UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS DO MAR E DO AMBIENTE

Geographic variation in sardine population traits: implications for stock assessment

Tese para a obtenção do grau de doutor
no ramo de Ciências e Tecnologias das Pescas,
especialidade Avaliação de Recursos

Maria Alexandra Cordeiro de Almeida e Silva

Orientador: Doutor Manuel Seixas Afonso Dias
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Abstract

This study integrates information on geographical and temporal variability of body morphometry, maturity-at-length, growth and population dynamics, to describe population structure of sardine within the Atlanto-Iberian stock region and adjacent waters and assist the definition of stock units. Biological information was collected regularly at main fishing ports and in acoustic surveys within the Iberian Peninsula since the 1980s and more recently in the French Gulf of Biscay. Spatial variation of morphometric and biological characteristics was also analysed across most of the species' range using samples collected in 1999 - 2004. Morphometric and biological characteristics showed clinal variation across the northeast Atlantic and differed between Atlantic and Mediterranean populations. These characteristics and population dynamics were homogeneous within the northern (east Cantabrian to northern Galicia), western (southern Galicia to southwestern Portugal) and southern (southern Portugal and the Gulf of Cadiz) regions of the Atlanto-Iberian stock. Differences among regions were obtained in some analyses but did not provide evidence of population discreteness when combined with other information from the area. Most characteristics were homogeneous across the limits of the Atlanto-Iberian stock. Overall, the results emphasized the need to explore alternative scenarios of stock structure, particularly in relation to the northern limit, and to consider geographical variation in biological traits and fisheries exploitation in future stock assessments.

Key-words: Growth; Iberian Peninsula; Sardine; Sexual maturity; Morphometry; Population dynamics.

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Resumo

Este estudo integra informação sobre variabilidade espaço-temporal da morfometria do corpo, comprimento de primeira maturação, crescimento e dinâmica populacional para descrever a estrutura populacional da sardinha na área do stock Atlanto-Ibérico e zonas adjacentes e apoiar a delimitação de unidades de stock. A informação biológica foi recolhida nos principais portos de pesca e durante campanhas de acústica na Península Ibérica desde os anos 80 e mais recentemente no Golfo da Biscaia. A variabilidade espacial das características morfométricas e biológicas foi também analisada ao longo da área de distribuição da espécie, utilizando amostras recolhidas entre 1999 e 2004. Observou-se uma variação clinal das características morfométricas e biológicas no Atlântico noroeste e diferenças entre populações Atlânticas e Mediterranicas. Estas características foram homogéneas dentro das regiões norte (Cantábrico este a Galiza norte), oeste (Galiza sul a sudoeste de Portugal) a sul (sul de Portugal e Golfo de Cádiz) do stock Atlanto-Ibérico. Observaram-se diferenças entre regiões em algumas análises mas não se obteve evidência de populações discretas quando os resultados foram combinados com informação adicional da mesma área. A maior parte das características mostrou estabilidade através dos limites do stock. Globalmente, os resultados apontaram para a necessidade de explorar cenários alternativos de estrutura de stocks, particularmente quanto ao limite norte, e de considerar a variabilidade geográfica das características biológicas e de exploração em futuras avaliações do stock.

Palavras-chave: Crescimento; Dinâmica populacional; Maturação sexual; Morfometria; Península Ibérica; Sardinha.

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Chapter 1

General Introduction

Widely distributed fish species are likely to show variable morphology, life history traits and demographic parameters across their range, reflecting genetic diversity, phenotypic plasticity or a combination of both factors (Swain et al., 2005). Genetic differences between groups of individuals from the same species may arise by genetic drift, the random fluctuation in allele frequencies that may cause any allele and corresponding biological trait to become more common (or rare) over successive generations. Natural selection, the tendency of alleles conferring higher fitness to become more common over time and detrimental ones less common, may also promote genetic differentiation. On the contrary, gene flow, resulting from the movement of gametes between conspecific groups will tend to reduce or prevent differentiation (Swain et al., 2005). Plasticity in morphological and life history traits results from the capacity of a given genotype to produce different phenotypes across an environmental gradient i.e., the reaction norm (Swain et al., 2005). Reaction norms of phenotypic traits provide information on the magnitude of plasticity and on the interaction between genetic and environmental influences for different genotypes.

Geographical differences in morphology, life history traits and demographic parameters have long been used to sub-divide species into populations and fisheries stocks. Populations may be viewed as ecological entities while stocks define operational units relevant to fisheries management but the two terms are often used as synonyms (Secor, 2005; Waldman, 2005). In its simplest definition, the term population refers to a group of individuals from the same species inhabiting a defined area at a given time but implicitly, populations must be reproductively isolated and self-sustainable to persist in time and space (Wootton, 1998). In the marine world, reproductive isolation has been associated with natal homing and retention of early-life stages, coupled with reduced viability of straying individuals (McQuinn, 1997). The view of completely isolated fish populations has been gradually challenged by evidence from studies of genetic variability, migration and phenotypic plasticity (McQuinn, 1997). Furthermore, evidence that geographic variability in phenotypic traits may reflect adaptive genetic differences (Conover and Schultz, 1995) in face of gene flow (Swain et al., 2005) contributed to new concepts of population structure midway between classical alternatives of complete isolation or panmixia. One of such concepts, that of the metapopulation structure, has been adapted and extended from the field of theoretical biology to the field of fisheries biology to explain partial isolation between stocks (McQuinn, 1997; Stephenson, 1999; Smedbol et al., 2002; Kritzer and Sale, 2004). The definition proposed by Kritzer and Sale (2004), that a metapopulation is a regional group of local populations with asynchronous internal dynamics but linked by sufficient gene flow to establish demographic connectivity (i.e. the exchange of individuals between populations affects their mutual size, structure and life history traits), highlights its relevance for fisheries assessment and management.

The concept of fisheries stock has also evolved considerably in the last three decades (Ihssen et al., 1981; Kumpf et al., 1987; McQuinn, 1997; Begg et al., 1999a;

Kritzer and Sale, 2004; Cadrin et al., 2005). From definitions based on "what is caught", it gradually approached the concept of ecological entity, as reflected in the definition of Hilborn and Walters (1992): *A unit stock is an arbitrary collection of populations of fish that is large enough to be essentially self-reproducing (abundance changes are not dominated by immigration and emigration), with members of the collection showing similar patterns of growth, migration and dispersal.* The concept of stock is still a matter of debate and much of the discussion deals with finding a sufficiently general definition to encompass most empirical knowledge available in fisheries biology. One such definition is provided in Waldman (2005), and states that stock units have "certain characteristics that render them fundamental to management: (1) they occupy their own physical life history circuit, including spawning grounds that are geographically or temporally unique; (2) they experience their own demographic influences, such as mortality from a particular suite of predators; (3) their complete-to-partial isolation allows fine tuning of their morphological and genetic characteristics to their particular environmental circumstances; and (4) their abundances and life history characteristics respond to their own sets of unnatural influences, such as fisheries and contamination of their habitats". As proposed by Secor (2005, Figure 3-1), the definition of stock units must take into account both the biological organization and the spatial and temporal scales which are relevant to the fisheries of interest and may thus range from the brood to the species, passing through population and metapopulation levels.

The definition of unit stocks is fundamental to fisheries management since stock assessment models usually assume closed populations with homogeneous life history characteristics and similar responses to exploitation. Violation of the stock unit, if several closed stocks or a portion of a stock are modelled, may lead to erosion of genetic diversity, changes in biological attributes and productivity rates, overfishing, depletion of the less productive stocks, and unexpected responses to a given management regime (Begg et al., 1999b). Studies demonstrating such effects are scarce. However, on a simulation of the behaviour of a stock complex, Frank and Brickman (2000) show that failing to account for different stock recruitment relationships masked over-exploitation of some sub-stocks and lead to inaccurate reference points for fisheries management. On another simulation study, Daan (1991) demonstrates that trends in stock parameters are biased if two sub-stocks experiencing different fishing mortality are assessed as a single stock.

An alternative to these classical assessment models is provided by models accounting for spatially explicit dynamics and migration of fish populations. Several models of this type have been developed in recent years both for stock assessment (e.g. Quinn et al., 1990; Methot, 2000; Buckland et al., 2004) and management (e.g. Pelletier and Mahévas, 2005). However, their successful application is often strongly dependent on the availability of data on migration rates, generally obtainable from tagging experiments, which are difficult and expensive to implement for most fish species (Quinn et al., 1990).

In summary, assessment of commercial fish species distributed in broad geographic areas requires comprehensive knowledge on the structure and connectivity of their component populations. The study of spatio-temporal variability in phenotypic and demographic traits of these populations is fundamental for the definition of assessment units and for the selection of adequate models to describe their history and to predict their response to management actions. The remaining of this chapter provides an overview of methods to study fish stock structure (section 1.1), summarises current knowledge on sardine biology, population structure and fisheries (section 1.2) and describes the objectives and organization of this study (section 1.3).

1.1 Methods to address fish stock structure

A wide variety of methods is used to study fish stock structure, their strengths and weaknesses depending on the study species, on the definition of stock used and on the degree of spatial resolution required (Cadrin et al., 2005). It is generally accepted that employing a broad spectrum of complementary techniques to the same species ("holistic approach") often allows to resolve apparent discrepancies between methods and increases the chance of correctly defining stocks (Begg and Waldman, 1999; Swain et al., 2005). Stock identification methods can be assembled in three groups (Cadrin et al., 2005): methods based on life history traits such as distribution, growth, mortality and reproduction, methods based on natural marks, such as body morphology, otolith morphology or chemical composition, parasites and genetic characters and those based on applied marks such as tagging and otolith thermal marking. Genetic markers have generally been considered more powerful to identify fish stocks than phenotypic traits, since they directly reflect reproductive isolation between populations. However, the exchange of a small number of migrants per generation is sufficient to homogenize populations with respect to neutral genetic markers (such as allozymes, mitochondrial DNA or microsatellite DNA) but insufficient to prevent genetic differences associated with adaptive traits, such as morphology or life history characters (Koljonen and Wilmot, 2005; Magoulas, 2005; Swain et al., 2005). Furthermore, selection can result in rapid adaptive genetic divergence of recently separated groups within temporal scales which are relevant to fisheries management, whereas neutral genetic divergence occurs at evolutionary time scales. Thus, differences between populations revealed by neutral genetic markers provide evidence of separate stocks, although absence of neutral genetic differentiation does not demonstrate that there is a single stock. Phenotypic markers can therefore disclose recent reproductive isolation or evidence that gene flow is not sufficient to prevent persistent phenotypic differences which may be environmentally induced but need to be accounted for in stock assessment and management (Swain et al., 2005).

Morphometric methods aim to separate populations with persistent differences in body shape. Compared to other stock identification approaches, morphometric methods are inexpensive and do not involve complex sampling or processing technology, although they require large samples due to the use of multiple variables. Careful selection of sampled individuals and/or statistical analyses are also necessary to avoid counfounding of shape with fish size or biological traits such as stomach fullness or gonad volume (Cadrin, 2005). Differences in fish body shape may arise from population-specific allometric patterns of certain body dimensions or may simply reflect differences in size at age, i.e. different growth patterns between populations (Cadrin, 2000). Fish body shape often shows clinal variation; for example, the ratio between head size and body size in Pacific sardine declines with latitude possibly resulting from the combination of a negative head allometry with a latitudinal decline in fish size (Hedgecock et al., 1989). Body dimensions are mainly affected by environmental factors and certain body shapes are typically associated with specific ecological niches; for example slender bodies, longer heads and larger eyes are more common in pelagic environments and planktonic feeding modes (Swain et al., 2005). Nevertheless, morphometric variability may have a strong genetic component, as shown from common garden experiments with Atlantic cod originating from populations at different latitudes (Marcil et al., 2006).

As morphological characters, life history parameters result from a combination of genetic and environmental influences (Swain et al., 2005). Differences in growth and maturity patterns may indicate that populations occupy different environments and therefore separate territories across their life history (Begg, 2005), but may also reflect adaptive genetic differences between populations (Billerback et al., 2000;

Kokita, 2004). Moreover, growth and maturity rates affect population productivity and yield which are fundamental parameters in stock management and must therefore be taken into account in the delineation of stock units (Swain et al., 2005).

Vital population parameters such as abundance, recruitment, demographic structure and survival reflect the underlying population dynamics and provide information on the association and geographic continuity of adjacent groups of fish (Begg, 2005). One advantage of using life history traits to delineate fish stocks is that samples allowing their estimation are often collected as part of routine surveys to provide input data for stock assessment (Begg, 2005). Since all phenotypic traits show large plasticity in response to changing environmental conditions and/or exploitation, a key aspect of morphometric and life-history stock identification approaches is the assessment of spatial and temporal stability of differences among putative stocks.

1.2 Sardine biology, populations and stocks

The sardine (*Sardina pilchardus*, Walbaum, 1792) is a small pelagic clupeoid distributed in the northeast Atlantic Ocean and the Mediterranean Sea. In the Atlantic sardine extends from the southern Celtic Sea and North Sea to Mauritania and Senegal, with residual populations off the Azores, Madeira, and the Canary Islands (Parrish et al., 1989). The limits of sardine distribution have been observed to expand and contract periodically. Between the mid-1960s and the mid-1970s, the southern limit extended from Morocco to Senegal, coinciding with the intensification of upwelling in the area, and retreated to the north in the following years (Lluch-Belda et al., 1989). Temporal changes in the northern limit have also been reported, with increased abundance off the southern North Sea in the early 1990s (Corten and Van de Kamp, 1996) and more recently off the northern North Sea, possibly associated with the transport of warmer Atlantic waters across the English Channel (Beare et al., 2004). The Atlantic range of sardine encompass diverse topographic and oceanographic conditions, although the core distribution area is located in one of the five eastern boundary upwelling systems of the world, the Canary Current (Lluch-Belda et al., 1989; Cole and McGlade, 1998; Mason et al., 2006). Sardine is also found across all the Mediterranean and adjacent Seas, being less abundant in southeastern Mediterranean areas, from the coastal waters of Tunisia to Turkey (Larrañeta, 1960). In contrast to the northeast Atlantic, Mediterranean waters, especially in the southeastern range, are rather oligotrophic with neither substantial upwelling nor alternative oceanographic conditions promoting high productivity (Stergiou et al., 1997).

The life history strategy of sardine is broadly characterized by rapid growth, small life-span and size, rapid maturation, large fecundity, and high mortality (Rochet, 2000; Rose et al., 2001). Sardine attains 14 years of age and 27 cm total length, growing to 90% of their maximum length during the first year and maturing within the first two years of life, although both growth and maturity show large variability among areas (e.g. Monteiro and Jorge, 1982; Pérez et al., 1985; Alemany and Álvarez, 1993; FAO, 2001a). Growth in length, somatic condition and lipid reserves show strong seasonal patterns with maximum values outside the spawning season (Bandarra et al., 1997; Ganias et al., 2007). Spawning is temperature-dependent (mainly between 12 and 18°C) and extends throughout most of the continental shelf (Larrañeta, 1960; Ettahiri et al., 2003; Coombs et al., 2006; Bernal et al., 2007). The duration of the spawning season increases from north (1-2 months) to south (6 months) in the northeastern Atlantic while peak activity shifts from late-spring to winter (Ettahiri et al., 2003; Coombs et al. 2006; Stratoudakis et al., 2007). In the Mediterranean Sea, spawning also extends for 6 months and shows a maximum in winter (Abad and Giraldéz, 1993; Ganias et al., 2007). Sardine is an indeterminate, batch spawner (Zwolinski et al., 2001; Somarakis et al., 2006) and produces pelagic eggs and larval stages (Russell, 1976). Other than temperature, body size and energy reserves of spawning females are major factors affecting the reproductive dynamics of sardine (Zwolinski et al., 2001; Riveiro et al., 2004; Ganias et al., 2007). Sardine feeds on phytoplankton, zooplankton and fish eggs with the relative importance of these prey groups depending on area and season (Varela et al. 1988; Garrido et al., in press).

Various hypothesis of sardine population structure (considering races or "forms") were proposed based on early analyses of morphologic and meristic characters (see Fage, 1920; Furnestin, 1943; Andreu, 1969; Furnestin and Furnestin, 1970). Parrish et al. (1989) revised information from these studies, assuming races and stocks as synonyms, and suggested four stocks of sardine within northeastern Atlantic waters: the septentrional Atlantic stock, distributed from the North Sea (57°N) to the Cantabrian coast of Spain (43°N), the Iberian or meridional Atlantic stock, distributed from western Galicia to the Gulf of Cadiz (from 43°N to 36°N), the Moroccan stock, distributed from Cap Spartel (36°N) to Cap Juby (28°N), and the Saharian stock, distributed from Cap Juby to Levrier Bay (21°N). However, this delimitation was never assumed for fisheries assessment and management across the area. More recently, several studies addressed the genetic structure of sardine populations using various genetic markers (Ramon and Castro, 1997; Kasapidis et al., 2004; Chlaida et al., 2005; Atarhouch et al., 2006, 2007; Laurent et al., 2007). Most genetic markers evidence significant differences between Atlantic and Mediterranean sardine populations but more limited genetic differentiation across the northeast Atlantic waters. However, peripheral regions such as Azores, Madeira and Mauritania were separated from the remaining Atlantic areas by allozymes and microsatellite DNA (Kasapidis et al., 2004; Laurent et al., 2007) and evidence of an isolated population off Safi, northern Morocco was also obtained (Chlaida et al., 2005, Atarhouch et al., 2006, 2007). A recent study of otolith chemical composition corroborates the existence of gene flow among Iberian sardine populations and further suggests that population mixing increases across life (Castro, 2007).

For fisheries assessment and management, a single sardine stock is considered in the European Atlantic waters, the Atlanto - Iberian stock (ICES, 2006a), three stocks are considered off northwest Africa (GFCM, 2006) and eight stocks are considered within the Mediterranean Sea (GFCM, 2006). Catch levels and acoustic survey assessments of these stocks indicate that the largest populations and more productive sardine fisheries are located in central Morocco; in recent years, annual landings within this area fluctuate around 500 thousand tons and biomass estimates range between 1 and 5 million tons, corresponding to 6 times the landings and 8 times the biomass estimated for the Atlanto-Iberian sardine stock. Total landings from the Mediterranean stocks (ca. 80 thousand tons in recent years) are close to those from the Atlanto-Iberian stock. Regular estimates of abundance are unavailable for most Mediterranean areas but recent acoustic data suggests that the largest Mediterranean stocks are located off the northern Spanish waters and Gulf of Lyons. As most small pelagic fish stocks, sardine stocks show wide temporal changes in abundance reflecting recruitment variations which are possibly environmentally-induced (Santos et al. 2001, 2005; Guisande et al., 2004).

The Atlanto-Iberian sardine stock is delimited by the French/Spanish border in the north and by the Strait of Gibraltar in the south (ICES Divisions VIIIc and IXa). Across this area, commercial sardine fisheries have subsisted for centuries (Alvarez et al., 1986; Pestana, 1989; Assis and Amaro, 2006; Mendes and Borges, 2006). Purse seine fisheries expanded in the early 20th century, following the introduction of "traineiras", small wooden vessels allowing more efficient and cost effective fishing than traditional sardine trap nets ("armações") and large "American type" purse seiners (Ramalho, 1933; Filgueiras, 1994). Besides gradual improvement of vessels and fishing gear, major technological advances in purse seine fisheries were the introduction of echosounder and sonar for the detection of schools in the 1950s and mechanical hauling devices in mid-1960s (Pestana, 1989; Filgueiras, 1994).

Sardine landings in the Iberian Peninsula reached an historical maximum of 246 thousand tons in 1961 (Figure 1.1). At present, sardine continues to be targeted by Spanish and Portuguese purse seine fleets and represents a significant part of total fish production in both countries and an important supply for the canning industry. In the Portuguese fisheries, sardine corresponds to 40% in weight and 17% in first-sale value of the fish production from national waters (e.g. DGPA, 2006) and make up 80% of purse seine landings. Six thousand workers are either directly involved in the fishery or associated with the sardine canning industry (EU-DGF, 2000; DGPA, 2006). Within the stock area, fisheries regulations include limitations to mesh size, gear dimension, fishing areas, use of attraction lights, and a minimum landings size (11 cm). Since 1997, additional restrictions to fishing effort, annual catches and seasonal closures have been gradually enforced by both countries (ICES, 2000).

Landings from the Atlanto-Iberian sardine stock were halved since the mid-1980s, spawning stock biomass (SSB) peaked in the mid-1980s and the mid-1990s following strong recruitments but both SSB and recruitment peaks show a declining trend across the period (ICES, 2006a). Furthermore, despite a reduction in fishing mortality since 1997, due to a gradual enforcement of fishing restrictions, and a strong

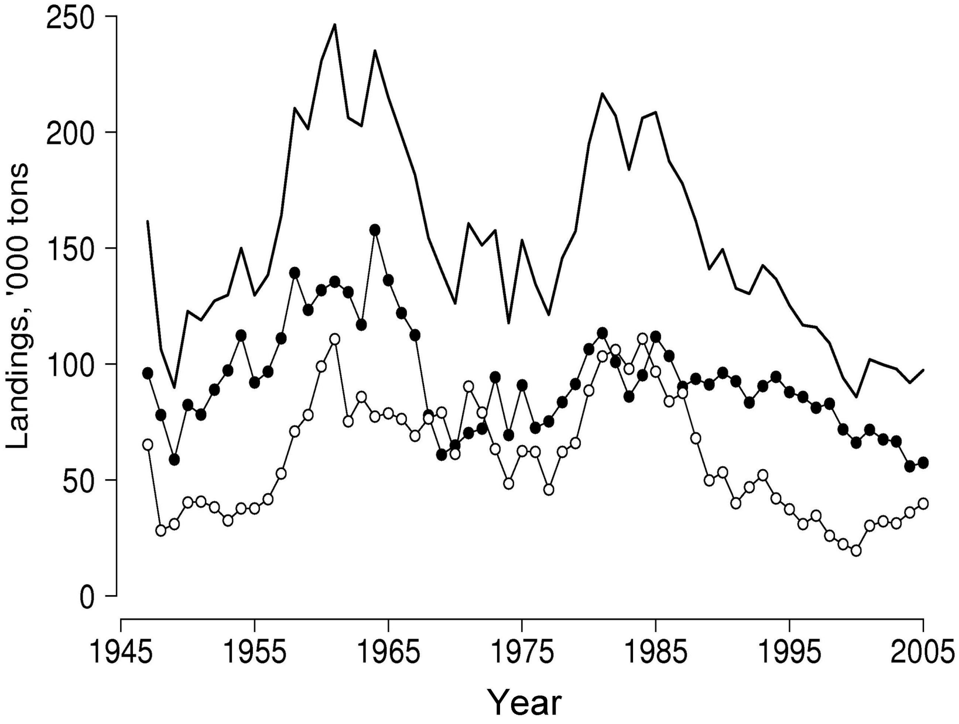

Figure 1.1: Historical landings of sardine in the Iberian Peninsula (from ICES, 2006a). Portuguese landings (black symbols); Spanish landings, including the Gulf of Cadiz since 1978 (white symbols); total landings (line).

recruitment in 2000, SSB merely recovered to average historical values, indicating that the stock has a low "buffer" biomass and is increasingly dependent on incoming year-classes. Examination of area-disaggregated information suggested that stock and catch declines were more pronounced off the northern Spanish waters where a major contraction of the spawning and distribution areas was also observed (ICES, 2000). In addition, both fisheries and survey data evidenced complementary age structures off northern Spain and Portugal, with mainly older sardines in the former area and the reverse in the latter area, suggesting that age-related northward migrations could have an important role in the dynamics of the stock (Porteiro et al., 1986). Several hypotheses, assuming variable degrees of connectivity between local populations, were raised to explain geographical differences in catch and abundance trends within the stock area (Carrera and Porteiro, 2003), while recent research suggested that sardine spawning areas are continuous across the northern stock boundary (Bernal et al., 2007). Geographical variability in catch and abundance signals has been difficult to reconcile in successive assessments of the stock increasing the uncertainty of fishing mortality and stock estimates, while the historical perspective shows sensitivity to which survey data and assumptions on fisheries and survey selectivity are included in the assessment model (ICES, 2000, 2001, 2002, 2003a). Overall, the reliability of sardine assessment has been affected by uncertainty about stock boundaries and homogeneity in the dynamics of populations, emphasizing the need for further research on the structure of populations distributed within and in the neighborhood of the Iberian Peninsula.

1.3 Objectives and structure of the study

The main objective of this study is to describe the population structure of sardine within the Atlanto-Iberian stock area and adjacent waters, integrating information from fish body morphometry, life-history and dynamics. The results are expected to assist the revision of stock units within this area but also to improve knowledge on factors affecting sardine growth, reproduction and population variability and to support the improvement of biological sampling design off the Portuguese waters.

Chapter 2 presents an overview of the data sources and laboratory protocols used in the subsequent chapters. In Chapter 3 the variability of sardine body morphometry across the northeast Atlantic and Mediterranean Sea is analysed and groups with significant differences in body shape are identified. Chapter 4 describes broad scale geographical variation in the length at first maturity and relates temporal variability of this parameter and of spawning seasonality with environmentally-induced changes in fish condition across the Iberian areas. Chapter 5 provides an overview of the variability of length-at-age across most of the distribution range of the species and describes persistent differences in growth patterns within the Iberian Peninsula. In Chapter 6, the dynamics of local sardine populations and fisheries within Iberian waters and the adjacent Gulf of Biscay are described and demographic links between populations are analysed. Finally, Chapter 7 integrates and discusses the results from the previous studies and relates them to the available information from other stock identification approaches.

The research studies presented in this thesis were part of the workplan of the EU Project SARDYN (Contract Q5RS-2002-000818) which aimed to investigate the structure and dynamics of sardine in the northeastern Atlantic.

Chapter 2

Overview of sampling and laboratory protocols

2.1 Introduction

The analyses presented in this thesis are based on three main sources of biological information for sardine:

1. Samples collected across the northeast Atlantic and the Mediterranean Sea in the period 1999 - 2004;
2. Data on abundance and biological characteristics collected annually during acoustic surveys in the coastal waters of the Iberian Peninsula (1984 - 2006) and the French Gulf of Biscay since 2000;
3. Data on landings and biological characteristics collected regularly at the main sardine fishing ports off the Iberian Peninsula in the period 1981 - 2005 and off the French Gulf of Biscay since 2002.

The first set of samples was used to study broad-scale spatial variation of sardine population traits in recent years. Most samples were obtained within the remit of projects PELASSES (EU Study 080/99) and SARDYN (EU Q5RS-2002-000818). The latter project aimed to study sardine stock structure within the northeast Atlantic, and particularly within the Iberian - Biscay region, integrating several methods (genetic, parasite and otolith chemistry analyses), apart from morphometric and life history approaches presented in this thesis. Additional samples from the Mediterranean Sea, Azores, Madeira and the northwest African waters were obtained through collaboration with researchers from local laboratories (see the Acknowledgements section).

The second and third data sets were used to describe geographic and temporal variation of life history traits and the dynamics of local sardine populations within the Iberian - Biscay region. These data sets were obtained from national databases, stored at IFREMER (France), IEO (Spain) and IPIMAR (Portugal) laboratories and correspond to data used in the annual assessment of the Atlanto - Iberian sardine stock. Area-disagregated data from Spanish and Portuguese areas were compiled and revised within the Project SARDYN (Anon., 2006).

Different sub-sets of the samples and data listed above are used in each of the analyses and descriptions of specific material and methods are found in the corresponding chapters. Here, an overview of these sub-sets is provided to facilitate the perception of spatial and temporal ranges and the link among the results from different studies. In addition, more comprehensive descriptions and revisions of sampling designs and biological sampling protocols common to the different analyses are presented.

2.2 Samples for broad-scale spatial analyses

A total of 66 samples of sardine (4582 individuals) were collected across the northeast Atlantic and Mediterranean Sea in the period 1999 - 2004 (Table 2.1; Figure 2.1). The collection of samples was opportunistic, depending on surveys planned within the projects and on collaboration with laboratories outside the main project areas, and therefore the final distribution of samples among areas and years is unbalanced and mainly concentrated within the Iberian - Biscay region (Figure 2.1). Samples collected during 1999-2000 were used to study sardine morphometric variability (Chapter 3), those collected within the spawning season across the period were used to describe sardine maturity-at-length outside the Iberian Peninsula (Chapter 4) and almost all samples were used to describe length-at-age variations across the species range (Chapter 5). Samples collected off the western North Sea comprise immature age 1 fish with 9.5 to 15.1 cm total length. Although information on sardine maturity-at-length and growth could not be obtained from these samples, they provide interesting information on the occurrence of juvenile sardine in the area.

Most samples (57 out of 66) were collected with pelagic trawls during research surveys. The remaining samples were collected from local commercial fisheries: samples 4-6 (English Channel) were collected from drift-net landings off Newlyn, samples 44, 46 and 64 from purse seine landings off Larache, Casablanca (north Morocco) and Nador (Mediterranean Morocco), and samples 50-52 (Azores, S.Miguel island) and 53 (Madeira) from tuna bait fisheries with small purse seine nets. Apart from a few samples (9, 18, 22 and 30), individuals were randomly collected from the catch. Survey samples were frozen within 1-2 hours after capture while samples from commercial catches endured a longer period before freezing; this should not have exceeded 12 hours, considering that drift-net operations and purse seine fish-

Table 2.1: Summary information of samples collected in the period 1999 - 2004 across the northeast Atlantic and Mediterranean Sea, and identification of samples used in Chapters 3 (morphometry), 4 (maturity) and 5 (growth).

	Area		ICES /FAO	Month	Year	Latitude	Longitude	Chapters: Morphometry		Maturity		Growth		Total
								n	Sample no.	n	Sample no.	n	Sample no.	n
1	North Sea	NSEA	IVb	1	2004	56° 17.0 N	00° 57.0 W							50
				2	2004	53° 49.9 N	02° 35.0 E							96
				2	2004	54° 47.0 N	01° 17.4 E							101
2	English Channel	CHAN	VIIe	1	2003	50° 01.0 N	05° 22.0 W					93	1	93
				2	2003	50° 14.0 N	04° 13.0 W					48	1	48
				11	2003	50° 03.0 N	05° 28.0 W					100	1	100
3	Northern France	NFRA	VIIIa	3	2000	48° 30.0 N	05° 26.0 W	70	1	84	1	83	2	84
				5	2000	46° 51.0 N	04° 56.7 W	78	2	100	1	100	2	100
				5	2001	46° 38.0 N	03° 02.8 W			103	1	50	2	103
				6	2001	46° 03.7 N	04° 09.9 W			100	1	50	2	100
				6	2003	46° 32.6 N	03° 46.0 W					88	2	88
				6	2003	46° 23.9 N	01° 48.5 W					45	2	45
				6	2003	47° 15.0 N	05° 17.1 W					43	2	43
4	Southern France	SFRA	VIIIb	4	2000	45° 09.5 N	02° 02.8 W	98	3	100	2	97	3	100
				5	2001	45° 03.3 N	02° 08.3 W			100	2	48	3	100
				6	2003	45° 01.7 N	01° 53.2 W					59	3	59
5	Eastern Cantabria	ECAN	VIIIc-Ee	4	2000	43° 25.3 N	01° 44.5 W	72	4			99	4	99
				4	2001	44° 15.4 N	01° 39.4 W					50	4	50
				4	2001	43° 31.7 N	03° 24.1 W					75	4	75
				4	2003	43° 30.4 N	02° 50.9 W					85	4	85
6	Western Cantabria	WCAN	VIIIc-Ew	4	2000	43° 40.3 N	06° 30.1 W	87	5			96	5	96
				4	2001	43° 46.0 N	06° 31.0 W					50	5	50
				4	2003	43° 55.4 N	06° 43.1 W					100	5	100
7	Northern Galicia	NGAL	VIIIc-W	3	2003	43° 20.0 N	08° 31.5 W					102	6	102
				3	2000	43° 20.0 N	08° 21.5 W					98	6	98
				4	2001	43° 45.1 N	08° 11.4 W					50	6	50
				4	2001	43° 29.1 N	08° 20.4 W					91	6	91
8	Southern Galicia	SGAL	IXa-N	3	2000	42° 21.9 N	08° 47.7 W	72	6					72
				3	2003	42° 20.0 N	08° 53.0 W					99	7	99
				4	2001	42° 21.9 N	08° 46.9 W					50	7	50
9	Northern Portugal	NPOR	IXa-CN	2	2003	41° 05.0 N	08° 59.9 W					97	8	97
				3	2000	40° 59.8 N	08° 42.8 W	99	7			100	8	100
				4	2001	41° 26.1 N	08° 49.7 W					47	8	47
10	S.western Portugal	SWPOR	IXa-CS	3	2000	38° 34.7 N	09° 18.4 W	87	8			96	9	96
				3	2001	38° 30.5 N	09° 12.8 W					41	9	41
				3	2003	38° 32.0 N	09° 13.6 W					101	9	101
11	Southern Portugal	SPOR	IXa-SA	3	2000	37° 05.2 N	08° 30.5 W	94	9			98	10	98
				3	2001	36° 57.2 N	08° 03.1 W					50	10	50
				3	2001	37° 04.6 N	08° 30.7 W					49	10	49
				3	2003	37° 03.1 N	08° 28.0 W					100	10	100
12	Gulf of Cadiz	CAD	IXa-SC	3	2000	36° 33.3 N	06° 23.6 W	96	10			98	11	98
				3	2001	36° 50.0 N	06° 41.7 W					72	11	72
				3	2003	36° 30.2 N	06° 30.9 W					70	11	70
13	North Morocco	NMOR	34.1.11	1	2000	33° 30.0 N	07° 40.0 W	97	11	100	13	97	12	100
				1	2003	35° 10.9 N	06° 09.9 W			39	13	39	12	39
				1	2003	35° 12.0 N	06° 09.9 W					48	12	48
				1	2003	33° 36.6 N	07° 35.4 W					38	12	38
14	Mauritania	MAU	34.3	3	2003	18° 25.3 N	16° 21.8 W					36	13	36
				12	2003	20° 38.0 N	16° 12.0 W			89	14	89	13	89
15	Azores	AZO	X	5	2000	38° 30.0 N	26° 00.0 W	93	14	99	15			99
				10	2003	38° 32.0 N	26° 00.0 W			26	15	26	14	26
				11	2004	38° 34.0 N	26° 00.0 W			99	15	95	14	99
16	Madeira	MAD	XI	3	2003	38° 36.0 N	26° 00.0 W			64	16	82	15	82
17	Gulf of Lyon	LYON	37.1.2	7	2003	42° 40.5 N	03° 16.7 E					46	18	46
				7	2003	42° 51.2 N	03° 26.4 E					22	18	22
				7	2003	42° 53.4 N	03° 43.9 E					32	18	32
				12	1999	40° 50.2 N	00° 12.3 E	43	13	50	12	49	18	50
				12	1999	40° 31.7 N	00° 58.8 E	10	13	10	12	10	18	10
18	Western Mediterr.	WMED	37.1.1	12	1999	39° 04.2 N	01° 37.2 E					48	17	48
				12	1999	37° 59.3 N	00° 36.7 E					44	17	44
19	S.western Mediterr.	SMED		12	1999	36° 46.6 N	02° 19.0 W	49	12	49	10	49	16	49
				12	1999	36° 43.0 N	03° 04.6 W	30	12	30	10	28	16	30
				12	1999	36° 34.0 N	04° 26.3 W	27	12	27	10	26	16	27
20	Morocco Mediterr.	MMED	37.1.1	10	2004	35° 12.0 N	05° 00.0 W			46	11			46
21	Aegean Sea	AEG		6	2003	40° 50.8 N	24° 23.9 E					34	19	34
				6	2003	40° 14.0 N	23° 10.9 E					42	19	42
	Total							1202		1315		4195		4582

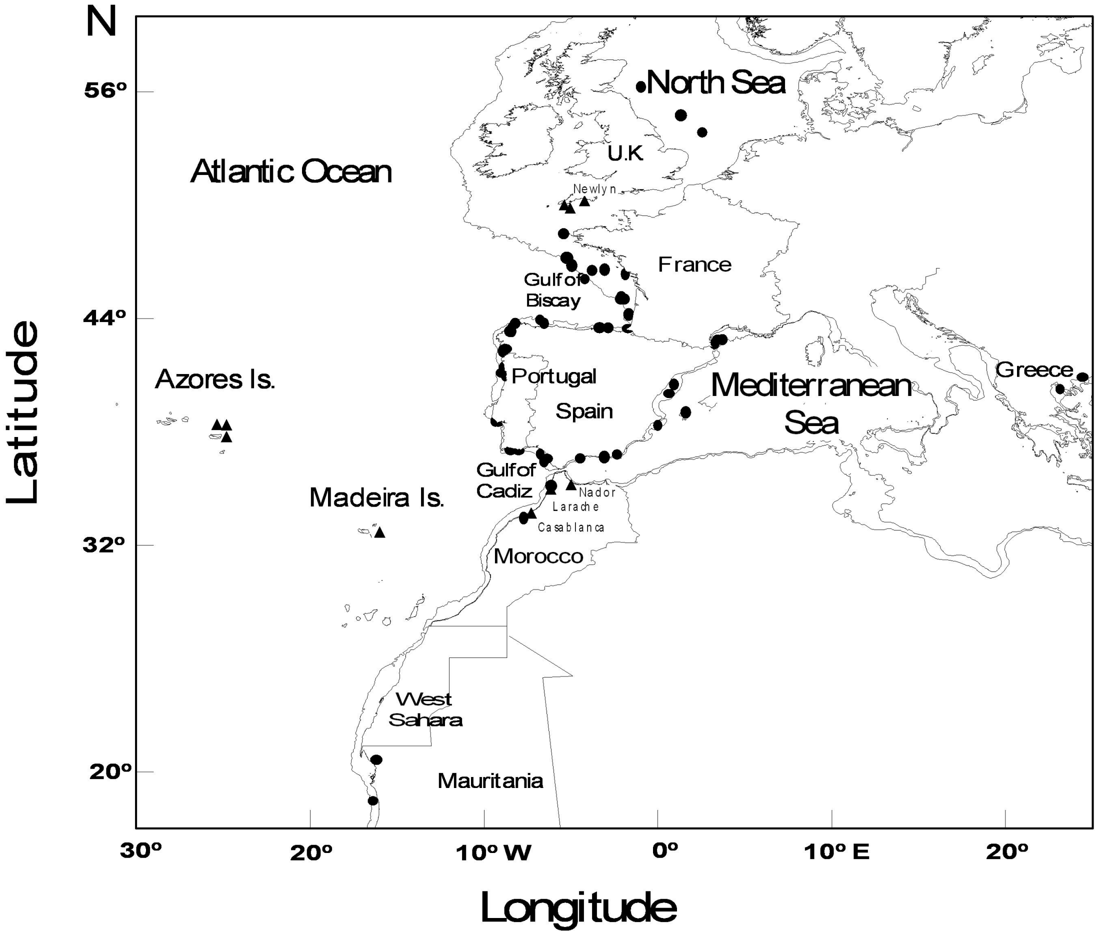

Figure 2.1: Location of samples collected in the period 1999 - 2004 across the northeast Atlantic and Mediterranean Sea. Circles represent samples collected within research surveys and triangles represent samples collected from landings. Bathimetric line = 200 m.

ing trips take generally less than half a day (e.g. Stratoudakis and Marçalo, 2002; Wise et al., 2007). All samples were transferred frozen to the laboratory of IPIMAR and processed for the collection of morphometric and biological characteristics (see below).

