SCRS

Utility of *CaM* gene marker to determine the boundary between the north and south Atlantic swordfish stocks

Seinen Chow and Motoki Nakadate
National Research Institute of Far Seas Fisheries
5-7-1 Shimizu- Orido, Shizuoka 424-8633, Japan

SUMMARY

Genetic variation at the calmodulin gene locus (*CaM*) was investigated for a total of 109 Atlantic swordfish (*Xiphias gladius*) individuals collected in 2002. We confirmed the results of previous studies which have revealed the presence of genetically distinct two swordfish stocks in the Atlantic. Forty-two of 109 individuals were collected between 10N and 20N, supposedly a boundary zone. The western sample (48W) (n=18) showed allele frequencies intermediate between the north and south stocks, and the eastern sample (28W to 37W) (n=24) showed more affinity with those of the south. Thus, the boundary zone between the north and south Atlantic stocks may shift somehow lower latitude toward west from east.

KEYWORDS

Atlantic swordfish, nuclear gene locus, stock boundary

INTRODUCTION

Although the swordfish (*Xiphias gladius*) is considered to be a highly migratory cosmopolitan fish species, conventional restriction and nucleotide sequence analyses of the mitochondrial DNA (mtDNA) have revealed the population to be structured not only between but also within ocean basins (Kotoulas et al., 1995; Rosel and Block, 1995; Alvarado-Bremer *et al*., 1996; Chow *et al*., 1997). Chow (1998) designed universal primers which amplify 4th intron of calmodulin gene (*CaM*) of wide variety of animals and found a single nucleotide polymorphism (SNP) in the swordfish. This SNP marker in the swordfish is detected by a restriction analysis, where two alleles (designated *A* and *B*) are determined. Using the SNP assay, Chow and Takeyama (2000) found highly significant difference in the allele frequencies between the north (20N-43N) and south (8N-33S) Atlantic samples. Subsequent effort on sample collection followed by the genetic analysis confirmed that the difference has been maintained during relatively long time (1992 to 2002) (Chow *et al*., 2001; Nohara *et al*., 2003). Although the genetic data from these studies indicate that the boundary between the north and south stocks may locate around between 10ºN and 20ºN, very few Japanese fishing vessels has operated in the latitudinal range making sample collection very difficult.

In 2002, Fishery Agency of Japan sent RV Shoyo-Maru to the Atlantic under the mission of biological survey for tuna and billfish (Okamoto *et al*., 2003). Furthermore, Federation of Japan Tuna Fisheries Co-operative Associations (The Japan Tuna) has managed to collect swordfish samples from vessels operating in the target area. In this report, we introduce the preliminary results of our genetic analysis on these swordfish samples.

MATERIALS AND METHODS

Nine commercial vessels have volunteered to collect a small piece of muscle tissue of swordfish, and the tissues were frozen and sent to the laboratory through the Japan Tuna. These samples were caught from February to March partially in April of 2002. Muscle tissues

collected in R/V Shoyo-Maru cruise during August to October 2002, were preserved in ethanol and brought back to the laboratory. All samples were accompanied with exact catch locality. Procedures for DNA extraction, and PCR amplification and genotyping for *CaM* gene locus are described elsewhere (Manniatis *et al*., 1982; Chow, 1998; Chow and Takeyama, 2000).

RESULTS AND DISCUSSION

CaM fragment was successfully amplified and genotyped in 109 out of 112 individuals sent to the laboratory. Genotype data were sorted according to the catch locality regardless of the month, body length and sex. Pie graph representations of the genotype frequency of each local sample are shown in Fig. 1. The results were concordant with the previous findings by Chow and Takeyama (2000), Chow *et al*. (2001), Chow and Nohara (2002) and Nohara *et al*. (2003), in which the *B* allele was observed at much higher frequency in the north samples than in the south. Chow and Takeyama (2000) concluded that there are at least two genetically distinct and isolated swordfish populations in the Atlantic and suggested that the boundary may locate between 10N and 20N. In this study, two local samples were obtained between 10N to 20N; one (n=18) by R/V Shoyo-Maru at 14N and 48W and the other (n=24) by commercial vessels operated in the range of 10N to 20N and 28W to 37W. The former sample represented intermediate allele frequencies (*A*=0.667, *B*=0.333) between the north and south samples, while the later showed more affinity (*A*=0.896, *B*=0.104) with those of the south. One sample (collected in the range of 7N to 15N and 24W to 35W) analyzed by Chow and Nohara (2002) also shared similar allele frequencies (*A*=0.911, *B*=0.089) with those of the south. It seems, therefore, that the boundary zone may shift somehow lower latitude toward west from east.

Because of the simple variation and substantial difference in the allele and genotype frequencies between the stocks, the *CaM* marker appears to be useful not only for delineating stock structure of the Atlantic swordfish but also for locating boundary zone between the north and south stocks. In the present study, however, the sample size was not large enough for spatiotemporal slicing. To further locate the boundary between the stocks, intensive Atlantic-wide sampling of swordfish is necessary in the range of 10N to 20N.

