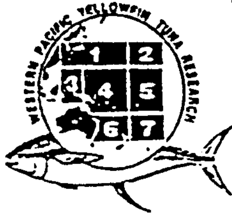

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Progress report on the use of otolith microchemistry, allozyme and mitochondrial DNA analyses for the delineation of Western Pacific Yellowfin tuna stocks*.

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Introduction

In 1992 CSIRO undertook a pilot study investigating the use of a number of new techniques in the delineation of yellowfin tuna stocks of the Western Pacific. The research was funded by the Australian Fisheries Research and Development Council which is interested in research on yellowfin stocks of the eastern Australian AFZ, which are currently exploited by both domestic and Japanese longline fleets, the latter operating under bi-lateral agreements between Australia and Japan.

The objectives of the one-year study were:

1. To provide an allozyme-based taxonomic key to the early life history stages of Thunnus species occurring in eastern Australian waters. This key would provide, for the first time, an accurate and efficient means of separating larval, post-larval and small juvenile specimens of the principal commercial tuna species (e.g. yellowfin, bigeye, albacore, southern bluefin, skipjack).

2. To assess the genetic variability in larval/juvenile yellowfin tuna collected from four different locations; one off the north-east coast of Australia, two in other parts of the Western Pacific and one in the Indian Ocean,

3. Using the specimens for which genetic data have been collected, to assess the extent of variability in otolith microchemistry, particularly in that region of the otolith that is deposited during the early stage of larval development (i.e. while the larvae are close to the spawning area). Data on the variability among and within samples from the four areas will provide a means of assessing whether yellowfin tuna otoliths constitute a natural tag that will allow the identification of spawning areas from which recruits to domestic and foreign fisheries within the AFZ originate.

Results

The laboratory processing of all samples and statistical analyses of data from the project are 95% complete and it is planned to submit manuscripts for publication from the work within the next 12 months.

(i) Genetic species identification

Molecular techniques are being developed for the identification of tuna species. These techniques involve both the examination of nuclear DNA markers (through allozyme electrophoresis) and restriction enzyme analysis of mitochondrial DNA. At present large samples of yellowfin and southern bluefin tuna have been examined, along with smaller numbers of bigeye, northern bluefin, Atlantic bluefin, albacore and skipjack. Absolute identification of all individuals in the present study, from small fragments of fresh or frozen tissue, has been achieved.

Future research aims to quantify levels of intra- and inter-species variation and possible overlaps, permitting identification of tuna on a global basis. Faster methods of identification will also be developed that can be carried out by minimally trained personnel, from fresh, frozen or alcohol-stored fragments of white muscle.

These techniques will permit the unequivocal identification of carcases from which fins, livers and gill rakers have been removed, and the unequivocal identification of tuna larvae collected for biological spawning surveys.

(ii) Allozyme and mitochondrial DNA analyses of geographic variation

Samples of yellowfin tuna from the western, central and eastern regions of the Pacific Ocean were examined for genetic variability. Four polymorphic allozyme loci (*ADA**, *GPI-S**, *GPI-F** and *FH**) were examined in all samples and a fifth polymorphism (*GDA**) examined in western and central samples only. All samples were also screened for mitochondrial DNA variation following restriction analysis by two enzymes (BcII and EcoR1) detecting polymorphic cut sites. Eighteen mtDNA haplotypes were revealed. No significant spatial heterogeneity was detected for alleles at the *ADA**, *GDA**, *GPI-S** and *FH** loci nor for the mtDNA haplotypes. Significant heterogeneity was detected for *GPI-F**. At this locus, the two eastern samples (southern California and northern Mexico) were not significantly different from each other but were significantly different ($P<0.001$) from the five western/central samples (Coral Sea, Philippines, Kiribati, Hawaii-91 and Hawaii-92). *GPI-F*B* was the most common allele in eastern and central regions, *GPI-F*C* the most common in western samples. The Hawaii-91 (comprising mostly juvenile fish) and Hawaii-92 (comprising mostly adult fish) were significantly different ($P=0.013$) from one another at the *GPI-F** locus.

There is clearly much genetic variation in yellowfin tuna that can be used for stock delineation research. The results of this pilot study demonstrate significant spatial genetic heterogeneity on a pan-Pacific scale for one allozyme locus. The lack of similar heterogeneity for mitochondrial DNA polymorphism was disappointing, given that the mitochondrial DNA molecule evolves faster than nuclear DNA and, being haploid and maternally inherited, is expected to be more sensitive to the effects of genetic drift.

Proposed future work on genetic stock delineation in this species involves the analysis of larger sample sizes (up from around 50 to 100 per sample to around 200) from a wider range of locations together with further research into restriction enzymes capable of detecting additional mitochondrial DNA variants.

(iii) Geographic variation in otolith microchemistry

Samples of very young juvenile yellowfin collected in Hawaii, the Philippines and Indonesia were used to examine the extent of variation in spawning area chemical signals. All fish were less than 35cm LCF (<100days old) and were assumed not to have moved far from their natal area. Otoliths are not reworked, and thus provide a time series of chemical information for each fish, starting at birth and incrementing daily until capture. To characterise the spawning area chemical signature, the chemistry of daily increments 6-10 was analysed using two microprobes - a wave dispersive electron probe for elements present in concentrations of >200 ppm, and a proton probe for elements present in concentrations of 1-200 ppm.

In addition to samples of small fish from Hawaii, the Philippines and Indonesia, a sample of otoliths from larger fish (45-60cm LCF) caught in the Coral Sea were analysed. These fish were part of a large aggregation of yellowfin and bigeye tuna that each year form off cairns in the NE Coral Sea. Since the work of MacPherson in the 1980's the aggregation has been though of as a spawning aggregation and a possible source of recruits to the Australian east coast yellowfin fisheries. The age of the Coral Sea samples analysed in this study was assumed to be in the order of one year and thus that the potential/ likelihood of movement from their natal area was much higher than for the smaller fish collected from other areas of the Western and Central Pacific.

The results of the chemical analyses showed significant differences among collection sites in the concentrations of many elements present in the otoliths. Of 13 elements present in concentrations greater than the minimum detection limits of the probes used, 7 differed significantly.

Discriminant function analyses of data from the three sites at which small juvenile fish were collected resulted in 92%, 74% and 83% correct classification of specimens from the Philippines, Indonesia and Hawaii respectively. When data from the Coral Sea samples are included in the analyses there is a slight reduction in the correct classification of Philippino fish (84%) while Indonesian and Coral Sea fish are correctly classified to 79% and 77% respectively. The Hawaiian classification suffered significant misclassification (only 57% correct), with almost all of the leakage being to the Coral Sea. Similarities in the concentrations of two key elements caused the misclassifications.

The significantly differences in concentrations of elements within the otoliths of yellowfin from different parts of the Western Pacific are evidence that otoliths may provide a means of "typing" a fish's spawning area on the basis of its otolith chemistry. However, further work on the temporal stability of the chemical signal within an area, and the extent of fine scale variation are needed before the method can be effectively appraised as a means of stock discrimination.