2.3 Samples for the analyses of geographic and temporal variation within the Iberian-Biscay region

Acoustic surveys

Biological samples collected within acoustic surveys were used to study sardine maturity-at-length (Chapter 4) and growth patterns (Chapter 5) while estimates of abundance-at-age were used to describe the dynamics of populations from adjacent areas within the Iberian - Biscay region (Chapter 6). Acoustic surveys for the assessment of the Atlanto-Iberian sardine stock have been carried out by Spain and Portugal since the mid-1980s (Table 2.2; Figure 2.2). Since 2000, France started to collect information on sardine abundance and distribution in the Gulf of Biscay, within acoustic surveys directed to the assessment of anchovy (Table 2.2; Figure 2.2). Off Portugal, surveys have been carried out in spring, summer and autumn with variable regularity between seasons and the survey area was extended to the Gulf of Cadiz in 1992 (Dias et al., 1996; Marques et al., 2004). The summer series was abandoned in the late 1990s while the spring series became the most systematic one and is currently used to tune the assessment, combined with the Spanish survey in a single relative abundance index (ICES, 2006a). The total survey area is divided in ten sub-areas, broadly corresponding to ICES sub-divisions, used to stratify biological sampling and estimation of sardine abundance (Figure 2.2).

Acoustic surveys have been coordinated within dedicated ICES groups and comprehensive descriptions of sampling design and methods for acoustic abundance estimation were reported in ICES (1986, 1997a, 1998, 2006b). Inter-calibration between surveys has not been carried out and preliminary attempts to compare catchability and selectivity between Spanish and Portuguese surveys were inconclusive (Marques et al., 2004; ICES, 2006b). Nevertheless, standardization between surveys gradually improved and presently, similar acoustic equipment and survey design are used by the three countries while the same ship and fishing gear are used by Spain and France (Table 2.2). Differences persisting in survey methodology are due to practical contraints or to the need to adapt to characteristics of the local pelagic community (ICES, 2006b).

Several changes in survey equipment, design and acoustic data analyses have been introduced over time in order to improve fish accessibility and data quality. Some of them may affect the comparability of abundance and length/age structure estimates across the period but their net effect is difficult to assess (Marques et al., 2004; Marques, 2005). Major changes in acoustic surveys over time are listed in

Table 2.2: Summary information of acoustic surveys design and sampling methods across the study period and identification of data used in research studies of Chapters 4 (maturity), 5 (growth) and 6 (dynamics). VO: Vertical opening; PS: purse seine stations; BT: bottom trawl stations; LS: Length-stratified sampling; SR: simple random sampling. ALK: age-length key. N: sample size range. Biological characteristics: L: total length, A: age, TW: total individual weight; S: sex; M: maturity stage; F: fat stage; GW: gutted weight; GOW: gonad weight. Sampling depth is based on surveys in the period 2000 - 2006; IQR: inter-quartile depth range.

Survey Period	Series	Area	Vessel	Echo-sounder	Survey track	Pelagic gear VO (m)	Trawling speed (knots)	Median sampling depth (IQR)	No stations	ALK		Biology			Chapters			Notes
										Sampling stations	n	Sampling stations	n	Character.	3	4	5	
French																		
2000-2006	spring	French Gulf of Biscay	Thallassa	Simrad ER60	Parallel; day	18-24	4.0-4.5	115 (65-140)	11-42	LS; 2-4 fish per class/haul	300-1300	LS; 2-4 fish per class/haul	300-1300	L, A, TW, S, M, F		X	X	Uncomplete coverage of northern area in 2005
Spanish																		
1986-1989	spring	Northern Spain	Cornide de Saavedra	Simrad EK400	Zig-zag; day&night	8	3.5-4.0		11-19		420-720	SR; 40 fish per haul	440-850	L, A, TW, S, M, F		X	X	Galicia unsampled in 1996 and 1997 for biology/ALK
1991-1993	spring	Northern Spain	Cornide de Saavedra	Simrad EK500	Zig-zag; day&night	18-24	4.0-4.5		7-12	SR; 40 fish per class/haul	380-420		450-900	L, A, TW, S, M, F		X	X	Parallel, day&night in 1997
1996-2006	spring	Northern Spain	Thallassa	Simrad EK500	Parallel; day	18-24	4.0-4.5	72 (43-117)	9-38 (2-14 PS)		630-1400		680-2600	L, A, TW, S, M, F		X	X	Parallel, day&night in 1997
Portuguese																		
1984-1988	spring (1986, 1988)	Portuguese coast	Noruega	Simrad EK38S	Zig-zag; day&night	20	3.5		11-26 (5-6BT)	LS; 10 fish per class/area	550-710	LS; 10 fish per class/haul	1200-2600	L, A, TW, S, M, F		X	X	
	summer (1985-1988)																X	Parallel in 1988
	autumn (1984-1987)														X			
1992	autumn	Portuguese coast	Noruega	Simrad EK400	Zig-zag; day&night	20	3.5		19 (12 BT)		926		2000	L, A, TW, S, M, F	X		X	Mixed zig-zag and paralell design.
1995-2006	spring (1996-2006)	Portuguese coast and Gulf of Cadiz	Noruega	Simrad EK500	Parallel; day	10	4.0	36 (28-50)	15-41 (3-9 BT)	LS; 10-20 fish per class/area	500-1000		1700-8400	L, A, TW, GW, GOW, S, M, F		X	X	1996 survey design and gear as in previous series; 65% of Cadiz covered in 2001, unsampled in 1997; no survey in 2004.
	summer (1995, 1996)																X	mixed zig-zag and parralel in 1996
	autumn (1997-2001, 2005)														X		X	Day&night in 1997

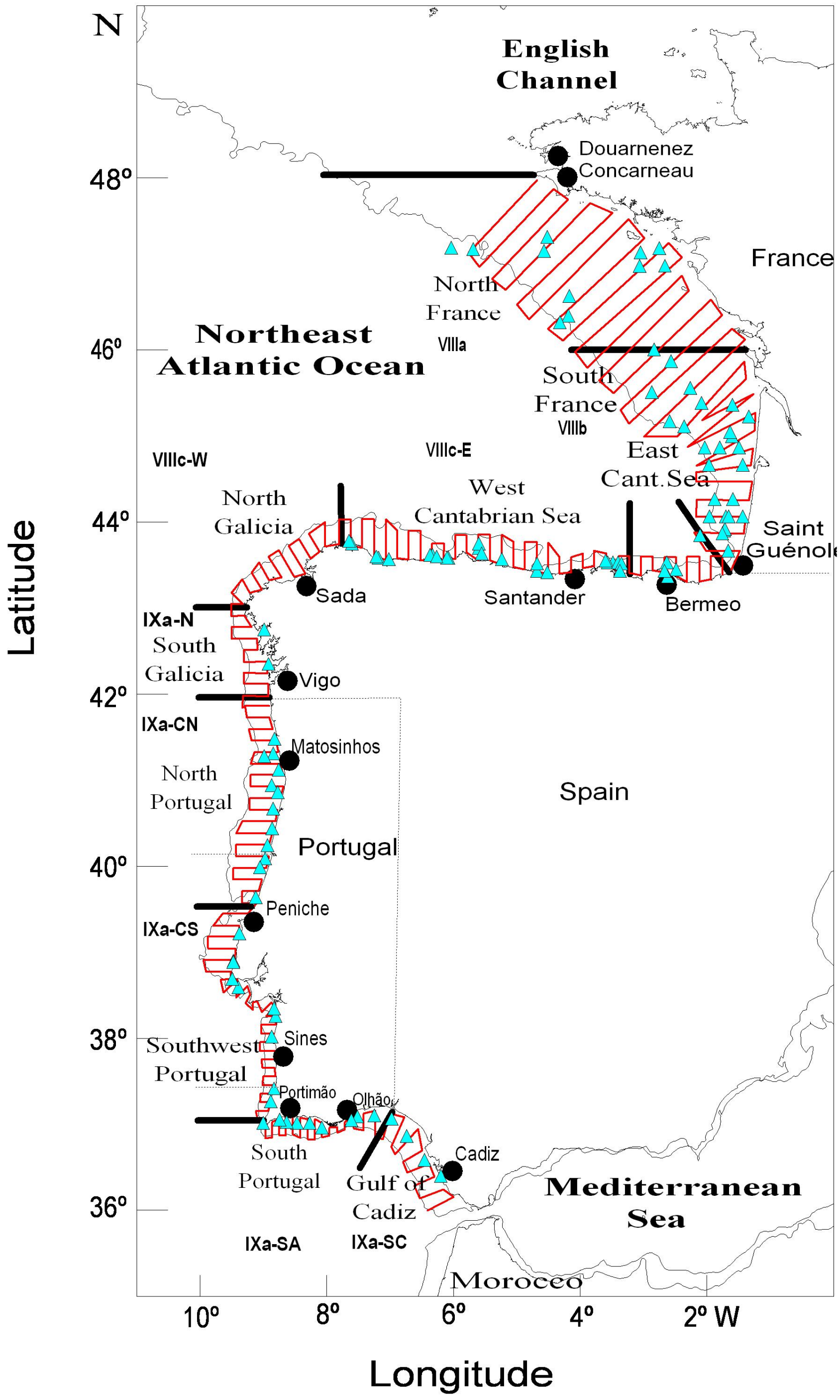

Figure 2.2: Map showing sub-areas considered in acoustic surveys, ICES sub-divisions and ports where sardine catches were sampled. An example of the acoustic track used in recent years and fishing stations with sardine presence is shown. Bathimetric line = 200 m.

Table 2.2. According to Marques et al. (2004) and Marques (2005), their potential influence on catchability and selection is the following:

(i) from zigzag to parallel grid design (1995 in Spain and 1997 in Portugal); possible decline of total catchability and selection of young age classes since zig-zag tracks provide higher sampling intensity near inshore turning points where juvenile sardine are preferentially distributed and overall abundance is higher;

(ii) from day and night surveying to daytime surveying only (1998 in both countries); possible increase of catchability since during the night, sardine schools spread on bottom becoming difficult to detect while in daytime dense schools are formed in midwater;

(iii) size of pelagic fishing gear (1991 in Spain and 1997 in Portugal); total catchability and selection of younger individuals may have declined in the Spanish survey, due to the shift to a larger gear which limits trawling in shallower waters. The use of an auxiliary purse seiner to sample inshore areas in Spain (since 1997) may have partly compensated this effect. In the Portuguese surveys, the shift from a larger to a smaller fishing gear may have increased catchability and selection of younger fish.

A summary of biological sampling design within acoustic surveys is presented in Table 2.2. The collection of otoliths for age-length keys (ALKs) is based on random samples in Spanish surveys and length-stratified samples in Portuguese and

French surveys (using 0.5 cm length classes). In Portuguese surveys, the allocation of sampling effort by length class changed in 1998, in order to improve sampling of older age groups with more variable length. In Spanish surveys, random samples are completed with fish from the tails of length distributions, resulting in a combination of simple random sampling and length stratified sampling. Sampling intensity and sample size increased across the study period in both surveys.

Biological sampling design and intensity in acoustic surveys reflect a compromise between practical constraints and the need to obtain representative samples for the estimation of multiple parameters. Selecting the best sampling strategy for the estimation of age composition, growth and maturation characteristics is both complex and disputed (Castro and Lawing, 1995). Length stratified samples provide unbiased age compositions and maturity-at-age estimates provided that age-length keys or age-length-maturity keys are weighted by the population length-frequency distribution (ICES, 1994; Morgan and Hoenig, 1997). On the other hand, mean lengths-at-age obtained with the same method are biased, the magnitude of bias declining as the size of length and age samples increase (ICES, 1994). Castro and Lawing (1995) illustrate this effect for sardine-like species on a simulation study: the authors observed that asymptotic length (L_∞) was overestimated and growth coefficient (K) underestimated when length stratified sampling was used while unbiased estimates were obtained with simple random sampling. In addition, fixed allocation (sampling of a fixed number of otoliths by length-class) resulted in larger bias and lower precision when compared to proportional allocation. These general considerations suggest that sardine growth based on acoustic survey samples may be biased to some extent, mainly in French surveys and in the earlier and less intensively sampled Portuguese and Spanish surveys.

Landings

Biological samples collected at the main sardine landing ports of the Iberian Peninsula were used to study spatial and temporal variation in maturity-at-length and spawning seasonality (Chapter 4) and to model sardine seasonal growth in recent years (Chapter 5) (Table 2.3; Figure 2.2). Catch biomass and catch-at-age data were used to describe sardine fisheries and population dynamics by area (Chapter 6). Catch length distributions and biological properties of sardine were sampled from purse seine landings at the main ports of the Iberian Peninsula since 1978 and from purse seine and pelagic trawl landings off the French Gulf of Biscay since 2002 (Figure 2.2). Catch sampling has been coordinated within ICES assessment working groups (e.g. ICES 1980, 1992) and EU Sampling Programs (Pestana et al., 1998; FIEFA, 2000; SAMFISH, 2002; EU Data Collection Regulation). Detailed information on sampling programs can be found in these reports and is summarised in Table 2.3. Statistical methods for catch-at-age estimation are described in Jardim et al. (2004). Sampling design and intensity of sardine catches have not been fully evaluated but a perception of the overall uncertainty in catch-at-age is provided in

Jardim et al. (2004) using 1999 data. The authors report coefficients of variation (CVs) below 20% up to age 8 but increasing sharply at older age classes (up to 60% at age 10). In area-disaggregated data, CVs below 20% were limited to ages 1-6 years.

Individual biological characteristics (length, weight, sex, maturity and fat stage) were collected from monthly or fortnightly random samples of the catch while otolith sampling was length stratified (by 0.5 cm length class). The collection of biological characteristics was systematic and consistent since 1981, apart from a shift in 2002 to length stratified sampling in the Portuguese ports, while sampling intensity and geographic coverage improved across the period (Table 2.3). During the 1980s, length samples and age-length keys were pooled across the northern Spanish areas (VIIIc-W and IXa-N) and across the western Portuguese areas (IXa-CN and IXa-CS) providing aggregated catch-at-age data. Independent catch-at-age data by ICES sub-division is available since 1991, apart from the Gulf of Cadiz, where length and age sampling started in 1998 and 2002, respectively. For the period 1998 - 2001, catch-at-age for the Gulf of Cadiz was calculated from age-length keys collected off southern Portugal since sardine growth is comparable in the two areas (Silva et al., 2004). Catch sampling off southern Portugal moved from Olhão, a port on the eastern part of the area (close to the Gulf of Cadiz) to Portimão, a port on the western part of the area, in 1991. The influence of this change in the catch composition for the area is uncertain. However, recent data indicate that smaller

Table 2.3: Summary information on sampling programs of sardine catches within the Iberian Biscay waters, and identification of data used in Chapters 4 (maturity), 5 (growth) and 6 (dynamics). LF: length frequency; LS: Length-stratified sampling; SR: simple random sampling. ALK: age-length key. n: sample size range. Biological characteristics: L: total length, A: age, TW: total individual weight; S: sex; M: maturity stage; F: fat stage; GW: gutted weight; GOW: gonad weight.

			ALK		Biology			Chapters			
Period	Country	Sampled areas	Sampling design	n	Sampling design	n	Characteristics	Maturity	Growth	Dynamics	Notes
1981-1990	Spain	VIIIcW, IXa-N	LS; semestral	500-1100	SR; monthly	300-2000	L, A, TW, S, M, F	X (IXa-N)		X	(1)
	Portugal	IXa-CN, IXa-CS, IXa-SA	LS; semestral	1300-2300	SR; monthly	3500 -6500	L, A, TW, GW, GOW, S, M, F	X		X	(2)
1991-2005	France	VIIIa, VIIIb	LS; quarterly	Not availble	Not availble		L, A, TW, S, M, F			X	(3)
	Spain	VIIIcE, VIIIcW, IXa-N, IXa-SC	LS; semestral	700-3200	SR; monthly	800-4500	L, A, TW, S, M, F	X (IXa-N)	X (VIIIcE)	X	(4)
	Portugal	IXa-CN, IXa-CS, IXa-SA	LS; quarterly	2500-8500	SR/LS; monthly	5000-10000	L, A, TW, GW, GOW, S, M, F	X	X (IXa-CN, IXa-SA)	X	(5)

(1) Pooled ALK and LF for IXa-N and VIIIcW; LF sampling starts in 1986 and ALK sampling in 1990 in VIIIc-E .
(2) Pooled ALK and LF for IXa-CN and IXa-CS; sampling of GW and GOW started in 1986.
(3) Sampling since 2002; LF and ALK combined for the whole area.
(4) X & LF sampling started in 1998 and ALK sampling in 2002 in IXa-SC.
(5) X & LS biological sampling since 2002

sardine are generally distributed closer to the Gulf of Cadiz while larger sardine predominate in the western part of the area (e.g. ICES, 2006b).

2.4 Protocols for morphometric and biological sampling

Sardine collected for morphometric analyses were placed individually in trays layered by plastic sheets, to preserve body shape and minimize dehydration and subsequently defrosted in the laboratory. Digital photographs and biological properties were recorded from each fish, as decribed in Chapter 3. Laboratory procedures were extensively tested and improved in order to obtain reliable digital photographs, with low distortion and identifiable landmarks. Fish were placed on sheets of milimetric paper and calibration of measurement units were carried out in each image (pixels to cm). Care was taken to avoid tilt and deformation of zoomed images. Apart for small adjustments in contrast and brightness, no other image processing tools were applied to digital photographs. Landmarks were digitized using the software TNPC Base V6 (Imagesp).

Complete biological sampling included measurement of total length with a precision of 0.5 mm, total weight and gutted weight (weight without viscera and gonads) with a precision 0.1 g, gonad weight with a precision of 0.01 g, identification of sex (male or female), classification of macroscopic maturity and visceral fat stages and extraction of otoliths, from each individual sardine. All properties were recorded in samples for broad-scale spatial analyses and those recorded within surveys and landings samples are listed in Tables 2.2 and 2.3.

Sex identification and classification of macroscopic maturity was carried out by visual examination of the gonads. In a small proportion of juveniles (generally below 10 cm) and in large, possibly senescent adults, these characteristics could not be determined. The classification of macroscopic maturity was based on the scales of Pinto and Andreu (1957) for female and Pinto (1957) for male sardine (Table 2.4), considered standard scales for the collection of maturity data on the Atlanto Iberian sardine stock (ICES, 1982). The males scale has been modified with an additional stage representing partial post-spawning individuals. Alternative scales have been used in studies of sardine reproduction within Iberia (Pérez et al., 1985) and in other areas of the species range (Rodríguez-Roda, 1970; Parnell, 1974; Krzeptowski, 1983; Mata et al., 1997).

Macroscopic maturity classification provides a fast and cost-effective method of obtaining information on size-at-maturity and seasonal reproductive cycles of fish, but requires considerable experience on the part of the observer, precision within and among observers and validation by histological examination of a range of developmental stages (West, 1990). Sardine maturity scales were developed following extensive research on sardine sexual maturation using macroscopic and histologic criteria (Andreu, 1955; Pinto and Andreu, 1957; Pinto, 1957). The accuracy of fe-

Table 2.4: Scales used to classify macroscopic maturity of female and male sardine in IPIMAR and IEO laboratories.

	Stage	Macroscopic aspect of the ovaries	Macroscopic aspect of the testes
1	Juvenile/resting	Tiny translucent, almost transparent; delicate but well defined blood vessels.	Small and asymmetric, laminar blade-like testes; thin distinct blood vessels; greyish or ivory
2	Developing	Beginning of opacification; several opaque oocytes are visible through the ovarian membrane; pink or yellow	Firmer and larger than I but with similar shape and colour; blood vessels visible as a network of red strings
3	Pre-spawning	Totally opacified; oocytes clearly visible through the ovarian membrane, laid out regularly in parallel bands; no hyaline zones; pink or yellow	Very firm; opaque; blood vessels no longer distinguishable but terminate on the testes surface as red star-shapes structures; dissected testis show fluid semen
4	Spawning	Distended and gelatinous; oocytes in oblique bands; transparent oocytes among opaque oocytes visible through the ovarian membrane	Large and plump but soft; filled with viscous semen that easily flows from vent; whitish and shiny
5	Partially spent	Very similar to stage III but oocytes are laid out irregularly with scattered hyaline zones in between; red to salmon	Very similar to stage II but much larger; reddish star-like structures at the surface of the testes
6	Spent	Very flaccid; haemorrhagic zones and sometimes, whitish nodules corresponding to residual oocytes reabsorbing; red	Thin and flabby with striations at the surface; red spots due to ruptured blood vessels; pinkish colour; small amounts of semen in the spermaducts

male macroscopic classification has been recently re-examined by histological analysis (ICES, 2003b). This study indicates that agreement between macroscopic and microscopic classifications is low (56%), mainly due to difficulty in separating pre-spawning (stage 3) from partially spent (stage 5) individuals (accounting for 20% of misclassification) and similar results were obtained from the study of male gonads (I. Afonso-Dias, personal communication). Stage 3 and 5 gonads have the same type of reproductive cells and are macroscopically differentiated by features such as color and texture (redish and flaccid in stage 5) which may result from poor sampling conditions. Correct classification of juvenile/resting individuals within the spawning season was 75%, with most misclassifications corresponding to individuals maturing for the first time in life (juvenile). Overall, the results from this study advised against the use of detailed macroscopic data on sardine maturity but also indicate that uncertainty drops considerably by pooling data across some maturity stages, such as stages 3 and 5.

The agreement between sardine maturity classifications by observers from IPIMAR and IEO has been recently evaluated using gonad samples collected within two acoustic surveys (spring 2000 and 2001). In this exercise, two separate random samples of sardine gonads were collected from the same fishing hauls; one sample of the pair was observed and classified on-board by an observer from IEO and the other was frozen and transported to IPIMAR for subsequent observation. The two observers involved in this exercise are responsible for regular sampling of sardine within sardine acoustic surveys. Classification of the 2001 samples showed almost 100% agreement between observers from the two laboratories (Figure 2.3). In the 2000 samples, the IPIMAR observer classified 34% more gonads as spent (stage 6) than partially spent (stage 5) compared to the IEO observer, a discrepancy which is possibly explained by the effects of freezing on the external appearance of gonads. These results provide some support to the standardization of recent maturity data collected on the two laboratories. However, they can not be generalised to previous years or other periods of the year.

Classification of sardine fat stage, an index of the amount of fat in the visceral cavity, was based on a four stage scale developed by Furnestin (1943) from studies of seasonal variation in average lipid content of sardine flesh (Table 2.5).

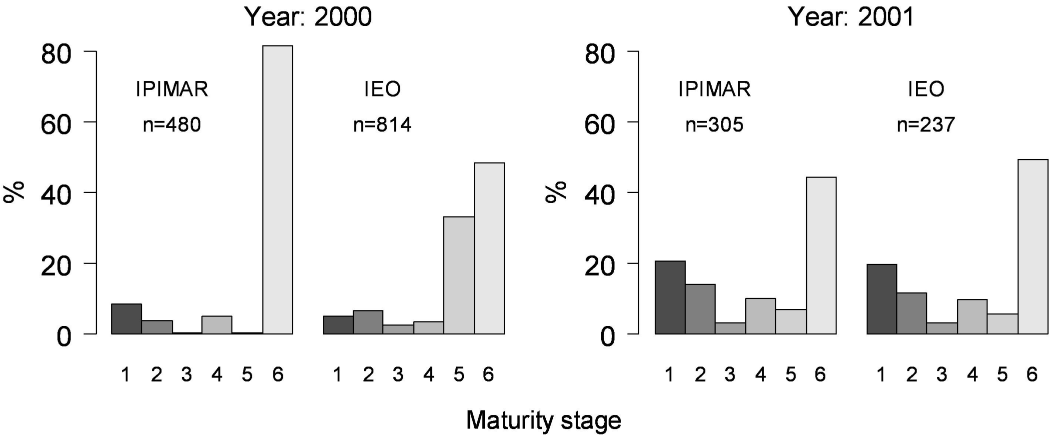

Figure 2.3: Frequency distributions of maturity stage classifications by IPIMAR and IEO observers in samples collected within acoustic surveys. Maturity stages: 1: juvenile/resting; 2: developing; 3:pre-spawning; 4: spawning; 5: partially spent; 6: spent.

Table 2.5: Scale used to classify the stage of visceral fat of sardine in IPIMAR and IEO laboratories.

Stage	Description
1	No fat or very thin discontinuous thread of fat
2	Thin but continuous thread of fat
3	Thicker thread of fat surrounding the gut
4	Fat filling up the visceral cavity

Sardine age determination was based on the analysis of growth rings in otoliths (*sagittae*). Standard methods of otolith preparation and age reading criteria for the Atlanto - Iberian sardine stock have been established within international age reading workshops since the late 1970s (e.g. FAO, 1978, ICES 1982, 1997b; Soares et al. 2004, in press). A summary of these methods as well as the biological information underlying the main criteria for age determination are presented in Chapter 5. One of the concerns regarding sardine age data is the implication of several age readers. Inter-calibration exercises have been carried out regularly, based on otolith collections from different areas, periods and age classes and involving readers with variable experience (FAO, 1978, ICES 1982, 1997b; Soares et al. 2004, in press). These exercises provided a wide range of outcomes which are difficult to compare, but some general conclusions regarding sardine age determination can be drawn:

- the structure of sardine otoliths shows increasing complexity from north to south across the northeast Atlantic waters, as the contrast between alternating opaque and translucent zones deteriorates and false rings become more frequent;

- the main difficulties in sardine age determination are: (i) identification of the first annual ring, which affects fish of all ages but generates a large relative ageing error in younger age classes, (ii) the discrimination of closely packed translucent rings in otoliths of older individuals (above 5-6 years) and (iii) the identification of the otolith margin (mainly during the winter to summer transition);

- agreement between experienced readers depends on the geographic area, age and season; agreement is higher for older fish, off north Iberia in winter (70-90%, 1-7 years old), declines in June-July samples of the same age range (50-70%) and in older fish (10-50%, >7 years); off south Iberian areas, the percentage agreement drops 10-20% for similar sets of otoliths;

Figure 2.4 shows age-bias plots (see Campana, 2001) obtained in the most recent sardine otolith workshop, as an example of age reading precision for two experienced readers and two samples of lower (northern Spain) and higher (southern Portugal) difficulty. Overall, systematic differences between readers and poor consistency within readers have been reported in various workshops and are still a matter of concern (e.g. Soares et al., 2004). A full evaluation of their influence on age composition or length-at-age estimates warrants further investigation.

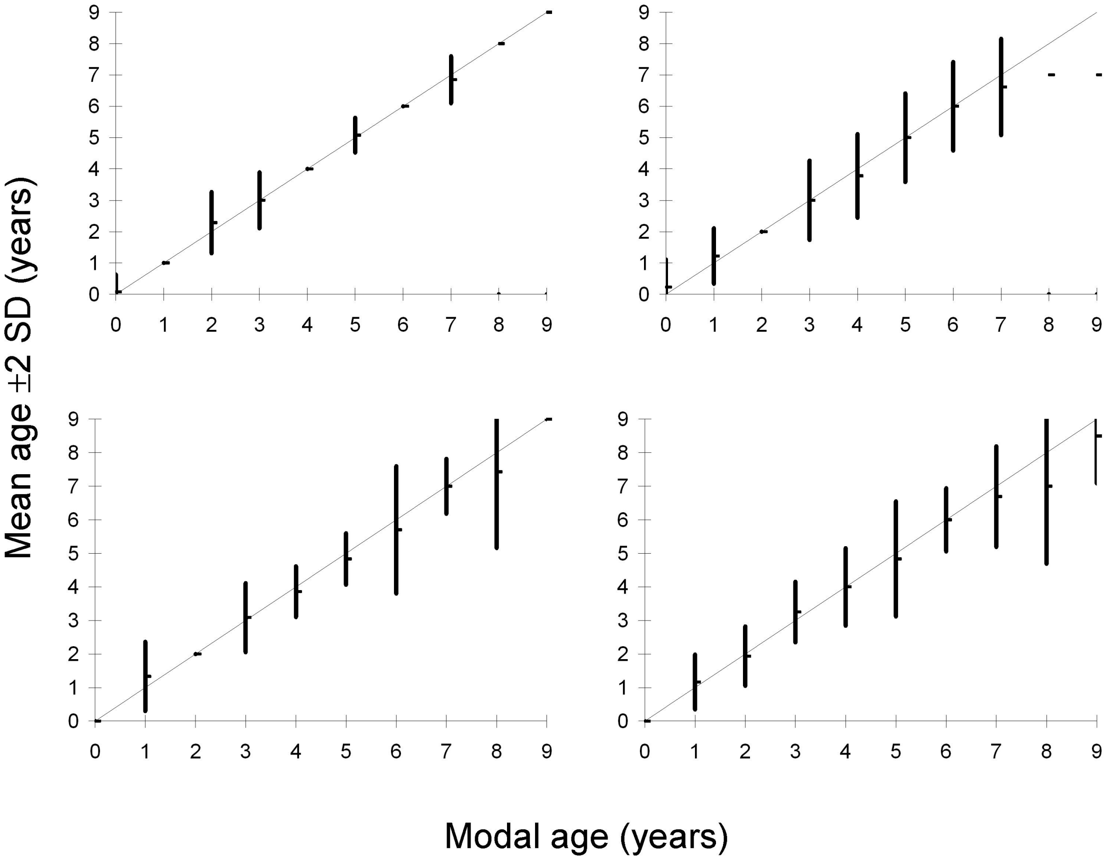

Figure 2.4: Age-bias plots of readings from two experienced observers (left and right panels) for otolith sets collected off northern Spain (upper panel) and southern Portugal (lower panel) obtained in a recent sardine otolith workshop (Soares et al., in press). Each plot represents the the mean age±2 standard deviation (SD) attributed by a reader to the otoliths of the different modal ages. Modal ages were determined using ages attributed by the five most experienced readers participating in the workshop.

Chapter 3

Body morphometry*

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3.1 Introduction

The sardine (*Sardina pilchardus*, Walbaum 1792) is a clupeoid whose distribution in the northeastern Atlantic extends from the southern Celtic Sea and North Sea to Mauritania and Senegal, with residual populations also off the Azores, Madeira, and the Canary Islands (Parrish et al., 1989; Figure 3.1). It is also found throughout most of the Mediterranean Sea, although the degree of mixing between Mediterranean and Atlantic populations is unknown. Abundance is greatest in the coastal waters of Morocco, where annual purse-seine catches exceeded 400 000 t throughout the 1990s (FAO, 2001b), but the species is also abundant in the Atlantic waters of the Iberian Peninsula, where it is the main target of the purse-seine fleets of Portugal and Spain. Total sardine catches in those countries peaked at around 250 000 t in the mid-1960s, but have declined during the past 15 years, and are currently at some 100 000 t (ICES, 2003a). Sardine catches north of the Iberian Peninsula are comparatively small, although a dedicated fishery with annual catches of some 15000-20000 t existed in French waters up to the mid-1960s (ICES, 1979). Sardine fisheries also exist throughout the Mediterranean, but they have traditionally contributed less than 20% of the total catch there (Andreu, 1969).

Attempts to differentiate sardine populations date from the 1920s (for reviews, see Andreu, 1969, and Parrish et al., 1989), with most studies using univariate analyses of meristic characters (counts of vertebrae and gill rakers) or morphometric variables (e.g. the ratio of head to total length: the cephalic index). Differences in

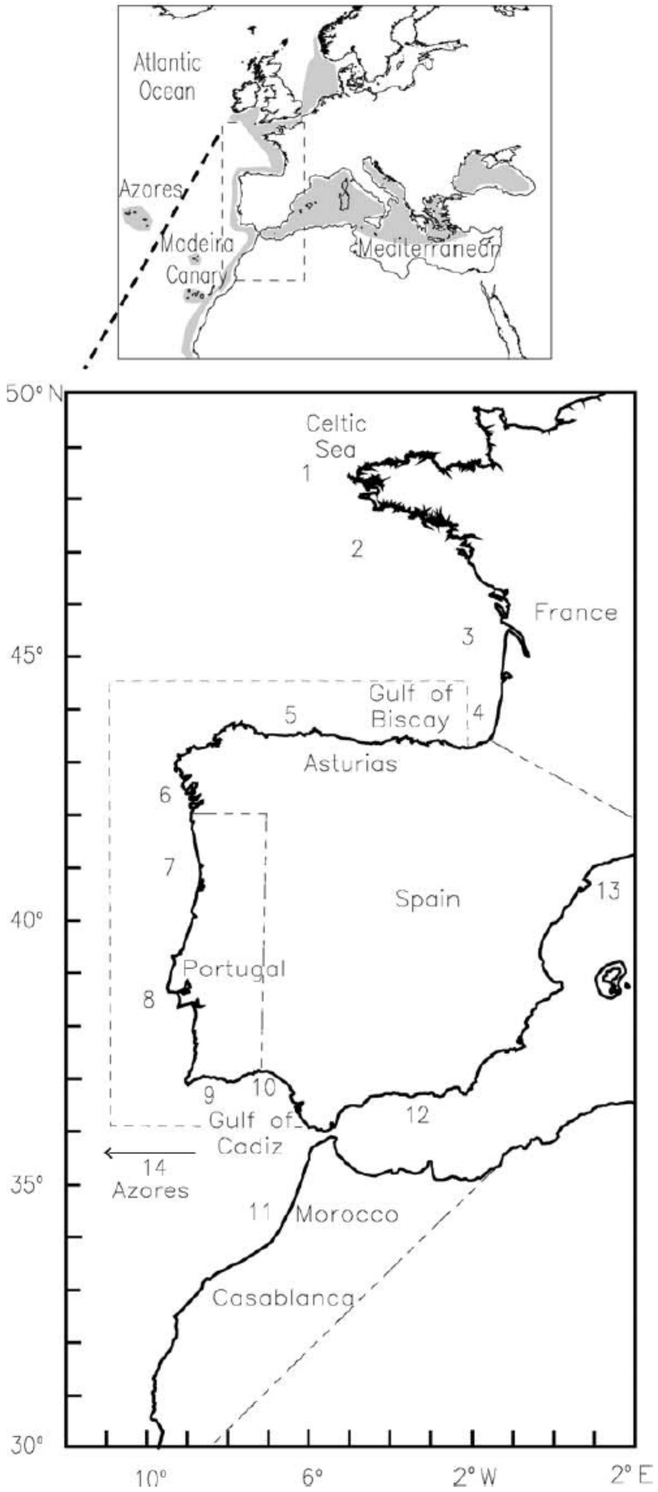

Figure 3.1: Locations of sampling sites (numbers) and delimitation of the stock area of Atlanto-Iberian sardine (dashed line). The map on the top shows the area of distribution of the species.

mean vertebral counts and the cephalic index were studied from restricted geographic areas and/or periods and led to various definitions of sardine races or geographical groups. However, failure to demonstrate persistent and significant phenotypic differences within a wide geographic area prevented consensus on the structure of sardine populations within its area of distribution. In the most thorough and geographically extensive study to date, the allometric relationship between the number of gillrakers and sardine length was considered the most appropriate index to differentiate populations. That analysis could only distinguish between sardine from the Atlantic continental shelf and those in the Mediterranean, and around Madeira and the Canary Islands (Andreu, 1969). Within the Atlantic, the most widely accepted subdivision is based on the early vertebral count study of Fage (1920), and it considers the existence of four sardine groups: the septentrional Atlantic group, distributed from the North Sea (57°N) to the Cantabrian coast of Spain (43°N), the Iberian or meridional Atlantic group, distributed off the Spanish and Portuguese coasts (from 43 to 36°N), the Moroccan group, distributed from Cap Spartel (36°N) to Cap Juby (28°N), and the Saharian group, distributed from Cap Juby to Levrier Bay (21°N; Parrish et al., 1989).

For management purposes, sardine in European Atlantic waters have always been considered to belong to a single stock, although the geographical limits have changed over time. Exploratory sardine assessment began in 1978, using biological, fisheries, and survey data from France, Spain, and Portugal (ICES, 1978). At that time the stock was delimited within ICES Divisions VIIIc (Bay of Biscay and Cantabrian Sea) and IXa (western and southern Iberia). However, the biological criteria available for stock delimitation were not considered adequate and emphasis was placed on the need to clarify the relationships between populations within the assumed stock area (ICES, 1978). The northern border of the stock was redefined in 1980 (ICES, 1980), giving rise to what is currently known as the Atlanto-Iberian stock of sardine, delimited by the France/Spain border in the north, and by the Strait of Gibraltar in the south (ICES Divisions VIIIc and IXa). Routine assessment of the Atlanto-Iberian stock of sardine has been conducted annually since 1982 under the auspices of the International Council of the Exploration of the Sea (e.g. ICES, 2003a).

Throughout the 1980s, the biological and fisheries data did not raise serious concerns about the area of delimitation or the biological homogeneity of the Atlanto-Iberian stock. However, recently there has been growing evidence of distinct regional changes in sardine distribution within the known stock area, increasing uncertainties in the estimates of stock size and in identification of trends in fishing mortality and abundance over time (ICES, 2000). This, together with recent observations indicating lack of discontinuities in the distribution of sardine eggs at the edges of the known stock area (ICES, 2000; Stratoudakis et al., 2003), casts new doubts on the hypothesis that the Atlanto-Iberian stock of sardine is a panmictic, closed population. The issue was addressed in a multivariate morphometric analysis of sardine samples from the area (ICES, 2000) that showed that head dimension increases from north to south. There is a latitudinal gradient from small head and large body size of sardine in the north (inner Bay of Biscay) to a large head and small body size in the south (southern Portuguese waters, and the Gulf of Cadiz). It was not possible to identify geographical areas with distinct sardine morphotypes in the study, and the need to increase the size and geographical range of samples was acknowledged (ICES, 2000).

In this paper, I examine the morphometric variability of sardine sampled from a broad geographic range (from the Celtic Sea to Morocco, and from the western Mediterranean to the Azores). The results are derived from a large number of fish per sample, and state of the art methods of morphometric data collection (digital images) and analysis (combining multivariate and geometric morphometrics; Marcus et al., 1996; Cadrin and Friedland, 1999; Cadrin, 2000). The analysis explores the homogeneity of sardine shape within the Atlanto-Iberian stock area, its relation with adjacent areas to the north (France), south (Morocco), and west (western Mediterranean), and makes comparisons with a distant population (Azores). Finally, distinctive characters for discrimination among sardine morphotypes are identified, and the output of traditional multivariate analysis of morphometric data is compared with that obtained from recent geometric methods. In geometric methods, the analysis takes into account the geometry of configurations, providing additional information on shape differences that is not available from multivariate methods (Rohlf and Marcus, 1993). Therefore, combination of multivariate and geometric methods is expected to increase the chances of detecting the small morphometric differences that are anticipated at an intraspecific level.

3.2 Material and methods

A total of 14 samples of sardine (Table 3.1) was collected between December 1999 and May 2000 within an area between the Celtic Sea and the Moroccan coast, and from the western Mediterranean to the Azores (Figure 3.1). All samples were collected during research surveys, apart from those off Morocco and the Azores, which were obtained from commercial vessels at the port of landing. In those cases, samples were collected about six hours after capture, but were kept in a good condition. Samples were frozen soon after collection and defrosted for laboratory analysis, which took place about two months later to ensure that all fish were analysed following a similar period of freezing. Digital photographs were taken with a Nikon Coolpix 950 (image resolution: 28.3 pixels cm-1) on the right side of each fish, and 15 landmarks were defined and recorded as two-dimensional coordinates (Figure 3.2). Landmarks were selected to provide a homogeneous coverage of the whole shape, but their homology and clarity in each fish were also taken into account. Ten of these landmarks (1-10) were used to calculate body distances on a truss network (Strauss and Bookstein, 1982), and 13 (1-10, 13-15) were used to describe body shape for geometrical analysis (Rohlf and Slice, 1990). Data were examined using bivariate scatter plots of truss variables against fish total length, and plots of superimposed

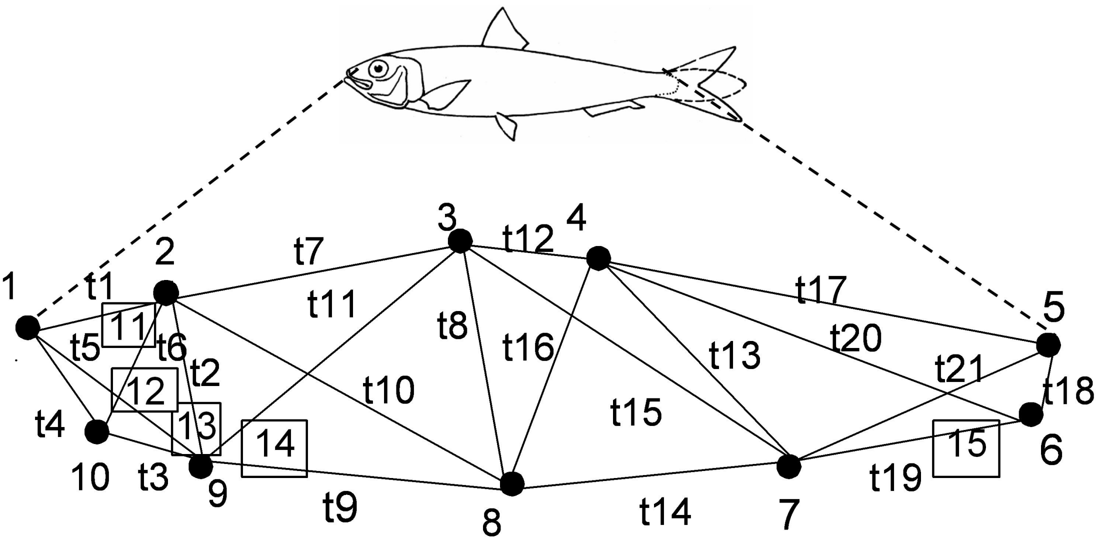

Figure 3.2: Location of anatomic landmarks and design of the truss network used in sardine morphometric analysis. Distances used in multivariate methods (t1–t21) were defined by landmarks 1–10 (truss network) and 11 and 12 (eye diameter). Landmarks 13–15 (indicated by squares) were only included in the geometric analysis.

landmark configurations within each sample. From the total number of fish collected (1344), some were excluded from further analysis owing to incomplete (96) or clearly outlying measurements (49 in the truss analysis, 72 in the geometric analysis). The multivariate approach was applied to 1199 of the fish analysed, and the geometric method to 1176 fish; final sample sizes ranged from 53 to 103 fish (Table 3.1).

Total length and biological parameters (sex, stage of sexual maturity, fat content, and stomach weight) were recorded for each sardine photographed. Macroscopic maturity was classified according to a six stage scale (adapted from Pinto, 1957, and Pinto and Andreu, 1957): juvenile/immature (1), maturing (2), pre-spawning (3), spawning (4), post-spawning (5), and recovering (6). Fat content (quantity of fat in

Table 3.1: Summary information of sardine samples (date, location, geographic area, and number of fish, n) used in morphometric analysis. Exact location is unknown for samples 11 and 14, which were obtained from commercial vessels. n_1 and n_2 indicate sample size for multivariate and geometric approaches, respectively.

Sample	Date	Latitude	Longitude	Country-Area	n_1	n_2
1	20 March 2000	48° 30.0 N	05° 26.0 W	France-Celtic Sea	70	67
2	11 May 2000	46° 51.0 N	04° 56.7 W	France-NW coast	78	77
3	22 April 2000	45° 09.5 N	02° 02.8 W	France-SW coast	98	96
4	10 April 2000	43° 25.3 N	01° 44.5 W	Spain-Bay of Biscay	72	77
5	6 April 2000	43° 40.3 N	06° 30.1 W	Spain-Asturias	87	84
6	25 March 2000	42° 21.9 N	08° 47.7 W	Spain-S Galicia	72	67
7	15 March 2000	40° 59.8 N	08° 42.8 W	Portugal-NW coast	99	96
8	21 March 2000	38° 34.7 N	09° 18.4 W	Portugal-SW coast	87	85
9	29 March 2000	37° 05.2 N	08° 30.5 W	Portugal-S Algarve	94	94
10	6 March 2000	36° 33.3 N	06° 23.6 W	Spain-Gulf of Cadiz	96	95
11	January 2000	-	-	Morroco-Casablanca	97	93
12	December 1999	36° 41.0 N	03° 00.0 W	Spain-SW Mediterranean Sea	103	100
13	December 1999	40° 41.0 N	00° 30.0 E	Spain-NW Mediterranean Sea	53	53
14	May 2000	-	-	Portugal-Azores	93	92
Total					1199	1176

the abdominal cavity) was visually evaluated on an ordinal scale from 1 (no fat) to 4 (abdominal cavity full of fat; Furnestin, 1945). Stomach weight refers to the wet weight of the whole stomach, including the stomach wall. Otoliths were extracted from each fish and the age was subsequently estimated using standard methods and criteria (ICES, 1997b). Preliminary analyses of the impact of biological characteristics on morphometric variables showed that the maturity stage considerably affected abdominal volume, introducing confounding effects in the comparison of samples containing fish of different spawning condition. Abdominal volume affected the vertical position of landmark 8, and therefore of two derived truss variables that correspond to nearly vertical distances (variables t8 and t16; Figure 3.2). Both the landmark and the truss variables associated with the abdomen were excluded from further analysis.

In all, 19 truss variables (t1-t7, t9-t15, t17-t21) and the eye diameter were analysed by multivariate methods, and the coordinates of 12 landmarks (1-7, 9, 10, 13-15) were used in a Procrustes analysis (Rohlf and Marcus, 1993). Truss variables (log-transformed) were corrected for size using the Burnaby method (Burnaby, 1966; Rohlf and Bookstein, 1987). This method requires that variables be log-transformed, and it assumes that the first eigenvector of the within-group covariance matrix of log-morphometric variables is a multivariate index of the size of fish. The effect of size is corrected by projecting the truss variables on the sub-space orthogonal to the space spanned by the size vector. The existence of a size vector common to all samples was tested by comparing the angle between the first eigenvector of the covariance matrix of each sample with the common size vector in the dataset. Bootstrap 95% confidence limits (Manly, 1997) indicated that the angle between the common size vector and the individual size vectors was not significant for any of the samples, suggesting that a common size transformation would standardize all samples. After size correction, the correlation between truss variables and fish total length decreased considerably, confirming that the influence of size was effectively eliminated. In the geometric method, the correction for size was incorporated in the process of superposition of configurations (Rohlf and Slice, 1990), by dividing the coordinates of each landmark by the centroid size for each fish (the sum of squared distances between all landmarks and the centroid of the configuration).

Principal components analysis was applied to size-corrected truss variables to outline groups of samples and to identify influential variables (Johnson and Wichern, 1998). Principal components were extracted from the covariance matrix. Mean values (centroids) and 95% asymptotic confidence limits of the scores of individual sardine on the first two principal components were computed for each sample. In the geometric analysis, the landmark data determining the configuration of individuals in each sample were superimposed by generalized orthogonal Procrustes analysis (Rohlf and Slice, 1990). Configurations were centred, scaled, and rotated in order to minimize the sum-of-squared distances (Procrustes distances) between homologous landmarks of all individuals. Mean shapes (consensus configurations) were calculated for each sample as arithmetic means of optimally superimposed configurations. The matrix of Procrustes distances among mean shapes was analysed by hierarchical clustering using complete linkage (Johnson and Wichern, 1998). This algorithm is recommended for data that are anticipated to show a cline of poorly separated clusters (Kaufman and Rousseeuw, 1990).

Two main groups of samples were suggested by the morphometric analyses, and these groups were discriminated using Fisher linear discriminant analysis of truss variables (Johnson and Wichern, 1998). Differences among the group means in the discriminant space were tested using the Mahalanobis distance, and the consistency of the groups was evaluated by computing the misclassification rates of new individuals (Johnson and Wichern, 1998). A total of 150 sardine (100 from the larger group and 50 from the smaller group) was randomly withdrawn before the discriminant analysis to establish a sample for validation, and the remaining fish (training sample) were used to estimate the discriminant functions. The scores of individual fish and the corresponding Euclidean distances from the group centroids were calculated for the validation sample. Each sardine was assigned to the group with the nearest centroid, and the percentage of individual fish from one group correctly classified was computed. All calculations were carried out with the software S-Plus 2000 for Windows (Statistical Sciences, Inc.).

3.3 Results

Table 3.2 summarizes the biological characteristics of the 14 sardine samples used in the analysis. Total length in the pooled samples ranged between 12.0 and 24.7 cm (mean 17.9 cm), corresponding to ages of 1-10 years (mean 2.3 years). Length and age distributions generally overlapped considerably among samples. Sardine in the Mediterranean and Celtic Sea samples were overall the smallest (mean lengths around 15 cm). The largest sardine were caught close to the boundaries of the Atlanto-Iberian stock area, with a mean length of 22.1 cm in Asturias, 20.3 cm in Algarve (south Portugal), and 22.0 cm off northwestern France. Sampling dates were intended to follow the south-to-north progress of the spawning season. Samples were mainly spawning fish, as indicated by both advanced maturity and low fat content, except off the Azores, where the biological data suggest a resting condition, and from the Celtic Sea, where fish were possibly in a pre-spawning state.

The principal components analysis of size-corrected truss variables showed that the first two principal components (PC) account for 50.2% of the total variance (33.1% for PC1, 17.1% for PC2). The first PC is essentially a contrast between the dimensions of the fish head (t1-t6 and ED), and of the fish body (t7, t9-t15, t17-t21; Figure 3.3). A group of three samples, from the Algarve, the Gulf of Cadiz, and Morocco (samples 9-11, respectively), segregates from the remaining samples on this PC (Figure 3.3), the first group having a larger head and smaller body dimensions than the latter. PC2 is dominated by variables t12 and t13, indicating differences in

Table 3.2: Summary of biological characteristics of sardine in each sample. The percentage of fish spawning corresponds to fish in pre-spawning and spawning condition (maturity stages 3, 4, and 6). Sex ratio is the percentage of females, and s.d. is the standard deviation.

Sample	Mean length (cm) and (range)	Mean age, (range) (years)	Sex ratio (%)	Percentage of fish spawning	Mean stomach weight/ body weight±s.d.	Dominant fat stage, (%)
1	15.6 (12.0-18.7)	1, (1-2)	57.1	22	4.6 ± 1.2	1, (98)
2	22.1 (19.3-24.7)	4.3, (2-8)	51.3	100	6 ± 1	1, (78)
3	16.3 (14.0-19.0)	1.1, (1-2)	42.9	66	6.3 ± 1.2	1, (78)
4	17.8 (15.8-20.2)	1.5, (1-3)	45.2	100	5.1 ± 0.7	1, (99)
5	22.1 (20.2-24.7)	4.4, (2-8)	44.8	100	6.2 ± 1.0	1, (100)
6	17.2 (15.0-22.8)	1.4, (1-3)	30.6	100	5.2 ± 0.7	1, (68)
7	19.1 (16.6-22.1)	2.7, (1-6)	68.7	100	8.1 ± 0.9	1, (100)
8	17.3 (15.0-19.9)	1.5, (1-4)	43.2	67	7.0 ± 1.1	1, (74)
9	20.3 (18.0-22.4)	5.8, (2-10)	38.3	96	5.9 ± 0.9	1, (96)
10	17.2 (14.5-20.7)	2.3, (1-6)	43	54	4.3 ± 0.9	1, (57)
11	16.7 (12.0-18.5)	2, (1-4)	49.5	98	5.5 ± 0.4	1, (74)
12	15.3 (13.3-20.6)	1.2, (1-3)	25.2	100	4.0 ± 1.1	2, (71)
13	15.6 (13.5-19.4)	1.5, (1-3)	60.4	51	3.5 ± 0.6	1, (79)
14	16.7 (13.7-18.3)	1.1, (1-2)	61.3	0	6.3 ± 0.9	4, (70)
Total/ Mean	17.9 (12.0-24.7)	2.3, (1-10)	46.8	76.6	5.7	1

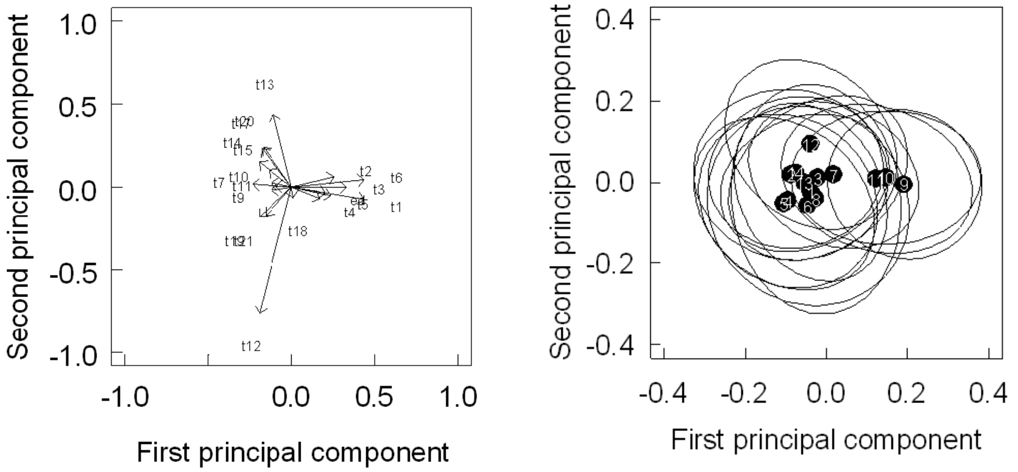

Figure 3.3: Main results of the principal components analysis on size-corrected truss variables. Vectors indicate (a) the loadings and (b) centroids (black dots) and 95% confidence ellipses of the scores for each of the samples on the first two principal components.

the length of the dorsal fin base. There is more overlap among samples on this PC, however, fish from sample 12 (southwestern Mediterranean) showing slightly larger mean values of dorsal base length.

The cluster analysis of distances among mean sardine shape of each sample supports the two-group structure highlighted by the principal components analysis (Figure 3.4), but in this case there is some additional evidence that samples from the Mediterranean and the Azores (samples 12-14) form a separate cluster from the French coast-northern Iberia group (samples 1-8). Figure 3.5 shows the average sardine for each of the three groups of samples, French coast-northern Iberia (samples 1-8), southern Iberia-Morocco (samples 9-11), and Mediterranean-Azores (samples 12-14), and the configuration of the overall mean fish. The most evident difference between the mean shape of sardine from the southern Iberia-Morocco cluster and

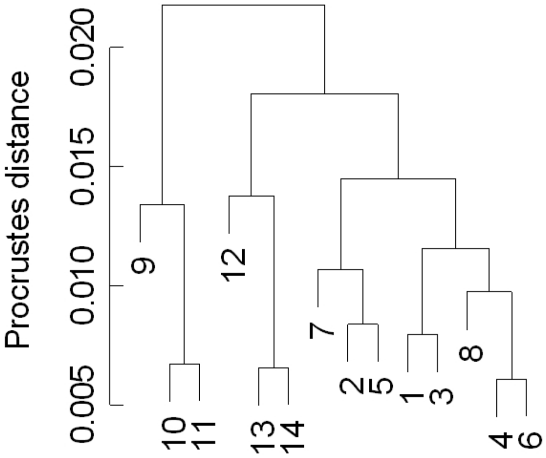

Figure 3.4: Dendrogram resulting from cluster analysis of Procrustes distances among sardine mean shape for each of the samples.

sardine from the other two groups is the larger absolute head dimension and the higher proportion of head to overall body size. On the other hand, sardine from the French coast-northern Iberia group have a more slender body and a more posterior insertion of the dorsal fin, and these differences are larger than when comparing sardine from the Mediterranean-Azores group with sardine from southern Iberia.

The discriminant function based on the two main groups, northern Atlantic-Mediterranean and southern Iberia-Morocco, suggested by both multivariate and geometric analyses, has a discriminant correlation of 0.77, indicating that a considerable proportion of total variability in the discriminant space corresponds to within-group variability (41.5%; Table 3.3). However, the Mahalanobis distance between the two group means (8.06) is significant at the 1% level (Figure 3.6), and the

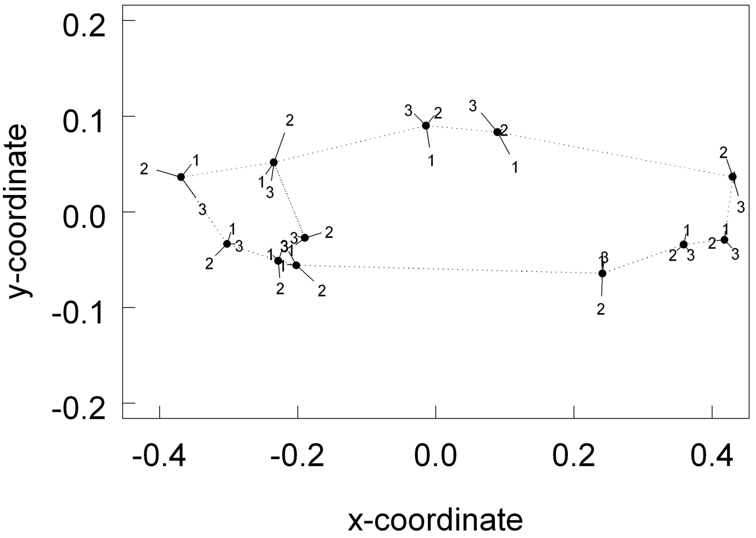

Figure 3.5: Landmark coordinates of the average individuals from the three groups of samples suggested by cluster analysis of Procrustes distances among sample means. The black dots are the coordinates of the overall mean individual. Segments correspond to the difference between the mean individuals of each group and the overall mean individual. The length of the segments was enlarged six times to show the differences among landmark positions. Fish head is on the left and the dorsal side on top of the plot. Group 1: French coast-northern Iberia (samples 1-8), Group 2: southern Iberia-Morocco (samples 9-11), Group 3: Mediterranean-Azores (samples 12-14).

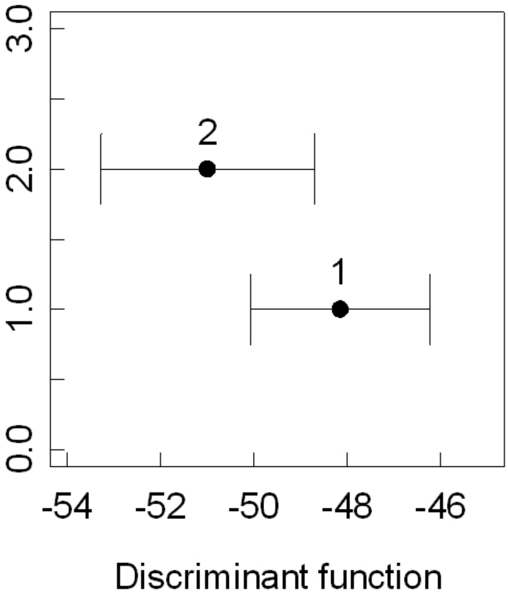

Figure 3.6: Plots of the centroids of the groups of sardine in the discriminant function. The error bars correspond to two standard deviations of the group observations on the discriminant function. Group 1: North Atlantic-Mediterranean (samples 1-8 and 12-14), Group 2: southern Iberia-Morocco (samples 9-11).

high percentage of correct classification of new fish (87% for the northern Atlantic-Mediterranean group, 86% for the southern Iberia-Morocco group), provides support to the differences between the groups, and highlights their homogeneity (Table 3.4). The pattern of correlation of the original variables with the discriminant function is similar to the pattern of loadings on the first PC, indicating that the dimensions of the head and the eye diameter explain most of the morphometric differences between sardine from the northern Atlantic-Mediterranean and those from southern Iberia-Morocco.

Table 3.3: Coefficients of correlation of the morphometric variables (body distances on a truss network) with the discriminant function. The corresponding discriminant capacity is shown at the bottom of the table.

Morphometric variable	Discriminant function
t1	-0.61
t2	-0.71
t3	-0.44
t4	-0.45
t5	-0.60
t6	-0.76
t7	0.51
t9	0.26
t10	0.30
t11	0.26
t12	0.36
t13	0.19
t14	0.49
t15	0.51
t17	0.57
t18	-0.19
t19	0.52
t20	0.59
t21	0.40
Eye diameter	-0.61
Discriminant capacity (%)	58.5

Table 3.4: Percentage of new individuals re-allocated in each group in validation of the discriminant function. Group North Atlantic-Mediterranean: samples 1-8 and 12-14; group Southern Iberia-Morroco: samples 9-11. Classification was of 100 sardine from the first group and 50 sardine from the second group.

	Re-allocation group	
Original group	North Atlantic-Mediterranean	Southern Iberia-Morocco
North Atlantic-Mediterranean	87%	13%
Southern Iberia-Morroco	14%	86%

3.4 Discussion

The distance and landmark morphometric data of sardine used in this study led to the identification of two morphological types, with geographic coherence within the northeastern Atlantic and the Mediterranean. Both morphometric methods indicated that sardine from southern Iberia (Algarve, Gulf of Cadiz) and northern Morocco had a morphotype distinct from the remaining area. Discrimination of the two morphotypes was confirmed statistically by the significant difference between group centroids and by the high percentage of correct classification (>85%) of new fish. Sardine from the northern Atlantic and from the Mediterranean area had a smaller head, eye diameter, and head-to-body ratio than those from southern Iberia and northern Morocco (Figures 3.4, 3.6). These differences reinforce the results of a recent multivariate study of sardine morphometry in Iberian waters that suggested a southerly increasing latitudinal gradient in the absolute and relative size of the head (ICES, 2000). Unlike observed here, that study provided no evidence of a discontinuity in sardine shape along the study area. This lack of agreement is not fully understood, but the smaller sample size and less precise morphometric measurements used by ICES (2000) possibly decreased the chance of detecting differences among samples that have considerable variability and overlap. Earlier univariate analyses on the cephalic index (Andreu, 1969; Fréon and Stéquert, 1982) also indicated an increase in the head-to-body ratio from north to south within the Atlantic.

Apart from the identification of two morphotypes based on head-to-body ratios, there is also some evidence for the identification of distinct morphological groups, mainly on the basis of the position of the dorsal fin (Figure 3.4). This character separates sardine from northern Iberia and the Mediterranean, consistent with earlier studies that have identified phenotypic differences between sardine in Mediterranean and Atlantic European waters (Parrish et al., 1989). It should also be noted that the sample from the Azores is grouped in the same morphotype as sardine from the Mediterranean. This is a counter-intuitive association if one takes into account the geographic relationship between the areas, but it has some support from the few existing studies that have analysed samples from the Azores. The local population had closer affinities with the Mediterranean than with the Atlantic populations in terms of cephalic index and growth rate of gillrakers (Ramalho, 1929, Andreu, 1969).

However, the separation of the Mediterranean/Azores group from sardine from northern Iberia was only depicted by the geometric analysis, so the result should be viewed with caution. The discrepancy between the results from the traditional multivariate and geometric methods regarding this separation may be explained partly by allometric differences between fish from the two groups, because a Procrustes analysis only corrects isometric size (the Mediterranean/Azores group has globally smaller sardine; Klingenberg, 1996). This is partly supported by the similarity between the pattern of sample clustering based on Procrustes distances, and on the analysis of truss distances corrected only for isometric size.

Morphometric features may also vary among cohorts, because the environment at the time of spawning and juvenile development changes between years (Meng and Stocker, 1984; Austin et al., 1999). The samples analysed in this study had different age compositions and were therefore represented by different cohorts. However, in this case the cohort composition does not seem to have affected the pattern of similarity among samples. In fact, the morphometric groups formed in the analyses are largely independent of the cohorts represented in their component samples.

Overall, the population structure suggested here increases doubts on the correct definition of the Atlanto-Iberian sardine stock, both regarding current boundaries and the homogeneity within the stock area. The lack of a boundary in the Bay of Biscay indicated by sardine morphology is compatible with the continuous distribution of sardine eggs and adults along the Cantabrian and French coasts (Anon., 2001). The situation is different for another small pelagic fish (*Engraulis encrasicolus*), for which populations in the Bay of Biscay segregate from populations in adjacent waters of Cantabria, possibly because oceanographic processes favour retention of eggs and larvae (Junquera and Perez-Gándaras, 1993). The discontinuity between sardine populations across the Strait of Gibraltar supports that feature as the eastern limit of the stock, but the link between sardine populations from the Gulf of Cadiz and northern Morocco implied here highlights the need to investigate the southern stock boundary further.

It is now commonly accepted that morphological variation has both environmental and genetic components, but that stable differences in shape among groups of fish may reveal different growth, mortality, or reproductive rates that are relevant for the definition of stocks (Swain and Foote, 1999; Cadrin, 2000). Here I used advanced image-processing techniques, the combination of two recent morphometric approaches (multivariate and geometric), and appropriate analytical methods to uncover differences in sardine from a wide geographic area. However, the temporal stability of morphometric groups depicted here has still to be confirmed, and samples from the 2003 spawning season will be analysed with that goal, focusing on areas of transition between the currently defined morphological groups. Comparison of the morphological variability in spawning and feeding seasons will provide additional insight into the temporal stability of the groups, and possibly of seasonal movements between areas. Finally, integrating morphometric information with the output of other phenotypic approaches, such as analysis of life history properties or otolith chemistry, and with genetic data, is essential for efficient stock identification (Begg and Waldman, 1999; Cadrin, 2000). Revision of the current stock definition should follow only if the morphological groups shown here prove to be persistent and coherent with other phenotypic and genetic structures of sardine populations.

Chapter 4

Maturity-at-length*

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4.1 Introduction

Spatial and temporal trends in maturation have been documented for many fish species. On wide geographic scales, maturation tends to co-vary with growth in such a manner that the ratios between length/age at first maturity and maximum length or age remain approximately stable across the distribution range of species (Beverton, 1992). Populations inhabiting colder habitats generally grow more slowly, live longer, and delay maturation when compared with those from warmer areas of the distribution, the differences reflecting the ability of the species to adapt to large-scale patterns in environmental conditions (Beverton, 1992). As is the case for other life history traits, maturation expresses the interaction between the genetic background and environmental influences, and provides evidence of geographic and/or reproductive isolation of fish populations (Begg, 2005). Therefore, differences in maturation have been taken as evidence for population discreteness within holistic approaches for the identification of fish stocks (Begg and Waldman, 1999; Begg et al., 1999b; Berg and Albert, 2003). Apart from their value in stock identification, spatial variations in maturation within the boundaries of a stock should be taken into account in stock assessment if they are associated with large spatial differences in population abundance (Hilborn and Walters, 1992).

Temporal trends in fish maturation have often been interpreted as compensatory density- dependent effects that regulate population growth (Rose et al., 2001). Density-dependent changes in maturation arise from food limitation attributable to increased intraspecific competition. The length or the age of maturation may be directly affected through changes in the quantity of energy reserves available for gonad development (Morgan, 2004), or indirectly via changes in growth which in turn influence the triggering of maturation (Engelhard and Heino, 2004). Density-dependent effects may explain the decrease in length/age at first maturity in many fish populations that have shown extensive declines in biomass (Armstrong et al., 1989; Nash et al., 2000; Engelhard and Heino, 2004). As most of these populations belong to heavily exploited fish stocks, fishing mortality has been suggested as the selective pressure inducing a rapid evolution of maturation characteristics (Barot et al., 2005). However, it has often proved difficult to disentangle the direct influence of population density from that of long-term environmental trends, and this is further confounded by the effects of environmental conditions on population abundance, which in turn can trigger density-dependent maturation (Rose et al., 2001).

The sardine (*Sardina pilchardus*) is a commercially important clupeoid distributed in the northeastern Atlantic from the North Sea to Senegal and throughout most of the Mediterranean Sea (Parrish et al., 1989). Its largest populations and the more productive sardine fisheries are situated off Morocco (stock biomass 1-5 million tones; catches around 600 000 t in recent years; FAO, 2003), whereas abundance and fishing intensity are low in the Mediterranean (http://www.faocopemed.org). Within Atlantic European waters, the sardine is considered to belong to a single stock for assessment purposes, the Atlanto-Iberian stock, with biomass around 500 000 t and catches around 100 000 t in recent years (ICES, 2005a).

The Atlanto-Iberian sardine stock is delimited by the French/Spanish border in the north, and by the Strait of Gibraltar in the south (ICES Divisions VIIIc and IXa). It is exploited mainly by the purse-seine fleets of Spain and Portugal, and it has been assessed since the early 1980s assuming that it consists essentially of self-reproducing populations that share homogeneous life history properties. However, recent studies provide evidence of geographic differences in morphometric characters, age structure, and population dynamics within the stock area, which challenge the assumption of a single management unit (ICES, 2000; Carrera and Porteiro, 2003; Silva, 2003). The stock boundaries have also been questioned on the basis of evidence of a continuous distribution of sardine eggs and adults extending into French waters and of possible mixing among populations across the southern border of the stock (ICES, 2000; Stratoudakis et al., 2003). Uncertainty regarding the definition of the stock unit has affected the level of confidence in stock estimates and knowledge of long term trends in abundance, and has highlighted the need for biological information that can both assist in defining management units and provide data to pursue stock assessment at finer spatial scales (ICES, 2005a).

The main goal of this manuscript is to study the spatial and temporal variability of sardine maturation patterns, and to discuss their contribution to the definition of stock structure. Data collected recently across most of the area of distribution of the species are used to describe large-scale geographic variability in life history traits such as longevity, maximum length, and maturity at length.

Despite the narrow temporal scale and the limited sampling intensity, the samples provide an opportunity to compare sardine maturation over a wide geographic area, using data collected and analysed with similar methodology. Data on length, weight, sex, maturity, fat content, and age routinely collected by Spain and Portugal since the early 1980s for the assessment of the Atlanto-Iberian sardine stock are used to compare sardine maturation patterns among stock areas, and to assess the temporal stability of such patterns by following interannual changes in the length at first maturity (L_{50}). Data on population abundance (from landings and estimated biomass) of the Atlanto-Iberian sardine stock are used to explore the existence of potential relationships with size at maturity (positive if both parameters represent a response to environmental conditions; negative if there is density-dependent competition). Finally, fish condition (weight and fat content at length) are used to explore the relationship between the accumulation of energy reserves during summer feeding and the size at first maturity.

4.2 Material and methods

Sardine samples collected within the remit of various international projects during the period 1999-2004, mainly for studying stock structure, are used to describe broad geographical patterns in sardine maturation. Overall, 51 samples of sardine (generally 100 fish) collected from the north (English Channel to southern France), south (northern Morocco to Mauritania), east (Azores and Madeira), and west (western Mediterranean) of the distribution of the Atlanto-Iberian stock were used (Figure 4.1). All samples were collected within the spawning season, either in the ports (5 samples) or during research surveys, but the final distribution of samples among areas and years is very unbalanced (e.g. 28 samples in south France and only one sample in Mauritanian waters). Samples were transferred frozen to the laboratory of IPIMAR, where fish were thawed and processed following routine biological sampling (see below).

Biological samples from sardine commercial landings and acoustic surveys have been regularly collected from the area since the early 1980s, and are used to describe temporal trends and small-scale spatial variation in the maturation pattern of the stock. Samples from landings by purse-seiners were collected once or twice a month between 1981 and 2003 at the main ports of the Iberian Peninsula (Figure 4.1). Sampling started more recently and has been less regular in the Cantabrian Sea, North Galicia (1993), and Cadiz (2000), providing around 800 fish by port and year. Ports located in southern Galicia, and in northern, southwestern, and southern Portugal account on average for 80% of the total landings of the stock. Sampling regularity in these ports was generally good, with an average of 1200 fish collected per port per year, except during periods of low sardine abundance or seasonal closures of the fishery. Each sample consists of six fish per half-centimetre class (Spanish ports)

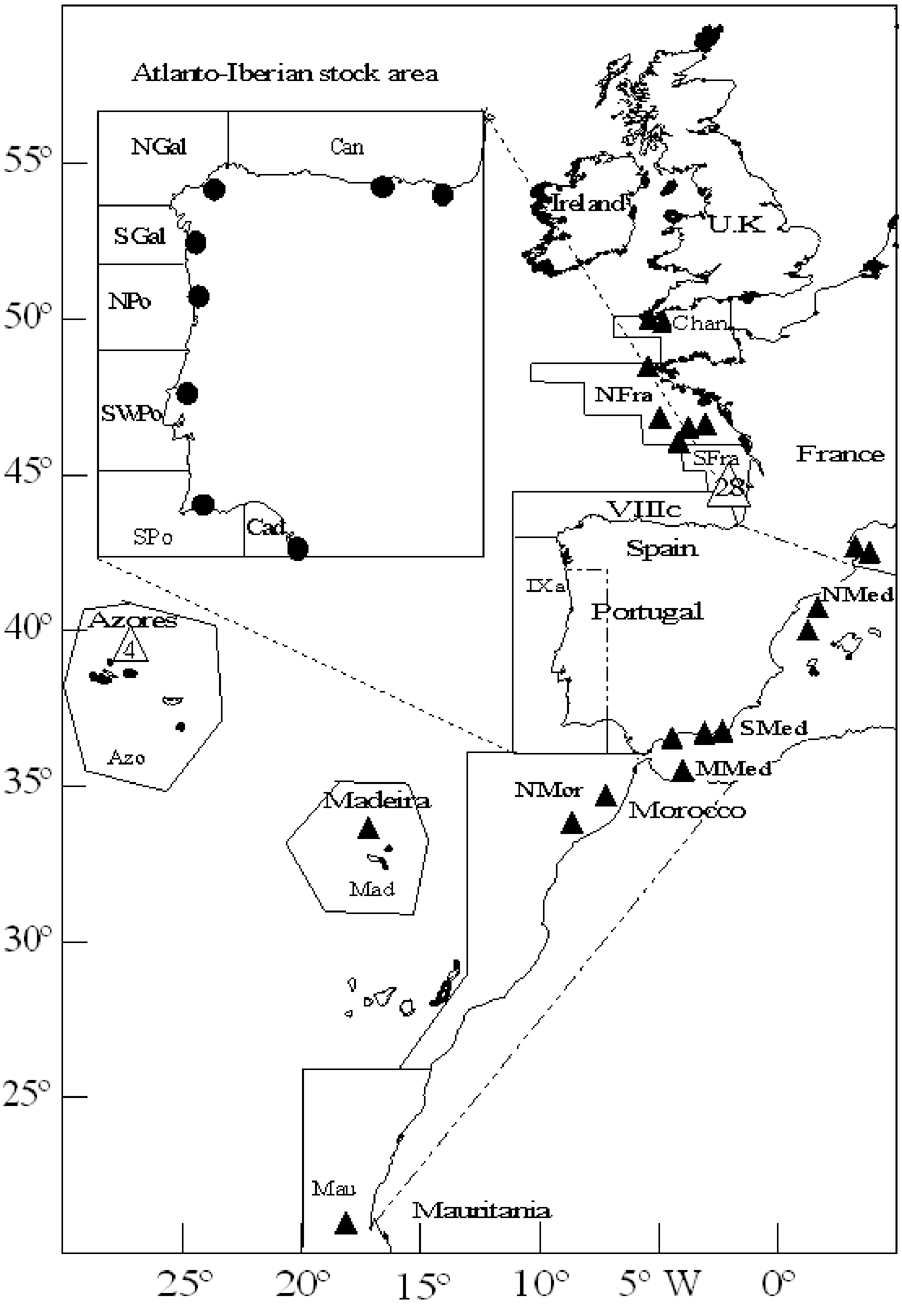


Figure 4.1: Map of the study area showing boundaries of ICES divisions and FAO divisions. The location of samples collected for broad spatial-scale analyses is indicated by the black triangles; larger symbols show the number of samples collected from close locations. The area of the Atlanto-Iberian sardine stock with the corresponding ICES subdivisions is enlarged on the top left of the map. Ports where samples for small-scale spatial analyses were collected are represented by black circles.

or a random collection of 100 fish (Portuguese ports). Samples from acoustic surveys have been collected irregularly since 1984 in spring (Spanish and Portuguese waters) and autumn (Portuguese waters). During such surveys, random collections of 40 fish (Spanish survey) or length stratified samples of 10-15 fish per half-centimetre length class (Portuguese surveys) were taken from each fishing haul.

Total length (cm), macroscopic maturity, and visceral fat stage were recorded for all fish. Total weight (g) was recorded for all market samples, but gutted weight (Wgut) and gonad weight (Wgon) are only available since the mid-1980s in sardine samples collected at Portuguese ports. Age data from survey samples collected within the stock area and from opportunistic samples collected outside this area are used to summarize the age structure of sardine populations across the study area. Macroscopic maturity stage was determined by visual examination of the gonads using a six-stage key: 1 - immature, 2 - developing, 3 - pre-spawning, 4 - spawning, 5 - spawning/recovering, 6 - post-spawning (Pinto, 1957; Pinto and Andreu, 1957). Fish at maturity stage 2 and above were considered to be mature (here used as a synonym for "adults", i.e. potentially part of the spawning population) and those in stages 3-5 were assumed to be showing spawning activity. The classification of the fat stage (1 - no fat visible, 2 - thin thread of fat surrounding part of the gut, 3 - thicker layer of fat partially surrounding the gut, 4 - thick layer of fat surrounding the gut) was based on the key proposed by Furnestin (1943).

Length-based maturity ogives were obtained from samples grouped into areas roughly according to ICES or FAO divisions. For a broad geographic comparison of maturity, ogives were obtained from interannually pooled samples from the areas outside the stock and from pooled survey data (separately for the 1980s and the 1990s-2000s) for areas within the stock region. To explore temporal trends in maturity within the stock area, maturity ogives were estimated from market samples pooled for each spawning season (October-January, spanning the first part of the spawning season up to the peak). The sampling period used to estimate maturity ogives was based on an analysis of sardine spawning seasonality as a function of fish length in the 1980s and 1990s. The probability of spawning (sp) was modelled as an anisotropic bi-variate function *(f(.))* of fish length class (l) 12-18 cm and month (mo) (September-August, coded with numbers 1-12) within each area and period using Generalized Additive Models with a binomial error distribution and a logit link (Wood, 2000). The general form of the models is

$$\mathit{logit}(E[\mathit{sp}]) = f(l, mo).$$

Eight models were fitted to data for each combination of area/period providing an explained deviance of 30-62%, with sample size ranging from 7130 to 21 205 observations. These models were used to predict the probability of spawning of fish 14 cm (mean L_{50}), 15.5 cm (mean L_{75}), and 18 cm long throughout the year. The last length class was assumed to represent the spawning seasonality of larger fish.

Generalized linear models (GLMs; McCullagh and Nelder, 1989) with a binomial error distribution and a logit link were fitted to the proportion of fish mature (m) by length class (l). The model has the general form

$$logit(E[m]) = a + bl$$

where a and b are the intercept and slope of the ogive, respectively. Estimates of the length at 50% maturity (L_{50}) and of the slope of the ogive at L_{50} (slope) were derived from the model parameters as

$$L_{50} = -\frac{a}{b}$$

$$Slope = \frac{b}{4}$$

The standard error of L_{50} was estimated using the formula for the approximate variance of a ratio of two random variables (Mood et al., 1974):

$$s.e._{L_{50}} = (\frac{a}{b})^2(\frac{var(a)}{a^2} + \frac{var(b)}{b^2} - 2\frac{cov(a,b)}{ab}),$$

and the s.e. of the slope at L_{50} is

$$s.e._{slope} = \frac{s.e._b}{4}.$$

Standard errors of the parameters were corrected to account for the inflation of variance (relative to the nominal binomial variance) attributable to the combination of data from different samples (see below). This correction was carried out by multiplying the parameter standard errors by the square-root of the replication estimate of overdispersion (McCullagh and Nelder, 1989; Millar et al., 2004). Asymptotic confidence intervals were calculated using the t-statistic. Geographical differences and temporal trends in L_{50} within western Iberian waters were tested for the period 1985-2003 by linear regression analysis. The effects of area (factor variable), year (continuous variable), and their interaction were tested by F-test using a forward procedure (McCullagh and Nelder, 1989). The inverse standard errors of L_{50} were used as weighting factors. The northern and southern Iberian areas were not included in this analysis owing to a lack of data. All calculations were carried out with R 1.9.1 (R Development Core Team, 2004).

4.3 Results

Large-scale spatial variation in life history parameters

Sardine total length in all samples used in this study ranged between 7.0 and 29.5 cm, corresponding to ages of 0-13 years (Table 4.2). Length and age range decreased from north to south in the northeastern Atlantic and were generally smaller in the Mediterranean Sea. Cape Finisterre at the northwestern corner of the Iberian Peninsula seems to form a boundary in the length and age distribution of samples from the Atlantic, with higher median lengths and ages in areas to the north ($\approx$ 20 cm and 3 years) than to the south ($\approx$ 17 cm and 1 year old). The sample from

Mauritania was an exception to this trend, showing a similar length distribution to northern areas but a reduced age range. Samples from the western Mediterranean and from the Atlantic islands of the Azores and Madeira had smaller length and age ranges than those from southern Iberia.

Table 4.2 summarizes information on sardine maturation by area. Point estimates of L_{50} varied between 10.9 and 16.8 cm (mean 14.3 cm, CV 10%), and slopes in L_{50} ranged from 0.13 to 0.53 cm^{-1} (mean 0.30 cm^{-1}, CV 42%). There is some evidence of an overall increase in the percentage of small sardines mature from northern France to the Gulf of Cadiz but, unlike for length and age distributions, there is no clear boundary at Cape Finisterre. Instead, L_{50} is considerably higher in northern France (16.8 cm) but comparable among areas to the south of this region. At the southern extent of the Iberian Peninsula (Gulf of Cadiz), L_{50} was 2.4 cm lower than the overall mean, and transition to maturity is clearly steeper than in more northerly areas (slope in L_{50} 0.52 cm^{-1}) in recent years. The area of transition from a higher L_{50} in the west to a lower L_{50} near Cadiz is not clear because maturity parameters from southern Portugal were closer to those from Cadiz in recent years but similar to the western Iberian areas during the 1980s. Therefore, the most evident discontinuity in the maturation pattern of sardine within the Atlanto-Iberian stock area occurred in the Gulf of Cadiz.

Maturity ogives could not be estimated for the western Channel, the Mediterranean, or North Moroccan areas, because immature fish were either absent or

Table 4.1: Data used to summarize sardine maturation patterns by area within northeastern Atlantic and western Mediterranean waters. Information on sardine length and age in samples and mean landings and abundance by area is also provided. Area codes: Chan - English Channel; NFr - North France; SFr - South France; CanE - East Cantabrian Sea; NGal - North Galicia; SGal - South Galicia; NPo - North Portugal; SWPo - Southwest Portugal; SPo - South Portugal; Cad - Gulf of Cadiz; SMed - Southwest Mediterranean; Nmed - Northwest Mediterranean; Mmed - Moroccan Mediterranean; Nmor - North Morocco; Mau - Mauritania; Azo - Azores; Mad - Madeira. *a* total Can and NGal landings.

Area	ICES FAO area	Period	Month	n	Median length		Median age		Landings ('000 t)	Abundance ('000 t)
					(range)	(cm)	(range)	(years)		
Chan	VIIe	2003	Jan, Nov	180	23.5	(18.0-27.0)	6	(2-12)	12.6	-
NFr	VIIIa	2000-2003	Mar-Jun	387	19.0	(11.5-24.5)	3	(1-8)	11.2	281
SFr	VIIIb	1997 - 2001	Mar-Jun	2.050	19.0	(11.5-26.0)	2	(1-10)		
Can	VIIIc-E	1986 - 1989	Mar, Apr	979	22.0	(11.5-27.0)	4	(1-11)	33*a*	61
		1990 - 2003	Jan-May	6.800	21.5	(11.5-29.0)	4	(1-13)	8.5	60
NGal	VIIIc-W	1986-1989	Mar, Apr	598	21.5	(11.0-25.5)	5	(1-10)	33*a*	57
		1990-2003	Mar-May	2.279	21.0	(11.0-26.5)	3	(1-12)	12.0	8
SGal	IXa-N	1986-1989	Feb-Apr	633	18.5	(12.0-25.5)	3	(1-10)	51.8	11
		1990-2003	Mar, Apr	3.284	17.5	(12.0-29.5)	2	(1-12)	13.4	10
NPo	IXa-CN	1984-1988	Nov, Mar	4.707	17.0	(9.5-25.5)	1	(0-8)	50.3	197
		1996-2003	Nov, Mar	15.808	15.0	(7.0-24.0)	1	(0-10)	40.9	149
SWPo	IXa-CS	1984-1988	Nov, Mar	2.804	17.0	(8.0-23.0)	2	(0-8)	31.8	118
		1996-2003	Nov, Mar	13.183	17.5	(7.0-24.0)	2	(0-9)	27.7	115
SPo	IXa-AS	1984-1988	Nov, Mar	2.262	17.5	(12.0-22.5)	1	(0-8)	16.3	74
		1996-2003	Nov, Mar	9.495	18.5	(9.5-23.0)	2	(0-9)	18.0	83
Cad	IXa-SC	1996-2003	Nov, Mar	9.491	16.5	(9.5-25.0)	2	(0-9)	5.3	139
SMed	37.1.1	1999	Dec	106	14.5	(13.0-20.5)	1	(1-3)	4.7	37
MMed	37.1.1	2004	Oct	46	13.5	(12.5-16.0)	0	(0-1)	14.0	-
NMed	37.1.1/2	1999	Dec	60	15.0	(13.5-19.0)	1	(1-3)	11.0	76
NMor	34.1.11	2000-2003	Jan	139	17.0	(12.0-19.0)	2	(1-4)	14.6	22
Mau	34.3	2003	Feb-Apr	89	23.0	(13.5-26.5)	3	(0-6)	11.5-37.5	670
Azo	Xa	2000-2004	Jun, Nov	324	16.5	(11.5-18.5)	0	(0-2)	<1	-
Mad	XIa	2003	Mar	64	17.0	(16.0-19.5)	1	(1-4)	<1	-

Table 4.2: Maturation parameters of sardine summarized by area and period within the northeastern Atlantic and western Mediterranean. Crude estimates of the mean length at age 1 are also provided. Area codes: NFr - North France; SFr - South France; CanE - East Cantabrian Sea; NGal - North Galicia; SGal - South Galicia; NPo - North Portugal; SWPo - Southwest Portugal; SPo - South Portugal; Cad - Gulf of Cadiz; SMed - Southwest Mediterranean; Nmed - Northwest Mediterranean; Mmed - Moroccan Mediterranean; Nmor - North Morocco; Mau - Mauritania; Azo - Azores; Mad - Madeira. * minimum length in samples.

Area	Period	Month	L_{50}[CI] (cm)		Slope in L_{50} [CI] (cm^{-1})		Minimum length at maturity (cm)	Mean length ± s.d. at age 1 (cm)
NFr	2000 - 2003	Mar - Jun	16.8	[16.4, 17.2]	0.21	[0.16, 0.25]	14.5	15.1 ± 1.63
SFr	1997 - 2001	Mar - Jun	14.0	[13.7, 14.4]	0.23	[0.19, 0.28]	12.5	15.8 ± 1.44
Can	1986 - 1989	Mar, Apr	14.6	[13.9, 15.3]	0.25	[0.13, 0.37]	13.5	15.6 ± 1.18
	1990 - 2003	Jan - May	13.8	[13.5, 14.0]	0.29	[0.26, 0.33]	11.5*	15.3 ± 2.21
NGal	1986 - 1989	Mar, Apr	15.6	[15.1-16.1]	0.34	[0.23-0.46]	13.5	13.8 ± 1.89
	1990 - 2003	Mar - May	13.3	[12.2-14.3]	0.18	[0.12-0.23]	12.0	16.4 ± 1.58
SGal	1986 - 1989	Feb - Apr	13.4	[12.8, 14.0]	0.53	[0.32, 0.74]	13.0	15.6 ± 3.95
	1990 - 2003	Mar, Apr	13.9	[13.5, 14.4]	0.23	[0.19, 0.28]	12.0*	17.0 ± 4.12
NPo	1984 - 1988	Nov, Mar	14.6	[14.4, 14.8]	0.25	[0.22,0.28]	12.0	15.5 ± 3.93
	1996 - 2003	Nov, Mar	14.3	[14.1, 14.4]	0.23	[0.21, 0.26]	11.0	15.0 ± 3.87
SWPo	1984 - 1988	Nov, Mar	14.7	[14.3, 15.1]	0.17	[0.14, 0.20]	10.5	15.9 ± 3.98
	1996 - 2003	Nov, Mar	14.3	[14.1, 14.6]	0.20	[0.18, 0.22]	11.5	14.2 ± 3.77
SPo	1984 - 1988	Nov, Mar	13.9	[13.6, 14.3]	0.25	[0.20, 0.30]	12.5	16.4 ± 4.05
	1996 - 2003	Nov, Mar	12.4	[12.0, 12.9]	0.24	[0.20, 0.27]	11.0	16.3 ± 4.03
Cad	1996 - 2003	Nov, Mar	10.9	[9.7, 12.1]	0.52	[0.07, 1.03]	10.0	15.5 ± 3.93
SMed	1999	Dec	-	-	-	-	13.0*	15.6 ± 1.00
MMed	2004	Oct	-	-	-	-	12.5*	15.2 ± 1.40
NMed	1999	Dec	-	-	-	-	13.5*	14.6 ± 0.83
NMor	2000 - 2003	Jan	-	-	-	-	12.0*	14.9 ± 1.79
Mau	2003	Feb - Apr	16.2	[14.7, 17.7]	0.13	[0.06, 0.20]	13.5*	17.8 ± 1.15
Azo	2000 - 2004	Jun, Nov	15.7	[14.9, 16.5]	0.21	[0.07, 0.36]	14.5	16.5 ± 1.82
Mad	2003	Mar	-	-	-	-	16.0*	17.8 ± 0.66

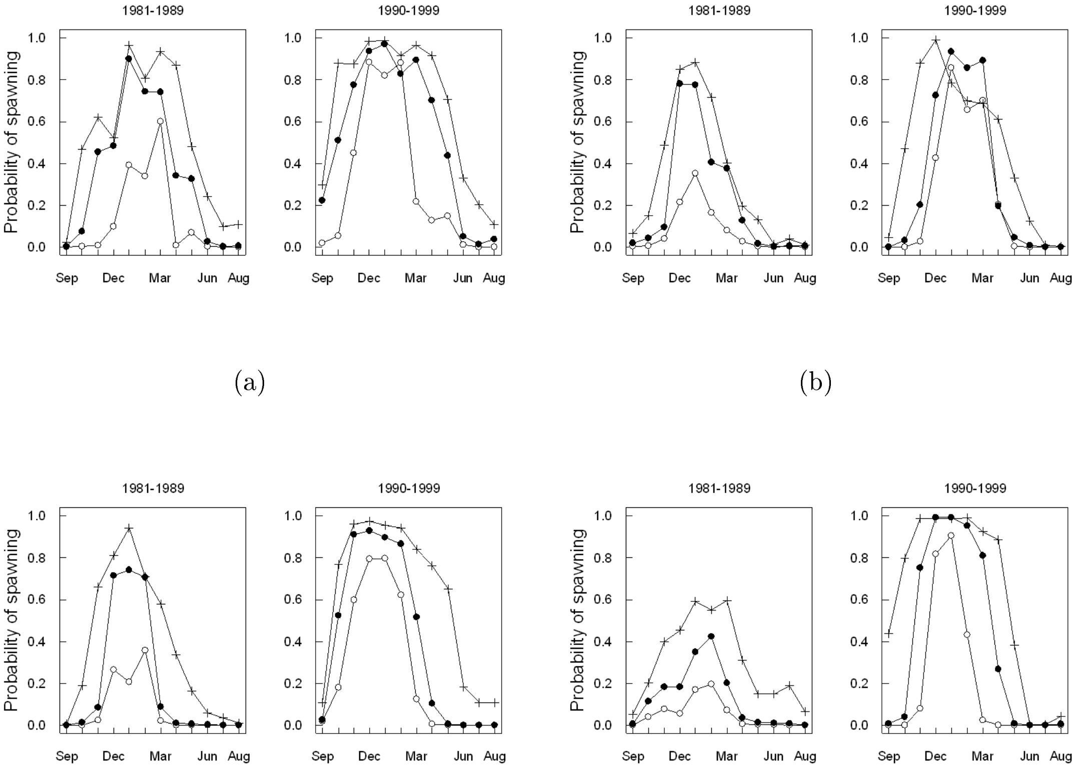
present in only small numbers within the samples. This would be expected for the Channel where the minimum length of fish sampled was 18 cm. Conversely, the small fish (12-13.5 cm) in the Mediterranean and Moroccan samples were mostly adults, providing some indication that L_{50} values in those areas would be at least as low as those in northwestern Iberian areas. The samples from Mauritania (L_{50} 16.2 cm) and Azores (L_{50} 15.7 cm) suggest that sardine length-at-first maturity may increase towards the southern and the western limits of distribution of the species.

Spatio-temporal variation in maturity-at-length along the Iberian Peninsula

Annual maturity ogives are seldom defined for the northern stock areas (Cantabria and north Galicia) because samples are dominated by large fish (median length 21 cm, median age ≈ 4 years; Table 4.1). Pooled survey data provided estimates of L_{50} between 13.3 and 15.6 cm, within the range of variation of L_{50} in the more southerly areas (Table 4.2). Similarly, most of the sardines sampled in the Gulf of Cadiz were mature, in both survey (Table 4.2) and market samples. However, unlike in northern Spanish waters, small sardine were found in the area during all spawning seasons. Four maturity ogives were defined based on survey data from the Gulf of Cadiz, providing the lowest pooled estimate of L_{50} within the stock area (see above).

Off western and southern Iberia, maturity-at-length data covered both juvenile and adult fish, defining clear maturity ogives in most spawning seasons. Patterns of spawning seasonality suggested no major differences among size classes, areas or periods that could hamper the comparison of maturity ogives (Figure 4.2). In general, spawning activity extended from late autumn to late spring, with a peak between December and March. Smaller fish have shorter spawning seasons, but maximum activity of the different size classes was synchronized. In addition, there was a pronounced increase in the duration of the season and in the intensity of peak spawning in all areas from the 1980s to the 1990s, particularly for smaller fish. Acoustic survey data (not shown) support the above changes in spawning seasonality between decades. Although the spawning season expanded between decades, neither the peak nor the overall pattern of activity showed marked temporal shifts. The above results confirm that samples used to construct maturity ogives generally cover the progress of the spawning season up to the peak of spawning (January) of fish of different size in each area and period. In two cases (south Galicia and south Portugal during the 1980s), the spawning season shifted slightly towards spring, possibly introducing a positive bias in L_{50}.

Figure 4.3 shows annual estimates of L_{50} based on GLM parameters for the spawning seasons 1981-2003 off western and southern Iberia (from south Galicia to south Portugal). L_{50} was not significantly different among the three western areas ($p=0.18$). Overall maturation patterns were comparable in the four areas, as indicated by the similarity among the lengths of 25% ($\approx$ 12.0 cm), 50% ($\approx$ 14 cm), and 75% ($\approx$ 15 cm) maturity, based on data pooled for the study period. Figure 4.3



(a) (b)

(c) (d)

Figure 4.2: Probability of spawning of 14 cm (white circles), 15.5 cm (black circles), and 18 cm (crosses) sardine in the 1980s and 1990s off (a) southern Galicia, (b) northern, (c) southwestern, and (d) southern Portugal.

also suggests that L_{50} decreased in the four areas; this is corroborated by decadal survey data (Table 4.2). In fact, when the period from 1985 is considered, there is a significant linear decline in L_{50} ($p<0.001$), and differences in this trend are not significant among the three western areas ($p=0.85$). Downward shifts of L_{50} were observed also for the northern areas of the stock (Cantabrian Sea and north Galicia), resulting in a decline in the average length at first maturity for the Atlanto-Iberian stock of sardine, from 14.5 cm in the 1980s to 13.3 cm in the 1990s.

Annual changes are similar in southern Galicia and northern Portugal, where L_{50} rose from the early to the mid-1980s and decreased towards the late 1990s. Off southern Galicia, there was an associated increase in the slope of the maturity ogives (not shown). Off southwestern Portugal, L_{50} declined after a period of high values during the mid-1980s. The series of L_{50} for southern Portugal has large temporal gaps owing to the lack of small immature fish in samples during most of the 1990s, precluding analysis of temporal trends. For the early 1980s, L_{50}s are within the range of variation of values for the other stock areas. However, there is indication mainly from survey data that L_{50} was significantly lower in the late 1990s (Table 4.2). Data on gonad weight provide some support to maturity data, given the significant negative correlation between L_{50} and the gonadosomatic index of maturing fish (in the range L_{25}- L_{75}) in western Portuguese areas (Figure 4.4).

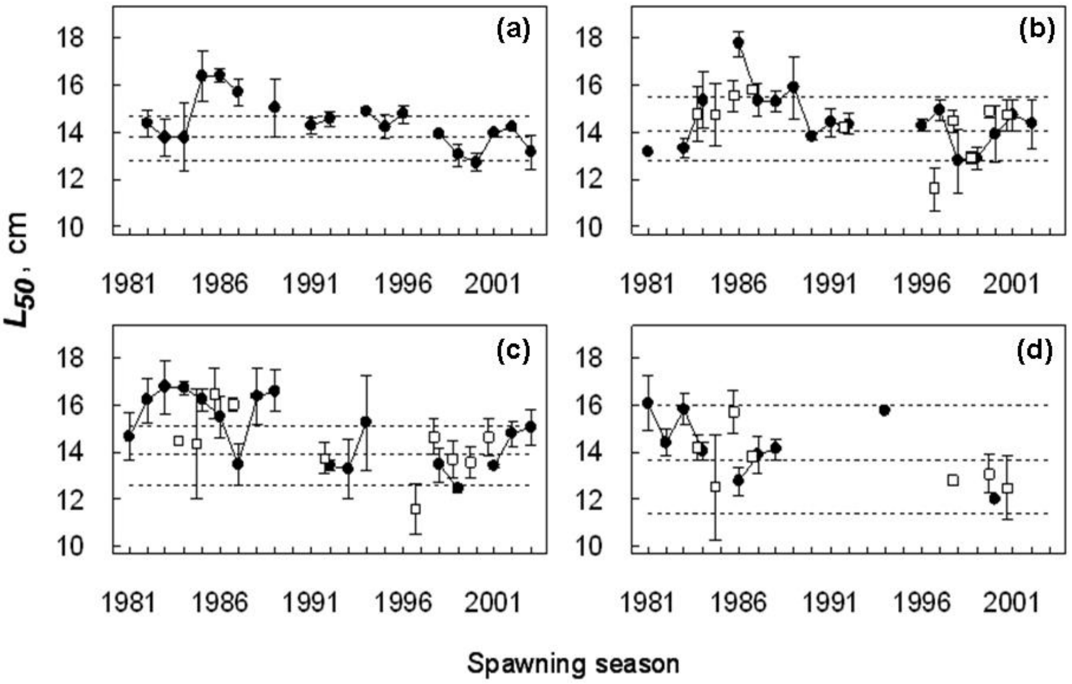


Figure 4.3: Variations in L_{50} in the spawning seasons of 1981-2003 by area. (a) IXa-N, (b) IXa CN, (c) IXa-CS, (d) IXa-SA. Circles, data from market samples; squares, data from autumn acoustic surveys. Bars represent 95% confidence limits. Dashed lines show L_{25}, L_{50}, and L_{75} based on data pooled for the study period.

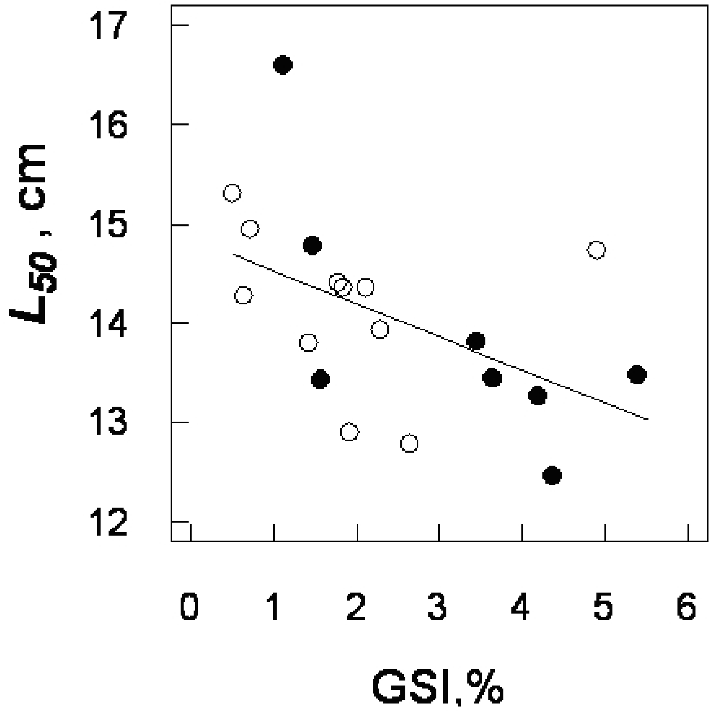


Figure 4.4: Relationship between L_{50} and the mean gonadosomatic index (GSI) of maturing fish (12-15.9 cm) in corresponding spawning seasons for 1987-2003 off northern (white circles) and southwestern (black circles) Portugal. A linear regression line is superimposed on the data to illustrate the trend.

Relationship between L_{50}, condition, and abundance

The influence of fish body condition on maturation was explored using data from the western Iberian areas on the mean total weight and the percentage of fish with medium/high visceral fat (fat stage >2; % fat) in summer (July and August). This is when plankton production and sardine body condition peak. Therefore, fish weight and fat reserves in summer are a reliable index of the energy made available for reproduction in the current spawning season (Koops et al., 2004), and possibly also for the spawning season of the following year (Hunter and Leong, 1981). As the mean weight and the % fat showed similar temporal variations in the different length classes, 18 cm fish (the best sampled length class during the study period) were used, assuming to be representative of the average condition of the whole population. The two variables showed an increasing trend through the period in all areas: pooled data for 1982-1985 and 2000-2003 showed increases of 8-11% in mean weight and of 26-58% in the percentage of fish with fat. L_{50} was significantly negatively related to the mean weight-at-length (Spearman rho = -0.35, p = 0.01), and to the percentage of fish with fat (Spearman rho = -0.47, p = 0.005) in the current summer season. However, the correlation improved when L_{50} was related to fish weight (Spearman rho = -0.51, p = 0.0003) and % fat (Spearman rho = -0.61, p = 0.0003) in the previous summer, indicating that condition has a prolonged effect on the length at first maturity (Figure 4.5).

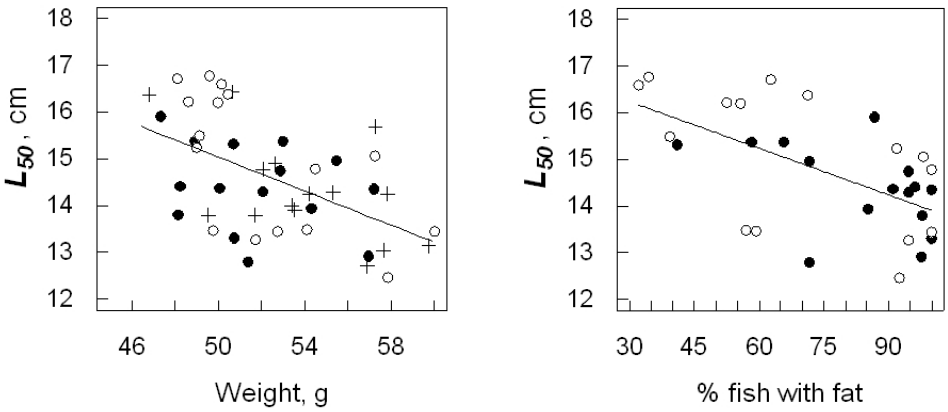


Figure 4.5: Relationship between L_{50} in a given spawning season (October-January) and the mean weight at length (left panel), and the % of fish in fat stage >2 in the summer of the preceding year (July and August) (right panel) off northern Portugal (black circles), southwestern Portugal (open circles), and southern Galicia (plus signs; only weight data available). The mean weight and % fish in fat stage >2 is shown for the best sampled length class in the areas, 18 cm. A linear regression line is superimposed on the pooled data to illustrate the trend.

The influence of population abundance on the length at first maturity was also studied, using data on sardine landings and biomass (from acoustic surveys) off western Iberia. The long-term trends in maturation do not show an association with local annual landings or survey abundance indices in Portuguese waters. Such a result was expected considering there were no major changes in sardine abundance within those areas during the study period. However, off southern Galicia L_{50} was significantly correlated with landings ($r = 0.48$, $p = 0.02$; Figure 4.6). Landings are considered to be representative of the large decrease in sardine abundance in this area (Table 4.1; Carrera and Porteiro, 2003), so the above relationship provides some evidence of a density-dependent effect on maturation. There is also some indi-

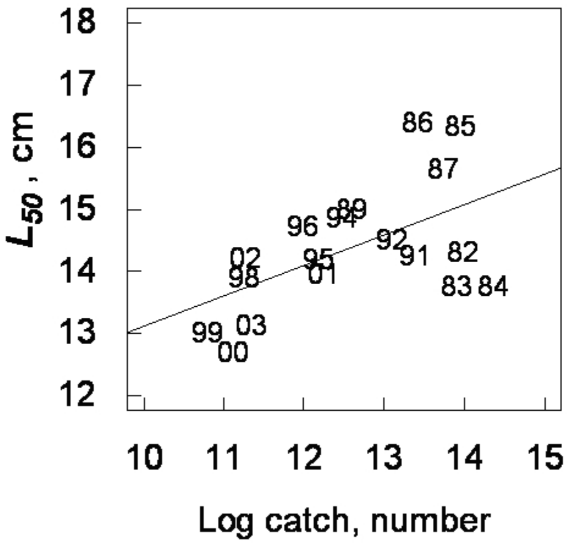


Figure 4.6: Relationship between L_{50} and landings in southern Galicia in the period 1982-2003. Text numbers show the year. A linear regression line is superimposed on the data to illustrate the trend.

cation that population abundance may influence maturation on a shorter time scale, because recent shifts in sardine abundance have been followed by wide variations in L_{50}. In fact, owing to the strong incoming 2000 year class, sardine abundance increased by a full order of magnitude in northern Portugal from 1999 to 2000 (ICES, 2002). Maturation length rose 2.0 cm in the area and increased also in the adjacent areas (south Galicia and southwest Portugal) the following spawning season, to where the cohort spread during its first year of life (Figure 4.3).

4.4 Discussion

All data analysed in this study were collected using the same macroscopic criteria for maturity, so the only concern with data quality relates to the accuracy of macroscopic staging. The reliability of macroscopic maturity data is supported by the significant correlation between L_{50} and the gonadosomatic index of maturing individuals observed since 1987 off western Portugal (Figure 4.4). GSI data further strengthen the hypothesis of a decline in the length at first maturity in the area. Additional information on the accuracy of maturity staging is limited to a few recent intercalibration exercises, which point to some uncertainties in maturity classification. A recent intercalibration of macroscopic staging of sardine ovaries with histological criteria showed that 24% of stage 1 gonads were misclassified (they were in fact stage 2), and 27-36% of post-spawning fish were wrongly identified as pre-spawning (early spawning season), or spawning/recovering (late spawning season; ICES, 2003b). These results indicate that both the proportion of mature fish and the proportion of active individuals may have been slightly underestimated in the past.

The samples analysed across the area of distribution of sardine suggest an increase in the percentage of small sardine that are mature from north to south within the northeast Atlantic. There is also evidence of earlier maturation in the Gulf of Cadiz, and in Mediterranean and Moroccan waters and, possibly, delayed maturation towards the northern (north France), southern (Mauritania) and western (Azores) limits of distribution (Table 4.2). Earlier results on sardine maturation support the broad spatial trends in maturity described here, corroborating the upward shift in L_{50} from southern to northern France, and the low length at first maturity of sardine off Cadiz compared with northwestern Iberian areas (Table 4.3). Further, they support the increase in maturation length towards the southern (Saharan Morocco) and western (Canary Islands) extent of sardine range. Large-scale spatial variation of sardine length at maturity shows an overall positive relationship with longevity and maximum fish size (Tables 4.2 and 4.3), and bears some direct association with growth during the first year (Andreu and Plaza, 1962; Morales-Nin and Pertierra, 1990; FAO, 2001b). A similar geographic pattern of maturation has been reported for horse mackerel (*Trachurus trachurus*) (Abaunza et al., 2003): L_{50} varies from 15 to 23 cm in north African waters, and 16 cm in northwestern Mediterranean to 16-19 cm off southern Portugal, and increases gradually to the northern areas, attaining 20-25 cm in the North Sea and English Channel. Length at maturity is also higher in sardine populations with greater maximum size. These broad spatial patterns suggest that maturation has an inverse relationship with the potential growth span of the species across its range, conforming to the general principles of life history strategy (Beverton, 1992; Rose et al., 2001).

In Atlantic waters, the most striking discontinuities in the length at first maturity of sardine are at the southern (south Morocco/Mauritania) and western (Atlantic Islands) limits of distribution, and also between the southern and the northern French

Table 4.3: Literature information on sardine maturation within the northeastern Atlantic and western Mediterranean. Area codes: CMor-Central Morocco; SMor-South Morocco; CanI-Canary Islands; Med-Mediterranean Sea; see also legend of Table 4.2. Notes: (1) Maturity key from Pinto and Andreu (1957); (2) Maturity stage 2 (initial development) not included in fraction mature; (3) Age readings from scales; (4) Maturity key from Hjort (1910), (5) Maturity key not referred to; (6) Age readings from otoliths; M-male; F-female.

Area	Period	Month	Length range (cm)	Age range (year)	L_{50} (cm)	Min. length at maturity (cm)	Maturation age (year)	Notes	Reference
Chan	1935-1938	-	18.0-26.0	2-8	≈ 19	-	3-5	-	Hickling, 1945.
	-	-	-	-	20.5	-	4	-	Hodgson & Richardson, 1949
NFr	1928-1929	Spawn. season	9.0-20.0	0-5	17.0-18.0	-	During 3^{rd} year	3, 4	Le Gall, 1930
SFra	1941-1943		8.0-20.0	0-4	15.5	-	End 2^{nd} year		Furnestin, 1943
SGal	1951-1954	-	12.0-20.0	-	14.5	12	0-1	1, 2	Andreu, 1955
	1981	Jan - Mar	11.0-23.5	1-8	14.5	13.5	1.3		Pérez et al., 1985
Cad	1958-1964	Spawn. season	7.0-20.0	0-3	-	10.5	-	1, 2	Rodríguez-Roda 1970
SMed	1949-1955		8.0-16.0	-	11.5	-	1	3, 4	Larrañeta, 1976
	1989-1991	Oct - Jan	8.0-23.0	-	12.6-13.8	-	-	1, 2	Abad & Giraldéz, 1993
Med	Synoposis		22 (max)	8 (max)	10-14	-	0-2		Larrañeta, 1960
NMor	1959-1964	Oct-May	11.0-21.0	0-3	-	11.5	-	1, 2, 3	Rodríguez-Roda 1971
CMor	1967-1968		12.0-21.0	-	M: 13.0	11.8	-	5	Sedletskaya, 1973
					F: 15.0	12	-		
SMor	1967-1968	Spawn. season	12.0-23.0	-	M: 13.5	11.8	-	5	Sedletskaya, 1973
					F: 16.5	13.4	-		
	1974-1975		11.0-24.0	1-6	-	M: 13.0	-	5, 6	Krzeptowski, 1983
					-	F: 15.0	-		
CanI	1995-1996	Nov-Mar	6.0-23.0	-	15	-	-	2	Mata et al., 1997

waters. At the southern limit of the species, there is some evidence of differences in morphological characters, age structure, spawning areas, growth, and maturation between north/central Morocco and south Morocco/Mauritania, pointing to the existence of separate sardine populations (FAO, 2001a). Additionally, ongoing studies on sardine genetics indicate that sardine from the Azores and Madeira (western) and Mauritania (southern) are distinct from those in the Atlantic between southern Iberia and the English Channel (Kasapidis et al., 2004). By contrast, the abrupt increase in L_{50} between southern and northern French waters is neither explained by genetic differentiation (Kasapidis et al., 2004) nor by major discontinuities in the distribution of sardine between the areas (Bellier et al., 2004; ICES, 2005a). The predominance of large mature sardine in northern French waters and in the English Channel (Furnestin, 1943; Hickling, 1945) supports the hypothesis that nursery grounds of these sardine populations are located in the south, possibly in the Bay of Biscay (Furnestin, 1943). The French waters north of the Gironde Estuary (French coast, 45°30´N) are distinctive oceanographically from those of the Bay of Biscay, with considerably lower temperature attributable to a persistent cold water mass (Puillat et al., 2004). Colder water conditions also prevail in the northern areas of the English Channel and North Sea, restricting sardine spawning activity to a few months during summer and autumn (Stratoudakis et al., 2004). Such conditions may influence the migration of juveniles to southern areas, and also constrain the reproductive development of the few small fish that remain in the areas.

Atlanto-Iberian sardine stock maturation is comparable among the western areas of distribution, the mean L_{25}, L_{50}, and L_{75} being around 12, 14, and 15 cm, respectively. There is limited information on maturation length in northern Spanish waters (north Galicia and Cantabria), which is dominated by large adults, possibly immigrants from adjacent recruitment areas off northwestern Iberia or the Bay of Biscay (Carrera and Porteiro, 2003). Nevertheless, decadal L_{50}s for these areas are similar to those for western Iberia. The long-term average maturation pattern of sardine off southern Portugal is uncertain because of the scarcity of data, but recent information points to similarity with sardine maturation in the Gulf of Cadiz. In the latter area, sardine mature smaller than the general pattern, which in turn is similar to that observed in the southwestern Mediterranean. A similar spatial pattern was demonstrated for sardine morphometry, the similarity extending also to northern Morocco (Silva, 2003). However, earlier studies on meristic characters distinguished European Atlantic from Northwest African sardine populations (Parrish et al., 1989), whereas Mediterranean and Northwest African sardine have been considered a different subspecies from the European Atlantic sardine (Bauchot and Pras, 1980). The lack of genetic distinction among sardine populations around the Iberian Peninsula (Kasapidis et al., 2004) suggests that maturation differences are mainly driven by environmental conditions common to both Mediterranean and Atlantic waters of southern Iberia, which are themselves distinct from the prevailing conditions off western Iberia. Differences in maturation may be mediated by growth, because lower mean lengths at age have been observed in both the Gulf of Cadiz and Mediterranean waters than off Galicia and central Portugal (Rodriguez-Roda, 1970).

The long-term samples off western Iberia show an increasing trend in the percentage of small sardines maturing each spawning season since the early 1980s, along with associated increases in spawning activity (mainly of smaller fish) and in the duration of the spawning season between the 1980s and the 1990s. These changes suggest an improvement in the overall reproductive potential of sardine, and may be the result of enhanced fish condition during that period. In fact, for other clupeoid species, most of the energy spent in a specific spawning season comes from fat reserves stored during the current and the previous season (Hunter and Leong, 1981). This hypothesis is supported here by the significant correlation between mean sardine weight and % fat at length with the length at first maturity. Temporal changes in sardine condition may have been caused by environmentally driven variation in food availability. There is evidence of environmental change off western Iberia during the last two decades (Santos et al., 2001, 2005; Borges et al., 2003; Guisande et al., 2004), and these seem to be part of wider temporal and spatial variations in the hydroclimatic conditions of the whole North Atlantic (Ottersen et al., 2001). Based on satellite-derived sea-surface temperature data, Santos et al. (2005) document a shift in the upwelling regime off western Iberia, from a weak intensity in the 1980s to stronger in the 1990s, during both winter and summer. This increased upwelling intensity may have enhanced plankton productivity in the area and hence food availability for sardine and other clupeoid populations. Sardine condition and therefore L_{50} may also be partially regulated by density dependence, as observed for other pelagic species (Parrish and Mallicoate, 1995). The fact that the long term decline in L_{50} was inversely related to abundance off southern Galicia (Figure 4.5, an area that experienced a great decrease in its sardine population during the last two decades (Carrera and Porteiro, 2003), strengthens this possibility. It is also likely that variations in both sardine condition and abundance were affected by changes in environmental conditions observed in the past two decades off western Iberia.

In spite of the above evidence of environmentally driven changes in the length at first maturity, the hypothesis of an evolutionary trend in sardine maturation is not excluded. In fact, the pronounced changes in the abundance and age structure of sardine populations in mainly the northern areas of the stock (Galician and Cantabrian waters; ICES, 2000) might have provided scope for the selection of early maturing phenotypes. It can be argued that selective changes would not likely take place over such a short time ($<$20 years), because most experimental studies suggest that 10-20 generations are needed for selection to occur (Swain et al., 2005). However, only four generations were needed for the growth rate of Atlantic silverside (*Menidia menidia*) a short-lived pelagic species (Connover and Munch, 2002), to evolve. Sardine have moderate longevity (7-8 years), but they mature during the first two years of life, as do other short-lived pelagic species. Therefore, the possibility that the maturation of sardine evolved over just two decades warrants further investigation.

Maturation reaction norms (Heino et al., 2002; Barot et al., 2004) are a useful tool in exploring evolutionary trends in maturation, because they allow disentangling the influence of growth and mortality (assumed to reflect environmental variation) on maturation. Estimation of reaction norms for sardine maturation could complement these analyses, because variation in growth may explain the geographic and temporal changes in maturity at length described. Age data are available from commercial and survey length-stratified samples of sardine covering part of the area and period analysed here, and they can be used in future to pursue such an approach. Nevertheless, the estimation of reaction norms for sardine requires careful consideration in relation to sampling design, consistency of age determinations, and also to whether age and length distributions derived from commercial samples are representative of the whole populations.

Overall, there is clear evidence that sardine may attain sexual maturity at a wide range of lengths across its distribution area, varying from 11 cm to almost 17 cm (mainly age groups 0 and 1), in relation to broad patterns of growth and longevity. Within the Atlanto-Iberian stock, maturation patterns by length are comparable across the western areas (from southern Galicia to southwestern Portugal), and there is no evidence of pronounced differences towards the northern areas (northern Galicia and Cantabria). On the other hand, sardine mature smaller in the Gulf of

Cadiz than in the remaining stock areas, and there is no indication of a different maturation pattern north of the stock boundary (southern French waters). As is the case with other life history traits, length at maturity does not provide direct evidence of stock structure, so these results are not sufficient to review the identity of the sardine stock in Atlantic European waters. However, they do assist in delineation of areas with distinct phenotypic groups of sardine, and this information can be utilized along with evidence from other approaches to stock identification for management purposes.

Chapter 5

Growth patterns*

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Silva, A., Carrera, P., Massé, J., Uriarte, A. D., Santos, M. B., Oliveira, P.B., Soares, E., Porteiro, C., Stratoudakis, Y. Geographic variability of sardine growth across the northeastern Atlantic and the Mediterranean Sea. Fisheries Research.

5.1 Introduction

The sardine (*Sardina pilchardus*) is a small pelagic clupeoid distributed in the northeastern Atlantic from the North Sea to Senegal and throughout most of the Mediterranean Sea (Parrish et al., 1989). It has high commercial importance, being targeted by purse-seine fisheries across most of its distribution area and by pelagic trawlers mainly within the African waters. Sardine abundance and fishing intensity are generally lower in the Mediterranean than in the Atlantic waters (GFCM, 2006). Within the northeastern Atlantic, the largest populations are situated off northwestern Africa, while the second most important area spans the Iberian - Biscay waters (from northern France to the Gulf of Cadiz) (FAO, 2004; ICES, 2006a). For assessment and management purposes, eight sardine stocks are considered across the Mediterranean Sea, the most abundant and productive ones being located off northern Spain and the Gulf of Lyons (GFCM, 2006). Three sardine stocks are considered off the Atlantic coast of western Africa (FAO, 2004); the largest of these stocks, the central stock, shows acoustic biomass estimates between 1 and 5 million tons and annual landings of 600 thousand tons in recent years. Across the Iberian-Biscay region, sardine is mainly distributed off the western Portuguese waters and in the French Gulf of Biscay (ICES, 2006a). A single sardine stock is considered in the European Atlantic waters for assessment and management, the Atlanto-Iberian stock, delimited by the French/Spanish border in the north, and by the Strait of Gibraltar in the south (ICES, 2006a). Catches from this stock have fluctuated around 95 thousand tons in recent years and estimates of spawning biomass range between 250 and 400 thousand tons. The delimitation of this stock, particularly the location of the northern boundary, has been challenged in recent years and several studies were carried out to assist its revision in the near future (e.g. Anon., 2006; ICES, 2006a; Bernal et al., 2007). Off the English Channel and North Sea, sardine is apparently much less abundant than in neighbour French waters (ICES, 2006a) although there is evidence that sardine abundance is increasing in the northern limit of its distribution (Beare et al., 2004).

Sardine populations show large variation in size and age structure across the northeastern Atlantic and Mediterranean waters. Geographic differences in the mean length-at-age often associated with differences in size and age structure, may reflect size-specific migrations between areas (Nöttestad et al., 1999; Villamor et al., 2004) or alternatively, separate growth patterns (c.f. Begg, 2005 and references therein). Over broad spatial scales, growth patterns are often observed to vary with latitude such that larger and faster growing populations occur in more northern areas, in apparent contradiction with general environmental gradients expected to influence growth (Conover, 1992). Growth trends corresponding to a geographical pattern of genotypes (countergradient variation), may have developed as an adaptation to environmental seasonality, enabling populations to survive longer, colder and resource-poor winters at higher latitudes. On the other hand, persistent differences in growth trajectories, often combined with differences in maximum length, maximum age or age distributions may indicate some degree of separation between fish populations within a given life phase and be used to define or corroborate stock structure (Begg and Waldman, 1999).

This study describes spatial and temporal variations in sardine growth across the northeastern Atlantic and Mediterranean waters. Samples collected opportunistically in recent years across the species range are used to explore variations in sardine length-at-age and growth performance at a broad geographic scale. A more detailed description of the variations in sardine growth between areas, decades and seasons is presented for the Iberian - Biscay region, using acoustic survey data collected annually since the mid-1980s and monthly samples from the landings in 2004 and 2005. The observed broad scale geographic variability in sardine growth is then related to existing knowledge in population structure, migration patterns and latitudinal trends in temperature seasonality and plankton production. The implications of spatial growth differences to the definition and assessment of the Atlanto-Iberian stock are also discussed.

5.2 Materials and methods

Samples

Samples collected opportunistically within the remit of various international projects in the period 1999-2004 were used to describe the broad-scale variation of sardine mean length-at-age and growth performance across the northeastern Atlantic and the Mediterranean Sea. Overall, 42 samples of sardine (3827 fish) were collected in the coastal Atlantic waters between the English Channel and Mauritania, off the Atlantic Islands of Azores and Madeira, in the western Mediterranean waters from the Balearic Islands to the Gulf of Lyon and in the Aegean Sea (Table 5.1, Figure 5.1). Most of the samples were collected with pelagic trawls during research surveys, although samples from the English Channel were collected from beach seine landings and those from Azores, Madeira, north Morocco and Mauritania were collected from purse seine landings. In all cases, samples consisted of random collections of individuals from survey hauls or landings; despite the weak size selectivity of purse and beach seine nets some undersampling of the smallest individuals is expected in samples from the commercial fisheries due to the market preference for sardines above 15-16 cm for fresh consumption and the canning industry and a minimum landing size of 11 cm. Samples were transferred frozen to the laboratory of IPIMAR for routine biological sampling and extraction of otoliths.

Samples collected during spring acoustic surveys in the Atlantic coastal waters from Brittany (France) to the Gibraltar Strait (Gulf of Cadiz, Spain) were used to describe in more detail the spatio-temporal variation of sardine growth within the Iberian - Biscay region (Table 5.2, Figure 5.1). Surveys were carried out in February-April off Portugal and Cadiz, March-April off northern Spain and April-June in French waters, broadly following the latitudinal progression of sardine spawning

Table 5.1: Data used to describe large-scale spatial variation of sardine growth within the northeastern Atlantic and Mediterranean Sea. Note: the age range refers to observed ages and not to true ages calculated from birth-date and sampling month.

Area			Sampling period		Length range	Age range	Sample
			Year	Month	(cm)	(years)	size
1	English Channel	CHAN	2003	Nov, Jan, Feb	18.0-27.2	2-14	241
2	North France	NFRA	2000-2003	Mar, May, Jun	11.7-24.7	1-8	459
3	South France	SFRA	2000-2003	Apr, May, Jun	14.0-23.6	1-7	204
4	East Cantabria	ECAN	2000-2003	Apr	15.3-24.3	1-9	309
5	West Cantabria	WCAN	2000-2003	Apr	18.7-25.1	2-9	246
6	North Galicia	NGAL	2000-2003	Mar, Apr	14.8-24.8	1-8	341
7	South Galicia	SGAL	2000-2003	Mar, Apr	12.1-21.8	1-5	149
8	North Portugal	NPOR	2000-2003	Feb-Apr	10.9-22.1	1-6	244
9	S.W. Portugal	SWPO	2000-2003	Mar	12.9-19.9	1-4	238
10	South Portugal	SPOR	2000-2003	Mar	15.0-23.4	1-10	297
11	Gulf of Cadiz	CAD	2000-2003	Mar	12.7-20.7	1-6	240
12	North Morocco	NMOR	2000, 2003	Jan	12.0-21.0	1-5	222
13	Mauritania	MAU	2003	Dez, Mar	14.4-27.0	0-7	120
14	Azores	AZO	2003,2004	Oct, Nov	11.5-18.7	0-4	121
15	Madeira	MAD	2003	Mar	16.2-19.8	1-4	82
16	Western Mediterranean	WMED	1999	Dec	13.0-21.0	0-7	149
17	Gulf of Lyon	LYON	1999, 2003	Jul, Dec	13.0-18.2	0-5	96
18	Aegean Sea	AEG	2003	Jun	11.0-16.0	1-4	69

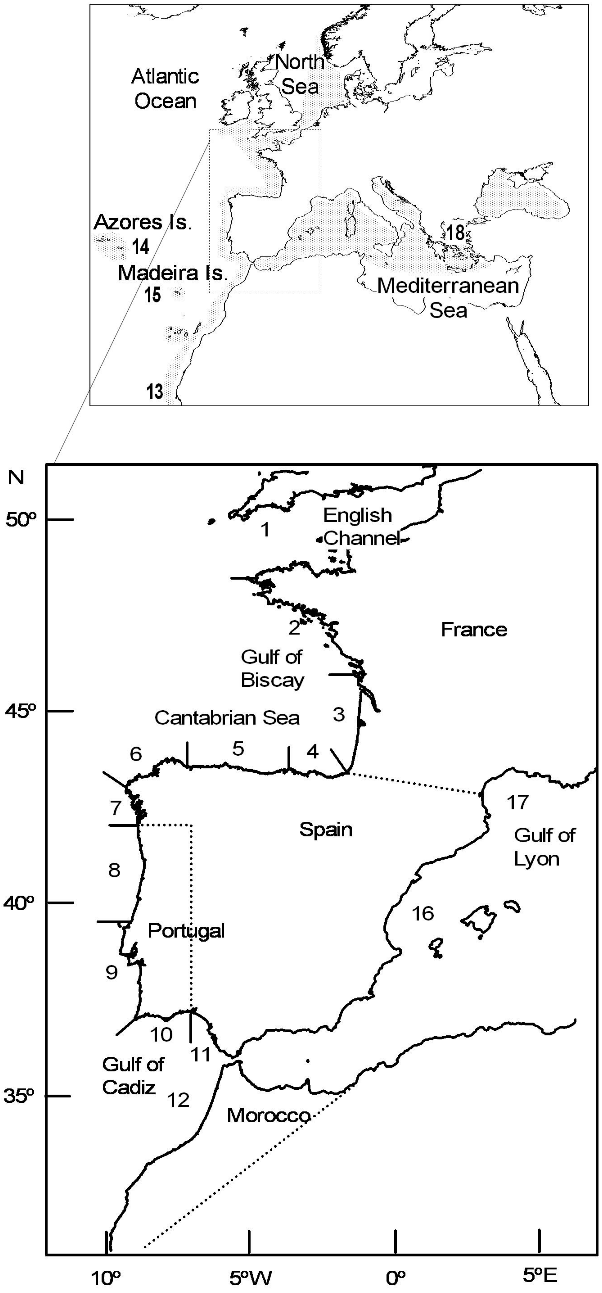

Figure 5.1: Map of the study area showing regions considered when pooling data for the analyses of growth. 1 English Channel, 2 North France, 3 South France, 4 East Cantabria, 5 West Cantabria, 6 North Galicia, 7 South Galicia, 8 North Portugal, 9 Southwest Portugal, 10, South Portugal, 11 Gulf of Cadiz, 12 North Morocco, 13 Mauritania, 14 Azores Is., 15 Madeira Is., 16 Western Mediterranean, 17 Gulf of Lyon, 18 Aegean Sea.

season (Coombs et al., 2006; Stratoudakis et al., 2007). Length and age data are available from the French waters since 2000 while the northern region of the Iberian Peninsula was sampled since 1986 with gaps in some years. Off Portugal and the Gulf of Cadiz, regular sampling started in 1996 with two surveys covering only Portuguese waters in the mid-1980s. Otoliths were collected from length-stratified samples in the Portuguese (10-15 otoliths per half-centimetre in each area, see Figure 5.1) and French surveys (2-4 otoliths per half-centimetre in each haul) and from random samples of 40 fish per fishing haul in Spanish surveys. The average number of otoliths collected by year and area for ages 1 to 6 years (used for growth modelling) ranged between 158 and 521 (Table 5.2).

Monthly or bi-monthly samples of sardine collected within the Iberian waters from the landing harbours were used to study sardine seasonal growth and to explore relationships between growth and biological/environmental variables during recent years (2004-2005). Length-stratified samples (5-10 fish by half-centimetre) were collected at the main fishing ports of the Cantabrian Sea (including catches from southern French waters), north and south Portugal (Figure 5.1). The final number of fish sampled by month and year is variable both within and between areas, ranging between 30 and 250 in the Cantabrian Sea, 100 and 300 in north Portugal and 50 and 150 in south Portugal, with gaps in a few months. The monthly length distribution of catches was not available for some of the areas, preventing to account for the length-stratification of samples on the estimation of length-at-age.

Table 5.2: Summary information of length and age data collected during spring acoustic surveys within the Iberian - Biscay area. N: average number of otoliths collected by year and area for ages 1 to 6 years. S.D. standard deviation.

Area			Years	N	Length (cm)		Age (years)	
					Range	Mean±S.D.	Range	Mean±S.D.
2	North France	NFRA	2000-2005	241	13.0-26.5	20.5±2.3	1-13	3.2±2.0
3	South France	SFRA	2000-2005	521	12.0-26.5	19.6±3.7	1-9	2.2±1.5
4,5	Cantabrian Sea	CAN	1986-1988, 1990, 1993, 1996-2005	333	11.0-27.6	21.2±2.4	1-12	3.7±1.7
6	North Galicia	NGAL	1987-1988, 1990, 1992, 1992-1993, 1998-2005	158	11.0-25.6	21.1±2.3	1-10	3.5±1.5
7	South Galicia	SGAL	1988-1988, 1990-1993, 1996-2005	203	10.9-24.4	18.3±2.5	1-12	2.5±1.5
8	North Portugal	NPOR	1996-2003, 2005	234	8.6-23.4	16.4±2.9	1-10	2.0±1.5
9	S.W. Portugal	SWPO	1986, 1988, 1996-2003, 2005	270	7.5-24.0	17.4±3.4	1-9	2.7±1.8
10	South Portugal	SPOR	1986, 1988, 1996-2003, 2005	204	9.6-23.4	18.6±2.4	1-12	3.3±2.0
11	Gulf of Cadiz	CAD	1996-2003, 2005	199	9.6-23.5	17.3±2.5	1-9	2.5±1.6

Biological sampling

Total length (cm), sex (female, male) and age were recorded for all sardines sampled. Sex could not be determined in a few juvenile (total length below 10 cm) and some large (above 22-23 cm) individuals. In samples collected from the market, total weight (g) and macroscopic maturity stage (Pinto and Andreu, 1957) were also recorded. Otoliths (*sagittae*) were extracted from the fish, cleaned in water and mounted in black polyester plates using a synthetic resin. Otoliths were viewed on standard dissecting microscopes at 20-30x magnification. Age determination of sardine from acoustic surveys or market samples within the Iberian-Biscay region was carried out in national laboratories (IPIMAR for Portugal, IEO for Spain and IFREMER for France) while otoliths from recent opportunistic samples were read by a single experienced reader from IPIMAR. In both cases, age readers followed the guidelines of international workshops held within the study period (ICES, 1997b; FAO, 2001a; Soares et al., 2004). Standard protocols for sardine age determination assume the 1st of January as the average birth-date of the species and consider that a set of one opaque zone, laid down during the fast growing period, and one translucent zone, formed during the slow growing period, correspond to annual growth zone, annulus. Fast growing periods usually coincide with the period of planktonic blooms, which may occur between late-winter (e.g. in the Mediterranean) and early summer (e.g. northern Atlantic areas) whereas slow growing periods take place in the less productive seasons. Age, in years, is thus determined as the number of complete translucent zones. During the first semester, the last translucent zone is considered complete, and hence counted, whereas during the second semester the translucent zone in the otolith edge is considered to be in formation and it is not counted.

The birth-date convention matches the mid-spawning season across the area from western Iberia to Mauritania and in the Mediterranean waters (Abad and Giráldez, 1993; Coombs et al., 2006; Stratoudakis et al., 2007; Ganias et al. 2007). On the other hand, a lag of 3-5 months is observed relative to the spawning peak in the northern Atlantic areas (from the English Channel to the Cantabrian Sea). To our knowledge, data on sardine spawning are not available for the Atlantic islands of Azores and Madeira, however, the application of the 1st of January birth-date to these areas may not introduce serious bias since peak spawning off the Canary Islands is also known to occur in winter (Mata et al., 1997).

Absolute age based on annual growth rings has not been validated in most areas of the sardine distribution. However, daily growth studies carried out on sardine recruits from Galician, Cantabrian and northwest Mediterranean waters provide a detailed description of growth during the first year of life, permitting to identify the location of the first true annual translucent ring (Alvarez and Alemany, 1997; Alemany et al., 2006). In addition, the periodicity of otolith growth zones was shown to be annual off northwest Spain, Portugal, west Africa and northwestern Mediterranean, from studies of the monthly evolution of the otolith edge (Jorge and Monteiro, 1980; Álvarez and Porteiro, 1981; Krezptowski, 1983) or length frequency analysis (Pertierra and Morales-Nin, 1989). Annual growth rings were observed in sardine scales within the same areas (Andreu and Plaza, 1962; Bravo de Laguna et al., 1979) and shown also for sardines sampled off the English Channel (Hickling, 1945) and the French Gulf of Biscay (Furnestin, 1943). The annual pattern of alternating translucent and opaque rings formed during winter and spring-summer respectively, further suggests that sardine grows mainly during spring and summer in most areas.

It must be highlighted that the clarity of sardine otoliths declines from north to south in the Atlantic waters and is also poor in the Mediterranean Sea, due to decreasing contrast between opaque and translucent zones and to the appearance of false rings (Pertierra and Morales-Nin, 1989; Soares et al. 2004). In a recent sardine age reading workshop, otoliths from southern Iberia and west Africa with poor readability were shown to decrease the agreement between readers and the precision of mean length-at-age estimates (although bias was not observed). Age determination in the southern areas of the Atlantic and in the Mediterranean waters is further complicated by the broad spawning season; since spawning may extend for several months, the first winter ring forms at variable distances from the otolith nucleus or may be preceded by a clear translucent zone which confounds the identification of the true annual ring (Alemany and Álvarez, 1993; ICES, 1997b).

Data analyses

To describe the broad scale spatial variation of sardine growth, recent samples collected across the Atlantic and Mediterranean waters were grouped into areas, roughly according to ICES or FAO Divisions (Table 5.1). Von Bertalanffy growth curves (see subsection on Growth models) were fitted to length-at-age data by area and used to predict length-at-age 2 and age 4 years. Growth modelling was restricted to areas where the number of length-at-age observations and the range of lengths and ages sampled were sufficient to describe the growth trajectory. Since samples were collected in different periods of the year, age in years of each individual fish was converted to age in decimal years for growth modelling, taking into account the average birth-date in the area and the sampling date. Based on Stratoudakis et al. (2007, and references therein), assumed birth-dates were May for the French areas (NFRA, SFRA), March for the northern Spanish waters (ECAN and NGAL) and January for the remaining areas (NPOR, SPOR, CAD, NMOR, MAU and WMED). For the remaining areas, the mean length-at-age 2 or age 4 (whichever was best represented) was calculated.

The study of spatio-temporal variability in sardine growth across the Iberian-Biscay region was based on survey samples pooled into nine areas (Figure 5.1 and Table 5.2): north France, south France, Cantabrian Sea, north Galicia, south Galicia, north Portugal, southwest Portugal, south Portugal and Gulf of Cadiz. These areas are routinely used for estimation of sardine abundance and length structure by acoustic methods (ICES, 2006a,b). Age-length keys (ALKs) were constructed for each area, year and sex using biological samples and subsequently raised to the population using length frequency distributions. The resulting matrices of fish abundance by length class and age in each area, year and sex (population ALKs) were used to calculate the mean and variance of length-at-age using standard formulas for grouped data (Zar, 1996). Estimates of mean length-at-age and variance for combined sexes, areas or periods were based on pooled population ALKs. Differential growth by sex was explored using annual estimates of mean length-at-age across the Iberian-Biscay waters between 2000 and 2005. To compare growth among areas and over time, data were pooled into three periods: 1986-1993 containing data for northern Spain (from the Cantabrian Sea to south Galicia), 1996-1999 containing data for the Iberian areas and 2000-2005 containing data for the whole study area (Iberian-Biscay waters). Growth curves were fitted to the mean lengths-at-age in each period and area using inverse variances as weighting factors to account for the decrease of variance with age (Kimura, 1980).

The seasonal growth of sardine was described using length-at-age data from each individual fish in market samples. The monthly length increments of age 2 individuals were calculated from seasonal growth models fitted separately to data from the Cantabrian Sea, north and south Portugal. Seasonal variations in condition were evaluated by changes in total weight of a given length class in each area. As the monthly mean total weight showed similar temporal variations in the different length classes, data for 20-20.9 cm fish (the best sampled length classes across areas during the study period) were used, assuming to be representative of the average condition of the whole population. The percentage of fish spawning was calculated as the % individuals in pre-spawning and spawning maturity stages. Spearman rank correlation coefficients (Zar, 1996) were used to test the significance of the relationships between the seasonal cycles of biological and environmental variables.

Monthly averages of sea surface temperature (SST) and Chl a in 2004 - 2005 were estimated from daily remote sensing data for the fishing areas from which sardine were sampled for seasonal growth analyses (Figure 5.1). Chl a estimates were extracted from the 9km resolution SeaWiFS (Sea-viewing Wide Field-of-view Sensor) Chl a concentration standard mapped images (L3SMI product, Thomas and Franz, 2005). SST averages were computed from EUMETSAT's Ocean and Sea Ice Satellite Application Facility (O&SI SAF) "Regional SST" product, for the "CANA" and "GASC" regions. These SST estimates are computed using data from the AVHRR (Advanced Very High Resolution Radiometer) instrument on board the polar orbiting NOAA satellites, and are comparable to the in situ measurements at night (Brisson et al., 2001).

Growth models

Growth was modelled using the Von Bertalanffy (VB) curve with the parameterization proposed by Schnute (1981). Compared with the traditional VB model, this parameterization provides estimators with better statistical properties (close to obeying asymptotic properties and less correlated), ensures faster convergence from departing initial estimates and provides parameters of simple biological interpretation (Schnute, 1981; Ratkowski, 1986). Annual growth is described by the expression (Schnute, 1981):

$$L_t = l_i + (l_j - l_i)\frac{1 - e^{[-k(t-t_i)]}}{1 - e^{[-k(t_j-t_i)]}}$$

where k (growth coefficient), l_i (expected length at age i) and l_j (expected length at age j) are model parameters, t_i and t_j are reference ages, in years, and L_t is the expected length at age t (years). The proportion between two successive annual length increments is given by $e^{(-k)}$ such that large/small k values indicate a fast/slow declining growth with age (Schnute and Fournier, 1980).

To describe seasonal growth, the annual growth model was modified to include sinusoidal variation in length-at-age as suggested by Quinn and Deriso (1999):

$$L_t = l_i + (l_j - l_i)\frac{1 - e^{[-k(t-t_i-(F(t)-F(t_i))]}}{1 - e^{[-k(t_j-t_i-(F(t_j)-F(t_i))]}}$$

where

$$F(tx) = (\frac{C}{2\pi})sin[2\pi(t_x - t_s)]$$

and

$$t_x = t, t_i, t_j$$

C is a dimensionless non-negative constant expressing the amplitude of the seasonal growth oscillation, t_s is the phase shift of the growth oscillation in relation to the start of the year, expressed as a fraction of the year, and t is age in decimal years. For an annual cycle, a phase shift t_s=0 (or 0 plus any integer) indicates that minimum growth occurs in January and the growing period ranges from April (t_s+0.25 year) to October (t_s+0.75 year) (Quinn and Deriso, 1999). The value of C indicates the magnitude of growth around the yearly time of minimum growth, ranging from zero for continual growth (nonseasonal) to 1 for null growth and to values above 1 for negative growth. No attempt was made to constrain the value of C to avoid negative growth; models with free and constrained (=1) C gave similar estimates of the remaining parameters while the former provided a better fit to the data.

Models were fitted by nonlinear least squares using the Gauss-Newton algorithm and 95% approximate inference intervals were calculated for growth parameters and expected values of length-at-age (Bates and Watts, 1988). Ages 1-6 years were used to model seasonal and annual growth of sardine from the Iberian-Biscay region. In annual models, ages t_i and t_j were taken to be 1 and 5 years old (thus, l_i=l_1 and l_j=l_5). In seasonal models, reference ages were adjusted to the beginning of April to match the average survey dates and therefore, t_i=1.25 and t_j=5.25 years. In the study of broad-scale variability of growth, older ages were poorly represented in some areas therefore, growth models were fitted to ages 1-5 years and reference ages were taken to be 2 and 4 years old (thus, l_i=l_2 and l_j=l_4). In a few cases, the growth coefficient of the VB model was not significantly different from zero suggesting linear growth across the range of observed ages and a linear regression model was fit to the respective length-at-age data.

To study sardine growth variability within the Iberian-Biscay region, area-based VB curves were compared by likelihood-ratio tests (Kimura, 1980). The hypothesis of separate curves for each pair of areas was set as a base case and tested against a sequence of hypotheses assuming that some of the growth parameters are shared. The first of these hypotheses tested simultaneous equality of the three growth parameters (*i.e.* whether the curves are coincident and therefore a single growth curve fits best the data). When this hypothesis was rejected, further tests were carried out to evaluate differences between curves in each growth parameter at a time (l_i, l_j or k). Depending on the most parsimonious model fitting the data, we classified the relationship between two growth curves, 1 and 2, in one of the following categories:

i. parallel, when k1=k2 and ($l1_i$>$l2_i$ and $l1_j$>$l2_j$ or vice-versa);

ii. convergent, when $l1_i \neq l2_i$ and $l1_j$=$l2_j$;

iii. divergent, when l1i=$l2_i$ and $l1_j \neq l2_j$

iv. separate, when either all three parameters were significantly different or when lengths at reference ages were such that growth trajectories crossed (i.e. , l1i>$l2_i$ and $l1_j$<$l2_j$ or vice-versa).

Significance levels were 0.01 in the comparison of sex-specific growth curves and 0.001 in comparison of growth curves for different areas, to account for multiple comparisons.

Growth patterns obtained from broad-scale samples collected within the northeastern Atlantic and the Mediterranean were compared between areas and contrasted with literature information using the relationship between $\log_{10}k$ and $\log_{10}L_\infty$ (auximetric plot; Cury and Pauly, 2000). For most species, this relationship is linear with a slope of -2 and an intercept which defines an average species-specific growth index, ϕ' (for a review see Pauly, 1991). The growth index may be viewed as the theoretical growth coefficient, k, for individuals of 1 unit length and be used to compare the growth performance between populations and species. L_∞ values were derived for each area from the parameters of the fitted VB models as (Schnute, 1981):

$$L_\infty = \frac{l_i - l_j e^{[-k(t_j - t_i)]}}{1 - e^{[-k(t_j - t_i)]}}$$

Eighty estimates of k and L_∞ were compiled from studies of sardine growth in several periods and areas across the northeastern Atlantic and the Mediterranean Sea (Guerrault, 1980; Monteiro and Jorge, 1982; Krzeptowski, 1983; Morales-Nin and Pertierra, 1990; Alemany and Álvarez, 1993; FAO, 2001a; Voulgaridou and Stergiou, 2003; and references therein). A linear regression model was fitted to these estimates and used explore the application of the growth index formula to sardine (Pauly, 1991) :

$$\phi' = log_{10}k + 2log_{10}L_\infty$$

All calculations were carried out with R 2.4.0 (R Development Core Team, 2006).

5.3 Results

Sex-specific growth

Sex-specific growth curves for sardine, based on pooled survey data from the Iberian-Biscay region in 2000-2005, are not coincident ($p=0.004$) due to significantly larger female l_5 ($p=0.005$) as indicated by log-likelihood ratio tests. The most parsimonious growth model provided l_5 ($\pm$S.E.) estimates of 21.4 ± 0.10 cm for females and 21.0 ± 0.10 cm for males but common estimates of l_1 and k (14.4 ± 0.10 cm and 0.73 ± 0.041 year^{-1}). These results indicate that male and female sardines have diverging growth trajectories, however differences in expected lengths are small up to age 6, as shown by overlapping 95% inference intervals (Figure 5.2).

Broad-scale spatial variation

Sardine total length in the broad-scale samples ranges between 10.9 and 27.2 cm, corresponding to ages of 0-14 years (Table 5.1). Off the English Channel, sampled individuals were older than 2 years, showed the oldest age and length among all samples (27.2 cm and 14 years) and the largest length-at-age 4 across the Atlantic areas apart from Mauritania (Table 5.1, Figure 5.3). Samples from the northeastern Atlantic suggest a latitudinal decline of mean length-at-age from north

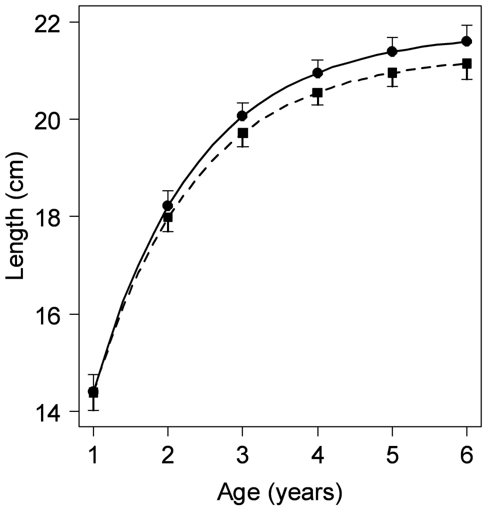

Figure 5.2: Average growth curves for female (solid line) and male sardine (dashed line) based on survey data collected across the Iberian-Biscay area in 2000-2005. Bars represent 95% asymptotic inference intervals for length-at-age.

France to north Morocco and a sharp increase off Mauritania. Sardines sampled in the western Mediterranean show lower lengths, at ages 2 and 4 years, than those sampled in the Atlantic apart from the Gulf of Cadiz and north Morocco. There is also some indication that sardine length-at-age declines from the western to the eastern Mediterranean (Aegean Sea) while the length of age 2 sardines from the Atlantic islands is similar to that of southern Iberia and western Mediterranean individuals (Figure 5.3). Geographical variations are generally smooth but result in substantial differences when distant areas are compared; in fact, at similar ages, sardines from north France are larger than those from north Portugal by ca. 2 cm, and the latter are larger than those from the Gulf of Cadiz, western Mediterranean and north Moroccan by ca. 1 cm. Differences in length-at-age are maximum (ca. 3

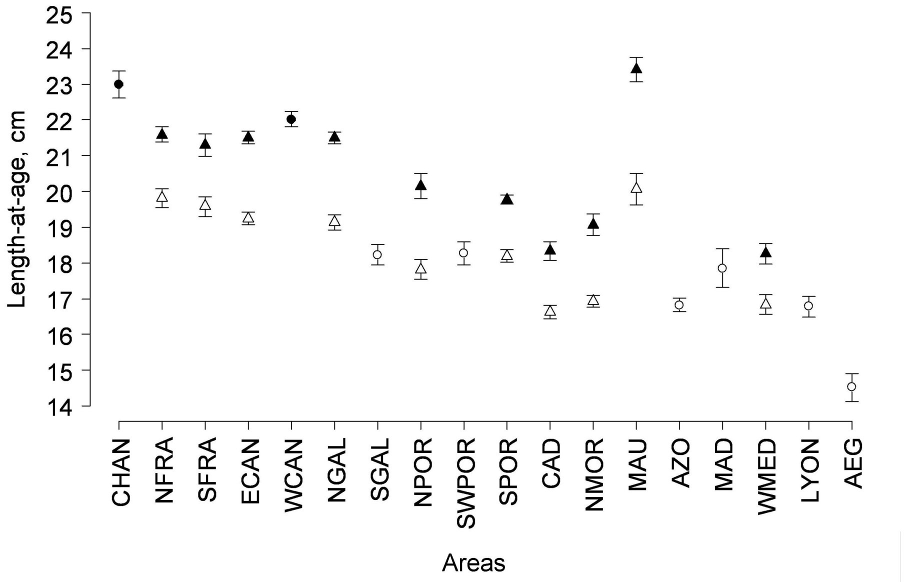

Figure 5.3: Mean lengths observed (circles) or predicted by VB models (triangles) for age 2 (open symbols) and age 4 (filled symbols) sardine in each area, using recent samples collected across the northeastern Atlantic and Mediterranean Sea. Bars represent two standard errors of the mean.

cm) when sardines sampled off north Morocco or off the western Mediterranean are compared with those from Mauritania.

VB growth curves explained more than 70% of the variance in length-at-age data from each area. The auximetric plot indicates that growth parameters obtained in this study are generally within the range of values reported in earlier studies (Figure 5.4). Pairs of $\log_{10}$k and $\log_{10}L_\infty$ are widely scattered around a regression line with slope -2.04 (S.E.=0.40) and intercept 2.46 (S.E.=0.54) which explains a low (but significant) percentage of total data variance ($r^2=0.22$, N=90, $p<0.05$) (Figure 5.4). Although the slope estimate provides some support to the growth index formula (Munro and Pauly, 1983) the model shows clearly a poor fit to the main cluster of points. This cluster contains almost exclusively data from the northeastern

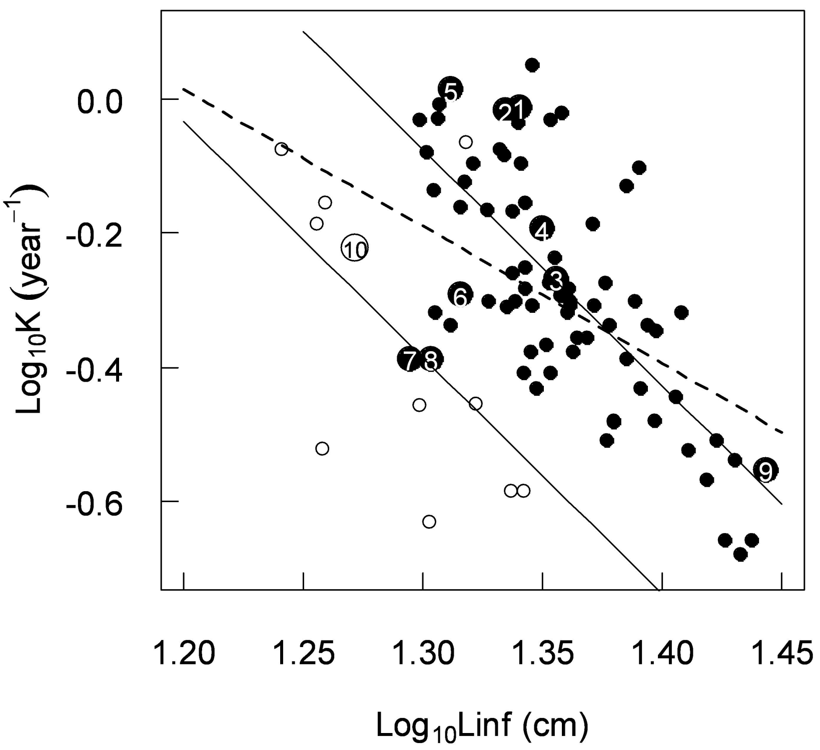

Figure 5.4: Auximetric plot of sardine growth parameters, L_∞ and K, using data compiled from earlier studies within the eastern and western Mediterranean (white circles) and the northeastern Atlantic waters (black circles) (see text for references). Numbers represent parameters obtained in this study for North France (1), South France (2), East Cantabria (3) North Galicia (4), North Portugal (5), South Portugal (6), Gulf of Cadiz (7), North Morocco (8), Mauritania (9) and west Mediterranean (10). Solid lines correspond to the linear regression model assuming separate intercepts for Atlantic and Mediterranean areas and the dashed line represents the common regression model.

Atlantic areas (but also 2 points from the southwestern Mediterranean) and suggests that a model with both a steeper slope and a larger intercept would be preferred. In addition, the sparser data from the western and eastern Mediterranean seem to align along a parallel axis raising the hypothesis that two linear regressions with a common slope but different intercepts for the Mediterranean and Atlantic areas would provide a better description of data.

This hypothesis was tested against the hypothesis of a common linear regression model; an indicator variable with levels for Atlantic and Mediterranean areas was added to the common model and the two models were compared by an F-test. The test showed that the model with a common slope of -3.24 (S.E.=0.40) but separate intercepts, 4.13 (S.E.=0.54) and 3.84 (S.E.=0.52) for the Atlantic and the Mediterranean areas respectively, provided a better fit to the data (F=33.9 on 1 degree of freedom, $p<0.05$) and improved the percentage of total explained variance to 43%. This result suggests that sardine from the Mediterranean Sea (apart from the southwestern area) and the northeastern Atlantic may represent two groups with separate average growth performances (given by the intercepts of the $\log_{10}k$-$\log_{10}L_\infty$ regression lines) and indicates that the slope of this relationship is steeper than the value (-2) assumed to represent a cluster of stocks from a single species (Pauly, 1991; Cury and Pauly, 2000).

Table 5.3 presents for each area the values of Munro and Pauly (1983) growth index and of a modified index (GI) obtained when the species constant (-2) is replaced by the slope from separate $\log_{10}$k-$\log_{10}$L$_\infty$ regression lines for Atlantic and Mediterranean waters. Although the two indices are positively correlated and suggest that the growth performance of sardine is higher off the northern Atlantic areas (French waters) and poorer in southern Iberia, north Morroco and west Mediterranean waters, they provide a distinct perspective of sardine growth performance off Mauritania; ϕ' suggests that sardine growth performance off Mauritania and western Mediterranean are comparable while GI suggests a higher similarity between Mauritania and the northern areas.

Spatio-temporal variation within the Iberian-Biscay region

Sardines between 9.6-27.6 cm total length and 1-13 years were sampled within the Iberian-Biscay region since the mid-1980s (Table 5.2). Maximum length declines from north to south corroborating the trend detected in the broad-scale samples (see previous section). Mean length shows a similar geographical trend but lower mean ages are associated with recruitment areas such as northern Portugal/south Galicia, Gulf of Cadiz and south France. A VB model fitted to annually pooled samples for 2000-2005 across the region showed that sardine grows from 15.3 cm (S.E. = 0.41 cm) at age 1 to 23.0 cm (S.E.= 0.02 cm) at age 10. Annual growth increments decline 64% in each successive age (k=0.44 year^{-1}, S.E.= 0.06 year^{-1}) such that individuals attain 90% of their maximum length at age 4.

Table 5.3: Growth index, ϕ' of sardine in each area based on recent samples collected across the northeastern Atlantic and western Mediterranean and compiled from literature information. Values of the modified growth index (GI) are also shown for each area.

Area	ϕ'			Reference	GI
	This study	Other studies	N		
NFRA	2.67	[2.41 - 2.68]	5	Guerrault, 1980; and references therein	4.70
SFRA	2.65	[2.30, 2.45]	2	Guerrault, 1980; and references therein	4.67
ECAN	2.44	-	-	-	4.50
NGAL	2.51	-	-	-	4.55
NPOR	2.64	[2.28 - 2.51]	4	Monteiro and Jorge, 1982; and references therein	4.63
SPOR	2.34	[2.29 - 2.43]	3	Monteiro and Jorge, 1982; and references therein	4.34
CAD	2.20	-	-	-	4.16
NMOR	2.22	[2.26 - 2.60][a]	14	FAO (2001a); and references therein	4.20
MAU	2.34	[2.19 - 2.48]	10	FAO (2001a); and references therein	4.53
WMED	2.32	[1.97 - 2.41]	7	Morales-Nin and Pertierra, 1990; Alemany and Álvarez, 1993; Voulgaridou and Stergiou, 2003	4.25

[a] - Estimates for Moroccan stock A (south of Casablanca)

VB growth curves fitted significantly to length-at-age data from most areas and periods; the percentage of length variance explained by the models ranged between 68 and 97% and absolute values of correlations between model parameters did not exceed 0.63. In two cases, Gulf of Cadiz in 1996-1999 and south Galicia in 2000-2005, sardine growth was best described by a linear model indicating a constant growth increment across the observed range of ages. Figure 5.5 shows the parameters of growth models fitted to sardine samples pooled by area and period and Table 5.4 summarises the results of log-likelihood ratio tests comparing growth curves. 95% inference intervals of growth parameters from different periods show considerable overlap in all areas, suggesting that sardine growth did not change significantly across the study period. Overall, growth curves are coincident across the area from north France to north Galicia and between north and southwest Portugal. Furthermore, growth curves from these two broad regions are generally parallel while l_1 estimates are significantly larger in the former region. In the period 1996-1999, the growth curve for south Galicia is divergent from those of northern areas and convergent with those from the west Portugal. A similar pattern occurs in recent years, as shown by predicted lengths-at-age from the linear model (Figure 5.5) indicating that length-at-age 1 in south Galicia is more typical of northern areas but length-at-age 5 resembles that off the west Portuguese waters. Growth curves from south Portugal and Cadiz show variable relationships with other areas with no clear spatial or temporal patterns. Nevertheless, sardine from south Iberia show significantly larger

Table 5.4: Summary of the results of the likelihood-ratio tests comparing sardine growth curves among areas within periods. *C* coincident, *P* parallel, *S* separate, *Co* converging, *D* diverging. Upper diagonal shows the results for the period 1996 - 1999 (uppercase letters) and 1986 - 1993 (lowercase letters) and lower diagonal shows the results for the period 2000 - 2005.

Areas	NFRA	SFRA	CAN	NGAL	SGAL	NPOR	SWPOR	SPOR	CAD
SFRA	**C**								
CAN	**C**	D		**C/c**	D/d	P	P	D	P
NGAL	**C**	**C**	**C**		d				
SGAL	D	D	D	D		Co	Co	D	D
NPOR	P	P	P	P	Co		**C**	**C**	**C**
SWPOR	P	P	P	P	Co	**C**		Co	Co
SPOR	P	D	S	D	**C**	Co	S		**C**
CAD	P	P	S	D	Co	P	P	Co	

length-at-age 1 than sardine from west Portugal and significantly lower length-at-age 5 than sardine from north Iberia- Biscay, in most pair-wise comparisons.

Overall, sardine growth across the Iberian-Biscay waters follows the large-scale latitudinal decline detected across the northeastern Atlantic waters. The statistical comparison of growth curves evidenced three patterns of length-at-age across the region (Figure 5.6): (i) large lengths across ages, typical of north Iberia-Biscay waters; (ii) large lengths at young ages but small lengths at old ages, observed both in south Galicia and south Iberia and (iii) small lengths across all ages, typical of west Portuguese waters. In spite of comparable length-at-age patterns, sardines from south Galicia have generally larger lengths-at-age than those from south Iberian areas.

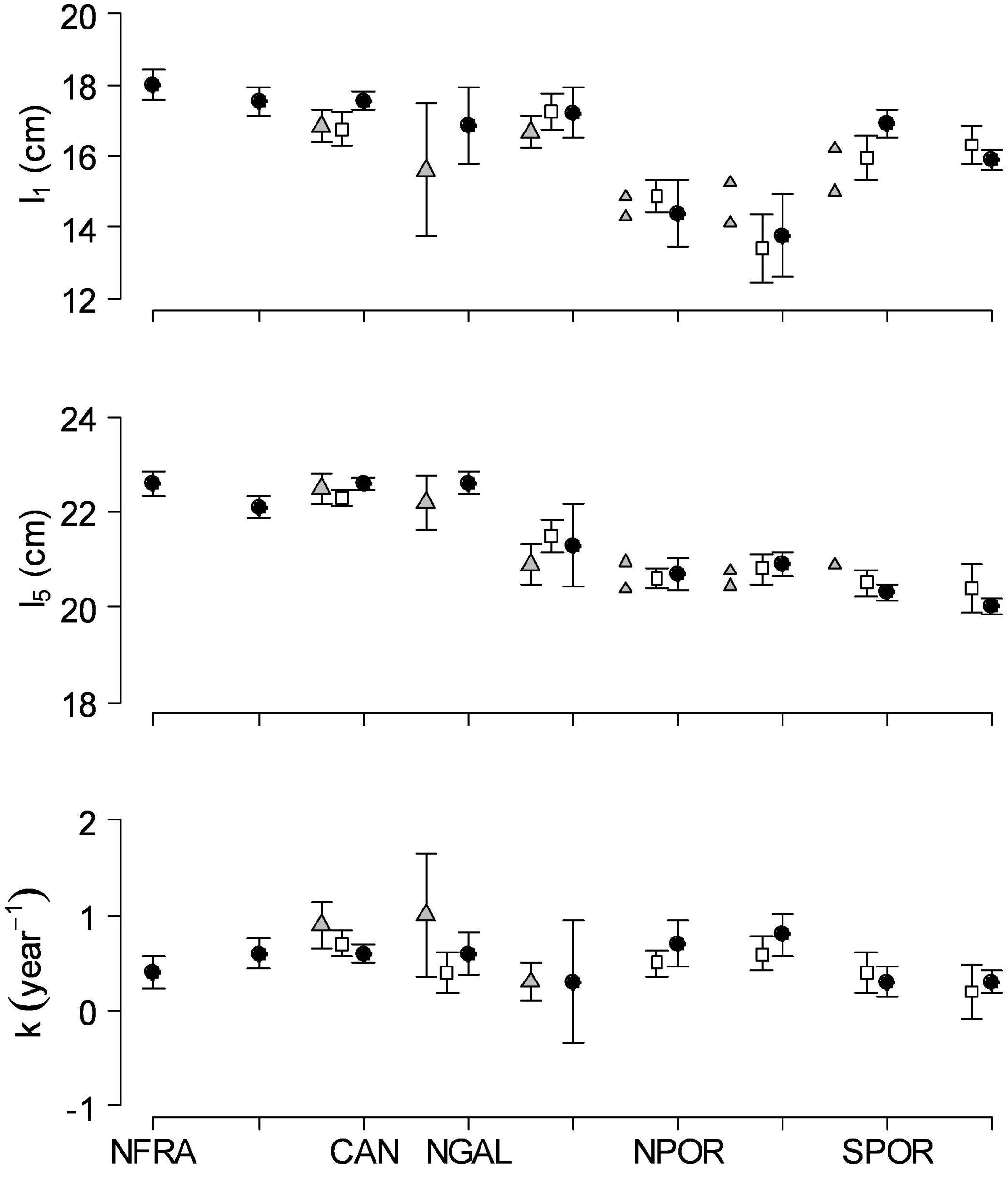

Figure 5.5: Variations in growth parameters for each area within the Iberian-Biscay waters in 1986-1993 (triangles), 1996-1999 (squares) and 2000-2005 (circles). Bars represent 95% inference intervals for the parameters. Smaller triangles represent the mean length of age 1 and age 5 sardine in Portuguese surveys from the 1980s. For the Gulf of Cadiz 1996-1999 and south Galicia 2000-2005, l_1 and l_5 represent the predicted lengths-at-age 1 and 5 and k the slope parameter of linear regression models.

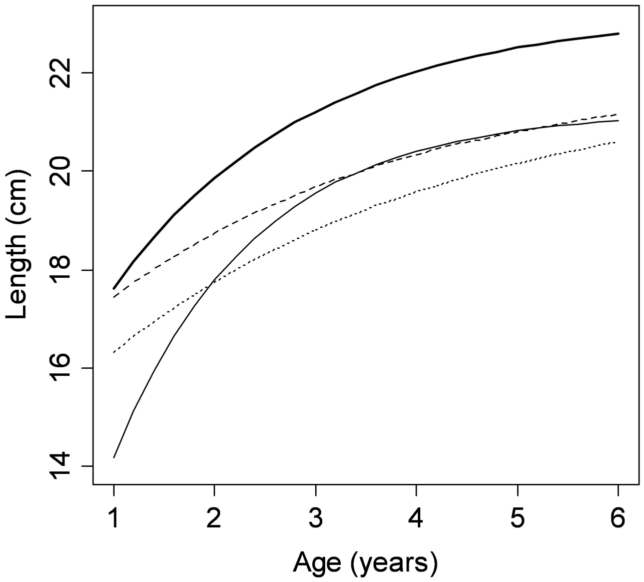

Figure 5.6: Von Bertalanffy growth curves fitted to pooled data from spring surveys from North Iberia-Biscay (thick solid line), South Galicia (dashed line), West Portugal (thin solid line) and South Iberia (dotted line), in the period 2000 - 2005.

Seasonal growth and relationships with biological and environmental variables

The seasonal growth of sardine is adequately described by the VB model in the three areas off the Iberian Peninsula (Table 5.5). Parameters l_1, l_5 and k estimated by the seasonal models are broadly in agreement with those estimated by the annual models in corresponding areas, apart from the estimate of k for south Portugal. Sardine length-at-age shows strong seasonal variation in all areas, as indicated by the amplitude parameter, C. This parameter is significantly above 1 in models for all areas (but particularly in the Cantabrian Sea and south Portugal), implying decreasing length in the yearly time of minimum growth (winter). This apparent

Table 5.5: Parameters of the Von Bertalanffy models describing the seasonal growth of sardine by area. *S.E.* standard error; *MMG* month of minimum growth, *GP* growing period, *D.F.* model degrees of freedom, *%EV* percentage of length variance explained by the model.

Parameters (S.E.)	Cantabrian Sea	North Portugal	South Portugal
l_1 (cm)	16.7	15.4	17.0
	(0.08)	(0.05)	(0.07)
l_5 (cm)	22.0	20.8	20.4
	(0.03)	(0.03)	(0.04)
k (year^{-1})	0.6	0.6	0.1
	(0.02)	(0.02)	(0.03)
C	2.7	1.4	2.5
	(0.18)	(0.09)	(0.22)
t_s (years)	1.06	0.87	1.07
	(0.01)	(0.01)	(0.01)
MMG	January	November	January
GP	April - October	February - August	April - October
Deviance	2082	6567	1678
D.F.	2506	5581	2272
%EV	70.1	72.7	55.6

negative growth suggests bias in the estimation of length-at-age, which may be a consequence of overestimation of age in the early months of the year if the true birth date is later than the assumed birth date. Other factors, such as seasonal changes in the availability of larger fish to the fishery may have affected the estimation of length-at-age. The phase shift parameter, t_s, shows that minimum growth occurs in late autumn-winter and maximum growth occurs six months later, in late spring-summer, being slightly earlier in the year off north Portugal than in the two other areas (Table 5.5).

Figure 5.7 presents the average monthly variation of sardine growth, body condition, spawning activity, SST and Chl $\underline{a}$, in the three areas during 2004-2005. Samples used in this study suggest that sardine condition and spawning activity have com-

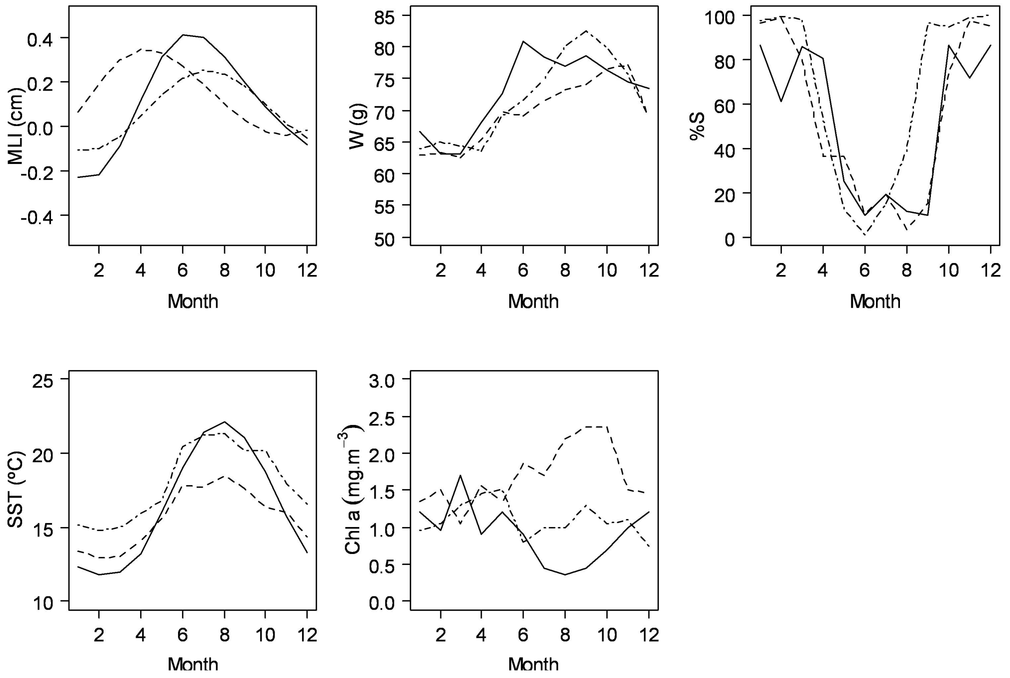

Figure 5.7: Seasonal variation of sardine monthly length increment at-age-2 (MLI), mean weight of 20.0-20.9 cm individuals (W), percentage of fish spawning (%S), sea surface temperature (SST) and Chlorophyll a (Chl a) in the Cantabrian Sea (full line), north Portugal (dashed line) and south Portugal (dotted-dashed line), based on pooled market samples from 2004 and 2005.

parable seasonality in the three areas. Condition is generally lower during winter, when spawning activity is at a maximum, and increases between spring and summer-autumn with the cessation of spawning. In spite of an overall inverse relationship between condition and spawning, the former remains relatively high during the first part of the spawning season, disrupting the correlation between the two variables (Table 5.6). SST is maximum during summer and minimum during winter but presents different amplitude and absolute values in each area. In the Cantabrian Sea, SST values are lower in the winter (average of January-March=12.2°C) and higher in the summer SST (average of July-September=21.4°C) than in the other two areas. Winter SST in north Portugal is slightly above that in the Cantabrian Sea (13.5°C) but summer values are 3°C below (17.8° C) while in south Portugal SST is the highest during winter months (15.2 °C) and closer to that in the Cantabrian Sea in the summer (20.4° C). Chl a shows a spring peak in the Cantabrian Sea and low values during summer, showing a significant inverse correlation with the seasonal cycle of SST. On the other hand, a direct relationship between SST and Chl a is observed off north Portugal since Chl a shows higher values in the summer due to seasonal upwelling. In south Portugal, Chl a levels are low across the year.

The monthly growth of sardine in the Cantabrian Sea and south Portugal is positively correlated with SST and weight-at-length and negatively correlated with spawning activity indicating that sardine grows in length and improves condition concurrently during the summer period, when temperature reaches the highest an-

Table 5.6: Spearman rank correlation coefficients between monthly length increment at-age-2 (*MLI*), percentage of fish spawning (*%S*), mean weight of 20-20.9 cm individuals (*W*), sea surface temperature (*SST*) and Chlorophyll a (*Chl a*). Cells in bold indicate significant correlation ($P<0.0125$).

Area	Variable	MLI	%S	W	SST
Cantabrian Sea	%S	**-0.74**			
	W	**0.78**	-0.64		
	SST	**0.82**	-0.64	**0.91**	
	Chl a	-0.55	0.55	-0.71	**-0.77**
North Portugal	%S	-0.27			
	W	-0.59	-0.31		
	SST	-0.24	**-0.80**	0.72	
	Chl a	-0.32	-0.61	0.68	**0.73**
South Portugal	%S	**-0.80**			
	W	0.62	-0.17		
	SST	**0.89**	-0.64	**0.78**	
	Chl a	0.05	-0.11	-0.13	-0.26

nual values and spawning activity is minimal (Table 5.6). The growing season is earlier in the year off north Portugal and although most of the growth occurs in the end of the spawning season when temperature is still rising, correlations are generally weak. Unlike the other two areas, growth in length precedes the increment of body condition which occurs in late summer as in the other two areas. A lag of 3-4 months is observed between the peak of primary productivity and maximum annual growth and body condition off the Cantabrian Sea. In north Portugal, growth in length takes place when Chl a shows low values but condition is positively (although not significantly) correlated with primary production.

5.4 Discussion

All sardine age data analysed in this study were obtained using the same type of calcified structure, preparation method and age reading criteria (e.g. FAO, 2001a; Soares et al., 2004). The main assumptions underlying age determination, the 1st of January birth-date and the annual periodicity of otolith growth rings, apply reasonably well across most of the species range. Validation of absolute ages is still a matter of concern but verification of increment periodicity for most populations and corroboration of the first growth ring for a few populations from the study area provide a minimum support to the accuracy of age data (Campana, 2001). Nevertheless, other potential sources of error, such as the implication of several age readers, the use of different sample sources and sampling periods may have affected the accuracy and precision of the present results (Campana, 2001) whereas the quality of age data is certainly lower in areas where otolith interpretation is more difficult, such as off south Iberia, Mauritania and Mediterranean waters.

Broad-scale samples suggest that maximum length and length-at-age of sardine decline across the northeastern Atlantic from the English Channel to north Morocco, show low values in western Mediterranean waters, and are substantially higher off Mauritania. Data compiled from earlier studies support these broad geographic patterns and support the general growth index formula (using a slope of -2) (Figure5.4). However, the auximetric plot also indicates that a linear model with a steeper slope and assuming different average growth performances for Atlantic and Mediterranean populations provide a significantly better description of sardine growth parameters. These results suggest that the relationship proposed by Pauly (1979) may vary below the species level, possibly separating groups of populations with larger genetic distance. In fact, sardines distributed in the Atlantic and in the Mediterranean Sea are reproductively isolated, as shown by recent studies of allozyme frequencies (Ramón and Castro, 1997) and mitochondrial DNA markers (Atarhouch et al., 2007) and mixing between populations from the two regions is limited, as indicated by significant differences in meristic (Andreu, 1969) and morphometric characters (Silva, 2003).

The auximetric plot suggests that sardine from Mediterranean areas have a lower average growth performance than sardine from most Atlantic areas. Apart from a lower growth, sardines from the Mediterranean Sea also show lower fecundity and spawning frequency than those from the Atlantic (Ganias et al. 2003, 2004). Lower values for several life history properties and lower abundance of Mediterranean sardine populations may thus be due to the pronounced oligotrophy of this Sea (Stergiou et al., 1997) in comparison to the Atlantic waters. Samples collected off north Morocco and the Gulf of Cadiz (this study) and southwestern Mediterranean (Alemany and Álvarez, 1993) are exceptions to the general growth patterns and may represent transition areas. In fact, some mixing possibly occurs between Atlantic and Mediterranean populations around the Gibraltar Straight, as suggested by the southeastern transport of ichthyoplankton along the Gulf of Cadiz shelf (Rubín et al., 1999). It is also plausible that the boundary between populations from the two seas changes over time since the genetic break has been associated with the Almeria-Oran front in the southwest Mediterranean (Alborán Sea) and this front changes its location closer to the Gibraltar Straight in some years (Atarhouch et al., 2007).

Sardine populations are genetically homogeneous across northeastern Atlantic from the English Channel to north Morocco (Kasapidis et al., 2004) although spatial differences in morphometric characters (Silva, 2003) and length of first maturity (Silva et al., 2006) suggest some degree of spatial population structuring. Differences in sardine growth patterns between some of the areas may be explained by size- and age-related migrations. This is the case of the English Channel where the absence of young sardines and the larger length of older individuals are consistent with a gradual immigration of the largest individuals from neighbouring recruitment areas in the Bay of Biscay. This hypothesis has been suggested by Furnestin (1943) to explain the predominance of juvenile sardines in the southern part of the Gulf of Biscay, the mixture of young and old fish in the central area and the exclusive presence of large (>18 cm) and old sardines (mostly 4-5 years) in the English Channel (Hickling, 1945). Size-specific migrations of sardines recruited off the northwest Iberian areas may be responsible for the predominance of large and old sardines off the Cantabrian Sea (Carrera and Porteiro, 2003) and explain the apparently larger growth in this area as shown by our results. This hypothesis has received some corroboration in recent years since strong year-classes originating in the north of Portugal were observed to expand to north Galicia and west Cantabria in the following years (ICES, 2006a).

However, differences in sardine length-at-age between recruitment areas such as south Biscay, north Portugal and the Gulf of Cadiz-north Morocco cannot be explained by migration. Instead, these differences suggest a latitudinal gradient in growth across the northeastern Atlantic, as observed for several temperate fish species, including clupeoids (Conover and Present, 1990; Lapolla, 2001). Latitudinal growth trends may have a genetic basis (countergradient growth variation; Conover and Present, 1990) as a consequence of selection pressures related to temperature seasonality and winter survival (Conover, 1992); increased juvenile growth enables populations to sustain longer, colder and often resource-poorer winters at higher latitudes and large body size increases the ability to store energy to reproduce in the beginning of the next growing season. Although countergradient variation cannot be proved from this study, growth differences between sardine populations in different latitudes are consistent with latitudinal gradients of temperature cycles (Coombs et al., 2006). Higher growth during the first year and larger lengths-at-age are observed in the Gulf of Biscay, where seasonal temperature gradients are steeper with temperature falling below 12 °C in winter but reaching high values (19-20 °C) during the short summer season (Coombs et al., 2006). Lower sardine growth occurs off west Iberia and north Morocco, where minimum winter temperatures are around 14-15°C but summer temperatures do not increase above 20-21°C due to the influence of seasonal upwelling. Furthermore, the latitudinal decline in the duration of the spawning season and the shift from winter to summer of sardine populations across the northeastern Atlantic (Coombs et al., 2006; Stratoudakis et al., 2007) are possibly additional adaptations to temperature seasonality in order to match the preferred SST range for spawning.

Geographic variations in sardine growth across the northwest African waters are well documented (see FAO, 2001a and references therein). The mean length-at-age of sardine increases from north Morocco to Sahara and Mauritania and there is a trend towards lower L_∞-higher k in the northern part of the region (north of 26-28°N). Sardine from the northern, (Morocco) and southern (Sahara) areas are considered two different populations separated around the 28°N parallel, based on information on body morphology, seasonal migrations, maturity and growth rates (FAO, 2001a). Recent studies showed a genetic discontinuity around 30°N (Agadir) by allozymes (Chlaida et al., 2005) and DNA (Atarhouch et al., 2007) indicating that Moroccan and Saharan populations are reproductively isolated. The significantly higher growth of sardine off Sahara and Mauritania is possibly due to the combination of high prevailing temperature and productivity (Coombs et al., 2006) and may also represent an adaptation to temperature seasonality. The high summer temperatures (reaching 25-26°) may prevent growth and force the growing period to months of comparatively lower temperatures (although still close to the peak summer temperatures of more northerly areas) when upwelling is more intense and therefore plankton production is higher. In fact, an earlier study indicates that sardine feeding intensity and growth are higher from March to June in west Saharan waters, when spawning activity is declining and temperature has not yet reached the peak summer values (Krezptowski, 1983).

Sardine growth showed marked seasonality in the northern, western and southern areas of the Iberian Peninsula. As other small pelagic fish (Cubillos et al., 2001), sardine from the Iberian areas grows and improves condition outside the main spawning season, when the allocation of energetic resources to gonad development ceases and temperature and food availability are increasing or close to the annual peak. Seasonal growth occurred mainly in summer in the Cantabrian Sea and south Portugal but slightly earlier in the year (spring) off northern Portugal. The reason for this difference is not clear from the data but may be related to the predominance of younger or older individuals in each area, with slightly different growing periods. As other species (William and Bedford, 1974) sardine may delay the main growing period as they grow older and it is plausible that both spring and summer are important growing seasons in the three areas. Association of sardine seasonal growth in the Iberian waters with food availability/quality is complicated by the lack of comparative studies of plankton abundance and sardine feeding at this temporal and spatial scale. Overall, spring growth is consistent with higher feeding intensity in both the north and the south Portuguese waters (Garrido et al., 2006) while both spring and summer growth are in agreement with the periods of higher plankton production off the western Iberian waters due to seasonal upwelling (which extends to the western part of south Iberia) (Cunha, 2001; Moita, 2001).

In spite of the above indications that latitudinal gradients in temperature seasonality, productivity and size-related migrations are important in determining spatial variation in sardine length-at-age, other processes, such as density-dependence and size-selective mortality (Sinclair et al., 2002), may generate geographical differences on the apparent growth trajectory. Size-selective mortality may result from fishing gear and/or fishermen selectivity and cause evolutionary change in growth through persistent removal of the larger individuals from populations (Law, 2000). Although both mechanisms have mainly been associated with temporal changes in life-history traits, they may act on a local scale (Rose et al., 2001). Swain et al. (2003) provide an example that temperature, density dependent effects and size-selective mortality may have a variable influence on the length-at-age of neighbour fish stocks depending on their abundance and exploitation levels. In the case of sardine, both overall abundance and catch levels vary extensively across the study area (FAO, 2004; ICES, 2006a) suggesting that influences of population density and fishing mortality on growth patterns warrants further investigation.

Overall, sardine growth evidences large spatial variability that needs to be taken into account for stock assessment and stock structure analyses. Our results showed persistent differences in length-at-age among the northern, western and southern areas of the Atlanto-Iberian stock, with similar differences anticipated in weight and maturity-at-age. Weight-at-age is expected to vary directly with length-at-age, while maturity-at-age may show a more complex spatial pattern since it also depends on the length at first maturity. Such differences support the current procedure of area-stratification used in biological sampling and calculation of catch, weight and maturity-at-age for assessment purposes (e.g. ICES, 2006a), but also suggest that fewer areas may be considered within the stock area. The discontinuity in growth off the northwestern tip of Iberia (Cape Finisterra) is consistent with a size-related north/easterly migration from western recruitment areas and supports the hypothesis that sardine from west and north Iberia belong to the same stock. However, the extent of such migration is unknown and the larger mean length at age in northern Iberia may also result from south/westerly movements from the Bay of Biscay recruitment area, where growth is significantly higher than western Iberia. The discontinuity in growth at the southwestern tip of Iberia (Cape St. Vincent) may either indicate populations with limited mixing or a similar size-related emigration from the recruitment area in the inner Gulf of Cadiz where growth is significantly lower than western Iberia. However, the implications of growth differences in sardine stock structure analyses should be considered concurrently with the other phenotypic and genetic evidence from the area.

Chapter 6

Area-based dynamics*

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* In preparation to be submitted as:

Silva, A., Skagen, D., Massé, J., Santos, M. B., Mesquita, C., Marques, V., Beillois, P., Pestana, G., Porteiro, C., Stratoudakis, Y., Area-based dynamics of sardine in the Iberian-Biscay region.

6.1 Introduction

The sardine (*Sardina pilchardus*, Walbaum, 1792) is a small clupeoid distributed in the northeast Atlantic and the Mediterranean Sea. In the Atlantic, sardine extends from the southern Celtic Sea and North Sea to Mauritania and Senegal, with residual populations off the Azores, Madeira, and the Canary Islands (Parrish et al., 1989). The Atlantic range of sardine encompasses diverse topographic and oceanographic conditions, although the core distribution area is located around one of the five eastern boundary coastal upwelling systems of the world, the Canary Current (Lluch-Belda et al., 1989; Cole and McGlade, 1998; Mason et al., 2006). As for other small pelagic fish inhabiting such systems, shifts in distribution range and inter-annual variation in productivity of sardine populations have been associated with environmental variability, namely large-scale changes of upwelling regime intensity (Beare et al., 2004; Guisande et al., 2004; Santos et al., 2005).

Phenotypic structuring of Atlantic sardine populations has long been recognized from studies of body morphology and meristic characters (see Fage, 1920; Furnestin, 1943; Andreu, 1969; Furnestin and Furnestin, 1970). A revision of this information (assuming races and stocks as synonyms) led to the delimitation of four biological stocks of sardine within the northeastern Atlantic waters (Parrish et al., 1989): the septentrional Atlantic stock, distributed from the North Sea (57°N) to the Cantabrian coast of Spain (43°N), the Iberian or meridional Atlantic stock, distributed from western Galicia to the Gulf of Cadiz (from 43°N to 36°N), the

Moroccan stock, distributed from Cap Spartel (36°N) to Cap Juby (28°N), and the Saharian stock, distributed from Cap Juby to Levrier Bay (21°N). More recently, multivariate analyses of morphometric data collected across the continental Atlantic shelf between the Celtic Sea and Mauritania suggest three sardine morphotypes separated off the southwestern tip of the Iberian Peninsula and off the Morrocan coast (Silva, 2003), although temporal stability of these boundaries has been subsequently questioned (Anon., 2006).

Geographic variability in sardine life-history traits has also been abundantly demonstrated. Silva et al. (2004, 2006) integrated earlier information on sardine growth and maturity-at-length with data collected recently across the northeast Atlantic and Mediterranean Sea and analyzed small scale spatio-temporal variability of these traits among Iberian sardine populations. Clinal variation was observed in both traits, with populations from northern areas showing higher length-at-age and higher L_{50} within the European waters and some evidence of an opposite trend along northwest African waters. Within Iberian waters, sardine from the southern coast showed persistently significant differences in growth and maturity while those from western and northern coasts had comparable L_{50} but differential growth patterns, consistent with the hypothesis of size-related northward migrations. Furthermore, patterns of growth and maturity of sardine from boundary regions of the Iberian Peninsula were similar to those of sardine from adjacent areas.

On the other hand, most studies of neutral genetic markers have shown limited population differentiation across the northeast Atlantic waters, apart from peripheral populations off Azores, Madeira and Mauritania, and from a local population off Safi, northern Morocco (Kasapidis et al., 2004; Chlaida et al., 2005; Atarhouch et al. 2006, 2007; Laurent et al., 2007). However, some genetic structuring, consistent with the hypothesis of isolation by distance, is suggested by the relationship between allozymic differences and geographic distances between populations (Laurent et al., 2007). Genetic differentiation may thus be captured when only distant populations are sampled; significant differences between sardine from the Gulf of Biscay and those of the Morrocan coast observed by Atarhouch et al. (2006) may be an example of this effect. Finally, evidence of mixing between sardine from northern Portugal with those from boundary areas of the Iberian Peninsula has been provided in a recent study of otolith element composition (Castro, 2007).

Within the European Atlantic waters, sardine is mainly fished along the coastal waters of the Iberian Peninsula by Spanish and Portuguese purse-seiners, while a smaller French fishery traditionally operates in the Gulf of Biscay (ICES, 2006a). International coordination for the assessment and management of these fisheries started in late 1970s within ICES (International Council for the Exploration of the Sea) (ICES, 1978). The management unit of interest, initially defined as the fishing areas of the three countries, was soon restricted to the Iberian Peninsula waters on the assumption that catches from the French waters had negligible relative importance (in harmony with prevailing stock concepts, Waldman, 2005) (ICES, 1980). The same stock delimitation, from the French/Spanish border in the Gulf of Biscay to the Strait of Gibraltar in the south (ICES Divisions VIIIc and IXa) is currently accepted (ICES, 2006a) but both the internal homogeneity and the self-sustainability of sardine populations within the stock area have been questioned since the mid-1990s.

Uncertainty about stock identity was triggered in the 1990s by the crisis of the sardine fishery in northern Spain, and particularly in southern Galicia, where the largest traditional Iberian fishery, producing almost half of the total annual landings, operated (Carrera and Porteiro, 2003). Total stock landings halved from the mid-1980s to the mid-1990s while spawning biomass (SSB) peaked in these periods following strong recruitment but both SSB and recruitment show a declining trend across the period (ICES, 2006a). Examination of area-disaggregated survey data corroborated that shifts in abundance were more pronounced off the northern Spanish waters, where a major contraction of the spawning and distribution areas was also observed (ICES, 2000). Different hypotheses, assuming variable degrees of connectivity between local populations, and combinations of fishery and environmental effects, were raised to explain geographical differences in catch and abundance trends within the stock area (Carrera and Porteiro, 2003). Furthermore, evidence of a continuous distribution of sardine eggs and adults across the northern stock boundary and observations of large abundance in the French waters (ICES, 2005a; Bernal et al., 2007) increased concern on the exchange across the northern stock limit. The southern stock boundary was also questioned by the continuity of the spawning area beyond the Gulf of Cadiz (Bernal et al., 2007) and the potential for advection of eggs and larvae from the Gulf of Cadiz to northern Morocco (Oliveira and Stratoudakis, in press). However, existing information suggests that no large populations are distributed in both southwestern Mediterranean and northern Morocco (ICES, 2003; FAO, 2004; GFCM, 2006) decreasing the risk of large impacts on the Iberian sardine stock.

Abundance signals provided by Spanish and Portuguese acoustic surveys have been difficult to reconcile in successive annual assessments of the stock, increasing uncertainty about fishing mortality and SSB estimates, while the historical perspective was shown to be sensitive to which survey data and patterns of fisheries and survey selectivity-at-age were assumed in the model (e.g. ICES 2000). To minimize these difficulties, abundance-at-age data from Spanish and Portuguese acoustic surveys were pooled in the latest sardine assessment, although the extent to which data from the two surveys are comparable and leakage across the stock boundaries remain unresolved issues in this assessment (ICES, 2006a). Area-based models were developed in recent years as an alternative way to cope with regional stock dynamics (Skagen, 2005; Anon., 2006; ICES, 2006a). These models showed reasonable fits to the data and provided useful exploration of alternative migration hypotheses but the lack of data on sardine migration to validate model output was considered a major flaw of area-based approaches (ICES, 2006a).

Considering that lower spatial resolution than that provided by the current stock assessment approach is necessary to understand sardine dynamics, the main aim of this study is to describe local dynamics of sardine populations, in terms of abundance, recruitment variability, total mortality/migration, exploitation and selectivity pattern, using area disaggregated catch and abundance data from the Iberian Peninsula and adjacent Gulf of Biscay since the early 1980s. Multiple linear regression models are fitted to log abundance-at-age from acoustic surveys and more flexible Generalized Additive Models are fitted to log catch-at-age data to describe spatial and temporal dynamics of year-classes. Migration is not explicitly modeled although it is discussed based on spatial demographic patterns and on the rates of year-class depletion obtained from the models. The geographic distribution of abundance and catch, location of recruitment areas, synchronicity in year-class strength and possible migrations are discussed in relation to stock identity.

6.2 Material and methods

Sampling and data sources

Two sources of data are used in this study, acoustic surveys and catches. Acoustic survey data has been collected by Spain and Portugal since the mid-1980s, for the annual assessment of the Atlanto-Iberian sardine stock (Table 6.1; Figure 6.1). In 2000, France started to collect information on sardine in the Gulf of Biscay, within acoustic surveys directed to the annual assessment of anchovy (*Engraulis encrasicolus*) (Table 6.1; Figure 6.1). Acoustic surveys have been coordinated and gradually standardized within a dedicated ICES Planning Group (e.g. ICES, 1998) and more recently within the ICES WGACEGG (ICES, 2006b). Details of survey design and abundance estimation as well as major methodological changes across the period are described in the above reports and also in Marques et al. (2004) and Anon. (2006). Sardine landings within the Atlanto-Iberian stock area have been sampled since the late 1970s. Annual catch biomass data were available since 1978 while regular sampling of length frequency distributions and age-length keys (ALK) at the main sardine landing ports started in 1981 (Figure 6.1). Catch sampling has been coordinated within ICES assessment Working Groups (e.g. ICES 1980, 1992) and since the mid-1990s within EU Sampling Programs (see Jardim et al., 2004 and references therein; EU Data Collection Regulation) (Table 6.2). Detailed information on sardine sampling programmes is presented in the above reports while statistical methods for catch-at-age estimation are described by Jardim et al. (2004).

Area-disaggregated estimates of biomass and number of fish by age class obtained in Spanish and Portuguese acoustic surveys were used to describe variations in sardine abundance, age structure, recruitment strength and mortality/migration in the Iberian Peninsula since the mid-1980s. Data on sardine biomass and relative

Table 6.1: Surveys used for the analysis of sardine distribution and year-class dynamics along the Iberian-Biscay region with summary of main changes introduced in survey methodology over time. LF: length frequency; LS: length-stratified sampling; ALK n: number of observations in age-length key; RV: research vessel.

Survey Season	Years	Months	Area	No. positive stations	LF sampling design	Sampling depth (m)	ALK sampling design	ALK n	Main changes in survey methodology
French									
Spring	2000-2004, 2005-2006	May-June	French Gulf of Biscay	11-42	40 fish/haul	35-250	LS; 2-4 fish/class/haul	500-1000	RV Thalassa; Simrad ER 60 echosounder; parallel transects; day surveying; 20 m vertical opening pelagic gear
Spanish									
Spring	1986-1988, 1990-1993, 1996-2006	Feb-May	Northwestern and northern Spain	7-32	40 fish/haul	20-150	LS; 5-10 fish/class/haul	400 -1400	RV Cornide Saavedra; RV Thalassa since 1997; change from Simrad EK400 to EK500 echosounder in 1991; change from zig-zag day-night transects to parallel day transects in 1997; change from 8 to 18 m vertical opening pelagic gear in 1991; purse-seiner sampling in SGAL since 1997.
Portuguese									
Spring	1986, 1988, 1996-2003, 2005-2006	Mar-May	Portuguese coast; Gulf of Cadiz since 1996	11 -41	100 fish/haul	20-100	LS; 10-15 fish/class/area	300-1300	RV Noruega; RV Capricórnio in summer 1996 and 1999; change from SIMRAD EK 38S to EK400 echosounder in 1992 and to EK 500 in 1995; change from zig-zag day-night transects to parallel day transects in 1997; change from 20 to 10 m vertical opening pelagic gear in 1996.
Summer	1985-1988, 1995, 1996, 1999	May-Aug	Portuguese coast; Gulf of Cadiz in 1995 and 1996						
Autumn	1984-1987, 1992, 1999-2001, 2005, 2006	Nov-Dec	Portuguese coast; Gulf of Cadiz in 1992, 1998 and since 2000						

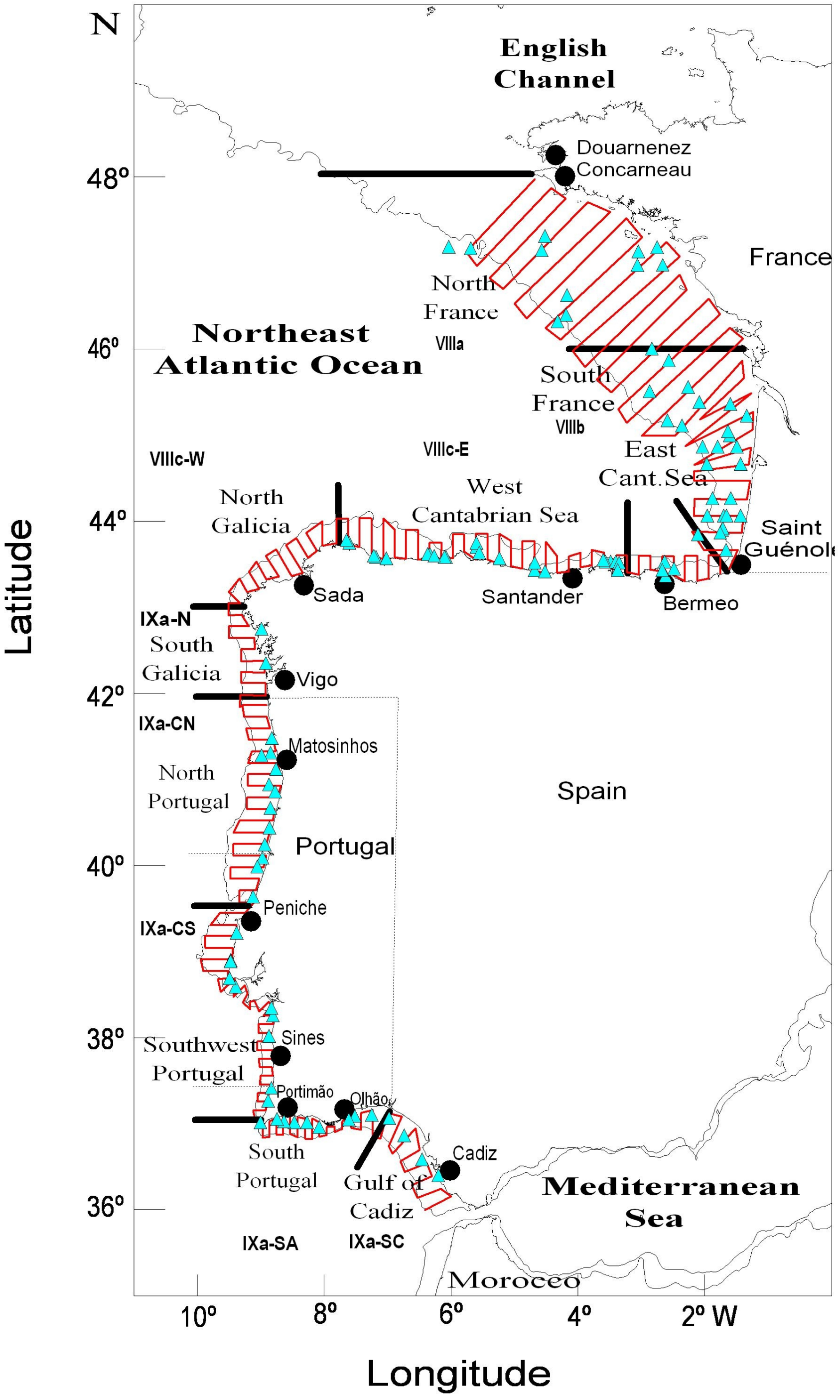

Figure 6.1: Map of the study area, showing sub-areas considered for acoustic estimation, ICES sub-divisions and ports where sardine catches are sampled. As an example, the location of survey stations with sardine in spring 2006 is shown (triangle). Bathimetric line= 200 m.

Table 6.2: Spatio-temporal disagregation of catch-at-age data used for the analysis of sardine distribution and year-class dynamics. LF: catch length frequency distribution; LS: length stratified sampling; ALK: age-length key.

Period	Country	ICES areas sampled	ALK		Notes
			Sampling design	n	
1981-1990	Spain	VIIIc-W, IXa-N	LS; semestral	500-1100	Pooled ALK and LF for IXa-N and VIIIc-W; LF sampling starts in 1986 and ALK sampling in 1990 in VIIIc-E ;.
	Portugal	IXa-CN, IXa-CS, IXa-SA	LS; semestral	1300-2300	Pooled ALK and LF for IXa-CN and IXa-CS
1991-2005	France	VIIIa, VIIIb	LS; quarterly		Sampling since 2002; LF and ALK combined for the whole area.
	Spain	VIIIc-E, VIIIc-W, IXa-N, IXa-SC	LS; semestral	700-3200	LF sampling started in 1998 and ALK sampling in 2002 in IXa-SC
	Portugal	IXa-CN, IXa-CS, IXa-SA	LS; quarterly	2500-8500	

abundance of fish by age class obtained in French acoustic surveys since 2000 were used to explore the relationship between sardine dynamics in Iberian waters and the adjacent Gulf of Biscay. Spanish and French surveys are carried out in early and late spring, respectively while Portuguese surveys (covering the Gulf of Cadiz since 1992) are carried out in spring, summer and autumn, with variable regularity (Table 6.1; Figure 6.1). The same methodology was used in the three seasonal Portuguese surveys (Marques, 2005) and data were pooled for subsequent analysis. For the purpose of this study, we consider three series of surveys, French, Spanish and Portuguese, and ten areas, corresponding to the geographic strata used for abundance and age structure estimation (e.g. ICES, 2006a). Data from the Spanish and Portuguese series were available in number of fish by age class and area. Data from the French series were available in proportion of fish by age class and area, calculated from the cumulative abundance along the track surveyed each year (total square nautical miles). Although these proportions are representative of the relative abundance of sardine by age class in each year and area, they did not provide information on spatio-temporal variation of abundance. Age classes in the Spanish and French series range from 1 year to a maximum of 12 years. In the Portuguese series, age-classes range from 0 to 5 years (fully disaggregated age data was not available for earlier surveys); age-classes from summer and autumn surveys were shifted by plus 0.42 and 0.67 years, broadly corresponding to the lag relative to spring surveys.

Sardine fisheries within the Iberian Peninsula were described using catch biomass and catch-at-age (in number) by ICES sub-division from the last two decades. Landings were assumed to represent catches since there is no evidence of systematic discarding or slipping practices (Stratoudakis and Marçalo, 2002). Catch at-age data were used to corroborate survey information on area-based population dynamics and to compare the variability of catches, exploitation rates and patterns between areas. Catch levels and exploitation patterns in Iberian areas were compared to those in adjacent waters of the French Gulf of Biscay (ICES Divisions VIIIa,b) using annual catch biomass since 1978 and catch-at-age data since 2002. Crude estimates of exploitation rates were obtained as the percentage of catch over survey biomass. Data on the number of purse-seine fishing trips per year and catch per trip off southern Galicia and Portugal were compiled from ICES (1980, 2000) for the period 1978-1998 and updated for Portugal with similar data for 1999-2005. Fishing trips were obtained from records of daily first sales at the main fishing ports and thus, include only trips which provided marketable fish. Despite reservations on the use of such data (e.g. Hilborn and Walters, 1992), they were assumed to provide broad indications of differences in fishing effort and catch-per-unit effort (CPUE) between southern Galicia and Portugal.

Sampling intensity and geographic coverage improved across the period which implies variable spatio temporal disaggregation over time (Table 6.2); from 1981 to 1990 catch-at-age data is available for three Iberian areas, with older ages pooled into a 6+ age group; in the most recent period, data are disaggregated by quarter and fully disaggregated by ICES subdivision (apart from the Gulf of Cadiz) and age. In the Gulf of Cadiz, length sampling started in 1998 and ALK sampling in 2002; catch-at-age data were calculated for 1998-2001 using ALKs from southern Portugal, where sardine growth is similar (Silva et al., 2004). Catch sampling in southern Portugal moved from a port on the eastern (Olhão, close to Gulf of Cadiz) to a port on the western (Portimão) part of the area in 1991. Smaller sardine are generally distributed off Olhão (e.g. ICES, 2006b) and thus, catch structure in the area is anticipated to have changed between periods.

Models for abundance-at-age from acoustic surveys

Linear regression models were fitted to log abundance-at-age from Spanish and Portuguese surveys and to log proportion-at-age from French surveys, using *area*, *year-class* (c) , *age* (a) and *year* as explanatory variables, following the approach by Cotter et al. (2007) for the analyses of fleet CPUE data. These models derive from the simple negative exponential model of year-class depletion $\mathrm{U}_{a,c}=\mathrm{U}_{0,c} \times e^{D}$. The linearized form of this model, termed year-class curve, has an intercept which estimates relative recruitment strength ($\mathrm{U}_{0,c}$) and a slope which estimates average year-class depletion rate (D) , when fitted to an index of abundance (Cotter et al., 2007). D is a negative value usually assumed to represent the average rate of total mortality, Z (fishing and natural) of a year-class. However, it includes selectivity and migration (see below). In the present study, year-class curves were modeled by area, separately for each survey series, assuming that (i) mortality is constant across the life of each year-class; (ii) selectivity is described by the term $\log(a+1)$; (ii) selectivity is constant in time and equal across survey areas and (iii) the fraction of total abundance by area is constant in time. Several candidate models (Table 6.3) are used to explore hypotheses of geographical differences in total mortality and relative recruitment strength and temporal variation in total mortality. The most parsimonious of candidate models has the general form (Model 1, Table 6.3):

$$logU_{a,c} = logU_{0,c} + Z'a + Slog(a+1) + Varea + e_{a,c}$$

where $U_{a,c}$ is abundance of year-class c at age a, $U_{0,c}$ is abundance of year-class c at age 0, V$area$ is a scaling coefficient providing an estimate of the average abundance of year-classes in each survey area, and $e_{a,c}$ represent independent normally distributed errors. In this model, D was partitioned in two components; Z' representing year-class depletion due to total mortality and migration and S, the coefficient for selectivity. For a given area, emigration tends to increase Z' and immigration to reduce it. A positive estimate of S indicates that younger fish are proportionately less caught than older fish, whereas a negative estimate indicates the opposite, with effects increasing as the absolute value of S increases. Nevertheless, estimates of Z' and S will tend to be highly correlated (and therefore biased) since both depend on age (Cotter et al., 2007). More reliable estimates of Z' can be obtained from

Table 6.3: List of candidate year-class (Yclass) models fitted to abundance-at-age in acoustic surveys.

Model terms	Biological meaning of model
1. Area+Selectivity+Yclass+Age	Mortality signal is the same for all areas and year-classes Year-class signal is the same for all areas
2. Selectivity+Age+Area/Yclass	Mortality signal is the same for all areas and year-classes Year-class signal differs between areas
3. Selectivity+Area/(Yclass+Age)	Mortality signal varies between areas but is the same for all year-classes Year-class signal differs between areas
4. Selectivity+Area/(Yclass+Age*year)	Mortality signal varies between areas and varies linearly over time Year-class signal differs between areas
5. Selectivity+Yclass+ Area/(Age)	Mortality signal varies between areas but is the same for all year-classes Year-class signal is the same for all areas
6. Selectivity+Yclass+ Area/(Age*year)	Mortality signal varies between areas and varies linearly over time Year-class signal is the same for all areas

log-index ratios (see below) for age-classes at which full selectivity is thought to occur.

Model selection was based on the Akaike Information Criterion using a modified criterion, the small sample AIC (AIC$_c$), recommended when the number of observations, n, is less than 40 times the number of parameters, K (Burnham and Anderson, 1998). AIC$_c$ is related to the usual AIC as:

$$AIC_c = AIC + \frac{(2K \times (K+1))}{(n-K-1)}$$

Values of AIC$_c$ were calculated for all candidate models to identify the best model (lowest AIC$_c$ model). Differences in AIC$_c$ were calculated for each alternative model

i relative to the best model, Δ_i = AIC$_{ci}$-minimum AIC$_c$. The alternative, usually simpler model i was selected if $\Delta_i \leq 2$ (Burnham and Anderson, 1998). In addition, residuals by age, year and year-class were inspected for each candidate model and used as additional criteria to decide between models with similar Δ_i.

Log index ratios (LIR), log U$_{a,c}$-log U$_{a+1,c}$, were calculated for 3 pairs of age classes: 1-2, 3-4 and 5-6 using predicted values from best models. LIRs provide an estimate of the rates of "appearance" (negative values) or "disappearance" (positive values) of year-classes between two given ages and reflect a combination of selectivity, mortality and migration (ICES, 2004). Negative values are expected in LIRs between younger age classes due to increasing selectivity with age, although age-related immigration to the area may have a similar effect. Positive values of older, fully selected age classes are indices of total mortality plus, eventual, emigration.

Models for catch-at-age from the fisheries

Generalized Additive Models (GAM) were fitted to the logarithm of catch-at-age using year-class, age and year as predictor variables, and assuming normally distributed errors and an identity link function (Wood, 2006). Separate models were fitted to data from each area within two periods, 1981-1990 and 1991-2005, due to different spatio-temporal disaggregation of data (see previous section; Table 6.2). The base model for the period 1981 - 1990 had the general form:

$$E[log(Ca)] = Yclass + f(Age, Year)$$

where Ca is annual catch-at-age, Yclass is an indicator variable for the year-class, Age and Year are numerical variables and $f()$ represents an anisotropic bi-variate smooth function.

For 1991-2005, quarterly catch-at-age (Cq) was modeled by adding an indicator variable of the Quarter to the model:

$$E[log(Cq)] = Yclass + Quarter + f(Age, Year)$$

The base models were used to start a backward stepwise procedure for the selection of the most parsimonious model using Generalized Likelihood Ratio Tests (Wood, 2006). These models assume variation in year-class strength and total catch by quarter (only for the recent period) and covariation between Age and Year, allowing for temporal variation in both the age profile (selectivity) and in the overall level of mortality. Alternative models were explored by replacing the smooth interaction term by smooth additive Age and Year effects and subsequently, simplifying the model by excluding one term at a time. Models with additive Age and Year effects do not allow temporal variation in selectivity but permit the overall level of catches to change over time. In fish stock assessment terms (and if natural mortality can be assumed constant over age and time) such a model indicates that fishing mortality is separable. Excluding terms on Year or Age imply assuming temporally constant mortality or age-constant selectivity, respectively, whereas non-significant Year-class effects indicate that year-classes are equally strong across the period.

Similar to the log-index ratios for survey data, log-catch-ratios, LCR were calculated for age classes 1-2, 3-4 and 5-6 years of the landings data, using model predicted catches-at-age:

$$LCR = logC_{a,c} - logC_{a+1,c}$$

where a is the age class and c is the year-class, and used to explore temporal and spatial variation in selection and mortality. The above models provide a framework to analyze catch-at-age data which is similar to the year-class curve models used in analyses of abundance-at-age from acoustic surveys. However, they also provide a more flexible tool to explore changes in selectivity and mortality with time, which have been previously suggested for the Iberian sardine fishery (ICES, 2000).

All calculations were carried out with R 2.4.0 (R Development Core Team, 2006). Generalized Additive models were fitted using library Mgcv 1.3-19 (Wood, 2000) while package YCC (Cotter et al., 2007) was used to analyze year-class curves.

6.3 Results

Abundance and age structure by area

The biomass of sardine is, on average, substantially higher in Portuguese surveys (433 000 t) than in Spanish surveys (105 000 t) and has intermediate levels in recent

French surveys (234 000 t). Within northern Spain, biomass is highest in western Cantabrian Sea (47 000 t, CV=0.70) and lowest in southern Galicia (14 000 t, CV=0.67), and shows intermediate and more variable levels in eastern Cantabrian Sea and northern Galicia (20 000 t, CV=0.89 and 24 000 t, CV=0.98, respectively) (Figure 6.2). In the central part of the Spanish area, biomass peaked in the late 1980s, declined during the 1990s, and increased again in the early 2000s, while in eastern Cantabrian Sea and southern Galicia, no obvious peak occurred in the 1980s. Within Portuguese waters, the average biomass declines from north to south (north: 210 000 t, CV=0.56; southwest: 111 000 t, CV=0.44; south: 65 000 t, CV=0.52) and increases again off the Gulf of Cadiz (125 000 t, CV=0.54), being generally less variable than off northern Spain (Figure 6.2). Biomass levels in the mid-1980s were close to the long-term average but temporal trends are difficult to detect due to gaps in the survey series. A plot of the proportion of total sardine biomass in Portuguese waters by year (not shown) provides some indication of a southward shift in the distribution of sardine from the mid-1980s to the mid-1990s. The earlier distribution is gradually re-established in the 2000s but seems to shift again in the two most recent years.

Age frequency distributions from the acoustic survey estimates show three distinct geographical patterns (Figure 6.2):

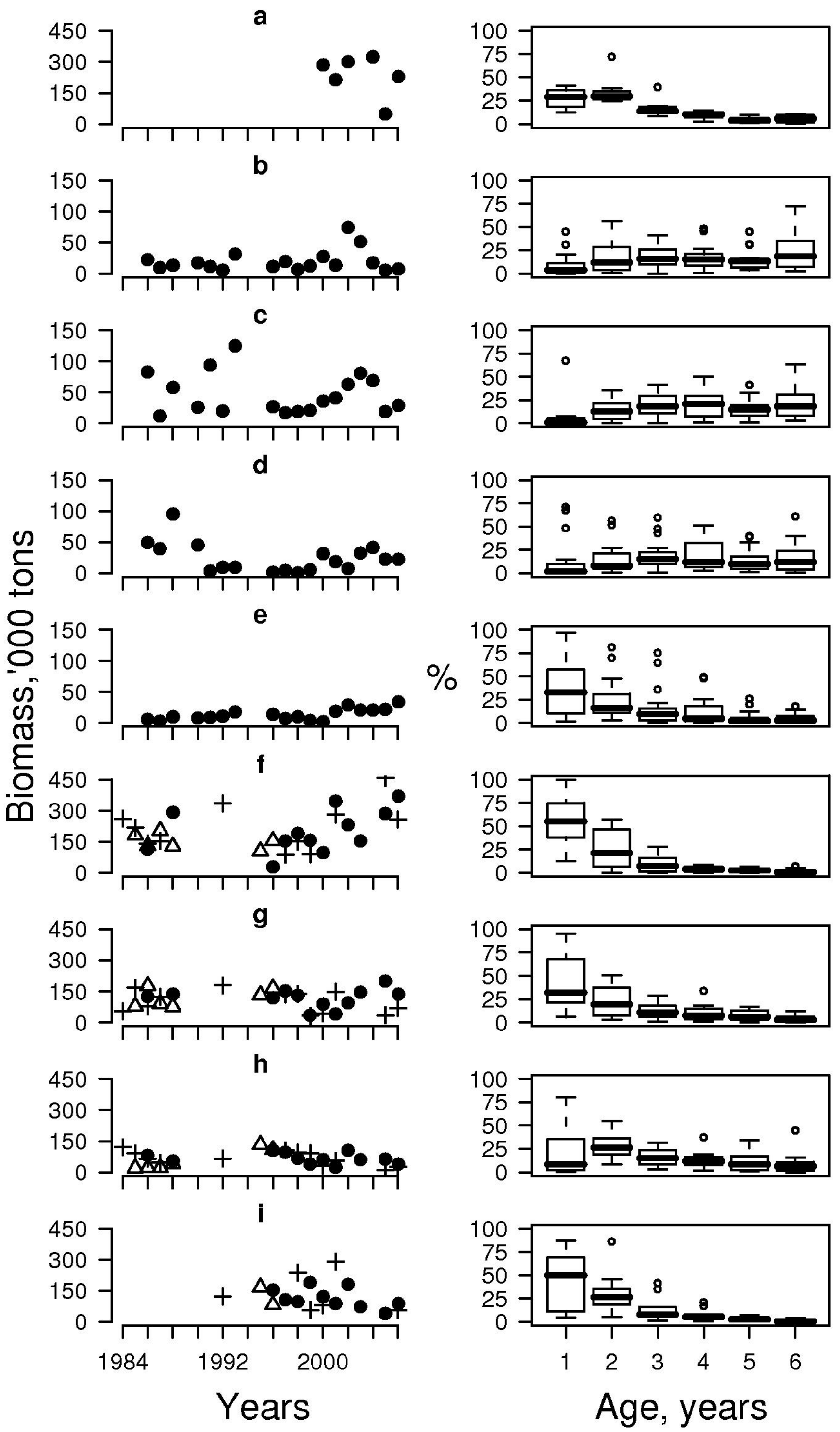

Figure 6.2: Total biomass (left panel) and boxplots of percentage of fish by age-class (right pannel) in each area, in spring (circles), summer (triangles) and autumn (cross) acoustic surveys. a: French Gulf of Biscay, b: eastern Cantabrian Sea, c: western Cantabrian Sea, d: northern Galicia, e:southern Galicia, f:northern Portugal, g: southwestern Portugal, h: southern Portugal, i: Gulf of Cadiz.

- predominance of younger age classes (1 and 2 years old) and a clear decline of the proportions over age in France, south Galicia, west Portugal and Gulf of Cadiz;
- low proportion of 1 year olds, declining over age from age 2 onwards but larger proportion of older fish than in previous areas in south Portugal;
- low proportion of younger age classes, increasing abundance over age but balanced contribution of fish older than 3 years in Cantabrian Sea and north Galicia

Survey models

The most parsimonious among candidate models (Model 1, Table 6.3) was rejected in all survey series, suggesting that some parameters are different among areas. The best models according to Δ_i indicate the same recruitment variation in areas covered by each survey series (Table 6.4). Thus, according to these models, areas differ by a scaling factor, i.e. on the overall level of recruitment. However, trends in year or year-class residuals were obvious in some areas suggesting a departure from the general trend for the survey series. This was the case of southern Galicia, where year-classes from the late-1980s and early 1990s showed closely packed positive residuals, and of the Gulf of Cadiz where a convex year effect was evident. Such trends were not observed when different recruitment variation by area was assumed, i.e. if the *Year-class* effect was nested within variable *Area*. Models including this assumption were therefore used to explore synchronicity in recruitment between areas within each survey series.

According to the best models, average recruitment strength and its temporal variability were substantially different between the Portuguese and Spanish survey series (Figure 6.3). A 50-fold difference is observed on the geometric mean abundance of age 1 sardine and some recruitment pulses differ between surveys; strong recruitments are observed in 1983 and 2000/2001 in the Spanish survey area and in 1991/1992 and 2000 in the Portuguese survey area. Some strong year-classes spread across adjacent years possibly reflecting broad recruitment seasons derived from protracted spawning seasons.

Selectivity coefficients are positive showing increased selection over age in all survey series. The S coefficients are lower and similar for the Portuguese and French survey series indicating that younger sardine are better caught and selection is flatter (Table 6.4). LIRs for young age-classes corroborate this pattern although differences between areas suggest poorer selection of younger sardine in the Cantabrian Sea compared to southern Galicia, and better selection of younger sardine in northern Portugal and Cadiz than in the remaining Portuguese areas. (Table 6.5).

LIRs of older ages (5-6 years, assumed to be fully selected in all survey series), increase from eastern Cantabrian Sea to the northwestern Iberian areas and decline southwards but increase again in the Gulf of Cadiz to the level of the northwestern areas, reflecting spatial differences in total mortality and/or migration among areas

Table 6.4: Summary of the best models fitted to abundance-at-age data from acoustic surveys within the Iberian-Biscay region. p: number of model parameters; AIC$_c$: small sample AIC (Akaike Information Criterion); Δ_i: change in AIC$_c$ of model i relative to minimum AIC$_c$ model; S: selectivity coefficient; Q: area effect coefficient; Z': slope of the year-class curve. SE represent standard error for each estimated parameter. NFRA: northern France; SFRA: southern France; ECAN: east Cantabrian Sea; WCAN: west Cantabrian Sea; NGAL: northern Galicia; SGAL: southern Galicia; NPOR: northern Portugal; SWPOR: southwestern Portugal; SPOR: southern Portugal; CAD: Gulf of Cadiz.

Survey	Model	p	AIC$_c$	Δ_i	S	SE$_S$	Area	Q	SE$_Q$	Z' (year^{-1})	SE$_{Z'}$	Comments and reservations
French	3	36	361	21	1.9	0.71	NFRA	0.3	0.96	-1.0	0.14	Too many parameters
							SFRA	-1.3	0.90	-1.1	0.15	Negative trend in residuals off SFRA
	5	21	341	1	2.0	0.73	NFRA	-0.4	0.93	-1.0	0.14	Close to lowest AIC$_c$ model; parsimonious
							SFRA	0.4	0.59	-1.2	0.15	No evidence of trends in residuals
Spanish	4	133	2317	60	5.1	0.40	ECAN	2.9	1.87	-1.1	0.09	Lowest AIC$_c$ among models with variable year-class strength; too many parameters
							WCAN	2.0	1.87	-1.0	0.10	Trends in age residuals off SGAL
							NGAL	9.5	1.94	-1.5	0.10	Significant negative trends in Z' in ECAN and WCAN
							SGAL	9.8	1.23	-1.6	0.10	
	6	44	2257	0	5.2	0.42	ECAN	4.4	1.12	-1.2	0.09	Lowest AIC$_c$ of all models; parsimonious
							WCAN	4.7	1.41	-1.1	0.09	Trends in year-class and age residuals off SGAL
							NGAL	5.0	1.42	-1.3	0.09	Significant negative trends in Z' in all areas; larger in NGAL
							SGAL	6.5	1.43	-1.6	0.10	
Portuguese	3	117	1794	72	1.7	0.57	NPOR	12.8	1.15	-1.2	0.17	Too many parameters
							SWPOR	13.1	1.15	-1.0	0.17	No evidence of trends in residuals
							SPOR	12.5	1.21	-0.8	0.17	
							CAD	12.4	1.15	-1.2	0.18	
	5	36	1723	2	1.7	0.63	NPOR	14.4	0.78	-1.2	0.22	Close to lowest AIC$_c$ model; parsimonious
							SWPOR	13.2	0.83	-0.9	0.22	Convex year effect on residuals off CAD
							SPOR	11.9	0.83	-0.6	0.22	
							CAD	13.8	0.85	-1.1	0.22	

Table 6.5: Log-index ratios (LIR) and log-catch ratios (LCR) for age classes 1-2, 3-4 and 5-6 years by area, obtained from predicted values of year-class models fitted to survey abundance and catch-at-age data. Standard deviations (SD) are shown when LIR and LCR change over time. ECAN: east Cantabrian Sea; WCAN: west Cantabrian Sea; CAN: Cantabrian Sea; NGAL: northern Galicia; SGAL: southern Galicia; NPOR: northern Portugal; SWPOR: southwestern Portugal; SPOR: southern Portugal; CAD: Gulf of Cadiz; NSPAIN: Cantabrian Sea and Galicia; WPOR: western Portugal.

Data source	Period	Area	Age range(years)					
			1-2		3-4		5-6	
Spanish survey	1986-2006	ECAN	-1.0	(0.10)	0.0	(0.10)	0.4	(0.10)
		WCAN	-1.0	(0.12)	0.0	(0.12)	0.4	(0.12)
		NGAL	-0.8	(0.25)	0.2	(0.25)	0.7	(0.25)
		SGAL	-0.5	(0.10)	0.5	(0.10)	0.9	(0.10)
Portuguese survey	1984-2006	NPOR	0.1		0.8		0.9	
		SWPOR	-0.2		0.5		0.6	
		SPOR	-0.4		0.3		0.4	
		CAD	0.1		0.7		0.9	
Catch	1981-1990	NSPAIN	0.0		0.3			
		WPOR	-0.1	(0.22)	0.8	(0.22)		
		SPOR	-0.1		0.8			
	1991 -2005	CAN	-0.7	(0.69)	0.0	(0.21)	0.6	(0.29)
		NGAL	-0.2	(0.27)	0.1	(0.22)	0.8	(0.41)
		SGAL	0.3	(0.56)	0.5	(0.62)	0.8	(0.74)
		NPOR	0.1	(0.61)	0.9	(0.38)	1.4	(0.43)
		SWPOR	-0.4	(0.23)	0.5	(0.24)	1.2	(0.38)
		SPOR	-1.0	(0.99)	0.5	(0.43)	1.2	(0.35)
		CAD	0.3	(0.46)	1.1	(0.66)	1.1	(0.68)

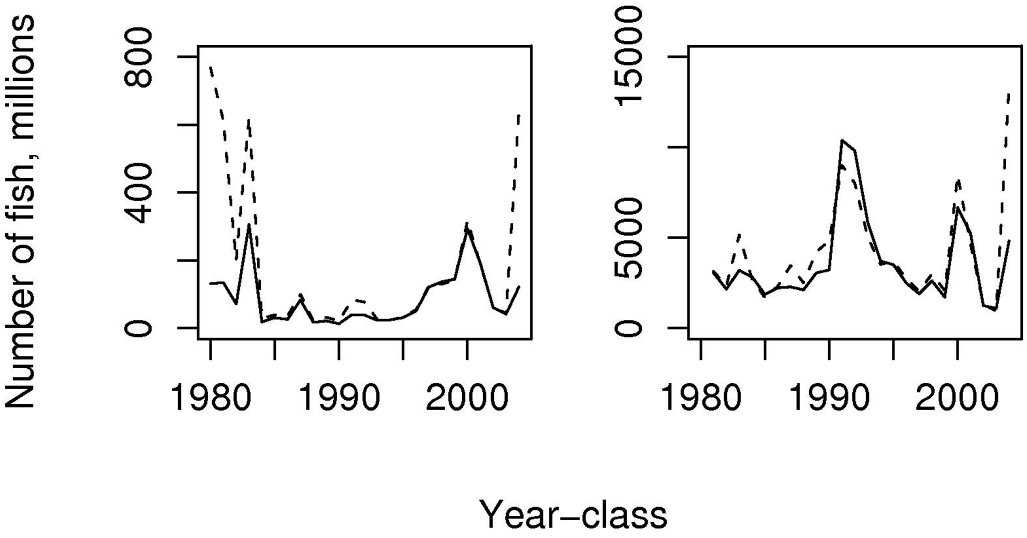

Figure 6.3: Abundance of 1 year-old sardine in Spanish (left panel) and Portuguese (right panel) acoustic surveys, predicted by year-class models assuming the same year-class strength across areas (solid line) or different year-class strengths by area (dashed line).

(Table 6.5). The inclusion of year effects on Z' provided minimum AIC models for all survey series, although the model was clearly supported by the Δ_i criteria only for the Spanish series. Trends in Z' are negative in all Spanish areas and larger in northern Galicia, indicating that total mortality increased or immigration decreased across the period.

Models assuming different year-class variation by area suggest that the geometric mean 1-year old abundance is the highest off northern Portugal and the Gulf of Cadiz (around 1300 million fish) and declines across the Portuguese coast. The estimates for Spanish areas are almost two orders of magnitude lower and decline from southern Galicia to the northern areas. Plots of year-class variability by area indicate that the 1983 year-class appears stronger from eastern Cantabrian Sea to northern

Portugal while the 1991/1992 year-class appears stronger off southern Iberia but is also noticeable in the western areas (Figure 6.4). The 2000/2001 year-class shows the widest geographical extent of all. In the Cantabrian areas, there is some evidence of increasing year-class strength during the 1990s with some strong year-classes in 1997-2000 being also observed in the French Gulf of Biscay. Off southern Iberia an upward recruitment trend is noticeable from mid-1980s to 1992 although uncertainty is high due to data sparseness. There is also some evidence of an abundant 2004 year-class in northwestern Iberia. Spearman rank correlation coefficients indicate a significant positive association ($p<0.001$) between the abundance of 1 year-olds in eastern and western Cantabrian Sea, southern Galicia and northern Portugal, southern and southwestern Portugal and southern Portugal and Cadiz (Table 6.6). Moderate positive correlations ($p<0.05$) are also observed between southern Galicia and areas to the north and between western Portuguese areas while negative correlations are evident between some northern and southern Iberian areas. Finally, it is worth noting that the perspectives of recruitment variability across the whole study area provided by models with and without area-based year-class effects are comparable, despite minor changes in the relative strength of the different recruitment pulses (Figure 6.3).

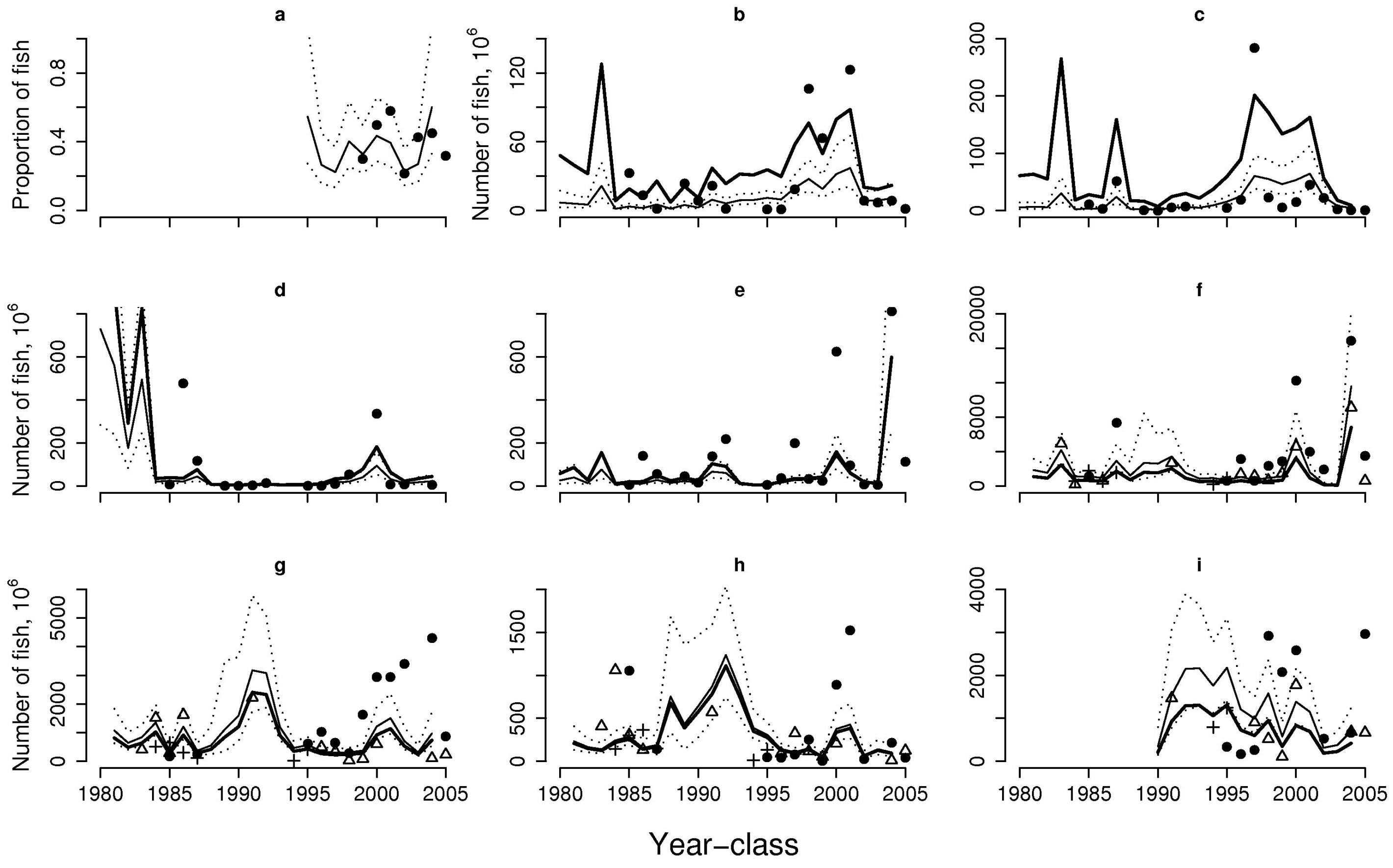

Figure 6.4: Temporal variation of sardine abundance at age 1 (solid thin line) and age 3 (solid thick line) by area in acoustic surveys, predicted (anti-logged) by -class models assuming variable year-class strength by area. Dotted lines shows predicted values±1SE for age 1 abundance. Symbols represent observed values in spring (circle), summer (cross) and autumn (triangle) surveys. a: southern France, b: eastern Cantabrian Sea, c: western Cantabrian Sea, d: northern Galicia, e: southern Galicia, f: northern Portugal, g:southwestern Por tugal, h: southern Portugal and i: Gulf of Cadiz

Table 6.6: Spearman rank correlation coefficients between year-class abundance in each area, using predicted age 1 abundance from models assuming area-based year-class effects (models 3 for French and Portuguese surveys and model 4 for the Spanish survey, see also Table 6.4). Values in bold are significant at the 0.001 level and values underlined are significant at 0.05 level. ECAN: east Cantabrian Sea; WCAN: west Cantabrian Sea; NGAL: northern Galicia; SGAL: southern Galicia; NPOR: northern Portugal; SWPOR: southwestern Portugal; SPOR: southern Portugal; CAD: Gulf of Cadiz;.

	NFRA	SFRA	ECAN	WCAN	NGAL	SGAL	NPOR	SWPOR	SPOR
SFRA	0.49								
ECAN	0.43	0.30							
WCAN	0.08	-0.28	**0.85**						
NGAL	0.27	0.30	0.17	0.33					
SGAL	0.09	0.41	0.50	0.36	0.42				
NPOR	0.18	0.54	0.03	-0.18	0.34	**0.71**			
SWPOR	-0.04	0.54	-0.17	-0.39	-0.13	0.29	0.52		
SPOR	0.19	0.38	-0.30	-0.42	-0.50	-0.12	0.23	0.59	
CAD	0.53	0.50	-0.09	-0.20	-0.62	-0.26	0.07	0.33	**0.76**

Catch, effort and catch-at-age structure

Mean catches of sardine in the period 1978-2005 are higher in northern Portugal (42 000 t, CV=0.26) and decline both to the north and to the south of this area. Catches in the two boundary areas, Gulf of Biscay and Gulf of Cadiz, are relatively low but show an increasing trend across the period (Figure 6.5). A crude estimate of the exploitation rate, obtained by the ratio of catch over spring survey biomass by area (not shown), suggests lower exploitation in the latter areas (median around 5% year^{-1}), higher and comparable among the remaining areas (median around 23% year^{-1}) and the highest off southern Galicia (median of 130% year^{-1}). The geographic distribution of catches changed extensively across the main fishing areas over the study period. During the late 1970s and 1980s, half of the catches were taken in the northwestern Iberian waters and were evenly distributed between southern Galicia and northern Portugal (ca. 26% each), while ca. 17% of catches were taken in both Cantabrian Sea and southwestern Portugal. In the early 1990s, the core fishing area moved southwards with 50% of catches off western Portugal and only 16% and 8% off northern Spain and southern Galicia, respectively. After an initial sharp increase, catches declined in all major fishing areas during the second half of the 1980s but most dramatically off southern Galicia where a drop of 75% (60 000 t) took place within 5 years (Figure 6.5). In the latter area, the number of fishing trips declines sharply in the early period of increasing catches, and is followed by a period of stable effort, such that catch variation reflect CPUE changes across the period (Figure 6.6). Catch biomass also reflects CPUE variation off Portugal but unlike in southern Galicia, fishing effort is suggested to increase from the early 1980s to the early 1990s and decline gradually thereafter. The latter decline is more pronounced in northern and southern Portugal than off the southwestern area, as indicated by area disaggregated effort data (not shown). Off northern Galicia, the number of fishing trips (not shown) declined consistently from 1988 to 1998 but there is no obvious association between catches and CPUE.

The age structure of sardine catches by age class is shown in Figure 6.5. In general, 0-group fish are poorly represented in catches although median proportions around 20% occur in southern Galicia, northwestern Portugal and Gulf of Cadiz. Catch-at-age patterns in each area closely agree with those obtained from survey data (Figures 6.2 and 6.5) although younger age classes (2 year-old and mainly 1 year-olds) are better represented in surveys, suggesting some extent of age selectivity

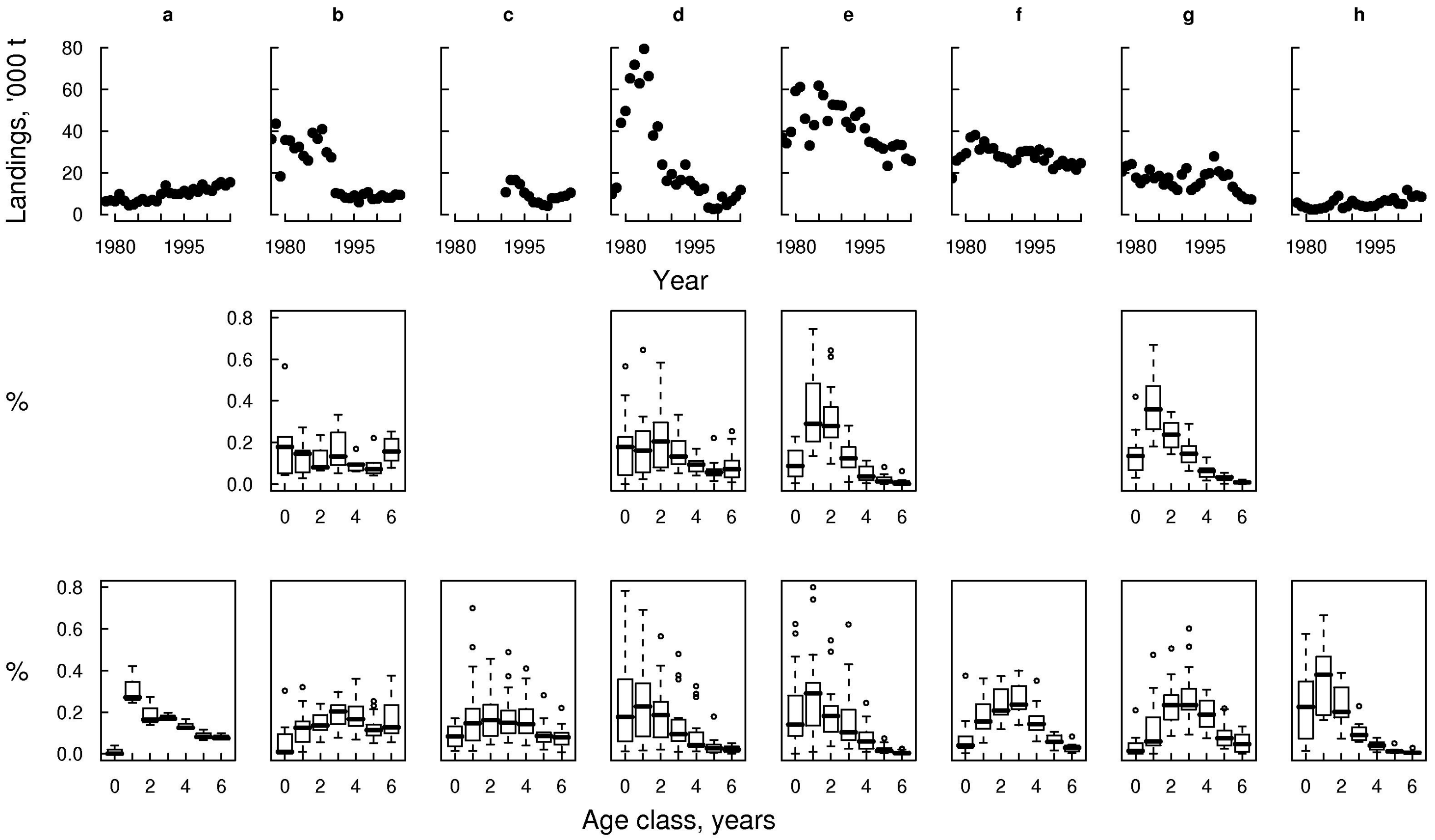

Figure 6.5: Annual catches (top panel) and boxplots of the percentage of catch by age-class in the period 1978-1990 (middle panel) and 1991-2005 (bottom panel) by stock area. a. French waters, b. Cantabrian Sea, c. northern Galicia, d. southern Galicia, e. northern Portugal, f. southwestern Portugal, g. southern Portugal, h. Gulf of Cadiz.

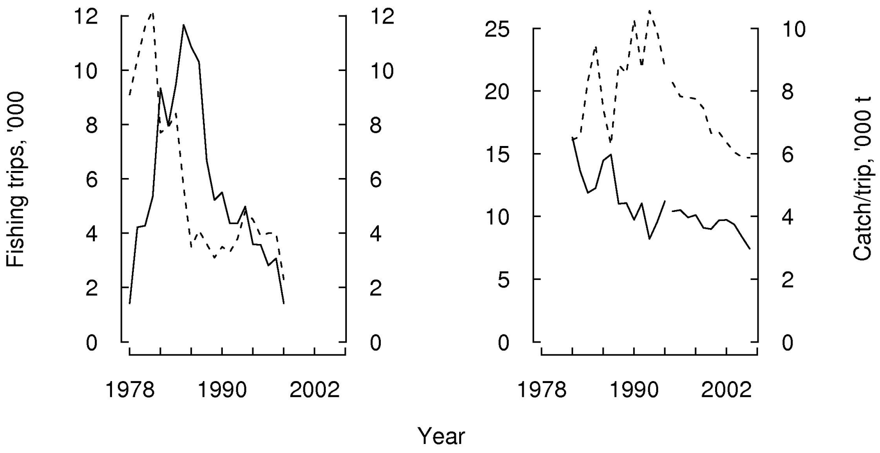

Figure 6.6: Annual variation in fishing effort (dashed line) and CPUE (solid line) in southern Galicia (left panel) and Portugal (right panel).

in all fisheries. The only noticeable difference between local age structure in surveys and catches is off southwestern Portugal and arises from the existence of a juvenile area close to Lisbon, which is surveyed but not exploited by the fishery. Changes in catch-at-age between periods are slight in most areas, the most pronounced being a decline in the percentage of 0-group fish off northern Spain (Figure 6.5). The apparently major change in the catch structure off southern Portugal probably reflects the change of sampling ports, as suggested by the similarity between the structure of catches off southern Portugal (1978-1990) and the Gulf of Cadiz (1998-2005).

Catch-at-age models

Models fitted to catch-at-age data from 1981-1990 are simpler than those fitted to 1991-2005 what may be partly due to lower level of data dis-aggregation (Table 6.7). In the earlier period, the age effect suggests that 0-group sardine are less well caught by the Portuguese fisheries but full selection occurs at a younger age (2 years) than in northern Spanish fisheries (3 years) (Figure 6.7). Log-catch ratios at ages 3-4 years are substantially lower off northern Spain (0.3) than in the Portuguese areas (0.8) (Table 6.5) and this difference persists in LCR_{4-5} (not shown), corroborating the spatial pattern observed in survey analyses. Year effects are not significant in models for northern Spain and southern Portugal, suggesting that combined effects of mortality, migration and selection are constant during the early period. The additive year effect off northern Portugal indicates that selection-at-age is constant but total mortality/migration vary across the period (Figure 6.7). Year-class abundance shows significant variation off northern Spain and western Portugal but not off southern Portugal (Figure 6.8). The 1983 year-class has the strongest signal in catch-at-age data from northern Spain while other earlier year-classes and the 1987 are also above average. The same year-classes are noticeable off western Portugal but with more similar contribution to the catches.

Catch-at-age data in the recent period, 1991-2005, are described by the base model in all areas. Quarterly effects (not shown) are significant in all areas and more pronounced from the Cantabrian Sea to northern Portugal. Opposite seasonal

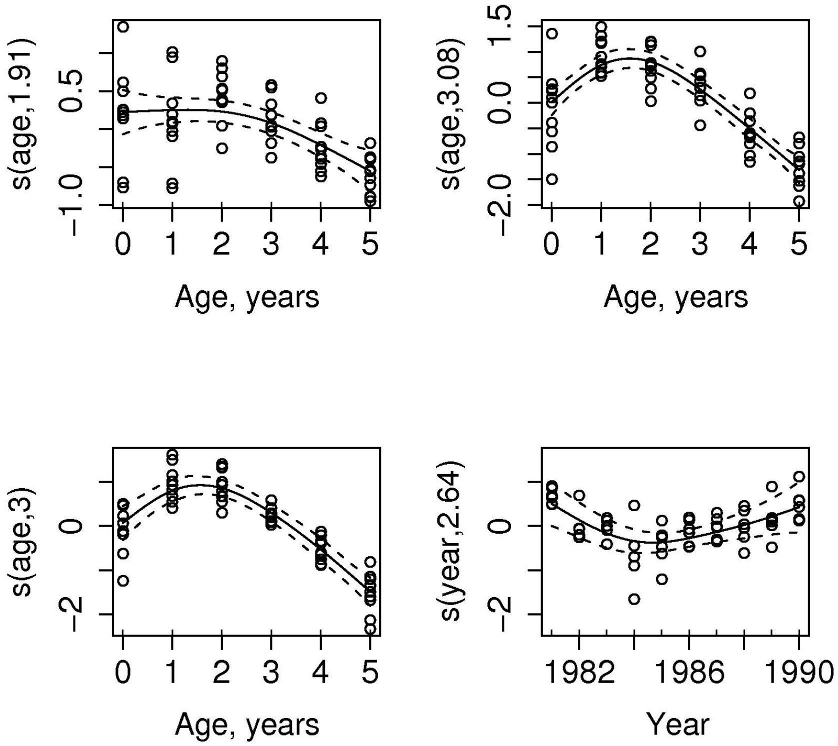

Figure 6.7: Partial effects of age and year for GAM models fitted to catch-at-age data from the 3 stock areas in the period 1981-1990. Upper panel: age effects for northern Spain (left) and southern Portugal (right); bottom panel: age (left) and year (right) effects for western Portugal. Solid lines: predicted values, dashed lines: 95% confidence intervals; symbols: residuals.

Table 6.7: Final year-class (Yclass) models fitted to catch-at-age data from each area and period. %ED: percentage explained deviance; d.f.: residual degrees of freedom.

Period	ICES Division	Model	% ED	d.f.
1981-1990	VIIIc-W, IXa-N	Yclass+s(Age)	78.2	42
	IXa-CN, IXa-CS	Yclass+s(Age)+s(Year)	88.3	39
	IXa-SA	s(Age)	73.2	56
1991-2005	VIIIc-E	Yclass+Quarter+te(Age,Year)	76.3	535
	VIIIc-W		79.3	532
	IXa-N		82.1	505
	IXa-CN		88.2	405
	IXa-CS		84.9	416
	IXa-SA		79.5	391
	IXa-SC		88.3	179

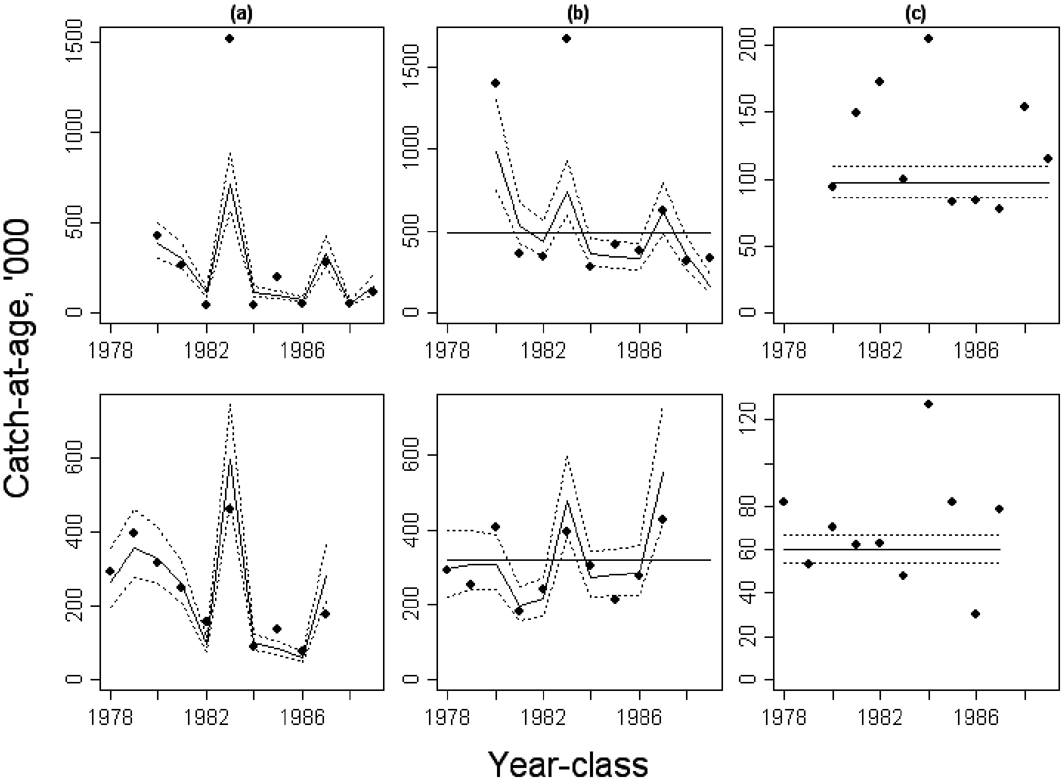

Figure 6.8: Variation in sardine year-class strength in the period 1978-1988 in northern Spain (a), western Portugal (b) and southern Portugal (c) as indicated by catches of age 1 (upper panel) and age 3 sardine (lower pannel).

patterns are observed in the Cantabrian Sea compared to the remaining areas, with most catches taking place in the first (43%) and fourth (28%) quarters of the year. Off Galicia and northern Portugal, a low proportion of the annual catch is taken in the first quarter (around 10%) and the majority of catches occur either in the middle quarters (65%, peak in 2^{nd} quarter, in Galicia) or in the second semester (60% , peak in 3^{rd} quarter, northern Portugal). In southern areas, seasonality is weak but larger catches are taken during the first semester.

Interactions between age and year were retained in models for the recent study period (Figure 6.9). A marked change in selection during the 1990s is suggested by non-parallel LCR trajectories in several areas; this change appears more marked off the Cantabrian Sea, southern Galicia and southern Portugal where selection of younger age classes (1 and 2 years) declined sharply from 1991 to 1995 and increased to previous values in the late 1990s.

Geographical patterns of LCRs and LIRs of younger age-classes show good agreement suggesting that differences in selection between areas are possibly explained by geographical differences in population demography (Table 6.5). Area-based LCRs of older age classes (3-4 and 5-6) are also consistent with LIRs, although estimates for the oldest age groups in Portuguese catches (5-6 years) are generally higher; such a difference may occur if older fish distribute outside the fishing area. Temporal changes in selection patterns between periods are difficult to evaluate due to the different level of spatial aggregation of data. Nevertheless, earlier LCR patterns for

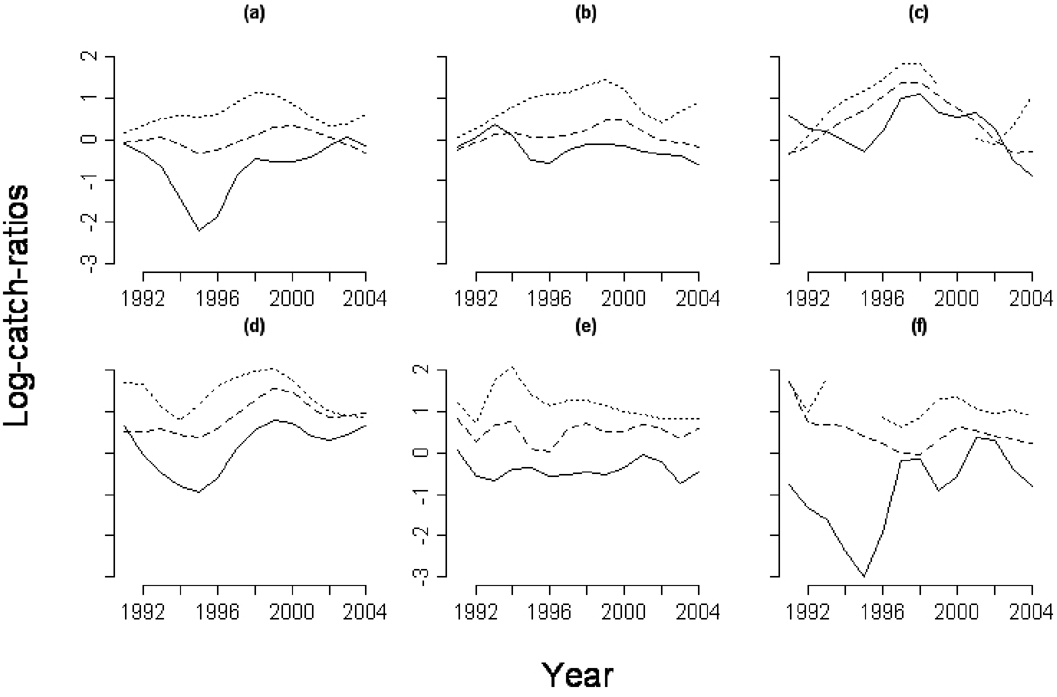

Figure 6.9: Log- catch-ratios between age classes 1 and 2 (solid line), 3 and 4 (dashed line) and 5 and 6 (dotted line) using predicted catch-at-age by area from GAM models in 1991-2005. Areas are: (a) Cantabrian Sea, (b) northern Galicia, (c) southern Galicia, (d) northern Portugal, (e) southwestern Portugal and (f) southern Portugal.

aggregated areas in the earlier period are broadly consistent with average area-based patterns in the recent period suggesting that no major changes occurred between periods. The change in LCRs off southern Portugal possibly reflects the change in sampling areas. The slope of log-catch by age class in the French waters (0.6) is comparable to LCR_{5-6} off the Cantabrian Sea.

Year-class effects are significant in all areas and variable between areas (Figure 6.10). Recruitment pulses indicated by catch based models are broadly consistent with those obtained from survey models although the perspective of the relative importance of pulses is different in some areas. This is the case of the Galician areas, where contrary to survey data, catches suggest a stronger 1991/1992 year-class compared to the 2000 year-class. The apparently strong 1996 year-class off southern Portugal has no parallel in other areas or in surveys and may be due to some artifact in the data.

6.4 Discussion

In this study, year-class curve models (YCC) and Generalized Additive Models were fitted to sardine abundance and catch-at-age data, respectively, to describe spatial variation in population dynamics. YCC models were preferred to other methods for estimation of fish abundance and mortality (e.g. Cotter et al., 2004; Methot, 2000) since they provided opportunity to analyse area-disaggregated data without assumptions on migration or estimation of migration rates. At present, the

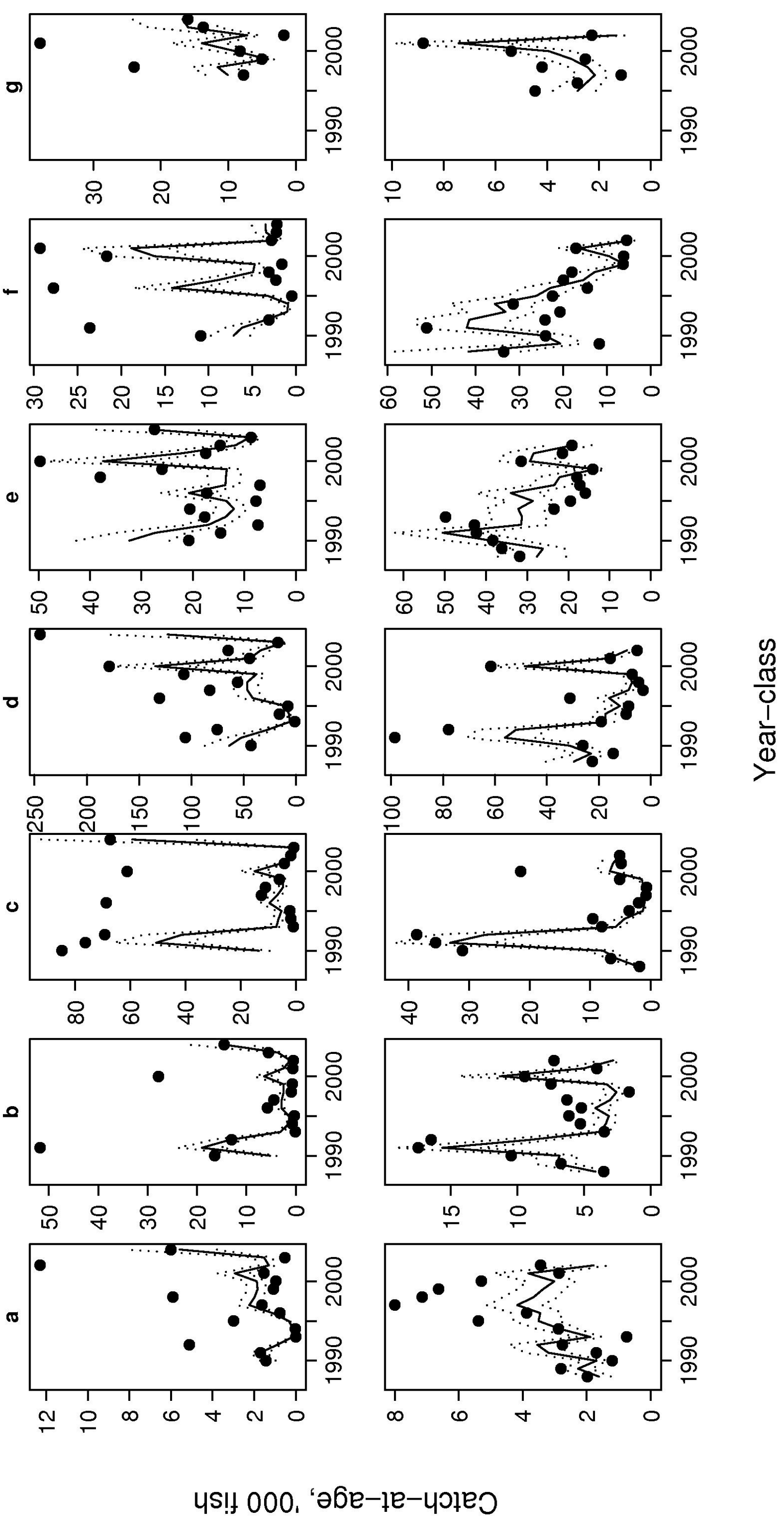

Figure 6.10: Sardine catches by year-class for ages 1 (top panel) and 3 (bottom panel) on the 2^{nd} quarter of the year observed (symbols) and predicted (anti-logged, solid line) from GAM models a: Cantabrian Sea; b: northern Galicia; c: southern Galicia; d: northern Portugal;e: southwestern Portugal; f: southern Portugal; g: Gulf of Cadiz.

lack of data on sardine migration is the main limitation to the use of other area-based methods. Additional appealing features of YCC models are the simple statistics (multiple linear regression), the facility to explore alternative model structures with standard statistical software and the possibility to model survey data separately from catch data (Cotter et al., 2004). The final models obtained in this study provided reasonable fits to the data (as judged by the distribution of model residuals) and were generally parsimonious.

However, compared to other methods YCC models are rigid (e.g. Cotter et al., 2004). In the case of sardine, assumptions of constant depletion rate for each year-class and of linear variations of the depletion rate over time are possibly too stringent. For example, fishing mortality estimated in the annual stock assessment is observed to double or halve in 5-6 years (ICES, 2006a). The selectivity function used within YCC models is a monotonic increasing function and therefore it assumes that survey selectivity increases continually with age. Selectivity functions more sensible in biological grounds are generally flat-topped, i.e. fish are assumed to be fully selected beyond a certain age. Finally, the AIC statistic used in model selection may be invalidated due to correlation among observations across different ages within a year and within an area (Cotter et al. 2007). Although there are ways to reduce this problem (Cotter et al. 2007) none was attempted here. GAMs were used to analyse catch-at-age data in order to obtain a framework similar to YCC models but allowing higher flexibility to describe changes in selection and mortality over time. Such changes have been suggested in earlier assessments of the sardine stock (e.g. ICES, 2000). Final models fitted to each period and area explained a considerable proportion of total deviance (73-88%). However, the low number of observations by year-class in the earlier period (1981-1990) may have limited the complexity of fitted models.

A major assumption underlying the analysis of acoustic survey data is that catchability is constant over time. The reliability of comparisons between areas from separate surveys also depends on the comparability of this parameter between surveys. Among the methodological changes introduced over time in Spanish and Portuguese acoustic surveys (see Table 6.1 for a summary), changes in survey grid design, diel survey time and fishing gear are the most likely to affect total catchability and selectivity over age (Marques et al., 2004). However, no severe bias in total abundance and length distributions between surveys was evidenced from limited comparisons of survey performance (Marques et al., 2004) and contrast with abundance estimates from DEPM surveys (ICES, 2006b). In addition, the fact that contrasting local age structures are obtained in this study from the same vessel and fishing gear, e.g. southern Galicia and French waters versus the Cantabrian Sea, provides some support to the hypothesis that demographic differences in acoustic survey estimates result from variations in the local availability of age-classes.

The analyses of area-disaggregated biomass estimates from acoustic surveys and catches evidenced considerable geographic variation in sardine abundance and exploitation across the Iberian waters and the adjacent Gulf of Biscay. Acoustic data suggest three main areas of biomass concentration within the Iberian waters, western Cantabrian Sea-northern Galicia, western Portugal and the Gulf of Cadiz. An area of persistently low sardine biomass in southern Galicia is also evidenced and is consistent with the observation of repeatedly low spawning biomass in the area obtained in DEPM surveys (ICES, 2006b) and with the location of an almost permanent gap in sardine spawning area (Bernal et al., 2007). However, conflicting information provided by catch and survey data from this area has still to be resolved (see below). Recent French surveys show large sardine biomass within the Gulf of Biscay and detailed spatial distributions of biomass (e.g. ICES, 2005a) indicate a wide but inter-annually variable distribution across the whole shelf area with generally low concentrations in the Adour area, close to the Spanish border.

Catch biomass data provide a different perspective of sardine distribution from acoustic biomass data due to variable exploitation rates across the area, apart from southern Galicia where alternative explanations must be investigated. Median exploitation ratios are substantially lower in the Gulf of Biscay and in the Gulf of Cadiz (5%), where sardine is occasionally and/or seasonally fished and anchovy is the main target of purse-seiners (Carrera and Porteiro, 2003; ICES, 2006a) and comparable in the remaining areas (around 23%). The exploitation rate obtained in southern Galicia (133%) is not viable while CPUE data suggest a much smaller discrepancy in sardine abundance between southern Galicia and the Portuguese areas than that obtained from surveys. As suggested by Carrera and Porteiro (2003), spring survey abundance may not reflect the actual sardine abundance available for the fishery, with most catches (peaking in summer/autumn) being taken from individuals migrating northwards across the area. Nevertheless, other explanations such as the possibility that some catches are taken outside the area (e.g. off northern Portugal) or exceptionally low sardine availability to the survey deserve further investigation.

Area-disaggregated age frequency distributions of sardine show that younger sardine (1 and 2 years old) predominate in the French Gulf of Biscay, across western Iberian waters and in the Gulf of Cadiz, while older age classes dominate in the northern Spanish waters and southern Portugal. Young sardine occur both in the northern and in the southern Gulf of Biscay and are the predominant age classes off the southern inshore areas (below 100 m depth) (ICES, 2005a). A similar structure in sardine demography is evident from the spatial distribution of female mean weight in DEPM surveys and explains differences in mean batch fecundity between areas; off the Cantabrian Sea, female mean weight is approximately double that in the remaining Iberian waters and the lowest mean weights across the area are observed off northern Portugal and the Gulf of Cadiz (e.g. ICES 2006b). Despite some recruitment across most Iberian areas, relative abundance of age 1 sardine is substantially higher off northern Portugal and the Gulf of Cadiz than in the remaining areas. Southwestern Portugal is the third most important recruitment area, although with mean age 1 abundance around 60% that in the Gulf of Cadiz. Recruits also occur regularly in southern Galicia, but predicted mean recruitment is almost two orders of magnitude below that off northern Portugal. Off the French waters, the large biomass estimated for the whole Gulf of Biscay combined with large proportions of 1 year olds in the southern area provide some evidence that this may also be a major recruitment area for sardine.

The pattern of localised recruitment contrasts with the continuity of sardine spawning across the area (Bernal et al., 2007). Furthermore, strong recruitment in the Gulf of Cadiz is consistent with significant spawning preference in the area but the relative importance of the Cantabrian Sea and northern Portugal for spawning and recruitment is apparently contradictory. Circulation patterns may explain the absence of recruits off the Cantabrian Sea or their concentration off northern Portugal. On the other hand, spawning preference in the Cantabrian Sea, may reflect differences in population demography and growth patterns; in fact, the predominance of older and also larger-at-age (Silva et al., 2004) sardine in the former area, forming a population of 100% potential spawners (ICES, 2006a), with larger daily specific fecundity may result in a net higher spawning intensity than off north Portugal where mostly young fish occur.

The results from this study suggest stronger connectivity in year-class variability between neighbouring areas than among distant areas of the Iberian-Biscay region, with some widely spread recruitment pulses and some local pulses suggesting that both large and small spatial- scale environmental and biological factors affect recruitment strength (Koslow, 1984; Cohen et al., 1991). The 2000/2001 year-class is noticeable across the whole Iberian-Biscay region, the 1983 year-class has a more northern distribution (northern and western Iberia) than the 1991/1992 year-class (western and southern Iberia) and the 2004 year-class appears outstanding off north-western Iberia. Catch-at-age data support survey indication of strong year-classes but provide a different perspective about their relative importance in some areas. The two sources are broadly consistent in the Cantabrian Sea and Portuguese waters, while striking differences are obtained in Galicia concerning the relative importance of the 1991/1992 and the 2000 year-classes. Despite corresponding to high percentages of total abundance (Porteiro et al., 1993), absolute numbers of the 1991/1992 year-class were low in acoustic surveys compared to other strong year-classes. The discrepancy between catch and survey signals seems to reflect the pronounced decline of the fishery in the area across the period and uncoupling between catches and trends in population abundance, although the possibility of changes in survey catchability cannot be excluded.

Year-class synchronicity between recruitment areas and areas of predominantly older sardine may reflect size-related migrations, a hypothesis which is not novel for the Iberian sardine stock. Porteiro et al. (1986) were first to refer to size-related northward migrations as a plausible explanation to the complementary age distribution of sardine in Portuguese and Spanish surveys and catches. More recently, two area-based assessment models were used to explore alternative hypothesis of sardine stock structure and migrations with the same data used in this study (Anon., 2006). In the AMCI model (Skagen, 2005) migration was explicitly modelled with the simplifying assumption that age-related emigration could take place from western to northern and/or southern Iberian coasts. The results suggested an increase of migration rate across the period in both directions and changes in predominant directions, with an increased trend for southward movement in the mid-1990s (Anon., 2006; ICES, 2006a). In the other model, a Bayesian state-space model, migration probabilities between areas were fixed *a-priori* using "expert knowledge", i.e. input from scientists closely working with the stock, and robustness tests were performed to evaluate the likelihood of migration patterns (Anon., 2006). In one of the trials, absence or diffusive movement of 0 group and 1 year old sardine and directional movement of 2+ adults from northern Portugal to southern Galicia and towards the southern stock boundary, were assumed and the results broadly agreed with those from the AMCI model. In another trial, immigration of 1-year old sardines from the French Gulf of Biscay to eastern Cantabrian Sea, providing a 19% increase of biomass in the latter area, was shown to be a likely hypothesis (ICES, 2006a).

The location of recruitment areas off northern Portugal and the Gulf of Cadiz and the pattern of correlations in year-class variability between-areas obtained in the present study, suggest that age-related migrations are more plausible from the Gulf of Cadiz to southern Portugal and from northern Portugal to southern Galicia extending possibly to northern Galicia. The observed spatial pattern in year-class slopes is consistent with these migration patterns. Although the possibility of spatial differences in total mortality cannot be excluded, exploitation rates obtained in this study do not justify strong differences in fishing mortality between most Iberian areas. Immigration into the Cantabrian Sea is strongly supported by the increase in year-class abundance over age (see Figure 6.4) and low year-class depletion rates; the hypothesis that Cantabrian sardines originate off northwestern Iberia is supported by the 1983 and 2000/2001 year-classes while the absence of the 1991/1992 year-class could be explained by a change in migration patterns, as suggested by area-based assessment models (Anon., 2006, see above). Increased mixing over age of sardine from the southeastern Gulf of Biscay, northwestern Iberia and the Gulf of Cadiz is suggested by a study of otolith elemental composition (Castro, 2007). On the other hand, recent data suggests that some strong year-classes observed in the Cantabrian areas (1997 -1999) could have migrated from the French Gulf of Biscay. Nevertheless, a firm conclusion about the relationship between sardine from northwestern Iberia, Cantabrian Sea and Gulf of Biscay depends on additional knowledge becoming available from the French Gulf of Biscay.

Our results corroborate the occurrence of spatio-temporal variation in sardine abundance and exploitation rate within Iberian waters since the mid-1980s (ICES, 2000; Carrera and Porteiro, 2003). The more pronounced decline in sardine catch and abundance off the northern Spanish waters than off the Portuguese waters may be partly explained by the exceptionally strong and persistent 1983 and 1987 year-classes and the failure of the 1991/1992 strong year-class to reach the Cantabrian Sea. However, despite a more stable situation off Portugal (see also Stratoudakis et al., 2003), the decline in CPUE, the drop of almost 50% in catches across this period indicate a more global stock decline. Data from ichthyoplankton surveys corroborate these changes since spawning areas were reduced during the 1990s both off the northern and northwestern Iberian areas, while marginal increases of the spawning area occurred off southwest and south Portugal (Stratoudakis et al., 2003; Bernal et al., 2007). The recent recovery of sardine biomass is clearly related to the strong 2000/2001 recruitment and its wide spatial influence although earlier year-classes may have also contributed to recovery in the Cantabrian Sea. Recent declining trends in sardine biomass and catches off southern Portugal are an exception to the global stock improvement and may reflect decreased recruitment in the Gulf of Cadiz.

Overall, this study provided evidence of geographic variation in sardine population dynamics and exploitation within the Iberian - Biscay region which needs to be taken into account in the delimitation of stocks and decisions about modelling approaches for fisheries assessment and management. Areas of clearly separate population dynamics, justifying sub-division of the Atlanto-Iberian stock, were not evidenced although higher independence is apparent between northern and southern Iberia than between these areas and western Iberia, from recruitment variability and population trends. Our data indicated that some recent year-classes distributed in the Cantabrian Sea cannot be traced off western Iberia and may alternatively have immigrated from the Gulf of Biscay, increasing doubts on the validity of the northern stock border. However, the relationship between sardine from northwestern Iberia, Cantabrian Sea and Gulf of Biscay can only be clarified when longer time series of data are available for comparison. Thus, models accounting for observed spatial structuring in population demography and consequently vital rates such as growth and fecundity, year-class strength and variability, exploitation rates and migration are possibly better suited in the future for the assessment of sardine within the Iberian-Biscay region.

Chapter 7

General Discussion

In the present study, the geographic variability of sardine body morphometry, maturity-at-length, growth and population dynamics were examined. The main aim of these analyses was to describe the population structure of sardine and thus, to assist the definition of fisheries stocks. Temporal variability in biological traits was described, when possible, to assess the persistence of geographical patterns. The analyses focused on the Iberian Peninsula and adjacent areas, where sampling intensity was higher and thus more robust results were obtained. Some information was obtained across the distribution range of the species (northeast Atlantic and Mediterranean Sea), from samples collected opportunistically in recent years. Literature information was used to corroborate the large-scale spatial patterns obtained from the analysis of these samples.

In this chapter, the main findings of each analysis are summarized, results concerning sardine population structure are integrated, and implications for the definition of the Atlanto-Iberian stock are considered, concurrently with other evidence on genetic, phenotypic and population dynamics from the area. A conceptual framework for pelagic fish life cycle dynamics is used to describe sardine population structure within and around the Iberian Peninsula. Finally, topics for further research on sardine population structure, stock identity and fisheries assessment are identified.

7.1 Conclusions from each population structure method

The analysis of morphometric variation among sardine populations from the northeastern Atlantic and the western Mediterranean (Chapter 3; study area in Figure 3.1) indicated:

- existence of two sardine morphotypes, one distributed in southern Iberia and northern Morocco and the other distributed across the remaining European Atlantic areas and in the Mediterranean Sea;
- the two morphotypes are distinguished by head size, eye diameter and head-to-body ratio; these are larger in sardine from southern Iberia and northern Morocco;
- a shallower differentiation is evident between sardine from the northern Atlantic and the Mediterranean Sea, mainly on the basis of the position of the dorsal fin;
- sardine from the Azores have closer affinities with the Mediterranean than with the Atlantic populations.

Overall, the morphometric data support the eastern limit of the Atlanto-Iberian stock in the Strait of Gibraltar, suggests the separation of a southern population from the remaining (western and northern) