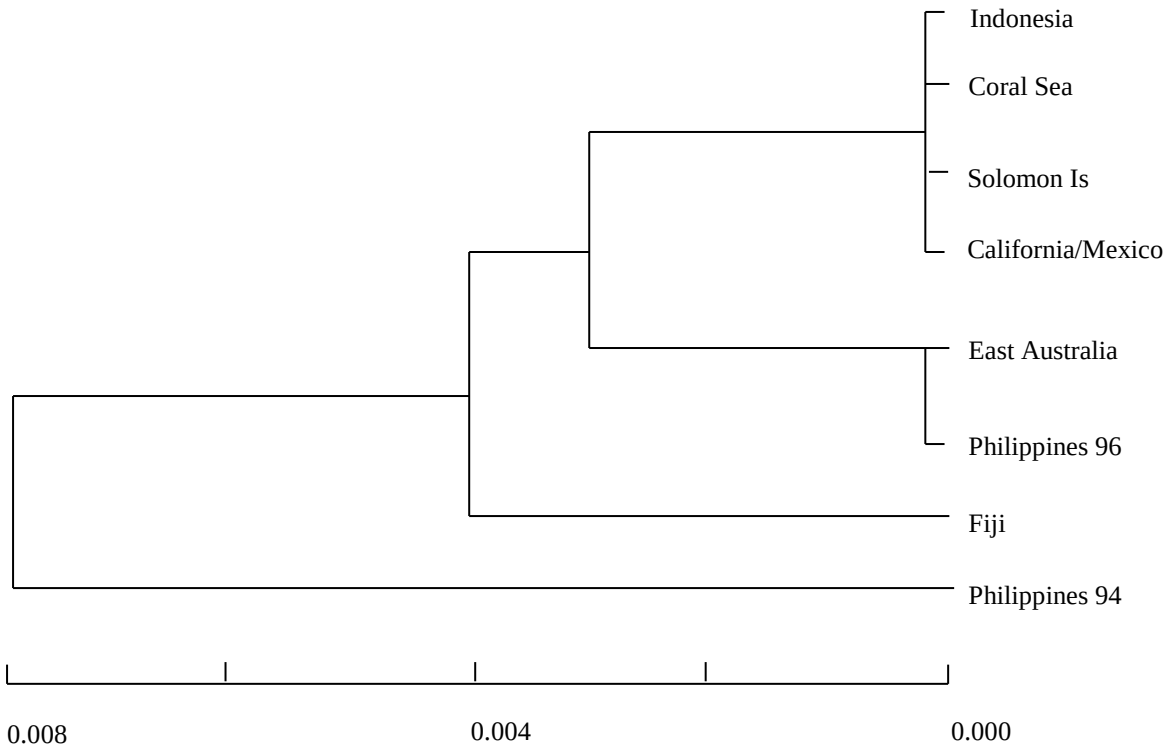

Figure 6.3.1. UPGMA cluster analysis among the eight Pacific Ocean yellowfin tuna populations using Nei (1978) unbiased genetic distance estimate (California/Mexico= California/Mexico 91-92; Coral Sea=Coral Sea 91-96; East Australia=East Australia 94-98; Fjij=Fiji 95-96; Indonesia=Indonesia 96-97; Philippines 94=Philippines 94-95; Philippines 96=Philippines 96-97; Solomon Is=Solomon Islands 95-96)

6.4 Discussion

In the current study, we used genetic variation at five dinucleotide microsatellite loci to investigate the population structure of yellowfin tuna within the western and eastern Pacific Ocean basin. Sample sizes were large, totalling over 1300 fish among eight populations. As demonstrated in previous DNA studies in yellowfin tuna (mtDNA nucleon diversity; 0.840, Scoles and Graves 1993 & microsatellite H_{obs}; 0.618, Takagi *et al.* 1999), considerable variation was detected in all populations based on both mean per locus observed heterozygosity (0.589) and overall mean observed heterozygosity (0.619) estimates.

Genotype frequencies in each population (following pooling of temporal samples from the same spatial area if allele frequencies were not significantly different) at each locus was tested for conformity to Hardy-Weinberg expectations. A significant deviation from expectation was only detected at locus *208* in Coral Sea 91-96 (after Bonferroni corrections to significance levels). In this instance, the deviation was caused by a small excess of homozygotes, and indeed there

was some evidence of a small general excess of homozygotes (~2.5%). Homozygote excess for microsatellite loci has also been reported in vermillion snapper (Bagley *et al.* 1999), whiting (Ricco *et al.* 1997), Atlantic cod (Bentzen *et al.* 1996), chinook salmon (Scribner *et al.* 1996).

There are many possible causes for homozygote excesses, including mis-typing, the presence of non-amplifying alleles, mixing of genetically discrete populations, and selection. Mis-typing is not as likely at locus *208* as at some other loci, as this locus had relatively few alleles and bands showed little stuttering. The presence of non-amplifying or "null" alleles (causing mis-scoring of expressed allele/null allele heterozygotes as homozygotes) is always a possibility with microsatellites and is a well-established phenomenon. Null alleles frequently reflect changes in one or the other of two PCR priming sites, so that a primer no longer binds efficiently thus blocking amplification during the polymerase chain reaction. Examples where mutations are known to produce null alleles include a single base pair transversion (Egglestontott *et al.* 1997), a 1 base pair insertion (Band and Ron 1997), a 4 base pair deletion (Jones *et al.* 1998) and an 8 base pair deletion (Callen *et al.* 1993).

The mixing of genetically discrete populations (such as differing year classes or those from different breeding grounds) will also generate a homozygote excess through the Wahlund effect, but the evidence for genetically discrete populations of yellowfin tuna from our study is very limited (and absent for locus *208*). Selection leading to a homozygote excess is also unlikely; these loci are from non-coding regions of the genome which are likely to be under minimal selection pressure, and in any case selection for homozygote excess is generally unstable and leads to loss of variation rather than maintenance of variation. We believe therefore that the homozygote excess noted for this one locus in one population is probably a stochastic artefact, with no biological significance. Overall, it can be assumed that mating within the yellowfin tuna populations was random.

Heterogeneity chi-square analysis of allele frequencies showed limited differentiation among the populations with only locus *161* displaying significant heterogeneity. The source of this differentiation could not be attributed to any clear spatial differences between geographical areas within the Pacific. Pairwise comparisons showed no clear geographic division. Much of the difference was due to significant heterogeneity between the Philippines 94-95 and Philippines 96-97 populations. We suspect that the genetic differences that were detected at this locus *161* were due to sampling or scoring errors; this locus was particularly prone to stuttering.

For future applications in fisheries stock structure, development and analysis with tetranucleotide repeats should help to reduce the levels of band stuttering observed at dinucleotide repeats and therefore reduce possible mis-identification of alleles. Tetranucleotide repeats are also known to amplify more faithfully than dinucleotide repeats (Hughes and Queller 1993) although they maybe more difficult to isolate and clone than GT or CT repeats (O'Reilly and Wright 1995; Paetkau and Strobeck 1995). Levels of polymorphisms at tetranucleotides as compared to di and tri nucleotide repeats may also be expected to be higher (Hughes and Queller 1993; O'Reilly and Wright 1995; Jarne and Lagoda 1996).

Whatever the explanation for the significant heterogeneity at locus *161*, it does not confirm the

earlier conclusion of Ward *et al.* (1994) that yellowfin tuna in the Pacific Ocean are made up of two reproductively isolated groups in the Pacific; western and central Pacific, and eastern Pacific. Allele frequencies in the most easterly population in the current study, California/Mexico 91-92, were not significantly different from those in any of the more western yellowfin tuna populations at locus 161 or any of the other four microsatellite loci. Ward *et al.* (1994) had previously demonstrated significant allele frequency differences at *GPI-1** between the eastern (California and Mexico) and western (Coral Sea and Philippines) Pacific. The lack of similar differentiation for the five microsatellite loci and for three other allozymes and mtDNA (Scoles and Graves 1993; Ward *et al.* 1994) suggests that the significant differentiation at *GPI-F** may have been the result of local selective pressures (Ward *et al.* 1994, 1997).

Based on tag data, Hampton and Gunn (1998) have found that yellowfin tuna in the Coral Sea and Western Tropical Pacific do mix to some extent. In addition, based on otolith chemistry data, yellowfin caught in the Coral Sea, south eastern AFZ and the Solomon Islands were not significantly different (Campbell 1999). Genetic data from the current study are consistent with this finding. No significant differences in allele frequencies at any locus between yellowfin tuna from the Coral Sea, East Australia (eastern AFZ) and the Solomon Islands were detected. This is also consistent with claims by Hampton and Gunn (1998) that yellowfin tuna spawning in the North West Coral Sea contribute to recruitment in the eastern AFZ or that fish from the AFZ originate from an area with similar water chemistry to that of the Coral Sea (Gunn and Grewe, unpublished). It therefore seems plausible that based on the current genetic data, recruitment into the eastern AFZ yellowfin tuna fishery is from the Coral Sea/Solomon Islands areas.

On a wider Pacific scale however, microsatellite data provides only weak support for population heterogeneity; no consistent genetic differences were detected across the entire Pacific Ocean. Although only one locus of the five microsatellite loci showed significant heterogeneity of allele frequencies, largely attributable to possibly artefactual differences between the Philippines 94-95 and Philippines 96-97 populations, a combined probability test across all five loci gave significant results. This suggests that there may be some very weak population structuring within the Pacific, too weak to detect on a per locus basis given the present sample sizes, and too weak to ascribe to particular populations. It appears therefore that there is sufficient gene flow throughout the Pacific to prevent the formation of genetically discrete populations, at least as can be ascertained with the markers and sample sizes that have been used so far.

A lack of genetically detectable stock separation between yellowfin tuna populations may be caused by sufficient gene flow between populations or the occasional recruitment of individuals from distant populations, that maintain panmixia. Genetic diffferentiation in marine fish is affected by their dispersal capability and large population sizes (Gyllensten 1985; Waples 1987; Ward *et al.* 1994). Species with larval stages that are carried by the ocean currents across large distances or marine populations that have few geographic barriers to dispersal will probably show little stock structure (Waples 1987). This is a realistic situation with the yellowfin tuna as they are a pan-tropical species found in all oceans with few geographical barriers to minimise stock separation and are characterised by large effective population sizes and highly mobile adults.

There are no diagnostic or fixed alleles among any of the populations, and small genetic distance estimates among the eight populations were observed. The F_{ST} statistics indicate very low levels of population differentiation (~0.2%) even over large geographic distances. This suggests that significant numbers of migrants are exchanged among yellowfin tuna populations. The sample sizes used in this study were quite large, and the total data set of >1300 makes this one of the largest microsatellite studies of stock structure carried out on any fishery. We believe that samples of at least 200 per population for each of at least two years will be required in any future genetic study if the issue of the genetic connectivity of Pacific Ocean yellowfin populations is to be finally resolved. This poses its own problems, not only concerning the resources required for such a study, but also the need to check and repeat all genotyping extremely carefully. If genetic differences are expected to be very small (as our results indicate), then discriminating a true small signal from confounding artefact noise will be difficult.

The issue of the number of alleles observed at a locus is also an important one. Ferguson and Danzmann (1998) suggest that genetic marker systems such as microsatellites which are characterised by large numbers of alleles may not be capable of detecting significant differences between genetically similar populations. Locus *161* has more than twenty alleles segregating with some very low allelic frequencies. The large number of alleles at these microsatellite loci also suggests that a larger sample size (≥200) would be needed to confirm if the observed levels of genetic differentiation are real. While a high proportion of microsatellite loci screened in fish are polymorphic (O'Reilly *et al.* 1996; Nielsen *et al.* 1997; Ricco *et al.* 1997; Bagley *et al.* 1999; Takagi *et al.* 1999), loci with only a few alleles tend to be more suitable for population studies while those with greater numbers of alleles are best suited for parentage and linkage studies (Carvalho and Hauser 1994; O'Reilly and Wright 1995). In the current study, there was no significant heterogeneity detected at loci that were characterised by smaller numbers of alleles.

The lack of differentiation in the yellowfin tuna could also be a product of the type of genetic marker used in the current study. Carvalho and Hauser (1994) suggest stabilizing selection arising from exposure to similar environments may result in populations not being genetically differentiated. However, it is well accepted that microsatellites are selectively neutral (see references in Introduction). In addition, the dinucleotide microsatellites may have failed to detect population differences due to the sample size employed in the current study, or the mutation rate of the microsatellites may be so high that population differences brought about by restricted gene flow are obscured.

We also wish to comment that the conservative Bonferroni adjustments, made to the α levels at which *P* values are compared to protect against Type I error, may make it difficult to detect heterogeneity among closely related yet significantly differentiated populations (Ferguson and Danzmann 1998). In the current study, if the α level remained at 0.05 instead of being lowered, temporal heterogeneity would have been observed between Coral Sea 91-92 and 95-96 at locus *125* and Philippines 94-95 and 96-97 at loci *161* and *208*. Similarly, the chi-square analysis of spatial heterogeneity produced significant comparisons at loci *113*, *125* and *161* with $P<0.05$. We may need to address the use and suitability of Bonferroni adjustments, otherwise failure to detect differentiation may lead to multiple stocks being managed as a single unit (Ferguson and Danzmann 1998).

The current study is only one of three microsatellite variation analyses undertaken in *Thunnus* species (Broughton and Gold 1997; Takagi *et al.* 1999; for this yellowfin study see Appleyard *et al.* 2001). Contrasting results concerning tuna stock relationships have so far been demonstrated. Takagi *et al.* (1999) used four dinucleotide microsatellites to investigate population structure of northern bluefin tuna (*T. thynnus*). All markers were considered highly polymorphic but as in the current study, were not successful in detecting differentation between north west Atlantic and Mediterranean samples of Atlantic northern bluefin (*T. thynnus thynnus*) (Takagi *et al.* 1999). As in the current study, Takagi *et al.* (1999) suggest using much larger sample sizes for comparing local populations (within ocean basins) when using highly polymorphic markers. In contrast however, Broughton and Gold (1997) detected significant allele frequency differences at several microsatellite loci among geographic samples of *T. thynnus* (both *orientalis* and *thynnus*). Additionally, both studies showed evidence of heterospecific amplication in other tuna species such as albacore (*T. alalunga*), bigeye (*T. obseus*) and yellowfin (*T. albacares*) using northern bluefin tuna primers (Broughton and Gold 1997; Takagi *et al.* 1999). Similarly, primer pairs utilised in the current study have been used successfully in an analysis of microsatellite variation in Indian Ocean bigeye tuna populations (Appleyard *et al.* submitted).

To summarise, we do not believe that the current genetic data (allozymes, microsatellites, mtDNA) enable us to reject the null hypothesis of a single panmictic yellowfin tuna population in the western Pacific Ocean. However, this does not mean that the null hypothesis is true, just that there is insufficient evidence to reject it. Waples (1998) contends that if components of a stock complex exhibit high gene flow, then management should not be based on genetic data alone. We would also suggest that the minimal level of microsatellite heterogeneity observed in the yellowfin tuna populations be approached carefully until other biological data such as tagging, morphology and otolith chemistry can help to determine management units. If the yellowfin tuna within the Pacific Ocean are truly panmictic, then managing the fishery as a single stock will not affect recruitment from overfished areas. If, however, different yellowfin tuna populations do exist, management as a single stock will mean that over-exploitation in certain areas will lead to reductions in effective population size and yield in these areas. The current genetic results also confirm previous findings, that it is likely the source of yellowfin tuna recruitment into the eastern AFZ is from the Coral Sea or Solomon Islands.

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Appendix 6A - Allele frequencies at five microsatellite loci in yellowfin tuna populations from the Pacific Ocean

Locus *113*

Allele	California 91-92	Coral Sea 91-92	Coral Sea 95-96	East Australia 94-95	East Australia 95-96	East Australia 96-97	East Australia 97-98	Fiji 95-96	Indonesia 96-97	Mexico 91-92	Philippines 94-95	Philippines 96	Solomon Islands 96
107	0.015	-	-	-	-	0.004	-	-	-	-	0.005	-	-
109	0.015	0.100	0.057	0.078	0.040	0.056	0.061	0.061	0.038	0.088	0.033	0.026	0.026
111	0.029	0.021	0.042	0.026	-	0.032	0.011	0.072	0.016	0.038	0.005	0.031	0.037
113	0.029	0.021	0.021	0.017	0.030	0.004	0.011	0.017	0.016	0.013	0.016	0.010	0.005
115	0.044	0.021	0.042	0.026	0.040	0.063	0.034	0.056	0.022	0.038	0.049	0.036	0.032
117	0.279	0.232	0.245	0.246	0.250	0.274	0.228	0.211	0.283	0.188	0.269	0.229	0.200
119	0.162	0.100	0.099	0.082	0.170	0.091	0.144	0.117	0.109	0.150	0.110	0.115	0.111
121	0.132	0.121	0.135	0.125	0.080	0.135	0.149	0.178	0.179	0.113	0.137	0.120	0.184
123	0.103	0.137	0.089	0.108	0.080	0.111	0.103	0.100	0.120	0.125	0.121	0.135	0.116
125	0.015	0.042	0.049	0.056	0.010	0.048	0.065	0.033	0.033	0.063	0.044	0.042	0.053
127	0.059	0.100	0.057	0.082	0.070	0.075	0.094	0.050	0.071	0.063	0.060	0.099	0.105
129	0.074	0.042	0.052	0.056	0.050	0.032	0.036	0.061	0.049	0.063	0.049	0.068	0.053
131	0.029	0.021	0.023	0.034	0.070	0.028	0.029	0.011	0.027	0.025	0.011	0.031	0.032
133	-	0.016	0.018	0.013	0.020	0.004	0.011	0.006	-	-	0.005	0.010	-
135	-	-	-	0.004	-	-	0.006	0.017	-	0.013	0.011	0.010	0.011
137	-	-	-	0.009	0.010	0.012	0.004	-	-	-	0.011	0.005	-
139	-	0.005	0.003	0.004	-	0.004	-	-	0.011	-	-	-	0.005
141	-	-	0.013	-	-	-	0.002	-	0.011	-	-	-	-
143	0.015	0.005	0.010	-	0.010	-	0.004	-	0.005	0.013	0.027	-	-
145	-	0.011	0.005	0.009	0.030	0.008	-	0.011	0.005	-	0.005	0.010	0.016
147	-	-	0.018	0.009	0.030	0.008	0.002	-	0.005	-	0.011	0.010	0.005
149	-	-	0.003	0.009	0.010	0.012	0.002	-	-	0.013	0.016	0.005	0.005
151	-	-	0.016	0.009	-	-	0.002	-	-	-	-	-	0.005
153	-	-	0.003	-	-	-	-	-	-	-	-	0.005	-
155	-	0.005	-	-	-	-	-	-	-	-	-	-	-
Allele count	68	190	384	232	100	252	522	180	184	80	182	192	190
Total fish	34	95	192	116	50	126	261	90	92	40	91	96	95

Locus *125*

Allele	California 91-92	Coral Sea 91-92	Coral Sea 95-96	East Australia 94-95	East Australia 95-96	East Australia 96-97	East Australia 97-98	Fiji 95-96	Indonesia 96-97	Mexico 91-92	Philippines 94-95	Philippines 96	Solomon Islands 96
148	-	-	-	-	-	0.004	0.004	-	-	-	0.005	-	-
154	-	-	-	-	0.010	-	0.004	-	-	-	-	0.005	-
156	-	-	0.003	-	-	0.004	-	-	-	-	0.011	-	0.005
158	0.015	0.010	-	0.004	-	0.008	0.013	0.011	-	-	0.005	-	-
160	0.015	0.016	0.005	-	-	-	0.009	0.023	0.029	0.025	0.005	-	-
162	0.603	0.573	0.677	0.685	0.760	0.649	0.663	0.608	0.640	0.725	0.537	0.641	0.668
164	0.235	0.313	0.253	0.254	0.170	0.286	0.263	0.295	0.265	0.200	0.362	0.266	0.268
166	0.015	0.036	0.016	0.009	0.020	0.012	0.011	0.040	0.007	0.013	0.011	0.021	0.005
168	-	0.016	0.003	0.009	0.010	0.004	0.011	0.006	-	0.013	0.021	0.010	0.021
170	-	-	0.003	-	-	-	0.002	-	-	-	-	-	-
172	0.118	0.026	0.039	0.039	0.030	0.028	0.017	0.017	0.051	0.025	0.043	0.057	0.032
174	-	0.010	0.003	-	-	0.004	0.002	-	-	-	-	-	-
176	-	-	-	-	-	-	-	-	0.007	-	-	-	-
Allele count	68	192	384	232	100	248	528	176	136	80	188	192	190
Total fish	34	96	192	116	50	124	264	88	68	40	94	96	95

Locus *144*

Allele	California 91-92	Coral Sea 91-92	Coral Sea 95-96	East Australia 94-95	East Australia 95-96	East Australia 96-97	East Australia 97-98	Fiji 95-96	Indonesia 96-97	Mexico 91-92	Philippines 94-95	Philippines 96	Solomon Islands 96
166	-	-	0.005	-	-	-	-	-	-	-	-	-	-
168	-	-	-	0.004	-	-	-	-	-	-	0.005	-	-
170	-	-	-	-	-	-	-	0.006	-	-	-	-	-
172	-	0.016	0.031	0.008	-	0.060	0.030	0.034	0.021	-	0.026	0.016	-
174	0.924	0.900	0.870	0.856	0.900	0.844	0.836	0.843	0.888	0.900	0.837	0.802	0.900
176	0.076	0.084	0.094	0.127	0.100	0.096	0.133	0.118	0.090	0.100	0.132	0.182	0.100
178	-	-	-	0.004	-	-	-	-	-	-	-	-	-
Allele count	66	190	384	236	100	250	532	178	188	80	190	192	190
Total fish	33	95	192	118	50	125	266	89	94	40	95	96	95

Locus *161*

Allele	California 91-92	Coral Sea 91-92	Coral Sea 95-96	East Australia 94-95	East Australia 95-96	East Australia 96-97	East Australia 97-98	Fiji 95-96	Indonesia 96-97	Mexico 91-92	Philippines 94-95	Philippines 96	Solomon Islands 96
180	-	0.005	0.005	0.004	-	-	0.002	-	-	0.013	0.011	-	-
182	-	-	-	-	-	0.004	-	-	-	0.013	0.005	-	-
184	-	0.005	0.005	0.004	-	0.008	0.004	0.006	0.005	0.013	-	0.016	0.010
186	0.020	0.022	0.018	-	0.040	0.020	0.019	0.011	0.005	0.025	0.032	0.021	0.021
188	0.030	0.016	0.010	0.013	0.020	0.028	0.002	0.034	0.016	-	0.011	0.031	0.005
190	0.015	0.016	0.018	0.026	0.010	0.012	0.021	0.023	-	0.013	0.011	0.016	0.016
192	0.015	0.016	0.016	0.022	0.020	0.032	0.013	0.023	0.038	-	0.022	0.005	0.005
194	0.030	0.027	0.029	0.026	0.020	0.024	0.032	0.023	0.022	0.038	0.043	0.036	0.021
196	0.061	0.011	0.016	0.013	0.010	0.016	0.017	0.017	0.016	0.050	0.032	0.016	0.010
198	0.152	0.121	0.076	0.069	0.020	0.084	0.090	0.075	0.065	0.025	0.038	0.052	0.057
200	0.061	0.209	0.104	0.103	0.100	0.172	0.176	0.132	0.194	0.138	0.231	0.141	0.156
202	0.167	0.093	0.109	0.142	0.140	0.084	0.116	0.149	0.070	0.100	0.113	0.151	0.120
204	0.030	0.060	0.094	0.043	0.040	0.072	0.055	0.040	0.086	0.063	0.022	0.031	0.083
206	0.015	0.016	0.039	0.052	0.030	0.036	0.029	0.115	0.022	0.038	0.043	0.010	0.031
208	0.045	0.099	0.063	0.043	0.090	0.080	0.059	0.086	0.059	0.038	0.054	0.031	0.073
210	0.061	0.055	0.063	0.047	0.080	0.040	0.046	0.023	0.048	0.038	0.032	0.057	0.047
212	0.061	0.077	0.036	0.034	0.030	0.036	0.046	0.052	0.032	0.025	0.043	0.042	0.036
214	0.030	0.027	0.039	0.052	0.050	0.024	0.050	0.040	0.065	0.025	0.016	0.063	0.052
216	0.076	0.022	0.036	0.039	0.020	0.016	0.032	0.040	0.016	0.088	0.038	0.021	0.036
218	-	-	0.008	0.013	0.030	0.028	0.050	0.029	0.016	0.063	0.065	0.021	0.016
220	0.061	0.033	0.042	0.034	0.050	0.048	0.023	0.006	0.070	0.038	0.054	0.052	0.031
222	0.055	0.038	0.073	0.095	0.080	0.056	0.073	0.040	0.065	0.050	0.038	0.094	0.083
224	0.015	-	0.026	0.034	0.040	0.020	0.008	0.017	0.043	0.038	0.011	0.016	0.021
226	-	0.005	0.026	0.030	0.030	0.028	0.017	0.011	0.011	0.025	-	0.042	0.021
228	-	0.005	0.013	0.034	0.020	0.008	0.015	-	0.005	0.025	0.011	0.016	0.005
230	-	0.016	0.021	0.013	0.010	0.012	0.004	0.006	0.022	-	0.016	0.005	0.031
232	-	-	0.010	0.009	0.010	0.004	0.002	-	0.005	0.025	-	0.005	0.010
234	-	-	0.003	0.004	-	0.008	-	-	-	-	-	-	-
236	-	-	0.003	-	-	-	-	-	0.005	-	-	0.005	-
238	-	-	-	-	0.010	-	-	-	-	-	0.010	0.005	-
Allele count	66	182	384	232	100	250	524	174	186	80	186	192	192
Total fish	33	91	192	116	50	125	262	87	93	40	93	96	96

Locus *208*

Allele	California 91-92	Coral Sea 91-92	Coral Sea 95-96	East Australia 94-95	East Australia 95-96	East Australia 96-97	East Australia 97-98	Fiji 95-96	Indonesia 96-97	Mexico 91-92	Philippines 94-95	Philippines 96	Solomon Islands 96
135	-	-	-	-	-	0.004	-	-	0.005	-	-	-	-
137	0.061	0.016	0.039	0.017	0.020	0.016	0.026	0.022	0.027	0.025	0.021	0.010	0.058
139	-	-	0.005	-	-	-	0.013	0.011	0.022	-	-	-	-
141	0.606	0.689	0.625	0.681	0.700	0.671	0.709	0.717	0.620	0.675	0.605	0.698	0.642
143	0.030	0.021	0.023	0.026	0.020	0.052	0.026	0.027	0.027	0.063	0.032	0.010	0.037
145	0.182	0.195	0.151	0.147	0.120	0.143	0.143	0.125	0.185	0.100	0.174	0.167	0.137
147	0.106	0.053	0.130	0.112	0.090	0.099	0.073	0.092	0.092	0.125	0.105	0.104	0.111
149	0.015	0.016	0.018	0.017	0.020	0.016	0.008	0.005	0.022	0.013	0.042	-	0.016
151	-	0.011	0.008	-	0.020	-	0.002	-	-	-	0.021	0.010	-
153	-	-	-	-	0.010	-	-	-	-	-	-	-	-
Allele count	66	190	384	232	100	252	532	184	184	80	190	192	190
Total fish	33	95	192	116	50	126	266	92	92	40	95	96	95

7 Otolith Chemistry

7.1 Introduction

Otoliths, the calcified “ear-stones” of fish, contain a chemical record that can be interpreted in relation to the past life of an individual fish (Thorrold *et al.* 1997; Thresher 1999; Gao *et al.* 2001; Rooker *et al.* 2001a,b). This record is determined in part by the incorporation of elements and isotopes from the surrounding water in concentrations that reflect the physical and chemical aspects of the ambient environment (Wells *et al.* 2000) and in part by the physiology of the fish (Kalish 1990; Fowler *et al.* 1995; Secor *et al.* 1995).

There have been few studies of the otolith chemistry of tuna, and those there are, focus primarily on bluefin tuna life history patterns (Radtke & Morales-Nin 1989) and stock structure Secor & Zdanowicz 1998; Proctor *et al.* 1995; Rooker *et al.* 2001a). Rooker *et al.* (2001b) included yellowfin tuna otoliths in their investigations of the composition of tuna otoliths and the effects of post-mortem handling, however yellowfin tuna otolith chemistry had not been used as a tool for determining stock structure. In this study we investigate the variation in otolith chemistry of yellowfin tuna spawned in different parts of the western Pacific Ocean and subsequently examine the hypothesis that recruits to the East Coast Tuna and Billfish Fishery are derived from the spawning grounds in the Coral Sea.

Since the commencement of this study there have been significant advances in the techniques used to investigate otolith chemistry. At the time we began our work, there were several techniques available and careful consideration was given to choosing the most appropriate methods for our analysis. The choice of instrument and method depended on the type and concentration of elements we were intending to measure. In general, the concentration of elements in otoliths can be described as ‘macro’, >10% by weight, such as Ca, C and O; ‘micro’, 100-5000 ppm, including Na, Sr, K, S and Cl; and ‘trace’, less than 50-100 ppm, including Zn, Br, Se, Ni and Pb. In addition to these elements, otolith research has included analyses of stable isotopes of carbon and oxygen because they are potential indicators of environmental temperature and hence can reveal information about on movement and migration (Kalish 1991; Edmonds & Fletcher 1997; Thorrold 1997, Edmonds *et al.* 1999).

The effectiveness of two analytical techniques in determining variation in otolith chemistry of yellowfin tuna spawned in different parts of the western Pacific Ocean was examined in the pilot study for this project Gunn & Ward (1994). Wave dispersive electron microprobe analysis (WD-EM) and proton induced x-ray emission spectroscopy (PIXE) were successfully used to differentiate spatially separated groups so the two techniques were again used in the current study.

After the pilot project was completed, a newly emerging technique, stable isotope mass spectroscopy (SIMS), was evaluated to determine if it could provide additional information to that gained from the WD-EM and PIXE analyses. Initial tests found that the analysis of stable isotopes of oxygen and carbon that are incorporated into the otolith structure could be used to detect differences in yellowfin tuna spawned at different sites. Due to this success, and the establishment of a state-of-the-art facility at the University of Wollongong, the technique was included in the current study.

7.2 Otolith Elemental Chemistry (WD-EM and PIXE)

7.2.1 Methods

Otolith preparation

There are three pairs of otoliths and the largest of these are the sagittae. Sagittae were used for this study because of the three otolith-pairs they are the easiest to sample and prepare for analysis. Sagittal otoliths were removed from fish either immediately after capture or unloading from fishing vessels, or from frozen samples sent back to the CSIRO laboratories. In the laboratory otoliths were washed in double-distilled deionized water ($DDDH_2O$), dried at 28°C and stored dry in polyethylene embedding capsules.

To measure the otolith chemical "fingerprint" deposited when fish were in their natal area we analysed the portion of the otoliths that was deposited during the first weeks of life. This involved sectioning the otoliths to expose the inner region of the otolith by sectioning, and subsequently grinding and polishing the otoliths. The method used for preparing samples for WD-EM and PIXE analyses is labour-intensive and time-consuming — approximately half a day is required to prepare each specimen for analysis.

We chose one sagitta from each pair to be prepared for WD–EM and subsequent PIXE analysis. Either the left or right sagitta was chosen at random; previous studies have shown that the chemistry of otolith pairs is not significantly different (Iacumin *et al.* 1992; Thorrold *et al.* 1997; Chesney *et al.* 1998; Rooker *et al.* 2001. All otoliths were prepared and analysed identically to allow quantitative comparisons among specimens.

Before they were sectioned, otoliths were embedded in epoxy resin — Araldite M resin with hardener HY951 — and left to harden for a minimum of 24 hours. The resulting resin blocks containing the specimens were sectioned on an Accutom rotary saw with two Buhler diamond-edged blades mounted parallel to cut transverse sections. Sections, which contained the primordium, were approximately 0.8 mm thick (Figure 7.2.1). During cutting $DDDH_2O$ was used as a coolant and run across the blade and specimen.

The resulting sections were mounted on glass rounds using wax, then ground down by-hand to expose the growth axis using two progressively-finer grades of silicon carbide wet-and-dry paper (1000 and 2400 grit) that were lubricated with $DDDH_2O$. The sections were then turned over and adhered permanently to the glass slides using Araldite M. The grinding was repeated on this side of the section using the two grades of wet-and-dry paper. Polishing was done with two progressively finer grades of Kemet diamond compound (6 and 3 micron), on a "Kent" lapping machine. A final polish by hand using 'Linde B' 0.5 μm aluminium oxide powder removed any small scratches, resulting in a flat, featureless surface, which was required for WD-EM and PIXE probe analysis. After each grade of wet-and-dry paper and polishing compound the specimens were cleaned ultrasonically for 3 minutes: one minute in each of 3 beakers of $DDDH_2O$.

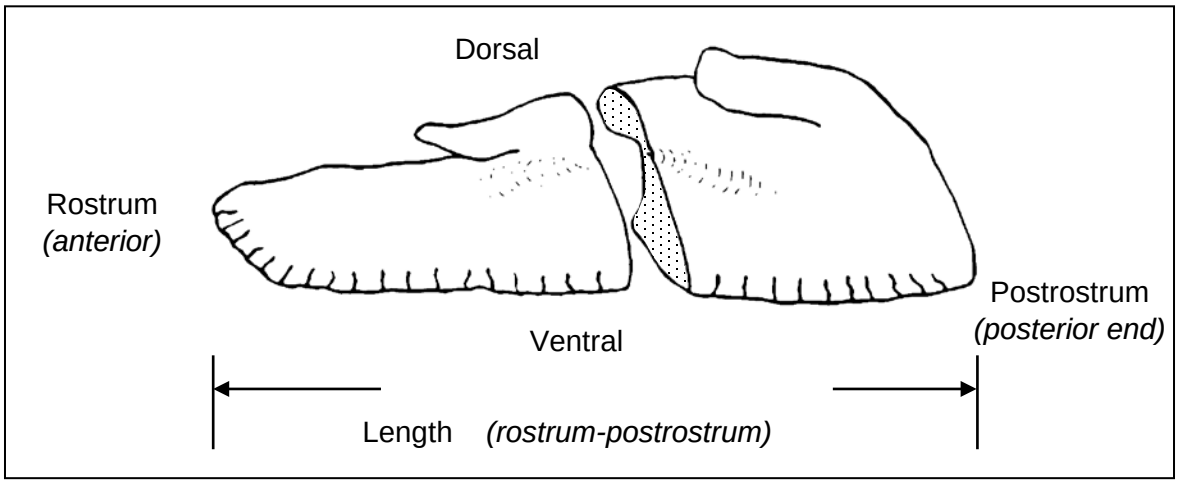

Figure 7.2.1. Sagittal otoliths were sectioned along the transverse axis, producing an 8-mm thick section that contained the primordium.

Prior to microanalysis specimens were heated on a hot-plate at 80°C for 10 minutes to remove any residual moisture. To minimize charging specimens were coated with a 25-30 nm carbon coat, and then stored under vacuum prior to analysis. Otoliths were chosen at random from the prepared specimens to ensure that the order in which the otoliths were analysed did not bias the variation of elemental concentrations among sites.

After microanalysis in the WD-EM, each specimen was inspected to determine if it had sustained beam damage, evident as pits, scratches or cracks. Although some surface damage is inevitable from WD microanalysis, excess 'pitting' may have affected the measured concentrations (such damage to a flat, polished specimen can partially deflect the beam from the surface and result in spurious concentration levels). If the surface was badly damaged we did not reanalyse the specimen in the PIXE probe. PIXE analysis was completed after WD-EM analysis, as the former is a high-energy, destructive technique that leaves large pits in the otoliths sections, rendering them unsuitable for any further analyses that require polished sections.

Probe Analysis

We analysed 992 otoliths in 4 batches using WD-EM and 987 otoliths were analysed using PIXE (table 7.2.1). The slight discrepancy was due to 5 specimens being rendered too damaged by the WD-EM analysis for further analysis.

WD-EM data were acquired using a JOEL 8900R electron probe fitted with 5 wavelength dispersive detectors. We used an accelerating voltage of 15 kV and a current of 50 nA with the beam defocused to a diameter of 30 µm, producing a beam power density of 1.06 µW μm^{-2}**,** which was low enough to minimise damage to the surface of the specimen so PIXE analysis could be conducted on the same area of the otolith surface. The beam was set at approximately 50 µm from the primordium towards the ventral margin. Based on interpretation of daily microincrements on yellowfin otoliths we know that this area is deposited during the first 10-20 days of life. We measured the concentrations (as weight fractions) of six elements: Ca, Na, Sr, K, S and Cl.

PIXE analysis was carried out using the Heavy Ion Analytical Facility at the CSIRO Division of Minerals following the procedures described in Sie & Thresher (1992). We measured the concentrations (in ppm) of 18 elements: Ca, Cr, Mn, Fe, Ni, Cu, Zn, Br, Sr, Rb, Mo, Pb Hg, Cd, Ga, Se, Co and Ba. The PIXE data was normalised to 40% calcium to account for the effect of depth averaging (Sie & Thresher 1992).

Table 7.2.1. Samples from 6 locations were analysed using WD-EM and PIXE probes. The WD-EM analysis was carried out on 4 occasions.

	WD-EM					PIXE
Sampling Location	**Aug 96**	**Mar 97**	**Nov 97**	**Sept 98**	**Total**	
Coral Sea	57	0	26	0	83	82
Fiji	78	0	0	0	78	78
Indonesia	0	74	71	0	145	143
Philippines	0	78	77	0	155	154
Solomon Islands	75	0	76	0	151	151
NSW/ Sth Qld Coast	68	0	74	238	380	379
Total from each analysis	278	152	324	238	992	987

Data Analysis

We performed univariate and multivariate statistical analyses of the WD-EM and PIXE probe data using the statistical software 'Systat 9.0'. The statistical tests we used to test if elements differed among locations and among cohorts assumed that the data were normally distributed. To determine if the data from the WD and PIXE analyses were normally distributed we pooled data from all locations, examined the frequency distributions of individual elements and tested for skewness and kurtosis. To meet the parametric assumptions it was necessary to perform ln-transformations on the chlorine values.

The frequency distributions of each element were also used to identify some data points as obvious outliers and these were excluded from further analysis. If a value was identified as an outlier in the distribution of one element the results of all elements for that sample were excluded. A number of outliers appeared in the distribution of zinc concentrations and we were reluctant to remove them because, following our method, we would have removed the values of all elements measured in those samples. After investigating the analysis of zinc on the PIXE probe we discovered the outliers occurred because of an analytical problem that did not affect the measurement of other elements. Hence, to remove their large influence on the sample mean but to be able to keep them in the analysis, we adjusted the zinc values that were atypical of the rest of the sample. This involved a small group of samples collected in NSW, which had measured concentrations of zinc much higher than the rest of the NSW samples; they were assigned the mean value of zinc for NSW (calculated with those outliers removed). Secondly, any negative values were assigned a value of zero. In this way we were able to retain the measurements of the other elements from the samples in our analysis.

PIXE data were examined in relation to the minimum detection limits (MDL), defined at 99% confidence level. MDLs were recorded for each sample and the mean level for each element was calculated. These were compared to the concentrations measured in the samples; when measured levels were lower than the MDL elements were discarded (excluded from further analysis).

PIXE analysis provides one-to-two orders of magnitude more sensitivity than WD and hence very low concentrations can be measured. However, the high sensitivity of PIXE analysis also means that even low levels of contamination can affect results. However, the examination of the distributions before proceeding with statistical analysis identified samples that had been possibly contaminated and they were excluded from the analysis.

Analysis of variance (ANOVA) was used to test for differences in otolith elemental concentrations among locations and cohorts. If statistically significant differences were not detected we excluded the element from further analysis. The remaining elements were entered into a multivariate analysis of variance (MANOVA) to determine if interannual variation existed in samples collected at the same location.

Principal components analysis (PCA) was used to determine the interrelationships in the data from a matrix of pairwise correlations. PCA grouped variables as 'factors' and determined how much of the variance was explained by each of the factors.

Linear discriminant factor analysis (LFDA) was used to 'classify' NSW samples into the group (cohort) they resembled most closely. Firstly the data from locations other than NSW were examined to describe the discriminant functions. The linear combination of variables that best discriminated between the cohorts was determined using interactive forward stepwise LDFA, which meant that elements were added to the model in order of their discriminating power. At each step the tolerance measure was examined to avoid including variables highly correlated with others already in the model. Each sample was classified into the group where the value of its classification function was the largest and the results were compiled in a classification matrix. Jackknifed classification matrices indicated the classification success of individual cases to their natal area and the proportion of NSW samples (entered into the classification as coming from an "unknown" location) classified to each location. The between groups F matrix, calculated from canonical distances from group means, indicated the pairs of cohorts that differed most and the pairs that differed least. We also examined the position of points in the canonical space using mahalanobis distances to determine the likelihood of samples being classified to each location. Similarly, posterior probabilities, which were calculated from mahalanobis distances, indicated the probability that each of the samples would be assigned to the locations.

7.2.2 Results

There was a wide range in the distributions of measured concentrations for all elements (figures 7.2.2 and 7.2.3 and table 7.2.2). All elements except lead differed significantly among locations (table 7.2.3). Table 7.2.4 provides a summary of the ANOVA post-hoc tests comparing location means; table 7.2.22 (Appendix 7A) summaries the ANOVA post-hoc tests comparing location means using WD-EM data not standardised to calcium. Further

details of the post-hoc tests showing matrices of pair-wise comparison probabilities can be found in table 7.2.23.

Ten cohorts from 6 locations were included for analysis (table 7.2.5). Again we saw a wide range in the distributions of measured concentrations for all elements (figures 7.2.4 and 7.2.5; tables 7.2.6 and 7.2.7). All elemental concentrations, including lead, varied significantly between cohorts (ANOVA, $p<0.05$) (table 7.2.8). This meant that in addition to the variation in otolith chemistry being significantly different between locations, there were significant differences between the otolith chemistry of cohorts, i.e. fish sampled at the same location during different years. The post hoc tests determined that the differences lay not only between cohorts from different locations but also from cohorts sampled at the same location (table 7.2.9 and 7.2.10).

Calcium concentrations measured using WD-EM varied significantly between cohorts, as it had done between locations. This was an unexpected result — 38% is the approximate expected level of calcium in otoliths. Calcium measurements can be affected by surface topography of specimens prepared for probe analysis (Proctor & Thresher 1998). However, all specimens were prepared in the same way and were then analysed in random order to ensure that specimens from one site were not analysed consecutively. During WD-EM analysis the calcium levels were monitored for any deviation from normal and calcium standards checked at the beginning of every group of analyses, hence WD-EM elemental concentrations were standardised to calcium for use in further analyses.

After examining the distributions of elemental concentrations and results of initial analysis of variance (ANOVA, $p<0.05$) we retained for analysis six elements measured by PIXE: Cu, Zn, Rb, Pb, Hg and Co; and 5 elements from WD-EM, standardised for calcium: Na, Sr, K, S, ln Cl.

Measures of skewness and kurtosis indicated that the distributions of most of the elemental concentrations approximated normal but were shifted slightly from the normal symmetric bell-shape of continuous data. The distribution of chlorine however, was positively skewed and had a high kurtosis value due to measured concentrations in the right hand ‘tail’, equivalent to chlorine distributions in previous studies of otolith chemistry (Thresher *et al.* 1994). Assuming that chlorine contamination did not occur, the high chlorine levels found in some samples are real and the distributions a true representation of chlorine in otoliths within a population. Consequently, we ln-transformed the Cl data so that it conformed to the parametric assumptions.

Since this study was conducted chlorine has been excluded from some studies, being considered a ‘universal contaminant’ (see Thresher 1999). Principal components analysis indicated that chlorine was correlated with Na and K (section 7.2 and figure 7.2.6) and Proctor and Thresher (1998) suggest this may be due to the elements being labile and hence affected by specimen handling. However, Proctor and Thresher (1998) found no evidence that Cl levels at the primordium were affected by the two factors to which we subjected the otoliths in this study. In one experiment, Proctor and Thresher (1998) compared Cl concentrations in otolith pairs, one of which was extracted immediately after death and the other left in the frozen fish for a period before extraction. They found that Cl levels

immediately adjacent to the primordium (equivalent to our probed point) were not significantly different. In our study we included otoliths extracted from frozen fish collected in the Philippines. In another experiment, they found there was no significant effect on Cl levels in otoliths that had been immersed in distilled water, which we used as a lubricant and cleaning agent. These results support our decision to include Cl in the data analysis but we view the results from the chlorine data with some caution, as there might be additional effects introduced during the preparation stage that Proctor and Thresher (1998) could not completely separate from the effect of preservation.

Iron was removed from the data set because there was evidence that a small but significant number of samples had been contaminated. We saw extremely high results throughout the data set (about 40-50% of samples), from all locations. This level of iron and the presence of molybdenum in some samples indicate that the contamination might have been due to the stainless steel specimen holder used during the grinding and polishing stage of otolith preparation (Sie & Thresher 1992). However, there was no evidence that the contamination had affected the remaining elements so while Fe was removed the remaining elements were retained.

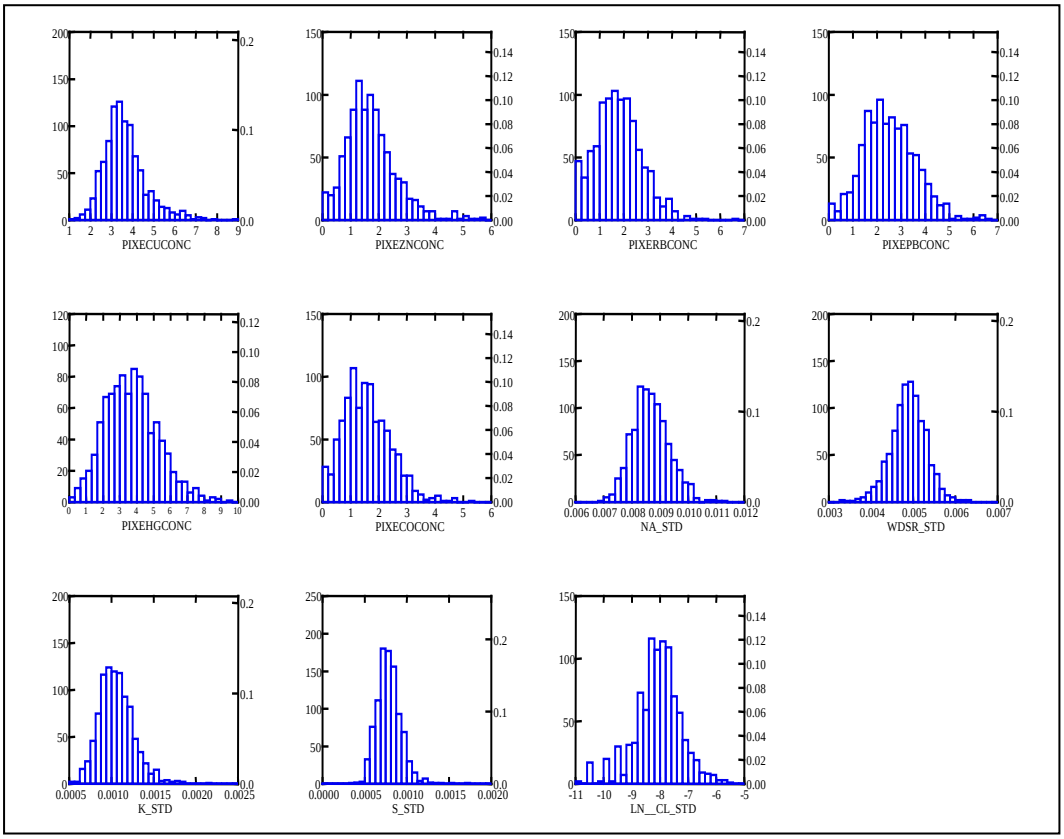

Figure 7.2.2. Frequency distributions of the 11 elements retained for analysis: left hand axes show the count, right hand axes the proportion per bar. WD-EM measurements are a ratio of element concentration to calcium concentration and PIXE measurements are in ppm.

Table 7.2.2. Means table for sampling locations

method	element		NSW/Sth Qld	Fiji	Indonesia	Philippines	Solomon Islands	Coral Sea
PIXE	Cu	number	366	78	142	153	151	75
		mean	3.681	3.335	3.303	3.332	4.023	4.120
		std deviation	0.970	0.719	0.837	0.866	1.192	1.159
	Zn	number	366	78	142	153	151	75
		mean	1.655	2.488	1.738	1.838	1.680	1.640
		std deviation	0.928	1.343	1.066	1.325	0.829	0.908
	Sr	number	366	78	142	153	151	75
		mean	2440.650	2488.096	2393.709	2395.677	2517.092	2478.147
		std deviation	259.297	241.056	218.987	220.019	222.561	232.333
	Rb	number	366	78	142	153	151	75
		mean	1.700	1.813	1.873	2.089	1.551	1.789
		std deviation	0.917	1.083	0.927	0.930	0.903	0.990
	Pb	number	366	78	142	153	151	75
		mean	2.502	2.744	2.403	2.417	2.590	2.618
		std deviation	1.087	1.152	1.077	1.045	1.098	1.114
	Hg	number	366	78	142	153	151	75
		mean	3.630	3.203	3.788	3.742	3.723	4.166
		std deviation	1.570	1.302	1.602	1.507	1.533	1.760
	Co	number	366	78	142	153	151	75
		mean	1.647	1.554	1.522	1.353	1.722	1.490
		std deviation	0.880	0.817	0.806	0.776	0.791	0.813
WD	Ca	number	367	78	143	155	151	76
		mean	38.658	39.488	39.150	39.117	38.767	38.723
		std deviation	0.583	0.502	0.594	0.572	0.431	0.480
	Na	number	367	78	143	155	151	76
		mean	0.333	0.317	0.352	0.338	0.345	0.333
		std deviation	0.024	0.020	0.026	0.025	0.019	0.021
	Sr	number	367	78	143	155	151	76
		mean	0.188	0.190	0.192	0.195	0.191	0.185
		std deviation	0.017	0.013	0.013	0.017	0.015	0.019
	K	number	367	78	143	155	151	76
		mean	0.039	0.037	0.042	0.045	0.044	0.043
		std deviation	0.007	0.007	0.006	0.010	0.008	0.008
	S	number	367	78	143	155	151	76
		mean	0.028	0.038	0.034	0.032	0.030	0.028
		std deviation	0.004	0.008	0.005	0.004	0.006	0.004
	Cl	number	367	78	143	155	151	76
		mean	0.018	0.016	0.015	0.014	0.020	0.015
		std deviation	0.019	0.013	0.014	0.009	0.018	0.009

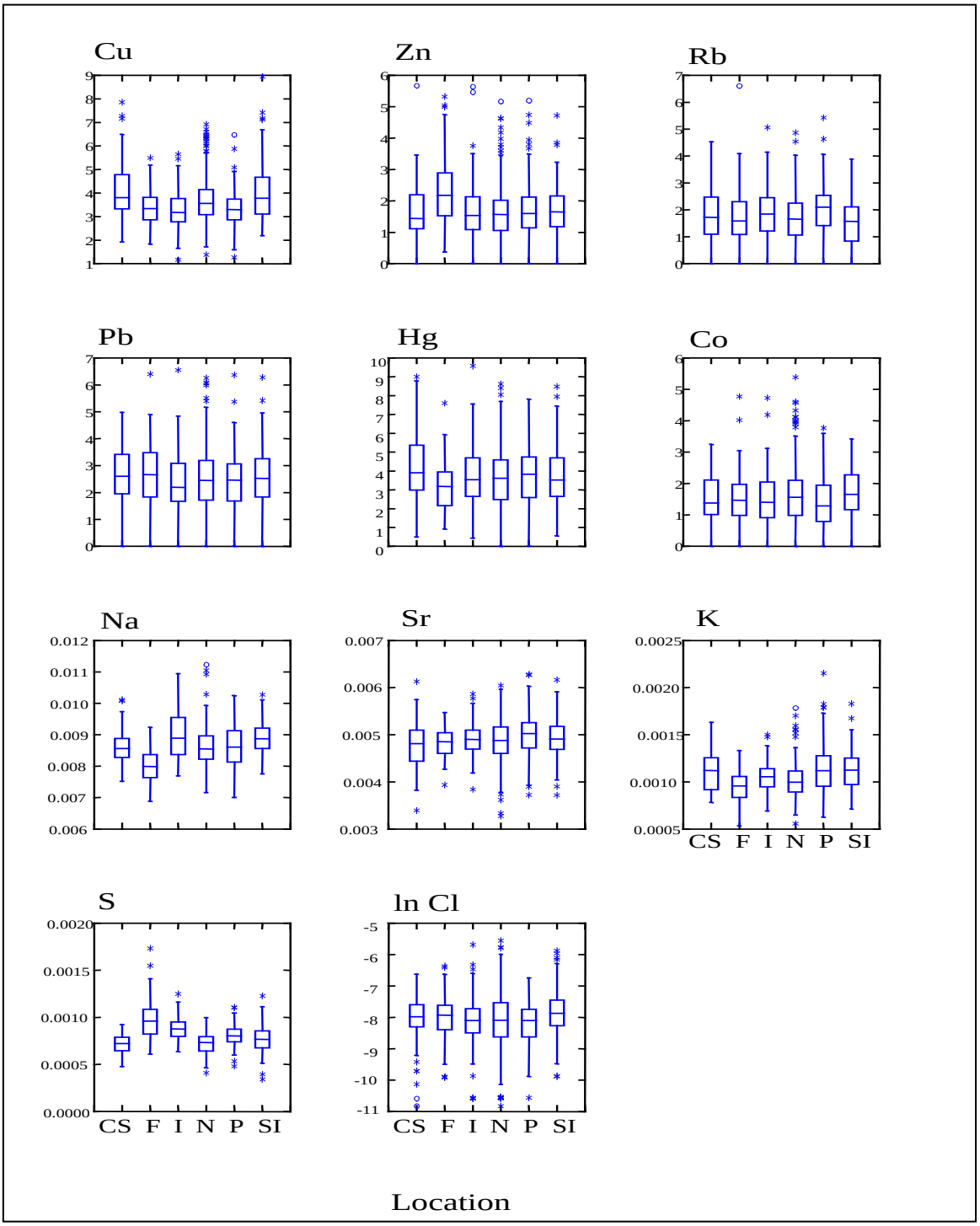

Figure 7.2.3. Element concentrations by sampling location. The boxes indicate the 25th, 50th (median) and 75th percentiles.

Table 7.2.3. Results from ANOVA between sampling locations.

method	element	df	F-value	P-value	
PIXE	Cu	5	16.425	<0.001	***
	Zn	5	8.881	<0.001	***
	Sr	5	6.204	<0.001	***
	Rb	5	5.946	<0.001	***
	Pb	5	1.53	0.178	NS
	Hg	5	3.201	0.007	***
	Co	5	3.993	0.001	***
WD	std Na	5	30.149	<0.001	***
	std Sr	5	3.363	0.005	***
	std K	5	22.196	<0.001	***
	std S	5	70.536	<0.001	***
	ln std Cl	5	3.302	0.006	***

Table 7.2.4. Summary of ANOVA Bonferroni post-hoc tests indicating group (location) means that differ significantly. * indicates p<0.05; otherwise p<0.01.

	Coral Sea	**Fiji**	**Indonesia**	**NSW/Sth Qld**	**Philippines**	**Solomon Islands**
Coral Sea						
Fiji	Cu Zn Hg Na std S std K std					
Indonesia	Cu Na std S std	Zn Na std S std K std				
NSW/Sth Qld	Cu K std	Zn Na std S std	Cu Na std S std K std*			
Philippines	Cu S std Sr WD std*	Zn Na std S std K std	S std Na std K std	Cu Rb Co Ca K std S std Sr WD std*		
Solomon Islands	Na std	Cu Zn Na std Sr-WD S std K std	Cu Sr-PIXE S std ln Cl std	Cu Sr-PIXE * S std Na std K std ln Cl std	Cu Sr-PIXE Co Na std ln Cl std	

Table 7.2.5. Samples from each cohort analysed by WD-EM and PIXE analysis and included in statistical analysis.

Location	Cohort	Number	LCF
Coral Sea	CS94	76	50-100
Fiji	F94	78	51-61
Indonesia	Ind95	74	25-45
	Ind96	70	20-41
Philippines	Phi94	78	22-47
	Phi96	77	26-45
Solomon Islands	SI95	75	29-53
	SI96	76	36-51
NSW/ Sth Qld Coast	NSW94	168	59-140
	NSW95	96	92-120

Table 7.2.6. Means table for cohorts (WD data not standardised to calcium)

			NSW92	NSW93	NSW94	NSW95	Phi94	Phi96	SI95	SI96	CS94	Ind95	Ind96	F94
method	element													
PIXE	Cu	number	20	83	167	96	77	76	75	76	75	73	69	78
		mean	3.694	4.113	3.514	3.596	3.239	3.425	4.650	3.403	4.120	3.416	3.184	3.335
		std deviation	0.774	1.198	0.892	0.805	0.756	0.96	1.278	0.667	1.159	0.964	0.663	0.719
	Zn	number	20	83	167	96	77	76	75	76	75	73	69	78
		mean	1.994	1.817	1.591	1.554	1.751	1.925	1.785	1.577	1.640	1.954	1.511	2.488
		std deviation	0.78	0.962	0.804	1.094	0.870	1.666	0.909	0.732	0.908	1.270	0.74	1.343
	Sr	number	20	83	167	96	77	76	75	76	75	73	69	78
		mean	2401.025	2484.860	2399.347	2482.531	2390.431	2400.992	2515.681	2518.484	2478.147	2412.027	2374.329	2488.096
		std deviation	270.894	267.084	238.693	275.155	242.156	196.544	231.941	214.439	232.333	249.547	180.994	241.056
	Rb	number	20	83	167	96	77	76	75	76	75	73	69	78
		mean	1.588	1.803	1.708	1.62	2.170	2.007	1.489	1.613	1.789	1.986	1.753	1.813
		std deviation	0.84	0.840	0.959	0.925	0.942	0.917	1.011	0.783	0.990	1.023	0.802	1.083
	Pb	number	20	83	167	96	77	76	75	76	75	73	69	78
		mean	2.839	2.670	2.344	2.562	2.273	2.562	2.845	2.338	2.618	2.309	2.502	2.744
		std deviation	1.281	1.067	1.047	1.104	0.944	1.126	1.173	0.961	1.114	1.208	0.917	1.152
	Hg	number	20	83	167	96	77	76	75	76	75	73	69	78
		mean	3.805	4.046	3.472	3.508	3.620	3.865	3.678	3.768	4.166	3.665	3.919	3.203
		std deviation	1.38	1.637	1.592	1.46	1.459	1.554	1.565	1.509	1.760	1.347	1.835	1.302
	Co	number	20	83	167	96	77	76	75	76	75	73	69	78
		mean	1.862	1.746	1.545	1.696	1.264	1.443	1.751	1.694	1.490	1.566	1.474	1.554
		std deviation	0.981	0.898	0.858	0.871	0.737	0.809	0.791	0.795	0.813	0.916	0.673	0.817
WD	Ca	number	21	84	168	94	78	77	75	76	76	74	69	78
		mean	38.629	38.566	38.725	38.627	39.321	38.911	38.548	38.983	38.723	39.337	38.95	39.488
		std deviation	0.57	0.478	0.624	0.59	0.568	0.501	0.371	0.376	0.480	0.565	0.562	0.502
	Na	number	21	84	168	94	78	77	75	76	76	74	69	78
		mean	0.341	0.331	0.338	0.324	0.321	0.355	0.342	0.348	0.333	0.335	0.37	0.317
		std deviation	0.019	0.020	0.028	0.016	0.018	0.019	0.017	0.02	0.021	0.019	0.019	0.020
	Sr	number	21	84	168	94	78	77	75	76	76	74	69	78
		mean	0.189	0.181	0.189	0.192	0.191	0.198	0.184	0.197	0.185	0.192	0.191	0.190
		std deviation	0.015	0.015	0.016	0.019	0.017	0.017	0.014	0.014	0.019	0.013	0.011	0.013
	K	number	21	84	168	94	78	77	75	76	76	74	69	78
		mean	0.033	0.042	0.039	0.038	0.047	0.042	0.048	0.039	0.043	0.043	0.04	0.037
		std deviation	0.004	0.008	0.007	0.006	0.012	0.007	0.006	0.007	0.008	0.007	0.005	0.007
	S	number	21	84	168	94	78	77	75	76	76	74	69	78
		mean	0.029	0.027	0.029	0.027	0.033	0.03	0.027	0.033	0.028	0.035	0.034	0.038
		std deviation	0.003	0.004	0.004	0.004	0.005	0.003	0.004	0.005	0.004	0.005	0.004	0.008
	Cl	number	21	84	168	94	78	77	75	76	76	74	69	78
		mean	0.01	0.019	0.021	0.012	0.018	0.009	0.023	0.017	0.015	0.017	0.012	0.016
		std deviation	0.005	0.020	0.022	0.011	0.010	0.005	0.018	0.017	0.009	0.016	0.011	0.013

Table 7.2.7. Means table for cohorts (WD data standardised)

	NSW93	NSW94	NSW95	Phi94	Phi96	SI95	SI96	CS94	Ind95	Ind96	F94
number	84	168	94	78	77	75	76	76	74	69	78
mean	0.008571	0.008717	0.008384	0.008156	0.009121	0.008880	0.008931	0.008598	0.008516	0.009497	0.008041
std deviation	0.000529	0.000701	0.000426	0.000495	0.000505	0.000444	0.000519	0.000516	0.000493	0.000537	0.000534
number	84	168	94	78	77	75	76	76	74	69	78
mean	0.004698	0.004885	0.004976	0.004867	0.005103	0.004767	0.005064	0.004788	0.004886	0.004907	0.004816
std deviation	0.000372	0.000427	0.000472	0.000452	0.000439	0.000363	0.000370	0.000489	0.000356	0.000309	0.000317
number	84	168	94	78	77	75	76	76	74	69	78
mean	0.001078	0.000994	0.000990	0.001197	0.001090	0.001258	0.001000	0.001119	0.001098	0.001030	0.000943
std deviation	0.000211	0.000167	0.000144	0.000299	0.000171	0.000146	0.000174	0.000217	0.000173	0.000136	0.000169
number	84	168	94	78	77	75	76	76	74	69	78
mean	0.000699	0.000741	0.000712	0.000828	0.000784	0.000696	0.000841	0.000724	0.000886	0.000874	0.000968
std deviation	0.000104	0.000113	0.000103	0.000120	0.000086	0.000114	0.000125	0.000097	0.000135	0.000116	0.000214
number	84	168	94	78	77	75	76	76	74	69	78
mean	0.000488	0.000537	0.000318	0.000462	0.000235	0.000597	0.000433	0.000376	0.000439	0.000299	0.000395
std deviation	0.000524	0.000565	0.000279	0.000263	0.000126	0.000470	0.000433	0.000223	0.000401	0.000290	0.000319

Table 7.2.8. Results of ANOVA for cohorts. 'Std' indicates the elements standardised to calcium.

method	element	df	F-value	P-value	
PIXE	Cu	11	16.85	<0.001	***
	Zn	11	5.413	<0.001	***
	Sr	11	3.968	<0.001	***
	Rb	11	3.25	<0.001	***
	Pb	11	2.523	0.004	***
	Hg	11	2.425	0.006	***
	Co	11	2.527	0.004	***
WD	Ca	11	29.253	<0.001	***
	Na	11	38.295	<0.001	***
	Sr	11	8.286	<0.001	***
	K	11	21.853	<0.001	***
	S	11	46.098	<0.001	***
	Cl	11	6.148	<0.001	***
WD	Na-Std	10	45.729	<0.001	***
	Sr-Std	10	7.287	<0.001	***
	K-Std	10	21.512	<0.001	***
	S-Std	10	43.437	<0.001	***
	Cl-Std	10	6.285	<0.001	***
	ln Cl-Std	10	8.007	<0.001	***

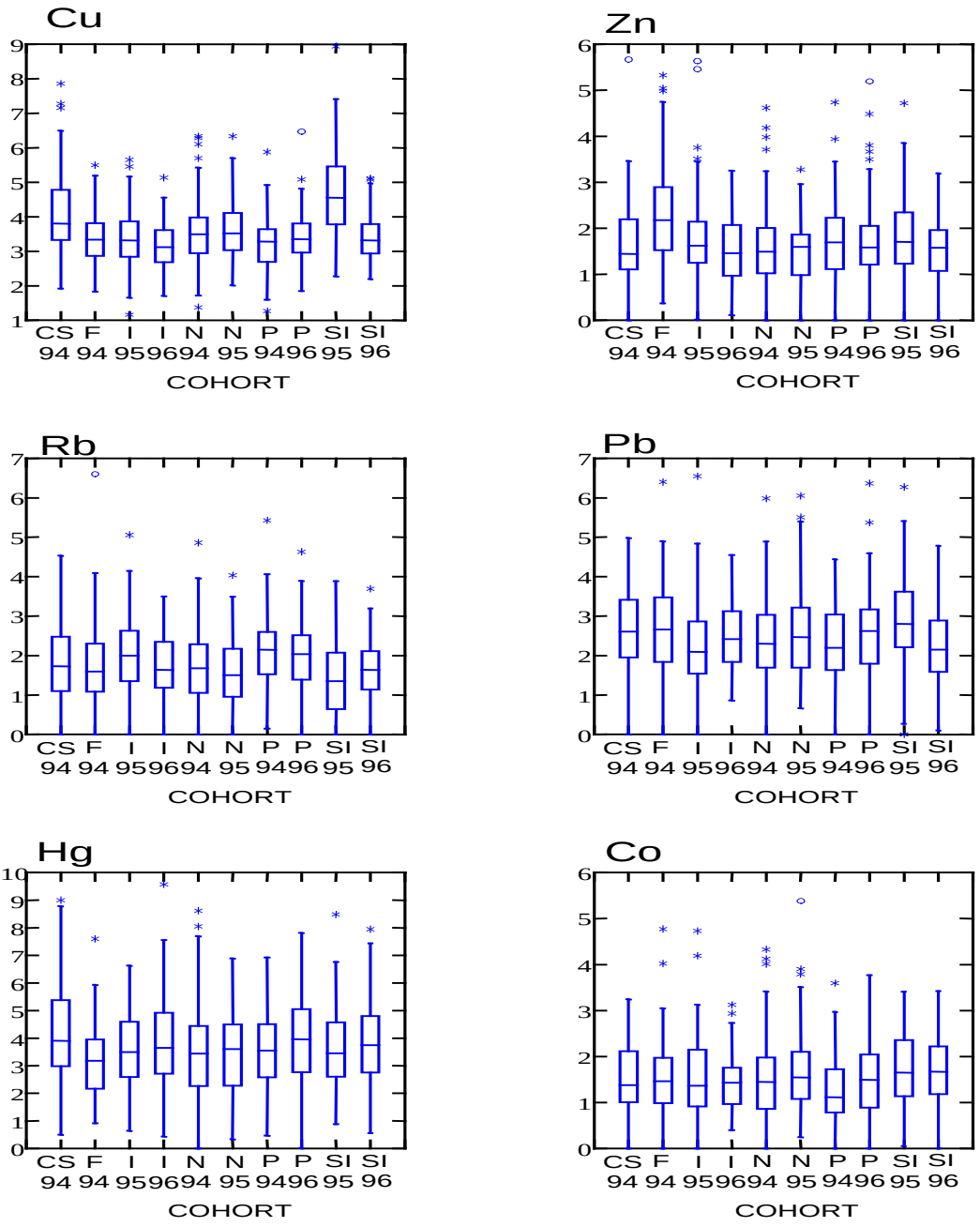

Figure 7.2.4. PIXE element concentrations by cohort. The boxes indicate the 25th, 50th (median) and 75thpercentiles.

