

Boundary between the north and south Atlantic populations of the swordfish (*Xiphias gladius*) inferred by a single nucleotide polymorphism at calmodulin gene intron

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Abstract Genetic differentiation of the Atlantic swordfish (*Xiphias gladius*) was investigated by a single nucleotide polymorphism (SNP) at the calmodulin gene (*CAM*) intron locus. Clearly distinct allele and genotype frequencies were observed between the north (20–41°N) and mid-south (10°N–33°S) Atlantic samples. Much lower frequency of *A* allele (37.5–57.1%) was observed in the north samples ($n = 160$ in total) than in the mid-south samples (83.3–92.6%; $n=354$), and homozygote *BB* was common in the north samples (23.4–31.3%) but very rare or absent (0–3.9%) in the mid-south samples. Very strong population subdivision was observed between the two groups ($F_{ST} = 0.34$, $P < 0.001$), while the allele and genotype frequencies within each ocean basin persisted over time (1990–2002 in the north, and 1994–2002 in the mid-south). Of two samples from the presumed boundary zone, one ($n = 18$) (14°N, 48°W) presented intermediate frequencies of the *A* allele (66.7%) and *BB* homozygote (11.1%), while the other ($n = 23$) (10–17°N, 28–37°W) shared similar frequencies of the *A* allele (89.1%) and *BB* homozygote

(4.3%) with those of the mid-south Atlantic samples. These results indicate that the gene flow and individual migration between the north and mid-south Atlantic populations are considerably restricted and that the current management boundary between the north and south Atlantic swordfish stocks of 5°N should be reconsidered.

Introduction

The swordfish (*Xiphias gladius*) is the sole member of the family Xiphiidae, a large epipelagic scombroid fish attaining to over 4 m and 500 kg, and observed in open waters of tropical and temperate waters of all oceans (Nakamura 1985). Although the swordfish is thought to be highly migratory, recent molecular genetic analyses based on the sequence variation in mitochondrial (mt) and nuclear (n) DNA have indicated that the global population of swordfish (*Xiphias gladius*) may be subdivided into at least four populations (Mediterranean Sea, North Atlantic, South Atlantic and Indo-Pacific) (Kotoulas et al. 1995; Alvarado Bremer et al. 1996, 2005; Rossel and Block 1995; Chow et al. 1997; Chow and Takeyama 2000; Nohara et al. 2003). Genetic structuring of the swordfish population within the Pacific Ocean is still controversial (Grijalva-Chon et al. 1994, 1996; Chow et al. 1997; Chow and Takeyama 2000; Reeb et al. 2004), while the swordfish population in the Atlantic is distinctly structured. Although mtDNA analysis revealed large genetic heterogeneity between the Mediterranean and Atlantic populations (Kotoulas et al. 1995; Chow et al. 1997; Chow and Takeyama 2000), it could detect little genetic difference between the north and south Atlantic swordfish samples (Alvarado Bremer et al. 1996; Chow et al. 1997; Chow and Takeyama 2000). On the other hand, much more pronounced differences between the north and south Atlantic swordfish

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samples were revealed by a single nucleotide polymorphism (SNP) observed at an intron locus of the calmodulin gene (*CAM*) (Chow and Takeyama 2000). Only two alleles (*A* and *B*) were observed at this locus, but the difference in the allele and genotype frequencies between samples from northwest (20–41°N) and mid-south (8°N–33°S) Atlantic was substantial enough to allow us to presume very little gene flow and individual migration between these ocean basins.

Understanding stock structure is very important to manage fishery resources, and the International Commission for the Conservation of Atlantic Tunas (ICCAT) has managed the north and south Atlantic swordfish stocks on the basis of a separation at 5°N (see Miyake and Ray 1989). Although the results obtained from the *CAM* locus suggested that the actual boundary may be located further to the north than this ICCAT working hypothesis (Chow and Takeyama 2000), no sample from a key area (10–20°N) was available to further investigate the boundary and its dynamics.

We have collected swordfish samples from this key area (10–20°N) to explore the boundary zone, as well as supplementary samples from the north and south Atlantic in 1998–2002, to investigate temporal variation within each ocean basin. In this study, we provide molecular evidence that the genetic difference persists over time and suggest the location of a boundary as well as potential mechanisms of isolation between the populations.

