

UNIVERSITÀ DEGLI STUDI DELLA TUSCIA DI VITERBO
DIPARTIMENTO DI ECOLOGIA E SVILUPPO ECONOMICO SOSTENIBILE

Dottorato di ricerca in “Ecologia e Gestione delle Risorse Biologiche”
ciclo XXII

**THE USEFULNESS OF A MULTIDISCIPLINARY APPROACH TO
INVESTIGATE THE DIFFERENTIATION OF ATLANTIC
SWORDFISH (*Xiphias gladius* L. 1758)**

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Viterbo

2011

“When we build,
let us think that we build forever.”

John Ruskin (English art critic)

To Maria.

ACKNOWLEDGEMENTS

The present work was undertaken at the Institute for Fisheries and Sea Research (IPIMAR, Portugal), in cooperation with “Tuscia”, University of Viterbo (Italy) and the Department of Public Health and Infectious Diseases from “La Sapienza”, University of Rome (Italy). During the study period the present candidate hold a PhD grant from the Portuguese Foundation for Science and Technology (FCT: SFRH/BD/25391/2005). In addition, this study benefited from the Portuguese fisheries data collection programme in course at IPIMAR, which was developed within the scope of the EU Data Collection Framework.

The development of a study like this obviously depends on the involvement and contribution of several people and institutions, to whom I would like to express my sincere acknowledgements:

- To Dr. Carlos Costa Monteiro (President of the IPIMAR, Portugal) for accepting and providing all the necessary conditions for the development of this study at IPIMAR;
- To Dr. Giuseppe Nascetti (Pro-rector of “Tuscia” University of Viterbo, Italy) for accepting and providing all the conditions necessary for the development of this study at the laboratory of Tarquinia;
- To Dr. Miguel Neves dos Santos (IPIMAR, Portugal) for believing in this PhD project since the beginning and particularly for the permanent interest and support. Above all for his friendship and for trusting me;
- To Dra. Simonetta Mattiucci (“La Sapienza” University of Rome, Italy) for kindly providing this opportunity and for accepting the supervision of this project, especially for the constant support and advice on parasitology. I’m sorry for the stressing days;
- To Dr. George Tserpes (Hellenic Center for Marine Research, Crete, Greece) for gently supporting and advising on ageing study;
- To Dra. Roberta Cimmaruta (“Tuscia” University of Viterbo, Italy) for accepting the supervision of this project, especially for the support and advice on genetics;
- To AMAP and Pescarade, in particular to Mr. Humberto Jorge and Mr. António Teixeira for their commitment in facilitating the access to pelagic long-liners. Special gratitude is owned to the crew of F/V “Príncipe das

- Marés”, “Alma Lusa”, “Paula Filipa” and “Mar Português” for helping on sample collection. Thanks a lot for the good times spent onboard;
- To João Regala, Sérgio Amorim, Marco Cerqueira, Paulo Freitas, Rolando Machado and Fernando Fontaínhas for their assistance on samples collection. Special recognition is also due to Sérgio Amorim for his help on spine cuts;
 - To Dra. Silvia Cecchetti (“Tuscia” University of Viterbo, Italy) for her assistance in the laboratory of Tarquinia and for maintaining a joyful working environment;
 - To the Italian colleagues, particularly to Simone Damiano, António Faranda, Valerio Sbragaglia and Michela Paoletti for their contribution in laboratory sampling procedures;
 - To Dr. Miguel Gaspar, Dra. Manuela Falcão and Dra. Panagiota Peristeraki for their friendly support and constructive suggestions;
 - To Tibério Simões, José Luis Sofia, Fernando Martins, Maria de Lurdes Santos, Luz Abreu and Lina Oliveira for the permanent encouragement and the pleasant working environment;
 - To many friends and colleagues that work or worked in IPIMAR and contributed in some way to keep looking forward, such as Alexandre Pereira, Ana Moura, Dalila Serpa, Fábio Pereira, Francisco Leitão, Hugo Saldanha, Israel Ribeiro, João Regala, Jorge Ramos, Paula Moura, Paulo Vasconcelos, Pedro Lino, Sérgio Abreu and Sérgio Amorim. Special gratitude is also owned to Pedro Lino for the technical support on computing, together with Francisco Leitão for the words of enthusiasm during those difficult moments;
 - To many good friends for believing and listening me whenever asked, particularly Cinara Alves, Cláudia Zambujeiro, Heloísa Colaço, Marta Braga and Teresa Guerreiro;
 - To my parents and brothers, without their constant encouragement, dedication and support it would never had been made;
 - To Nuno, for his endless patience and support and to Maria for their infinite and contagious energy, which altogether are the main reasons to keep carrying on.

Thank you all!

ABSTRACT

The swordfish (*Xiphias gladius* L. 1758) is a highly migratory species that are harvested over its entire distribution range. For management purposes, the International Commission for the Conservation of Atlantic Tunas (ICCAT) established two distinct stocks within the Atlantic, the north and south, separated at 5°N. The existing stock boundaries were established at a time when biological evidence for stock structure was conflicting and inconclusive and were not intended to represent discrete populations. Thus, the actual boundary was arbitrarily placed on 5°N for the practical purpose of alignment with ICCAT's existing statistical reporting areas. Taking in consideration the uncertainties concerning the Atlantic swordfish stock limits as well as the implications that such doubts have for management purposes, the present work aimed to evaluate the accuracy of the location of the current boundary between the north and south Atlantic stocks and assess the degree of subdivision and discreteness between them. In order to achieve such goals, a multidisciplinary (holistic) approach was carried out which incorporated a set of different aspects of the swordfish biology (parasitology; genetic mtDNA, morphometric and age and growth). The study of parasites allowed to gather new information on the relationship between infection levels and host size and sex and indicated which parasite taxa could be use as "biological tags" for stock discrimination. The mtDNA sequencing showed the levels of differentiation and variability within and between areas, while the morphometric analysis of the fin spine allowed inferring about the occurrence of phenotypic divergence between areas. The age and growth study allowed estimating the periodicity of band formation as well as modelling growth for Atlantic swordfish. Finally, the overall information gathered by the different techniques used revealed that migratory movements between the northern and southern areas are occurring and that mixing is happening especially in the equatorial area. Although no precise boundaries could be established for Atlantic swordfish stocks, the current data does not support the need to change the current fishery management boundary, placed at 5°N.

Key-words: *Xiphias gladius*, Atlantic, parasites, mtDNA D-loop, morphometric, age and growth.

RIASSUNTO

Il pesce spada (*Xiphias gladius* L. 1758) è una specie altamente migratoria che viene pescata in tutto il suo areale di distribuzione. Ai fini della sua gestione, la Commissione Internazionale per la Conservazione dei Tonnidi dell'Atlantico (ICCAT), ha stabilito l'esistenza di due stocks distinti di pesce spada all'interno l'Atlantico, il Nord e il Sud, separati alla 5° N. I confini di questi stocks tuttavia sono state definiti senza aver definito vari aspetti biologici della specie ittica. Pertanto, il limite reale è stata arbitrariamente posto al 5° N, quale allineamento con le aree esistenti ICCAT di segnalazione statistica. Prendendo in considerazione le incertezze sull'esistenza di due stocks distinti di pesce spada in Atlantico, il presente lavoro mira a valutare l'accuratezza della posizione del confine attuale tra il Nord e il Sud Atlantico. Al fine di raggiungere tale obiettivo, è stato effettuato un approccio multidisciplinare (olistico) che include lo studio di differenti aspetti della biologia del pesce spada (parassiti, struttura genetica a livello del DNA mitocondriale, dati morfometrici ed parametri di crescita legata all'età della specie ittica). Lo studio dei parassiti ha permesso di raccogliere nuove informazioni sul relazione tra i livelli di infezione e taglia del pesce e con il sesso; inoltre ha dato indicazioni sul possibile utilizzo di alcuni taxa come "biological tags" per la discriminazione degli stocks. La diversità genetica delle popolazioni di pesce spada è stata studiata anche mediante analisi genetica del gene mitocondriale del D-loop. A livello morfologico, i pesce spada catturati nelle acque tropicali centro-equatoriali e nell'Atlantico meridionale sono stati valutati utilizzando caratteri morfometrici del secondo raggio della pinna anale. L'età e la crescita del pesce spada ha permesso stimare la periodicità della formazione di band così come la modellazione di crescita. Infine, le informazioni raccolte in generale mediante le diverse metodologie utilizzate sembrano che indicare che ci siano movimenti migratori tra le zone nord e sud e che la miscela di popolazioni avvenga soprattutto nella zona equatoriale. L'insieme dei dati sembra supportare l'esistenza di due stocks in Atlantico, e poiché il limite dell'esistenza degli stocks sembra non avere confini precisi, i dati attuali non indicano la necessità di cambiare l'attuale limite di gestione della pesca, posto al 5 ° N.

Parole chiave: *Xiphias gladius*, Atlantico, parassiti, mtDNA D-loop, dati morfometrici, età e crescita.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
ABSTRACT	iii
RIASSUNTO	iv
TABLE OF CONTENTS	v
GENERAL INTRODUCTION AND OBJECTIVES	1
PART I: THE SWORDFISH AND THE FISHERIES	7
CHAPTER 1. The swordfish: biology and ecology	8
CHAPTER 2. The swordfish fisheries	17
PART II: MULTIDISCIPLINARY APPROACH FOR ATLANTIC SWORDFISH STOCKS DIFFERENTIATION	27
CHAPTER 1. The metazoan parasites of swordfish from Atlantic tropical-equatorial waters	28
CHAPTER 2. Metazoan parasites of swordfish, <i>Xiphias gladius</i> (Pisces: Xiphiidae) from Atlantic Ocean: implications for host stock identification	48
CHAPTER 3. Population structure of Atlantic swordfish (<i>Xiphias gladius</i> L. 1758) using mitochondrial DNA analysis: implications for fisheries management	67
CHAPTER 4. Fin ray morphometrics for swordfish (<i>Xiphias</i> <i>gladius</i> L. 1758) from the Atlantic Ocean	83
CHAPTER 5. Age and growth of swordfish (<i>Xiphias gladius</i> L. 1758) from the eastern Atlantic waters using anal fin spines	97
PART III: GENERAL DISCUSSION AND CONCLUSIONS	118
REFERENCES CITED IN THE THESIS	125

GENERAL INTRODUCTION AND OBJECTIVES

The highly migratory species that include billfishes, tunas and others are physiologically and morphologically well adapted to their habitat showing thus a wide distribution range (Nakamura, 1985; Turner, 1999; Ward *et al.*, 2000). The internal and external morphological characters used in the classification of these species are, in general, functional and reflect the degree of adaptation to the environment in which they live. The capability for fast swim is mainly related to the efficiency of the caudal fin as it is characterized by the insertion of the rays directly on the last vertebrae of the backbone, it is rigid and crescent-shaped (Azevedo, 1990).

Because these fishes migrate widely and have high commercial value, they are harvested over its entire range, representing an important source of revenue to many coastal nations around the world (Turner, 1999; Ward *et al.*, 2000). The exploitation of highly migratory pelagic fishes has long provided important fisheries, particularly swordfish and tunas. The early fishing methods started thousands of years ago as near-shore subsistence activities in subtropical areas (Ward *et al.*, 2000). These fishing methods, targeting highly migratory species, mostly involved the harpooning, longlining, trolling and driftnetting (Nakamura, 1985; Ward *et al.*, 2000). Actually the most important fishing methods is the surface longline which was gradually developed by Japanese, Chinese, Taiwan and Korean fleets originally operating in the northwestern Pacific and then extending to other Pacific areas, as well as to the Indian and Atlantic Oceans (Nakamura, 1985).

The global interest for the exploitation of highly migratory species resulted in the need to create an organisation that could compile all the information coming from the different fleets, deepening the knowledge of each fishery. Thus, in 1969 it was created the International Commission for the Conservation of Atlantic Tuna (ICCAT), the Office of the United Nations responsible for the management and conservation of tuna fisheries and related species in the Atlantic Ocean and adjacent seas. Faced with a dramatic increase in catches, mostly recorded between 1980 and 1990, and the resulting impact on the resources conservation status, ICCAT recognised the need to change its original purpose, assuming the management of the fisheries. Currently, ICCAT has responsibility over 30 species, including the swordfish. To assess the global status of each species, ICCAT members have the responsibility to develop multidisciplinary studies with special focus on the effects of fishing on the target and by-catch species.

For swordfish management purposes ICCAT established two distinct stocks within the Atlantic, the north and south, separated at 5°N (Anonymous, 2007). The existing stock boundaries were established at a time when biological evidence for stock structure was conflicting and inconclusive and were not intended to represent discrete populations (Anonymous, 1987). In fact, the current stock boundary was adopted due to the lack of available evidences on mark-recapture studies, catch distribution and larval distribution that support other division (Nielsen *et al.*, 2007). Although the choice was not clear, it was reasonable to consider one north Atlantic stock for management purpose, particularly due to the inability to decompose the large catches from north Atlantic in different small areas (e.g. east and west, as acknowledged by ICCAT scientists). Moreover, it was decided that a single south Atlantic stock should be consider, mainly due to the lack of information supporting another scenario. The actual boundary between the north and south Atlantic stocks was arbitrarily placed on 5°N for the practical purpose of alignment with ICCAT's existing statistical reporting areas (Anonymous, 1988).

Nowadays, questions about Atlantic swordfish stock boundaries and migrations persist as themes of discussion, despite the knowledge so far acquired on different aspects of the species: biology (Carey, 1982; Taylor and Murphy, 1992; Arocha, 2002; Fritsches *et al.*, 2005; Chancollon *et al.*, 2006), morphology (García-Cortés and Mejuto, 2002; Arocha *et al.*, 2003; Govoni *et al.*, 2003) and genetic structure (Rosel and Block, 1996; Reeb and Block, 1997; Chow *et al.*, 2002; Reeb *et al.*, 2003; Alvarado Bremer *et al.*, 2005a,b). Indeed, ICCAT has always been concerned to check whether this division corresponds to different biological stocks or not, recognizing the uncertainties in the distribution limits and the lack of biological evidences supporting such stock units.

The correct delimitation of fish stock boundaries and the knowledge of their structure are extremely important for the effective management of a fishery (Begg *et al.*, 1999). The application of management measures based on incorrect boundaries can lead to serious problems of stock depletion, as each stock may have different responses to different levels of exploitation and/or rebuilding strategies (Begg and Waldman, 1999). Moreover, when the conservation of a fish species is a concern, the identification of distinct evolutionary units is also informative and can help in the prioritization of areas of high value for conservation (Begg and Waldman, 1999). Thus, to optimize the commercial yield and conduct the assessment effort in a sustainable way, each stock

must be managed separately and its biological identity and conservation status should be clarified.

For an accurate biological identification of the stocks, the scientific knowledge must be focused on several demographic parameters such as: growth rates, recruitment indices, natural and fishing mortalities and migratory behaviour, i.e. the spatial and temporal dynamics and mixing rates (MacKenzie and Abaunza, 1998; Ward *et al.*, 2000). These parameters can be gathered from a broad spectrum of methodologies and studies, such as: phylogeographic (Awise, 1998; Bermingham and Moritz, 1998; Stepien *et al.*, 2001; Faulks *et al.*, 2008; Rocha *et al.*, 2008), mark-recapture (Pradel, 1996; Villella *et al.*, 2004; Labonne and Gaudin, 2005; Rosenberger and Dunham, 2005), holistic (Coppola and Pascoe, 1998; Xiao, 1998; Prager, 2002; Mueter and Megrey, 2006) and analytical or age-structured models (Miller *et al.*, 2003; Xiao, 2006).

Fish stocks are identified on the basis of differences in characteristics between them. Investigation of any single characteristic will not necessarily reveal stock differences even when “true” stock differences exist (Type I error). To overcome this difficulty a holistic approach of fish stock identification, involving a broad spectrum of techniques, appears to be pertinent (Begg and Waldman, 1999). In fact, integration of the results reached by each method used for stock identification purposes maximizes the likelihood of correctly defining stocks. This could be more profitable if a wide range of stock identification techniques could be used on the same samples, i.e., in a single study as it could reduce discrepancies inherent to each method as well as spatial and temporal driven factors that may affect biological features (Begg and Waldman, 1999).

Population genetics background can provide quick and reliable tools in assessing the status of fish stocks, as biomass exploitation can indeed cause the loss of genotypic arrays, thus reducing genetic diversity within populations. This process, called genetic erosion, can be accomplished in few years, with a loss of genetic variation even heavier and faster than biomass decline (Gharret and Thomason, 1987; Smith *et al.*, 1990; Nielsen *et al.*, 1997; Rocco *et al.*, 2007). The investigation of genetic variation levels is therefore a quick and reliable method to monitor the status of fish stocks. In other words, if the biodiversity (at the genetic level) of an exploited fish population is monitored through the time (and it is easier and less expensive than to monitor the numerical trend of the population itself), a loss of its genetic variability could highlight an incoming over-exploitation at its first stage. On the contrary, with the tools presently used in fishery research it is impossible to determine the potential yield from a fish

stock without severely over-exploiting it (Hilborn and Walters, 1992). Moreover the genetic variation approach is based on data that are completely independent from catch data and consequently encompass the errors due to the under-reporting that is rife among fishermen, namely when a TAC is enforced, as they may exceed their nominal quotas. Another innovative, reliable and not expensive methodology to identify fish stocks and assess their status is the study of fish parasites. To be a good marker, a parasite should have significantly different levels of infection in the subject host in different parts of the study area, should have a long life-span, should be easily detected and identified and should have no marked pathological effects on the host (MacKenzie, 1983, 2002). In marine fish, the most frequently used as tags have been the larval anisakid nematodes. These include the genera *Anisakis*, *Pseudoterranova* and *Hysterothylacium* that are the most common parasites of marine fishes (MacKenzie, 1983). Moreover, in the last years it became possible to identify their larvae at the specific level using allozyme electrophoresis (Mattiucci *et al.*, 1997, 2001, 2002, 2005). Since these biological species have different genetic structure, life cycle and geographic distribution, they can be fruitfully used as “biological tags”. Indeed they have provided valuable results in identifying geographic patterns and migrations in many fish species, such as the: European hake (Mattiucci *et al.*, 2004), herring (Campbell *et al.*, 2007), redfish (Marcogliese *et al.*, 2003), etc. However, to the best of our knowledge there are no such studies on highly migratory pelagic fish species. It has been moreover suggested that in host-parasite systems, high levels of polymorphism in the host seem to be necessary to recognise and resist to parasite infection (Frank, 1994; Tellier and Brown, 2007). In general, relatively high levels of genetic variability appear to be needed for populations and species not only to successfully track spatial and temporal environmental heterogeneity, but also to efficiently resist the attacks of parasites and other pathogens (“Red Queen Hypothesis”, Van Valen, 1973, in: Castrodeza, 1979) by which the parasite, that becomes adapted to the host most common genotypes, must face the rise and selective advantage of new genotypes in the host. A further methodology to access for intraspecific variation among fish stocks is the morphometric analysis, which has been successfully used in this context during the last decades (Cadrin and Friedland, 1999; Turan, 2004). Features containing stock-specific information such as those related with growth are biologically interpretable and thus used as a complementary technique for stock discrimination. Morphometric stock identification employs the precise definition of phenotypic stock - a group of individuals within a species that maintain

common characters that are environmentally-dependent. Therefore, morphometric differences may effectively identify phenotypic groups, but inferring reproductive isolation from morphometric patterns could be misleading. Thus, patterns in morphometric variations can detect ontogenetic changes and geographic variations among fish stocks that could complement other approaches to stock identification (Cadrin and Friedland, 1999). Life history parameters (age and growth studies) could also contribute with valuable biological information routinely collected for stock assessment purposes. Differences in life history parameters are taken as evidence that fish populations are geographically and/or reproductively isolated (Ihssen *et al.*, 1981).

Taking in consideration the uncertainties concerning the Atlantic swordfish stocks limits, structure and their status, as well as the implications that such doubts have for management purposes, the present work aimed to: 1) evaluate the accuracy of the location of the current boundary between the north and south Atlantic stocks (placed on 5°N); and 2) assess the degree of subdivision and discreteness between stocks. In order to achieve such goals a multidisciplinary approach was set with following specific objectives:

- i) describe the swordfish parasite fauna composition and the infection levels in different areas; interpretation of the results achieved according with host biology; and evaluation the viability to use the parasites taxa recovered as “biological tag” for stocks discrimination;
- ii) identify the levels of genetic variability within and among areas; estimate the genetic divergence; and assess the genetic relationships among areas;
- iii) describe the morphometric structure of the 2nd spine of the anal fin, used for ageing; and verify the degree of phenotypic variation among areas;

According with the specific objectives the present thesis is structured in three parts, two of them sub-divided into chapters, namely:

- Part I: The swordfish and the fisheries
 - Chapter 1: The swordfish: biology and ecology
 - Chapter 2: The swordfish fisheries
- Part II: Multidisciplinary approach for Atlantic swordfish stocks differentiation
 - Chapter 1: The metazoan parasites of swordfish from Atlantic tropical-equatorial waters

- Chapter 2: Metazoan parasites of swordfish, *Xiphias gladius* (Pisces: Xiphiidae) from Atlantic Ocean: implications for host stock identification
- Chapter 3: Population structure of Atlantic swordfish (*Xiphias gladius* L. 1758) using mitochondrial DNA analysis: implications for fisheries management
- Chapter 4: Fin ray morphometrics for swordfish (*Xiphias gladius* L. 1758) from the Atlantic Ocean
- Chapter 5: Age and growth of swordfish (*Xiphias gladius* L. 1758) from the eastern Atlantic waters using anal fin spines
- Part III: General Discussion and conclusions

PART I

THE SWORDFISH AND THE FISHERIES

Chapter 1

The swordfish: biology and ecology

MORPHOLOGY AND TAXONOMY

The swordfish (*Xiphias gladius* L. 1758) (Figure 1), also known as broadbill, belongs to the suborder Xiphoidei of the order Perciformes (Nakamura, 1985). Among the Xiphoidei, swordfish is the only living species of the family Xiphiidae which the most obvious distinctive character is the extension of the upper jaw into a long bill or rostrum. The same feature is shared by species belonging to the family Istiophoridae which earned them the common name of billfishes (Nakamura, 1985).

The swordfish is characterised by a large, robust and cylindrical body from the head until the caudal peduncle. The body surface is blackish-brown above and whitish below without scales and teeth in both jaws. The swordfish have two dorsal fins broadly separated in adults (continuous in immature specimens). The first dorsal has a short base, is high and triangular in shape (34-49 rays) while the second is very small (4-6 rays). The swordfish has also two separated anal fins in adults (continuous in immature specimens) being the first one high and triangular (13-14 rays), while the second (3-4 rays) is very small. The insertion of the second anal fin is slightly advanced considering the position of second dorsal fin. The swordfish has no pelvic fins and pectoral fins are rigid and located on the lower body flank presenting a total of 16-18 rays. The caudal fin is large and has a half moon shape. A large caudal keel on each side of the caudal peduncle could be found perpendicularly to the caudal fin. The anus is located close to the beginning of the first anal fin. The swordfish skeleton has 15-16 pre-caudal vertebrae and 10-11 caudal vertebrae (Nakamura, 1985).

Taxonomically, the swordfish is classified by Nakamura (1985) as:

Phylum Chordata

Class Osteichthyes

Order Perciformes

Suborder Xiphoidei

Family Xiphiidae

Genus *Xiphias*

Xiphias gladius (Linnaeus 1758)

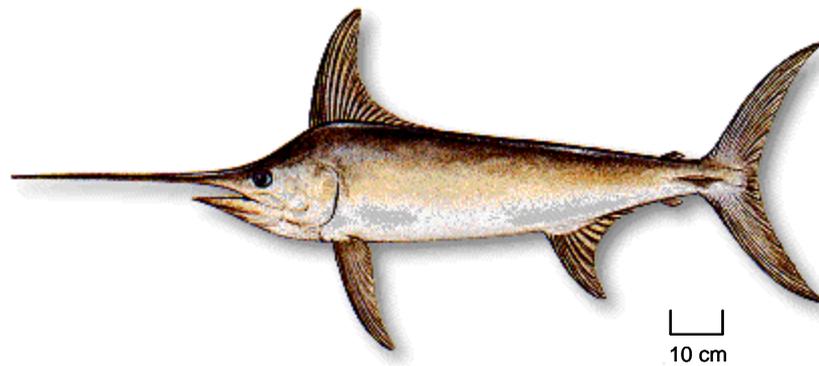


Figure 1. General aspect of an adult swordfish (*Xiphias gladius* L. 1758)

DISTRIBUTION, MOVEMENTS AND HABITAT

The swordfish is an epi- and mesopelagic oceanic species with a wide geographical distribution range (45°N - 45°S) within temperate-cold and tropical oceans and seas, including the Mediterranean Sea, Black Sea, Sea of Marmara and Sea of Azov (Carey, 1982; Nakamura, 1985; Draganik and Cholyst, 1988; Mejuto and Hoey, 1991, Ward *et al.*, 2000) (Figure 2). Their geographical distribution is roughly contained by the 13°C sea surface isotherm but tolerate a broad range of water temperatures. They have been reported from waters with temperatures ranging from 5° to 27°C (Nakamura, 1985).

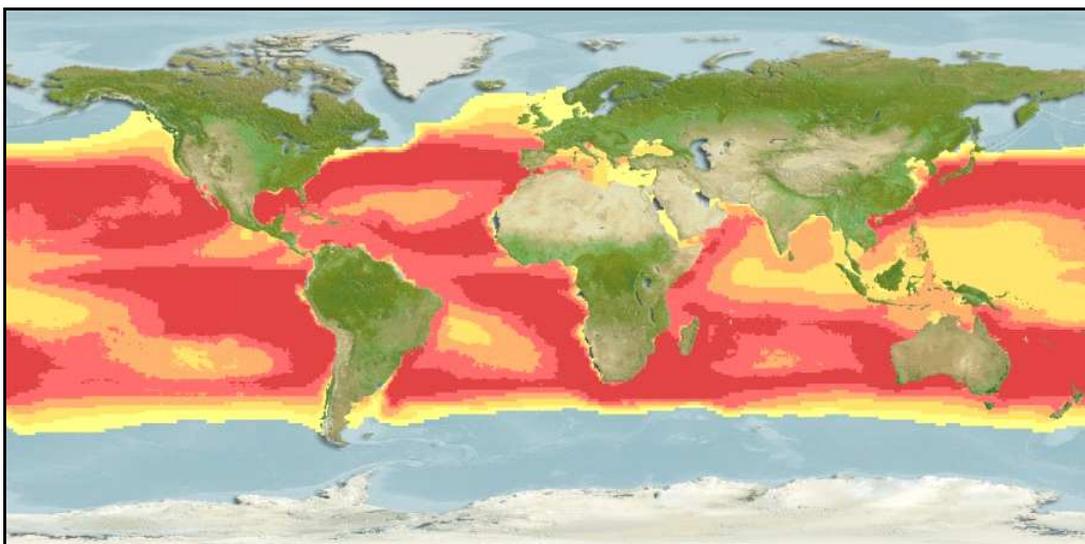


Figure 2. Geographic distribution range of swordfish. Probability of occurrence increase from yellow to red (Source: Kaschner *et al.*, 2008).

The horizontal distribution and the migratory ability of swordfish depend on size, sex, seasonal fluctuations in water temperature, currents and prey abundance. In fact, the larger specimens are conditioned by factors such as food availability and reproduction, despite their thermal preferences (Mejuto and Hoey, 1991). Their distribution shows a clear seasonality, varying from high latitudes to the north (areas of cooler waters where they feed during the summer months) and most southern latitudes (warmer waters to which they return in autumn for reproduction) (Nakamura, 1985; Mejuto and Hoey, 1991). The geographical segregation of sexes implies a mechanism that synchronises the migration of mature male and female swordfish to spawning areas.

