

## JIMAR ANNUAL REPORT FOR FY2000

### PROJECT PROPOSAL TITLE:

### **Genetic Analysis of Population Structure in Pacific Swordfish (Xiphias gladius) using Microsatellite DNA Techniques (2076/2129)**

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FUNDING AGENCY: JIMAR, Pelagic Fisheries Research Program

#### **1.) Purpose of the Project:**

This project attempts to develop and apply nuclear genetic markers (microsatellites) to determine whether populations of the swordfish in the Pacific Ocean are genetically subdivided. The intent of this project was to augment an earlier study, also funded by this agency, in which mitochondrial DNA (mtDNA) sequence was used to determine population structure in the Pacific. A paper submitted to Marine Biology in November of 1998 and officially accepted in October of 1999 is currently in press and will soon be available from the journal. In this paper we proposed two populations of swordfish divided into northern and southern populations in the western Pacific which overlap in the east. The overall purpose of the microsatellite project was to determine whether this same pattern was apparent in the nuclear genome. The importance of testing the mtDNA results with nuclear markers is that if the pattern is different, then it may suggest males have migratory behaviors that are different from females. This is because mtDNA is inherited maternally and records the historical patterns of gene flow in females only.

A second purpose of this project was to determine why the mtDNA was unable to distinguish populations in the eastern Pacific as belonging to either a northern or southern population. In all, the mtDNA data was difficult to interpret in the eastern Pacific because fish collected in Chile were genetically different from Japan but those in Mexico and California were not suggesting that population overlap was a probable complication. Given the fact that our genetic study, like most other pelagic fish studies, relied on the opportunistic sampling of adults from commercial fishing vessels, such a result was not unexpected as adults have the propensity for long distance dispersal. One piece of information supporting the possibility of mixture across the equator in the eastern Pacific came in the form of fishery statistics from Japanese longline vessels. Catch statistics averaged over a 15 year time period clearly showed a north/south continuity of catch in this region (Sousa-Nishizki and Shimizu 1991, Bull Far Seas Fish Lab 29:75-90). It was expected that our microsatellites might be able to unravel this putative overlap because they are somewhat faster evolving than mtDNA. In addition, different types of analysis can be performed with nuclear markers, assuming populations are in Hardy-Weinberg equilibrium, that will enable the detection of population admixture over populations that are actually interbreeding and behaving as a single genetic unit.

#### **2.) Progress during FY 2000**

The first accomplishment of note was the acceptance and inevitable publication of our mtDNA results in the journal *Marine Biology* for 2000 (136:1123-1131). The second accomplishment was to secure additional funding through a grant from CSIRO in Hobart, Australia in August of 1999 to further examine the genetic similarity noted in our mtDNA study between eastern and western Australian swordfish populations. Currently, these populations are managed as separate stocks. Because sample sizes from either group were small in our initial survey, we were forced to combine these samples in the analysis. The reviewers of our mtDNA paper criticized this approach. We were fortunate to have the opportunity to collaborate with Dr. Robert Ward in the Genetics division of CSIRO. Through this collaboration, we were recently able to address both the two stock hypothesis as well as the problem of small sample size in Australia using both mtDNA and microsatellites. The additional samples of Australian swordfish also enabled us to better analyze the microsatellite data in the Pacific. The third accomplishment for this fiscal year was the preparation of the microsatellite manuscript that will be submitted to *Molecular Ecology* shortly.

Readers of this report may remember from earlier accounts of our progress that our eleven microsatellite loci detected a slight divergence of Chilean and Ecuadorian swordfish from other locales sampled in the Pacific. However, there was no genetic divergence between northern and southern swordfish populations in the western Pacific. This observation was of great concern in the beginning until it was understood why mtDNA and microsatellites gave two different views of stock structure. The additional samples collected in Australia and those collected in California gave us better statistical power to determine the mechanisms at work in the east. In addition, a paper that was presented by Mike Hinton at the 50<sup>th</sup> Annual Tuna meetings held in Lake Arrowhead this year verified our discovery of a southeastern population. This revelation has provided us with sound reason to advocate with confidence the presence of three stocks in the Pacific, two detected by mtDNA and one found with microsatellites. The post-doc working on this project has recently returned from a meeting of the Standing Committee on Tunas and Billfish in the western Pacific where she presented this information and called for the recognition of three stocks in the Pacific which have an overlapping distribution in the east. This proposal was taken into consideration by being included in the summary statement prepared at the end of the meeting (see page 7 of the report available at the following web site: <http://www.spc.int/oceanfish/Html/SCTB/SCTB13/index.htm>).