Materials and methods

Of 16 swordfish samples used in this study, seven were derived from a previous study (Chow and Takeyama 2000)

(Table 1). Nine samples newly collected and analyzed in this study comprised of three from the north Atlantic (25–43°N, 27–71°W) in 1997, 2000 and 2002, two from mid Atlantic (10–17°N, 28–48°W) in 2002, and four from mid-south Atlantic (11°S–10°N, 1°E–33°W) in 1998, 1999 and 2002. According to the presumption by Chow and Takeyama (2000), the samples were categorized into three geographic areas; north Atlantic (NA) (north of 20°N), mid to south Atlantic (MSA) (south of 10°N), and presumed boundary zone (PBZ) (in the range of 10–20°N). One sample (PBZ02E) from PBZ was collected and donated by several Japanese commercial vessels upon request of the Japan Tuna Federation, and frozen muscle tissues were shipped to the laboratory. All other samples were collected by observers on commercial longline vessels or by researchers on R. V. Shoyo Maru, Fisheries Agency of Japan, so that the catch locality of individual fish is known with precision. Crude DNA was extracted using a standard phenol-chloroform method or a DNA extraction kit (GenomicPrep Cells and Tissue DNA Isolation Kit, Amersham Bioscience). Procedures for polymerase chain reaction (PCR) amplification of the *CAM* locus, endonuclease digestion by *Bst* UI (New England Biolabs, Ipswich, MA, USA), and agarose gel electrophoresis are described in Chow (1998) and Chow and Takeyama (2000). The Hardy–Weinberg exact test and *G*-like heterogeneity test between samples on the genotype frequency were performed using the GENEPOP 1.2 software (Raymond and Rousset 1995) and spatial genetic variation in allele frequency was quantified by estimating Wright's *F*_{st} using analysis of the molecular variance (AMOVA) (Excoffier et al. 1992)

Table 1 *Xiphias gladius*. Location of collection for 16 samples of Atlantic swordfish. [*N* sample size]

Area	Sample ID	Latitude	Longitude	Year	Month	<i>N</i>
North Atlantic (NA)	NA02	25–37°N	27–37°W	2002	March 2002	7
	NWA00	31–36°N	50–58°W	2002	February–March 2000	16
	NWA98	35–43°N	45–71°W	1998	February–March 1998	64
	NWA97 ^a	37–41°N	48–67°W	1997	April–June 1997	16
	NWA93 ^a	20–30°N	57–90°W	1993	January–May 1993	29
	NWA90 ^a	38–40°N	59–72°W	1990	June–November 1990	28
Presumed boundary (PBZ)	PBZ02W	14°N	48°W	2002	September 2002	18
	PBZ02E	10–17°N	28–37°W	2002	February–April 2002	23
Mid-south Atlantic (MSA)	TNA02	4–9°N	22–26°W	2002	February–March 2002	9
	TNA99	2–10°N	21–33°W	1999	December 1998–February 1999	84
	TNA97 ^a	5–8°N	8–21°W	1997	February–March 1997	30
	TSA02	7–11°S	2°E–11°W	2002	September 2002	22
	TSA98	7–11°S	1°E–11°W	1998	February 1998	30
	TSA97 ^a	5–11°S	2°E–8°W	1997	February–March 1997	51
	Brazil96 ^a	20–30°S	35–50°W	1996	September 1996	68
Brazil94 ^a	20–33°S	28–46°W	1994	October 1994–January 1995	60	

^a From Chow and Takeyama (2000)

implemented in Arlequin (Schneider et al. 1997). The Bonferroni method (Rice 1989) was used to correct for multiple comparisons.