LITERATURE CITED

ALVARADO-BREMER, J. R., J. Mejuto, T. W. Greig, and B. Ely. 1996. Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. J. Mar. Biol. Ecol. 197: 295-310.

CHOW, S., H. Okamoto, Y. Uozumi, Y. Takeuchi, H. Takeyama. 1997. Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. Mar. Biol. 127: 359-367.

CHOW, S. 1998. Universal PCR primer for calmodulin gene intron in fish. Fish. Sci. 64: 999-1000.

CHOW, S., and H. Takeyama 2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish (*Xiphias gladius*). J. Fish Biol. 56:1087-1098.

CHOW, S., K. Nohara, and Y. Takeuchi. 2001. Boundary between north and south Atlantic stocks of swordfish (*Xiphias gladius*): An implication from nuclear DNA analysis. ICCAT SCRS/01/146.

CHOW, S., and K. Nohara. 2002. Further implication on boundary between north and south Atlantic stocks of the swordfish (*Xiphias gladius*). ICCAT SCRS/02/141.

KOTOULAS, G., A. Magoulas, N. Tsimenides, and E. Zouros. 1995. Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, *Xiphias gladius*. Mol. Ecol. 4: 473-481.

MANIATIS, T., E. F. Fritch, and K. J. Sambrook. 1982. Molecular cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 545 pp.

NOHARA, K., H. Okamura, M. Nakadate, K. Hiramatsu, N. Suzuki, M. Okazaki, and S. Chow. 2003. Biological investigation on two types of bill internal structure of swordfish (*Xiphias gladius*) and genetic differentiation between the North and South Atlantic stocks. Bull. Fish. Res. Agency 7: 1-13.

OKAMOTO, H., K. Satoh, Y. Uozumi, H. Matsunaga, and Z. Suzuki. 2003. Brief report of the R/V Shoyo-Maru 2002 cruise for Atlantic bluefin spawning activity survey in the central north Atlantic in support of the BYP. ICCAT Col. Vol. Sci. Pap., 55: 1271-1277.

ROSEL, P. E., and B. A. Block. 1995. Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. Mar. Biol. 125: 11-22.

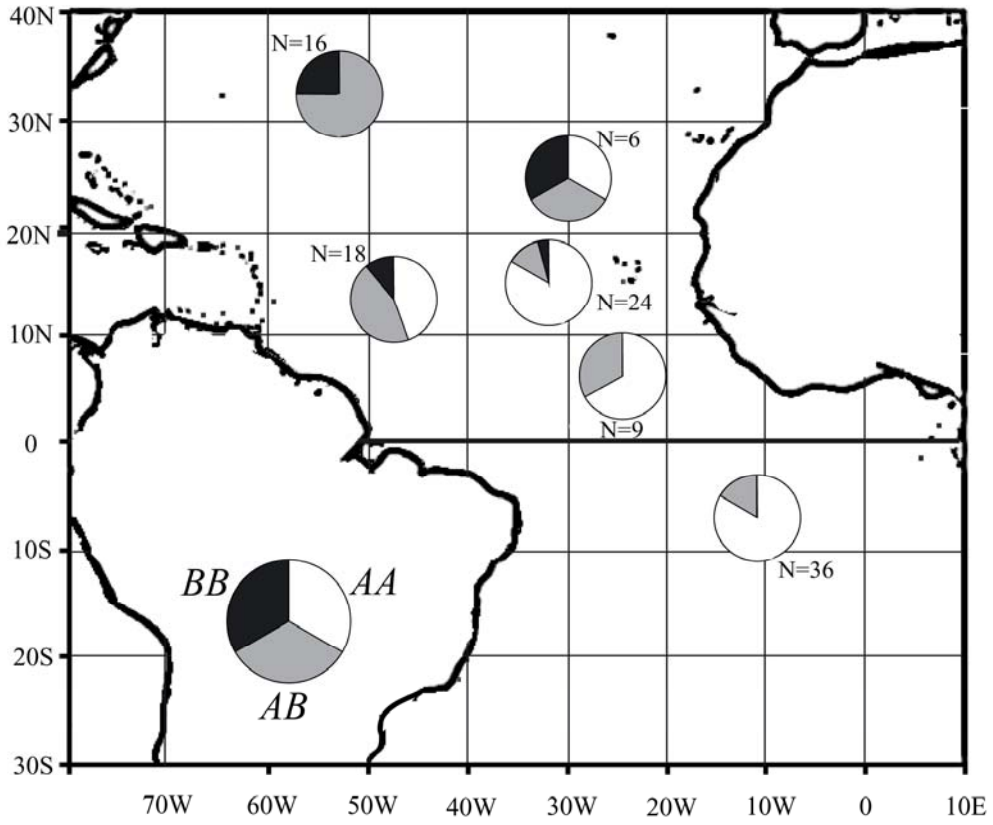

Figure 1. Pie graph representation of genotype frequencies at *CaM* gene locus of the swordfish samples collected in 2002.