

## THE 1995 ACTIVITIES OF BLUEFIN YEAR PROGRAM (BYP) IN THE WESTERN ATLANTIC

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The research activities relating to the Bluefin Year Program (BYP) were discussed and planned at the Meeting in Genoa on March 13-14, 1995 (SCRS/95/14). All reports presented at this meeting were presented to SCRS (SCRS/95/34-38). The activities of Canada and Japan after the Genoa Meeting are summarized in this report. In addition, the Research Planning Workshop for tagging studies was held in Miami during 1-4 August, 1995 (SCRS/95/95).

### The United States :

The activities of the United States in 1995 was summarized in the US National Report, SCRS/95/74.

### Canada :

The activities of Canada in relation to the BYP in 1995 was summarized in Table 1.

### Japan:

The Shoyo-maru, the Japanese Research Vessel, conducted a larval survey at two spawning grounds of Atlantic northern bluefin tuna, the Gulf of Mexico and the Mediterranean Sea, in 1994. The main objective of survey was to compare the genetic characteristics and productivity of both spawning grounds, to obtain an overall picture of occurrence of tuna larvae especially in the Mediterranean, and to establish a relative measure of sampling efficiency among different parties. The cruise activities was briefly explained in the SCRS/94/177.

A standard BONGO tow provided two samples for each station. During the cruise, one sample was always fixed and preserved with formalin but the other sample was preserved with three ways, with 90% ethanol, with saturated salt water mixed with DMSO (dimethyl sulfa-oxide) and in frozen to seek for an appropriate method for genetic analysis. Ethanol preserved samples were the easiest to handle among three methods but some of these had a trouble to amplify the long fragment. Some samples kept in a saturated salt water with DMSO were dissolved and rotten completely during the cruise.

Up to now, about 80% of formalin preserved samples were sorted and preliminary species identification was made for *Thunnus* for around 70% of sorted samples. Samples from the Tyrrhenian and Ionean Seas as well as samples from collaborative stations were processed in the first priority. We expected to complete sorting and species identification of tuna and tuna-like species for all formalin preserved samples within another six months.

Preliminary results of distribution of tuna larvae were shown in Figures 1 and 2. Dots and plus marks corresponded to the unsorted and sorted stations, respectively. Abundance of tuna larvae was shown in number per tow and was not adjusted with a filtered volume nor depth of tows. Whereas almost all tuna larvae other than bluefin were albacore in the

Mediterranean, yellowfin was a predominant species in other tuna of the Gulf of Mexico. Heavy concentrations of bluefin larvae were observed at south of Messina in the Ionean Sea. The maximum number of bluefin caught per tow was 272. The occurrence of bluefin larvae in the Gulf of Mexico seemed to be less patchy and less abundant comparing with the Mediterranean.

Though a big patch of tuna larvae could not be obtained in collaborative stations, it was expected that a certain proportion of positive tows made it possible to develop a comparison of sampling efficiencies among three parties.

All samples preserved in ethanol were sorted and tuna larvae were extracted for genetic analysis. Genetic analysis was made using PCR-based restriction fragment length polymorphism (RFLP) and nucleotide sequence analyses on the mitochondrial and nuclear genes.

PCR amplification for longer fragment (c.a. 2,000bp) containing the Dloop region was successful for three North Atlantic (adults: collected from an area of 33-65 W, 41-51 N in 1991-1993) and three Mediterranean samples (adults, juveniles and larvae: all collected in 1994). However, larval sample from the Gulf of Mexico (collected in 1994) could not be amplified for this fragment from unknown reason. For each sample, 29 to 101 individuals were subjected to the PCR-RFLP analysis. Monte Carlo simulation on the frequencies of the restriction types and of the genotypes composed by restriction types of five enzymes indicated no heterogeneity contrast among the North Atlantic samples as well as among the Mediterranean samples. In contrast, slightly significant heterogeneity was observed in 4 of 9 comparisons between the Atlantic and Mediterranean samples.

Shorter fragment (c.a. 450b) containing hyper-variable left domain of the Dloop region was successfully amplified in the larval samples from the Gulf of Mexico and the Mediterranean. The 360 nucleotides were sequenced for 16 individuals from the Gulf of Mexico and 20 individuals from the Mediterranean and were compared between the locations. The sequence analysis revealed no distinct sample-specific nucleotide substitution. The larvae derived from the two localities were observed to evenly distribute in the phylogenetic tree.

The Western Atlantic (n=31) and Mediterranean (n=34) samples were subjected to PCR-RFLP analysis on the 6th intron of nuclear creatine kinase gene, in which two alleles were interpreted by *Mbo*I digestion. Allele frequencies were similar between the samples, and the genotype proportions within each sample and of the pooled one were in accord with Hardy-Weinberg expectations.

These preliminary results indicate the needs of more intensive and extensive efforts such as comparison between year-classes within locality for adults, analyzing larger sample size in nucleotide sequencing for larvae, and use of highly variable nuclear gene such as microsatellite loci.