Results

Two alleles (designated *A* and *B*) at the *CAM* locus are unambiguously determined as previously reported. Genotype and allele frequencies of nine samples analyzed in the present study and those of seven samples obtained in previous study (Chow and Takeyama 2000) are shown in Table 2. All samples were in Hardy–Weinberg equilibrium ($P > 0.2$) except for one (NA02) ($P = 0.037$) probably due to the small sample size ($n = 7$). Allele frequency distribution was considerably heterogeneous among all samples (overall $F_{st} = 0.18$, $P < 0.001$). The frequency of the *A* allele in six samples ($n = 7$ to 64, 160 in total) of the north Atlantic (NA)(20–43°N, 27–90°W) ranged from 37.5 to 57.1%, while much higher frequencies of the *A* allele (84–91.7%) were observed in eight samples ($n=9–84$, 354 in total) in the mid-south Atlantic (MSA)(10°N–33°S, 2°E–50°W). No significant difference was observed among NA samples ($F_{st} = -0.009$, $P = 0.727$) and among MSA samples ($F_{st} = 0.0002$, $P = 0.388$). The six NA samples and eight MSA samples were pooled, respectively, and pairwise F_{st} values between samples were estimated (Table 3). Significant heterogeneity was observed between the pooled NA and MSA samples ($F_{st} = 0.34$, $P < 0.001$), between NA and PBZ02E ($F_{st} = 0.24$, $P < 0.001$), and between MSA and PBZ02W ($F_{st} = 0.18$, $P < 0.001$). Similar results were observed in the genotype frequency, where highly significant heterogeneity was observed between NA and MSA ($P < 0.001$), between NA and PBZ02E ($P < 0.001$), and between MSA and PBZ02W ($P < 0.001$). Although 25% of individuals in NA samples were *BB* homozygote, this genotype was observed to be very rare (1.5%) in MSA samples (Fig. 1). The sample sizes for PBZ02W ($n = 18$) and PBZ02E ($n = 26$) was relatively small. However, frequencies of the *A* allele (66.7%) and *BB* homozygote (11%) observed in the former sample appear to be intermediate between NA and MSA samples, whereas frequencies of the *A* allele (92.3%) and *BB* homozygote (0%) of the latter were similar to those of MSA samples. These results indicate that not only the gene flow but also individual migration between the north and mid-south Atlantic populations is considerably restricted, and the genetic difference between these two populations has been maintained over a long period of time. These results also suggest that the boundary between these populations may be located in the range of 10–20°N but may shift spatiotemporally, and that individuals from the north and south Atlantic populations may intermingle in the boundary zone.

Table 2 *Xiphias gladius*. Genotype and allele frequencies at the *CAM* locus (N sample size)

Area	Sample ID	Genotype			N	Allele	
		AA	AB	BB		A	B
NA	NA02	3	2	2	7	0.571	0.429
	NWA00	0	12	4	16	0.375	0.625
	NWA98	18	31	15	64	0.523	0.477
	NWA97 ^a	5	6	5	16	0.500	0.500
	NWA93 ^a	8	14	7	29	0.517	0.483
PBZ	NWA90 ^a	5	16	7	28	0.464	0.536
	PBZ02W	8	8	2	18	0.667	0.333
MSA	PBZ02E	19	3	1	23	0.891	0.109
	TNA02	6	3	0	9	0.833	0.167
	TNA99	68	15	1	84	0.899	0.101
	TNA97 ^a	25	5	0	30	0.917	0.083
	TSA02	17	5	0	22	0.886	0.114
	TSA98	24	6	0	30	0.900	0.100
	TSA97 ^a	38	11	2	51	0.853	0.147
	Brazil96 ^a	59	8	1	68	0.926	0.074
Brazil94 ^a	42	17	1	60	0.842	0.158	

^a Data derived from Chow and Takeyama (2000)

Table 3 *Xiphias gladius*. Pairwise F_{st} (below diagonal) and P values (above diagonal) between samples. Six north (NA) and eight mid-south (MSA) samples were pooled, respectively