The smaller specimens, less active and with reduce capacity to compensate large temperature variations, have their migratory movements driven by seasonal fluctuations of the isotherm, closely linked with latitude and ocean currents (Mejuto and Hoey, 1991). The highest concentrations of small swordfish are confined to the tropics that are influenced by prevailing currents between latitudes 10°S and 20°N. Larvae are more frequently encountered at temperatures above 24°C (Mejuto and Hoey, 1991; Govoni *et al.*, 2003).

The swordfish daily vertical shifts show a clear pattern that is related with dim illumination throughout the day and prey location (Carey and Robinson, 1981; Ward *et al.*, 2000). Overnight the swordfish remains in the surface layers (above 100 m) to feed and during the day shifts to greater depths. The diurnal movements follow the vertical migration of the “deep sound-scattering layer” where small shrimps, fish and squid assemblage are concentrate. The satellite tags data currently available are rated for a maximum depth of 650m (Sedberry and Loefer, 2001). Although the swordfish spend the daytime at greater depths, some individuals move to the surface which is called as “basking” behaviour (Carey and Robison, 1981). The areas were swordfish “bask” are characterised by a relatively shallow thermocline (Atlantic) or a layer where oxygen levels are low (north Pacific). The reason for basking behaviour is not well understood. Basking might then be a physiological response of swordfish to deep dives to anoxic or cold (<6°C) water below the termocline; after such a dive the swordfish must “bask” at the surface to warm vital organs or replenish oxygen. Another hypothesis is that basking increases body temperature to promote the digestion of food in the gut (Carey and Robinson, 1981).

The capacity to compensate large temperature variations presented by adult specimens was mainly due to the location of the different conductive tissues that permit

a reduction in heat loss. The swordfish brain (0.002% of body weight) is composed by a mass of tissue with large thermal capacity. The cranial endothermy involves the presence of specialised thermogenic tissue that develops from the eye muscle (Block and Finnerty, 1994). The central nervous system is thus protected from a rapid cooling of water temperature on its movements in depth (Carey, 1982). The amount of heat generated is such that in specimens marked and monitored by telemetry was possible to record skull temperature 10°-14°C higher than those observed in the surrounding water (Carey, 1982). The large capacity of physiological thermoregulation allows swordfish to keep active in a wide environmental temperature range (Carey and Robison, 1981).

REPRODUCTION

Swordfish rarely gather in schools. Fertilisation is external and it probably involves the pairing of solitary male and female swordfish within 75m from the sea surface, at temperatures around 23°C and salinity of 34-37‰ (Nakamura, 1985). Swordfish is highly fecund, with large females releasing several million eggs at a time. Swordfish do not necessarily have discrete spawning grounds and seasons. Generally, it is agreed that swordfish spawn in tropical and subtropical areas in the western parts of oceans, in localized areas relatively close to coastal waters (Taylor and Murphy, 1992; Arocha and Lee, 1995), and in spring and summer at higher latitude, when SST exceeds 24°C (Ward *et al.*, 2000). In the north Atlantic spawning occurs throughout the year in warm equatorial waters and also in the Caribbean Sea, Gulf of Mexico and off Florida with a peak in spring and summer months in these higher latitudes. In the south Atlantic Arfelli and Amorim (1985) documented spawning activities in the waters off the southeast coast of Brazil in January and February, while Neilson *et al.* (2007) reported spawning concentrations off the northeastern coast of Brazil from January to March. There was no evidence of spawning activity in the east Atlantic waters of the northern and southern hemispheres (Neilson *et al.*, 2007). In the central Pacific Ocean, it occurs in spring and summer (March to July), in western Pacific in spring (September to December) and in equatorial Pacific waters all year round. Arocha and Lee (1996) suggested that female swordfish in Eeuatorial waters of the Atlantic and Pacific Oceans spawn once every three days during a period of seven months. In the northwestern Indian Ocean spawning occurs within the second and third quarter of the year while in the south western in the

first and fourth quarter. Therefore, spawning in the Indian Ocean occurs at the same SST conditions and seasonal light regime (spring and summer) in the northern and southern hemispheres (Poisson and Fauvel, 2009). This pattern is progressively restricted to spring and summer at higher latitudes. The best known spawning grounds of swordfish are found in the Mediterranean Sea, off the southern part of Italian peninsula, Sicily and in the Strait of Messina. Adults are present all year round within the spawning grounds, except in January and February, and the peak occurs from the end of June to August.

AGE AND GROWTH

Swordfish exhibit phenomenal growth in their first year of life that could reach 90cm (c. 15kg) (Ehrhardt, 1992). Larval and juvenile swordfishes exhibit several growth phases (Figure 3). Growth is linear for small larvae and juveniles until 510 to 740mm LJFL and then became allometric for larger juveniles. Those phases are characterised by accelerated growth that slows forward from older juveniles until adults. The acceleration in growth rate follows an abrupt change in diet, and in jaw and alimentary canal structure (Govoni *et al.*, 2003).

The average maximum size for an adult swordfish is 190-217 cm for males and 246-364 cm for females. Estimated growth rates suggest that male swordfish would reach the maximum size at about 9 years old and female would reach maximum size at about 15 years old (Ward *et al.*, 2000). A probable explanation for this difference is that energy spends by males would be directed to reproductive activity rather than growth, as males mature earlier than females (Ehrhardt, 1992; DeMartini *et al.*, 2000). However, swordfish grow at varying rates, depending on the surrounding environment (e.g. food availability and water temperature), energy lost and genetic structure (Govoni *et al.*, 2003).

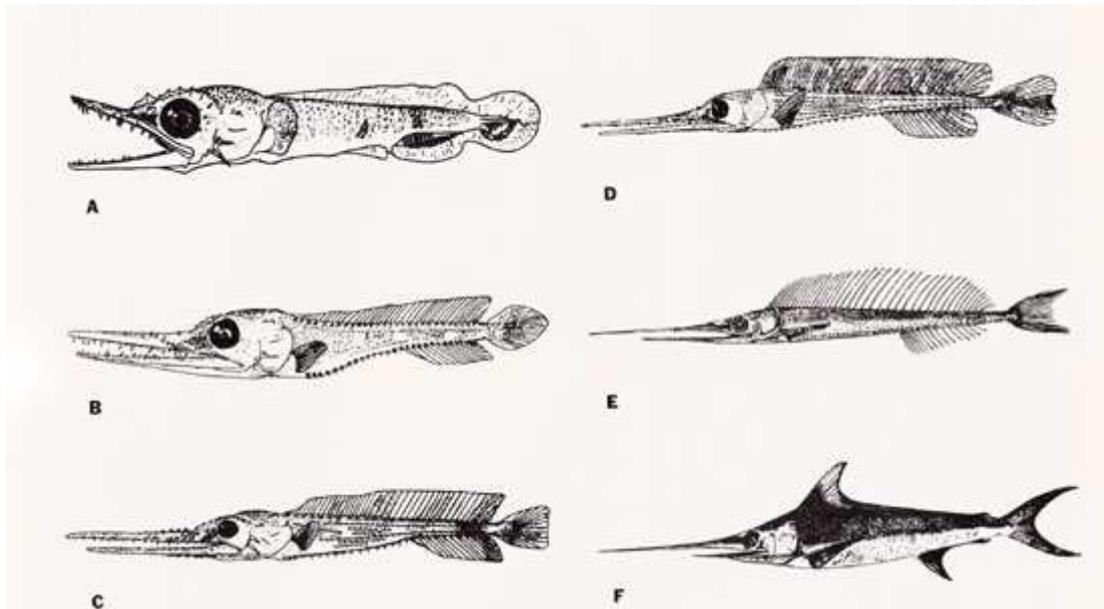


Figure 3. Swordfish larvae. A: 7.8mm (TL, Total Length); B: 14.5mm TL; C: 27.2mm TL; D: 68.8mm TL; E: 252mm BL (from the posterior edge of orbit to base of the caudal fin); F: 580mm BL. (Source: WWW.flmnh.ufl.edu/fish/gallery/descript/swordfish/swordfish.html)

FOOD AND FEEDING

Adult swordfishes are opportunistic feeders, known to forage solitarily from the surface to the bottom over a wide depth range (Nakamura, 1985; Chancollon *et al.*, 2006). Swordfish are visual predators, relying on their highly developed eyes to detect prey in water where light is poor. They use their rostrum to stun prey (Carey and Robinson, 1981). Adult swordfish diet comprised a wide variety of prey. Near the continental shelf or oceanic bank they spend daylight hours on the seabed, feeding on demersal fish. In deep oceanic waters adults feed primarily on squid, pelagic fish and occasionally crustaceans and octopus (Chancollon *et al.*, 2006). Their diet varies with location and the available prey items (Chancollon *et al.*, 2006). The young swordfish larvae are selective feeders that feed copepods on the first development phase changing then to other larval fishes following the development of their digestive tract (Govoni *et al.*, 2003; Chancollon *et al.*, 2006). Gradually the swordfish shift from copepods, neustonic fish larvae and as adults eat adult fishes, cephalopods and crustaceans (Govoni *et al.*, 2003; Chancollon *et al.*, 2006).

Optimal feeding conditions for swordfish are found around current boundaries and frontal zones where sharp gradients of temperature and salinity exist. Those boundaries concentrate prey species. Additionally the swordfish feeding activity increases during the full moon nights, particularly where the thermocline is deep. This is probably related to greater availability of light at depth (Draganik and Cholyst, 1988).

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Chapter 2

The swordfish fisheries

THE EVOLUTION OF THE SWORDFISH FISHERIES IN THE WORLD

The swordfish fisheries started thousand of years ago as near-shore subsistence activities of Mediterranean countries. The early fishing methods mostly involved the harpooning of large specimens, which took profit of their basking behaviour at the sea surface (Ward *et al.*, 2000). The harpooning was for many decades the only fishing method targeting swordfish in the Mediterranean Sea that gradually expanded to north and south American coastal areas. However, during 1980s harpoon fisheries began to decline as a result of the introduction of more efficient fishing gears, such as the driftnets and longlines (Ward *et al.*, 2000).

The driftnet was introduced by Japanese fleet in early 1960s to target pelagic species, particularly tunas. As it could be used over a wider geographical range and weather conditions, many subsistence and small-scale commercial fishing boats upgrade quickly to driftnets. The development of the monofilament nylon driftnets in mid-1980s and the distance-water fishing fleets from Japan and Taiwan increase substantially the swordfish fishing yields. Though, many nations banned this fishing technique as an outcry over wastage of incidental catch was produced (Ward *et al.*, 2000).

Distance-water longliners targeting swordfish and albacore started during the 1950s by Japanese fleet operating in the north Pacific (Ward *et al.*, 2000). Late 1950s, many distance-water longliners started targeting tuna throughout the Atlantic, such as yellowfin and bigeye for *sashimi* markets in Japan. In that period, more than half of the world's swordfish catches was taken as incidental catch of distance-water longliners targeting tuna. The few longliners targeting swordfish were mainly based in Hawaii, fishing in the north Pacific. The Hawaiian fleet caught mostly small sized specimens in subtropical and subarctic frontal areas of the north Pacific (Ward *et al.*, 2000). In the mid-1980s this technique became widespread and the techniques to target swordfish were well known (e.g. use expensive baits as squid, attached light, set at shallower water at night, etc.). Longline fishers realised that by using those fishing techniques and set the gear on areas where swordfish could be more abundant, the swordfish catch rates were higher than the catch rates of longliners targeting tuna (Ward *et al.*, 2000). Further, the introduction of nylon monofilament longline during the 1980s and the establishment of fresh-chilled fish market resulted in a substantial increased in the swordfish fishing yields (Ward *et al.*, 2000). Gradually, the number of fishing boats increases and geographical expansion of the main longline fleets occurred, which contributed to the

increased swordfish catches during the 1990s. Additionally, the continuous improvement of the fishing gear (single strand monofilament leaders, etc) and technique (light-sticks, fishing depth and topography, hauling speed, hydraulically powered reels, hooks spacing) also contributed to the increased of swordfish catch rates (Ward *et al.*, 2000). In each established fisheries, fishing effort continued to expand for several years highlighting the need for fishery managers to control expansion and overcapacity in the fleets (Ward *et al.*, 2000).

Nowadays, the swordfish is mainly caught with longline while other gears, such as the harpoon, and driftnet represent a small portion of the total fishing effort (Ward *et al.*, 2000). However, in many areas such as the Mediterranean Sea, the harpoon and driftnets fisheries still being in use to catch swordfish.

GLOBAL TRENDS OF SWORDFISH LANDINGS

The swordfish landings started to increase during the mid-1950's keeping this trend until 1970 when reached a peak of *c.* 39.000 ton (Figure 1). During this period the Pacific was responsible for 27% of the total swordfish landings, the Atlantic 7%, the Mediterranean Sea 4% and the Indian Ocean less than 0.5% (Figure 2). The swordfish landings decreased in 1971 which was attributed to the mercury contamination scare. In 1971 the United States Food and Drug administration (FDA), concerned about the level of swordfish, established a 0.5 ppm tolerance level for mercury in swordfish. Most swordfish exceeded this level, which caused demand to decline and effort to be reduced. However, in 1978 FDA revised its limits and from then swordfish landings increased exponentially, particularly in the Atlantic and Indian Oceans. Early 1990's the total swordfish landings stabilised being the contribution of the Atlantic fisheries 33%, the Pacific 30%, the Indian 22% and the Mediterranean Sea 4% (Figure 2). The redirection of the Taiwan fleet to Indian and Atlantic Oceans was the reason for the large increase of swordfish landings in those oceans (Ward *et al.*, 2000). The historical maximum of the swordfish landing was reached in 2003 (*c.* 119.000 ton) (Figure 1). After that a slightly decline on swordfish catches has been observed mainly due to the conservation and management measures implemented by ICCAT.

According to FAO (2010), in 2007 the world production of tunas and tuna like species was 5.1 million tons. Among this total, tunas represented 91.5% of the catches

while the swordfish 6.8% and the remaining billfishes 1.7%. If only the billfishes were considered the world production reached 428.000 tons. Among the billfishes the swordfish represented 79.8% and the remaining 20.2% are due to marlins. Actually, most of the world catches come from the Pacific Ocean reaching 41% of total swordfish landings. The Atlantic, Indian and Mediterranean Sea account 25%, 21% and 13% respectively (Figure 3).

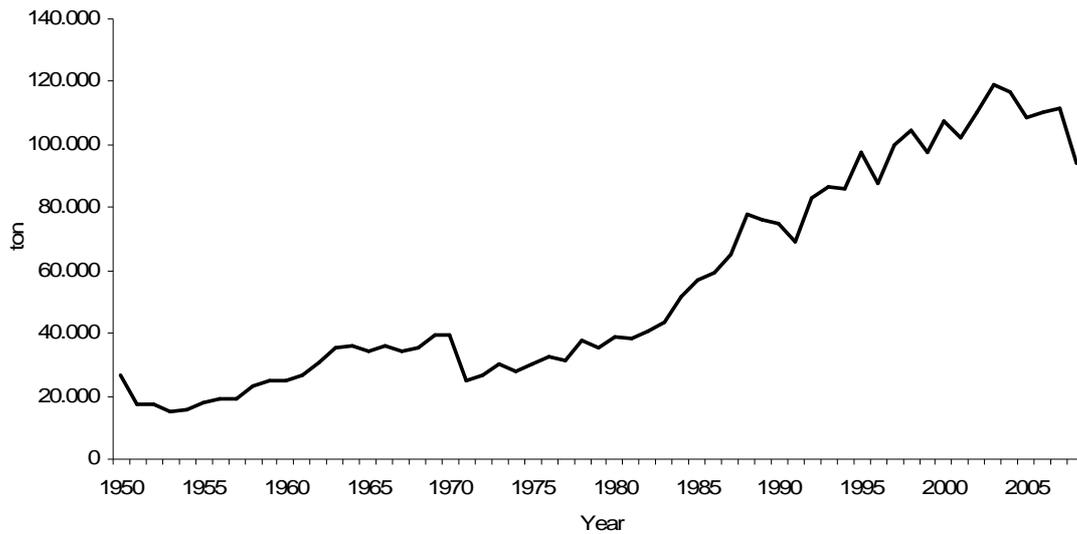


Figure 1. Global swordfish landings (Source: FAO, 2010).

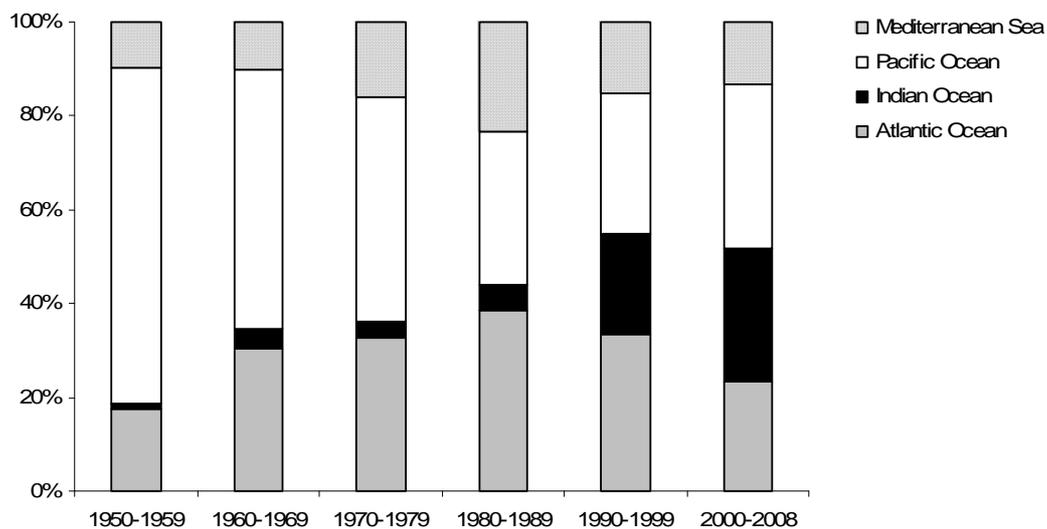


Figure 2. Global swordfish landings (10 years period) by ocean (Source: FAO, 2010).

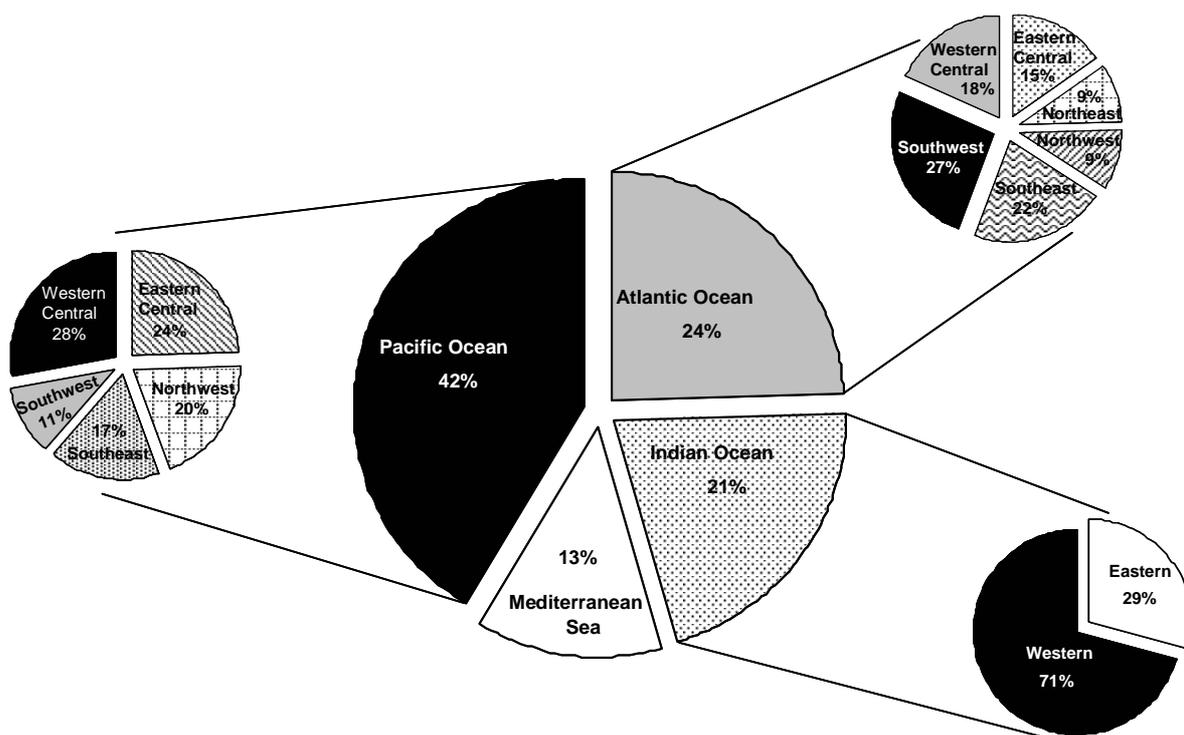


Figure 3. Proportion of swordfish landings by ocean basin (Source: FAO, 2010)

THE ATLANTIC SWORDFISH FISHERIES

Harpoon fishery in the Atlantic Ocean started thousand of years ago (Mejuto and Hoey, 1991) in north American Atlantic coast. This fishing technique quickly expanded to Canada in the Grand Banks (Tibbo *et al.*, 1961).

In 1956, the Japanese longline fleet started targeting tuna in central Atlantic waters and few years later the Russian fleet started targeting sharks (Hazin, 2006). In the early 1960s, the American and Canadian fleets fit their harpoon vessels to longliners, encouraged by the large swordfish catches reached by the Japanese and Russians in Central Atlantic. The swordfish landings increased significantly since 1970, which correspond to the large activity of the longline fleets in this water basin (Mejuto and Hoey, 1991). In 1991 the total Atlantic landings dropped as swordfish abundance decreased (Anon., 2000; Anon., 2005). The swordfish landings showed then a sharply increase until 1995 when reached the historical maximum of *c.* 43.000 ton. After that a decrease trend has been observed until 2008 reaching 21.859 tons that represented a significant decline from that reported in 2007 (27.941 tons) (Figure 4). The reduction on swordfish landings was related to the relocation of the main fleets for other oceans,

especially to Pacific, and to the management measures imposed by ICCAT (Anon., 2001).

Currently, at least 17 nations fished swordfish in Atlantic with Canada, Spain, United States, Portugal, Brazil, Namibia, Uruguay, South Africa and Venezuela taking the largest catches (Anon., 2009).

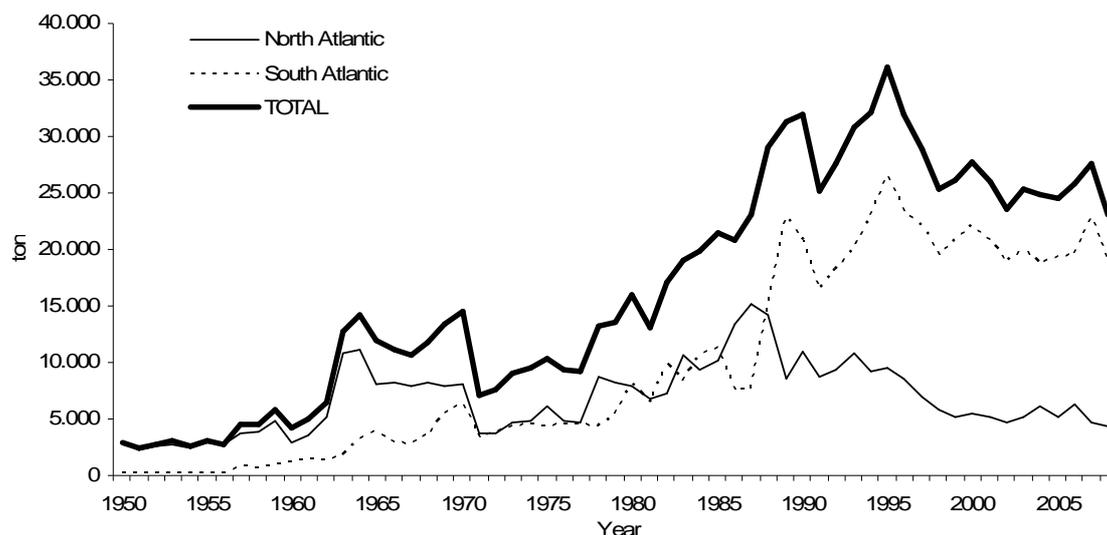


Figure 4. Swordfish landings in the Atlantic Ocean (Source: FAO, 2010)

The North Atlantic fisheries

The north Atlantic was the area where the majority of the fisheries were developed (Figure 4), which is why this stock has historically been subject to greater fishing effort. Since the beginning of the fisheries the swordfish landings showed an increase trend following the evolution patterns of the Atlantic total catches. In 1964 the swordfish landings reach a peak of *c.* 8.000 tons that correspond with the replacement to longlines by the American and Canadian fleets. In this year, those countries were responsible for 84% of the total landings. The swordfish landings showed then a stable trend and in 1971 decreased slightly followed by the gradual increase until 1987, when the historical maximum was reached (*c.* 15.200 tonnes). The Spanish and the United States were the fleets that contribute most for the total swordfish landings yielding 73% and 13%, respectively. Since 1988 a steady decline in swordfish landings from the northern area were registered mainly in response to changes in the distribution of the fleet,

particularly for the south Atlantic. Further, several fleets fishing within the north Atlantic area, such as the United States, Spain, Portugal and Canada have been changing occasionally the target species (e.g. tuna and sharks) to take advantage of market conditions, the high catch rates and also the reduction of swordfish abundance (Anon., 2002).

According with FAO (2010), in 2008 22% of the swordfish landings coming from the northern Atlantic area and were mainly exploited by the fleets of Canada (32%), Spain (31%), United States (16%) and Portugal (17%).

The South Atlantic fisheries

The swordfish landings in the south Atlantic were relatively low until the early 1980's. Since 1980's the swordfish landings progressively increased but substantial growth was observed since 1988 due to the displacement of the main fishing fleets to this area particularly from the northern area (Anon., 2002). The historical maximum (c. 26.500 tonnes) was reached in 1995 being the largest catches attributed to the Spanish (41%), Japanese (8%) Brazilian (22%) and Taiwanese (3%) fleets. The catches decline gradually after 1995, as seen for the north Atlantic, due to the regulatory measures implemented by the ICCAT, to the displacement of the fishing effort to other oceans and to shifts in the target species.

According with FAO (2010) in 2008, the biggest proportion of swordfish catches came from the southern Hemisphere reaching 78% of the total Atlantic. Further, the countries that contribute most for the total swordfish landed in this area were the Spain (27%), Brazil (22%), Japan (6%) and Namibia (3%).

GENERAL TRENDS OF ATLANTIC SWORDFISH MANAGEMENT MEASURES AND THEIR IMPACT ON STOCK STATUS

The north Atlantic swordfish is the most extensively researched and assessed stock as represents one of the most important fish resources in the area that are harvested since thousand of years ago. The swordfish assessment, as well as all other species managed by ICCAT is held by the respective working group under the Standing

Committee on Research and Statistics (SCRS) of ICCAT. The SCRS meets annually to monitor the state of exploitation of swordfish stocks and predicted whether the current level of exploitation is sustainable in the long term. Based on the catch and fishing effort statistics given by the different member states and upon presentation of scientific papers, the SCRS makes some proposals to the committee of ICCAT. By the opinion of SCRS and whenever the committee deems it, appropriate assessment is conducted on swordfish stocks. The last of these evaluations took place in 2009 is scheduled to close for the year 2011.