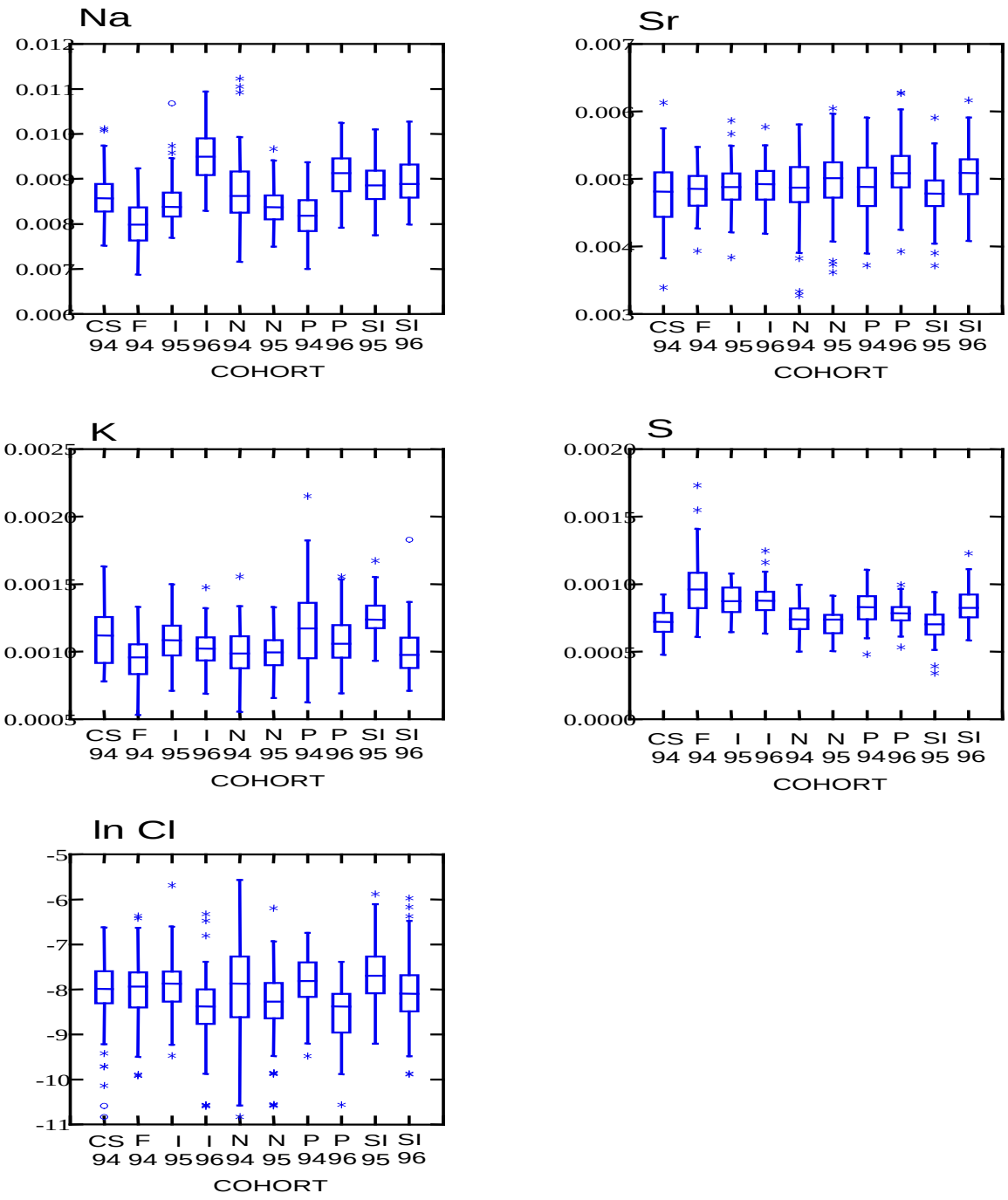

Figure 7.2.5. WD-EM element concentrations by cohort. The boxes indicate the 25th, 50th (median) and 75thpercentiles

Table 7.2.9. Summary of ANOVA Bonferroni post-hoc tests indicating group (cohort) means that differ significantly * = P<0.05; **<0.01. PIXE elements are standardised to 40% calcium, but WD elements are NOT standardised to calcium

	CS94	F94	Ind95	Ind96	NSW93	NSW94	NSW95	Phi94	Phi96	SI95
CS94	1									
F94	Zn** Ca** Cu** Na** Hg K** S**	2								
Ind95	Cu** Ca** S**	Na** S** K**	3							
Ind96	Cu** Na** S**	Zn** Ca** Na** S**	Ca** Na**	4						
NSW93		Zn** Ca** Cu** Na** Hg* WD Sr* S**	Cu** Ca** WDSr** S**	Cu** Ca** Na** WDSr* * S**	5					
NSW94	Cu** K**	Zn** Ca** Na** S**	Ca** S** K**	Na** S** Cl**	Cu** WDSr**	6				
NSW95	Cu* K**	Zn** Ca** S**	Ca** Na* S** K**	Ca** Na** S**	Cu* WDSr**	Na** Cl**	7			

Table 7.2.9. continued.

	CS94	F94	Ind95	Ind96	NSW93	NSW94	NSW95	Phi94	Phi96	SI95
Phi94	Ca** Na* S**	Zn** S** K**	Na**	Ca** Na** S**	Cu** Ca** Co* WDSr** S** K**	Rb* Ca** Na** K** S** Cl**	Rb** Ca** Co* K** S**	8		
Phi96	Na** WDSr** *	Ca** Na** K** S**	Ca** Na** S**	Na** S**	Cu** Ca** Na** S** Cl** WDSr**	Na** WDSr** * K**	Ca* Na** S** K*	Ca** Na** K** Cl**	9	
SI95	Cu* K** Cl*	Zn** Ca** Cu** Na** K**	Cu** Ca** S** K**	Cu** Ca** P-Sr* Na** S** Cl** K**	Cu* Na* K**	Cu** K** Pb**	Cu** Na** WDSr* K** Cl**	Rb** Ca** Cu** Na** Co* S** Pb**	Rb* Ca** Cu** Na** S** WDSr** K** Cl**	10
SI96	Na** WDSr** * S**	Zn** Ca** Na** S**	Ca** Na** K*	P-Sr* Na**	Ca** Na** WDSr** S**	Ca* Na* WDSr** *	Ca** Na** S**	Rb* Ca** Na**		Cu** Ca** WDSr** S** K**

Table 7.2.10. Summary of ANOVA Bonferroni post-hoc tests indicating group (cohort) means that differ significantly * = P<0.05; **<0.01. Data are for WD elements, which have been standardised to calcium.

	CS94	F94	Ind95	Ind96	NSW93	NSW94	NSW95	Phi94	Phi96	SI95	SI96
CS94	1										
F94	Na ** S ** K **	2									
Ind95	S **	Na ** S ** K **	3								
Ind96	Na ** S **	Na ** S **	Na ** ln Cl *	4							
NSW93		Na ** S ** K **	S **	Na ** S **	5						
NSW94	K **	Na ** S **	S **	Na ** S ** ln Cl *	WDSr * K *	6					
NSW95	K **	Na ** S **	S ** ln Cl *	Na ** S **	WDSr **	Na **	7				
Phi94	Na ** S **	S ** K **	Na **	Na ** K ** ln Cl **	Na ** S ** K **	Na ** S ** K **	S ** K **	8			

Table 7.2.10. continued.

	CS94	F94	Ind95	Ind96	NSW93	NSW94	NSW95	Phi94	Phi96	SI95	SI96
Phi96	Na ** WDSr ** ln Cl *	Na ** WDSr ** S ** K **	Na ** S ** ln Cl **	Na ** S **	Na ** WDSr ** S ** ln Cl **	Na ** WDSr ** ln Cl ** K *	Na ** S ** K *	Na ** WDSr * ln Cl ** K *	9		
SI95	ln Cl * K **	Na ** S ** K ** ln Cl *	Na ** S ** K **	Na * S ** ln Cl ** K **	Na * K **	K **	Na ** ln Cl ** K **	Na ** S **	WDSr ** S ** ln Cl * K **	10	
SI96	Na ** WDSr ** S * K **	Na ** WDSr ** S **	Na **	Na **	Na ** WDSr ** S **	S **	Na ** S **	Na ** K **		WDSr ** K ** S **	11

Interannual Comparison

We were able to further examine interannual variation using otoliths collected in Indonesia, Philippines and Solomon Islands. In these locations sampling had been conducted during the same month in two years. Multivariate analysis of variance (MANOVA, $p<0.001$) was used to determine if there were significant differences between the samples collected at the same locations but in different years. Included in the analysis were six PIXE elements: Cu, Zn, Rb, Pb, Hg and Co; and five WD elements: Sr, Na, K, S and Cl.

Interannual differences were detected between cohorts from each of the locations — the MANOVAs determined that there were highly significant differences between cohorts. The results of the univariate F-tests indicated which individual elements were significantly different (Table 7.2.11) and hence driving the results. WD-EM elements contributed more to the discrimination between the cohorts than did PIXE elements; potassium was a discriminating element in each of the 3 cohort comparisons. The significant difference between the Philippines cohorts was driven only by the WD-EM elements; no PIXE elements differed significantly. Therefore, much of the interannual variation between cohorts was due to the "biological" micro constituents rather than the trace elements (Table 7.2.11 and 7.2.20). However, copper also contributed to the variation: it was high in the Solomon Islands 95 samples.

Table 7.2.11. MANOVA determined that elements differed significantly among cohorts at 3 locations. The significant differences were due largely to the WD-EM elements.

		Solomon Islands 1995 and 1996	**Philippines 1994 and 1996**	**Indonesia 1995 and 1996**
Multivariate test statistics	Wilks' Lambda	<0.001	<0.001	<0.001
Univariate F-Tests $p<0.05$	PIXE elements	Cu, Pb		Zn
	WD elements	Sr, K, S, ln Cl	Na, Sr, K, S, ln Cl	Na, K, ln Cl

Principal components analysis (PCA)

PCA determined interrelationships in the data from a matrix of pair-wise correlations and defined groupings of variables as 'factors'. The groupings are displayed in the factor loadings plot (Fig 7.2.6). We examined the correlation structure of variables rather than covariance because the 'micro' (WD-EM) and 'trace' (PIXE) elements exist and are measured in otoliths at different orders of magnitude: micro elements are measured at 100-5000 ppm and the trace elements measured by PIXE are less then 50-100 ppm.

The major groups identified from PCA were:

- WD-EM variables Na and Cl, with WD-EM variable K closely related
- WD-EM S and Sr
- PIXE variables in 2 sub-groups: Cu, Co and Zn; Pb, Rb and Hg.

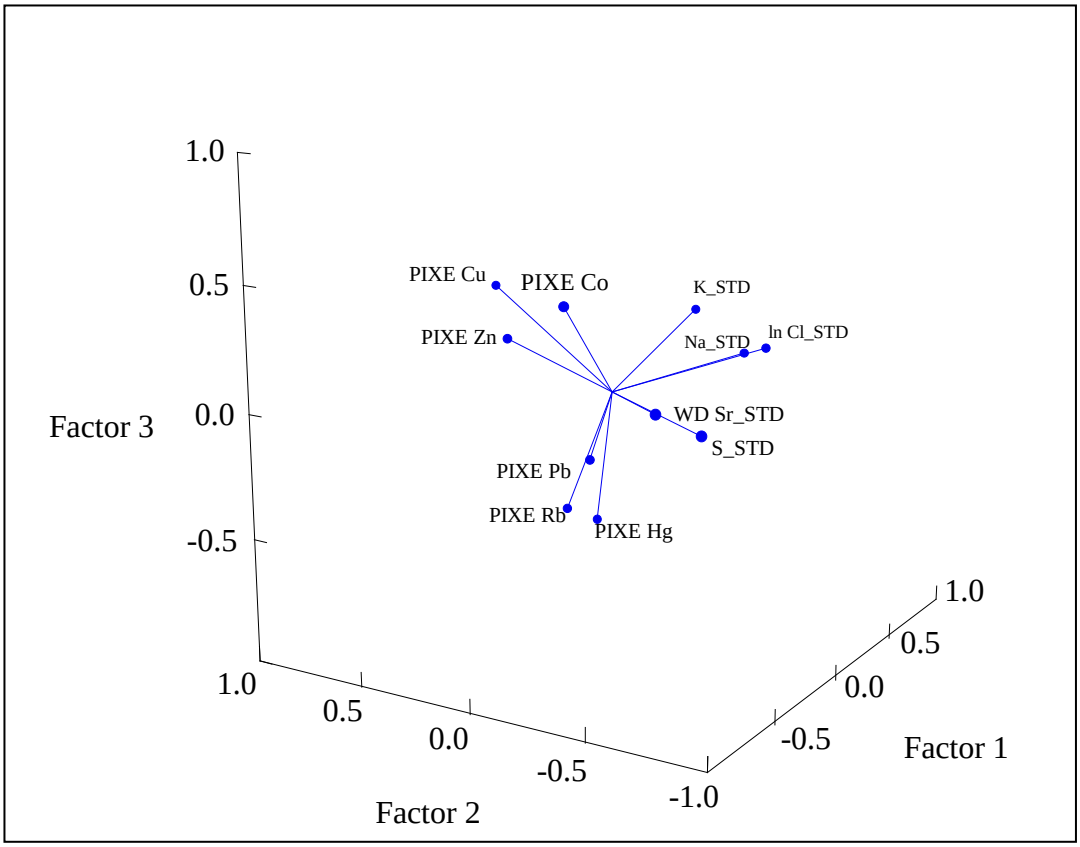

Figure 7.2.6. Factor loadings plot from a principal components analysis. The major identified were Na and Cl, with K closely related; S and Sr; and the PIXE elements in 2 groups: Cu, Co, Zn, and Pb, Rb, Hg.

Which cohorts are NSW samples most alike?

To determine which cohort the NSW samples most closely resembled, we used linear discriminant function analysis (LDFA). This technique established which elements had the greatest discriminatory power and from them identified 'elemental fingerprints' of individual cohorts. Each of the samples from the NSW cohorts was 'classified' by their elemental concentrations to the cohort they most closely resembled.

1994 Cohorts

Of the 167 samples in the 1994 NSW cohort, 11% were classified to the Fiji 94 cohort, 15% to Philippines 94 and 74% to Coral Sea 94 (table 7.2.12).

Table 7.2.12. Classification of NSW 94 samples to other 1994 cohorts. 74% of NSW 94 samples were classified to the Coral Sea 94 cohort.

	Fiji 94	Philippines 94	Coral Sea 94	Total
NSW94 classification	18 (11%)	25 (15%)	123 (74%)	166

Discriminant analysis also determined which groups were most alike and most different (table 7.2.13). Of the 1994 cohorts the pair that was 'most different' was the NSW 94 and Fiji 94 cohorts and the pair with the least difference, i.e. most alike, was Coral Sea 94 and NSW 94.

Table 7.2.13. Pair-wise relationships between 1994 cohorts with NSW included. Coral Sea and 'NSW cohorts were the 'most alike' pair.

		Between groups F-matrix value
Least alike	NSW 94 and Fiji 94	30.187
	Coral Sea 94 and Fiji94	27.226
↓	NSW 94 and Philippines 94	20.731
	Coral Sea 94 and Philippines 94	14.401
	Fiji 94 and Philippines 94	13.798
Most alike	Coral Sea 94 and NSW 94	6.100

As a part of the discriminant analysis, canonical variate analysis calculated canonical discriminant functions, the linear combination of variables that best discriminated among the groups. A plot of canonical scores (Figure 7.2.7) provided a visual display of the differences

between groups; the confidence ellipses in the canonical plots are centred on the means of the variables and the extent of the ellipse indicates 1 standard deviation. We plotted the scores of individual NSW samples to show how close they are in canonical space to the means of the other groups. The closer a point is to a group mean the more likely it is to be 'classified' by discriminant analysis to that group.

Discriminant analysis classified 74% of the 1994 NSW samples to the 1994 Coral Sea cohort. It was obvious in the canonical plot that many NSW samples lay within a standard deviation of the mean of the Coral Sea cohort but there were some NSW samples that lay outside confidence ellipse of any group. However, because of their position in canonical space, these samples were 'assigned' to the Coral Sea group. Despite being assigned to that cohort, their distance from the Coral Sea mean indicated that they might not be very similar to the majority of Coral Sea samples.

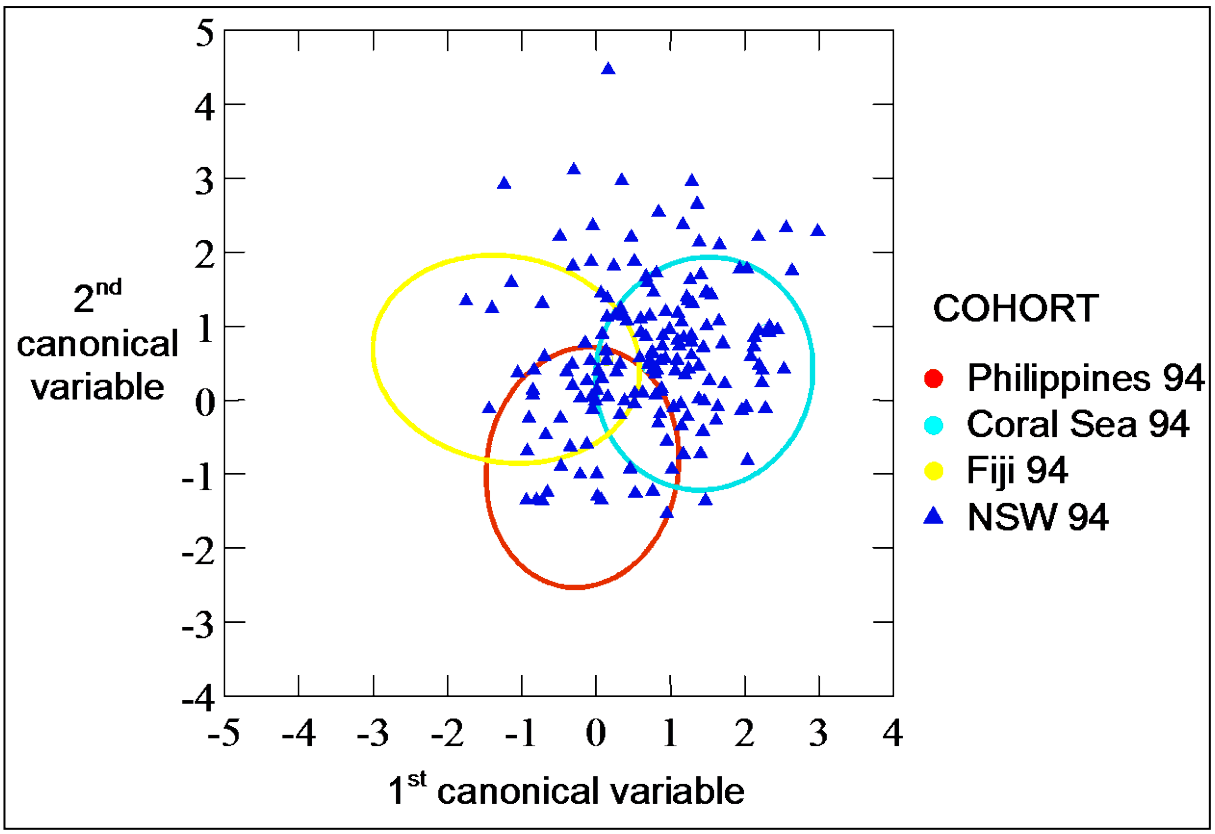

Figure 7.2.7. Canonical scores plot of the 1994 cohorts, showing NSW samples as individual points; the confidence ellipses in the canonical plots are centred on the means of the variables and the extent of the ellipse indicates 1 standard deviation.

The position of points in the canonical space (displayed in the 2-dimensional plot) were analysed using mahalanobis distances. These are the distances from the group means on the canonical variate axes. The mahalanobis distances to each group mean is calculated for each sample and the closer a case is to the mean of a particular group the more likely it is to be classified to that group.

'Posterior probabilities' were computed in Discriminant Analysis from mahalanobis distances, and provided a probability that a sample will be assigned the each group. For example, about 100 NSW samples had a low probability (0.1) of being assigned to the Fiji 94 cohort and only a small number of the smaller numbers of NSW samples had a higher probability of being assigned to the Fiji cohort. The bar and bubble charts of posterior probabilities (Figure 7.2.8 and 7.2.9) indicate that NSW samples are more likely to occur closer to the Coral Sea mean of canonical scores than either the Philippines mean or Fiji mean. At the high probability end the cohort to which the greatest proportion of NSW samples are assigned is Coral Sea 94.

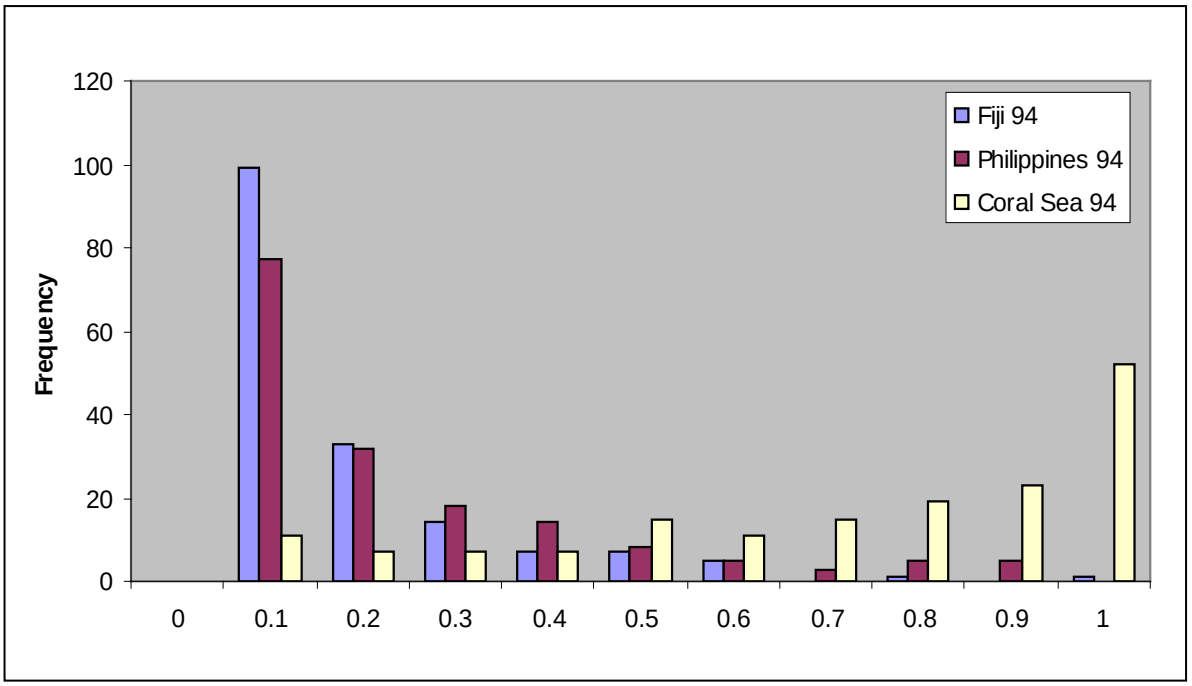

Figure 7.2.8. The proportion of posterior probabilities for assigning NSW 94 samples to 3 cohorts: Fiji 94, Philippines 94 and Coral Sea 94. The majority of NSW samples have a low probability of being assigned to the Fiji cohort and high probability of being assigned to the Coral Sea cohort.

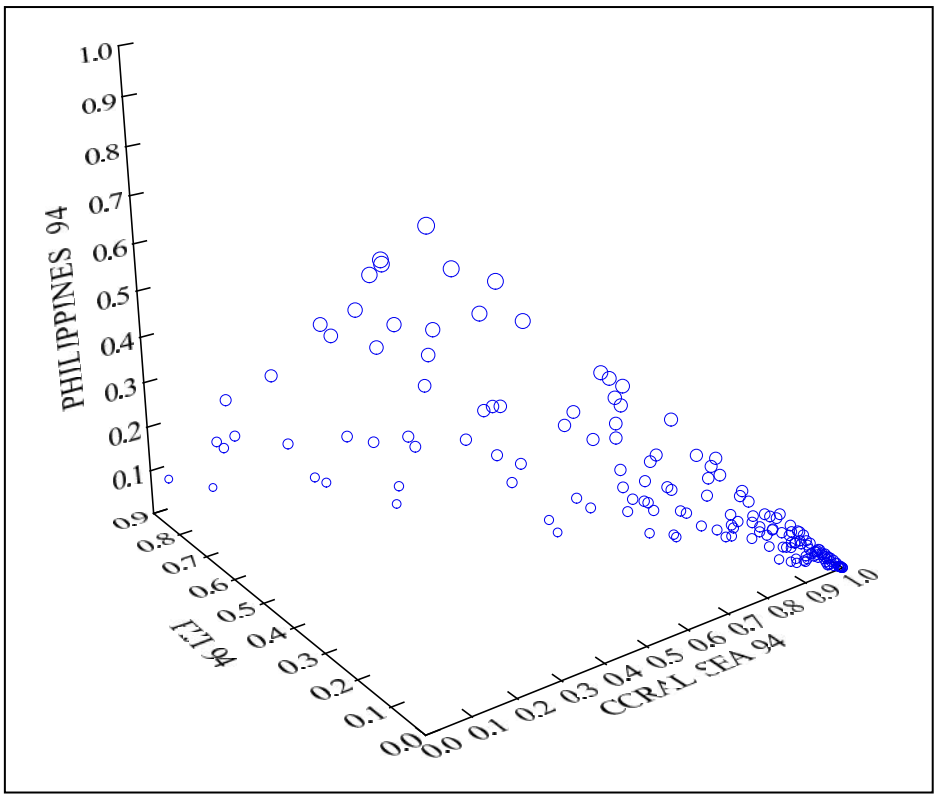

Figure 7.2.9. A 3-dimension plot of the NSW 94 posterior probabilities shows the large number of samples with a high probability of being assigned to the Coral Sea cohort.

1995 Cohorts

The classification of NSW samples to the cohort they most closely resembled was repeated for the 1995 cohort. The elemental concentrations in each NSW 95 sample were compared to the cohort signals determined from elemental concentrations in the samples from Indonesia 95, Solomon Islands 95 and, as we had not collected samples estimated to have been spawned in the Coral Sea in 1995, we used Coral Sea 94. As for the NSW 1994 cohort a majority of the NSW 95 samples (63%) were 'assigned' to the Coral Sea cohort. Only 10% of NSW samples were classified to the Solomon Islands cohort and 27% to Indonesia (table 7.2.14).

Table 7.2.14. Classification of NSW 95 samples to other 1995 cohorts and 1994 Coral Sea

	Indonesia 95	Solomon Islands 95	Coral Sea 94	Total
NSW 95 classification	25 (27%)	9 (10%)	59 (63%)	93

From a pair-wise comparison of cohorts, the Solomon Islands and Indonesian cohorts were determined to be most different, and the Solomon Island and Coral Sea as most alike (table

7.2.15). Despite the Solomon Islands and Coral Sea being the pair with the least difference, very few NSW samples were classified as Solomon Islands. The reason for this can be seen in a plot of canonical scores (Figure 7.2.10) — there was a lot of overlap between the confidence ellipses of the Coral Sea and Solomon Islands however most of the NSW samples had low canonical variables and lay at one end of the Coral Sea ellipse, the end that did not overlap with Solomon Islands.

Table 7.2.15. Pair-wise relationships between 1995 cohorts (including NSW 95) and 1994 Coral Sea

	Cohort pair	Between matrix F-value
Least alike	Solomon Islands 95 and Indonesia 95	20.579
	Solomon Islands 95 and NSW 95	16.935
↓	Indonesia 95 and NSW 95	14.621
	Indonesia 95 and Coral Sea 94	11.368
	NSW 95 and Coral Sea 94	5.840
Most alike	Solomon Islands 95 and Coral Sea 94	4.847

The position of points in the canonical space (displayed in the 2-dimensional plot, figure 7.2.10) were analysed using mahalanobis distances, the distances from the group means on the canonical variable axes. The large proportion of higher probabilities for the Coral Sea cohort (figure 7.2.11) indicates the NSW samples are more likely to be assigned as Coral Sea. Conversely, the proportion of probabilities for the Solomon Islands and Indonesian cohorts indicate that a majority of NSW samples are less likely to be assigned to these cohorts than to the Coral Sea.

Another display of the posterior probabilities, the 3-dimensional bubble plot (Figure 7.2.12), again shows that NSW samples are more likely to occur closer the Coral Sea mean of canonical scores than to either the Philippines mean or Fiji mean. At the high probability end (0.9) the cohort to which the greatest proportion of NSW 95 samples are assigned is Coral Sea 94.