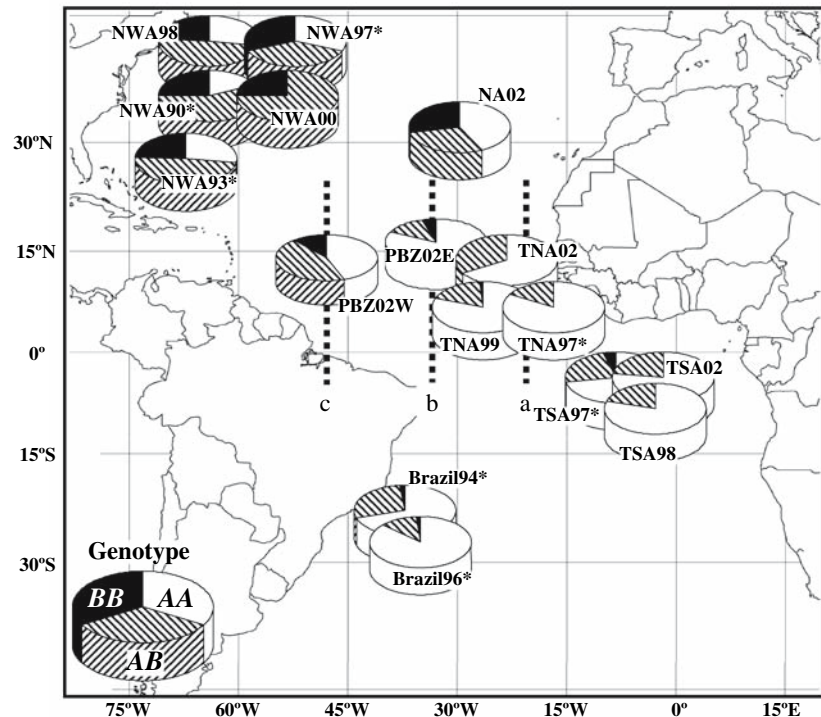
	NA	PBZ02W	PBZ02E	MSA
NA	–	0.109	<0.001*	<0.001*
PBZ02W	0.04	–	0.01	<0.001*
PBZ02E	0.24	0.12	–	0.99
MSA	0.34	0.18	–0.01	–

* Significant after Bonferroni correction

Discussion

The present study confirmed and corroborated the results of previous investigation for the Atlantic swordfish stocks (Chow and Takeyama 2000). The presence of a strong genetic break between swordfish populations in the Atlantic Ocean would be highly unexpected, as it would be located in the open sea, and there is no evidence for disjunction in swordfish distribution throughout the Atlantic. In cases where substantial genetic structuring in populations of large marine pelagic fishes has been documented, for example in several tuna species (Chow and Ushiyama 1995; Alvarado Bremer et al. 1998; Chow et al. 2000; Takeyama et al. 2001; Viñas et al. 2004; Durand et al. 2005; Nakadate et al. 2005), all of these findings pertain to genetic differentiation between samples from different ocean basins, i.e. between Atlantic and Indo-Pacific or between Mediterranean and

Fig. 1 *Xiphias gladius*. CAM genotype frequency of the 16 Atlantic swordfish samples showing number of individuals analyzed and year sampled for each sample. Data for seven samples (with asterisk) were derived from Chow and Takeyama (2000). Three dotted lines (a, b and c) indicate positions of longitudinal sections, where horizontal oceanographic profiles of temperature, salinity and dissolved oxygen were constructed (see Fig. 2)



Atlantic. To date, no genetic discontinuity between the north and south Atlantic populations has been observed in other large pelagic fish such as tunas and marlins (Chow et al. 2000; Graves and McDowell 2003; Ely et al. 2005).

Several aspects of swordfish biology would seem to act against the formation and maintenance of genetically discrete populations. Swordfish are widely distributed in all oceans including the Mediterranean Sea and are thought to be highly migratory (Nakamura 1985). Swordfish also possess a specialized heating system to warm the eyes and brain (Carey 1982; Fritsches et al. 2005), enabling this species to tolerate rapid temperature changes and dive into deep, cold water (Carey and Robison 1981; Takahashi et al. 2003) probably to search for prey. This characteristic would suggest that swordfish are less likely to be restricted in their habitat by thermoclines.

Despite these expectations, mtDNA analysis has revealed that the Mediterranean swordfish population is genetically distinct from the Atlantic population (Kotoulas et al. 1995; Chow et al. 1997; Alvarado Bremer et al. 2005). It is believed that the Strait of Gibraltar and the endemic spawning ground in the Mediterranean Sea may be functioning to ensure a distributional disjunction between the Mediterranean and Atlantic populations and to maintain the genetic isolation. It appears this may be also the case for albacore (Viñas et al. 2004; Nakadate et al. 2005).

Since there is no conspicuous geographic structuring in the mid Atlantic, oceanographic and biological factors which may correspond to the observed genetic population

structuring in the swordfish were investigated. Temperature data collected by expendable bathythermographs (XBT) in 1980–1988 showed that vertical sections of temperature between 15°N and 6°S were different from other areas, where the vertical temperature gradient was considerably reduced and thermocline was very shallow (Reverdin et al. 1991). Furthermore, Hastenrath and Merle (1987) found that the shallow thermocline observed between 15°N and 10°S in the mid Atlantic tends to be deeper in the western basin as compared to the eastern basin. Using data from the World Ocean Atlas (Boyer et al. 2002; Locarnini et al. 2002; Stephens et al. 2002), we constructed vertical longitudinal sections of temperature, salinity and dissolved oxygen from 40°N to 20°S (Fig. 2, top, middle and bottom, respectively). These sections (Fig. 2a–c) are slices at three longitudes (20°30'W, 32°30'W and 48°30'W) (see dotted lines in Fig. 1 and small map in Fig. 2 as well). Data in June, March and September from 1977 to 1998 were collected and monthly averages were used to construct the vertical profiles. Sections at 32°30'W (Fig. 2b) and 48°30'W (Fig. 2c) correspond to the locations and seasons of our two presumed boundary area swordfish samples (PBZ02E and PBZ02W), and section at 20°30'W in June was adopted as an easternmost reference and as a mid-season sample between above two sections. As previously reported, a shallow thermocline and a reduced temperature gradient are observed between 20°N and 10°S (Fig. 2, top). In addition, sections for salinity and dissolved oxygen show similar profiles to those of temperature, with lower salinity and