Since 1988, ICCAT's Standing Committee on Research and Statistics (SCRS) has assessed the status of north Atlantic swordfish. In 1990, SCRS assessment showed a continuous decline in swordfish biomass since about 1980, with a failing prevision of the stock after 1990 (Anon., 2000; Anon., 2005). In response to the 1990 assessment session, ICCAT issue its first recommendation aiming the catch reduction, particularly the juvenile fraction of the population, to be taken by the major fleets fishing in the northern Atlantic area. Thus, in 1991 this measure started to be implemented by limiting the catch size (or weight) (TML) to at least 125 cm (or approximate value in live weight of 25 kg) with a tolerance of 15% in the total catch in number. In 1994, ICCAT introduced the total allowable catches (TACs), which are divided into country specific quotas, and maintained the minimum size limits (Anon., 2000). However, several fleets ignore those measures while for others were not effective. As a result of the continuous declining of swordfish catch rates, international TACs were established by year and country, which resulted in the relocation of some longliners to the south Atlantic and Pacific Ocean, while others opportunistically targeted tuna and shark to take advantage of market prices and locally high catch rates (Anon., 2000). In 1995, the annual quota allocated to each country is adjusted and incidental catches of swordfish caught by Japanese tuna longline fleet were also subject to regulation. ICCAT deliberated that the rejection of swordfish catches could not exceeded 8% in weight of the total catch. In 1996, quotas were kept and other alternative measure to TML was implemented that was the possibility to capture individuals with 119 cm, with 0% tolerance. Only the United States and Canada regulated their fisheries based on this measure (Anon., 2001). Since 1997, the restriction measures began to be more stringent as the level of exploitation reached could result in dramatic consequences for the recruitment and biomass of the northern Atlantic population (Anon., 2001). Thus, it was possible the application of sanctions to countries that capture beyond its quota, between assessment

periods. For these cases the over-exploitation had to be removed from the value of the quota for the subsequent evaluation period. This measure was also valid for countries that captured below the quota, the difference being added to the quota for the next evaluation period. Between 1997 and 1999 the north Atlantic swordfish population has shown recovery signs, as a result of a decrease in fishing mortality. Moreover, the good recruitment (age 1) observed between 1997 and 1999 was a result of the TML implementation and allowed to predict an optimistic scenario, if the juvenile fraction of the population was not heavily exploited. For this reason, the SCRS has suggested that the measure taken for the TML should be implemented more effectively trying not to exert pressure on the juvenile fraction of the population as they will contribute to the future parent stock (Anon., 2001).

The latest swordfish stock assessment (2009) revealed that the biomass of the northern stock was estimated to be at or above the value needed to produce the maximum sustainable yield (MSY) (Anon., 2009). The target stock size or the biomass at MSY previewed by the ICCAT rebuilding program, implemented since 1999, was achieved. In fact, since 2003 the catches have been below the TAC which increased the chances for stock recovery. However, the ICCAT alerted that the north Atlantic stock is nowadays less productive as it was in 2006 (Anon., 2009). Concerning the southern Atlantic swordfish, the ICCAT assessment results for 2006 were inconclusive, recognizing that further research was required on population biology and on the nature of the fisheries (Anon., 2007). In 2009, the assessment indicated that the stock was not over-fished despite the conflicting signals for several indices used (Anon., 2009).

Currently ICCAT keep the two management options for the TML, which are applied throughout the Atlantic (125 cm with 15% tolerance and 119 cm with 0% tolerance). In addition to this measure, the TACs still being implemented annually and distributed among the countries with exploitation interests on swordfish as well as those whose catches of swordfish contribute significantly to the total catch. The estimation of the TAC for the north Atlantic stock took in consideration the high recruitment observed in recent years and aimed to obtain biomass values that support the levels of MSY (up to year 2009). Thus, ICCAT recommended that catches (including discards) should be around 14.000 ton; otherwise the trajectory of the stock may decline. For the south Atlantic the number of available data showed contradictory trends leading to an unsafe estimate of the state of the stock. For this reason, the ICCAT warns of the need to

improve the quality of data on the major fleets fishing in the south Atlantic and recommends that catches must be around the 14.000-15.000 tons (Anon., 2002).

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PART II

MULTIDISCIPLINARY APPROACH FOR ATLANTIC SWORDFISH STOCKS DIFFERENTIATION

Chapter 1

The metazoan parasites of swordfish from Atlantic tropical-equatorial waters

Garcia, A., Santos, M.N., Damiano, S., Nascetti, G., Mattiucci, S. 2008. The metazoan parasites of swordfish from Atlantic tropical-equatorial waters. *Journal of Fish Biology*, 73: 2274-2287.

ABSTRACT

The metazoan parasite fauna composition of the swordfish (*Xiphias gladius*) is reported from 145 (72 females and 73 males) specimens caught in Atlantic tropical-equatorial waters during June to July 2006 and August 2007. Seventeen metazoan taxa (two monogeneans, two digeneans, one acanthocephalan, four cestodes, seven nematodes and one copepod) were identified by morphological and genetic methodologies. Among those identified *Anisakis brevispiculata*, *Anisakis paggiae* and the acanthocephalan *Bolbosoma vasculosum* are new host records. Dominant species were: the anisakid nematode larvae *Anisakis physeteris*, *Anisakis brevispiculata*, *Hysterothylacium incurvum* and *Hysterothylacium corrugatum* (s.l.), the adult cestode *Fistulicola plicatus*, the plerocercoid *Hepatoxylon squali* and the monogeneans *Tristoma integrum* and *Tristoma coccineum*, which showed high prevalences and intensities. The relationship between metazoan parasites and host size and sex was investigated for the first time. No statistically significant correlation was found between host sex and parasite burden. Infection levels with *T. integrum* and *T. coccineum* were observed to be significantly negatively correlated to host size. Infection levels by endoparasites observed in *X. gladius* from Atlantic tropical-equatorial area may be related to host feeding behaviour and the availability of the parasites' intermediate and final hosts in the area studied. Anisakids of the genus *Anisakis*, were identified genetically to species level, and were considered to be promising biological tags for the characterization of *X. gladius* stocks.

INTRODUCTION

Parasite community structure and the processes involved on their distribution within a fish host population have received considerable attention in recent years. Several studies concerning different host-parasite systems provide evidence that parasites exhibit heterogeneous distributions within their host populations as each individual host can provide different habitats for parasites according to their characteristics (Caillaud *et al.*, 2006; Ferrari *et al.*, 2007). Numerous explanations have been offered for such heterogeneity related to ecological and physiological differences between host

individuals that could affect the immune defence mechanisms and the levels of exposure to parasites (Zuk, 1990; Zuk and McKean, 1996; Klein *et al.*, 1999; Klein, 2000).

Differences in parasite distribution patterns have been compared with several host features. Host size or age and sex are frequently listed as factors that could lead to differences mainly for parasite load and species richness (Lo *et al.*, 1998; Poulin, 2000; Morand *et al.*, 2004; Krasnov *et al.*, 2005; Caillaud *et al.*, 2006; Christe *et al.*, 2007; Simkova *et al.*, 2008). From an ecological perspective, several mechanisms have been postulated for the observed differences in parasitic burden between host size or age and gender. One of the causes could be host mobility (home range) that could provide greater levels of exposure to different parasites. Host mobility could also influence the foraging pattern as the availability of food items could change in space (Caillaud *et al.*, 2006). The diversity and abundance of metazoan parasites could be more pronounced if the host has a larger geographical distribution, such as for large pelagic fishes. This could be more relevant for those parasite species showing complex life cycles, involving different organisms at different positions in marine food webs (Mattiucci and Nascetti, 2007). The same effect could be observed when the energetic requirements rise with the consequent increase in food consumption (Lo *et al.*, 1998).

Although there is knowledge about the influence of size or age on the metazoan fauna recovered from several fish hosts (George-Nascimento, 1996; Lo *et al.*, 1998; González *et al.*, 2001; González and Poulin, 2005; Campbell *et al.*, 2007), the influence of sex on infection levels is poorly documented for fishes (Rohde, 1993; González *et al.*, 2001; Skartstein *et al.*, 2001; Simkova *et al.*, 2008), which differ from mammals and birds in several aspects of their life-history traits (Charnov *et al.*, 2001).

Xiphias gladius L. is one of the most important highly-migratory fish resources in the Atlantic Ocean. Large pelagic fishes such as *X. gladius* undertake extensive and complex migrations across the oceans. Such migrations allow these fast-moving fishes to take advantage of the rich food areas. One of the most important factors that guide their migrations is water temperature (Palko *et al.*, 1981). Migration routes could be different in juveniles as well as female and male spawners. Different sexes could acquire different parasite species in a geographical area according to their migration route and their vertical distribution. Although the parasite fauna of some Atlantic populations of *X. gladius* have been studied (Illes, 1971; Hogans *et al.*, 1983; Gómez-Cabrera, 1987; Castro-Pampillón *et al.*, 2002a), there are no studies dealing with the aspects related to the parasitic infection levels and host size and sex. Indeed, studies on

their parasites from the Atlantic Ocean focused only on the northwest area (Illes, 1971; Hogans *et al.*, 1983), Gibraltar waters (Gómez-Cabrera, 1987) and the Gulf of Guinea (Castro-Pampillón *et al.*, 2002a). Comparison of the metazoan parasite fauna of *X. gladius* collected in the northwest Atlantic (Hogans *et al.*, 1983) and Gulf of Guinea was carried out by Castro-Pampillón *et al.* (2002b).

The aims of this study were to report the metazoan parasites of *X. gladius* from Atlantic tropical-equatorial waters, both quantitatively and qualitatively; to study the relationship between *X. gladius* length and parasitic infection levels; and to investigate if there is a relationship between parasitic infection levels and *X. gladius* sex.

MATERIAL AND METHODS

Sampling and parasitological examination

A total of 145 *X. gladius* (72 females and 73 males) were caught by a commercial longline vessel in June and July 2006 and August 2007 in Atlantic tropical-equatorial waters (latitudes 10° N and 5° S and longitudes 0° and 40° W) (Table 1). Data were collected within the Portuguese fisheries data collection programme.

For each specimen, the lower jaw fork length (LJFL) was taken to the nearest 1 cm, immediately following capture. The body surface of the fish was examined only for the presence of the mesoparasite copepod *Pennella* spp. on board the vessel. After autopsy, host sex was recorded, and the abdominal cavity examined for endoparasites. The fish were then cleaned, the internal organs and the gills were removed, stored and frozen at -20° C, until their shipment to, and examination in the laboratory. The branchial arches, stomach, intestine, mesenteries and serosa were examined separately for metazoan parasites, including those encysted in or outside the intestine and stomach walls. After macroscopic examination, the internal surfaces of the stomach and intestine were scraped and washed with saline for further stereoscopic examination (x20). The parasites were sorted, counted and preserved in 70% ethanol for morphological identification. The anisakid nematodes recovered were stored at -70°C for identification using molecular methodologies.

Genetic methodologies

Genetic markers obtained from multilocus allozyme electrophoresis (MAE) were used for identification to species of the 1979 *Anisakis* spp. larvae type II (Berland, 1961) recovered from the fish samples, according to the procedures reported by Mattiucci *et al.* (1997). Among the 20 enzyme-loci studied in *Anisakis* spp. (Mattiucci *et al.*, 1997, 2001, 2002, 2005), the following diagnostic loci were used to identify the larvae to species: malate dehydrogenase-1 (*Mdh-1*, EC 1.1.1.37), superoxide dismutase (*Sod-1*, EC 1.15.1.1), nucleoside phosphorilase (*Np*, EC 2.4.2.1), aspartate aminotransferase (*Aat-2*, EC 2.6.1.1), adenylate kinase (*Adk-2*, EC 2.7.4.3), leucine aminopeptidase (*Lap-1*, EC 3.4.11.1), leucine-leucine-leucine peptidase (*PepC-1*, *PepC-2*, EC 3.4.11), mannose phosphate isomerase (*Mpi*, EC 5.3.1.8), glucose phosphate isomerase (*Gpi*, EC 5.3.1.9) and phosphoglucomutase (*Pgm2*, EC 5.4.2.2). The staining procedures were the same as reported by Mattiucci *et al.* (1997). *Anisakis pegreffii* from the Mediterranean Sea was used as a reference population.

Table 1. Number of *Xiphias gladius* specimens examined by size (lower-jaw-fork-length, LFLJ) and sex.

Sex	LJFL classes (cm)						TOTAL
	< 120	120-140	141-160	161-180	181-200	> 200	
Female	10	9	9	20	12	12	72
Male	10	17	15	16	10	5	73
TOTAL	20	26	24	36	22	17	145

Data analysis

For each parasite species, infection levels were calculated for prevalence (P), mean intensity (MI) and its range according to Rozsa *et al.* (2000). For each individual host, the parasite species richness (González and Poulin, 2005) and the numbers of individual parasites were estimated by pooling ecto- and endo-parasites. First, the effect of individual host size on species richness and on the total number of individual parasites was investigated using Spearman's correlation coefficient (r_s). These analyses were carried out for the whole data set and within sexes, which provided a baseline to

proceed with the investigation between sex and the parasitic infection data, according to LJFL classes. For this purpose, the *X. gladius* specimens were grouped in to six LJFL classes comprising: the young-of-the-year (YOY) specimens (<120 cm), juveniles (120-140, 141-160 and 161-180 cm) and adults (181-200 and >200 cm) (Table 1).

Differences in parasite MI between host sexes were assessed by the Mann-Whitney *U*-test, while differences in P were tested with the χ^2 -test. The statistical analyses were computed with GraphPad Prism software v5 (San Diego, CA, U.S.A.) with a 95% CL ($\alpha = 0.05$).

RESULTS

Xiphias gladius parasites of Atlantic tropical-equatorial waters

Because no significant differences were found in the parasite fauna composition in fish collected during the two sampling years, the data obtained were combined according to host size and sex (Table 2). Seventeen parasite taxa were recovered from the *X. gladius* examined including: one mesoparasite (copepod), two ectoparasites (monogeneans) and 14 endoparasites (two digeneans, four cestodes, seven nematodes and one acanthocephalan; Table 2). Individual fish harboured a mean \pm S.D. parasite taxa of 5.8 ± 1.6 (range 0-9) and a mean \pm S.D. parasite burden of 66.5 ± 59.0 (range 0-340). No correlation was found between individual host size with respect to species richness and the number of parasites, either when both sexes were pooled or when considered separately.

Among all the endoparasites recorded, the most abundant and frequent species were: the anisakid nematodes *Anisakis physeteris*, *Anisakis brevispiculata*, *Hysterothylacium incurvum* and *Hysterothylacium corrugatum* (*s.l.*), the adult cestode *Fistulicola plicatus* and the plerocercoid *Hepatoxylon squali* which accounted for c. 90% of the parasite burden and showed high levels of P and MI in all the samples (Table 2).

Table 2. Prevalence (P%), mean intensity (MI, \pm S.D.) and range (in parentheses), of infection by parasite taxa collected from *Xiphias gladius* from Atlantic tropical-equatorial waters, according to host LJFL classes and sexes (see Table I)

Parasite taxa	Host sex	LJFL-class (cm)											
		< 120		120-140		141-160		161-180		181-200		>200	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
MONOGENEA													
<i>Tristoma coccineum</i>	P (%)	40.0	20.0	22.2	29.4	33.3	13.3	5.0	18.8	-	20.0	16.7	-
	MI \pm SD	4.8 \pm 2.8 (2-8)	7.5 \pm 2.1 (6-9)	4.5 \pm 0.7 (4-5)	2.4 \pm 1.5 (1-4)	2.0 \pm 1.0 (1-3)	1.0	1.0	1.7 \pm 0.5 (1-2)	-	1.0	1.0	-
<i>Tristoma integrum</i>	P (%)	60.0	80.0	77.8	88.2	44.4	60.0	60.0	43.8	41.7	50.0	50.0	-
	MI \pm SD	7.3 \pm 4.5 (2-4)	10.1 \pm 7.8 (1-25)	5.6 \pm 4.2 (1-13)	5.6 \pm 4.9 (1-20)	13.3 \pm 7.8 (2-20)	2.2 \pm 1.7 (1-5)	4.8 \pm 4.2 (1-14)	3.0 \pm 1.8 (1-6)	1.4 \pm 0.9 (1-3)	3.0 \pm 1.4 (1-4)	1.7 \pm 1.6 (1-5)	-
DIGENEA													
<i>Hirudinella ventricosa</i>	P (%)	-	10.0	-	5.9	44.4	-	25.0	31.3	50.0	20.0	16.7	60.0
	MI \pm SD	-	1.0	-	1.0	1.5 \pm 0.6 (1-2)	-	2.4 \pm 3.1 (1-8)	1.4 \pm 0.5 (1-2)	1.7 \pm 0.5 (1-2)	1.0	1.0	1.3 \pm 0.6 (1-2)
<i>Opisthadenia dimidia</i>	P (%)	-	-	-	-	-	13.3	-	-	-	16.0	-	-
	MI \pm SD	-	-	-	-	-	3.0 \pm 2.8 (1-5)	-	-	-	1.0	-	-
CESTODA													
<i>Fistulicola plicatus</i>	P (%)	90.0	100.0	100.0	100.0	77.8	93.3	95.0	81.3	83.3	100.0	75.0	100.0
	MI \pm SD	7.7 \pm 6.9 (1-19)	6.8 \pm 5.6 (1-19)	13.6 \pm 8.9 (2-27)	16.5 \pm 10.3 (3-38)	13.3 \pm 6.2 (6-24)	15.4 \pm 11.9 (1-39)	10.2 \pm 7.7 (1-29)	11.5 \pm 9.3 (1-33)	23.5 \pm 14.4 (3-46)	16.2 \pm 14.3 (4-49)	8.6 \pm 5.2 (1-18)	11.6 \pm 2.6 (8-15)
<i>Hepatoxylon squali</i>	P (%)	70.0	70.0	88.9	70.6	77.8	86.7	70.0	75.0	66.7	14.0	58.3	60.0

	MI±SD	2.6±1.5 (1-5)	2.1±1.5 (1-5)	3.6±3.7 (1-10)	3.3±2.9 (1-8)	4.4±2.9 (1-8)	7.2±8.0 (1-31)	3.6±3.5 (1-14)	3.8±2.2 (1-8)	3.5±2.7 (1-8)	9.1±15 (2-46)	3.1±3.2 (1-10)	1.0
<i>Sphyricephalus viridis</i>	P (%)	-	20.0	-	5.9	11.1	6.7	15.0	12.5	16.7	15.0	25.0	-
	MI±SD	-	1.0	-	1.0	1.0	1.0	1.0	1.0	1.5±0.7 (1-2)	1.0	1.0	-
Tetraphyllidea	P (%)	-	-	11.1	5.9	33.3	-	5.0	-	8.3	-	17.0	100.0
	MI±SD	-	-	1.0	4.0	2.3±1.5 (1-4)	-	1.0	-	4.0	-	1.0	1.0
NEMATODA													
	P (%)	60.0	60.0	66.7	52.9	77.8	80.0	80.0	63.5	75.0	60.0	33.3	-
<i>Anisakis physeteris</i>	MI±SD	9.7±11 (1-30)	8.2±10.7 (1-29)	4.2±4.4 (1-13)	27.2±27.8 (2-80)	19.3±18.1 (2-47)	11.7±12.8 (1-47)	5.2±3.7 (1-11)	6.2±5.6 (1-17)	18±14.9 (1-35)	17.3±14.1 (2-37)	9.0±7.3 (3-18)	-
	P (%)	30.0	30.0	33.3	52.9	77.8	66.7	60.0	56.3	41.7	20.0	8.3	-
<i>Anisakis brevispiculata</i>	MI±SD	15.0±10.5 (5-26)	3.7±1.5 (2-5)	4.3±3.5 (1-8)	21.0±14.2 (4-41)	11.6±17.1 (2-50)	13.5±11.5 (1-40)	6.8±8.8 (2-33)	10.2±6.1 (1-20)	16.8±17.0 (2-45)	22.5±14.8 (12-33)	70.0	-
	P (%)	10.0	10.0	11.1	-	-	-	-	-	-	-	-	-
<i>Anisakis paggiae</i>	MI±SD	1.0	1.0	1.0	-	-	-	-	-	-	-	-	-
	P (%)	-	30.0	11.1	-	11.1	-	15.0	6.3	8.3	12.0	8.3	-
<i>Anisakis sp.2</i>	MI±SD	3.7±1.2 (3-5)	-	2.0	-	3.0	-	1.3±0.6 (1-2)	2.0	5.0	1.0	2.0	-
	P (%)	60.0	90.0	100.0	94.0	100.0	93.3	90.0	81.3	100.0	14.0	58.3	60.0
<i>Hysterothylacium incurvum</i>	MI±SD	31.2±44.5 (1-99)	15.9±20.5 (1-63)	25.9±19.7 (2-57)	20.6±22.9 (3-86)	42.9±26.4 (4-88)	45.3±51.1 (1-197)	26.8±27.5 (1-100)	16.6±36.6 (1-134)	30.3±39.6 (1-130)	47.8±58.0 (1-169)	45.7±41.8 (1-114)	1.0
	P (%)	50.0	60.0	100.0	94.1	100.0	86.7	85.0	56.3	83.3	15.0	50.0	-
<i>Hysterothylacium corrugatum (s.l.)</i>	MI±SD	11.3±14.1 (1-30)	7.0±6.5 (1-19)	7.8±5.9 (1-17)	6.2±6.9 (1-26)	12.9±7.9 (1-26)	14.6±15.5 (2-59)	8.5±8.3 (1-30)	7.1±12.8 (1-40)	10.8±12.3 (1-39)	18.4±17.8 (1-51)	16.0±12.1 (1-34)	-

<i>Oncophora melanocaephala</i>	P (%)	1.0	-	-	-	-	-	-	-	-	-	-	-
	MI±SD	1.0	-	-	-	-	-	-	-	-	-	-	-
ACANTHOCEPHALA													
<i>Bolbosoma vasculosum</i>	P (%)	-	-	22.2	11.8	33.3	26.7	10.0	18.8	16.7	10.0	-	-
	MI±SD	-	-	2.0±1.4 (1-3)	2.0±1.4 (1-3)	3.0±2.0 (1-5)	3.5±4.4 (1-10)	6.0±2.8 (4-8)	3.3±3.2 (1-7)	2.0±1.4 (1-3)	2.0	-	-
COPEPODA													
<i>Pennella instructa</i>	P (%)	-	20.0	-	-	-	-	-	-	-	10.0	8.3	-
	MI±SD	-	1.5±0.7 (1-2)	-	-	-	-	-	-	-	1.0	1.0	-

The genetic identification of the 1979 *Anisakis* spp. larvae of type II (Berland, 1961) recovered allowed the recognition of the following species as parasites of the *X. gladius* from tropical-equatorial Atlantic waters: *A. physeteris*, *A. brevispiculata* (Mattiucci *et al.*, 2001), *Anisakis paggiae* (Mattiucci *et al.*, 2005). Moreover, some of the larval *Anisakis* type II corresponded genetically to the taxon indicated as *Anisakis* sp. 2 (Mattiucci *et al.*, 2007, 2008); this represents a gene pool genetically distinct from all the *Anisakis* spp. so far detected (Mattiucci *et al.*, 2008).

Among the *Anisakis* spp. identified, *A. physeteris* and *A. brevispiculata* showed high relative proportions (55.5% and 40.2%, respectively) in the fish; while, *A. paggiae* and *Anisakis* sp. 2 rarely occurred in the fish (Figure 1). Mixed infections by all four species of *Anisakis* were observed in several fishes.

Because it has been suggested that *H. corrugatum* includes the presence of two sibling species, namely *H. corrugatum* sp. A and *H. corrugatum* sp. B (Mattiucci *et al.*, 1994; unpubl. obs.), whose recognition is based on the use of genetic molecular markers, the nematodes morphologically referred to as *H. corrugatum* collected from *X. gladius* in this parasitological survey were here indicated as *H. corrugatum* (*s.l.*) (Table 2).

Among the ectoparasites, the monogeneans *Tristoma integrum* and *T. coccineum* were also highly prevalent. Consequently, they were considered as dominant species within the *X. gladius* parasite community in the study area (Table 2).

Less abundant and rare species were: the copepod mesoparasite *Pennella instructa*, the adult nematode *Oncophora melanocephala*, the digeneans *Opisthadenia dimidia* and *Hirudinella ventricosa*, the acanthocephalan *Bolbosoma vasculosum*, the cestodes *Sphyricephalus viridis* (plerocercoid) and the larval stages of Tetracystida. These metazoan parasites accounted for c. 2% of the whole parasite fauna and thus were excluded from further analysis.

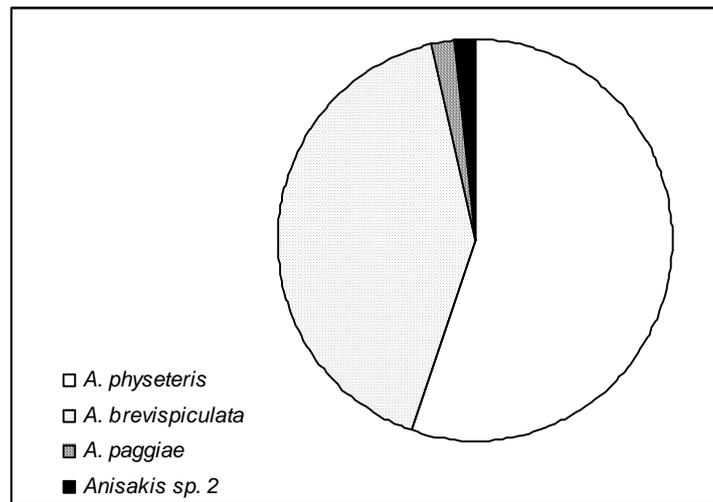


Figure 1. Relative proportions of *Anisakis* spp.: □ *A. physeteris*; ▒ *A. brevispiculata*; ▓ *A. paggiae*; and ■ *Anisakis sp. 2* larvae, identified by genetic markers, in *Xiphias gladius* from Atlantic tropical-equatorial waters.

Parasitic infection levels and host size and sex relationship

The results indicate that there are no differences between females and males in P (χ^2 -test, $p > 0.05$ for all taxa) and MI (Mann–Whitney *U*-test, $p > 0.05$ for all taxa) values of the infection by metazoan parasites.

Considering the intensities of infection for the dominant metazoan taxa, statistically significant and negative correlations were observed between host size and the infection levels for the two monogeneans *T. coccineum* (r_s , $n=145$, $p=0.01$) and *T. integrum* (r_s , $n=145$, $p < 0.01$). Intensities of infection by *T. integrum* in male fish were higher in YOY and small juveniles (<141cm LJFL) than in adults (>200 cm LJFL). In *T. coccineum*, this pattern was observed in both females and males (Table 2).

Despite the high number of larval *A. physeteris* and *A. brevispiculata* recovered and the high level of infection found in the fish examined (Table 2), no significant correlations were observed with respect to host size (r_s , both $p > 0.05$). With regard to the adult anisakid nematodes belonging to the genus *Hysterothylacium* (Table 2), parasites of the stomach and intestine of *X. gladius* there were no significant correlations in the intensity of the infection when related to host LJFL (r_s , $p > 0.05$ for both *H. corrugatum s.l.* and *H. incurvum*).

As for the adult cestode, *F. plicatus*, parasite of the intestine, high levels of prevalence and intensity of the infection were found (Table 2), but no significant correlation (r_s , $p>0.05$) was reported for the intensity of infection for this parasite according to host LJFL. A similar condition was found for the larval cestode, *H. squali* (r_s , $p>0.05$).

DISCUSSION

Most of the parasites recovered from the *X. gladius* caught in the Atlantic tropical-equatorial waters were already known for this species. The cystacanth of *B. vasculosum* found in the intestine and stomach of *X. gladius* is a new host record. Further, among the larval anisakid nematodes identified, *A. brevispiculata* and *A. paggiae* represent new host records for this species. Moreover, the data acquired on these nematodes enlarge the geographical range so far known for the *Anisakis* spp., being *A. physeteris*, *A. brevispiculata* and *A. paggiae* which have not been reported from tropical-equatorial Atlantic waters (Mattiucci and Nascetti, 2006). The species named *Anisakis* sp. 2, showing the larval morphology of type II, at allozyme level, has been demonstrated to have some loci fixed for alternative alleles with respect to the other species of *Anisakis* (Mattiucci *et al.*, 2008). Moreover, the sequence analysis of the mtDNA *cox2* gene performed on specimens of the *Anisakis* sp. 2, confirmed that this taxon represented a distinct phylogenetic unit (Mattiucci *et al.*, 2008). It is genetically more closely related to *A. physeteris*, *A. brevispiculata* and *A. paggiae* at both nuclear (allozymes) and mitochondrial levels (mtDNA *cox2*), rather than to the other species of *Anisakis* (Mattiucci *et al.*, 2008). The data also confirmed that the *Anisakis* species so far included in the *A. physeteris* complex (i.e. *A. physeteris*, *A. brevispiculata*, *A. paggiae* and *Anisakis* sp. 2) and forming a well separated cluster (Mattiucci and Nascetti, 2008), share the same larval morphotype II (Berland, 1961), and they can be recognized easily by the use of genetic molecular markers. Further, the relative proportions of the larval *Anisakis* spp. identified in the Atlantic tropical-equatorial *X. gladius* population (Figure 1) is different from that so far found in fish from the Mediterranean Sea and from northeast Atlantic areas (Mattiucci *et al.*, 2007; unpubl. obs.). In particular, the lack in the tropical-equatorial *X. gladius* population of some *Anisakis* spp. (i.e. *A. pegreffii* and *Anisakis simplex* s.s.) which were identified in *X. gladius* from Mediterranean and

northeast Atlantic waters, seems to indicate these parasites as promising biological tags for the characterization of stocks of *X. gladius* from Atlantic Ocean and from other water basins.