After a thorough analysis of the microsatellite data, it appears that our results are consistent with the conclusion of a third population of swordfish in the southeastern Pacific. The fact that mtDNA fails to reveal this third population clearly is due to an overlap to some extent of all three populations in the eastern Pacific. In the mtDNA study, our results were suggestive of a division between the southeastern Pacific and samples collected in the northwest. However, the fact that California and Mexico were not significantly different from either group made the analysis confusing to interpret.

One way to infer population admixture over population homogeneity is to assume populations should be in Hardy-Weinberg equilibrium. Under this model, if deficiencies of heterozygotes are observed, then this could be explained as a mixture of two separate populations, a phenomenon known as the Wahlund principle in population genetics. Heterozygote deficiencies were noted in 8 of the 11 loci assayed in California and suggest population admixture. Because

heterozygote deficiencies were also observed in all other populations for various loci, the presence of null alleles might also be problematic. However, California and Mexico had the highest number of loci with a deficiency and this would be expected under a population admixture scenario.

To further address population admixture in the northeastern Pacific, we isolated one locus in which one allele is in highest frequency in Chile/Ecuador while the remaining Pacific populations have another allele in highest abundance. Interestingly, the fish from California and Mexico have an intermediate allele frequency. This again corroborates the proposed mixing phenomenon. We had wanted to sample along the corridor connecting California and Mexico to at different times of the year during FY2000 with the hopes of catching a shift in allele frequency for this one locus as immigrants from Chile moved north or those from Japan and Hawaii moved east. Unfortunately, it has been difficult to acquire samples. Two years ago a sword boat out of Moss Landing whose captain had been collecting swordfish for our lab was lost at sea. Furthermore, our requests for tissue samples from NMFS in La Jolla are still pending. As of this writing, we have been unable to secure adequate sample sizes to make it worthwhile to begin this project. Recently, Dave Holts at NMFS in La Jolla has promised us that the California samples they currently have will be shipped to us within two weeks. If this is done, and there is adequate funding, we will pursue this project further.

In all, our progress for this year has been substantial in tying the story of swordfish population structure in the Pacific together into a nice package that can be useful for fishery management. It has also generated a number of new hypotheses, which can now be tested by other methods, including satellite tag technology. We even have one locus that can serve to periodically monitor the influx of swordfish into California from the south and northwest. With our development of novel genetic markers and our innovative methods of analysis, the project funded by the PFRP has revealed evidence of multiple stocks in Pacific swordfish showing a pattern of genetic connectivity between some populations and divergence in others. We are pleased that other labs have been able to duplicate some of our results and we are very happy to find that the Pacific is no longer considered a single panmictic stock making it somewhat easier to develop a basin-wide management plan. Because of PFRP's commitment to support our work year after year, we have finally, and with statistical confidence, identified three regions of swordfish production. Now it is up to others to design models, analyze data, and test hypothesis with these three regions in mind. This is what we were asked to do at the start of our project and we have delivered the results.

### **3.) Plans for the next Fiscal Year:**

Depending on the availability of funding, we may continue our work on California swordfish once our promised samples arrive from La Jolla. We will be submitting the microsatellite work for publication shortly and preparing a paper on Australian swordfish with our collaborators in Hobart at CISRO. We will then prepare a final manuscript tying the nuclear and mitochondrial data into one large paper for the Pacific, Atlantic, and Indian oceans and Mediterranean Sea that describes the genetic history and migration corridors of swordfish worldwide.

4.) List of Papers Published in Referred Journals during FY 2000

Reeb et al. 2000. Structure and Migration corridors in Pacific populations of the swordfish, Xiphias gladius, as inferred through analysis of mitochondrial DNA. *Marine Biology*, (136:1123-1131).

5.) Other Papers, Technical Reports, etc.

Reeb, C.A., L. Arcangeli, and B.A. Block. in prep. Global Population Structure of the Swordfish, *Xiphias gladius*, as Inferred through Analysis of Eleven Microsatellite Loci.

R. Ward, C. Reeb, and B. Block. Genetic analysis of australian swordfish with special emphasis on the east coast fishery. Report to CISRO, Division of Marine Research

6.) Students graduating with MS or Ph.D degrees during FY 2000; Dissertation Titles.

Ellen Freund, 1999. Comparisons of metabolic and cardiac performance in scombrid fishes: insights into the evolution of endothermy.

David Marcinek, 2000. The physiological ecology of myoglobin in scombrid fish.