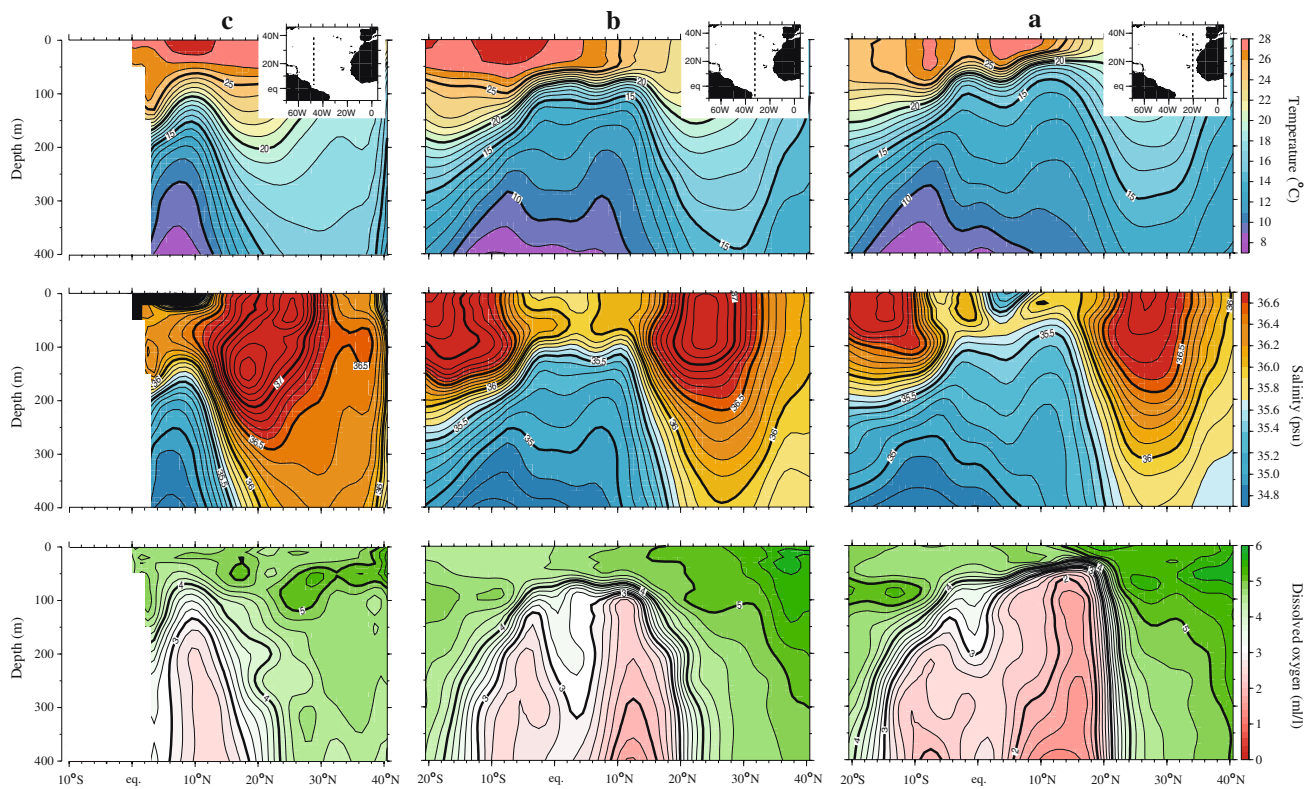


Fig. 2 *Xiphias gladius*. Longitudinal sections at 20°30'W (a), 32°30'W (b) and 48°30'W (c) in the Atlantic, showing vertical profiles of temperature (top), salinity (middle) and dissolved oxygen (bottom).

These images were drawn using monthly means of these values obtained in June, March and September, respectively (Boyer et al. 2002, Locarnini et al. 2002, Stephens et al. 2002)

dissolved oxygen water extending higher in the water column in the 20°N–10°S area relative to other areas (Fig. 2, middle and bottom, respectively). Steep gradients in temperature, salinity and dissolved oxygen are observed between 15–20°N in the eastern Atlantic (i.e. 20°30'W section, Fig. 2a) and are shifted to the south (10–15°N) in the western Atlantic (i.e. 48°30'W section, Fig. 2c). The same profiles were obtained using data for other months. The swordfish as a deep diver may be more sensitive to the degree of oceanographic variability in the water column in comparison to other shallow water fishes. Although these oceanographic conditions may act as slight impediments to swordfish migration, swordfish are caught throughout the presumed boundary zone. Therefore these conditions may not be the exclusive or even primary factor for the strong genetic break and other factors may also play a role. Tropical coastal ichthyofauna in the western Atlantic are known to be separated into two major clusters, the Caribbean and Brazilian Provinces (Floeter and Gasparini 2000), and massive discharge of freshwater from Amazon River may act as the barrier between these provinces. While we would not expect Amazon discharge to affect a wide-ranging pelagic fish, it will be important to consider other similar but more subtle factors which could drive the observed pattern.

Clear evidence of swordfish spawning grounds in the Atlantic is still scarce, but it appears there may be two spatially differentiated spawning grounds: one off the southeast coast of United States extending to the Gulf–Caribbean, and another within an equatorial band west of 10°W between 5°N and 5°S to off Brazil–Uruguay (Alvarado Bremer et al. 2005). However, even if the spawning grounds of the two populations are well separated, there must also be restriction of larval transportation. Information from lobster research may provide some useful insights here. The Western Atlantic spiny lobster, *Panulirus argus*, is distributed from the east coast of United States at North Carolina to Rio de Janeiro, Brazil, including the entire Gulf of Mexico and Caribbean Sea (Holthuis 1991). Spiny lobster larvae have a very long planktonic period extending from several months to more than one year (Matsuda and Yamakawa 2000), the larvae may be transferred from coastal areas to the open sea (Booth and Phillips 1994). However, molecular analysis revealed that the north and south Atlantic populations belong to completely different gene pools (Sarver et al. 1998). Therefore, there must be a potential barrier to prevent larval transportation between the northern and southern hemispheres. Since the swordfish larvae and juveniles are passively transported for a shorter period of time

(Sun et al. 2002; Govoni et al. 2003), such a barrier would have to act more efficiently on swordfish than on spiny lobster.

Conventional tag-recapture (Garcia-Cortes et al. 2003) and satellite telemetry tracking (Sedberry and Loefer 2001) have reported no trans-equatorial movement of the swordfish, and our results support the suggestion by these studies that the swordfish are much less migratory than previously believed. Although further investigation will be necessary from both the biological and oceanographic perspectives, we note that the presence of well differentiated spawning grounds, little larval transportation between populations as well as limited migration constitute *sine qua non* conditions to maintain the strong genetic break between the north and south Atlantic swordfish populations.

Inappropriate definition of management units must be avoided to prevent biases in population estimates and erroneous conclusions regarding population depletion or recovery. The present investigation indicates the current ICCAT management boundary between the north and south Atlantic swordfish stocks of 5°N should be reconsidered. This boundary issue is critical to the ongoing international rebuilding program for the north Atlantic stock, particularly given the setting of separate quotas for northern and southern stocks and existing concerns about the effects of these quotas on discard rates and fleet behavior in the two areas (NOAA 2003).

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