The monogeneans *T. coccineum* and *T. integrum* are host specific for *X. gladius* and were previously reported as parasites of *X. gladius* populations from the northwest Atlantic (Illes, 1971; Hogans *et al.*, 1983) and the Gulf of Guinea (Castro-Pampillón *et al.*, 2002a,b). In the present study, the intensities of infection by the large capsalid monogeneans and their prevalence decreased with LJFL, with young specimens being more infected than the adults and independent of host sex. A similar trend has been observed in other monogenean species. Grutter *et al.* (2002) hypothesized that the cleaner fish *Labroids dimidiatus* (Valenciennes) was responsible for the reduced level of intensity of the infection by the capsalid *Benedenia lolo* found in the larger fish species (Labridae), with respect to the lower LJFL classes of fishes, which were highly infected, suggesting that small fishes are more vulnerable to infection to monogeneans than larger ones. No data are so far reported about the existence of *L. dimidiatus* on ectoparasites of *X. gladius*. On the other hand, among host factors, host size was considered as the main factor influencing the composition and infections of temperate and tropical ectoparasite species (Buchman, 1989). Moreover, generally related species of monogeneans (congeners) exhibit different predilections when they co-occur on the same host and occupy adjacent sites (Rohde, 1993). In the present study, it has been observed that *T. integrum* and *T. coccineum* have a clear spatial segregation in relation to their microhabitats on the gills of *X. gladius*; *T. coccineum* was found mainly occupying the external surface of host gills, whereas *T. integrum* lives on the internal gill surface, probably resulting from interspecific competition between the two species. Kadlec *et al.* (2003) also attributed the difference of microhabitats generally observed in monogenean species to variations in host size. In the present study, the proportion of *Tristoma* spp. tended to decline with host growth. The effect of environmental condition or stress on parasites depends on the particular species of ectoparasites and host environment, which may increase the infections by some parasites, but may decrease in others (Lafferty and Kuris, 1999). Some environmental conditions (e.g. temperature and salinity) related to different habitats occupied by juvenile and adult *X. gladius* could lead to the differences observed in the two capsalid species in the different LJFL classes.

The endoparasite species richness was highly diverse in *X. gladius* and was maintained during its life history as any change was observed between the smaller and the larger specimens in the study area. Moreover, sex-related bias was not recognized for the endoparasites, in any phase of *X. gladius* ontogenetic development. Poulin (1996) did not find a sex-related bias in fish hosts but a slight consistent male bias in parasite infections in birds and especially in mammal hosts. Similar finding was observed by González *et al.* (2001), who did not find any difference between bigeye flounder *Hippoglossina macrops* (Steindachner) sexes. This could be true for a specific host-parasite system considering the particular ecological features. Parasites may infect the sexes differently, because male and female fishes often have different feeding habits (Rohde, 1993) which could be a consequence of their different mobility (Caillaud *et al.*, 2006). This could be more relevant for *X. gladius* inhabiting the tropical-equatorial area because it is an important spawning area for this species, i.e. the large females could have reduced mobility to concentrate their effort on reproduction. In the present study, it has been demonstrated that host sex did not have a significant influence on parasitic infections or in species richness, suggesting that habitat use and diet of *X. gladius* are similar for both sexes. These findings seem also to indicate that the tropical-equatorial area is the main feeding area for both juvenile and adult *X. gladius* as no differences in the infection levels by endoparasites, acquired by host feeding behaviour were observed in different LJFL classes in the present survey. Thus, the data obtained suggest that the *X. gladius* parasite fauna composition in Atlantic tropical-equatorial waters may be the result of its habitat and feeding behaviour and some abiotic factors.

The larval anisakid nematodes belonging to the genus *Anisakis* (*A. physeteris*, *A. brevispiculata*, *A. paggiae* and *Anisakis* sp. 2) were recovered from the *X. gladius* stomach, that acquired these parasites by preying on infected squids, which are probably the intermediate hosts of the *Anisakis* spp. (Mattiucci *et al.*, 2007; Mattiucci and Nascetti, 2008).

Xiphias gladius is the definitive host for the anisakid nematodes *H. incurvum* and *H. corrugatum* (*s.l.*). Both *H. incurvum* and *H. corrugatum* (*s.l.*) were previously reported as adults in fish fauna from the northwest Atlantic (Hogans *et al.*, 1983) at high prevalence and intensity levels, as well as from other geographical areas included in the host range, such as the Mediterranean Sea (Damiano *et al.*, 2006). The high infection levels reported for these anisakid nematodes suggest that they are dominant and specialist parasites for this fish host. Although no intermediate hosts are known for

Hysterothylacium spp. recovered, fish and squid are likely to be suitable intermediate hosts.

Xiphias gladius is the definitive host for the cestode *F. plicatus*, a parasite of the intestine. It is a specialist parasite for this host, and has been reported, at high levels of infection, also in the *X. gladius* population from the Mediterranean Sea (Damiano *et al.*, 2006), as well as from other Atlantic waters. No data are available for the intermediate hosts of *F. plicatus*.

Hepatoxylon squali, which shows high infection burdens in tropical-equatorial *X. gladius* showed very low level of infection (P=1.5%, MI=1.00) in the Mediterranean population (Damiano *et al.*, 2006; unpubl. obs.).

The biogeographical aspects of the recovered parasites could explain the record of some parasite taxa in the study area. Moreover, the availability of *X. gladius* parasite intermediate and final hosts might be responsible for the maintenance of the high levels of infection observed by those parasite taxa, which have been acquired by the fish during feeding in these Atlantic waters. Since anisakid nematodes of the genus *Anisakis* are heteroxenous parasites, the high infection levels by the *A. physeteris* and *A. brevispiculata*, seem to indicate a high stability in the marine trophic webs. Indeed, the high population densities of both definitive (cetaceans) and paratenic-intermediate hosts (mainly squid) of these parasites in the study area would allow the maintenance of the high parasite intensities observed in the sample of *X. gladius*. Low parasite infection level by *A. physeteris* has been reported in *X. gladius* from other geographical areas, such as the Mediterranean Sea (P=19.1%; MI=3.8) (unpub. obs.). On the other hand, parasite infection levels and genetic diversity of anisakid nematodes have been considered as possible indicators of food web stability and integrity of marine ecosystems (Mattiucci and Nascetti, 2007).

Finally, the detection of some *Anisakis* spp. in *X. gladius* populations from Atlantic tropical-equatorial waters may have important implications for *X. gladius* stock characterization. These genetically identified species have been used in recent years, as biological tags to recognize stocks of demersal and pelagic fish species (Mattiucci *et al.*, 2007).

ACKNOWLEDGEMENT

The authors express their gratitude to PESCARADE and the crew of F. V. Príncipe das Marés for their assistance in swordfish sampling. The first author holds a PhD grant from the Portuguese Foundation for Science and Technology (ref. SFRH/BD/25391/2005) in co-operation with Tuscia University, Viterbo, Italy. The authors thank two anonymous referees whose constructive comments improved the paper. The authors are grateful to A. Pike for his advice and suggestions and to H. Palm for his help with Trypanorhynch cestode identification.

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Chapter 2

Metazoan parasites of swordfish, *Xiphias gladius* (Pisces: Xiphiidae) from Atlantic Ocean: implications for host stock identification

Garcia, A., Mattiucci, S., Damiano S., Santos, M.N., Nascetti, G. 2011. Metazoan parasites of swordfish, *Xiphias gladius* (Pisces: Xiphiidae) from the Atlantic Ocean: implications for host stock identification. *ICES Journal of Marine Sciences*, 68(1): 175-182.

ABSTRACT

The metazoan parasite fauna composition of the swordfish, *Xiphias gladius*, is reported from 157 specimens caught in four areas of the Atlantic Ocean: northwest (NW), central north (CN), east tropical (ET) and central south (CS). Samples were collected in 2003, 2006 and 2007. In all, 18 parasite taxa were identified by morphological and genetic methods. These data, together with earlier analyses from the tropical-equatorial (TEQ) Atlantic revealed the association of some parasite taxa to distinct areas based on their presence/absence and infection levels. *Hysterothylacium corrugatum* (*s.l.*) and *Rhadinorhynchus pristis* were mostly associated with swordfish caught in the NW, and *Anisakis paggiae* and *H. incurvum* with fish caught in the southern areas (ET, TEQ and CS). *Anisakis simplex s.s.* could be used as a marker for all northern samples. Parasitological data on Atlantic swordfish seems to support the existence of at least two discrete stocks in the Atlantic, supporting swordfish genetic stock structure.

INTRODUCTION

Accurate delimitation of fish stock boundaries is helpful in fisheries management, especially when stocks respond differently to exploitation rates and/or rebuilding strategies. Moreover, the identification of distinct evolutionary units is informative and can help in the prioritization of areas of high value for conservation. Therefore, to target assessment effort sustainably, the biological identity and conservation status of each stock needs to be clarified. The study of parasites can contribute with valuable information on geographic distribution patterns, migrations and feeding habits of the hosts (George-Nascimento, 1996; Mattiucci *et al.*, 2004, 2008; Timi, 2007; Valdivia *et al.*, 2007). Recently, comparative studies of hosts and their parasites have expanded the understanding of the boundaries of the evolutionarily units of the hosts (Abaunza *et al.*, 2008). Guidelines on how to use parasites as biological tags in fish population studies have been reviewed and discussed in depth (MacKenzie, 2002; Lester and MacKenzie, 2009). Three main criteria determine the selection of parasites to be used as tags: 1) infection levels must be different among the host sampling areas; 2) the parasite should

be detectable in the host throughout the timescale of the study; and 3) the parasite should not be pathogenic to the fish host. One approach is to analyse the whole parasite assemblage recovered from each sampling area, instead of using a single parasite species, because this might yield a better picture of host stock structure (MacKenzie, 2002). In addition, the residence time of the parasite in the fish host must be well known for it to be an effective population marker (Lester and MacKenzie, 2009).

The swordfish, *Xiphias gladius*, is a pelagic and highly migratory species, with a range extending from tropical to cold-temperate oceans, including the Mediterranean Sea (Nakamura, 1985). It is a commercially valuable species and currently managed as two population units; the north and south Atlantic, separated by latitude 5°N (Anon., 2006). ICCAT (the International Commission for the Conservation of Atlantic Tunas) has always been concerned about this boundary, and despite what is known about swordfish biology (Taylor and Murphy, 1992; Arocha, 2002; Chancollon *et al.*, 2006), morphology (García-Cortés and Mejuto, 2002; Arocha *et al.*, 2003; Govoni *et al.*, 2003) and genetic structure (Chow *et al.*, 2002; Reeb *et al.*, 2003; Alvarado-Bremer *et al.*, 2005a,b; Muths *et al.*, 2009), questions persist about stock boundaries and migrations, and it is still unclear whether or not the management divisions corresponds to different biological stocks.

The aim of this study was to analyse the metazoan parasite composition of *X. gladius* from different sampling areas in the Atlantic Ocean and to compare it with data from tropical-equatorial (TEQ) waters (Garcia *et al.*, 2008), in an attempt to provide a more comprehensive analysis of swordfish metazoan parasites in Atlantic waters and to use the metazoan parasite fauna as a tool to investigate the possible existence of discrete population units.

MATERIALS AND METHODS

Samples and methodology

Swordfish samples were collected within the Portuguese fisheries data collection programme, between June and August of 2003, 2006, and 2007. In all, 157 swordfish were caught in five areas throughout the Atlantic aboard a commercial longliner: 42 from the northwest (NW) in 2006, 46 from the central north (CN) in 2003, 45 from the

eastern tropical (ET) in 2007, and 24 from the central south (CS) in 2007 (Figure 1, Table 1).

Immediately following capture, lower-jaw-fork-length (LJFL) was measured to the nearest centimetre and the body surface was examined for the presence of the mesoparasitic copepod *Pennella* spp. After dissection, the body cavity was examined for endoparasites and the internal organs (intestine and stomach) and gills removed, stored, and frozen at -20°C until subsequent parasitological examination in the laboratory. The gill arches, stomach, intestine, mesenteries, and serosa were examined macroscopically. Further stereoscopic examination (2×10) was carried out on the internal fluids of the stomach and intestine. All metazoan parasites collected from each fish were sorted, counted, and preserved in 70% ethanol for later morphological identification. The anisakid nematodes recovered in the samples were stored at -70°C for identification to species level using molecular genetic tools (Mattiucci *et al.*, 2007).

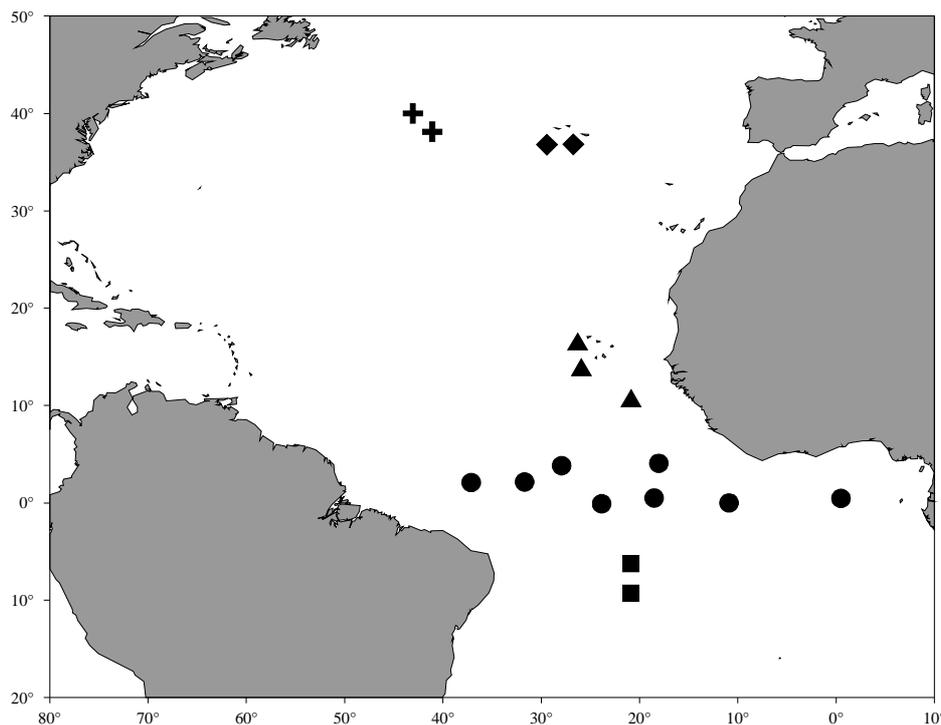


Figure 1. Swordfish sampling areas in the Atlantic Ocean, showing the locations of collection of NW (stars), CN (diamonds), ET (triangles), TEQ (ovals) reported in Garcia *et al.* (2008), and CS (squares) samples.

Data analysis

For each parasite taxon and area, infection levels were calculated for prevalence (P), mean intensity (MI) and its range, and abundance (A), according to Rozsa *et al.* (2000). Only parasite taxa selected as suitable for use as tags according to the criteria mentioned above were considered for statistical analysis.

As the host sample sizes were not equal in the different sampling areas, the relationship between swordfish sample size and P and MI for each of the selected parasite taxa was investigated by Spearman's correlation analysis (r_s ; Sokal and Rohlf, 1987). The same analysis was used to evaluate the effect of individual host size on species richness (González and Poulin, 2005), on the total number of parasites (ecto- and endoparasites) collected, and on the number of individual parasites by area.

The parasitic infection levels in the different areas were compared with those observed previously in swordfish from the (southeast) TEQ area (Garcia *et al.*, 2008). Therefore, the univariate and the multivariate analyses were performed on samples from NW, CN, ET, and CS, as well as TEQ. A Kruskal-Wallis ANOVA (H) was used to test for significant differences between mean intensity of infection by each parasite taxon from five different sampling areas. A Student-Newman-Keuls multiple comparison test was used to identify the source of any significant difference. Chi-squared analyses and a multiple comparison test for proportions were performed to test for statistically significant differences between prevalence values from different sampling areas. A stepwise linear discriminant analysis (LDA) was carried out to check for differences between areas and to determine among the parasite taxa selected which were responsible for the differences. LDA was computed after $\log(n + 1)$ transformation of the number of each parasite taxon recovered, by individual fish and area. The importance of each taxon was assessed using the backward stepwise method based on the Mahalanobis distances (Suzuki *et al.*, 2008). Multivariate LDA was performed by the Brodgar 2.5.1 package (Brodgar, 2006) and univariate analysis with Statistica 6.0 (StatSoft Inc., 2001).

Table 1. Sampling year, sample size, and mean length (lower-jaw-fork-length, LJFL) \pm s.d. (range) of swordfish caught between June and August in four different areas within Atlantic Ocean.

Area	Sampling year	n	Host LJFL (cm) mean \pm S.D. (range)
NW	2006	42	127.9 \pm 23.7 (85-180)
CN	2003	46	106.7 \pm 33.0 (81-240)
ET	2007	45	129.8 \pm 32.6 (78-223)
CS	2007	24	148.5 \pm 29.8 (110-234)

RESULTS

Swordfish parasite taxa and the host-size relationship by area

In terms of the host sample size by area, no significant correlations were found with parasite prevalence or with mean intensity ($p_{rs} > 0.05$), for any of the parasite taxa. Therefore, no correction for sampling effort was necessary. Further, no significant differences were found in the parasite fauna composition recovered from swordfish during the different sampling years, based on a comparison of fish captured in the NW in 2005 and 2006. As no differences were found and a more samples were collected in 2006, however, only data from 2006 were used for statistical analysis.

In all, 18 parasite taxa were recorded from 99% of the swordfish examined, including 1 mesoparasite (copepod), 2 ectoparasites (monogeneans), and 15 endoparasites (one digenean, four cestodes, eight nematodes, and two acanthocephalans; Table 2). Swordfish from the CN had the fewest individual parasites and taxa, with (mean \pm s.d.) 11.9 \pm 14.7 parasites and 3.3 \pm 1.8 taxa. Swordfish from the ET had the most parasites (89.1 \pm 92.1), and those from the NW had the most taxa (7.0 \pm 1.7). There were no statistically significant correlations ($p_{rs} > 0.05$ for all) between individual host size and parasite species richness in any area. In contrast, the total number of parasites increased significantly with host size in fish from the NW ($r_s = 0.40$, $p < 0.01$) and the CN ($r_s = 0.32$, $p = 0.03$), but not in swordfish from the ET ($r_s = 0.24$, $p = 0.11$) or the CS ($r_s = 0.18$, $p = 0.41$). The parasite taxa selected as suitable for use as biological tags were the plerocercoid cestodes *Hepatoxylon squali* and *Sphyricephalus viridis*, the acanthocephalans

Bolbosoma vasculosum and *Rhadinorhynchus pristis*, and the larval anisakid nematodes *Anisakis simplex* (s.s.), *A. paggiae*, *A. physeteris*, *A. brevispiculata*, *Anisakis* sp. 2, *Hysterothylacium incurvum*, and *H. corrugatum* (s.l.; Table 2). Among those endoparasites, the raphidascarid nematodes *H. incurvum* and *H. corrugatum* (s.l.), and the cestode *H. squali*, were recorded in fish from all areas at relatively high abundance and frequency, representing 69.9, 43.5, 79.2, and 67.9% of the parasite burden in the NW, the CN, the ET, and the CS, respectively (Table 2). The most abundant larval *Anisakis* recovered in fish samples from the ET area were genetically recognized as belonging to the species *A. physeteris* and *A. brevispiculata*. The larval stages of *A. simplex* (s.s.) were identified only in swordfish from the northern areas of the Atlantic Ocean (NW and CN). Additionally, a genetically recognized taxon, indicated here as *Anisakis* sp. 2 (Mattiucci *et al.*, 2007), was found only in the fish from the ET. The acanthocephalan *B. vasculosum* was recovered only from swordfish from the northern areas and *R. pristis* from the southern areas (ET and CS), as well as in the NW. Finally, the plerocercoid *S. viridis* was collected only from swordfish caught in the southern areas (Table 2). In terms of the number of individual parasites of the selected taxa, statistically significant correlations with host size were not frequent, though when found were generally not repeated across areas (Table 3). The plerocercoid *H. squali* was the only species that showed significant correlations in more than one area (Table 3), with larger fish less infested by this parasite than smaller ones.

Table 2. Prevalence (P, %), mean intensity (MI) \pm s.d. and range (in parenthesis) of the infection by the parasite taxa collected on *X. gladius* from four areas of the Atlantic Ocean.

Parasite taxa	NW		CN		ET		CS	
	P (%)	M.I. \pm s.d. (Range)						
MONOGENEA								
<i>Tristoma coccineum</i>	60	7.5 \pm 7.2 (1-23)	59	1.3 \pm 0.6 (1-3)	18	2.1 \pm 1.4 (1-4)	13	1 (1)
<i>Tristoma integrum</i>	76	7.9 \pm 8.2 (1-38)	22	1.4 \pm 0.5 (1-2)	80	6.4 \pm 5.0 (1-22)	54	3.5 \pm 3.4 (1-11)
DIGENEA								
<i>Hirudinella ventricosa</i>	41	1.8 \pm 1.0 (1-4)	2	3.0 (3)	11	1.6 \pm 0.9 (1-3)	33	1.3 \pm 0.5 (1-2)
CESTODA								
<i>Fistulicola plicatus</i>	91	5.1 \pm 5.6 (1-24)	67	3.4 \pm 2.8 (1-14)	82	7.7 \pm 5.4 (1-22)	100	16.1 \pm 10.1 (2-38)
<i>Hepatoxylon squali</i> *	71	5.4 \pm 6.8 (1-28)	57	5.6 \pm 14.3 (1-74)	76	5.9 \pm 6.7 (1-32)	63	2.7 \pm 1.8 (1-8)
<i>Sphyricephalus viridis</i> *	-	-	-	-	16	1.4 \pm 0.5 (1-2)	4	1.0 (1)
Tetraphyllidea	-	-	35	6.2 \pm 9.5 (1-37)	-	-	-	-
NEMATODA								
<i>Anisakis simplex s.s.</i> *	50	3.4 \pm 1.3 (1-5)	22	4.0 \pm 2.6 (1-10)	-	-	-	-
<i>Anisakis physeteris</i> *	21	2.1 \pm 1.3 (1-4)	9	1.3 \pm 0.5 (1-2)	44	7.5 \pm 7.9 (1-27)	17	6.0 \pm 10.0 (1-21)
<i>Anisakis brevispiculata</i> *	17	3.0 \pm 3.3 (1-10)	-	-	36	5.8 \pm 8.1 (1-28)	13	4.7 \pm 6.4 (1-12)
<i>Anisakis paggiae</i> *	-	-	-	-	2	1.0 (1)	17	1.0 (1)
<i>Anisakis sp.2</i> *	-	-	-	-	20	3.8 \pm 3.2 (1-10)	-	-
<i>Hysterothylacium incurvum</i> *	100	18.6 \pm 23.0 (1-124)	30	4.7 \pm 5.3 (1-16)	91	54.5 \pm 65.3 (2-375)	79	39.6 \pm 53.9 (1-147)
<i>Hysterothylacium corrugatum s.l.</i> *	100	25.7 \pm 33.2 (1-172)	11	5.6 \pm 5.4 (1-14)	91	18.0 \pm 21.8 (1-125)	71	14.1 \pm 18.0 (1-49)
<i>Oncophora melanocephala</i>	2.4	1.0 (1)	4	1.0 (1)	-	-	-	-
ACANTHOCEPHALA								
<i>Rhadinorhynchus pristis</i> *	43	4.1 \pm 3.7 (1-14)	-	-	11	1.0 (1)	4	1 (1)
<i>Bolbosoma vasculosum</i> *	31	1.5 \pm 0.9 (1-3)	4	1.0 (1)	-	-	-	-
COPEPODA								
<i>Pennella instructa</i>	-	-	7	1.0 (1)	2	2.0 (2)	-	-

-, No parasites

*, Parasite taxon selected as potential biological tag for swordfish stock identification in the Atlantic Ocean.

Table 3. Spearman's correlation coefficients (r_s) between the number of individual parasites and *X. gladius* length for the selected taxa in four areas of the Atlantic Ocean.

Parasite taxa	NW	CN	ET	CS
<i>H. squali</i>	0.44 *	0.17	0.32 *	0.29
<i>S. viridis</i>	-	-	0.35 *	0.26
<i>A. physeteris</i>	0.07	-0.03	0.07	0.07
<i>A. brevispiculata</i>	0.15	-	-0.07	0.28
<i>A. paggiae</i>	-	-	-0.08	-0.31
<i>A. simplex s.s.</i>	0.09	0.17	-	-
<i>Anisakis</i> sp.2	-	-	0.31*	-
<i>H. incurvum</i>	0.37 *	-0.15	0.16	0.32
<i>H. corrugatum s.l.</i>	0.38 *	0.25	0.16	0.35
<i>B. vasculosum</i>	0.25	0.03	-	-
<i>R. pristis</i>	0.09	-	0.20	0.11

* Significant at 0.05 level

Parasite infection levels in swordfish sampled from the different areas

Statistically significant differences were found between parasitic intensity values of the selected parasite species collected in swordfish samples from the NW, the CN, the ET, the CS, and the TEQ (Table 4). Except the cestode *S. viridis*, the intensity of infection by the remaining taxa showed that most differences were observed between the northern (NW and CN) and the southern (ET, TEQ, and CS) fish samples, with those from the southern areas more closely related to one other than those from the northern areas. Prevalence values for the cestodes *S. viridis* and *H. squali* and the nematode *Anisakis* sp. 2 did not differ significantly among areas (Table 4). Conversely, the prevalence values of infection by *A. physeteris*, *A. brevispiculata*, *H. incurvum*, and *H. corrugatum (s.l.)* were significantly different between areas, with higher values in ET and TEQ than in other areas (Table 4). Further, the prevalence of infection by *A. simplex (s.s.)*, *B. vasculosum*, and *R. pristis* were significantly different between areas, with highest values in samples from the NW, whereas prevalence of the larval nematode *A. paggiae*, which was parasitizing only the samples from the CS and the ET, had the highest values in the CS. The first two discriminant functions of the multivariate LDA

carried out on all swordfish samples accounted for 74.2% (eigenvalue=1.504) and 20.6% (eigenvalue=0.418) of the total variance (total explained=94.8%). A statistically significant group effect was found (Wilks $\lambda = 0.255$, $F_{24,315}$, $p < 0.01$). The spatial distribution of individual fish represented by the sample scores and the group means illustrated separation between three groups (Figure 2), one consisting of fish from the southern samples (ET, TEQ, and CS), which clustered together, and the other two represented by the samples from the NW and the CN, with the NW samples more separated from the remaining groups. The nematode *H. corrugatum* (s.l.) was the species that contributed most to the separation of fish samples, and it was found to be related to the fish from the NW (Figure 3). Similarly, *R. pristis* was associated with swordfish from the NW. *Anisakis paggiae* was associated with the swordfish from the southern areas (ET, TEQ, and CS) and inversely correlated with the occurrence of *A. simplex* (s.s.), which characterized the samples from the CN.

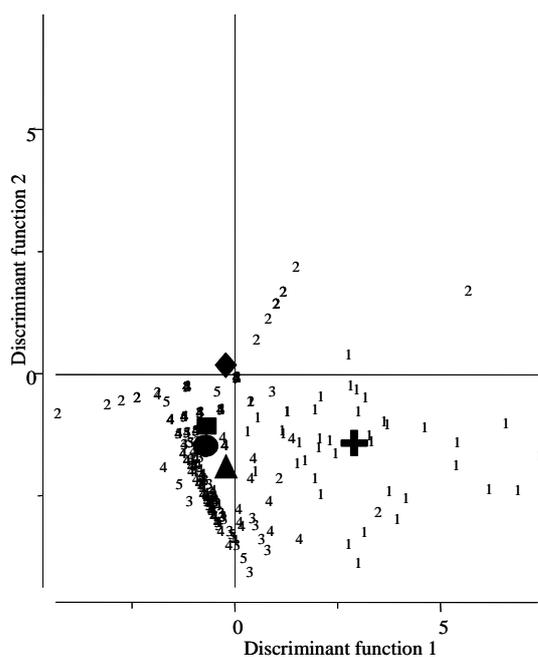


Figure 2. LDA on swordfish samples from five sampling areas in the Atlantic Ocean. Numerals represent samples from the NW (1), CN (2), ET (3), and CS (5), and data from the TEQ area (4) reported in Garcia *et al.* (2008). Symbol shapes for NW (star), CN (diamond), ET (triangle), TEQ (dot), and CS (square).

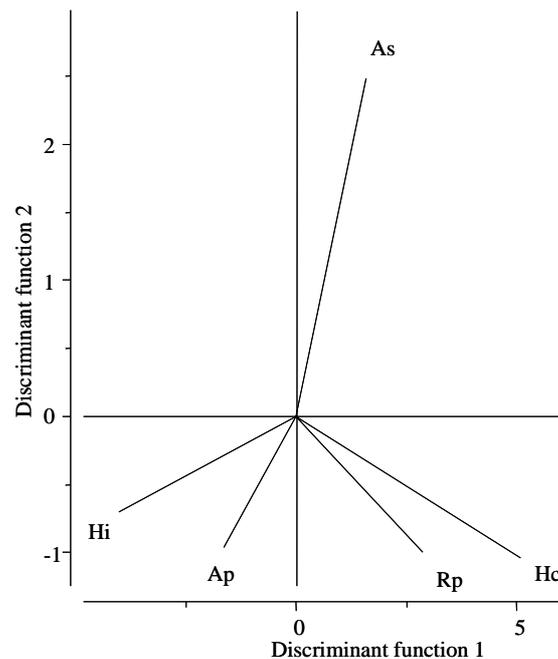


Figure 3. Correlation between the first two discriminant functions and the swordfish parasites selected as potential tags: Hi, *Hysterothylacium incurvum*; Hc, *Hysterothylacium corrugatum* (s.l.); Ap, *Anisakis paggiae*; As, *Anisakis simplex* (s.s.); Rp, *Rhadinorhynchus pristis*.

Hysterothylacium incurvum was the second most important species for samples discrimination, and its prevalence was associated with the fish from southern areas. *Bolbosoma vasculosum*, *S. viridis*, *H. squali*, *A. physeteris*, *Anisakis* sp. 2, and *A. brevispiculata* were excluded from the analysis because they were responsible for only slight changes in the Mahalanobis distance, and hence of little importance for discrimination purposes.

Based on the above five-category analysis, a second LDA was performed considering ET, TEQ, and SC as a single group. Re-analysis of the data revealed that two discriminant functions explained 100% of the overall variance, the contribution of the first being 80.4% (eigenvalue=1.494) and the second one 19.6% (eigenvalue=0.364). A statistically significant group effect was also found (Wilks $\lambda = 0.294$, $F_{48,803}$, $p < 0.01$).

Table 4. Kruskal-Wallis ANOVA (H) and Chi-squared (χ^2) comparisons of prevalence and mean intensity for the selected parasite taxa of *X. gladius* between five areas in the Atlantic Ocean [present data and from Garcia *et al.* (2008) for TEQ].

Parasite taxa			NW	NW	NW	NW	CN	CN	CN	TEQ	TEQ	ET
			vs	vs	vs	vs	vs	vs	vs	vs	vs	vs
			CN	ET	TEQ	CS	ET	TEQ	CS	ET	CS	CS
<i>H. squali</i>	H	11.23 ^{**}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	χ^2	1.19 ^{NS}	-	-	-	-	-	-	-	-	-	-
<i>S. viridis</i>	H	13.58 ^{NS}	-	-	-	-	-	-	-	-	-	-
	χ^2	1.71 ^{NS}	-	-	-	-	-	-	-	-	-	-
<i>A. physeteris</i>	H	67.43 [*]	NS	NS	NW<TEQ ^{**}	NS	NS	CN<TEQ ^{**}	NS	TEQ>ET ^{**}	TEQ>CS ^{**}	NS
	χ^2	26.03 [*]	NS	NS	NW<TEQ ^{**}	NS	CN<ET [*]	CN<TEQ [*]	NS	NS	TEQ>CS ^{**}	NS
<i>A. brevispiculata</i>	H	48.39 [*]	NS	NS	NW<TEQ ^{**}	NS	NS	CN<TEQ ^{**}	NS	TEQ>ET ^{**}	TEQ>CS ^{**}	NS
	χ^2	9.68 ^{**}	NW>CN [*]	NS	NW<TEQ ^{**}	NS	CN<ET [*]	CN<TEQ [*]	CN<CS ^{**}	NS	TEQ>CS ^{**}	NS
<i>A. paggiae</i>	H	28.83 [*]	Ab	NS	NS	NW<CS [*]	NS	NS	CN<CS [*]	NS	NS	NS
	χ^2	15.86 [*]	Ab	NS	NS	NW<CS ^{**}	NS	NS	CN<CS [*]	NS	TEQ<CS [*]	ET<CS ^{**}
<i>A. simplex s.s.</i>	H	99.97 [*]	NW<CN [*]	NW>ET [*]	NW>TEQ [*]	NW>CS [*]	CN>ET [*]	CN>TEQ [*]	CN>CS [*]	Ab	Ab	Ab
	χ^2	3.69 ^{NS}	-	-	-	-	-	-	-	-	-	-
<i>Anisakis sp.2</i>	H	21.48 [*]	Ab	NS	NS	Ab	NS	NS	Ab	NS	NS	NS
	χ^2	0.30 ^{NS}	-	-	-	-	-	-	-	-	-	-
<i>H. incurvum</i>	H	75.34 [*]	NW>CN ^{**}	NW<ET [*]	NS	NS	CN<ET [*]	CN<TEQ ^{**}	CN<CS [*]	TEQ<ET ^{**}	NS	ET>CS ^{**}
	χ^2	12.62 ^{**}	NW>CN [*]	NS	NS	NS	CN<ET [*]	CN<TEQ [*]	CN<CS ^{**}	NS	NS	NS
<i>H. corrugatum s.l.</i>	H	93.38 [*]	NW>CN [*]	NW>ET ^{**}	NW>TEQ [*]	NW>CS [*]	CN<ET [*]	CN<TEQ ^{**}	CN<CS ^{**}	NS	NS	NS
	χ^2	25.25 [*]	NW>CN [*]	NS	NS	NS	CN<ET [*]	CN<TEQ [*]	CN<CS [*]	NS	NS	NS
<i>B. vasculosum</i>	H	27.79 [*]	NS	NS	NS	NS	NS	NS	NS	NS	NS	Ab
	χ^2	10.24 ^{**}	NW>CN ^{**}	NW>ET [*]	NW>TEQ ^{**}	NW>CS [*]	NS	NS	NS	TEQ>ET ^{**}	NS	Ab
<i>R. pristis</i>	H	73.07 ^{**}	NW>CN [*]	NW>ET [*]	NW>TEQ [*]	NW>CS [*]	NS	NS	NS	NS	NS	NS
	χ^2	38.33 [*]	NW>CN [*]	NW>ET ^{**}	NW>TEQ [*]	NW>CS ^{**}	CN<ET ^{**}	NS	NS	TEQ<ET ^{**}	NS	NS

Ab, absent in both areas; *p<0.05; **p<0.01; NS, p>0.05.

DISCUSSION

The results of this study contribute important information on the parasite fauna of swordfish from the Atlantic Ocean. All the parasite taxa recovered were already known for the host species, but the genetic identification of the larval anisakid nematodes to species level enlarged the geographic range so far known for *A. physeteris*, *A. brevispiculata*, *A. pegreffii*, and *A. paggiae*, representing new geographic records for this host from the CS (Mattiucci and Nascetti, 2006, 2008).

The results showed both quantitative and qualitative differences between swordfish parasite fauna from the different areas. Among the selected taxa, the larvae of *A. simplex* (*s.s.*) were found only in fish from the NW and the CN, and *Anisakis* sp. 2 only in the ET and the TEQ. The other selected taxa were found in more than two areas and most showed different levels of infection, mainly in terms of MI. Most species showing differences between areas were parasites transmitted via trophic webs that can be accumulated in the host for a long period; this suggests that those parasites can be used in discriminating at least two distinct swordfish units in Atlantic waters studied so far. Lester *et al.* (1985) distinguished between permanent and semi-permanent, or temporary parasites, reflecting the probable longevity of each parasite taxon in its host. Among the dominant taxa identified in the present study, 11 were considered to be permanent because they have long lifespans in swordfish. These included two trypanorhynch (plerocercoid) species, *H. squali* and *S. viridis*, two acanthocephalans (cystacanths), *R. pristis* and *B. vasculosum*, and the larval anisakid nematodes, *Anisakis* spp. and *Hysterothylacium* spp. Trypanorhynchs are long-lived parasites at the plerocercoid stage (Chubb, 1980), because their life cycles include transmission through the food chain until they reach the final host, and this could cover a long period (Speare, 1995). The acanthocephalans generally have complex life cycles involving a number of hosts. Crustaceans are almost always the first intermediate host, and the swordfish an occasional host harbouring cystacanth stages that encyst in the body wall and could remain infective throughout the life of the host. Larval nematodes that have long lifespans in their intermediate/paratenic hosts tend to be more useful as biological tags than adult stages (Khan and Tuck, 1995). Those parasites are included usually with the groups suitable as biological tags for other fish (Mackenzie and Abaunza, 1998), including pelagic and demersal fish in the Atlantic (Mackenzie, 1983; Castro-Pampillón *et al.*, 2002b; Mattiucci *et al.*, 2004, 2007, 2008).

The remaining four dominant taxa were considered as temporary parasites because they have short lifespans in swordfish, surviving less than a year, so they were not able to discriminate any swordfish unit. They include the adult digenean *H. ventricosa*, the monogeneans *T. coccineum* and *T. integrum*, and the adult cestode *F. plicatus* (Speare, 1995; Oliva, 2001).

The intensity of infection by metazoan parasites commonly increases in older fish because they have had longer to accumulate parasites than younger fish (Poulin, 2000). Being larger, they also provide a larger area for parasite establishment, resulting in higher rates of infestation (Poulin, 2000). This could also be true for a specific host-parasite system considering particular ecological features, but other causes rather than host size/age could influence this relationship. In our study, there was no correlation between host size and the total number of parasites recovered in the ET and the CS, but in the NW and the CN, there was a positive correlation. A similar result was observed in *X. gladius* captured in the TEQ (Garcia *et al.*, 2008). In fact, the southern areas studied are important swordfish spawning and feeding grounds, and infection levels by endoparasites were similar in both juvenile and adult fish. In terms of the number of individual parasites, few positive relationships were observed with host size and no consistent pattern across all taxa in any area, suggesting that factors other than host size/age are responsible for the relationships observed. For example, *H. squali*, with a cumulative effect in the swordfish from the ET, was more abundant in the ET and the CN than the CS, where the fish were significantly larger. *Hysterothylacium squali* was also more abundant in the ET than in the NW, where there were no differences among host sizes. Despite showing a significant increase with fish size in the CS, *H. squali* was less abundant in smaller fish.

Differences in parasite distribution patterns within a host population could be the result of different foraging patterns, because the availability of food items changes in space (Caillaud *et al.*, 2006). This is particularly important for a highly migratory species such as swordfish, whose distribution covers a broad geographic range in the Atlantic Ocean. The swordfish parasite composition and burden is likely the combined result of host habitat and feeding behaviour, the occurrence and population size of the intermediate hosts of those parasites displaying an indirect life cycle in the sampling area, and the geographic distribution of some parasite species.

The multivariate techniques applied to the parasite species used here successfully discriminated at least two swordfish units in the Atlantic. The NW area was separated as

an independent group characterized by high levels of infection by *H. corrugatum* (*s.l.*) and *R. pristis*. The CN area was separated as a more heterogeneous group that could be explained by the influence of the Mediterranean swordfish population. *Anisakis simplex* (*s.s.*) could serve as a biological tag for both northern samples (NW and CN), although it was mainly associated with the CN, because the intensities of infection were higher in the CN than in the NW. The three southern areas (ET, TEQ, and SC) formed another independent group, whose integrity was especially evident after pooling fish from the ET, the TEQ, and the SC. *Anisakis paggiae* and *H. incurvum* were related to fish from all southern areas, whereas *Anisakis* sp. 2 did not help to distinguish the fish from the ET and the TEQ because of the lower levels of infection observed there.

Our results suggest the existence of several *X. gladius* units in the Atlantic Ocean. However, the boundary at latitude 5°N which is currently defined by ICCAT for managing Atlantic swordfish stocks might not be suitably adjusted to the stocks. Modern fisheries management is moving towards a precautionary approach to ensure sustainable utilization of marine resources. Begg and Waldman (1999) suggested a holistic approach to stock identification because the integrative effect of such an approach maximizes the likelihood of correctly defining fish stocks. The results so far acquired on the parasite fauna composition of Atlantic swordfish and the possible use of selected parasites as biological tags could improve fish stock definition. A multidisciplinary approach will allow future adjustment of the boundary between swordfish stocks in the Atlantic, leading to more efficient management and monitoring of the stocks.

ACKNOWLEDGEMENT

We thank PESCARADE and the crew of FV “Príncipe das Marés” for their assistance in swordfish sampling. The first author holds a PhD grant from the Portuguese Foundation for Science and Technology (ref. SFRH/BD/25391/2005) in cooperation with Sapienza-University of Rome and Tuscia-University of Viterbo, Italy. We are also grateful to Ken Mackenzie for his comments on an early draft of the manuscript, to two anonymous referees whose constructive comments improved the paper significantly, and to Harry Palm for his help with trypanorhynch identification.

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Chapter 3

Population structure of Atlantic swordfish (*Xiphias gladius* L. 1758) using mitochondrial DNA analysis: implications for fisheries management

Garcia, A., Cecchetti, S., Santos, M.N., Mattiucci, S., Nascetti, G., Cimmaruta, R. Population structure of Atlantic swordfish (*Xiphias gladius* L. 1758) using mitochondrial DNA analysis: implications for fisheries management. *Animal Biodiversity and Conservation* (submitted)

ABSTRACT

The swordfish (*Xiphias gladius* L. 1758) is a pelagic and highly migratory species inhabiting tropical and temperate waters from all oceans. Recent studies on the genetic structure of Atlantic swordfish have demonstrated significant heterogeneity within this water basin, but the precise boundary between populations remains to be identified. In this context, genetic diversity was investigated at the D-loop mitochondrial control region from 274 swordfish specimens collected from five different areas of the Atlantic Ocean (northwest, north central, northeast, northern tropical, and equatorial). The analysis of molecular variance (AMOVA) showed that genetic variation was mainly due to differences within rather than between the studied areas. Additionally, the phylogenetic analysis did not show evident relationships among haplotypes from all areas, as they are randomly allocated across the tree diagram. However, low but significant F_{ST} values are recorded when comparing equatorial samples with all the other from north Atlantic. These results do not support a need for changing the current management boundary for the Atlantic fishery.

INTRODUCTION

The swordfish (*Xiphias gladius* L. 1758) is a pelagic and highly migratory species inhabiting tropical and temperate waters, with a geographical range extending from 45°N to 45°S (Nakamura, 1985). The migratory ability of swordfish, combined with the lack of pronounced barriers to gene flow, has been thought to preclude the development of a strong signal of population genetic structure (Waples, 1998). However, studies on the genetic structure of swordfish populations (i.e. biological stocks) using both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) genome have evidenced significant inter-oceanic differentiation of Atlantic, Indo-Pacific and Mediterranean populations (Alvarado-Bremer *et al.*, 1995, 1996, 2005a; Kotoulas *et al.*, 1995; Rosel and Block, 1996; Chow *et al.*, 1997; Chow and Takeyama, 2000; Greig *et al.*, 2000; Jean *et al.*, 2006; Muth *et al.*, 2009). Within the Atlantic Ocean, the first studies failed to reveal any mtDNA population heterogeneity of swordfish populations (Alvarado-Bremer *et al.*, 1995; Rosel and Block, 1996), but as sampling effort increased a significant northwest *versus* south Atlantic genetic distinction was detected, both using

mtDNA (Alvarado-Bremer *et al.*, 1996, 2005a; Chow *et al.*, 1997) or nDNA markers (Greig *et al.*, 1999, 2000). Particularly, significant heterogeneity in mtDNA diversity was found between the northwest and south Atlantic regions as well as by means of both nuclear *loci* aldolase *B* (*aldB*) and lactate dehydrogenase *A* (*IdhA*) (Greig *et al.*, 1999, 2000). However, a large mtDNA survey failed to detect significant Atlantic heterogeneity, but a parallel study of nDNA calmodulin gene intron 4 (*CaM*) showed a sharp differentiation between northwest Atlantic swordfish with those from southern regions (Chow and Takeyama, 2000).

Despite the considerable advances in our understanding of Atlantic swordfish population structure that suggested the existence of genetic heterogeneity within the Atlantic, data so far acquired caused scientists and managers to question the validity of the 5°N hypothesis for the location of the boundary between the Atlantic populations. This is due to the fact that the genetic studies carried out so far share several limitations that still waiting to be solved (Alvarado-Bremer *et al.*, 2006). Firstly, the genetic homogeneity within north Atlantic (West versus East) swordfish has been poorly tested due to the limited sampling effort in the eastern region. As a further consequence, the degree of differentiation among swordfish from south and NE Atlantic has been poorly evaluated. Moreover, the differences between northwest and south Atlantic swordfish populations appear to be clear. Another point concerns the geographic boundary and the possible mixing of north and south Atlantic populations. Recently, Kasapidis *et al.* (2007) increased the sampling effort in northern Atlantic region and by means of microsatellites stated that genetic divergence among the swordfish specimens collected in NW, NE and mid-Atlantic areas appeared to be not significant. Also, these authors suggested a north-south reduction in gene flow within the Atlantic but recommended further studies that could elucidate the genetic structure of swordfish in this water basin.

Management implications of such questions are obvious, since the Atlantic swordfish is subject to intense harvest and to date managed as two stocks that are regulated by quotas assignments. Thus, univocal data supporting a sustainable management of the Atlantic swordfish are urgently needed, being the first step the genetic characterization of each population to assess the stock boundary and their “health” state. In fact, information on the population structure of commercially exploited fish stocks has now been widely acknowledged as crucial for their conservation and management (Hilborn *et al.*, 2003). They would indeed allow establishing conservation policies that can

protect locally adapted populations and drive the management efforts appropriately by regulating fishing activities.

In the present study, the highly polymorphic control region of the mtDNA (D-loop) was used to characterise the genetic diversity within and among swordfishes from different Atlantic areas and to examine their level of genetic divergence through: i) investigating the population structure of north and central Atlantic swordfish; and ii) testing the heterogeneity among swordfish specimens collected from distinct geographic areas.

MATERIAL AND METHODS

Sampling

Muscle tissue samples were collected from 274 swordfish specimens, onboard commercial longline fishing vessels, between May 2003 and November 2007 in five different areas throughout the Atlantic Ocean, namely: northwest (NW, n=39, July 2006), north central (NC, n=53, May 2003), northeast (NE, n=51, May 2003), northern tropical (TR, n=48, June-August 2007) and equatorial (EQ, n=83, June-November 2007) (Figure 1). A small portion of muscle tissue was taken from each specimen using a sterilized scalpel, stored in an eppendorf tube and kept frozen at -80°C until assayed.

DNA extraction

DNA extraction was based on CTAB (Cetyl Trimethyl Ammonium Bromide) method adapted from Murray and Thompson (1980) protocol, as follows. Approximately 200mg of tissue was placed in 1.5ml eppendorf tube and homogenized with 500µl of CTAB-buffer 2% (tris-HCl 0.1M, pH 8; NaCl 1.4M; EDTA 0.02M) and 5µl of Proteinase K (20 µg/ml). The tissue was then digested by incubation in water-bath for three hours at 55°C. After incubation, 500µl of phenol-chloroform were added and mixed by gently shaking the tubes, followed by centrifugation at 13,000 rpm during 15 minutes for protein precipitation. The supernatant was taken out and 500µl of chloroform were added to the lower phase. The tube was then manually shaken and

centrifuged again for 15 minutes at 13,000 rpm. The supernatant was again removed and 1000 μ l of ethanol (100%) and 20 μ l of NaCl (5M) were added to the lower phase, for precipitation at -20°C during 30 minutes. Then the solution was centrifuged for 15 minutes at 13,000 rpm and the upper phase discarded. A total of 500 μ l of ethanol (70%) were added, centrifuged and the supernatant removed. The DNA pellet was then dried in the thermoblock for 30 minutes and re-suspended in 100 μ l of TE-buffer (EDTA 1mM and tris 10mM, pH 8). DNA was then diluted (1:50) with distilled water and so suitable for polymerase chain reaction (PCR).

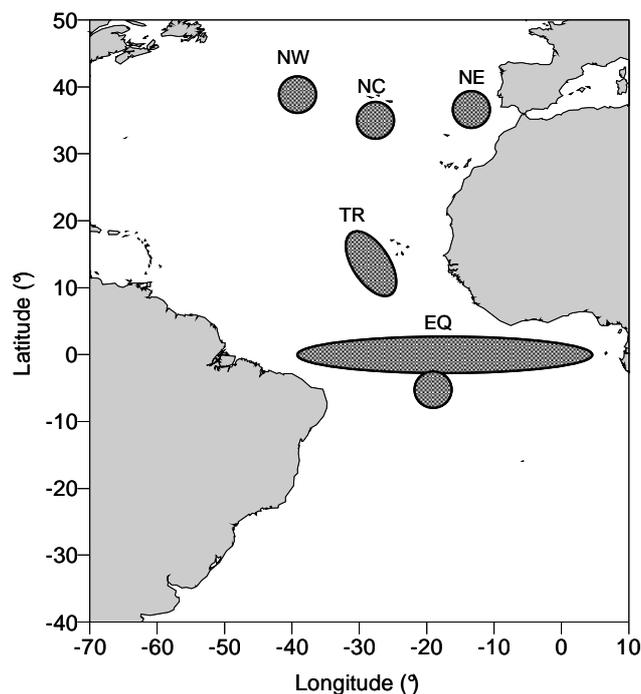


Figure 1. Swordfish sampling areas in the Atlantic Ocean: northwest (NW), north central (NC), northeast (NE), northern tropical (TR) and equatorial (EQ).

PCR amplification

The DNA amplification was achieved using the primers specifically designed for the control region of swordfish (Alvarado-Bremer *et al.*, 1995). The L-strand primer L15998 (5'-TAC CCC AAA CTC CCA AAG CTA-3') was used in combination with the H-strand primer H235 (5'-CGT GTG CAC TCT GAA ATG TCA-3') to amplify a DNA fragment composed by *c.* 525bp using a Perkin Elmer thermal cycler apparatus (GeneAmp 2400). The PCR reaction was carried out in 0.2ml eppendorf tube containing

45µl master mix (1µl of each primers, 2µl MgCl₂, 5µl EDTA-buffer, 1µl dNTP, 0.5µl Taq polymerase, and 34.5µl distilled water) and 5µl of extracted template DNA. The PCR reaction was then cycled for 4 min at 94°C followed by 35 cycles of 1 sec at 94°C (denaturing), 1 sec at 50°C (annealing), 1 sec at 72°C (extension) and the final (extension) cycle at 72°C for 10 sec. Negative controls without DNA template were prepared in every series of amplification in order to exclude the possibility of contamination of reagents or reaction buffers. The amplified product was checked by electrophoresis in 2.0% agarose gel using TBE-buffer (0.045M tris-borate; 0.001M EDTA, pH 8) stained with 0.01% ethidium bromide.

Restriction Fragment Length Polymorphism (RFLP) analysis

Five restriction enzymes (*Alu I*, *Dra I*, *Vsp I*, *Hpa II* and *Dde I*) were used to digest the amplified fragments. Digests were performed in 7.5µl final reaction volume with 5.0µl of PCR product, 0.5µl of restriction enzyme, 1.0µl of the manufacture's (Promega) recommended buffer and 1.0µl of bovine serum albumin (BSA). Two mineral oil drops were added to the reaction mixture to keep the final reagent concentration by avoiding their evaporation. After centrifuged the reaction mixture the digestions were incubated for 3h at 37°C for all the enzymes used in the study. The resulting restricted fragments were analysed by electrophoresis on 3.0% agarose gel using TBE-buffer (0.045M tris-borato; 0.001M EDTA, pH 8) stained with 0.01% ethidium bromide and finally photographed. One molecular weight marker and one reference PCR product was run along with the digested PCR products in order to estimate the sizes of the resulting mtDNA fragments.

Restriction patterns generated from each restriction endonuclease were labelled with letters (A, B, C and D) indicating variant digestion patterns (Table 1). Composite mtDNA haplotypes were constructed from all the enzymes used and arranged in the follow order: *Alu I*, *Dra I*, *Vsp I*, *Hpa II* and *Dde I*. Thus, to each fish was assigned a code of five letters that described its composition in terms of multi-enzyme haplotype.

Table 1. Restriction patterns of the mtDNA D-loop haplotypes recorded in 274 swordfishes from Atlantic Ocean (values show restriction fragments length in bp).

Restriction enzymes	Recognition sequence	Haplotype				
		A	B	C	D	E
<i>Alu I</i>	AG-CT	525	273	177	94	241
			252	348	177	
					254	
<i>Dra I</i>	TTT-AAA	25	227	25	525	
		294	298	500		
		206				
<i>Vsp I</i>	AT-TAAT	112	230	111		
		291	295	414		
		122				
<i>Hpa II</i>	C-CGG	490	262	525		
		35	224			
			39			
<i>Dde I</i>	C-TNAG	275	192	525	274	
		250	81		155	
			252		96	

Genetic data analysis

Chromatographic curves of forward and reverse sequences were edited in Chromas v.1.6. All sequences were then aligned by eye using ClustalX v.1.83 (Thompson *et al.*, 1997). The amount of sequence divergence for each geographical population was assessed by estimating the number of polymorphic sites (S), haplotype diversity (h ; Nei, 1987), nucleotide diversity (π , Nei, 1987) and average number of pairwise nucleotide differences (k ; Tajima, 1983) all performed using Arlequin v.3.1 (Excoffier *et al.*, 2005).

The level of genetic diversity within and among sampled areas was hierarchically evaluated by analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992). Significance of pairwise comparison was tested with 10,000 permutations. Samples were hierarchically divided in two groups (1: NW, NC, NE and TR; 2: EQ) to test the accuracy of the current management boundary set at 5°N (see Figure 1). Tajima (1989) D -test and Fu (1997) F_S -test were used to test the deviation from neutral molecular evolution in relation to mtDNA sequences. Significance was assessed in both tests by generating random samples (1000 simulated samples) under the hypothesis of selective

neutrality and population equilibrium. Both AMOVA and neutrality tests were performed with Arlequin v.3.1 (Excoffier *et al.*, 2005).

The phylogenetic relationships among haplotypes were graphically arranged in Mega v.4 (Tamura *et al.*, 2007) with unrooted neighbour-joining (NJ) dendrogram using the gamma corrected Tamura-Nei distance matrix. The statistical robustness of NJ distances was determined by 1,000 bootstrap replicates (Felsenstein, 1985).

RESULTS

Molecular attributes

A single fragment of approximately 525 bp was amplified from each specimen and no apparent size differences among them were observed. Restriction profiles obtained by each of the five endonucleases showed five patterns in *Alu I*, four in *Dra I*, three in *Vsp I*, three in *Hpa II* and four in *Dde I* (Table 1). The majority of the samples shared the most common restriction pattern for each endonuclease digestion, despite the difference in their frequencies. Pattern A in *Alu I* digestion was the most common in EQ, while pattern B prevailed in the remaining samples. Pattern A in *Dra I*, *Vsp I*, *Hpa II* and *Dde I* were the most frequent in all the sampled areas.

A total of 68 composite haplotypes were recorded but only one (BAAAA) was distributed randomly among the five areas with relatively high frequency (NW=38.5%; NC=49.1%; NE=35.3%; TR=45.8%; EQ=20.5%) and 38 were limited to one area, namely: 8 in NW, 2 in NC, 6 in NE, 7 in TR and 15 in EQ. A total of 84 polymorphic nucleotide sites were observed, from which 19 were singleton variable sites and 65 were parsimony informative. The substitution bias favoured transitions over transversions being their ratio 10.9. The overall relative nucleotide frequencies were: C=20.7%, T=31.2%, A=31.7% and G=16.5%.

For the pooled samples, genetic variability was high displaying a value of 0.860 ± 0.019 for haplotype diversity (h) and 0.022 ± 0.011 for nucleotide diversity (π). The h ranged between 0.935 ± 0.017 in EQ and 0.755 ± 0.063 in NC while π from 0.026 ± 0.013 (EQ) to 0.017 ± 0.009 (TR) (Table 2).

Table 2. Genetic variability of Atlantic swordfish within the five sampled areas.

Sampling area	n	H	S	$h \pm \text{s.d.}$	$k \pm \text{s.d.}$	$\pi \pm \text{s.d.}$	Tajima's D test	Fu's F_S test
NW	39	21	58	0.852 ± 0.056	11.178 ± 5.187	0.021 ± 0.011	-0.742	-1.988
NC	53	19	63	0.755 ± 0.063	11.144 ± 5.142	0.021 ± 0.011	-0.702	0.700
NE	51	23	65	0.868 ± 0.043	10.534 ± 4.880	0.020 ± 0.010	-0.914	-1.877
TR	48	20	61	0.781 ± 0.060	8.843 ± 4.150	0.017 ± 0.009	-1.288	-1.456
EQ	83	39	77	0.935 ± 0.017	13.748 ± 6.236	0.026 ± 0.013	-0.394	-6.050

n, sample size; H, number of haplotypes; S, number of polymorphic sites; h , haplotype diversity; k , mean pairwise nucleotide differences; π , nucleotide diversity

Population structure and phylogeny

The overall hierarchical AMOVA showed that the greatest genetic differentiation (97.03%, $p=0.005$) in swordfish control region was found within distinct geographical areas and a very small amount (0.02%, $p=0.354$) was due to divergence among distinct geographical areas within groups (Table 3). The amount of variance among groups was 2.95% and did not display statistically significant differences ($p=0.191$). If only one AMOVA group was considered in the analysis, the results showed again that the majority of the variance was explained by differences within areas (98.10%, $p=0.001$) rather than among them (1.90%). The highest pairwise F_{ST} values were found between EQ and NC (0.042, $p<0.05$) and the lowest between TR and NW (-0.002, $p>0.05$) (Table 4). It is worth noting that the only statistically significant on pairwise F_{ST} values were those occurring between EQ and the remaining areas (Table 4).

Table 3. Hierarchical AMOVA analysis on the Atlantic swordfish molecular data.

Source of variation	Variance components	Percentage of variation	Fixation indices
Among groups	0.012	2.95	$F_{CT} = 0.030$
Among areas within groups	0.000	0.02	$F_{SC} = 0.000$
Within areas	0.424	97.03	$F_{ST} = 0.030^*$

Group 1: NW, NC, NE and TR; Group 2: EQ; * Significant at 0.05 level

Table 4. Pairwise F_{ST} values among the five swordfish sampled areas in Atlantic Ocean. The F_{ST} values were calculated with 110 permutations.

	NW	NC	NE	TR	EQ
NW					
NC	-0.00329				
NE	-0.00213	0.00714			
TR	-0.00183	-0.00484	0.00938		
EQ	0.01689*	0.04195*	0.01633*	0.03336*	

*Significant at 0.05 level

The indices of neutral evolution (Tajima's D and Fu's F_S tests) yielded moderately low negative values in the large part of the sampled areas, but failed to detect statistically significant differences (Table 2).

The topology of the gene tree obtained using NJ analysis showed that there is no evident phylogeographic relationship among haplotypes from the different geographical areas, as they are randomly allocated across the tree diagram (Figure 2). No apparent latitudinal gradient of the proportion of each haplotype was observed, excepting the most common haplotype (BAAAA) that seems to decrease with latitude. Moreover, only 19 branches received values higher than 70%.

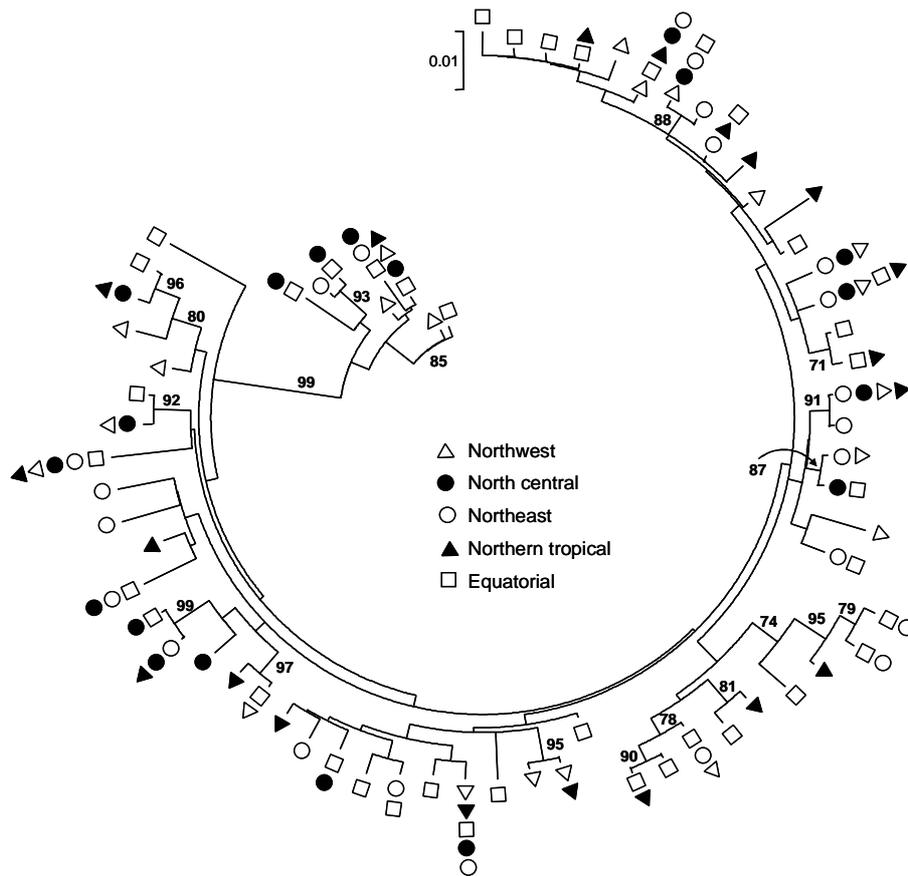


Figure 2. Neighbor-joining (NJ) tree of the 68 haplotypes from Atlantic swordfish. Bootstrap value support above 70% is shown by the branches and symbols are related with the geographic origin of each haplotype.

DISCUSSION

The mitochondrial control region sequence of swordfish showed moderately high levels of variation with 16% of the nucleotide positions being polymorphic. However, the substitution bias ratio ($r=10.9$) herein achieved for swordfish was smaller than those recorded by Alvarado-Bremer *et al.* (1997) in albacore ($r=9.0$) mitochondrial genome. All the samples were characterized by high levels of haplotype diversity, with 56% of the haplotypes as private of a single area. Such pattern was already reported for other scombrid species, such as albacore (Chow and Ushiana, 1995; Viñas *et al.*, 2004). The nucleotide diversity found was within the range reported for Atlantic swordfish by Alvarado-Bremer *et al.* (2005a), but lower than the values found for other highly migratory species, such as albacore (Viñas *et al.*, 2004) bigeye tuna (Martinez *et al.*, 2006) and bluefin tuna (Carlsson *et al.*, 2004). High genetic diversity within geographic

areas and low genetic differentiation among areas within the same ocean basin are commonly observed in large pelagic marine fishes and could be explained by their wide distribution range, that favour the gene flow, and their large population sizes (Avisé, 1998). Accordingly, the indices of neutral evolution recorded revealed that all mtDNA D-loop mutations are selectively neutral or in equilibrium, allowing to exclude a role of selective pressures.

In the present study, no appreciable variance was attributable to variation among groups (north *versus* south, divided at 5°N), despite the significant heterogeneity found within the sampling areas. The lack of evident structure among the north Atlantic samples (NW, NC, NE and TR) was also confirmed by the moderately low F_{ST} statistics, which pointed out that genetic exchange rate between them are sufficient to prevent genetic divergence. However, it is worth noting that F_{ST} values are significantly different when the equatorial samples (EQ) are compared with all the other from north Atlantic. This observation would confirm that the equatorial area (between 5°N and 10°S) may represent a zone of intergradation within the Atlantic Ocean. Such fact may also be supported by the finding that the higher levels of genetic divergence were recorded exactly within the equatorial samples. Unfortunately, the reduced sampling effort in the south Atlantic area probably did not allowed the detection of north-south stock differences. Therefore, the present results do not support a need for changing the current management boundary at 5°N.

The absence of an evident genetic differentiation among the NW, NC, NE and TR samples did not exclude the two-stocks hypothesis (separate the west and east) considered for managing the Atlantic swordfish fisheries (Miyake and Rey, 1989), as the sample coverage was limited to the East of 40°W. Kasapidis *et al.* (2007) also failed to detect a west-east difference. However, as in the present study, the west area was limited to longitude 47°W. Moreover, for the same reason, the possible subdivision between NW Atlantic (west of 40°W) and south Atlantic swordfish could not be excluded. In fact, such division was strongly supported by concordant results at both mtDNA and scnDNA data (Alvarado-Bremer *et al.*, 2005a). Furthermore, the extent of mixing between Atlantic swordfish samples should cover a large area southern to the current boundary location, but the present study does not suggest evidence for stock mixing northern than 5°N. Indeed, the results presented here did not revealed significant heterogeneity in mtDNA diversity between the samples collected southern and northern than 30°N, as suggested by Chow and Takeyama (2000) analysing CaM locus.

Kasapidis *et al.* (2007) also failed to detect genetic divergences among northern and mid-Atlantic areas.

At present, the main swordfish fisheries in the Atlantic Ocean are managed as two different units, north and south. The management unit definition recognizes genetically structured populations connected by limited gene flow. The results achieved with the present study did not give evidences to change the current management boundary for the Atlantic swordfish fishery, as slight genetic structuring was observed only for the equatorial area. Further examination of a larger number of samples from the southern Atlantic is needed to confirm and possibly quantify the extent of genetic differentiation reported herein.

ACKNOWLEDGMENTS

The authors express their gratitude to PESCARADE and to the crews of the fishing vessels “Príncipe das Marés” and “Paula Filipa” for their assistance in swordfish sampling. The first author holds a PhD grant from the Portuguese Foundation for Science and Technology (SFRH/BD/25391/2005) in co-operation with the University of Rome “La Sapienza” and University of Viterbo “Tuscia ” in Italy.

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Chapter 4

Fin ray morphometrics for swordfish (*Xiphias gladius* L. 1758) from the Atlantic Ocean

Garcia, A. and Santos, M.N. Fin ray morphometrics for swordfish (*Xiphias gladius* L. 1758) from the Atlantic Ocean. *Journal of Applied Ichthyology* (submitted)

ABSTRACT

Morphologic differentiation of swordfish caught in east tropical, equatorial and south central Atlantic waters was investigated using morphometric characters of the second ray of the anal fin. Regression analysis revealed that fin ray growth in width was less variable than growth in height and fin ray width increases more rapidly than fish length. Discriminant analysis suggested significant phenotypic heterogeneity among areas. The tropical samples were the most isolated, while the equatorial and south central samples were clustered together.

INTRODUCTION

Morphometric characters are important elements in the species identification and characterizing strains of the same species (Chondar, 1973; Jaiswar *et al.*, 2004). A mathematical representation of the relationships among different morphological characters derived from the study of different sexes from a particular area is very useful tool for the study of biology, physiology, ecology, population dynamics, fisheries assessment, and general condition of the study population (Jaiswar *et al.*, 2004). Therefore, the range of variation between morphometric characters of a fish species could also give useful information to delineate fish stocks (Turan, 2004). Phenotypic plasticity of fishes allows them to respond adaptively to environmental changes by modification in their morphology, reproduction or survival to mitigate the effects of these variations (Stearns, 1983; Meyer, 1987). Such phenotypic adaptations do not necessarily result in genetic changes and thus, the detection of phenotypic differences among populations can not usually be taken as evidence of genetic differentiation (Ihssen *et al.*, 1981; Allendorf, 1988). In fact, the ability of the populations to adapt and evolve their biological attributes according with environmental conditions is reported in several studies as it is frequently employed to measure stocks discreteness (Turan, 1999; Jaiswar *et al.*, 2004; Turan 2004; Omoniyi and Agbon, 2008).

Swordfish (*Xiphias gladius* L. 1758) is a widely distributed pelagic fish that can be found in all Oceans between latitudes 45°N and 45°S (Nakamura, 1985). The second anal fin ray has been extensively used in swordfish ageing studies, used by the International Commission for the Conservation of Atlantic Tuna (ICCAT) on stock

assessment models for this species (Tsimenides and Tserpes, 1989; Ehrhardt, 1992; Tserpes and Tsimenides, 1995; Uchiyama *et al.*, 1998; Sun *et al.*, 2002; DeMartini *et al.*, 2007). Despite the existence of a number of studies concerning several aspects of Atlantic swordfish populations (e.g. Govoni *et al.*, 2003; Chancollon *et al.*, 2006; Garcia *et al.*, 2008), to the authors best knowledge, there is no information on morphometric structure of anal fin rays, particularly the second ray of the anal fin. The main objective of the present study was to estimate relationships between body length and five fin ray measurements, for swordfishes caught in the Central Atlantic Ocean. Secondly, we assessed possible phenotypic differentiation between three areas, providing new data on stock identification.

MATERIAL AND METHODS

Swordfish samples were collected onboard commercial longline vessels, between 2006 and 2009 in three different areas throughout the Atlantic Ocean, namely: east tropical (TR), equatorial (EQ) and south east (SE) areas (Figure 1). For each specimen, the lower-jaw-fork-length (LJFL, to nearest centimeter) was taken, sex was determined and the second ray of anal fin was removed and frozen until assayed. In the laboratory each anal fin was thawed and immersed in boiling water for a few minutes to remove the second fin ray cleaned from skin and tissue.

The morphometric analysis was carried out with linear measurements of the second ray of the anal fin, namely: the total straight height (SH), basal width (TW), maximum width above the basal area (MaW) and minimum width above the basal area (MiW) (Figure 2). All variables were measured with a digital calliper to the nearest 0.01 mm. Besides, linear measurements on the an arc height (AH) were made to the nearest 1 mm using a tape measure, in order to better characterise the shape (particularly the curvature) of the fin rays (Figure 2).

Differences in swordfish mean length between sexes for the pooled areas were assessed by analysis of variance (ANOVA), while the estimation of the morphometric relationships between swordfish length (LJFL) and the second fin ray measurements was made by adjustment of a linear function to the data (Ricker, 1973):

$$\text{Log } Y = \text{Log } a + b \text{ Log } X,$$

where Y = Fin ray measurement (SH, TW, MaW, MiW or AH); X = size of the fish (LJFL); a = intercept (initial growth coefficient); b = slope (relative growth rate of variables).

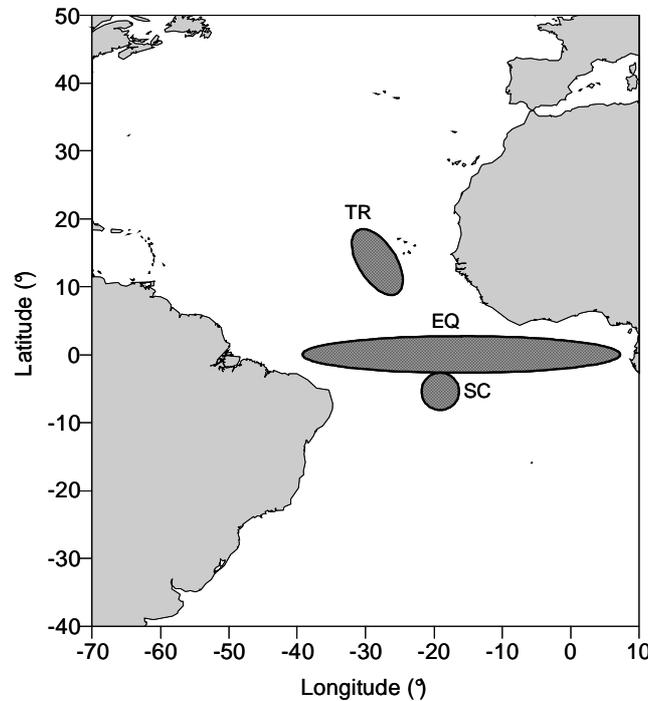


Figure 1. Swordfish sampling areas in the Atlantic Ocean: east tropical (TR), equatorial (EQ) and south central (SC).

The parameters a and b of the morphometric relationships were estimated through regression analysis (least squares method) and the association degree between variables was calculated by the correlation coefficient (r). The type of relationship was assessed through the allometry coefficient (b). If the b values obtained in the linear regressions were significantly different from the isometric value ($b=1$), a t -test ($H_0: b=1$) with a confidence level of $\pm 95\%$ ($\alpha=0.05$) was applied and expressed by the following equation (Sokal and Rohlf, 1987):

$$t_s = (b - 1)/s_b$$

where t_s = t -test value; b = slope (relative growth rates of variables); s_b = standard error of the slope (b). The intervals of b , the range of variation and the standard error were also estimated.

Subsequently, the comparison between the obtained values of t -test and the correspondent tabled critical values, allowed for determination of the statistical significance of the b values, and their inclusion in the isometric range ($b=1$) or allometric ranges (negative allometry: $b<1$; positive allometry: $b>1$). The t -test was also used to compare b values achieved for the same relationship between sexes, for the pooled samples, and between areas for the same sex.

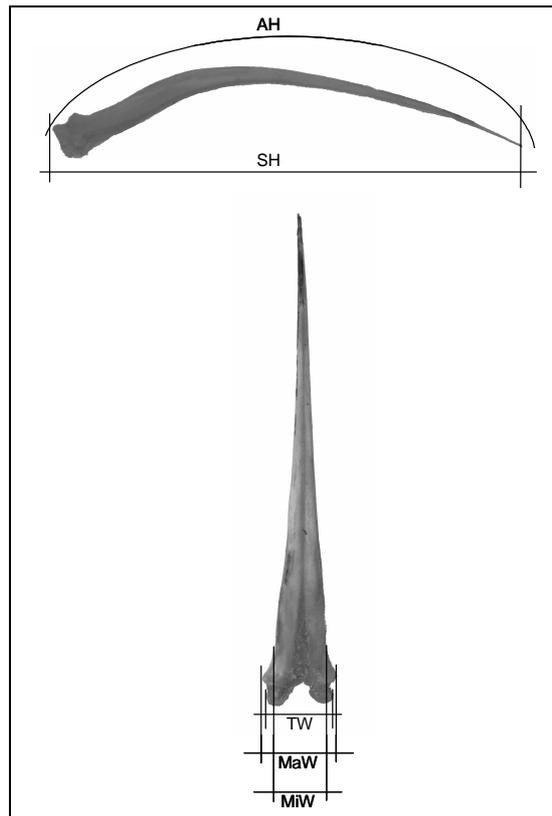


Figure 2. Schematic representation of the second ray of the swordfish anal fin measurements (AH, arc height; SH, straight height; TW, basal width; MaW, maximum width above the basal area; MiW, minimum width above the basal area).

A stepwise Linear Discriminant Analysis (LDA) was carried out to identify the degree of similarity among areas and the relative importance of each morphometric characters for group separation. In the present study, there were significant correlations between body length and morphometric characters. Therefore, transformation of absolute measurements to size-independent variables was carried out using the formula (Elliott *et al.*, 1995):

$$M_{\text{adj}} = M(L_S/L_0)^b$$

where M is the original morphometric measurement, M_{adj} the size adjusted measurement, L_0 the standard length of fish (LJFL), and L_S the overall mean of standard length of all fish from each variable. The b parameter was previously estimated by regression analysis.

The importance of each character was assessed using backward stepwise method based on Mahalanobis distances (Suzuki *et al.*, 2008). Multivariate LDA analysis was carried out in Brodgar 2.5.1. package (Brodgar, 2006), while regression analysis was conducted in Microsoft Excel[®] 2003.

RESULTS

A total of 667 swordfishes (277 males and 390 females) were sampled, ranging in size between 80 and 255 cm LJFL (Table 1). Statistically significant differences were found in swordfish mean sizes among sexes for the pooled areas (ANOVA: $F=184.768$, $p<0.001$) being the females larger than males.

The morphometric relationships determined by area and sex are given in Table 2. The results revealed that most correlation coefficient (r) were invariably higher for females than for males. The r values were greater than 0.87, except in the case of LJFL vs. fin ray height (AH or SH) relationships. Moreover, most regressions were highly significant ($P<0.001$). The fin ray morphometric relationships indicate a clear prevalence of positive allometry (60%) over negative allometry.

For the pooled samples the comparison between females and males showed that almost all b values achieved from the studied relationships were not significantly different ($p>0.05$) except the LJFL vs. TW relationship that display statistical significant differences among sexes ($p<0.05$). The comparison between the b values achieved for each area revealed the absence of statistically significant differences among specimens from the same sex ($p>0.05$). Thus, multivariate LDA analysis was carried out for sexes combined.

Table 1. Descriptive statistics of the morphometric measurements of Atlantic swordfish: LJFL, Lower-jaw-fork-length; SH, total straight height; AH, arc height; TW, basal width; MaW, maximum width above the basal area; and MiW, minimum width above the basal area.

Area	Morphometric measurements	Males			Females		
		N	Mean±s.d. (mm)	Range (mm)	N	Mean±s.d. (mm)	Range (mm)
Tropical	LJFL	61	1277±349	940-1890	66	1374±349	820-2230
	SH	60	111.54±38.88	50.05-160.77	64	119.91±39.21	58.94-216.00
	AH	60	119.42±42.24	51.00-173.00	64	127.89±43.63	59.00-239.00
	TW	59	16.11±6.72	10.33-27.12	63	18.41±6.72	7.68-34.62
	MaW	59	15.76±5.68	10.91-24.96	63	17.50±5.67	9.73-34.04
	MiW	53	10.64±4.64	6.23-18.62	60	11.88±4.64	4.78-21.29
Equatorial	LJFL	159	1390±338	800-2060	239	1624±339	820-2510
	SH	159	123.54±41.82	48.00-273.00	232	138.02±41.94	42.00-280.00
	AH	159	132.08±46.52	50.00-295.00	232	148.56±46.65	47.00-305.00
	TW	157	18.38±6.71	7.97-33.39	237	23.63±6.72	7.60-42.45
	MaW	156	18.00±5.67	9.99-29.41	238	22.24±5.67	8.61-40.01
	MiW	149	12.73±4.57	6.48-21.44	231	16.01±4.58	5.10-28.47
South Central	LJFL	57	1462±322	940-1900	85	1710±322	950-2550
	SH	56	130.70±43.23	58.15-212.00	84	148.56±43.20	52.11-280.00
	AH	56	138.89±47.74	61.00-227.00	84	159.09±47.81	57.00-304.00
	TW	57	20.05±6.42	10.83-29.78	85	25.20±6.43	13.60-40.05
	MaW	57	19.36±5.57	10.33-27.51	85	24.07±5.57	13.78-36.47
	MiW	45	13.92±4.44	7.62-19.76	80	17.09±4.44	8.60-29.79

The multivariate LDA showed that the first two discriminant functions explained 99.5% (eigenvalue = 1.729) and 0.5% (eigenvalue = 0.008), respectively. A statistically significant group effect was found (Wilks $\lambda = 0.363$, $F_{80,513}$, $P < 0.01$). The spatial distribution of individuals represented by the samples scores and the group means illustrated a clear separation between the TR samples and the samples from the other two areas (EQ and SC) (Figure 3). Each swordfish specimen was correctly assigned to one of the five sampling areas with an accuracy of 70.9%, being highest in the case of the TR samples (93.8%). The MiW was the variable that contributed most for the

Table 2. Parameters of the morphometric relationships established between swordfish length (LJFL) and measurements of the second ray of the anal fin (SH, AH, TW, MaW and MiW). [N = number of individuals; a = intercept (initial growth coefficient); b = slope (relative growth rate of variables); S.E. = standard error; and N.S. = not significant]

Area	Morphometric relationship	Sex	N	a	$b \pm$ S.E. (range)	Correlation coefficient (r)	F	p -value	t -test	Type of growth
Tropical	LJFL vs. SH	♂	60	99.781	0.014±0.256 (-0.498-0.526)	0.007	0.003	0.956 ^{NS}	3.85	- allometry
		♀	64	17.917	0.377±0.191 (-0.004-0.759)	0.243	3.907	0.053 ^{NS}	3.26	- allometry
		Combined	124	28.941	0.275±0.150 (-0.023-0.572)	0.163	3.348	0.070 ^{NS}	4.82	- allometry
	LJFL vs. AH	♂	60	108.116	0.011±0.269 (-0.527-0.548)	0.005	0.002	0.968 ^{NS}	3.68	- allometry
		♀	64	17.162	0.399±0.198 (0.003-0.795)	0.248	4.047	0.049	3.04	- allometry
		Combined	124	29.160	0.286±0.157 (-0.024-0.596)	0.163	3.336	0.070 ^{NS}	4.56	- allometry
	LJFL vs. TW	♂	59	0.053	1.177±0.089 (0.999-1.355)	0.868	174.776	<0.001	1.99	+ allometry
		♀	63	0.029	1.302±0.060 (1.183-1.422)	0.941	472.760	<0.001	5.05	+ allometry
		Combined	122	0.034	1.273±0.049 (1.176-1.371)	0.921	669.188	<0.001	5.56	+ allometry
	LJFL vs. MaW	♂	59	0.057	1.156±0.078 (1.000-1.313)	0.891	218.839	<0.001	2.00	+ allometry
		♀	63	0.065	1.133±0.063 (1.006-1.260)	0.916	319.449	<0.001	2.10	+ allometry
		Combined	122	0.061	1.145±0.047 (1.051-1.239)	0.911	582.051	<0.001	3.05	+ allometry
	LJFL vs. MiW	♂	53	0.016	1.335±0.109 (1.116-1.554)	0.864	149.667	<0.001	3.07	+ allometry
		♀	60	0.022	1.276±0.065 (1.145-1.407)	0.932	380.314	<0.001	4.22	+ allometry
		Combined	113	0.020	1.293±0.055 (1.184-1.403)	0.912	548.840	<0.001	5.31	+ allometry
Equatorial	LJFL vs. SH	♂	159	11.770	0.467±0.138 (0.194-0.740)	0.260	11.397	<0.001	3.85	- allometry
		♀	232	16.090	0.414±0.097 (0.224-0.605)	0.272	18.346	<0.001	6.06	- allometry
		Combined	391	12.092	0.467±0.075 (0.319-0.615)	0.300	38.571	<0.001	7.09	- allometry
	LJFL vs. AH	♂	159	10.523	0.502±0.142 (0.221-0.784)	0.271	12.435	<0.001	3.49	- allometry
		♀	232	14.313	0.451±0.099 (0.257-0.646)	0.289	20.973	<0.001	5.57	- allometry
		Combined	391	10.672	0.506±0.077 (0.354-0.657)	0.316	43.125	<0.001	6.42	- allometry
LJFL vs. TW	♂	157	0.047	1.206±0.051 (1.106-1.306)	0.887	569.928	<0.001	4.07	+ allometry	

	♀	237	0.025	1.345±0.033 (1.279-1.410)	0.935	1637.564	<0.001	10.37	+ allometry
	Combined	394	0.026	1.333±0.027 (1.279-1.386)	0.928	2437.233	<0.001	12.32	+ allometry
	♂	156	0.079	1.097±0.039 (1.020-1.174)	0.916	798.975	<0.001	2.50	+ allometry
LJFL vs. MaW	♀	238	0.056	1.174±0.030 (1.114-1.234)	0.929	1484.647	<0.001	5.71	+ allometry
	Combined	394	0.056	1.172±0.023 (1.127-1.217)	0.932	2611.157	<0.001	7.51	+ allometry
	♂	149	0.028	1.236±0.049 (1.138-1.334)	0.900	632.668	<0.001	4.77	+ allometry
LJFL vs. MiW	♀	231	0.018	1.328±0.033 (1.263-1.393)	0.936	1626.299	<0.001	9.95	+ allometry
	Combined	380	0.019	1.318±0.026 (1.267-1.370)	0.933	2525.194	<0.001	12.13	+ allometry
South Central	♂	56	15.615	0.419±0.240 (-0.061-0.900)	0.232	3.059	0.086 ^{NS}	2.42	- allometry
LJFL vs. SH	♀	84	58.279	0.171±0.199 (-0.224-0.566)	0.095	0.745	0.391 ^{NS}	4.17	- allometry
	Combined	140	27.009	0.317±0.142 (0.037-0.597)	0.187	5.001	0.027	4.83	- allometry
	♂	56	12.892	0.470±0.244 (-0.020-0.959)	0.253	3.696	0.060 ^{NS}	2.17	- allometry
LJFL vs. AH	♀	84	49.270	0.217±0.201 (-0.183-0.617)	0.118	1.164	0.284 ^{NS}	3.89	- allometry
	Combined	140	22.634	0.364±0.144 (0.080-0.648)	0.211	6.418	0.012	4.43	- allometry
	♂	57	0.045	1.224±0.073 (1.078-1.370)	0.915	282.734	<0.001	3.07	+ allometry
LJFL vs. TW	♀	85	0.039	1.255±0.057 (1.140-1.369)	0.923	477.439	<0.001	4.43	+ allometry
	Combined	142	0.036	1.273±0.042 (1.190-1.355)	0.932	929.100	<0.001	6.53	+ allometry
	♂	57	0.071	1.125±0.066 (0.992-1.258)	0.916	288.660	<0.001	1.89	+ allometry
LJFL vs. MaW	♀	85	0.071	1.130±0.056 (1.020-1.241)	0.912	412.815	<0.001	2.35	+ allometry
	Combined	142	0.059	1.165±0.040 (1.086-1.244)	0.926	848.150	<0.001	4.12	+ allometry
	♂	45	0.024	1.274±0.068 (1.137-1.411)	0.944	352.910	<0.001	4.04	+ allometry
LJFL vs. MiW	♀	80	0.023	1.287±0.058 (1.171-1.403)	0.929	490.838	<0.001	4.94	+ allometry
	Combined	125	0.022	1.294±0.042 (1.210-1.377)	0.941	947.772	<0.001	6.99	+ allometry

separation between areas, followed by MaW and TW (Figure 4). The importance of variables SH and AH was negligible in terms of Mahalanobis distances, thus were excluded from the analysis.

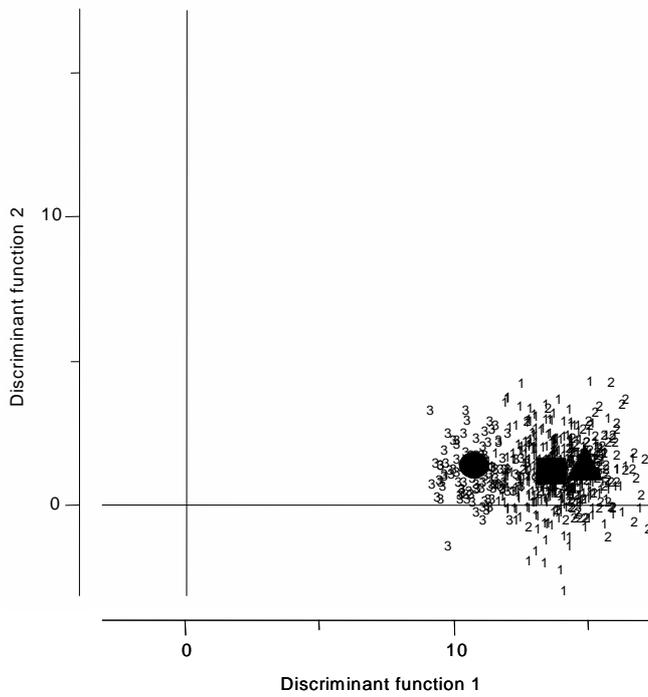


Figure 3. LDA on morphometric characters from swordfish second ray of the anal fin from three sampling areas in the Atlantic Ocean: 1 - equator; 2 - south central; and 3 - east tropical. Numbers represent each fish and Circle, Square, and Triangle represent group averages, respectively to east tropical, equatorial and south central areas.

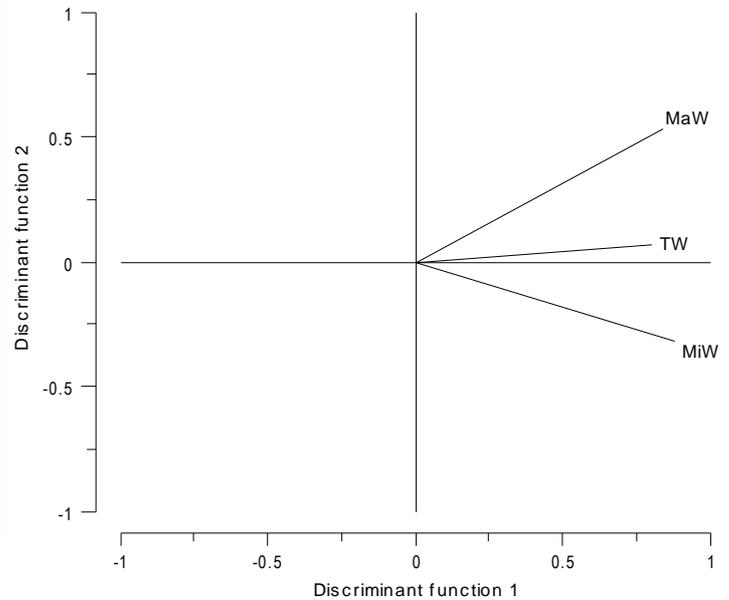


Figure 4. Correlation between the first two discriminant functions and the swordfish morphometric characters from the second ray of the anal fin: MaW, maximum width above the basal area; MiW, minimum width above the basal area; and TW, basal width.

DISCUSSION

Morphometric characters from the second ray of the anal fin showed significant phenotypic heterogeneity among Atlantic swordfish samples. The higher correlation coefficient obtained for the LJFL *vs.* TW, LJFL *vs.* MaW and LJFL *vs.* MiW relationships than for the LJFL *vs.* SH and LJFL *vs.* AH relationships, suggests that fin

ray growth in width is less variable than growth in height. Although teleost fin rays are highly pliable and resistant structures, they could be partially amputated, completely lost or severely injured which change the normal fin ray height. In these situations regeneration occurs as it constitute a general phenomenon in the fins of teleosts fishes (Becerra *et al.*, 1996). In fact, fins are able to complete self-restoration though a process of epimorphic regeneration (Santos-Ruiz *et al.*, 2002), thus the relationships with fin ray height could not be used accurately. Furthermore, swordfish consistently display positive allometries on length and fin ray width relationships, indicating that fin ray width increases more rapidly than fish length.

The results achieved by multivariate LDA suggested two phenotypically distinct populations. The differentiation may suggest the existence of phenotypic divergence, highlighting that migration among east tropical (TR) and the southern samples (EQ and SE) could be limited. On the other hand, there may be enough mixing among EQ and SE to prevent differentiation, which supports the homogeneity among those samples. Fishes are known to exhibit a large component of environmentally induced morphological variations (Wimberger, 1992). Therefore, Atlantic swordfish populations which experience specific environmental conditions such as temperature, salinity, food availability or prolonged swimming may determine their phenotypic differentiation. As morphology is especially dependent on environmental condition during early life history stages (Cheverud, 1988), morphological differentiations could indicate that the major part of fishes spent their lives in separate regions. Accordingly, significant morphological differences do not necessarily demonstrate restrictions of gene flow among populations but they could suggest that fishes may not mix extensively.

The results achieved suggested that there may be some population structuring among the studied areas being, the tropical area relatively isolated from the equatorial and south east areas. In fact, other morphometric relationships showed the same pathway, particularly length vs. weight relationships found by Mejuto *et al.* (1988). Although the present results suggest the accuracy of the current ICCAT stock boundary (at 5°N), for an accurate identification of Atlantic stocks a variety of methods should be used and compared (Begg and Waldman, 1999). In fact, integration of the results achieved by different method used for stock identification (i.e. genetics and parasitological fauna), maximizes the likelihood of correctly defining stocks.

ACKNOWLEDGMENTS

The authors express their gratitude to Sérgio Amorim for collecting fin rays. The first author holds a PhD grant from the FCT (ref. SFRH/BD/25391/2005).

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Chapter 5

**Age and growth of swordfish (*Xiphias gladius* L. 1758)
from the eastern Atlantic waters using anal fin spines**

ABSTRACT

The age and growth of the swordfish, *Xiphias gladius*, were estimated using the anal fin spines of 535 swordfishes caught in eastern Atlantic waters. Samples were collected in 2006, 2007 and 2009. Of the 535 anal fin spines sampled, 512 (96%) were aged successfully. The samples consisted of 12 age-classes, with age-classes 2 and 3 being the most represented. Marginal increment analysis showed that growth bands 1 to 7 were deposited annually. The monthly mean MIR estimated and the month effect on MIR provided by the GLM suggested that an annulus is formed in middle spring. The swordfish length-at-age was back calculated using two methods and estimated by the standard and generalised von Bertalaffy models. Overall, growth rates were higher during the first year of life and slowed appreciably afterwards for both sexes. However, females still growing faster than males and consequently reach larger sizes than males. The standard von Bertalaffy function provided a more realistic description of age-size relationship of swordfish over the age ranges considered. Growth parameters for the pooled sexes were described as: $L_{\infty}=332.104$, $K=0.077$; $t_0=-2.778$.

INTRODUCTION

Swordfish (*Xiphias gladius* L. 1758) is one of the most widely distributed pelagic fish, heavily exploited by several fleets within its entire distribution range (Nakamura, 1985). The high commercial value of swordfish in European markets has led to concerns about the ability of the fishery to sustain itself at current exploitation rates. The last Atlantic swordfish stock assessment made by ICCAT (International Commission for the Conservation of Atlantic Tuna), revealed that the biomass of the northern stock was estimated to be at or above the value needed to produce the maximum sustainable yield (MSY) (Anon., 2009). However, ICCAT also alerted that the north Atlantic stock is nowadays less productive than it was in 2006 (Anon., 2009). On the other hand the South Atlantic stock assessment did not show signs of over-exploitation, despite the conflicting signals from the several indices analysed (Anon., 2009).

Estimated growth rates are key elements on age-structured stock assessment based models for the species that could increase the effectiveness of management strategies

due to the valuable information provided on the status of fish stocks (Hilborn and Walters, 1992). Most billfishes' stock assessments have a high degree of uncertainty in their estimates, mainly due to the lack of biological information, particularly size-at-age (Kopf *et al.*, 2009). Despite the difficulties to collect adequate sample sizes, growth models have been developed for swordfish (Ehrhardt, 1992; Tserpes and Tsimenides, 1995; Sun *et al.*, 2002, DeMartini *et al.*, 2007). Swordfish age determination studies have been conducted using different hard parts, such as anal-fin rays (Price *et al.*, 1988; Tsimenides and Tserpes, 1989; Ehrhardt, 1992; Tserpes and Tsimenides, 1995; Ehrhardt *et al.*, 1996; Sun *et al.*, 2002; DeMartini *et al.*, 2007), otoliths (Castro-Longoria and Sosa-Nishizaki, 1998; Uchiyama *et al.*, 1998) and vertebrae (Esteves *et al.*, 1995; Uchiyama *et al.*, 1998). Several authors have pointed out that for many billfish species, the use of the fin rays for ageing are more practical, mostly due to being easier to collect and process (Tsimenides and Tserpes, 1989; Uchiyama *et al.*, 1998).

In many ageing studies the periodicity of the growth bands increment has seldom been validated. The analysis of the growth area (opacity *vs.* translucency) in the edge of the hard structures (edge analysis) and the variation of the increment of the last band over time (marginal increment analysis) are some of the most used techniques for validating age estimates (Tserpes and Tsimenides, 1995; DeMartini *et al.*, 2007; Sun *et al.*, 2002).

Given the importance of swordfish for commercial longline pelagic fisheries, the current needs and requests by managers for more information on age and growth that can be used for stock assessment and the current uncertainty regarding swordfish age validation, the main goals of the present study were to: 1) determine whether the growth increments in the anal-fin spines of swordfish are formed annually and 2) estimate the age and model the growth for the eastern Atlantic swordfish.

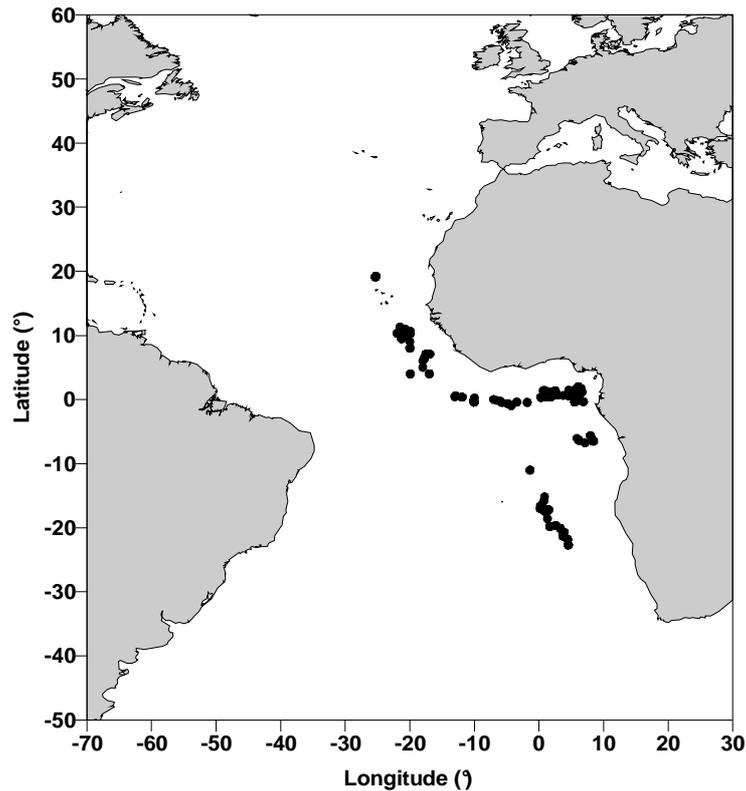


Figure 1. Swordfish sampling areas in eastern Atlantic waters.

MATERIAL AND METHODS

Sampling and spine preparation

Spine samples from 535 (288 females and 247 males) swordfishes caught in the eastern Atlantic waters were collected and used for this study (Figure 1). Sampling was undertaken onboard commercial longline vessels in 2006 (March, April and September), 2007 (between June and August and October) and 2009 (between May and September). For each specimen, the lower-jaw fork length (LJFL, to nearest centimeter) was taken, sex was determined and the anal fin was removed and frozen until assayed. In the laboratory, fins were thawed and sections of the second spine of the anal fin were prepared for reading according to the method described by Tsimenides and Tserpes (1989). Briefly, each anal fin was immersed in boiling water for a few minutes to remove the second spine and freed from skin and tissue. The spine was then cleaned with water and left to dry completely before being embedded in polyester resin. After drying (*c.* 12 hours), a total of four cross-sections of *c.* 0.7 mm thickness were obtained

from each spine, namely: two successive sections at the point where the spine flares (condyle) and two successive sections at the location equivalent to the condyle width measured above the line of the minimum condyle width. The cross-sections were cut using a low-speed precision saw and diamond wafering blade (BUEHLER® Isomet 1000).

Age estimation and precision

Spine sections were scanned using a previously calibrated scanner (HP® Scanjet 5530) and the digitalized image analysed with Image J 1.41 using dark background. Typically, broad translucent (slow growth) and narrow opaque bands (fast growth) could be seen in alternate positions from the core until the edge of the section. The total number of translucent bands (annulus) in spine sections was recorded and the distance from the focus to the edge of the section (spine radius) and to each annulus was measured. The first section over the condyle was firstly observed and used, while the second section was only used in cases where the first section was not clear. The selected section of each spine was read twice by the same reader about two to three months apart with no knowledge of fish length. When there was not an agreement between counts of translucent bands, spines were read for a third time. If agreement could not be reached, those spines were excluded from further analysis.

Estimated age was assigned to each swordfish on the basis of the number of annuli and the characteristics of the bands as described by Tserpes and Tsimenides (1995), namely the disappearance of the early growth rings and the existence of multiple bands in older fishes. The spine nucleus was frequently reabsorbed and replaced by vascularized tissue that hides the first rings in older fishes. Therefore, as suggested by Tserpes and Tsimenides (1995), the mean radius of those annuli in spine sections of young fishes was measured, allowing the estimation of their likely position in older fishes. In such cases, if the total spine radius was greater than the mean achieved for the first three annuli, one and two or three years, respectively, were added to the assigned age. The appearance of multiple bands in older fishes was described by Berkeley and Houde (1983) as those which form around the entire circumference of the spine with lesser distance from the preceding and the following bands. In these cases if the clearest band was possible to identify it was considered an annulus and the others were ignored,

otherwise the specimen was considered unreadable. Unreadable spines were also considered those which the opaque and translucent bands could not be identified.

To assess the reader precision, the average percentage error (APE) was computed and defined as (Beamish and Fournier, 1981):

$$APE_j = 100\% \frac{1}{R} \sum_{i=1}^R \frac{|x_{ij} - x_j|}{x_j},$$

where x_{ij} is the i th age estimation of the j th fish, x_j the mean age estimated for the j th fish, and R the number of occasions each fish was aged.

To improve the subsequent fit of growth curves to the age data, the ages of each fish were converted into absolute decimal age. The birth date was arbitrarily placed on 1st January, allowing for the minimization of the variability associated with a large sampling period (Coelho and Erzini, 2002). Absolute decimal age was calculated as the number of annual bands plus the percentage of the year (from 1 January) that had passed since the date of capture.

Validation and growth

The marginal increment ratio (MIR) was estimated to determine the time of band formation, according with the following formula (Tserpes and Tsimenides, 1995):

$$MIR = (S - r_n) / (S),$$

where S is the spine radius and r_n the radius of the most recent annulus.

The mean MIR (\pm s.d.) was computed by month and age, for sexes combined, in order to locate periodic trends in band formation. Specimens estimated to be age 0 were not included in MIR analysis because they lack formed bands. Likewise, fishes older than 7 years were also excluded from this analysis due to the lack of sufficient number of samples. Monthly variations of marginal increment ratio (MIR) estimates were analysed by means of Generalised Linear Modelling (GLM) techniques (McCullagh and Nelder, 1983). Apart from the variable “month”, the effect of “age” was also included in the model to account for MIR differences among ages. The final model was expressed as:

$$MIR_{i,j} \sim \text{Intercept} + \text{Month}_i + \text{Age}_j + \text{error}_{i,j}$$

Interaction among “month” and “age” was not considered due to lack of sufficient data.

Based on the deviance residuals plot, a model assuming a Gamma error structure with a “log” link function was found to be the most appropriate. Model fitting was accomplished under the R language environment (R Development Core Team, 2008) and statistical inference was based on a 95% confidence level.

Back calculation of length-at-age (Francis, 1990) was determined by the adjustment of linear and power functions to the relationship between spine radius (S) and length (LJFL) data. Thus, to back calculate lengths at previous ages the following two methods were used:

Method I: The relationship between LJFL and S was achieved through standard linear regression analysis ($LJFL = a + bS$) (Berkeley and Houde, 1983). The relationship parameters (a and b) and the distance from the focus to each annulus, which it was assumed to be annual, were used to estimate the length-at-age by the modified version of the direct proportion formula (Fraser, 1916; Lee, 1920):

$$LJFL_n - a = (S_n/S)(LJFL - a)$$

where $LJFL_n = LJFL$ when the annulus n was formed; $LJFL = LJFL$ at the time of capture; $a =$ intercept (initial growth coefficient) on length axis from linear regression of LJFL on S ; $S_n =$ distance from the spine focus to annulus n ; and $S =$ spine radius.

Method II: The relationship between LJFL and S was achieved through power function analysis ($LJFL = aS^b$) expressed in its linearised logarithmic form ($\ln LJFL = \ln a + b \ln S$) (Ehrhardt, 1992). The relationship parameters (a and b) were estimated through nonlinear least squares method and together with the distance from the focus to each annulus were used to estimate the length-at-age using the Monastyrsky formula (Bagenal and Tesch, 1978):

$$LJFL_n = (S_n/S)^b LJFL$$

where $LJFL_n = LJFL$ when the annulus n was formed; $LJFL =$ length at the time of capture; $b =$ the exponent of the regression of LJFL on S ; $S_n =$ distance from the spine focus to annulus n ; and $S =$ spine radius.

Sex differences in the LJFL vs. S relationships were tested for equality of slopes by means of Analysis of Covariance (ANCOVA) (Dixon and Massey, 1985). Statistical inference was based on $\pm 95\%$ confidence level.

Estimates of theoretical growth in length were obtained by fitting mean monthly observed decimal length-at-age data to two different forms of the von Bertalanffy growth equation: the standard form and the generalised one as proposed by Richards (1959) and described as:

Standard VB:

$$L_t = L_\infty \left(1 - e^{-k(t-t_0)}\right)$$

Generalised VB:

$$L_t = L_\infty \left(1 - e^{-K(1-m)(t-t_0)}\right)^{\frac{1}{1-m}}$$

where L_t = mean LJFL at age t ; L_∞ = asymptotic length; k and K = growth coefficients; t_0 = theoretical age at zero length; m = fitted fourth function parameters. The use of mean data instead of the raw ones, although it may not be a statistically recommended procedure, was preferred since it assigns equal weight to all observations. Growth parameters of both models for males, females and sexes combined were estimated iteratively using either the “Gauss-Newton” or the “grid-search” minimisation algorithms included in the “nls” and “nls2” R packages, respectively (Bates and Chambers, 1992; R Development Core Team, 2008).

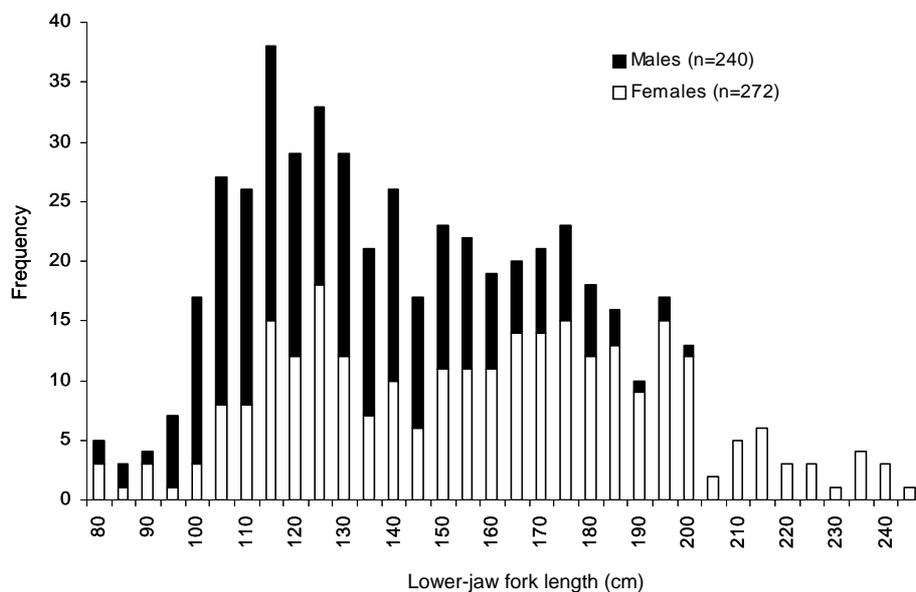


Figure 2. Length frequency distribution (5 cm intervals) of aged swordfish from the eastern Atlantic waters.

RESULTS

Of the 535 anal fin spines sampled, 512 (96%) (240 males and 272 females) were aged successfully. The overall average percentage error (APE) was 3.35% (2.62% for males and 4.01% for females). In 14 of the 24 unreadable spines, any annuli could be identified because the opaque-translucent area was unclear. The remaining 10 spines were considered unreadable because the existence of multiple bands which made the identification of annuli difficult or resulted in ageing discrepancies between readings.

Table 1. Descriptive statistics on sizes of aged swordfish from the eastern Atlantic waters. LJFL, lower-jaw-fork-length.

Age	Sample size	Mean LJFL (cm)	Standard deviation	LJFL range (cm)	Mean size increment
0	3	83.00	1.73	82-85	-
1	19	99.95	10.37	82-119	16.95
2	71	114.61	8.64	94-135	14.66
3	99	124.13	9.30	102-150	9.52
4	83	141.98	12.14	116-173	17.85
5	73	159.37	12.96	131-187	17.39
6	60	174.15	15.48	152-235	14.78
7	49	183.59	15.07	133-220	9.44
8	17	196.35	17.68	163-240	12.76
9	18	207.61	13.88	182-237	11.26
10	11	208.45	21.09	180-242	0.84
11	6	224.67	13.31	215-251	16.22
12	3	226.00	23.07	200-244	1.33

The length of the aged individuals ranged from 85 to 206 cm for males and from 82 to 251 cm for females (Figure 2; Table 1). The mean rings radius, standard deviation, range and the number of observations are shown in Table 2. In 150 spines sections the total radius was greater than the mean achieved for the first annulus, thus one year was added to the assigned age. In 81 sections, two years was added as the vascularized tissue area was greater than the mean achieved for the first and the second annuli. In this study the vascularization of the third annulus was also observed in 43 spine sections which mean that 3 years were added when the first measure was higher that the mean achieved for the first three annuli. The smallest estimated age was 0+ and the highest

12+ (sexes pooled). Age groups 2 and 3 were the dominant in the whole sample and account *c.* 33%.

Table 2. Number of observations (n), mean, standard deviation (s.d.), maximum (Max) and minimum (Min) diameter of translucent rings on the second spine of the anal fin of swordfish from the eastern Atlantic waters.

Ring	Mean (mm)	Max (mm)	Min (mm)	s.d. (mm)	n
1	1.612	2.098	1.045	0.227	235
2	2.258	2.985	1.458	0.300	365
3	2.902	3.794	1.641	0.383	358
4	3.627	4.766	2.463	0.416	306
5	4.275	5.456	2.721	0.514	231
6	4.879	6.397	3.253	0.600	153
7	5.364	7.806	3.452	0.697	95
8	6.057	8.631	4.895	0.785	50
9	6.537	9.112	5.106	0.907	36
10	7.043	9.663	5.316	1.037	16
11	7.889	10.624	6.018	1.404	7
12	7.015	7.475	6.554	0.651	2

Table 3. Analysis of deviance of the generalised linear model fitted to the MIR data.

Source of variation	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			452	163.48		
month	6	9.52	446	153.97	12.03	<0.001
age	6	87.05	440	66.91	110.03	<0.001

Marginal increment analysis showed that growth bands 1 to 7 were deposited annually. The analysis of deviance indicated that both factors (month and age) were statistically significant (Table 3). The monthly mean MIR estimated (Figure 3) and the month effect on MIR provided by the model (Figure 4) suggested that an annulus is formed in middle spring. The GLM provided a good fit to the data as it was demonstrated by the deviance residual plot.

The mean back calculated length-at-age obtained by Method I and II are showed in Table 4. The relationship between LJFL and S for males, females and sexes combined are described as follow:

Method I Males: $LJFL = 63.29 + 21.07(S)$ [$r^2=0.83$]

Females: $LJFL = 68.24 + 20.15(S)$ [$r^2=0.84$]

$$\text{Sexes combined: LJFL} = 65.80 + 20.56(S) [r^2=0.85]$$

$$\text{Method II Males: } \ln(\text{LJFL}) = 4.25 + 0.54(\ln S) [r^2=0.83]$$

$$\text{Females: } \ln(\text{LJFL}) = 4.21 + 0.58(\ln S) [r^2=0.87]$$

$$\text{Sexes combined: } \ln(\text{LJFL}) = 4.22 + 0.57(\ln S) [r^2=0.87]$$

ANCOVA did not revealed significant differences between sexes for both methods (Method I: $F_{1,507}=0.82$, $p=0.36$ for the slope; $F_{1,508}=1.25$, $p=0.27$ for the intercept; Method II: $F_{1,507}=3.32$, $p=0.07$ for the slope; $F_{1,508}=1.44$, $p=0.23$ for the intercept). Method I yielded moderately higher rates than Method II. Growth rates were higher during the first year of life in both methods, and slowed appreciably afterwards for both sexes. However, females still growing faster than males and consequently reach larger sizes than males.

Fitted standard and generalised VB growth using the observed mean length for males and females are shown in Figure 5 and the estimated parameters for each curve in Table 5. Both models provided a good fit to the data (Figure 5). However, the values achieved for the generalised VB model were higher than the values of the standard VB growth model.

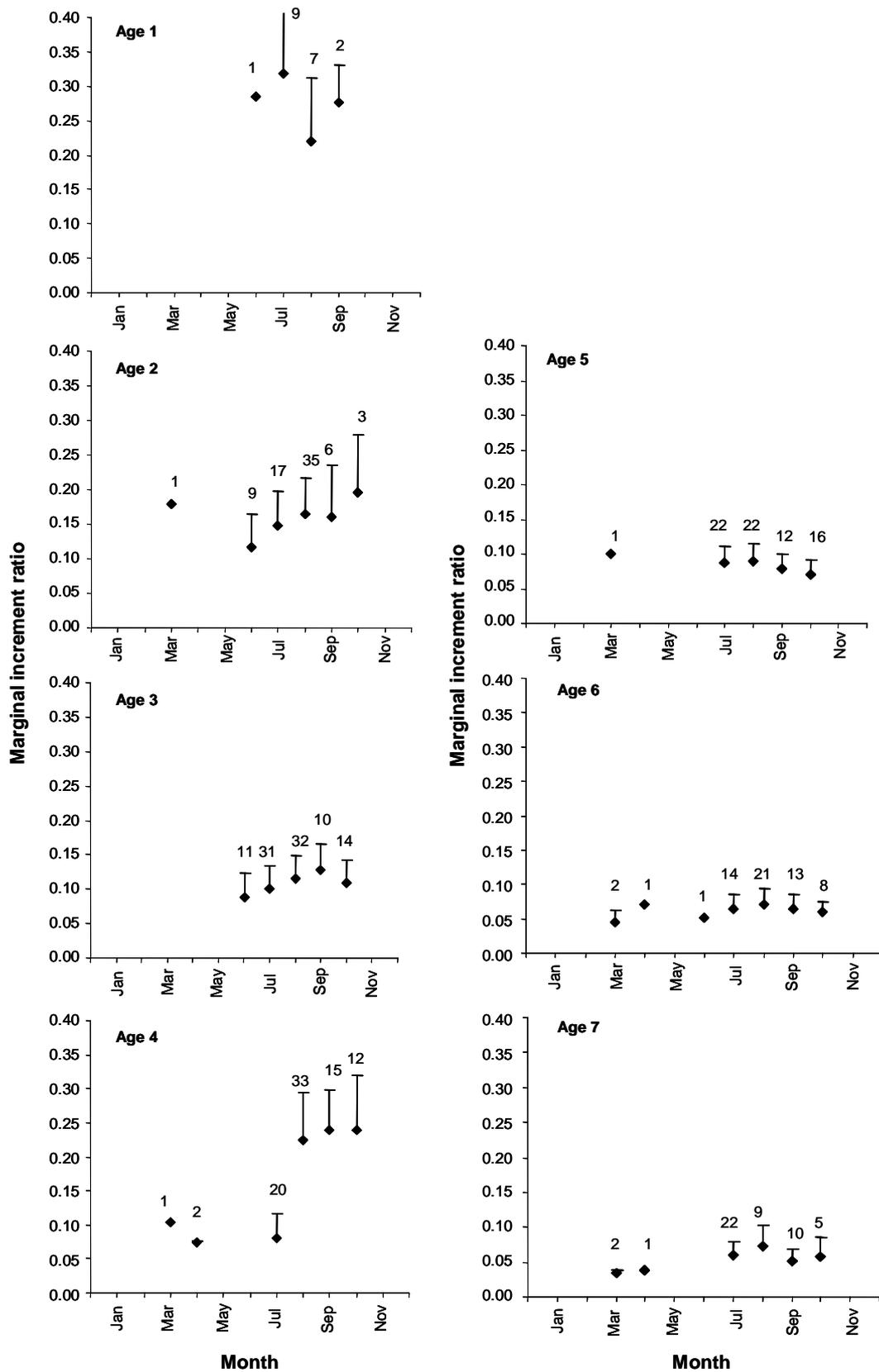


Figure 3. Mean monthly marginal increment ratio for swordfish aged 1-7 from the eastern Atlantic waters. Vertical bars represent s.d. and the number the sample sizes.

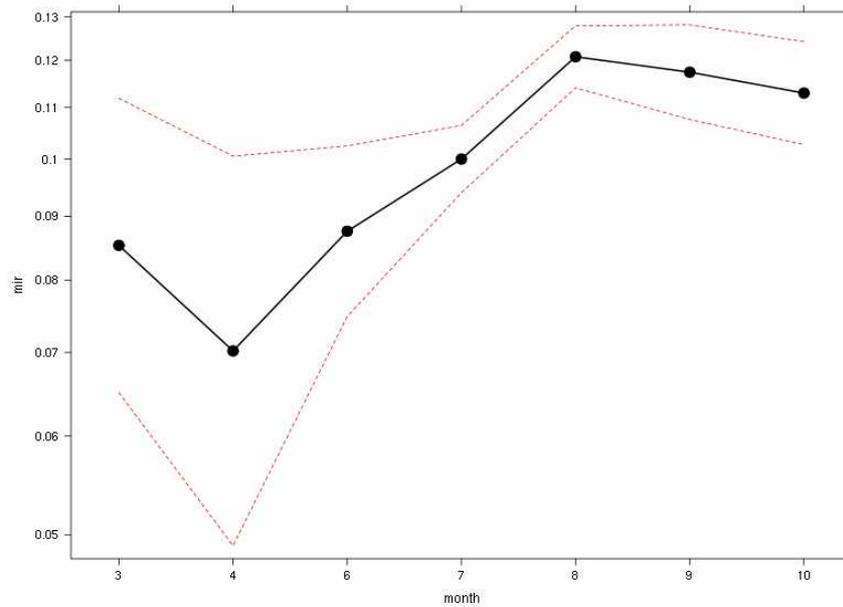


Figure 4. Generalised linear model derived effect of month on MIR. Dotted lines indicate standard errors.

Table 4. Back-calculated and estimated lower-jaw-fork-length (LJFL, cm) at age for swordfish from the eastern Atlantic waters.

Age	Back-calculated (Method I)		Back-calculated (Method II)		Predicted (Standard von Bertalanffy)		Predicted (Generalised von Bertalanffy)	
	Males	Females	Males	Females	Males	Females	Males	Females
1	89.87	89.41	81.82	83.59	83.30	82.88	98.17	126.80
2	107.44	106.00	104.82	102.58	102.00	102.14	119.64	147.94
3	117.59	117.74	116.04	116.32	119.38	119.85	138.02	166.03
4	134.31	135.02	133.76	134.45	134.71	136.13	153.73	181.50
5	147.48	155.48	147.42	155.79	148.25	151.10	167.18	194.74
6	162.39	169.59	162.78	170.24	160.19	164.86	178.69	206.07
7	176.59	176.60	177.15	177.40	170.74	177.51	188.53	215.76
8	182.01	192.79	182.87	193.68	180.04	189.15	196.95	224.05
9	195.42	201.68	192.09	202.69	188.25	199.84	204.16	231.14
10	174.03	205.15	174.58	206.14	195.49	209.68	210.32	237.21
11		219.13		220.20	201.89	218.72	215.60	242.40
12		220.64		221.59	207.53	227.04	220.11	246.85

Table 5. Parameters estimate for the standard von Bertalanffy and the generalised von Bertalanffy growth models for swordfish from the eastern Atlantic waters.

Parameters	Standard von Bertalanffy			Generalised von Bertalanffy		
	Males	Females	Sexes combined	Males	Females	Sexes combined
L_{∞}	249.904	321.913	332.104	246.84	273.158	291.579
k or K	0.125	0.084	0.077	0.156	0.100	0.156
t_0	-2.196	-2.544	-2.778	-2.250	-1.500	-3.000
m				0.111	-0.143	0.250

DISCUSSION

This study proved that the anal fin spines are useful for swordfish ageing studies. This finding is important as swordfish have no scales, have small otoliths that are not amenable to traditional ageing techniques (Ovchinnikov, 1971; Beckett, 1974) and the vertebrae collection is often difficult since might reduce the economic value of the fish. Several European fleets target swordfish during small (few days to a couple weeks) trips and sell the fish fresh, meaning that collecting the vertebrae is impossible. An important assumption for any ageing study based on the growth of hard structures of fishes, is that the hard structures exhibit a proportional growth throughout the life of the fish (DeVries and Frie, 1996). The growth of the second spine of the swordfish anal fin meets this requirement by exhibiting a positive correlation between the spine width and the body length (unpub. obs.).

As mentioned, the main problems associated with the use of the spines for ageing are the phenomenon of vascularization of the spine nucleus and the existence of multiple bands in larger fishes. Tserpes and Tsimenides (1995) noted that an experienced reader could overcome those problems by using the techniques described above, i.e., identifying the position of the missing annuli on sections from younger specimens and for the multiple bands checking whether the annulus continue around the entire circumference of the spine and also recording the distance from the preceding and following annuli. Similar approaches have also been used for swordfish in Atlantic Ocean (Berkeley and Houde, 1983), Pacific Ocean (Sun *et al.*, 2002; DeMartini *et al.*, 2007) and Mediterranean Sea (Tserpes and Tsimenides, 1995). In the present study, high reader precision was achieved yielding higher values for males than for females. According to Beamish and Fournier (1981) the average percentage error provides a

good estimate of readings precision as it describes the reproducibility of age estimates and not only the consistency among determinations.

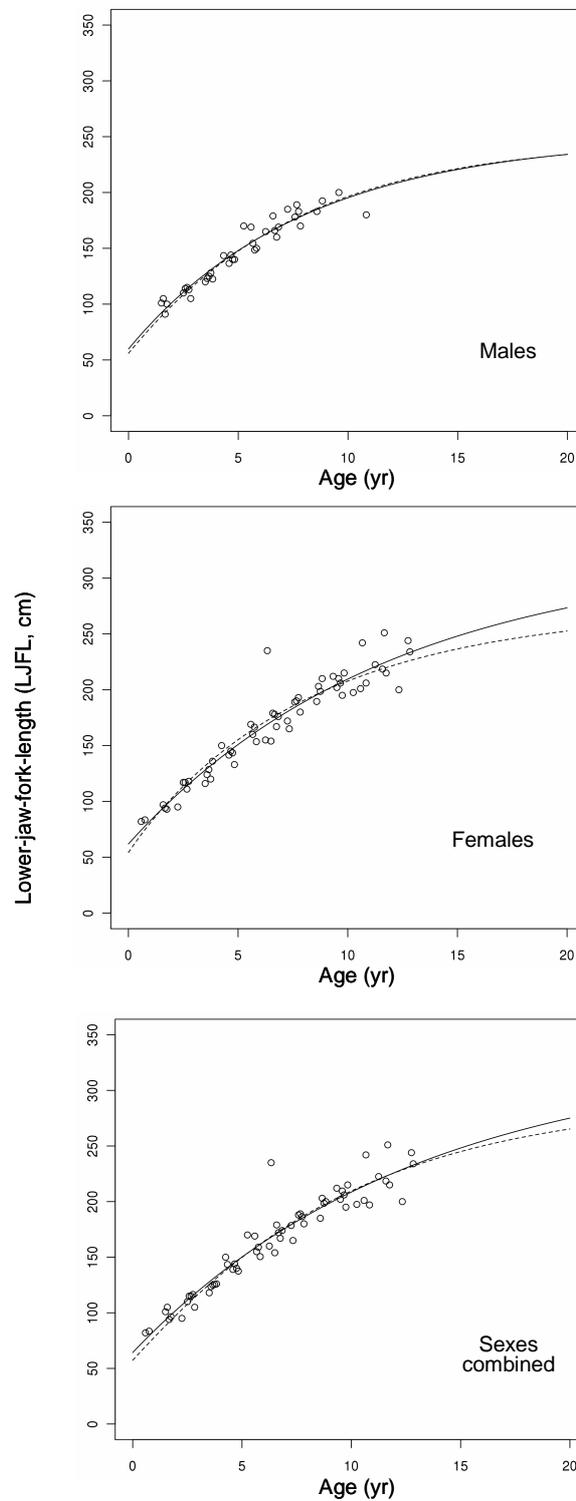


Figure 5. Standard and generalised von Bertalanffy (VB) growth curves for swordfish from the eastern Atlantic waters. Continuous line, standard VB; Dotted line, generalised VB.

Table 6. Estimated lower-jaw-fork-length (LJFL, cm) at age for swordfish from northwest Atlantic waters.

Age	Berkeley and Houde (1983)		Ehrhardt (1992)	
	Males	Females	Males	Females
1	97.2	98.0	89.7	89.8
2	118.5	119.9	117.0	118.9
3	136.0	139.7	137.3	142.9
4	150.4	157.8	153.4	161.3
5	162.3	174.3	168.9	177.2
6	172.0	189.3	181.8	189.6
7	180.0	202.9	195.3	204.4
8	186.6	215.3	206.1	214.7

Marginal increment analyses demonstrate successfully the seasonality of band formation and partially validate the method used (ages 1 to 7). In older fishes spine growth is considerably reduced, thus an accurate validation of the method requires usually mark-recapture techniques and capture of known age fish (Beamish and McFarlane, 1983). The growth band is formed every year in middle spring. Since it has been suggested for Atlantic swordfish that spawning activity are more frequently observed in the west part of the Atlantic, annulus formation may be related with drains on physiological condition, such as migration, as suggested by Berkeley and Houde (1983) for Atlantic swordfish and Tserpe and Tsimenides (1995) for eastern Mediterranean swordfish. The results of an event such as migration expose fish to different environments and place them under additional physiological stress (Campana, 1999). In fact, sampling location was mainly included in the transitional area between feeding and spawning grounds of Atlantic swordfish but also the Gulf of Guinea, where several samples were collected, was considered the main feeding area for the south Atlantic stock (Alvarado-Bremer *et al.*, 2005a). Several other studies using swordfish anal fin spines revealed that the spawning activity is the main mechanism regulating cyclic deposition of growth increments as the fish use more energy to produce gametes than for growth, resulting in a less calcified ring. In the north Pacific one annulus is formed during the swordfish spawning season, from July to September (Sun *et al.*, 2002; DeMartini *et al.*, 2007), while in south Pacific from December to March (Cerna, 2009).

Back calculation analysis based on method II provided a better fit to the data than method I. According with Ehrhardt (1992) and Tserpes and Tsimenides (1995) the nonlinear function is more realistic to express the relationship between spine radius and LJFL. Data obtained using method II was more closely related with the length-at-age data predicted by standard von Bertalanffy model. Ehrhardt (1992), Sun *et al.* (2002) and Chiang *et al.* (2004) favoured method II because they believed that it was more biologically realistic.

Female swordfish is typically larger for similar ages than males and grow faster than males. Similar results have been achieved for swordfish from the Mediterranean Sea (Tserpe and Tsimenides, 1995), Pacific (Sun *et al.*, 2002; DeMartini *et al.*, 2007) and Atlantic (Berkeley and Houde, 1983 Ehrhardt, 1992; Ehrhardt *et al.*, 1996) oceans. Therefore, the estimation of a common growth equation is not valid but may be useful for management purposes only under certain circumstances. Moreover, the sexual differences in body size and growth reinforce the argument that sex-specific, age based stock assessments are needed for swordfish. Swordfish growths differ between the estimates made by the standard and generalised von Bertalanffy equations. The standard growth function provides a more realistic description of age-size relationship of swordfish over the age ranges considered. Ehrhardt (1992) suggested that the generalised von Bertalanffy growth function does not represent adequately the swordfish growth as the parameters estimated do not have biological meaning.

When comparing overall growth curves of swordfish, estimated L_{∞} was similar in the current study than those recorded for the northwest Atlantic (Berkeley and Houde, 1983; Ehrhardt, 1992) but higher than those from the Mediterranean (Tserpes and Tsimenides, 1995), and the northwestern Pacific (Sun *et al.*, 2002). Oppositely, growth parameters achieved for swordfish caught in southeast Pacific showed higher values than those observed in this study (Cerna, 2009). However, the swordfish caught in northwest Atlantic grow in length faster than those caught in east Atlantic waters if the standard VB parameters were considered (Table 4; Table 6). For example the mean length at age 1 of swordfish caught in northwest Atlantic is about 98 cm LJFL (Berkeley and Houde, 1983) and 89 cm LJFL (Ehrhardt, 1992), whereas the mean length at age 1 in east Atlantic is about 83 cm LJFL. Differences in mean size at age observed within the Atlantic (NW *vs.* east Atlantic area) could be due to variations in growth patterns as the north and south Atlantic have been identified as different population units (Anon., 2007). The study area coincides with the grade of

differentiation between the two Atlantic swordfish populations which boundary was placed on 5°N (Anon., 2009). In this context the sampled area constitute an area of intergradation between both populations and thus specimens from the north and south populations could be found in these areas. Lower growth rates were also found for Pacific swordfish caught within an area where it is supposed to coincide with the grade of differentiation between populations (Cerna, 2009). However, life history information for different regions, e.g. the variation in growth rates, spawning season and grounds and environmental marks on stock structures are important for stock assessment purposes.

ACKNOWLEDGMENTS

The author expresses their gratitude to Sérgio Amorim for collecting fin rays. The first author holds a PhD grant from the FCT (ref. SFRH/BD/25391/2005).

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PART III

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION

The present study aimed primarily to evaluate if a multidisciplinary (holistic) approach incorporating a set of different aspects of the swordfish biology (parasitology; genetic mtDNA, morphometric and age and growth) could provide consistent results for differentiating Atlantic swordfish stocks, and assess their degree of subdivision and discreteness. In order to achieve such goals all these aspects were applied to samples from the same specimens to facilitate the interpretation of the results. Herein we summarize the main results achieved, and discuss these highlighting contradictions and coincidences.

The study of swordfish parasites in tropical-equatorial Atlantic waters widened the geographical range known for some parasite taxa, provided a more comprehensive analysis of the species metazoan parasites and allowed gathering new information on the relationship between infection levels and host size and sex (Part II: Chapter 1). The infection levels by the large ectoparasites recovered from the swordfish gills (*Tristoma* spp.) revealed that young swordfish are more vulnerable to infection than adults. These results suggested that juveniles experienced different environmental conditions (e.g. temperature and salinity) or habitats than adult swordfish. On the other hand, infection levels by endoparasites (mostly transmitted via trophic webs) were maintained during its life history, as no difference was observed between juvenile and adult specimens. When swordfish sex was considered, no statistically significant correlation was found either, with parasite burden indicating similar diet for both females and males. Overall, the tropical-equatorial area seems to be a main feeding ground for both juveniles and adults, which parasite fauna composition and burden is likely the combined result of host habitat, feeding behaviour and the availability of the parasites' intermediate and final hosts in the area. Furthermore, high parasitic infection levels and genetic diversity of anisakid nematodes may indicate food web stability and integrity of marine ecosystems. Additionally, the results provided indications on the use of some parasite taxa as biological tags for swordfish stock discrimination purposes. Thus, an additional study was conducted to verify which parasites could help to separate the Atlantic swordfish stocks (Part II: Chapter 2). The results suggested that Atlantic swordfish is sub-structured into at least two main groups: 1) northwest and north central; and 2) tropical, equatorial and south central. Based on parasites presence/absence and infection levels, it was possible to associate some taxa to distinct areas. *Hysterothylacium*

corrugatum (s.l.) and *Rhadinorhynchus pristis* were mostly associated to swordfish caught in the northwest area, whereas *Anisakis paggiae* and *H. incurvum* related to fish caught in the southern areas (tropical, equatorial and south central). *Anisakis simplex* s.s. could be used as a marker for all northern samples (northwest and north central). The occasional occurrence of *R. pristis* in some samples collected in tropical and south central areas, known to be more common in swordfish from northern areas indicated migration of swordfish to the south Atlantic.

The results obtained through mitochondrial DNA sequencing showed low levels of genetic differentiation, high levels of genetic variability and quite stable genetic structure in the different study areas (Part II: Chapter 3). All the samples were characterized by high levels of haplotype diversity and moderately high levels of variation in the mtDNA control region. Furthermore, mtDNA analysis revealed that most of the variation found in haplotypes was due to differences within areas. Additionally, the phylogenetic analysis did not show evident relationships among haplotypes from all areas. Despite the low F_{ST} values, statistical significant differences were recorded when comparing equatorial samples with all the other areas. Accordingly, equatorial area may represent an area of inter-gradation within the Atlantic Ocean, as higher levels of genetic divergence were recorded. No indication of genetic erosion of Atlantic swordfish was noted for any of the geographic areas sampled.

The morphometric study of the second spine of the anal fin (used for age and growth study) allowed to conclude that fin spine growth in width is less variable than growth in height, and that fin spine width increases faster than fish length (Part II: Chapter 4). The morphometric relationships between swordfish length and the second fin spine measurements [total straight height (SH), basal width (TW), maximum width above the basal area (MaW) and minimum width above the basal area (MiW)] indicate a clear prevalence of positive allometry (60%) over negative allometry (40%). The results achieved by linear discriminant analysis supported the separation of Atlantic swordfish in two phenotypically distinct groups: 1) tropical; and 2) equatorial and south east. The differentiation may suggest the occurrence of phenotypic divergence, highlighting that migration between east tropical and the southern areas (equator and south east) could be limited. On the other hand, there may be enough mixing between equator and south east that prevent differentiation, thus being responsible for the homogeneity among those

samples. The morphometric characters of the spine that contributed most for the samples separation were the MiW, followed by the MaW and the TW.

The age and growth study allowed estimating the periodicity of band formation as well as modelling growth for eastern Atlantic swordfish (Part II: Chapter 5). Marginal increment analysis (MIR) showed that growth bands (1 to 7) were deposited annually. The monthly mean MIR estimated and the month effect on MIR provided by the GLM, suggested that an *annulus* is formed in spring. The swordfish length-at-age was back calculated using two methods (linear regression and power function analysis) and estimated by the standard and generalised von Bertalanffy models. Overall, for both sexes growth rates were higher during the first year of life and slowed appreciably afterwards. However, there are evidences that females grow faster than males and reach larger sizes than males.

A variety of univariate and multivariate statistical tools were used to analyse the results provided by each technique. The application of a single statistical tool to a common data matrix was difficult due the heterogeneity of the data. Therefore, a critical comparison of the results provided by each technique was done. As summarised herein and detailed in previous sections of this thesis, some techniques showed differences between areas while others did not (chiefly the genetic technique). Similar results were achieved for *Trachurus trachurus* (Abaunza *et al.*, 2008), *Sebastes mentella* (Marcogliese *et al.*, 2003) and *Aphanopus carbo* (Gordo *et al.*, 2009). The absence of significant genetic structure can not always be equated as true panmixia hypothesis. It may take a long time following sub-division of a population for genetic markers to reveal genetic structure (Grosberg and Cunningham, 2001). Moreover, the rates of organism evolution (morphology, ecology and behaviour) may occur over too short a period of time to have allowed the evolution of structural proteins or other material used for genetic analysis (Clayton, 1981). Due to the random genetic drift, genetic differentiation may occur very slowly in the typically large population of commercial marine fish (Ward *et al.*, 1994). Genetic markers are generally oversensitive to a low level of gene flow, i.e., a relatively low level of exchange between stocks, negligible from a management perspective, may be sufficient to ensure genetic homogeneity (Carvalho and Hauser, 1994; Ward and Grewe, 1994). Therefore, molecular markers may not be sufficient to detect existing genetic variation among populations and also only a small proportion of DNA is analysed by molecular markers. Thus, the study of parasites and environmentally induced phenotypic variation may have advantages in

stock identification, especially when the time is insufficient for significant genetic differentiation to accumulate among populations. The lack of obvious physical barriers to dispersal in the ocean led to the consideration that species having broadly dispersing larvae or adults that show migratory behaviour, should exhibit little genetic structure across their range, particularly considering that gene flow rates of 1% would lead to genetic homogeneity (Ward, 2000). However, the findings concerning sample homogeneity are of little assistance to fisheries managers, who still need information to define stock boundaries. In this sense, the holistic approximation to stocks identification is more efficient (Waldman, 1999), as information with multiple perspectives can be obtained following the application of different techniques. Some of these techniques are costly and it is desirable to select the most useful approaches. Data on body morphometrics and the use of parasites as biological tags have been found to be especially useful in the differentiation of swordfish stocks. The holistic approach and the application of all the techniques on the same swordfish specimens allow reducing uncertainty, thus decreasing type I error in stock identification.

CONCLUSIONS AND FUTURE RESEARCH NEEDS

The results achieved by the different techniques used (the holistic approach) revealed that migratory movements between the northern and southern areas are occurring and that mixing is happening especially in the equatorial area. Accordingly, although no precise boundaries could be establish for Atlantic swordfish stocks, apparently there is no need for changing the current fishery management boundary, which has been placed by the management body (ICCAT) at 5°N.

Nevertheless, in order to promote a sustainable management of the Atlantic swordfish fisheries complementary studies must be undertaken in a near future. In fact, although the present study provided new knowledge on Atlantic swordfish, several unanswered questions persist, thus requiring further studies. These should focus on the same techniques but covering a wider geographical area, particularly the southern hemisphere and the northwest Atlantic areas. Furthermore, it would be very important that these studies are complemented with other studies, based on different techniques such as tagging and otolith microconstituent analysis. A carefully designed experiment using satellite archival tags and otolith microconstituent analysis will allow the

confirmation of two stocks in the Atlantic Ocean (north and south), their boundaries and mixing areas and the determination of their natal region. Additionally, data concerning the population dynamics could help on fisheries management, such as to determine the potential relationship between the oceanographic parameters and recruitment of swordfish. The application of other genetic techniques could also be useful for stock identification studies such as allozymes and microsatellite DNA analysis. In order to conduct a suitable analysis of the various results, the nature and time scale of the techniques must be taken into account.

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