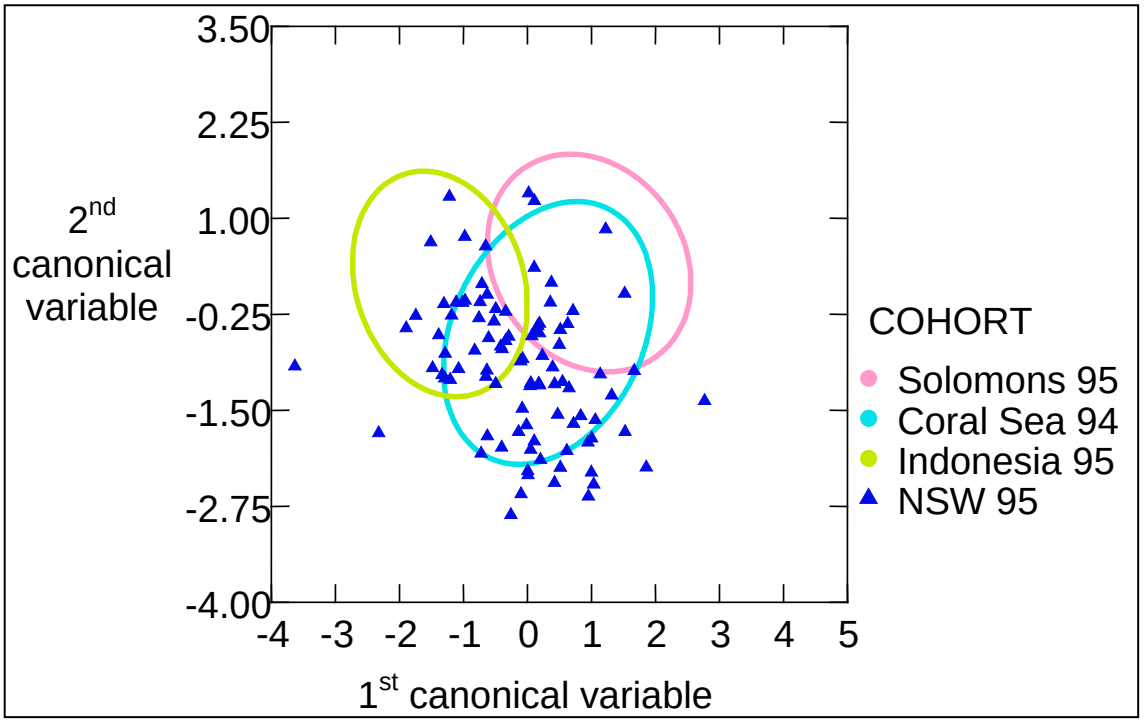

Figure 7.2.10. Canonical scores plot of the 1995 cohorts, showing NSW samples as individual points; the confidence ellipses in the canonical plots are centred on the means of the variables and the extent of the ellipse indicates 1 standard deviation.

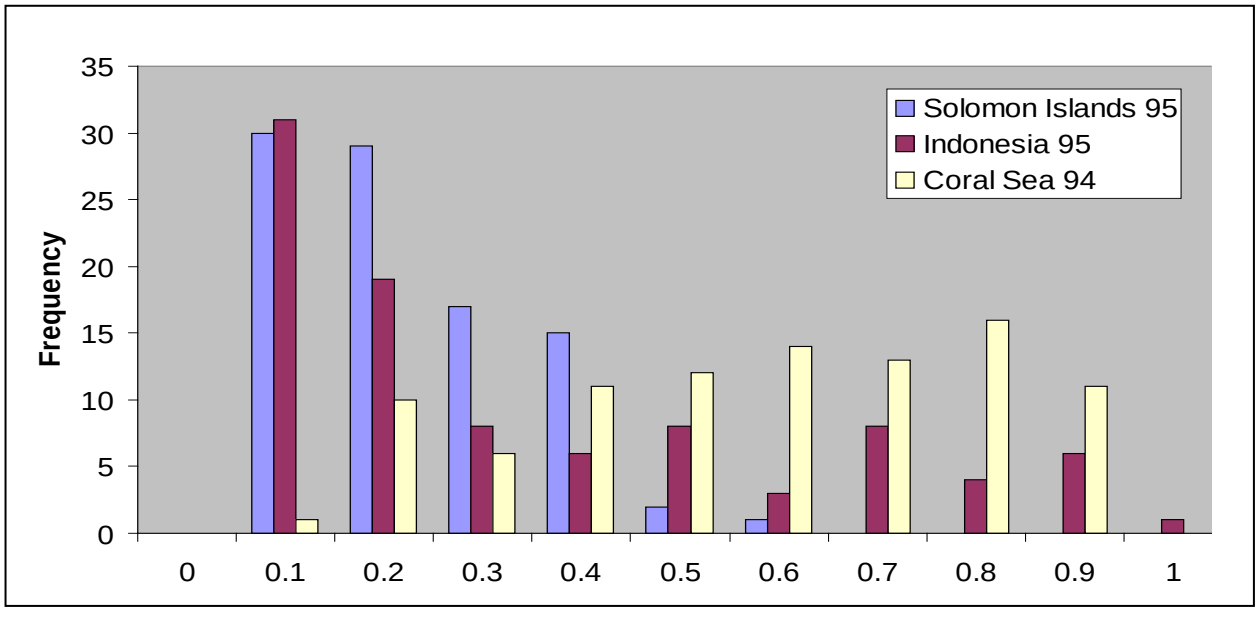

Figure 7.2.11. The proportion of posterior probabilities for assigning NSW 95 samples to 3 cohorts: Solomon Islands 95, Indonesia 95 and Coral Sea 94. The majority of NSW samples have a low probability of being assigned to the Indonesian and Solomon Islands cohorts and high probability of being assigned to the Coral Sea cohort.

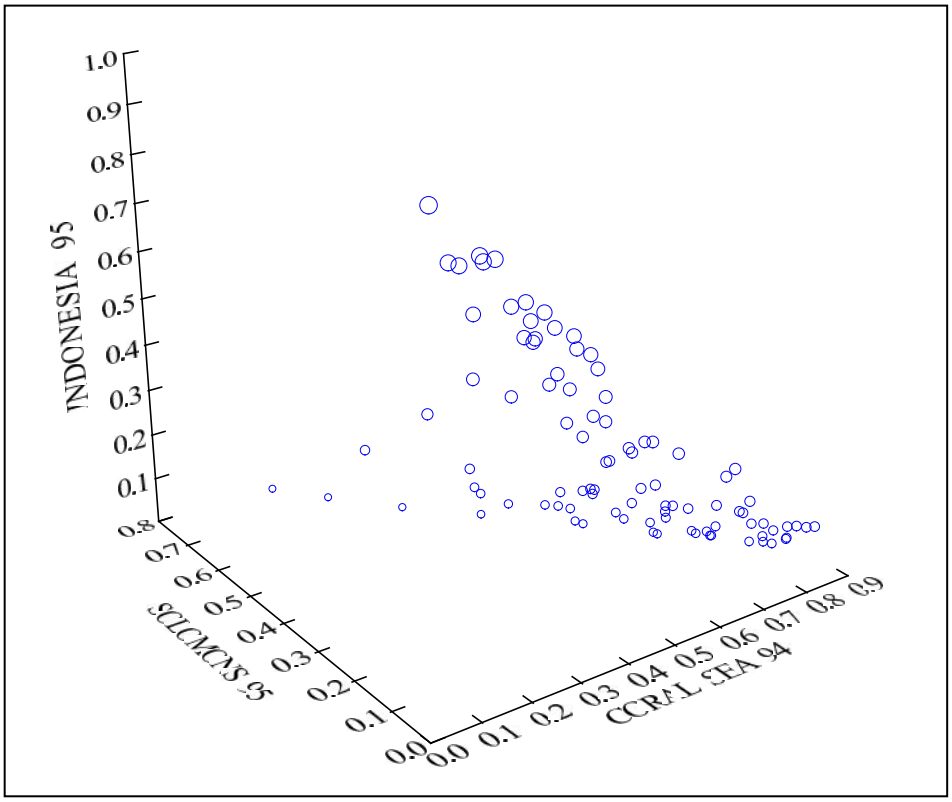

Figure 7.2.12. A 3-dimension plot of the NSW 95 posterior probabilities show the large number of samples with a high probability of being assigned to the Coral Sea cohort.

Exploring the similarity between NSW and Coral Sea

The results from discriminant analysis indicated that the elemental signal of otoliths from a majority of fish caught off NSW were most similar to the otolith signals from fish caught in the Coral Sea. We investigated differences between these groups to determine if the otolith signals were truly equivalent. When we examined the relationship between these cohorts we found highly significant differences between the Coral Sea cohort and each of the NSW cohorts (MANOVA, $p<0.001$, table 7.2.16.). A similar suite of PIXE and WD-EM elements contributed to the discrimination: individual elements that differed significantly in ANOVA between pairs of cohorts were Cu, Hg, K and Na ($p<0.001$) and Cl and Sr ($p<0.05$). There were significant differences between the 2 NSW cohorts, but due only to Na and Cl.

Table 7.2.16. Pair-wise comparison of Coral Sea and NSW cohorts. The MANOVA F-statistics and probabilities are given, and the elements that differed between cohorts.

	F-statistic	Probability	PIXE elements	WD elements
Coral Sea 94 and NSW 94	5.401	<0.001	Cu, Hg	K
Coral Sea 94 and NSW 95	4.143	<0.001	Cu, Hg	Na, K, Cl, Sr
NSW 94 and NSW 95	2.670	<0.01		Na, Cl

Using discriminant analysis we determined the proportion of Coral Sea samples that most closely resembled each NSW cohort. Using Coral Sea samples as "unknowns" to be classified to the 2 NSW cohorts, 57% of Coral Sea samples were classified to NSW 94 and the rest to NSW 95 (table 7.2.17). If the classification had been completely random we would have expected to see a 50/50 split, accordingly the 57/43 split, although not a large discrepancy, indicated that more of the Coral Sea otolith signals resembled the NSW 94 cohort. As we did not have samples from a 1995 Coral Sea cohort it is unclear whether this is because there was an interannual difference between the fish spawned in the Coral Sea in 1994 and 1995 or because more fish from the NSW 95 cohort were spawned in places outside the Coral Sea.

Cobalt was one of the elements that was included as an important discriminator in the multivariate discriminant analysis where as it had not differed significantly in the ANOVAs of the same cohorts.

Table 7.2.17. Classifying Coral Sea samples to NSW 94 and NSW 95 cohorts

	NSW 94	NSW 95	Strongest discriminating elements
Coral Sea 94	42 (57%)	32 (43%)	Na, Co

In a further analysis in which all cohorts were included except Coral Sea, 58% of NSW 94 samples were classified to the Philippines 94 cohort and 42% to Fiji 94. For the 1995 cohorts 73% of NSW 95 samples are classified to the Indonesia 95 cohort and 29% to Solomon Islands. When we excluded the Coral Sea cohort from the discriminant analysis, the WD-EM elements were still the strongest discriminators amongst the suite of 11 elements (tables 7.2.18 and 7.2.19).

Table 7.2.18. Classifying NSW samples to cohorts, excluding the Coral Sea cohort

a) 1994 cohorts

	Philippines 94	Fiji 94	Strongest discriminating elements
NSW 94	97 (58%)	69 (42%)	K, S

b) 1995 cohorts

	Solomons 95	Indonesia 95	Strongest discriminating elements
NSW 95	27 (29%)	66 (71%)	S, K

Table 7.2.19. Elements used to discriminate sites using discriminant analysis. These site-specific signals were subsequently used to 'assign' NSW samples to the site they most closely resembled (WD-EM elements bolded)

	1994 cohorts		1995 cohorts	
	CS excluded	CS included	CS excluded	CS included
Strongest	**K**	**S**	**S**	**S**
discriminators	**S**	**Na**	**K**	**K**
	Zn	**K**	Cu	Cu
↓	Rb	Cu	Zn	**Cl**
	Co	Zn	**Sr**	Pb
	Pb	Rb	Pb	Hg
	Hg	Hg	**Na**	Zn
	Na	**Cl**	Rb	Rb
	Sr	Co	**Cl**	**Na**
	Cu	Pb	Hg	Co
weakest	**Cl**	**Sr**	Co	**Sr**

7.2.3 Discussion

The delineation of stock structure using variation in otolith chemistry is based on three core assumptions (Campana *et al.* 2000):

1. there are characteristic and reproducible chemical markers or "fingerprints" for each group or "stock",
2. the marker or fingerprint remains stable over the interval between characterisation and mixing.
3. all possible groups contributing to the group mixture have been characterised.

The same suite of assumptions underpins use of genetic, morphometric or parasite fauna data in delineation of stock structure (Wood *et al.* 1989; Wirgin *et al.* 1997).

Where these assumptions can be validated, quantitative analysis of otolith composition can provide an indication of, among other things, stock delineation, natal source and stock mixing (Blaber *et al.* 1996; Secor 1999; Campana 2001; Thorrold *et al.* 2001). And, because otolith chemistry is believed to be phenotypically controlled, patterns of stock structure and dynamics can be derived even where there is no genetic heterogeneity within the fish sampled. Therein lies much of the attraction of using otolith chemistry to elucidate tuna stock structure.

Otolith chemistry is thought to be determined in part by the environmental conditions experienced by the fish, and in part by physiological processes. However, the study of otolith chemistry is a relatively immature science - the literature dates back only to the mid-1980's - and despite a significant number of observational and experimental studies over the last decade, the relative contributions of environmental conditions (temperature, salinity etc), ambient elemental concentrations and physiological processes (including physiological control of elemental uptake, otolith deposition) to observed variation in otolith composition remain largely un-quantified (see reviews by Thresher (1999) and Campana (1999)).

We know that absorption of elements into the otoliths is not direct; elements pass through the blood plasma and the endolymph (in which otoliths form) before being incorporated into the crystalline structure (Kalish 1991; Dove 1996) and in this process there is differential uptake of elements. This means that relationships between concentrations of elements in the environment and the otolith matrix are often not linear. In cases where elemental concentrations in the environment have been experimentally manipulated, and for marine fishes these have principally involved the manipulation of environmental strontium/calcium (see Campana (1999) for a review), linear correlations between the environmental and otolith Sr/Ca ratios have been demonstrated, although the distribution co-efficient has been much lower than 1, a clear indication that there is physiological discrimination of Sr. Similar discrimination has also been shown for Ba/Ca ratios in marine fish (Bath *et al.* 2000). A consistent feature of the experimental work is that the concentrations of the divalent ions appear more likely to be related to water concentrations that those of other ions (Milton and Chenery 2001; Rooker *et al.* 2001b).

The influence of temperature and salinity on otolith composition has been the subject of considerable research, but again primarily with respect to the deposition of Sr (reviewed by

Campana (1999)). Although there remains uncertainty over the relative importance of temperature and growth rates (themselves often influenced by temperature), it is clear that Sr deposition can be influenced significantly by either one or both of these factors.

The nature and extent of physiological control over the incorporation of elements is perhaps the most poorly understood of all the factors influencing otolith chemistry. Thresher *et al.* (1994), Proctor *et al.* (1995) and Campana *et al.* (2000) suggest that elements under strong physiological regulation appear to vary relatively little among fish populations. This is somewhat surprising, as there is evidence from our study and many others (including Thresher *et al.* 1994) of significant geographic variation in elements such as sodium, potassium, magnesium, and sulphur. Despite their observation of relatively little variation, Campana *et al.* (2000) note that if physiologically controlled elements do differ significantly among groups there is no reason to exclude them from the chemical 'fingerprint'.

In reviewing our data, we concluded that understanding all the factors and processes involved in yellowfin tuna otolith development is not essential for the delineation of their stock structure. Our first concern was whether the assumptions listed above are appropriate for our case. If they are valid, then the extent and nature of variation in yellowfin otolith chemistry, and how this relates to locality from which fish were sampled are the critical issues. Similar conclusions have been reached for a range of other species (Edmonds *et al.* 1999; Newman *et al.* 2000; Campana *et al.* 2000).

Assumption 1. There are characteristic and reproducible chemical markers or "fingerprints" for each group or "stock".

The collection phase of our study ran for 3 years and during this time we sampled otoliths from yellowfin tuna caught in 6 areas around the western Pacific Ocean. Five of these were known spawning sites for yellowfin tuna and at these we sampled small fish assumed to have remained close to their natal site; larger fish were sampled at the sixth and most southerly location, NSW.

Eleven of the elements analysed using the PIXE and WD-EMPA differed significantly among the locations. These included both minor and trace elements, those that are likely to be under "strict physiological regulation" and those that most likely reflect environmental variation. The large sample sizes for each area, strict analytical procedures designed to minimize any errors or biases that may result in spurious inter-site difference (e.g. randomised order for sample analysis) and the highly significant differences such a large number of elements, suggest that the assumption of characteristic and reproducible group "markers" or fingerprints is valid.

Assumption2. The chemical markers or fingerprints remain stable over the interval between characterisation and mixing.

This assumption pertains principally to the temporal variation within a chemical marker, and is an important but often overlooked element in stock delineation studies using otolith chemistry. However, another issue related to the stability of the chemical marker is that of the lability of the elements within the otolith matrix.

An uncertainty within our data are possible post-mortality effects on otolith composition. After the collection of all our samples, several studies investigated the influence of specimen handling and preparation on otolith chemistry (Proctor and Thresher 1998; Thresher 1998; Milton and Chenery 1998; Rooker *et al.* 2001b) and found that otolith composition is not entirely stable post-mortem. Our sampling of otoliths was standardised as much as possible. However, in the Philippines, contrary to the collection protocols used for the other sites, heads were collected and sent frozen to CSIRO for otolith extraction. Thus, we cannot rule out that this may have influenced the concentrations of elements in these otoliths. However, in our favour is the fact that we were analysing material from the core of the otolith. Milton and Chenery (1998) examined otoliths that had undergone different post-mortem handing including freezing and found few differences between treatments on measurements of elements at the primordium - mean concentrations of 2 of the 13 elements measured were significantly higher in otoliths that had been sampled from frozen fish. This is similar to the findings of Proctor & Thresher (1998) who found that, of 5 post-mortem treatments, freezing had the least effect on elemental concentrations.

Other possible influences that may have masked the real otolith chemistry and introduced confounding factors into this study were more easily minimised: storage, preparation and analysis of otoliths were carefully controlled. One advantage of using probe analysers over bulk analysers is the ability to compare otoliths at specific points in the life history (in this case the first weeks of life) and the added advantage is that surface contamination of the whole otoliths during collection and handling may not affect the primordium. We also checked for systematic errors: samples were chosen at random to preparing and analysing the samples from one cohort in a batch.

Thus, in considering the issue of lability of elemental composition, we established a standard set of post-mortem handling and analytical protocols designed to minimise any site-specific effects. The one breach of these protocols was in the handling of Philippines samples, but as we were analysing the primordial region of the otolith it seems unlikely that the freezing of these samples prior to otolith removal would have significantly affected our results.

To test for temporal stability in our otolith markers we sampled three of the natal sites on two occasions – Indonesia in 1995 and 1996, Philippines in 1994 and 1996, and the Solomon Islands in 1994 and 1995. On each occasion, otolith samples were collected from similar-sized fish, caught in the same areas, at the same time of year, and handled in the same way. As all fish sampled were less than 4 months old, we assumed that they had not moved significantly away from their spawning location. We compared the composition of the core regions of these otoliths to examine the temporal stability of otolith chemistry over inter-annual time periods. Multivariate analyses of variance (MANOVA), in which elemental concentrations are used as the dependent variables and cohort as the independent variable, revealed highly significant differences ($p<0.001$) at each of the sites between the samples collected at different times (tables 7.2.11 and 7.2.20). Both trace elements (copper and lead in the Solomons, and zinc in Indonesia) and elements thought to be under strong physiological control (strontium, sodium, potassium, sulphur and chlorine) showed significant temporal variation at one or more of the sites, although the suite of physiologically-controlled elements showed the greatest variation. In the case of potassium and chlorine, concentrations varied significantly between pairs of samples at all sites.

The significant differences in otolith chemistry with time within locations suggest that what ever causes a signal in the otolith, be it environmental or physiological variation, it is not stable over the time scales we have examined.

Table 7.2.20. Inter-annual variation in yellowfin tuna otolith elemental weight fraction concentrations for samples collected at three sites in the Indo-West Pacific (significant differences are shown in bold).

Element	Probe Analysis	Solomon Islands 95:96 (n=151)		Philippines 94:96 (n=153)		Indonesia 95:96 (n=142)	
		F	P	F	P	F	P
Copper	PIXE	56.645	**0.000**	1.777	0.185	2.770	0.098
Zinc	PIXE	2.408	0.123	0.657	0.419	6.359	**0.013**
Rubidium	PIXE	0.721	0.397	1.181	0.279	2.276	0.134
Lead	PIXE	8.463	**0.004**	2.943	0.088	1.148	0.286
Mercury	PIXE	0.131	0.718	1.012	0.316	0.890	0.347
Strontium	PIXE	0.004	0.948	0.089	0.766	1.036	0.311
Cobalt	PIXE	0.195	0.660	2.043	0.155	0.463	0.497
Sodium	WDEM	0.422	0.517	144.412	**0.000**	128.132	**0.000**
Strontium	WDEM	24.848	**0.000**	10.856	**0.001**	0.328	0.568
Potassium	WDEM	96.866	**0.000**	7.315	**0.008**	7.194	**0.008**
Sulphur	WDEM	55.631	**0.000**	6.759	**0.010**	0.387	0.535
Chlorine	WDEM	4.985	**0.027**	47.759	**0.000**	5.839	**0.017**

There is very little environmental data available for the three sites. However, there were inter-annual differences in the order of 1.0-1.5°C in sea surface temperature at each site (table 7.2.21). Also noteworthy are the very significant differences in temperature (2-4°C) between the three sites located in the 10°S to 10°N – Philippines, Solomon Islands and Indonesia - and the two located further south – Coral Sea and Fiji.

Table 7.2.21. Temperatures at the times spawning occurred in the sites at which juveniles fish were sampled. (These data were obtained from the NASA Physical Oceanography Distributed Active Archive Center at the Jet Propulsion Laboratory, California Institute of Technology)

Location	Approximate Position	Year	Month	Mean SST	Median SST	Max SST	Min SST	S Dev SST
Coral Sea	15°S 146°E	1994	10	25.41	25.35	25.65	25.20	0.11
Philippines	8°N 127°E	1994	11	28.40	28.35	28.65	28.05	0.15
Philippines		1996	10	29.51	29.70	30.15	28.35	0.47
Fiji	18°S 176°E	1994	11	26.06	26.10	26.40	25.65	0.20
Solomon Islands	8°S 159°E	1995	4	29.54	29.55	30.00	29.25	0.20
Solomon Islands		1996	3	28.55	28.50	29.55	28.05	0.46
Indonesia	3°S 128°E	1995	8	26.93	27.00	27.45	26.25	0.39
Indonesia		1996	8	28.45	28.50	28.95	27.60	0.34

Given the large sample sizes used for the comparisons, and the significant spread in birth dates for fish in each of the cohorts/samples analysed, the scale of inter-annual variation is considerable; meaning that, if one was attempting to use samples from a number of cohorts as a marker or fingerprint, the second core assumption would be invalid. In our case, the inference from these data is that classifications of natal ground markers need to be based on single cohorts, rather than on samples pooled across cohorts. There remains the possibility that the signal in also variable within a year, and quantification of this was beyond the scope of this study. If intra-annual variation is significant, then it would mean that samples taken from discrete time periods may not represent the full range of variation from that site.

This is a point often overlooked in otolith chemistry studies, where the assumption is made that signals are stable across time or, if not, that variance among sites is greater than that within samples from a number of cohorts taken at an individual site. Where the later is the case, ignoring annual variation may mask the nature and scale of spatial variation. In cases where no difference is apparent among sites in samples pooled across years (e.g. Proctor *et al.* 1995, for SBT) it may be that the signal is being masked by inter-annual variability.

Assumption 3. All possible groups or stocks contributing to the group mixture have been sampled and characterised.

Quite simply this is not possible in a pelagic species such as yellowfin tuna, which spawns over huge expanses of the tropical and sub-tropical Pacific Ocean. It is similarly unlikely to be realistic for any species that have widespread spawning sites. In our study, we focussed on sampling spawning areas that covered a range of plausible sources of recruits to the east coast yellowfin tuna fishery, acknowledging from the start that we could not be certain that a very close statistical relationship between say Fiji and NSW fish two years later indicated that fish had definitely recruited from Fiji.

Determining the origin of recruits to the NSW fishery

The significant differences in otolith markers among sites, and among cohorts at some sites, meant that in using natal site data to classify the origin of NSW fish only fish from the same cohort could be used. If 0+ fish had been available at all sites in all years, as per our original experimental design, this would have presented little problem for us. However, despite extensive efforts, 0+ fish were not caught in the Coral Sea in 1995 or 1996. Also the 1995 samples from the Philippines were destroyed in a Philippines research laboratory power failure. As a result, we were restricted to using a sub-set of the range of sites when examining the links between natal sites and the NSW recruits. As we were particularly interested in looking at the links between the Coral Sea and NSW fish, we opted to use 1994 Coral Sea chemical markers in the classification of the 1995 NSW cohort. This is obviously not optimal, particularly as we could not assume that the 1994 cohort data was representative of the 1995 signal.

The results of Linear Discriminant Function (LDF) classification of NSW samples indicated that for the 1994 cohort, the elemental signal of otoliths from a majority of fish caught off NSW were most similar to the otolith signals from fish caught in the Coral Sea. Using natal site data from the Coral Sea, Fiji and the Philippines, 74% of the 1994 NSW cohort were classified as being closest to the Coral Sea, 11% to Fiji and 15% to Philippines.

Using the Coral Sea 1994 markers with those from the 1995 cohorts from Indonesia and the Solomon Islands to classify the 1995 NSW cohort, we found that 63% of the 1995 NSW cohort were classified to the Coral Sea cohort, 27% to Indonesia and 10% to Solomon Islands.

What do these numbers mean in terms of the origin of recruits to the east coast/NSW fishery? Previous studies have interpreted results from LDF of otolith chemistry data to track stock movements, and even to quantify stock mixing, describe natal homing and deduce migration history (Thresher *et al.* 1994; Campana *et al.* 1995; Dove *et al.* 1996; Thorrold *et al.* 2001).

We'd suggest that a more cautious approach should be taken to interpreting LDF analyses of otolith chemistry data, particularly where you have not been able to sample all the range of possible markers or possible contributors to your mixed population (as with all but one of the above studies). Discriminant analysis is a powerful multivariate tool for classifying samples to "the most alike group". It's primary weakness is that even when an "unknown" sample differs very significantly from the range of possible discriminators, it will receive a classification to

the nearest marker. In the case of our data, canonical vector plots show cases where a fish is classified as coming from the Coral Sea, or one of the other natal sites, even when its chemical signal means it differs by significantly more than one standard deviation from the group mean. Thus, classification doesn't necessarily confirm that fish share a common history, only that they are more alike than other groups in the analysis.

The classification rates of NSW do confirm that the chemistry of the fish we caught in NSW was in both years closest to the chemistry we found in fish from the Coral Sea. That there was less similarity between NSW and the fish from Fiji, the Philippines, the Solomon Islands and Indonesia is also significant in a biological context. Our examinations of posterior probabilities of the LDF classifications show that in the majority of cases when either the 1994 or 1995 NSW fish were classified as being closest to the Coral Sea 94 signal, the probabilities were high (>0.6). This contrasted with the situation for the non-Coral Sea natal sites where the classifications were generally more equivocal with probabilities in the range of 0.35-0.6. So, the probability of a NSW fish being classified to the 94 Coral Sea cohort was greater than being classified to all other, non-Coral Sea cohorts. This provides confidence that the match between the Coral Sea and NSW otolith chemistry was in fact much closer than that between other natal sites, supporting our hypothesis that recruits to the NSW fishery derive from the Coral Sea. However, we can not categorically say that the fish that were classified as coming from any of these sites were in fact spawned in those sites; we know that yellowfin tuna spawn throughout the tropical western and central Pacific, at many more sites than we were able to sample.

Although we sampled at only a limited number of locations, these included sites from a range of tropical and semitropical latitudes. If otoliths were simply environmental recorders, and the otolith elemental fingerprint purely a product of chemical and physical environmental factors, we might expect to see the cohorts from locations at similar latitudes being alike, i.e. the cohorts from tropical sites would have similar elemental signals as would cohorts from the sub-tropical sites. However, this was not evident in our data: Coral Sea 94 and Fiji 94 samples were collected at similar latitudes (and water temperatures) but had significantly different elemental signals (low misclassification rates). From this we conclude that physiological differences between fish at different locations play a significant role in creating unique otolith compositions. This is supported by the fact that the otolith elements usually thought to be largely under physiological control were the ones that were powerful discriminators between cohorts.

In conclusion to this section of the report, we've been able to show that otolith chemistry does provide a natural tag for yellowfin tuna, and that chemical markers vary geographically. The scope of the study did not allow us to collect and analyse otoliths from throughout the range of yellowfin in the Western Pacific, and we ran into difficulties in collecting an adequate number of samples from some sites because yellowfin abundance fluctuates significantly on seasonal and inter-annual bases, particularly in the Coral and Tasman Seas. Thus, we can not say unequivocally that the classification of natal origin of recruits we derived in our analyses account for all possible scenarios. However, we believe that our results provide significant evidence that the north-western Coral Sea spawning area is a major source of recruits to the east coast fishery. The otolith data also suggest links between yellowfin caught off the east coast and populations across the western Pacific Ocean that would need further sampling and analysis before the extent of immigration from these areas could be quantified.

The most powerful use of the results we have produced here will be in their integration with all the other indicators of stock structure and migration collected by this study and others over the last ten years. This synthesis is provided in Chapter 8.

7.2.4 References

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Appendix 7A

Table 7.2.22. Data from WD-EM analysis; elements are not standardised to calcium. Summary of ANOVA Bonferroni post-hoc tests indicating group (location) means that differ significantly * = P<0.05; otherwise highly significant P<0.01.

	Coral Sea	**Fiji**	**Indonesia**	**NSW/Sth Qld**	**Philippines**	**Solomon Islands**
Coral Sea						
Fiji	Na Sr-WD S					
Indonesia	Na Sr-WD S	Na Sr-WD S				
NSW/Sth Qld		Na Sr-WD S	Na Sr-WD S			
Philippines	Na K S	Na Sr-WD S	Sr-WD S	Ca Na K S		
Solomon Islands	Sr-WD	Na Sr-WD S	Na S Cl *	Sr-WD S	Ca Na S * Cl	

Table 7.2.23 (a)-(m). Details of post hoc tests showing matrices of pairwise comparison probabilities.

Locations: 1 = Coral Sea
2 = Fiji
3 = Indonesia
4 = NSW / Sth Qld
5 = Philippines
6 = Solomon Islands

(a) copper (PIXE)

	1	2	3	4	5	6
1	1.000					
2	0.000	1.000				
3	0.000	1.000	1.000			
4	0.006	0.067	0.001	1.000		
5	0.000	1.000	1.000	0.003	1.000	
6	1.000	0.000	0.000	0.005	0.000	1.000

(b) zinc (PIXE)

	1	2	3	4	5	6
1	1.000					
2	0.000	1.000				
3	1.000	0.000	1.000			
4	1.000	0.000	1.000	1.000		
5	1.000	0.000	1.000	1.000	1.000	
6	1.000	0.000	1.000	1.000	1.000	1.000

(c) strontium (PIXE)

	1	2	3	4	5	6
1	1.000					
2	1.000	1.000				
3	0.200	0.076	1.000			
4	1.000	1.000	0.702	1.000		
5	0.215	0.082	1.000	0.757	1.000	
6	1.000	1.000	0.000	0.014	0.000	1.000

(d) rubidium (PIXE)

	1	2	3	4	5	6
1	1.000					
2	1.000	1.000				
3	1.000	1.000	1.000			
4	1.000	1.000	0.941	1.000		
5	0.353	0.520	0.719	0.000	1.000	
6	1.000	0.689	0.052	1.000	0.000	1.000

(e) lead (PIXE)

	1	2	3	4	5	6
1	1.000					
2	1.000	1.000				
3	1.000	0.397	1.000			
4	1.000	1.000	1.000	1.000		
5	1.000	0.464	1.000	1.000	1.000	
6	1.000	1.000	1.000	1.000	1.000	1.000

(f) mercury (PIXE)

	1	2	3	4	5	6
1	1.000					
2	0.002	1.000				
3	1.000	0.116	1.000			
4	0.100	0.422	1.000	1.000		
5	0.803	0.195	1.000	1.000	1.000	
6	0.665	0.250	1.000	1.000	1.000	1.000

(g) cobalt (PIXE)

	1	2	3	4	5	6
1	1.000					
2	1.000	1.000				
3	1.000	1.000	1.000			
4	1.000	1.000	1.000	1.000		
5	1.000	1.000	1.000	0.004	1.000	
6	0.715	1.000	0.580	1.000	0.002	1.000

(h) calcium (WD)

	1	2	3	4	5	6
1	1.000					
2	1.000	1.000				
3	1.000	1.000	1.000			
4	1.000	1.000	1.000	1.000		
5	1.000	1.000	1.000	0.004	1.000	
6	0.715	1.000	0.580	1.000	0.002	1.000

(i) sodium (WD)

	1	2	3	4	5	6
1	1.000					
2	0.000	1.000				
3	0.000	0.000	1.000			
4	1.000	0.000	0.000	1.000		
5	0.000	0.000	1.000	0.000	1.000	
6	1.000	0.000	0.000	0.585	0.000	1.000

(j) strontium (WD)

	1	2	3	4	5	6
1	1.000					
2	0.001	1.000				
3	0.000	0.000	1.000			
4	1.000	0.000	0.000	1.000		
5	1.000	0.000	0.000	0.364	1.000	
6	0.003	0.000	0.236	0.000	0.064	1.000

(k) potassium (WD)

	1	2	3	4	5	6
1	1.000					
2	0.964	1.000				
3	0.086	1.000	1.000			
4	1.000	1.000	0.369	1.000		
5	0.000	0.503	1.000	0.000	1.000	
6	0.299	1.000	1.000	1.000	0.302	1.000

(l) sulfur (WD)

	1	2	3	4	5	6
1	1.000					
2	0.000	1.000				
3	0.000	0.000	1.000			
4	1.000	0.000	0.000	1.000		
5	0.000	0.000	0.000	0.000	1.000	
6	0.147	0.000	0.000	0.002	0.041	1.000

(m) chlorine (WD)

	1	2	3	4	5	6
1	1.000					
2	1.000	1.000				
3	1.000	1.000	1.000			
4	1.000	1.000	0.779	1.000		
5	1.000	1.000	1.000	0.152	1.000	
6	0.231	0.711	0.049	1.000	0.008	1.0001

(n) sodium – standardised to Ca

	1	2	3	4	5	6
1	1.000					
2	0.000	1.000				
3	0.000	0.000	1.000			
4	1.000	0.000	0.000	1.000		
5	1.000	0.000	0.000	1.000	1.000	
6	0.005	0.000	1.000	0.000	0.002	1.000

(o) WD strontium – standardised to Ca

	1	2	3	4	5	6
1	1.000					
2	1.000	1.000				
3	0.983	1.000	1.000			
4	1.000	1.000	1.000	1.000		
5	0.012	0.054	1.000	0.049	1.000	
6	0.413	1.000	1.000	1.000	1.000	1.000

(p) WD strontium – standardised to Ca

	1	2	3	4	5	6
1	1.000					
2	0.000	1.000				
3	0.765	0.000	1.000			
4	0.000	0.173	0.029	1.000		
5	1.000	0.000	0.008	0.000	1.000	
6	1.000	0.000	0.085	0.000	1.000	1.000

(q) WD sulfur – standardised to Ca

	1	2	3	4	5	6
1	1.000					
2	0.000	1.000				
3	0.000	0.000	1.000			
4	1.000	0.000	0.000	1.000		
5	0.000	0.000	0.000	0.000	1.000	
6	0.165	0.000	0.000	0.004	0.151	1.000

(r) WD chlorine – standardised to Ca

	1	2	3	4	5	6
1	1.000					
2	1.000	1.000				
3	1.000	1.000	1.000			
4	1.000	1.000	0.605	1.000		
5	1.000	1.000	1.000	0.106	1.000	
6	0.228	0.522	0.038	1.000	0.005	1.000

7.3 Secondary Ion Mass Spectrometry (SIMS)

7.3.1 Introduction

Stable isotopes are non-radioactive forms of elements that are differentiated by their atomic mass. They occur naturally in the tissues of fish and have been measured in blood, muscle, endolymph (fluid surrounding the otoliths) and otoliths (Mulcahy *et al.* 1979; Schwarcz *et al.* 1998). The isotopes are deposited in the inorganic crystalline aragonite of otoliths; their ratio is determined partially by the surrounding environment and hence these ratios can reflect differences between areas (Dufor *et al.* 1998; Edmonds *et al.* 1999).

Because oxygen isotopes are thought to be deposited approximately in equilibrium with ambient sea water they can be accurate recorders of temperature (Devereux 1967; Kalish 1991a, b; Thorrold *et al.* 1997). It has been hypothesised that they can be used to identify separate water bodies in which ambient temperature differs and hence have be used to delineate stocks of a number of fish species (Edmonds and Fletcher 1997; Edmonds *et al.* 1999; Gao and Beamish 1999; Newman *et al.* 2000; Stephenson *et al.* 2001).

The deposition of the stable isotopes of carbon is more complex. Disequilibria between stable carbon isotopes and ambient sea water has been recorded in otoliths of several species, i.e. ^{13}C was depleted compared to DIC (Kalish 1991a; Iacumin *et al.* 1992; Radtke *et al.* 1996; Thorrold *et al.* 1997; Schwarcz *et al.* 1998). However in addition, Kalish (1991a) found that there was a strong correlation between $\delta^{13}C$ and temperature and between $\delta^{13}C$ and $\delta^{18}O$. He suggested that this was not likely to be a direct relationship; other factors may influence the ratio, such as metabolic rate and oxygen consumption. Further studies have reported other influences on the carbon isotope ratios including onogenetic changes, increased range of depth distribution, i.e. spending more time in colder water and dietary shifts (Mulcahy *et al.* 1979; Radtke *et al.* 1996; Schwarcz *et al.* 1998).

One possible influence on the results of our analysis is the ability of tunas to elevate their body temperature using vascular counter-current heat exchangers (Carey 1973; Holland *et al.* 1992). Unlike the endothermy of birds and mammals, this ability is subject to variation in tunas, and is affected by ambient (water) temperature and swimming velocity (Dewar *et al.* 1994). Among the tunas there are two types of heat exchange systems: one highly developed system present in the species that occupy colder water (bluefin tuna and bigeye tuna) and a system found in the small, tropical tunas such as skipjack tuna *Katsuwanis pelamis* (Block and Finnerty 1994; Block *et al.*1997) and yellowfin tuna morphology is an intermediate (Dewer *et al.* 1994; Brill and Bushnell 2001; Graham and Dickson 2001). Whereas bluefin and bigeye tuna spend time in cool water in temperate regions and/or below the thermocline, yellowfin tuna are limited by water temperature (Brill *et al.* 1999), suggesting that their thermoregulatory mechanisms are less effective than those of bluefin.

The fact that thermoregulatory mechanisms are less developed in yellowfin tuna compared with bluefin tuna suggests that their otolith isotope ratios will be more influenced by the surrounding water mass. Bluefin tuna otoliths have been used as recorders of environmental temperatures by Radtke and Morales-Nin (1989) who hypothesised that the changes in the Sr/Ca ratio recorded along the otolith reflected migrations across water masses of different temperatures. However, the ability of tunas to maintain body temperature above ambient

water temperature may obscure the temperature signal to some unknown degree and hence make their otolith stable isotope ratios unsuitable as a proxy for water temperature (Kalish 1991a; Campana 1999). On the other hand this does not preclude their use in discriminating between different groups of fish.

Although kinetic and metabolic factors and thermoregulation may affect otolith isotope ratios, we concur with Edmonds *et al.* (1999), that even without knowing the underlying causes of isotopic disequilibria it is still possible to use the measured differences between isotopes in otolith carbonate to differentiate groups of fish. Similar to our analysis of concentrations of elements in the otolith matrix (reported in the previous section) we aimed to use the information from isotope signals to determine whether young fish caught in different areas exhibited significantly different oxygen and carbon isotope ratios, from which it may be possible to determine at a later date their natal area. Unlike the otolith elemental analysis, stable isotopes are thought not to be susceptible to changes due to post mortem treatments or contamination from handling and analysis (Campana 1999).

Before undertaking this analysis we conducted a pilot study to determine if the SIMS technique could in fact detect differences between yellowfin tuna otoliths collected from fish caught in different areas: the Philippines, Coral Sea and Hawaii. Stable isotope analysis of the otoliths was carried out in the School of Earth Sciences at the Australian National University. The analysis differentiated the 3 groups; otoliths sampled from fish caught at the different locations had different mean isotope ratios. The $\delta^{18}O$ values followed the expected trend of fish from warmer areas having lower values, i.e. ^{18}O was depleted (Radtke *et al.* 1996). The $\delta^{13}C$ values also followed the expected trend that ^{13}C was more depleted in the warmer areas, and $\delta^{13}C$ lower, but also that $\delta^{13}C$ of otoliths from larger (older) fish were higher (Kalish 1991a; Radtke *et al.* 1996; Schwarcz *et al.* 1998). In the pilot project, fish from the Coral Sea (50-90 cm LCF) had higher $\delta^{13}C$ than the fish from Hawaii and the Philippines (30-45 cm LCF).

The ranges of $\delta^{13}C$ and $\delta^{18}O$ values measured in the pilot project were low compared with other marine fishes, even lower than the levels reported in otoliths of juvenile southern bluefin tuna *Thunnus maccoyii* (Kalish 1991a). Several factors could possibly explain this: the high metabolic rates of juvenile tunas; the thermoregulatory abilities of tunas to maintain body temperature above that of the ambient water; and the high temperature of the tropical waters in which juvenile yellowfin were spawned. Despite the environmental isotope signal possibly being obscured to some degree by the thermoregulatory mechanisms of the there were significant differences detected between the signals from different locations. In the current study we measured the oxygen and carbon isotopes from yellowfin tuna otoliths sampled from five locations in the western Pacific Ocean and the Coral Sea and compared these site-specific signals with the signal from fish collected off the eastern Australian coast.

7.3.2 Methods

The "sister" otoliths of a sub-sample of the specimens analysed using WD-EMPA and PIXE were prepared for SIMS analysis. We were interested only in the portion of the otolith deposited during the first month of life, i.e. before juvenile fish had moved far from the areas in which they had been spawned and before thermoregulatory system was fully developed. Therefore, an attempt was made to extract from the otolith the material deposited during the first month of life. This area was estimated by examining otolith sections using a scanning electron microscope, which revealed daily increments. We counted these to estimate the material that was deposited during the first month of life.

Two steps were required to sample the otolith material deposited in the first month of life and exclude otolith material deposited after this time from the analysis:

1. Chemical removal, or "etching", of otolith material from the medial and lateral faces, i.e. above and below the primordial area in the photographs below (Figure 7.3.1A and B)

2. Drilling the material from the primordial area to remove it from the remaining otolith. In otoliths from small fish, the amount of material to be removed was minimal, conversely in larger fish a larger amount was removed. (Figure 7.3.2).

The excess otolith material was chemically removed – "etched" – by immersing the otolith in 1N HCl, using a paint brush to remove bubbles from the medial and lateral surfaces (upper and lower surfaces in figure 7.3.1) as they formed (as a product of the chemical etching). Dipping the otoliths in bleach and rinsing in distilled water arrested the etching process.

Initially, we conducted trials to determine required etching times for otoliths from fish of various sizes. Etching times ranged from 5 to 90 seconds for otoliths from fish of 22 to 149 cm LCF respectively. After etching, each otolith was examined under light microscope to determine if etching had been adequate – when the etching had removed the over burden from the primordial area the innermost increments were obvious. If the over burden had not been removed completely, the otolith was etched again. However, there was no way to check if etching in the sulcus (medial face) had been adequate. After etching, the otoliths were dried at 30°C.

A dental drill fitted with a diamond burr ball was used to drill through the primordial area, an area of approximately 0.5 mm radius. The etched otoliths were fragile so the rostral tips were embedded in wax to give support during the drilling, and the drill was run at a slow speed(approximately 2000 rpm). Sample sizes were at least 200 mg, which allowed for a replicate sample.

SIMS analysis was carried out in the School of Geosciences at the University of Wollongong on a micromass PRISM III stable-isotope mass spectrometer. $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios were measured after the carbonate was decomposed to CO_2 using phosphoric acid. The stable isotope values are expressed as δ values relative to the international calcite standard, PDB (Epstein *et al.* 1953), i.e. as parts per thousand difference (‰) between isotope ratio of the

sample and that of the standard.

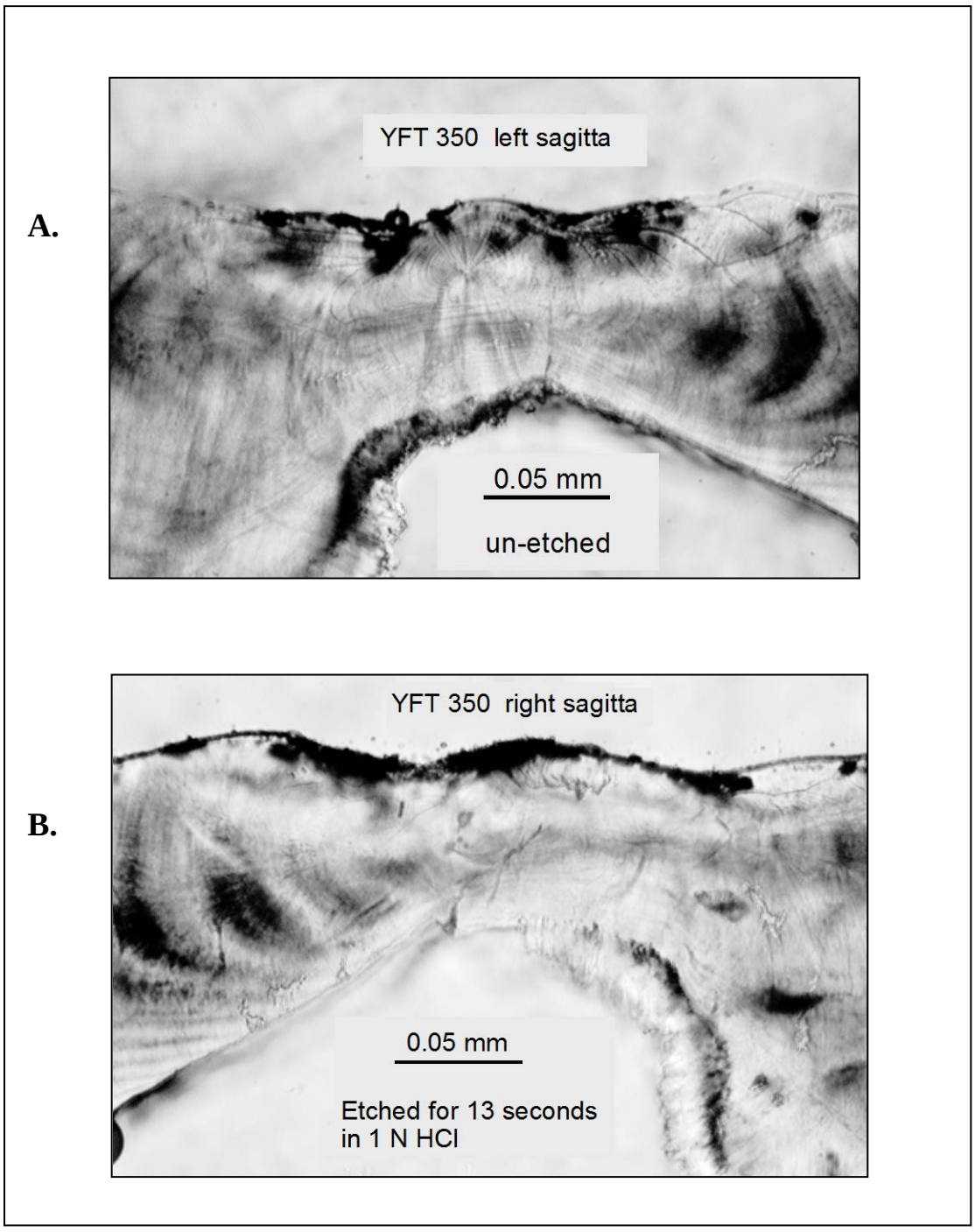

Figure 7.3.1. Otolith sections from 'sister' sagittae of a 43 cm fork length yellowfin tuna. A. The 'over burden' of otolith material deposited beyond the primordium can be seen on the lateral face (top). B. The 'sister' otolith after 13 seconds of etching shows a reduced amount of otolith material on both the lateral and medial margin.

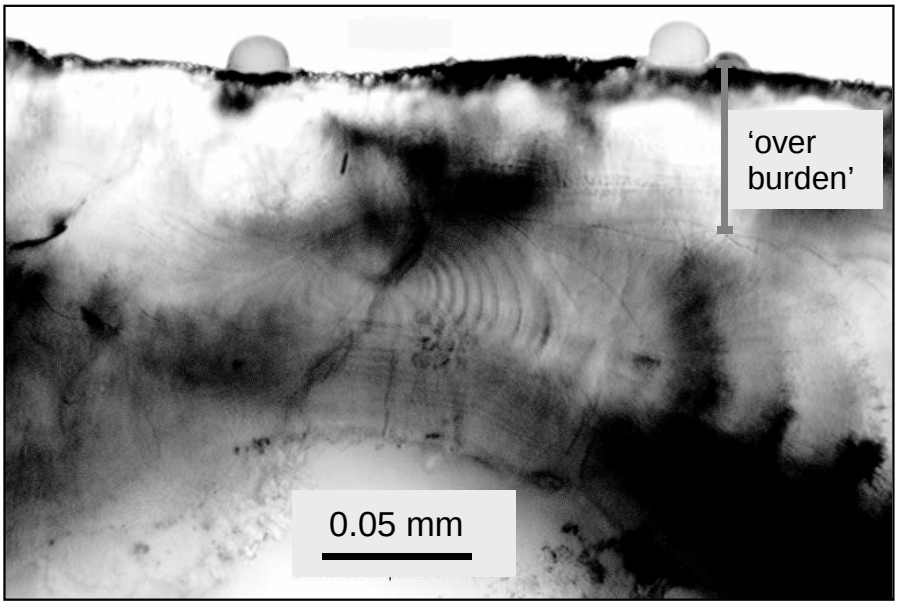

Figure 7.3.2. Sagittal otolith section from a 70 cm fork length yellowfin tuna, prior to etching. The increased amount of 'over burden' of otolith material in larger fish required more etching to expose the region around the primordium.

The $\delta^{18}O$ and $\delta^{13}C$ values from the 6 locations were compared using analysis of variance (ANOVA). The initial analysis of isotope values indicated that $\delta^{18}O$ and $\delta^{13}C$ values fell into groups in order of fish size so we investigated the relationship between fish length and isotope levels using linear regression and found that there was a significant relationship between each isotope and fish length, presumably because we had not been successful in sampling the same otolith material from fish of all sizes. Because the isotope ratios may have been influenced by factors related to the age, further analysis was conducted accounting for fish length (as a proxy for age).

To account for the effect of fish size in the variation we used an analysis of covariance (type I sum-of-squares) with fish length as the covariate. To examine the relationships between isotopes we standardised the isotope values to correct for the effect of fish size, by pooling the isotope data, finding the regression slope between the isotope values and fish length (Stephenson *et al.* 2001). Standardised isotope ratios were calculated using:

$$y = x - m.\ L$$

where y = the Standardised $\delta^{13}C$ value

x = the $\delta^{13}C$ value

m = the regression slope

L = fish length (LCF)

7.3.3 Results

The isotope values were low (–11.955 to –8.100 to for $\delta^{13}C$; –3.635 to –1.850 for $\delta^{18}O$, table 7.3.1) compared with other species of fish analysed for these stable isotopes. The highest mean levels of $\delta^{18}O$ and $\delta^{13}C$ were measured in the NSW/Sth Qld samples and the lowest values from locations at which the smallest fish were samples — Indonesia, Philippines and Solomon Islands (table 7.3.1).

Table 7.3.1. Summary of data, with mean, range and standard deviation (s) for each location

Location	*N*	LCF (cm)		$\delta^{13}C$			$\delta^{18}O$		
		mean	range	mean	range	s	mean	range	s
NSW/Sth Qld	108	120	60–160	-9.075	-10.190 to -8.100	0.451	-2.475	-3.340 to -1.850	0.303
Philippines	79	35	22–47	-9.772	-10.790 to -8.755	0.427	-2.956	-3.480 to -2.448	0.199
Solomon Islands	78	44	29–53	-9.600	-10.993 to -8.234	0.496	-2.882	-3.635 to -2.113	0.273
Coral Sea	25	69	52–90	-9.355	-10.316 to -8.607	0.450	-2.775	-3.635 to -2.113	0.280
Indonesia	73	31	20–45	-9.987	-11.055 to -8.352	0.530	-2.880	-3.360 to -2.428	0.179
Fiji	74	56	51–61	-9.143	-10.047 to -8.179	0.421	-2.767	-3.383 to -2.104	0.311
All samples	437	62	20–160	-9.475	-11.055 to -8.100	0.578	-2.769	-3.635 to -1.850	0.321

Fish length and isotope ratios

The initial analysis of isotope values indicated of $\delta^{18}O$ and $\delta^{13}C$ there was a relationship between fish length and both isotope ratios. Linear regression indicated that the relationship between fish length and isotope levels was significant (figure 7.3.4 and table 7.3.2). R-square was small and the variation within the samples large but, with the large sample sizes, the significant relationship was detected. In the absence of more information, we assumed a linear relationship for our data but the relationship between fish length and the carbon isotope ratio may be more complex.

The frequency distributions of fish length were not homogeneous between sites; in fact, in some cases there was no overlap (figure 7.3.3). This should not have been a confounding factor in the results if we had successfully sampled material from only the primordial area of the otoliths. This material would have been deposited during the same period for every fish: before the fish grew beyond about 15 cm LCF, during which time they would have remained near the spawning area. However, the isotope values showed a pattern similar to the distributions of LCF, i.e. they varied with LCF, which varied according to location (figures 7.3.5, 7.3.6 and 7.3.7). The possible explanations for this were that the variation with location was real; that there had been ‘contamination’ of younger otolith material in the samples and the variation was due to size (a proxy for age); or the variation was due to a combination of the two.

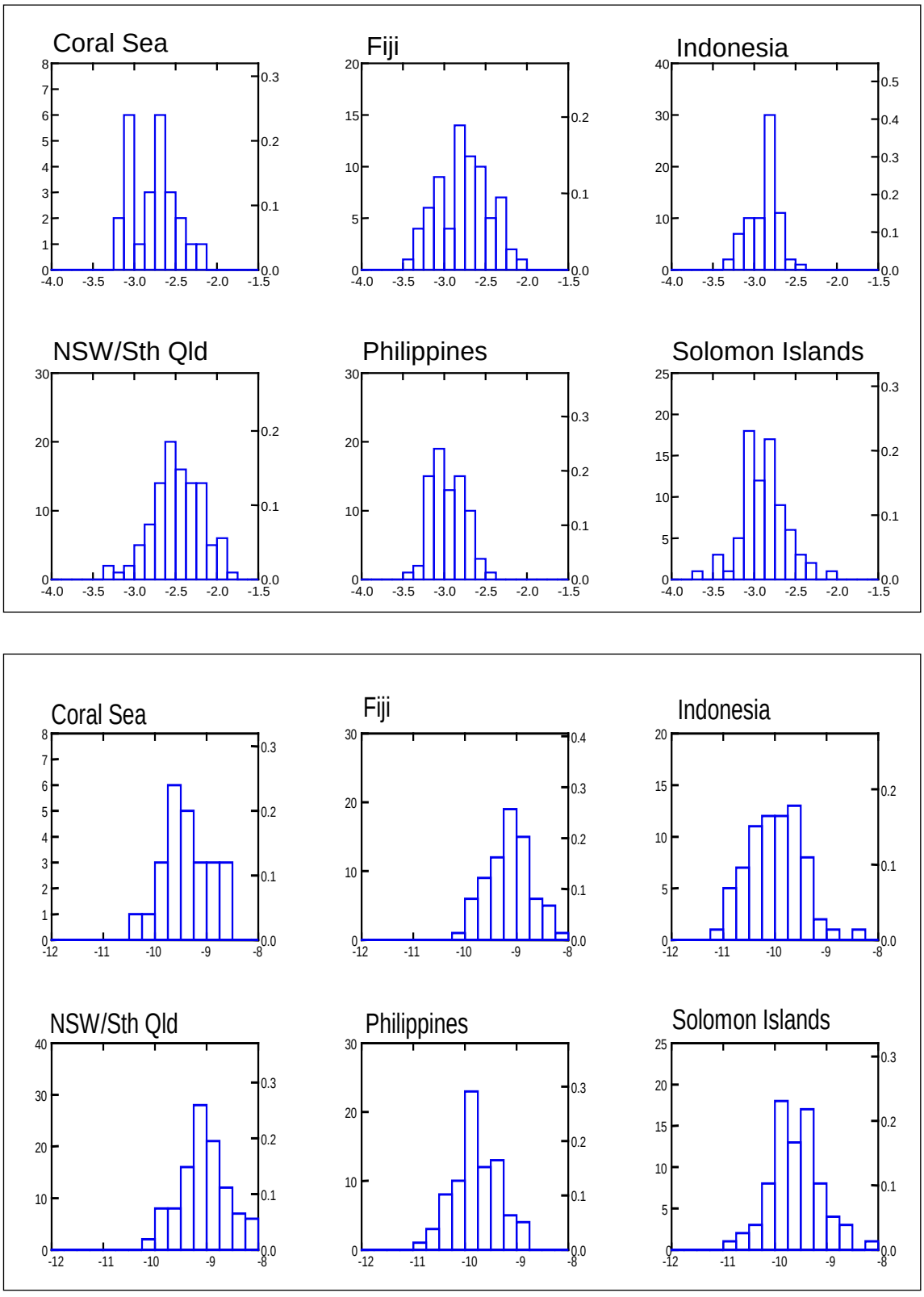

Figure 7.3.3. Frequency distributions of $\delta^{18}O$ (top) and $\delta^{13}C$ (bottom) values from each location. Left axes indicate the count and right axes the proportion per bar.

ANCOVAs among locations were run using type I sum-of-squares with LCF as the covariate; adding the fish length factor to the model before location (tables 7.3.3 and 7.3.4). There were significant differences among locations even when the effect of fish length was accounted for. Results from ANCOVAs showed that both fish length and location were highly significant for $\delta^{13}C$ ($p<0.001$) and for $\delta^{18}O$, fish length was highly significant ($p<0.001$) and location was significant ($p=0.012$). For both isotopes the length x location interaction was not significant.

In addition, we restricted the analyses to the three locations from which the smallest fish (less than 53 cm fork length) were collected (tables 7.3.5 and 7.3.6). Results of ANCOVAs with LCF as the covariate indicated that, for both isotopes, LCF was no longer a significant factor but there were still significant differences detected between locations. Table 7.3.7 provides a summary of the results of all the ANCOVAs.

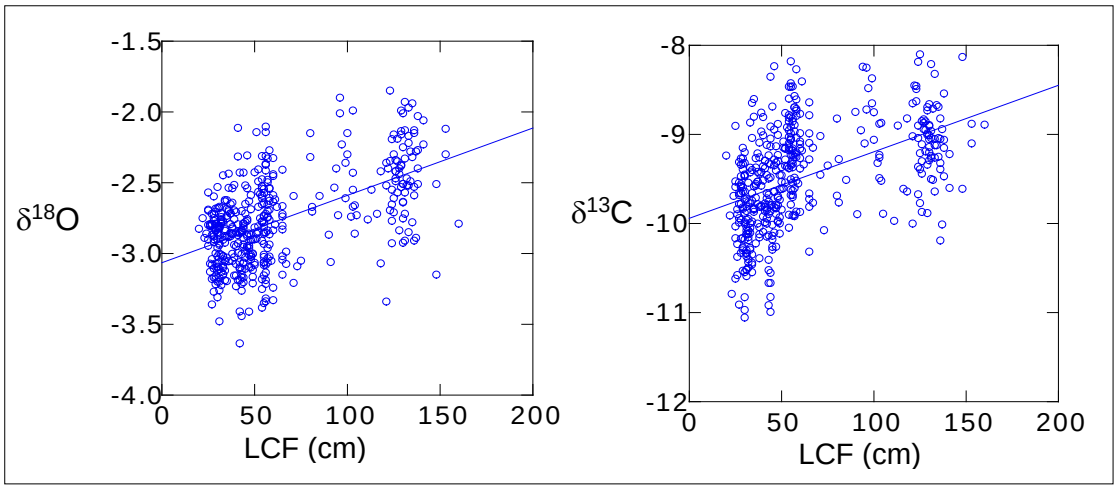

Figure 7.3.4. There was a significant relationship between fish length and both of the isotopes, $\delta^{18}O$ and $\delta^{13}C$. Regression lines are shown.

Table 7.3.2. Results of regression analysis examining the relationship between fish length (LCF) and isotopes $\delta^{18}O$ and $\delta^{13}C$. The size of fish from which otoliths were sampled was fish 20-260 cm LCF.

	$\delta^{18}O$	$\delta^{13}C$
Slope	0.00476	0.00746
P value	<0.001	<0.001
R-square	0.301	0.223

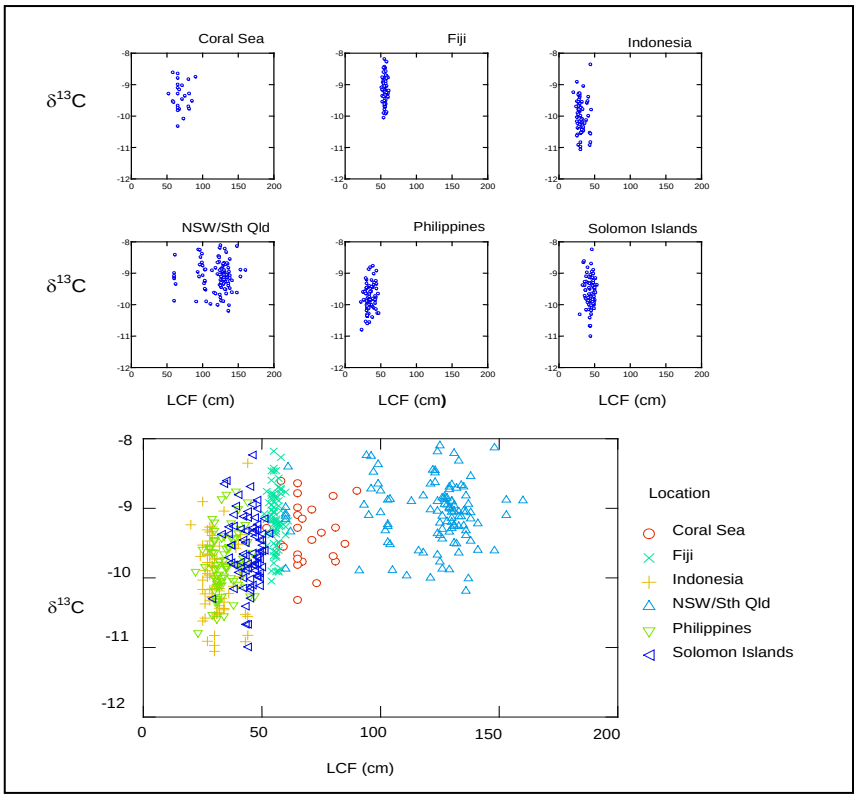

Figure 7.3.5. Length–to–caudal fork *vs* $\delta^{18}O$ for each location.

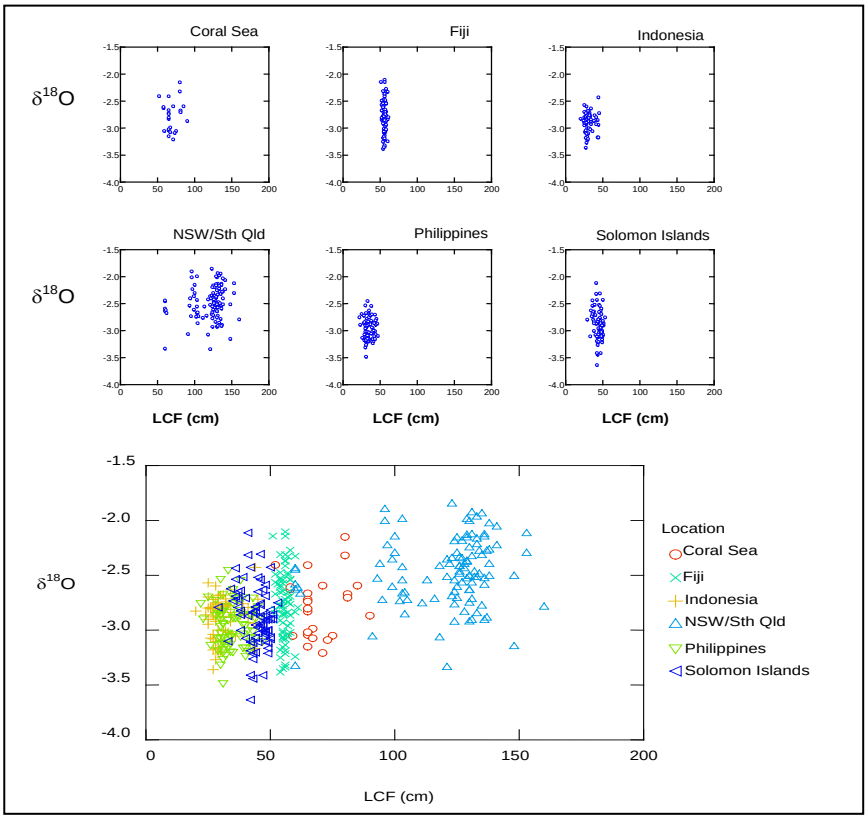

Figure 7.3.6. Length–to–caudal fork *vs* $\delta^{13}C$ for each location.

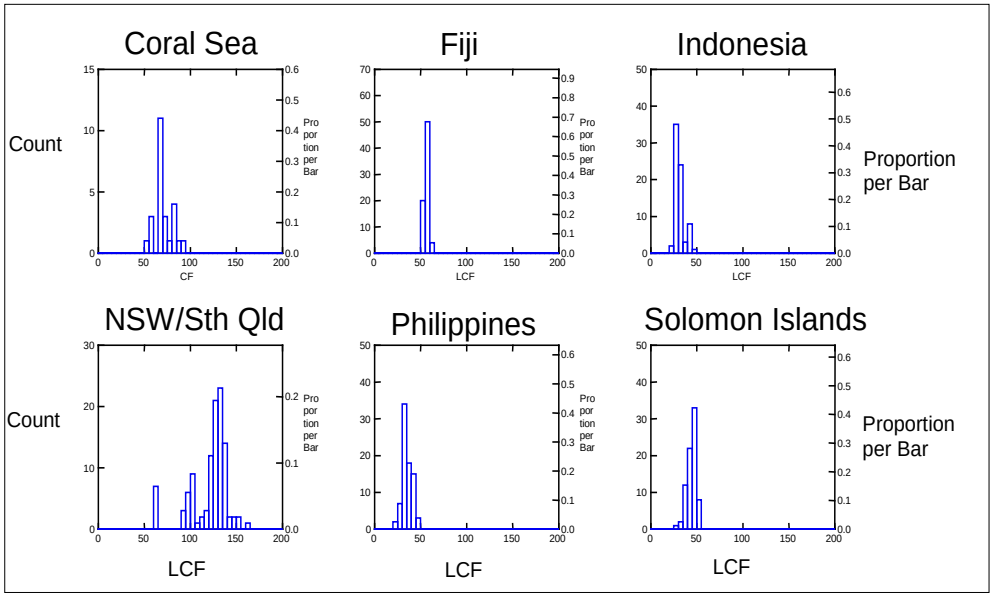

Figure 7.3.7. Frequency distributions of fish length for each location

Table 7.3.3. ANCOVA of the $\delta^{18}O$ values of the sagittal carbonate for 6 locations: NSW/Sth Qld, Coral Sea, Philippines, Fiji, Indonesia and Solomon Islands.

Source	df	S of S	M-S	F	p
LCF	1	13.20025	13.20025	191.3840	<0.0001
Location	5	1.02188	0.20438	2.9631	0.01213
LCF*Location	5	0.39221	0.7844	1.1391	0.3388
Error	425	29.26600	0.06886		

Table 7.3.4. ANCOVA of the $\delta^{13}C$ values of the sagittal carbonate for 6 locations: NSW/Sth Qld, Coral Sea, Philippines, Fiji, Indonesia and Solomon Islands.

Source	df	S of S	M-S	F	p
LCF	1	32.51322	32.51322	150.3057	<0.0001
Location	5	20.577	4.115	19.048	<0.0001
LCF*Location	5	0.97011	0.19402	0.8969	0.48297
Error	425	91.93343	0.21631		

Table 7.3.5. ANCOVA of the $\delta^{18}O$ values of the sagittal carbonate for 3 locations: Indonesia, Solomon Islands and Philippines (NSW/Sth Qld, Coral Sea and Fiji samples excluded)

Source	df	S of S	M-S	F	p
LCF	1	0.009292	0.0092924	0.257359	0.6126907
Location	1	0.212663	0.2126634	5.889870	0.0164217
Error	149	5.379888	0.0361066		

Table 7.3.6. ANCOVA of the $\delta^{13}C$ values of the sagittal carbonate for 3 locations: Indonesia, Solomon Islands and Philippines (NSW/Sth Qld, Coral Sea and Fiji samples excluded)

Source	df	S of S	M-S	F	p
LCF	1	0.42733	0.427332	1.851087	0.1757116
Location	1	1.38767	1.387673	6.011021	0.0153726
Error	149	34.39737	0.230855		

Table 7.3.7. A summary of the results of ANCOVA of $\delta^{18}O$ and $\delta^{13}C$ values.

Number of locations included	Locations excluded	Isotope	P-Value (LCF)	significance	P-value (Location)	significance
6	None	Standardised $\delta^{18}O$	<0.0001	***	0.01213	*
		Standardised $\delta^{13}C$	<0.0001	***	<0.0001	***
3	NSW/Sth Qld, Coral Sea, Fiji	Standardised $\delta^{18}O$	0.613	NS	0.016	*
		Standardised $\delta^{13}C$	0.176	NS	<0.015	*

Relationship between $\delta^{13}C$ and $\delta^{18}O$

To examine if there was a relationship between $\delta^{13}C$ and $\delta^{18}O$, the isotope ratios were firstly standardised to fish length to be able to compare data from fish of different sizes. Figure 7.3.8 displays the means and standard errors of the standardised $\delta^{13}C$ and $\delta^{18}O$ for each location. A scatter plot of $\delta^{18}O$ versus $\delta^{13}C$ (figure 7.3.9) shows that the two isotopes, standardised for fish length, are positively correlated. The plots also show the extent of the overlap between values from locations.

Results of the regression analyses (table 7.3.8) indicated that the correlation between the two variables was highly significant. The least squares linear regression line was $\delta^{18}O = -1.021 + (0.208 * \delta^{13}C)$. When the data were examined by location, the relationship was significant for all locations except Coral Sea. Regression lines for each location are shown in figure 7.3.10.

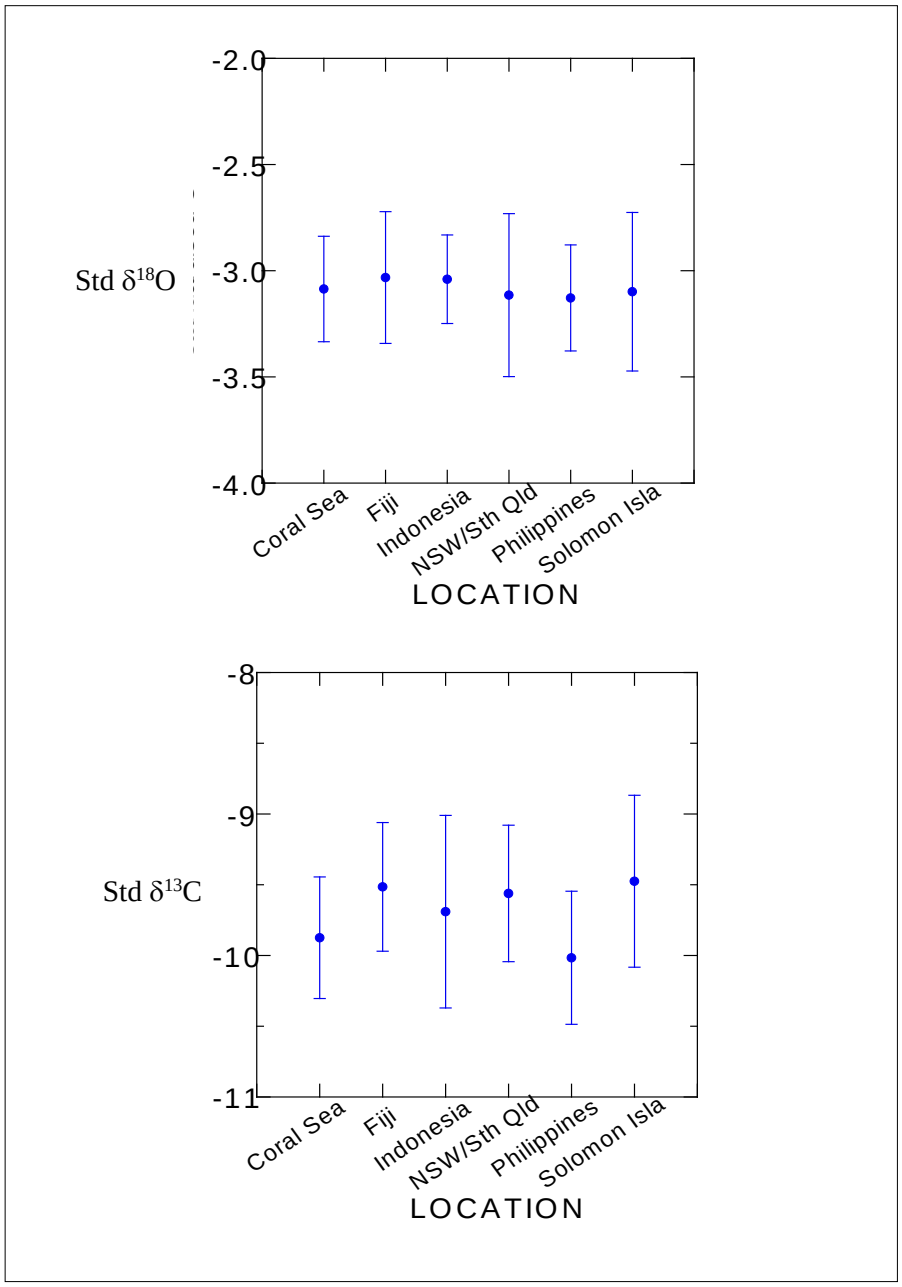

Figure 7.3.8. The mean values of $\delta^{13}C$ and $\delta^{18}O$ standardised for fish length (± S.E.) for each location.

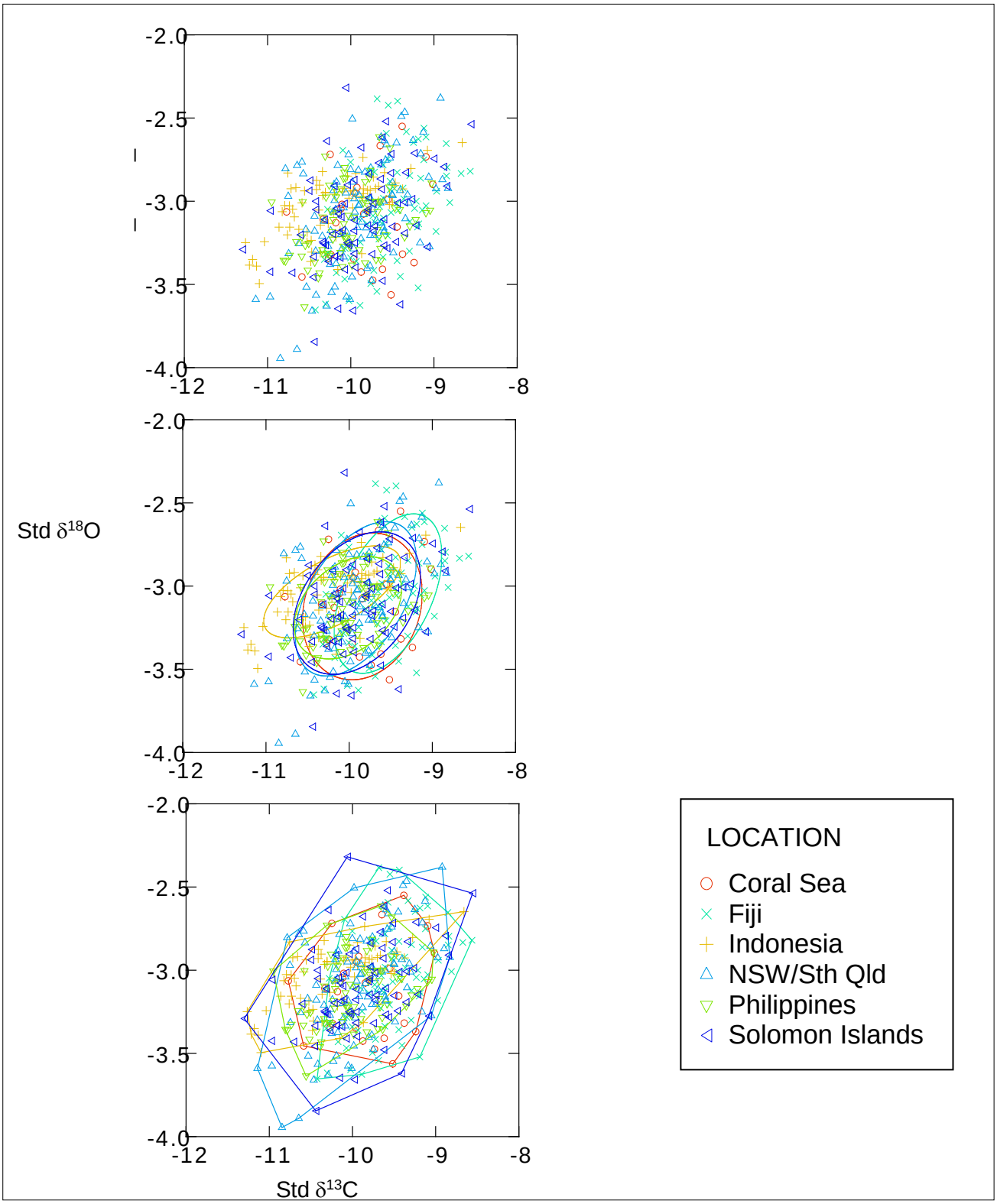

Figure 7.3.9. Standardised $\delta^{18}O$ vs standardised $\delta^{13}C$. The data are displayed in each of the three plots: in the second plot confidence ellipses are centred on the means of the variables in each location and the extent of the ellipse indicates 1 standard deviation; in the third plot of each row convex hulls are drawn around all points for each locations.

Table 7.3.8. Results from a least squares linear regression with standardised $\delta^{18}O$ as the dependent variable and standardised $\delta^{13}C$ as the independent variable. There is a significant relationship between the 2 variables in all locations except Coral Sea.

Location	Regression Slope	P value	R-square
All Locations	0.208	<0.001	0.160
NSW/Sth Qld	0.312	<0.001	0.241
Coral Sea	0.106	0.411	0.030
Fiji	0.351	<0.001	0.226
Indonesia	0.212	<0.001	0.398
Philippines	0.170	0.001	0.127
Solomon Islands	0.204	0.001	0.133

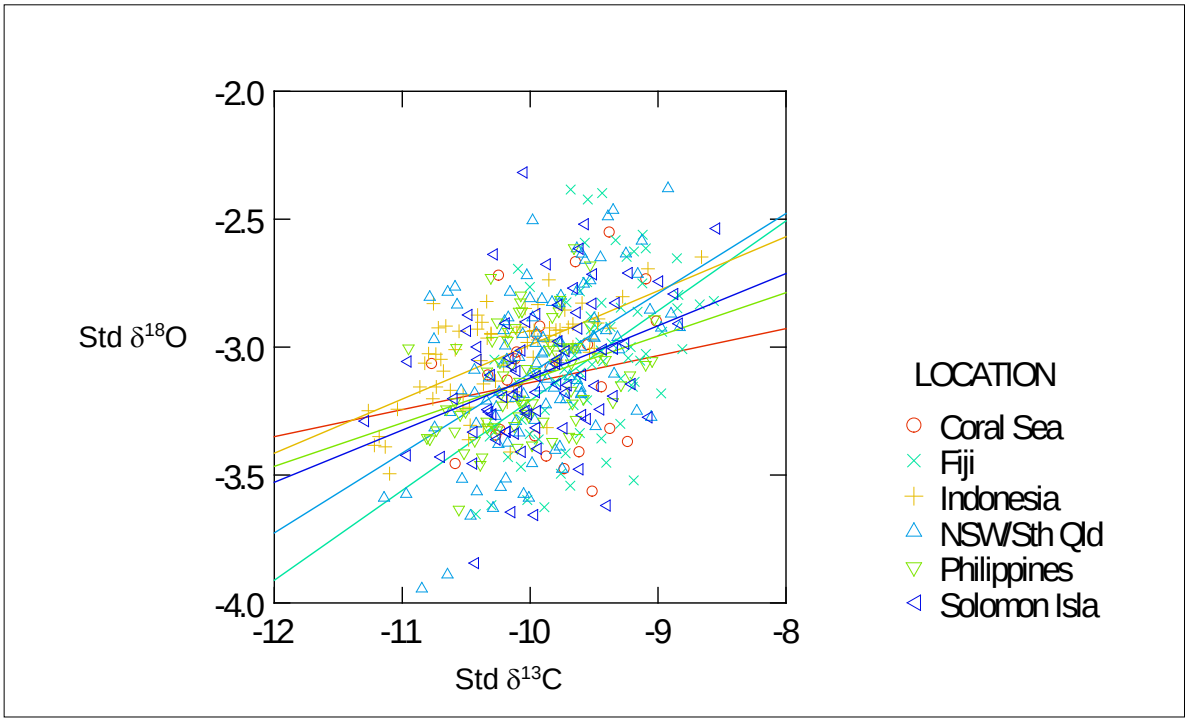

Figure 7.3.10. Standardised δ18O vs Standardised δ13C.

7.3.4 Discussion

Stable isotope ratios in otoliths have been used to differentiate groups of fish (Edmonds and Fletcher 1997; Thorrold *et al.* 1997; Newman *et al.* 2000; Gao *et al.* 2001a; Stephenson *et al.* 2001). In this study, groups of yellowfin tuna spawned at different locations were differentiated by comparing stable isotope signals in otoliths. However, these results were limited by the fact that we were not successful in sampling the core of the otoliths. We were interested in the part of the otolith deposited during the first month of life, before migrations from spawning areas and possible mixing with other groups of fish had occurred. So we attempted to extract the primordial part of the otoliths from fish of various sizes. However, the strong relationship between isotope values and fish size indicated that the otolith sampling technique had not been completely successful: otolith material deposited later in life contaminated the samples. The consequence of the contamination was that the signal from the otolith material deposited in the first month of life at a particular location was obscured to some unknown extent. The isotopic information stored in otolith material deposited later in life would have been influenced by shifts in ontogeny, diet and an extended range of vertical and horizontal movements through water masses of varying physical and chemical characteristics. In addition, migrations could have produced mixing and hence diluted the environmental signal from spawning areas.

As there was a relationship between fish length and the isotope ratios, it was necessary to account for fish size in the analyses. We did this in two ways: firstly by using fish length as a covariate in ANOVA, to determine if significant differences existed between locations. Secondly, in order to determine if a relationship existed between oxygen and carbon isotopes, we standardised the isotope values for fish length by using the slope of the regression lines.

In a study of goldband snapper (Newman *et al.* 2000) to determine location-specific signatures from stable isotopes measured in whole otoliths, otolith weight (a proxy for fish size) was found to be more important than location in determining isotope ratios. When the analysis was restricted to a particular range of otolith weight, location was more important than otolith weight. Likewise, we restricted the analysis by removing locations according to the mean size of the fish collected from the location. When we removed the three locations from which the largest fish were collected (i.e. excluding fish greater than 53 cm fork length) the relationships between $\delta^{18}O$ and LCF and $\delta^{13}C$ and LCF were no longer significant but the significant difference among locations was still evident.

$\delta^{18}O$ values have been used to reconstruct temperature histories (Kalish 1991a; Thorrold *et al.* 1997; Gao *et al.* 2001b); both $\delta^{18}O$ and $\delta^{13}C$ are influenced in part by water temperature. Higher temperatures are related to a depletion of ^{18}O and ^{13}C, which results in low $\delta^{18}O$ and $\delta^{13}C$ values. If ambient water temperature strongly influenced isotope ratios we would expect the lowest $\delta^{18}O$ values from fish collected in the warmest areas. In fact, the two lowest mean standardised $\delta^{18}O$ values are from two of the three warmest sites but the third warm site, Indonesia, had a higher mean than the spawning sites known to be cooler. There were obviously other factors that contributed to the isotope ratio of these samples.

The environmental spawning site signals may be obscured partially by other factors that influence the isotope ratios, including thermoregulatory mechanisms, both physiological and behavioural. Brill *et al.* (1999) hypothesized that the thermoregulatory abilities of yellowfin

tuna are more developed than previously believed because, although yellowfin tuna are restricted by water temperature, this may not be because the vascular system is less developed than in bigeye and bluefin tuna, but due to heart function. This ability of tunas to maintain body temperature above ambient water temperature may exclude the use of otolith oxygen isotope ratios for interpreting environmental temperatures (Kalish 1991a; Campana 1999). However, the aim of this study was not to determine the temperatures of the water in which the yellowfin were spawned but to determine significant differences among groups that could be used to differentiate fish spawned at different locations. Carbon isotope ratios are affected by another factor, metabolic rate, that contributes to depletion of ^{13}C: the high metabolic rates of juveniles result in low $\delta^{13}C$ values. If our technique had been completely successful in sampling otolith material deposited at precisely the same time of life for each fish, the confounding effect of shifts in metabolic rate may have been controlled.

Despite the limitations of our sampling technique we were able to detect differences between spawning sites of yellowfin tuna that exist in both oxygen and carbon isotope ratios. Micromilling techniques have been used recently to sample amounts of otolith material as small as 30 μg to investigate stable isotopes variation between years and between seasons (Schwarcz, *et al.* 1998; Gao and Beamish 1999; Gao *et al.* 2001b). At the time of sampling for this project the technology was less precise than now, it was very expensive and useful on flat, polished otolith sections but the technique has evolved in recent years and it has been used successfully in recent studies of otolith chemistry to isolate small areas around the core or sample material deposited during one season (Gao 1999; Gao *et al.* 2001b). Our results from SIMS analysis indicate that groups of yellowfin tuna can be differentiated from the spawning-site signal of otolith stable isotopes. Using a more precise sampling technique, it may be possible for future investigations to use the spawning site signal to classify fish to their natal stock. Consequently, these results cannot be used to accept or reject the null hypothesis, that yellowfin tuna caught in the NSW fishery are derived from the Coral Sea.

7.3.5 References

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8 Synthesis of Genetics and Otolith Chemistry Results

8.1 Introduction

This study has taken a multi-disciplinary approach to examining stock structure in yellowfin tuna. Following the success of our pilot work (Gunn and Ward 1994, Ward *et al.* 1994, 1997), in which we demonstrated ocean-basin scale variation in both phenotypic (otolith chemistry) and genetic (allozyme and mt-DNA) markers, we set out in this larger study to examine variation at a much finer scale – that of regions within the equatorial and south western Pacific Ocean. Our rationale was that if we could demonstrate heterogeneity or phenotypic variation at this scale, it would allow us to resolve fine scale structure within what are currently considered to be broadly distributed stocks. This information would in turn be very useful as a basis for management of catch/effort at regional or sub-regional scales, should there be concern about, or evidence for, localised depletion of resources.

Our particular interest was in examining whether markers could be found to resolve the natal origin of recruits into the longline fishery on the east coast of Australia. In developing the study we set out to collect data that would allow us to evaluate four hypotheses/ stock structure scenarios for yellowfin caught on the Australian east coast:

1. They are solely recruited from the Coral Sea, and the fish from the Coral Sea are completely isolated from fish in adjacent waters of the Pacific Ocean.

2. They are recruited predominantly from the Coral Sea, but there is some immigration from the adjacent Pacific.

3. They are recruited predominantly from the Western Pacific, but there is limited recruitment from the Coral Sea.

4. Considerable movement occurs between all areas within the Pacific and the Coral Sea, resulting in a single panmictic stock in our region.

In the discussion below we examine these hypotheses in light of the data we collected, and integrate these with data available on movement (from conventional tagging), catch per unit effort and size distribution (see table 8.1.1).

At the beginning of this discussion, it is worth emphasising that the tropical tunas could best be described as a “worst case scenario” for determining stock structure. They are broadly distributed – yellowfin for example are found in tropical and sub-tropical waters of all the worlds oceans – and, as they are capable of making extensive migrations, they have a potential to mix broadly throughout their range. Unlike the bluefin tuna species, which characteristically have discrete spawning grounds (Caton 1990), yellowfin and other tropical tuna species (eg. bigeye and longtail) spawn over very extensive areas in all the oceans they inhabit (Nishikawa *et al.* 1985). In the Pacific Ocean they have a continuous distribution from 40°N to 40°S, although in the sub-tropical and temperate latitudes abundance tends to vary seasonally as the warm currents with which they associate extend and contract.

8.2 Biological and Oceanographic considerations

8.2.1 Spawning

Although yellowfin in spawning condition have been reported in waters as cold as 22°C (Schaefer 1998), and larvae of *Thunnus* species have been caught in waters down to 24°C (Richards and Simmons 1971; Davis *et al.* 1990; Boehlert and Munday 1994), the vast majority of yellowfin spawning appears to take place in tropical latitudes, in water temperatures warmer than 26°C. Schaefer (1998) notes that yellowfin spawn across extensive areas of the world's oceans, and essentially continuously at tropical latitudes. His study of spawning in the eastern Pacific Ocean showed that between latitudes 0-20°N spawning occurs continuously with no seasonality, while at higher latitudes spawning is temporally restricted to the respective summer periods, when sea surface temperatures exceed 24°C. A slightly different pattern has been observed in the western and central Pacific (Itano, 2001). In his large study of the reproductive biology of yellowfin, Itano found that yellowfin in Hawaii show markedly seasonal spawning, at times when water temperatures were more than 24.5°C, whereas yellowfin in equatorial areas (10°N-10°S) showed more consistent spawning throughout the year (Itano, 2001). This study did not take samples in the Coral Sea region. However, McPherson (1991) documented seasonal spawning in the north-western Coral Sea off Cairns during the spring/summer months. The area where McPherson found spawning yellowfin (and bigeye) is at the northerly extreme, or "source" of the East Australian Current (EAC), which flows southwards from the Coral Sea for much of the year, but is strongest at southern latitudes during the summer months. The southward flow of the EAC can bring warm water (24 - 26°C), of tropical origin as far south as Sydney during the summer months, so it is feasible that spawning could occur on the east coast as far south as this. However, *Thunnus* larvae have been very rare in collections made along the central and southern NSW coast.

The fact that recruitment into NSW tends to occur as a pulse over a few months each year, and that this comprises fish of a limited size range, indicates that the supply of recruits into our fishery/region is discontinuous. Such a pattern is consistent with what could be expected if the recruitment is sourced from the spring/summer spawning of yellowfin in the Coral Sea. An alternative, or even complimentary explanation may be that the conditions that favour recruitment of young yellowfin from tropical areas, where spawning is continuous, lead to seasonal advection of larvae and very young juveniles, or migration of slightly older juveniles. This pattern may reflect seasonal and/or interannual variations in the oceanography of the area.

8.2.2 Oceanography

The EAC is a western boundary current within the South Pacific sub-tropical gyre and is therefore the south Pacific equivalent of the Gulf Stream in the North Atlantic and the Kuroshio in the North Pacific (Ridgeway and Godfrey 1997). Yellowfin extend their northerly distribution in the Pacific and Atlantic Oceans through associations with these currents, much as they do in our region through the EAC. The EAC differs from these other currents however in having very vigorous eddies that tend to result in recirculation of the water being transported along the east coast of Australia (figure 8.2.1). From a biological perspective, the eddies and resulting recirculation create retention mechanisms for

communities of animals that are adapted to tropical/sub-tropical waters (Brandt 1981). Yellowfin are closely associated with the EAC and associated eddies as they migrate down the coast (Young *et al.* 2001) – and association that is most likely driven by both physiological preferences for warm waters, and prey availability (Block *et al.* 1997). The association of yellowfin with the EAC and its eddies suggests that, as is the case for smaller prey species, there is a potential for retention of yellowfin for long periods within the system. However, that does not in itself provide a mechanism for a regionalisation of the yellowfin stock as it is rare to find yellowfin in spawning condition around the eddies.

Figure 8.2.1. East Australian Current (blue) and movements of yellowfin tuna (orange); larger, darker arrows indicate more common movements of yellowfin. Movement of yellowfin is generally along a north-south axis, possible movements of yellowfin in and out of the eastern AFZ are indicated by the shaded arrows.

The EAC exhibits a clear seasonal cycle at latitudes 25-45°S with the strongest southwards flow occurring during summer. The strong seasonal pulsing of water down the east coast is reflected in the seasonal pattern of CPUE (see above) and is associated with the recruitment of 0+ and 1+ yellowfin down the east coast. North of 25°S, there is a seasonal reversal of flow offshore, due to local wind forcing when the seasonal SE trades blow in the sub-tropical and tropical latitudes. There is no indication that this reversal of flow is fed from the south by the EAC (Ridgeway and Godfrey 1997).

Thus, it is apparent that the EAC provides a vector for movement of young yellowfin recruits down the east coast of Australia, and that its eddy systems act as retention mechanisms for both immature and mature size fish. As the EAC has its origins in the South Pacific sub-tropical gyre, it could also provide a vector for movement of fish into the Coral Sea from the Coral Sea rim and further east. Unfortunately, little is understood of the inter-annual variability in the strength of the EAC in the Coral Sea, and it is very difficult to quantitatively examine the links between the magnitude of yellowfin recruitment along the east coast and the strength of inflows from outside our region.

If regionalised migration and recruitment is occurring, as much of the data collected in this and other studies suggests, fish entrained in the EAC or its eddies would need to make return northwards migrations back into the Coral Sea, either outside or against the EAC. This is unlikely to be difficult for mature age yellowfin. Once into the sub-tropical latitudes, the northerly movement of water forced by the winter/spring trade winds would assist migrations back into the NW Coral Sea. The timing of spawning in this area – during late spring and early summer accords with this hypothesised movement associated with regional circulation patterns.

8.3 Conventional tagging data and movement patterns of yellowfin in the eastern AFZ

Four conventional tagging programs conducted over the last three decades (Pepperell and Diplock 1989; Itano and Williams 1992; Hampton and Gunn 1998) provide data on the movements of yellowfin within the eastern AFZ, and mixing between this region and the broader Western and Central Pacific Ocean that are pertinent to our study. Unfortunately, the vast majority of the fish tagged during these experiments were >50cm LCF at release, and thus the movement data collected tell us very little about the origins of recruits to the AFZ.

Pepperell and Diplock (1989) reported on programs conducted during the 1970's and 80's in which nearly 5000 yellowfin were tagged, principally off the NSW coast. The first involved recreational taggers, who between 1973 and 1988 released 3611 yellowfin, of which 64 recaptures have been reported. All of these recaptures were made within the Australian EEZ, with significant evidence for northwards and southwards movements along the coast but no evidence for emigration out into the wider Pacific where longline fishery catch and effort levels suggest that if movement were occurring that tags recaptures would be highly likely.

Since the 1989 report the recreational tagging program has grown significantly and, to late 2001, 10,000+ yellowfin had been released with 518 recaptures reported. The bulk of

releases have been in NSW waters, and the pattern of recaptures has remained very similar to that reported by Pepperell and Diplock. Figure 8.3.1 summarises the displacement distances, times at liberty and the relationship between these two for all recaptures where both the location of release and recapture are available. These data indicate that the majority of fish moved less than 500 miles from their point of release, regardless of their time at liberty. A handful of fish tagged in Australian waters have been recaptured in the tropical Pacific, and one fish tagged in Papua New Guinea moved to Sulawesi in Indonesia. Movement of fish in the Australian Fishing Zone has generally been along a north-south axis with approximately equal numbers moving in each direction (see figure 8.2.1). Only six fish released in north Queensland have been recaptured, all but one on the NSW coast.

The second program reported by Pepperell and Diplock (1989) involved 1041 fish tagged by scientists after being caught by longline (97 releases for 0 returns) and pole and line (944 releases for 24 returns). The fish were recaptured after 19-579 days at liberty, and all were recaptured along the east coast, inside the Australian EEZ.

The results of the two tagging programs strongly suggest some retention of yellowfin along the east Australian coast. Although non-reporting may explain the lack of recaptures in the large longline and purse seine fisheries east and north of Australia, the large numbers of recaptures within the AFZ, many after significant times at liberty, suggest that the tagged fish do not mix freely with fish in the tropical and equatorial regions to Australian north and north east.

The large “Skipjack Survey and Assessment Program” conducted between 1977 and 1980 by the South Pacific Commission (SPC) tagged 9464 yellowfin throughout the western Pacific, of which 322 were released in Australian waters (Itano and Williams 1992). Of the nine thousand tags released outside Australian waters, only one was recaptured within the Australian EEZ. Although there was no domestic longline effort in those days, there was significant Japanese effort off the east coast of Australia, with 9-12 million hooks being set each year during the early 1980’s (Ward *et al.* 1996). Given this level of effort, one would have expected larger numbers of recaptures if there was significant immigration of fish between the equatorial latitudes where the majority of the yellowfin were released by this program and Coral/Tasman Sea waters off the east coast of Australia.

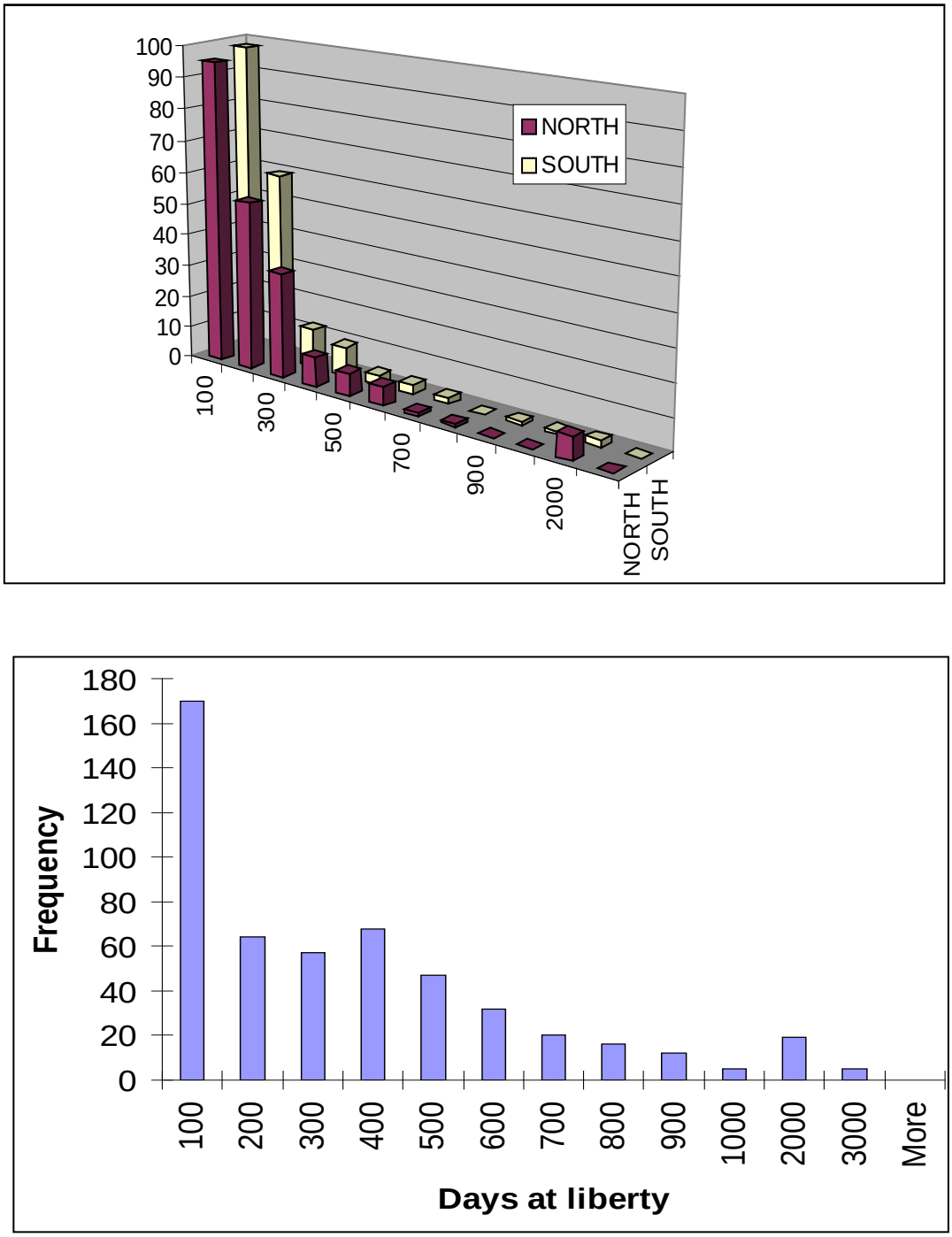


Figure 8.3.1. Displacement distances and directions (top) and times at liberty for fish recaptured after release by recreational anglers (bottom). Data source: Dr M. Lowry, New South Wales Fisheries Research Institute.

In the late 1980's and early 1990's, the SPC tagged 33,523 yellowfin in the Western Pacific, as part of their Regional Tuna Tagging Program (RTTP)(Kaltongga 1998). To date there have been 3,476 returns (10.4%). Only one of the 30,000 plus yellowfin released outside the Australian EEZ was recaptured inside the zone. During this period, the combined Japanese and Australian effort along the east coast averaged 8-10 million hooks per annum and catches included size classes encompassing those of the tagged fish. Thus one would have expected larger numbers of recaptures if there was significant immigration of yellowfin from the equatorial latitudes. The RTTP included two cruises into the Coral Sea, in 1991 and 1992. On these cruises, 2905 yellowfin were tagged, and 88 of these fish have been reported recaptured. Half of the recaptures have been made by the Australia domestic fleet in the eastern AFZ (the majority of these in the Cairns area), the remainder have been caught over a wide geographic range in the tropical and equatorial western Pacific Ocean. The pattern of recaptures shows clear links between the Coral Sea and the adjacent Pacific Ocean, as well as southerly movements of yellowfin. The latter suggest links between the Coral Sea and the stocks supporting the longline fishery in the south-eastern Australian Fishing Zone (Hampton and Gunn 1998). As we saw in the recreational tagging data, the distribution of displacements for fish tagged in the Coral Sea show that the majority of fish do not move more than a few hundred nautical miles from their point of release (figure 8.3.2), and those that have moved tend to have been caught in the Coral Sea rim archipelagos.

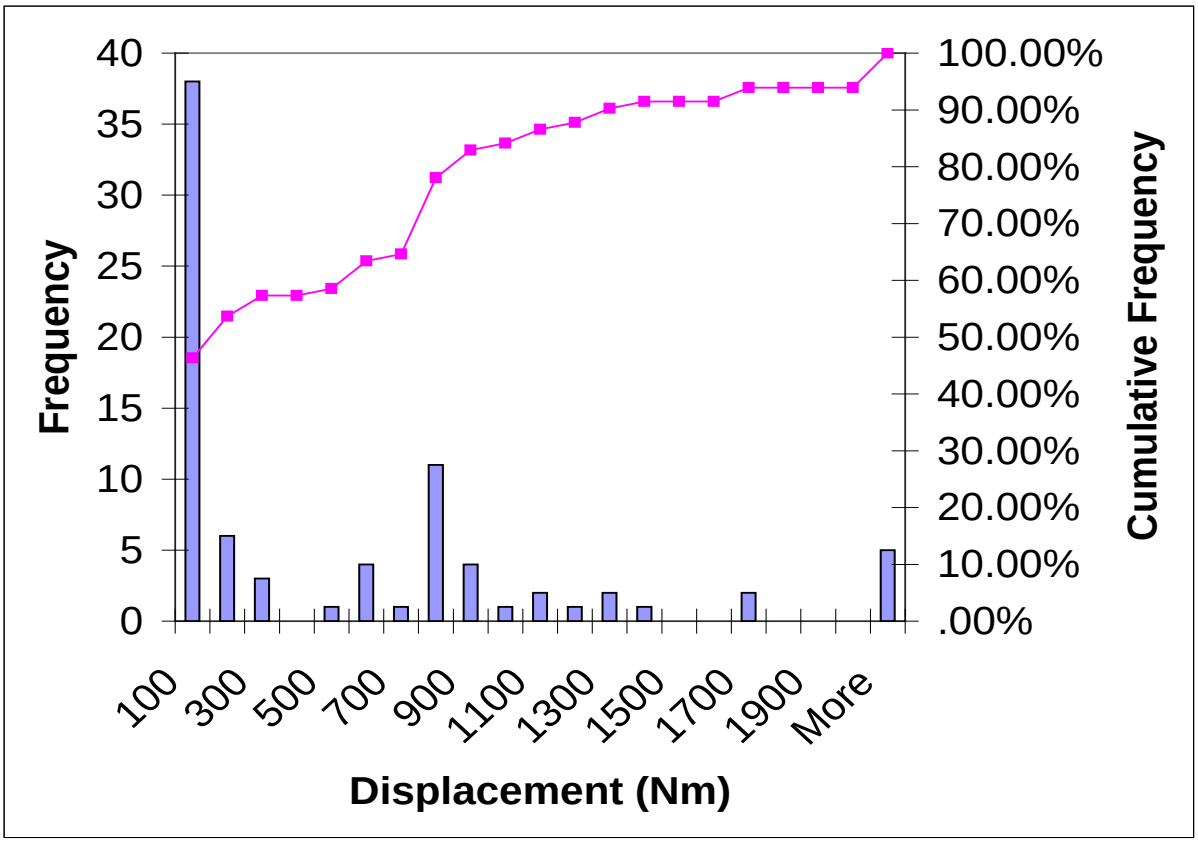


Figure 8.3.2. Displacement distances of yellowfin tuna tagged by the SPC RTTP in the Coral Sea in 1991 and 1992 and recaptured between 1991 and 2000.

8.4 Outcomes of the genetics study

Earlier genetic studies had not revealed any differentiation among western Pacific collections of yellowfin tuna using either allozyme or mtDNA markers (Scoles and Graves 1993; Ward *et al.* 1994; Ward *et al.* 1997). Since the current study was principally to assess the relationships of Coral Sea yellowfin tuna to other western Pacific samples, we decided not to use either of these two types of markers, but to use instead variation in microsatellite DNA. Microsatellite DNA is a hypervariable class of DNA which has already proved its worth in several recent fish population studies (O'Connell and Wright 1997; DeWoody and Avise 2000).

The null hypothesis we were testing was that there was no significant differentiation among collections of yellowfin tuna from the western Pacific, including the Coral Sea. We decided, additionally, to include in our examination some pre-existing yellowfin tuna samples from the eastern Pacific. These had earlier proven to be differentiated from western/central Pacific samples for one of four polymorphic allozyme loci, but not for mtDNA (Scoles and Graves 1993; Ward *et al.* 1994; Ward *et al.* 1997).

Five polymorphic microsatellite loci were examined, one of which showed small but significant differentiation among collections. This heterogeneity was largely attributable to slight allele frequency differences in one of the two Philippines collections, and may be an artefact; no significant differentiation was detectable among samples from the Coral Sea (two collections), east Australia (four collections), Fiji (one collection), Indonesia (one collection), Philippines (one of two collections), Solomon Islands (one collection) and eastern Pacific (California and Mexico, one small collection each).

The microsatellite data therefore throw little new light on the relationships among western Pacific yellowfin tuna populations. The overall evidence of genetic homogeneity in this region is inconsistent with any hypothesis that proposes reproductive isolation of any (examined) stock in this region, but the extent of gene flow among regions could vary from quite low to high and still maintain the appearance of homogeneity. Certainly the data are consistent with the hypothesis that the Coral Sea contributes the bulk of recruits to the Australian east coast fishery, but the hypothesis that some or indeed many recruits come from regions outside the Coral Sea cannot be excluded. It should also be pointed out that larger sample sizes might reveal evidence of small-scale (but nonetheless significant) population heterogeneity hidden in smaller studies: the power of any test increases as sample size increases.

The microsatellite data in fact proved less powerful than allozyme analysis in detecting sample differentiation. The five microsatellite loci (and the mtDNA analyses) failed to reveal the significant differentiation between western and eastern Pacific samples that one of four allozyme loci had shown. Why should this be so? It may be that the mutation rate for these microsatellite loci is very high, and that new mutations are wiping out any signal that might have arisen from population isolation. An alternative explanation is that the entire Pacific population of yellowfin tuna is panmictic, and that the divergence of just one allozyme locus is due to differential selective forces between regions.

In general, little genetic differentiation is expected among samples of highly migratory fish,

such as tunas, taken from within ocean basins. The yellowfin data accord with this expectation. Bigeye tuna in the Indian and Pacific Oceans similarly showed very little evidence of within-ocean differentiation for microsatellites and mtDNA (Grewe and Hampton 1998; Appleyard *et al.* 2002). Nevertheless, exceptions have been recorded; Indo-Pacific collections of swordfish showed evidence for a northern Pacific stock (differentiated by mtDNA), a southern Pacific stock (also differentiated by mtDNA), and a western Australia stock (differentiated by microsatellites) (Reeb *et al.* 2000; Ward *et al.* 2001). Surprisingly, substantial and highly significant differentiation was observed among samples of northern bluefin tuna from within the Mediterranean Sea (Broughton and Gold 1997). Instances of inter-ocean genetic differentiation are relatively common; mtDNA studies have for example established that Atlantic and Indo-Pacific populations of bigeye tuna and swordfish are well-separated (Alvarado-Bremer *et al.* 1998; Chow *et al.* 2000; Chow *et al.* 1997). While this is not the place for a full review of the uses and findings of the genetic techniques available to study pelagic migratory fish, it is clear that our understanding of the population structure of pelagic fish has benefited greatly from the deployment of genetic techniques (Hauser and Ward 1998).

8.5 How do tagging results aid in our interpretation of the genetics and otolith chemistry results?

From a genetics perspective, the tagging data show that within a generation there is likely to be enough mixing between yellowfin in the Coral Sea/Australian and those in the adjacent western Pacific to result in sufficient genetic flow to mask any genetic heterogeneity. The movement of yellowfin tagged by recreational fishers in Papua New Guinea to Sulawesi in northern Indonesia further emphasises this point. Thus, it is not surprising that we found no consistent pattern of heterogeneity among samples collected at the various sites around the Western Pacific. The apparent lack of movement from the adjacent areas of the Pacific into the Coral Sea and more southerly parts of the east coast tends to suggest emigration is more common than immigration (see figure 8.2.1). As the east coast of Australia is at the southern extreme of the range of yellowfin in the Pacific, this might be expected.

The recreational tagging data suggest that once fish have migrated onto the NSW coast many do not move significant distances, and that few migrate back up to the Coral Sea. However, this conclusion is subject to some significant caveats. Conventional tagging data provide only two points in the migration path of an individual, recaptures are only made where fishermen are fishing, and returns are only made by fishermen willing to cooperate with the tagging program. Thus, the apparent lack of returns outside the AFZ, and in the Coral Sea may simply reflect either lack of effort or co-operation. With this caveat in mind however, the pattern of returns for fish tagged on the NSW coast suggest that many may be “lost” to the spawning population as the accepted minimum temperature for spawning of yellowfin is 26°C, a limit that is rarely reached along much of the east coast of NSW. The only spawning habitat in this region would be within the east Australian current.

Our otolith data suggested strong links between the Coral Sea and NSW. The tagging data show that 1+-year-old fish move from the Coral Sea down the NSW coast. Once in NSW, there is also a consistent pattern of fish moving southwards down the east coast, from Coffs Harbour to Eden, and back the other direction. It would be useful to know if the fish do complete some kind of annual or periodic migration cycle, but to date there have been no

recoveries of fish tagged on the NSW coast back in the Coral Sea. It is likely that the only way to demonstrate such cyclic migrations would be to use archival or satellite tagging techniques.

8.6 Catch distribution and catch rates

Yellowfin catches and catch rates vary cyclically in the AFZ with both Japanese and domestic longline data indicating that periods of high catch rates are often followed by years of decreasing catch rates (Campbell 1999). Three-fold changes in catch rates are not uncommon, and it seems likely that this variation is due to cycles in abundance rather than changes in vulnerability to longline gear. Both the Japanese fleet that operated in the AFZ from the early 1950's to the mid-1990's, and the domestic fishery that has operated since the mid-1980's, used surface longlines, set during the day with fish baits to target yellowfin.

What causes cycles in yellowfin abundance in the AFZ remains largely unknown, but to a large degree our thinking on this issue is conditioned by where we think the fish recruit from. Among the plausible explanations for the cycles are:

1. The AFZ is a self recruiting stock (ie only a small proportion of the fish caught within the AFZ are spawned outside the AFZ) and the observed fluctuation in catch rates is the result of fluctuations in spawning activity and/or survivorship of larvae and juveniles in the spawning areas of the Coral Sea and warmer parts of the Tasman Sea and/or variation in the strength of the East Australian Current, which is considered to be the major vector for southward movement of fish along the east coast of Australia.
2. The yellowfin caught along the east coast originate principally from the Coral Sea, but immigration from outside the immediate region occurs sporadically. When high recruitment from the adjacent Pacific occurs, the abundance of yellowfin along the Australian east coast is high.
3. The yellowfin along the east coast originate from a broad geographic region (including the Coral Sea) and abundance along the east coast is driven by recruit abundance and environmental conditions that favour immigration into the AFZ (eg. a strong East Australian Current (EAC)).

If yellowfin in the AFZ were self recruiting, then following 20 years of industrial purse seine fishing on skipjack and young yellowfin in the equatorial Pacific one might expect to see a difference between the abundance of yellowfin in the AFZ and those of the broader Western Pacific. Unfortunately, full stock assessments for both regions are not available. However, Campbell (1999) examined trends and patterns in CPUE for the AFZ and compared these with Japanese catch rates in the equatorial region bounded by 0-9°S and 155-169°E. He found that "changes in catch rates (and inferred abundance levels of yellowfin tuna) are not correlated between the two regions studied, with declines in catch rates in the equatorial region not being apparent in the region off eastern Australia". Campbell (1999) noted, subject to a number of the common caveats that are required when interpreting CPUE data series, that such a pattern may indicate that the level of mixing between the two regions is "not as high as would be expected if these regions were part of a single, homogenous stock".

Another indicator of the origin of recruits may come from the temporal and spatial variation in recruitment to the fisheries. Pulses of small fish moving down the east coast from Cairns to

the NSW coast would allow us to follow the patterns of movement and assist in determining their origin. Unfortunately, the smallest fish retained in the commercial fishery tend to be late 1+ or 2-year-old fish. At this age fish would have had plenty of time to recruit into the Coral Sea from adjacent waters.

Campbell (1999) found little indication of a spatial signal in the Japanese size data he examined. However, more recent analysis of domestic data suggests that there is a clear link between pulses of recruitment in the Coral Sea, and those that appear either during the same quarters, or one quarter later, on the south eastern Queensland coast and further south along the NSW coast (Campbell *et al.* 2002). In years when the Coral Sea/Cairns fishery is having a high recruitment of 2-year-old fish, so are the Mooloolaba and NSW fisheries. Conversely in 1999, when the Coral Sea catch included very few 2-year-old recruits, there was no indication of recruitment in the fisheries to the south (figure 8.6.1). These congruent patterns suggest that the Coral Sea and east coast fisheries exploit the same recruits, but it is not possible to determine from these patterns where the recruits come from.

If the majority of the yellowfin recruits came from outside the AFZ, then one would expect to see parallels between the levels of recruitment and catch rates of small fish in the high abundance areas of the tropical Pacific and those of the AFZ. Using a MULTIFAN-CL model, in which the Central and Western Pacific was split up into seven “regions”, Hampton and Fournier (2001) derived estimates of recruitment for each region and found that the estimated average distribution of recruitment indicated that 80% of the total recruitment occurred in the three tropical regions. They noted both low- and high-frequency variation in regional and pooled recruitment signals and hypothesised that the low frequency variation was correlated to decadal-scale environmental variation whereas the higher frequency fluctuations “might be correlated to El Nino-LA Nina cycles, although these hypotheses have not yet been examined in detail. The times series of yellowfin recruitment estimated by Hampton and Fournier (2001) indicate that the recruitments of 1995, 1996 and 1998 were significantly higher than those of 1997, 1999 and 2000. Recruitment estimates for their region covering the Australian east coast do not show a clear signal in these years. However, if we use the size data described by Campbell (2001) to examine the relative abundance of 2 year olds in the east coast fishery (see figure 8.6.1), one sees that “recruitment” was strongest in 1998 and 2000, and weak in 1999. Thus, in some years we seem to have a signal similar to those estimated by Hampton and Fournier, while in 2000 there appears to be a mis-match. Hampton and Fournier (2001) note that as the cohorts of their most recent years have experienced relatively little fishing, the CV’s around their estimates are high for 1999 and 2000.

In conclusion, spatial and temporal variation in catch data provide conflicting pictures of the relationship between the yellowfin populations of the eastern AFZ and those of the broader western Pacific. There is evidence for both matches and mis-matches in trends and inter-annual fluctuations in abundance (as estimated from CPUE) and recruitment. We see in the recently collected size data from the domestic fleet a common pattern of recruitment along the entire east coast of Australia since 1997. This cannot be used to infer origin of recruits, but we should be mindful that whatever their origin, it is likely that the exploitation along the coast is likely to be focussed on a single recruitment pulse, rather than a continuous supply of recruits from an outside source.

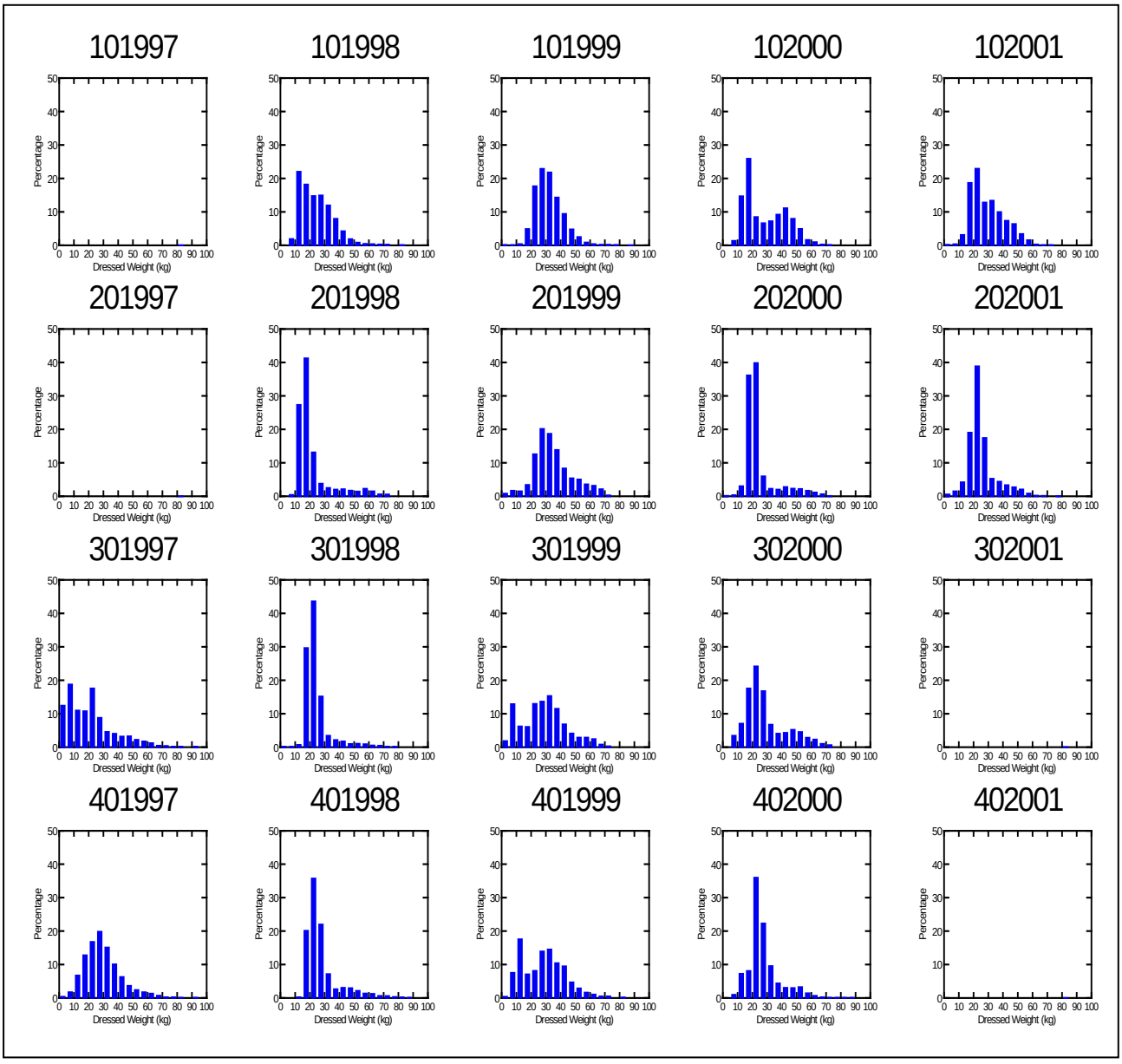
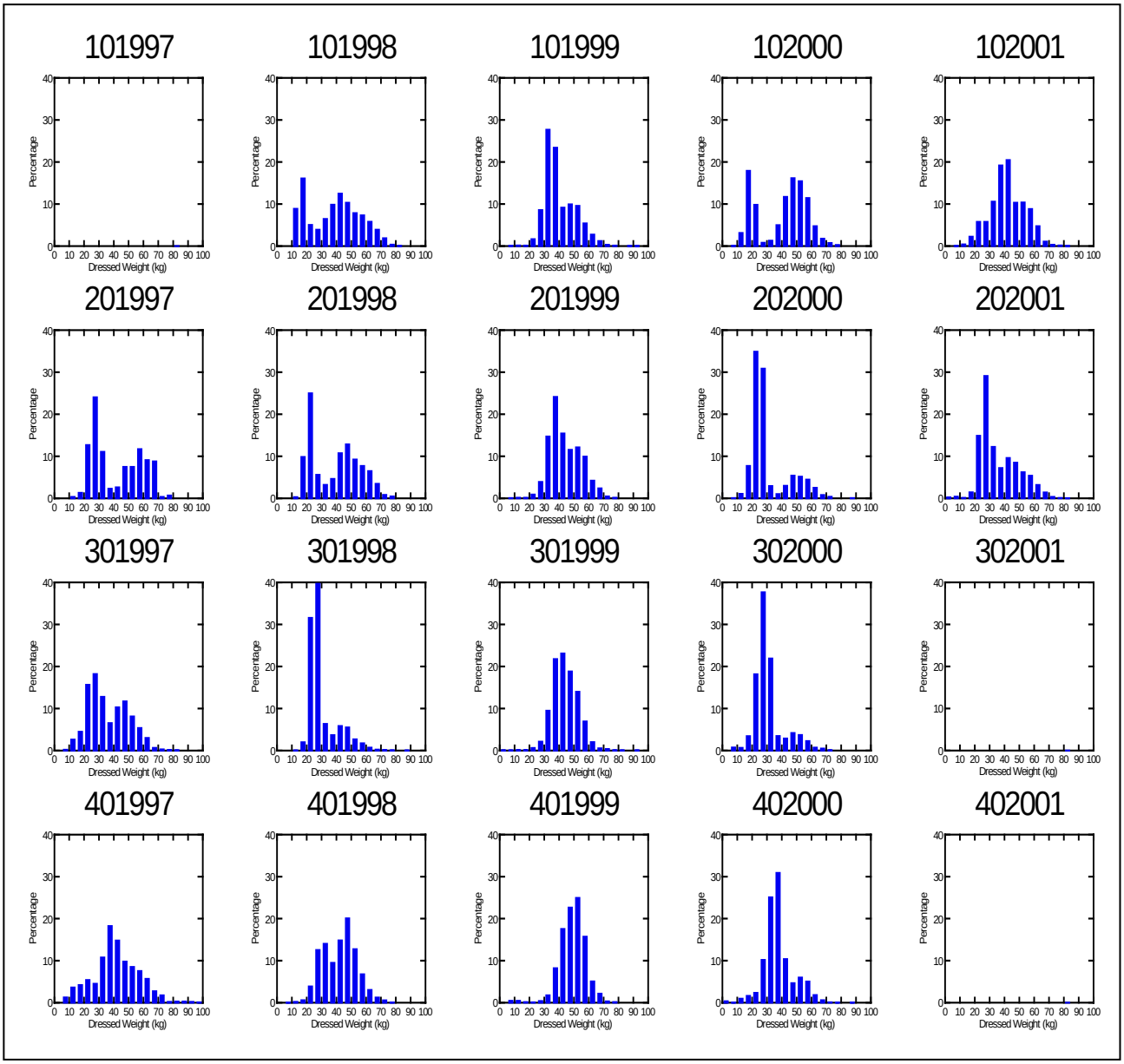
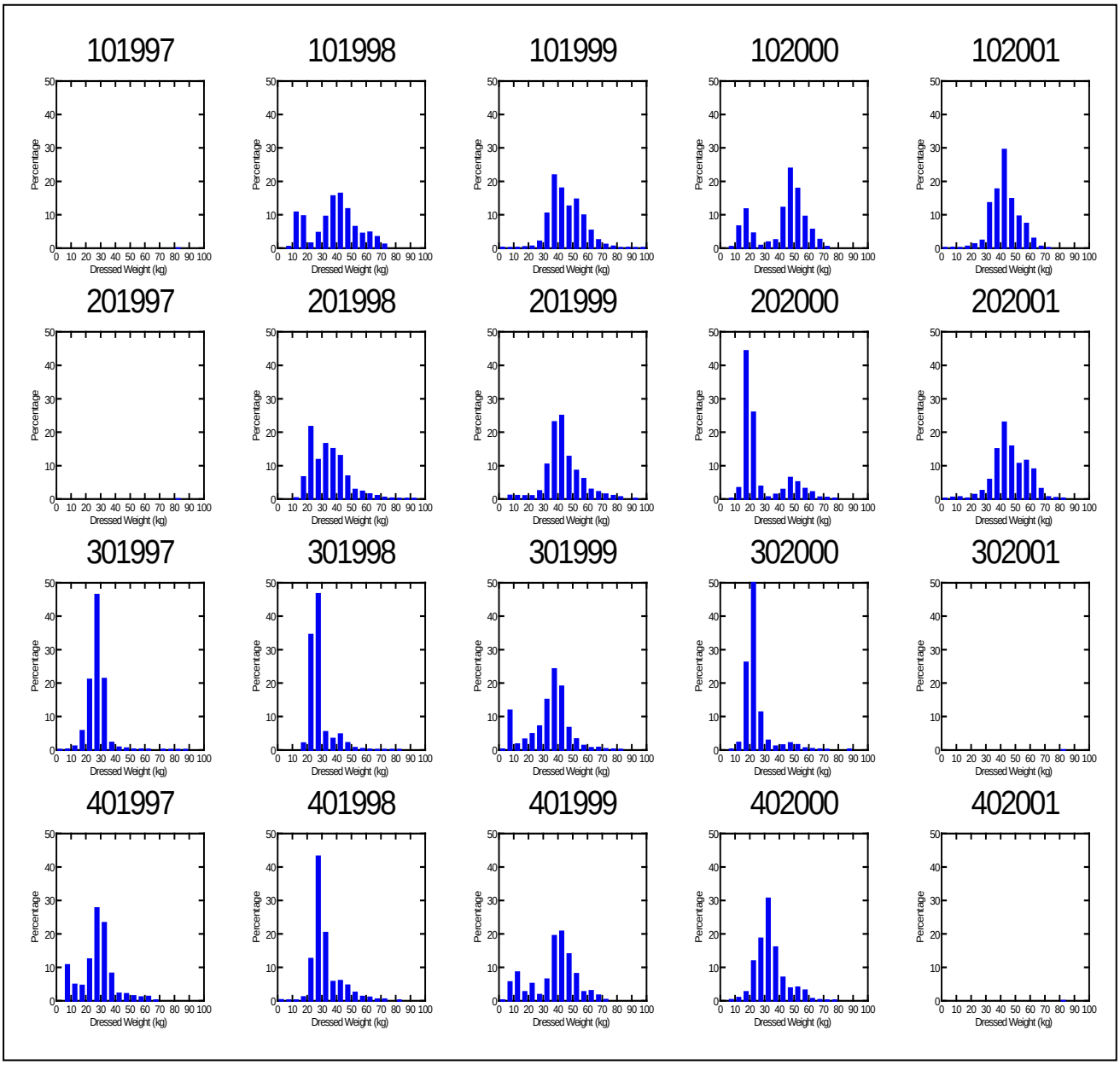


Figure 8.6.1. Weight frequency distributions for catches in three locations along the Australian east coast - (a) Cairns, (b) Mooloolaba and (c) NSW – for the period 1997-2000.

a. Cairns - Figure is split by quarter and year. 101997 = 1st quarter, 1997 etc.



b. Moololaba – Figure is split by quarter and year. 101997 = 1st quarter, 1997 etc.



c. NSW Coast - Figure is split by quarter and year. 101997 = 1st quarter, 1997, etc.

Table 8.1.1. Four possible scenarios for the stock structure of the Eastern Tuna & Billfish Fishery. The outcomes from 4 studies — genetics, otolith chemistry, tagging and CPUE analysis — were assessed to determine if they provided evidence supporting each of the scenarios.

	Possible scenarios for recruitment to the east coast tuna stocks			
	Scenario 1 Stock principally comprises fish from the **Coral Sea** *Implication: The parental biomass is smaller than for scenario 4 resulting in smaller recruitment levels.*	Scenario 2 Stock comprises both western Pacific and Coral Sea fish, with **Coral Sea dominant** *Implication: The parental biomass is between that of scenarios 1 and 4.*	Scenario 3 Stock comprises both western Pacific and Coral Sea fish, with **western Pacific dominant** *Implication: The parental biomass is between that of scenarios 1 and 4 but larger than for scenario 2.*	Scenario 4 Fish from the east coast are part of **a single large western Pacific stock**, including the Coral Sea *Implication: The parental biomass is large and will result in large recruitment levels.*
Genetics	not supported	supported	supported	supported
Otoliths	not supported	supported	supported	not supported
Tagging data	not supported	The small number of tag returns doesn't allow us to determine which area dominates but currently the returns indicate very low levels of movement into and out of the Coral Sea.		limited evidence
CPUE analysis		The localised view of the stock indicates pulses of recruitment.	The globalised view of the stock indicates continuous YFT distribution.	

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9 Benefits

This study has provided input into AFMA's Fisheries Assessment process and the management of yellowfin tuna in the Eastern Tuna and Billfish Fishery (ET&BF). AFMA is currently finalising a Management Plan for the ET&BF, and at the same time Strategic and Ecological Risk Assessments are being conducted. All of these processes have benefited directly from the results of the study. Although there remains uncertainty regarding the frequency of large recruitment events from the broader central and western Pacific, and we have no data on the exploitation rates for yellowfin in our region since the significant expansion of the domestic sector in the ET&BF, the results of the study suggest some caution when levels of catch and effort are determined. A precautionary approach to the exploitation of yellowfin until exploitation rates, and the patterns of recruitment across our region are better understood will likely provide medium to long-term benefits to the 200 longline fishing operators that each year target and catch yellowfin, as well as the significant number of sport and game fishermen that catch the species along the east coast of Australia.

10 Further development

In this study we used new otolith chemistry and genetic techniques to examine geographic variation in yellowfin genetic and phenotypic markers. In the process we have been able to evaluate the potential for use of these techniques for further work on tunas, and other fish species.

The use of genetic data for identifying population subdivision is a powerful technique guiding management decisions, especially when clear differences can be demonstrated between sampled areas. However, an inherent risk of using genetic data for management decisions for fish stocks, such as yellowfin tuna, under multinational jurisdiction is in the confusion surrounding the interpretation and use of wording to describe results of population genetic data. This confusion stems from limitations of data that do not demonstrate clear separation of fish sampled, for example, from two reasonably separated areas that putatively represent isolated populations. Lack of identifiable differentiation between the two samples may be consistent with two samples taken from a single genetic pool of individuals (i.e. a single genetic stock) but the data are also consistent with two samples taken from genetically isolated populations (two reproductively isolated stocks) given that the markers examined do not actually demonstrate differences between the two areas. All too often the lack of differentiation between two separated samples of fish is used by management authorities to imply there is strong evidence for panmictic populations; and consequently, conclusions are drawn that are potential hazards for safe management decisions. The danger in using the single gene pool interpretation of the data is that these samples may in fact represent reproductively isolated populations with very limited or no migration between spawning areas. If this is the case then using average catch statistics to set catch quotas could result in an irreversible extinction of breeding stock from one area, which may not recover in the foreseeable future, due to over-fishing of one stock while under-fishing of the other. Clearly management decisions in situations such as this should err on the side of caution and manage the separate areas as two genetically distinct populations until more data become available to provide better confidence in the interpretation of the data.

Gaining more data to describe population genetics of the areas sampled for this yellowfin project should lead to a better understanding and definition of whether or not the areas sampled by this study represent a single or multiple gene pools. Differences between populations occur over time as a result of random genetic drift of gene frequencies and introduction of new alleles through mutation, provided exchange of individuals between these areas is extremely limited. The probability of demonstrating existence of genetic differences between truly isolated populations is therefore directly related to both the amount of time the areas have been genetically separated and also by further increasing the number and types of genetic markers incorporated in the study. For the current study yellowfin tuna, a new class of markers, di-nucleotide microsatellites, was examined. These markers are known for their rapid mutation rates and were perhaps too variable for defining population parameters for a pelagic species such as yellowfin tuna. However, tetra-nucleotide microsatellites, which appear to be more stable and have lower rates of mutation, have been used to successfully demonstrate population differentiation in swordfish (Reeb *et al.* 2000). Such markers have been developed for tuna species and examination is underway to assess the degree of differentiation among our yellowfin samples. Should other markers become available it would be prudent for further examinations to use them as well.

The results from the Secondary Ion Mass Spectrometry - examining oxygen and carbon isotopes of the internal region of the otolith - were encouraging from the perspective that they showed significant difference among natal sites. However, as we were unable to prevent contamination of the otolith material laid down in the first few months of life with that laid down later in life when working with larger specimens, there was no scope for comparing isotope ratios of recruiting fish with those of the natal areas (as we were able to do with the probe-based analyses of other elements). We believe SIMS offer considerable promise in a range of applications, particularly at the range of sensitivity provided by the facility we used at the University of Wollongong. However, when comparing otoliths of different sizes (i.e. fish of different age), the issue of contamination must be successfully addressed. Our technique for removal of the surface layers was careful, and we believed would overcome the problem. However, it appears that even small amounts of contamination can significantly affect results. As we noted above, micro-milling techniques have been used successfully in recent studies of otolith chemistry to isolate small areas around the core or sample material deposited during one season (Gao and Beamish 1999; Gao *et al.* 2001), offering the promise that this more precise sampling technique may provide a means for future investigations on the use the spawning site oxygen and carbon signals to classify fish to their natal stock.

Our work with PIXE and WDEMPA probes confirmed the potential for use of otolith chemistry to examine phenotypic variation in pelagic fishes. Over the last few years laser ablation techniques have been successfully developed and applied in a number of studies with objectives similar to ours. Having reviewed the results offered by the range of techniques now available, we'd strongly suggest that any future studies of tuna or pelagic fish otolith chemistry use WD-EMPA in combination with LA-ICPMS (Thorrold *et al.* 1998, 2000). Despite the added requirements for clean-room techniques, the latter offers significant advantages over PIXE.

The inter-annual variability in otolith chemistry signals described in our study emphasises the need to examine spatial variation on appropriate temporal scales. This offers challenges to those working with pelagic animals as the supply of fish is often not continuous or predictable. Nevertheless, there appears significant scope to examine otolith chemistry variation in species such as bigeye tuna, where genetic analyses suggest sufficient gene flow to mask and regional sub-structure in stocks, yet tagging data suggest limited movement is common.

From a fisheries management perspective, the results of our study suggest that a priority for the next few years will be to determine the exploitation rates of yellowfin in the eastern AFZ/Coral-Tasman Seas. A conventional tagging program, releasing tags into the broader Central and Western Pacific at the same time as we release tags into the Coral Sea would provide an excellent foundation for examining movement patterns as well as determining exploitation rates. At the same time, it is important that the Australian Fisheries Management Authority continue to monitor closely the size distribution of yellowfin catches in the eastern AFZ, and ideally liaise with Authorities in New Zealand, New Caledonia, PNG and the Solomons to examine regional patterns in recruitment.

10.1 References

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11 Planned Outcomes

One of the primary planned outcomes for this project was to determine the most likely origin of yellowfin tuna recruits to the eastern tuna and billfish fishery (ET&BF). The results of otolith chemistry analyses provided evidence that not all recruits are derived from the spawning grounds in the Coral Sea. In the event of this result another planned outcome was to estimate the proportion of recruits to ET&BF that came from the Coral Sea, or from other areas of the central and western Pacific. The otolith chemistry analyses produced site- and cohort-specific markers that were used to classify yellowfin recruiting to the ET&BF and, for the years of the study, the results showed that the majority of yellowfin recruits were most likely to have come from the Coral Sea but the proportion varied from year-to-year.

Another planned outcome of this study was to assess the relationships of Coral Sea yellowfin tuna to other western Pacific samples. New genetic techniques were developed during the project and used to test if significant differences could be detected among yellowfin populations across the Pacific. Five polymorphic microsatellite loci were examined, one of which showed small but significant differentiation among collections but, overall, the microsatellite data revealed little about the relationships among western Pacific yellowfin tuna populations. The evidence of genetic homogeneity in this region is inconsistent with any hypothesis that proposes reproductive isolation of any (examined) stock in this region, but the extent of gene flow among regions could vary from quite low to high and still maintain the appearance of homogeneity. Possibly larger sample sizes might reveal evidence of small-scale (but nonetheless significant) population heterogeneity hidden in smaller studies: the power of any test increases as sample size increases.

A further output from the project was a synthesis of these results with other information on yellowfin biology; catch distribution and catch rates; tagging results and oceanography. The synthesis provided a broader perspective of the system and contributed to our investigation of what links exist between yellowfin from the ET&BF, the Coral Sea and the wider western Pacific Ocean.

These results have provided input into AFMA’s Fisheries Assessment process and the management of yellowfin tuna in the ET&BF. In 1998, 1999 and 2000, the ET&BF Fishery Assessment Group (FAG) used the information provided by this study on linkages between the Coral Sea and the ET&BF in its interpretation of catch rates and recruitment patterns of yellowfin. The project data were also provided at an important effort setting workshop in 1999 at which representatives from industry, science and management met to discuss the optimal levels of effort within the ET&BF. The likelihood that yellowfin recruitment into the ET&BF was strongly linked to the Coral Sea, rather than the alternative scenario that all recruits were sourced from the greater Western Pacific, was a significant contributing factor to a majority decision within the meeting to adopt a low risk position in setting an effort cap for the ET&BF. AFMA is currently finalising a Management Plan for the ET&BF, and at the same time Strategic and Ecological Risk Assessments are being conducted. All of these processes have benefited directly from the outputs of the study.

The outputs have also been used within a major AFMA report on the trends in abundance of

yellowfin on the east coast of Australia and in the SW Pacific Ocean (Campbell 1998). In this report an understanding of the probable sources of recruits to the ET&BF was used in the interpretation of observed inter-annual variation in abundance, and the development of hypotheses explaining these.

12 Conclusion

The objectives of our study combined development of new techniques (genetic micro-satellites and measurement of otolith oxygen 18/16 and carbon 13/12 ratios using Secondary Ion Mass Spectrometry) with the application of these and existing techniques to determine the structure of yellowfin stocks in the south west Pacific Ocean and if possible, to determine the origin of recruits to yellowfin fisheries in the eastern AFZ.

The development of genetic micro-satellites for yellowfin tuna was successfully completed (objective 1), and these were used to determine the extent of heterogeneity of samples collected from sites throughout the western Pacific, including the Coral Sea and NSW within the eastern AFZ. The data from micro-satellites, along with those for allozymes and mitochondrial DNA do not allow us to reject the null hypothesis of a single panmictic yellowfin tuna population in the western Pacific Ocean. However, this does not mean that the null hypothesis is true, just that there is insufficient evidence to reject it (objective 2). Waples (1998) contends that if components of a stock complex exhibit high gene flow, then management should not be based on genetic data alone. We would also suggest that the minimal level of micro-satellite heterogeneity observed in the yellowfin tuna populations be approached carefully until other biological data such as tagging, morphology and otolith chemistry can help to determine management units. If the yellowfin tuna within the Pacific Ocean are truly panmictic, then managing the fishery as a single stock will not affect recruitment from overfished areas. If, however, different yellowfin tuna populations do exist, management as a single stock will mean that over-exploitation in certain areas will lead to reductions in effective population size and yield in these areas. The current genetic results also confirm previous findings that it is likely the source of yellowfin tuna recruitment into the eastern AFZ is from the Coral Sea or Solomon Islands.

The development of techniques to examine oxygen and carbon isotopes was partially successful. We developed methods to extract material from small (<50cm FL) fish, which allowed us to demonstrate site-specific signals in the isotope ratios of both elements (objective 3). This finding suggests that these isotope ratios could be useful stock delineators. However, we were unable to successfully isolate material from the core of otoliths from larger fish. This meant that the isotope ratios of these were contaminated with material laid down when the fish was likely to have been away from its natal site. Attempts to correct for a size effect in the data were successful from a statistical viewpoint but we considered the biological meaning of statistical differences between size-standardised data to be questionable, and thus chose not to use the data as natal markers in the broader study.

Electron and proton micro-probe analyses revealed significant variation in the concentrations of a number of micro-constituents in the otoliths of young-of-the-year yellowfin tuna collected from sites throughout the Western Pacific, including the Coral Sea. The analyses also revealed significant inter-annual variation in the signals for three sites sampled on two different years. Given the temporal variability, the micro-constituent concentrations were used to establish site- and cohort-specific markers for the samples. The cohort specific markers were used to classify the origin of fish recruiting to the NSW coast as 2 year olds in 1996 and 1997. These classifications indicated that the NSW otolith chemistry was very similar to that for fish

spawned in the Coral Sea, and less similar to those spawned in the Philippines, Solomon Islands, Fiji or Indonesia. However, there were fish caught on the NSW coast that were very different to those sampled in the Coral Sea, a clear indication that recruits to the ET&BF are unlikely to have all come from one source.

The critical management question addressed by this study is what proportion of recruits to the ET&BF are likely to have come from the Coral Sea, or from other areas of the Central and Western Pacific (objectives 4 and 5). To answer this we synthesised the new data collected by this study on otolith chemistry and genetics, with data collected by conventional tagging studies over the past 30 years, catch, effort and size data collected for the domestic fishery by the Australian Fisheries Management Authority, catch and effort data from the Japanese fishery that operated in the eastern AFZ for over 40 years, and oceanographic data for current systems likely to influence recruitment of yellowfin into the ET&BF. We set up four hypotheses regarding the possible source of recruits to the ET&BF:

1. All yellowfin recruit from the Coral Sea

2. The majority of yellowfin recruit from the Coral Sea with the Central and Western Pacific playing a lesser role.

3. The majority of yellowfin recruit from the Central and Western Pacific, with the Coral Sea playing a lesser role.

4. All yellowfin recruit from the equatorial Central and Western Pacific

The results of this study allow us to reject hypotheses 1 and 4. Also, at least for the years in which we sampled, it would appear that Hypothesis 3 is also unlikely to have been the case. The conclusion we have reached on the data available to us is that Hypothesis 2 is a valid. We hasten to emphasize however, that large recruitment events from outside the Coral/Tasman Sea region are likely to significantly affect the ET&BF from time to time. Sporadically throughout the 1980's and 1990's we have seen evidence of large pulses of young of the year yellowfin moving down the east coast. Whether these are the result of strong year classes in the Coral Sea, or an indication of an influx of fish from the broader Central and Western Pacific remains uncertain.

In light of our findings, we suggest that a priority for the fishery (science, industry and management) should be to promote a program of conventional tagging in the Coral Sea to allow determination of exploitation rates for yellowfin in the eastern AFZ. There have been discussions recently at the Secretariat for the Pacific Community Standing Committee for Tuna and Billfish regarding the need for a large-scale tagging program on yellowfin and bigeye in the Central and Western Pacific. This provides a very useful opportunity to continue collection of data on movement and mixing of yellowfin in our region, while allowing estimation of exploitation rates on both broad and local scales.

Acknowledgements

We thank the Australian Fisheries Research and Development Corporation (FRDC) for funding this work (Project No. 94/022) and the following people who assisted us in collecting samples: Dave Armstrong, Sofri Baha, Graham Baker, Noel Barrett, Charles Bryce, Thor Carter, Robin Croxon, Tim Davis, Joe Grekko, Damian Heran, Bob Lamason, Mike Rowley, Karl Staisch, Filipe Viala, Kevin Williams and Ian Wilson. Thanks go Carl Bjorkland for assistance with the otolith microsampling and Thor Carter for otolith preparation and probe analysis. We also acknowledge the contributions of Ian Harrowfield, Soy Sie (CSIRO Minerals), Sue Wang and Allan Chivas (University of Wollongong), who provided the facilities and their expertise for the otolith chemical analyses. Michael Lowry of the NSW Fisheries Research Institute very kindly provided access to data from the FRI recreational fishery tagging program.

Appendix A - Staff

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Genetics

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Dr Sharon Appleyard	CSOF 3
Bronwyn Innes	CSOF 3

Appendix B. Intellectual Property

The intellectual data arising from this research are:

1. Genotype and allele frequency data.

2. Copyright in this report.

Appendix C. Database structures for storage of project data

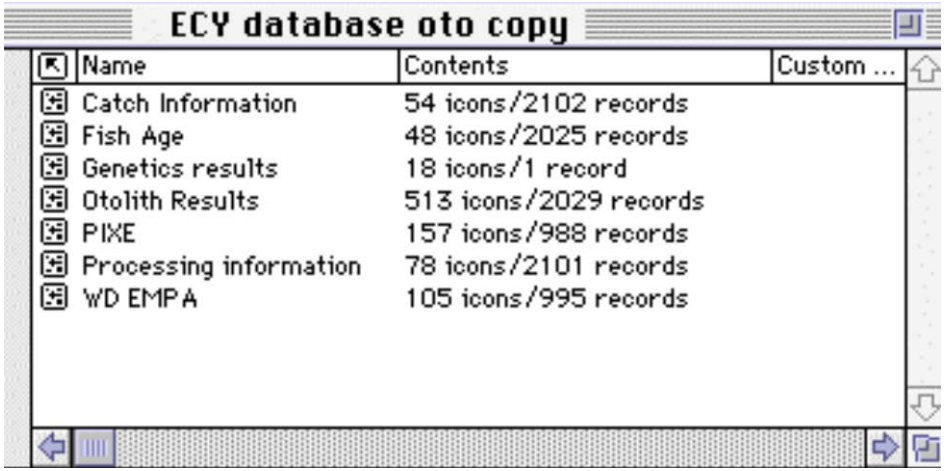


Figure B(i) Seven tables were designed and implemented to store data collected for the project.

Fields were established in each of the 7 tables to store data collected for the project. In the figures below the fields are displayed as they appeared on the forms designed for data entry (Figures B. ii-viii).

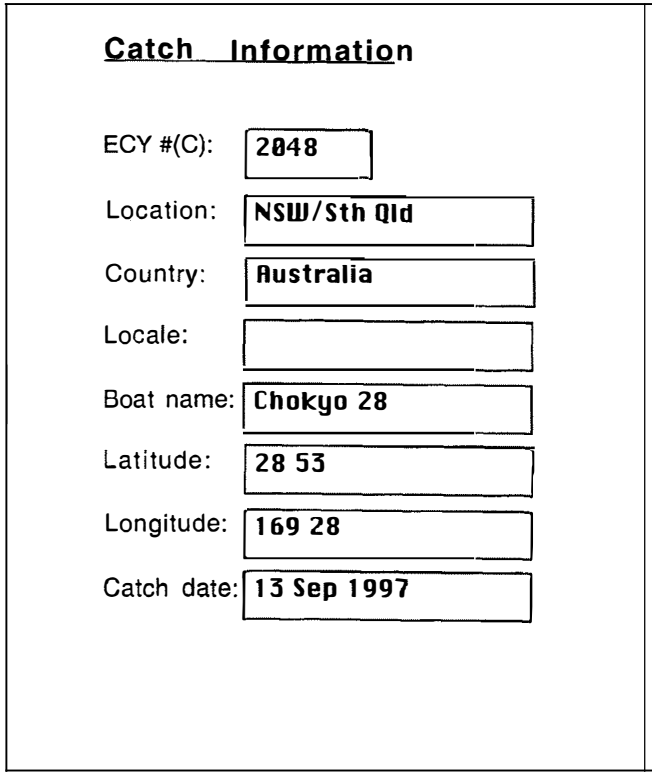


Figure B(ii). Fields in the table Catch Location.

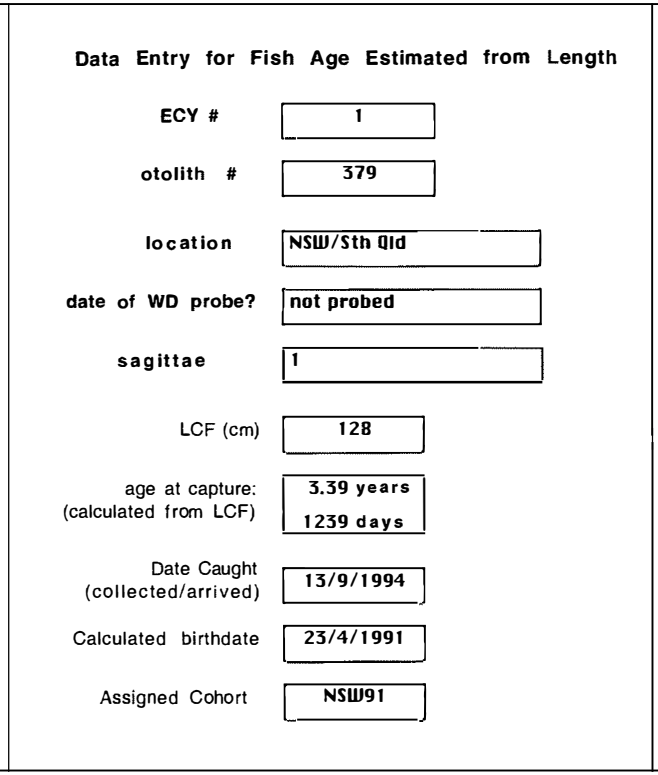


Figure B(iii). Fields in the table Fish Age.

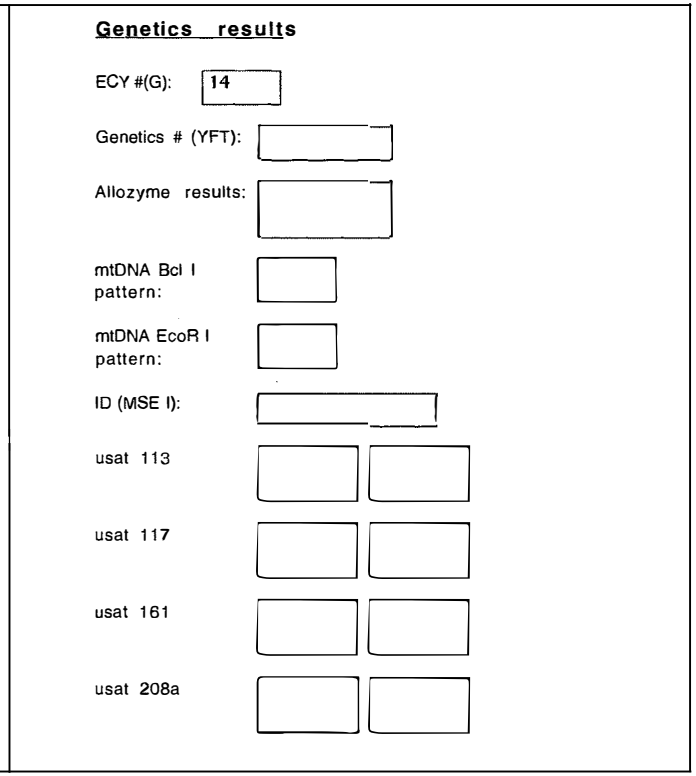
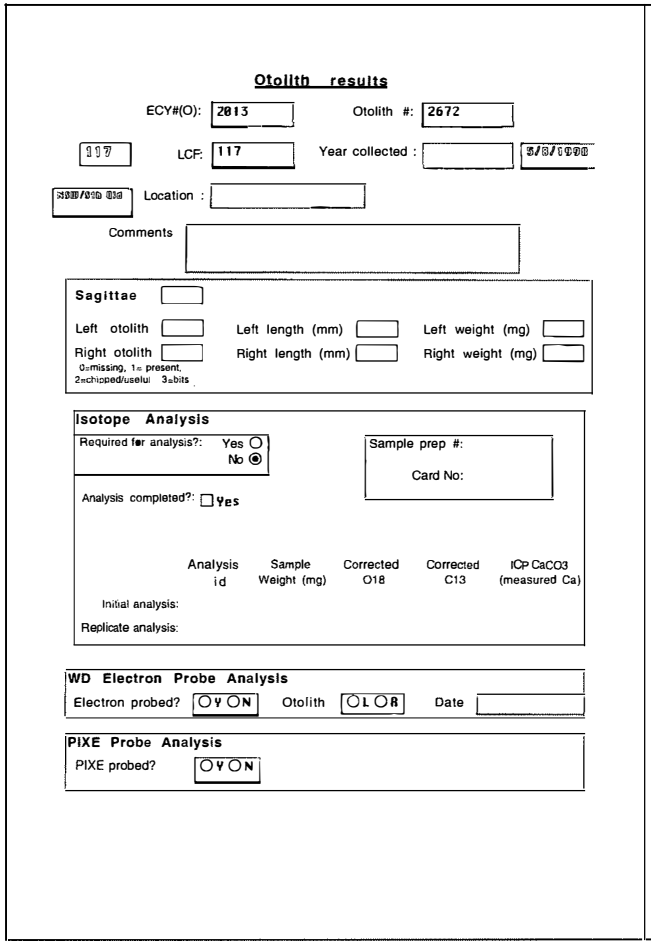


Figure B(iv) Fields in the table Genetics Results



Otolith results

ECY#(O): 2013 Otolith #: 2672

117 LCF: 117 Year collected :

Location :

Comments

Sagittae

Left otolith | Left length (mm) | Left weight (mg)

Right otolith | Right length (mm) | Right weight (mg)

0=missing, 1= present, 2=chipped/useful 3=bits

Isotope Analysis

Required for analysis?: Yes / No (●)

Sample prep #:

Card No:

Analysis completed?: ☐ Yes

	Analysis id	Sample Weight (mg)	Corrected O18	Corrected C13	ICP CaCO3 (measured Ca)
Initial analysis:					
Replicate analysis:					

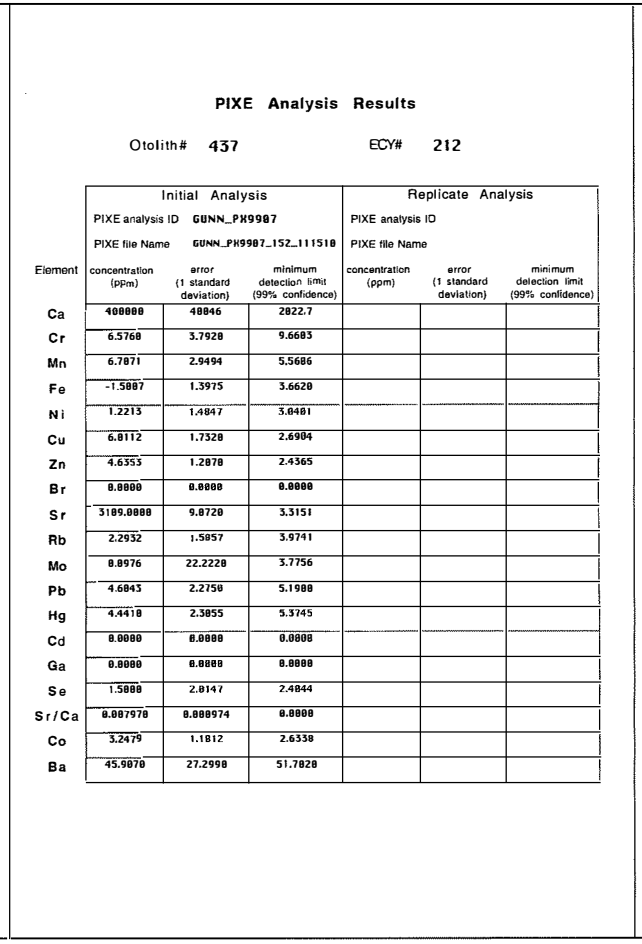
WD Electron Probe Analysis

Electron probed? O Y O N Otolith O L O R Date

PIXE Probe Analysis

PIXE probed? O Y O N

Figure B(v). Fields in the table Otolith Results.

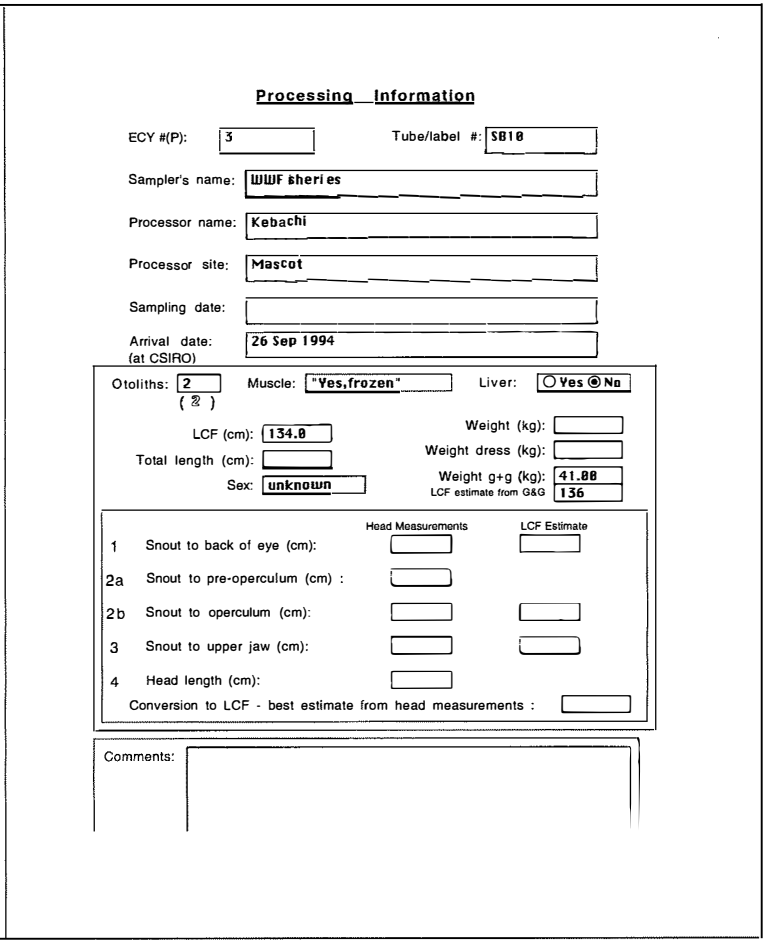


PIXE Analysis Results

Otolith# 437 ECY# 212

Element	Initial Analysis: concentration (ppm)	Initial Analysis: error (1 standard deviation)	Initial Analysis: minimum detection limit (99% confidence)	Replicate Analysis: concentration (ppm)	Replicate Analysis: error (1 standard deviation)	Replicate Analysis: minimum detection limit (99% confidence)
	PIXE analysis ID GUNN_PX9907			PIXE analysis ID		
	PIXE file Name GUNN_PX9907_152_111510			PIXE file Name		
Ca	400000	48046	2822.7			
Cr	6.5760	3.7928	9.6683			
Mn	6.7871	2.9494	5.5686			
Fe	-1.5887	1.3975	3.6620			
Ni	1.2213	1.4847	3.0401			
Cu	6.8112	1.7328	2.6904			
Zn	4.6353	1.2878	2.4365			
Br	0.0000	0.0000	0.0000			
Sr	3189.0000	9.0720	3.3151			
Rb	2.2932	1.5057	3.9741			
Mo	0.0976	22.2220	3.7756			
Pb	4.6043	2.2750	5.1988			
Hg	4.4418	2.3855	5.3745			
Cd	0.0000	0.0000	0.0000			
Ga	0.0000	0.0000	0.0000			
Se	1.5000	2.0147	2.4844			
Sr/Ca	0.007970	0.000974	0.0000			
Co	3.2479	1.1812	2.6338			
Ba	45.9070	27.2990	51.7020			

Figure B(vi). Fields in the table PIXE.



Processing Information

ECY #(P): 3 Tube/label #: SB10

Sampler's name: WWF Sheries

Processor name: Kebachi

Processor site: Mascot

Sampling date:

Arrival date: (at CSIRO) 26 Sep 1994

Otoliths: 2 (2) Muscle: "Yes,frozen" Liver: O Yes (●) No

LCF (cm): 134.0 Weight (kg):

Total length (cm): Weight dress (kg):

Sex: unknown Weight g+g (kg): 41.80

LCF estimate from G&G 136

		Head Measurements	LCF Estimate
1	Snout to back of eye (cm):		
2a	Snout to pre-operculum (cm) :		
2b	Snout to operculum (cm):		
3	Snout to upper jaw (cm):		
4	Head length (cm):		

Conversion to LCF - best estimate from head measurements :

Comments:

Figure B(vii). Fields in the table Processing Information.

WD EMPA Results

Otolith# **2682** ECY# **2023**

Concentration (weight percentage)

	Point Probed	Na	Sr	K	S	Cl	Ca	O	Total	Analysis#
PRIMORDIUM	1	0.30100	0.18000	0.04700	0.02000	0.01000	38.75900	48.38500	54.22370	150
	3									
	4									
	6									
	average	0.30100	0.18000	0.04700	0.02000	0.01000	38.75900	48.38500	54.22370	

1 *Primordium point to use for analysis*

APEX	2	0.35800	0.13800	0.04400	0.01600	0.01000	38.57300	48.49800	53.97710	151
	5									
	average	0.35800	0.13800	0.04400	0.01600	0.01000	38.57300	48.49800	53.97710	

2 *Apex point to use for analysis*

WD Probe trip **16/9/1998**

Notes

Figure B (viii). Fields in the table WD EMPA.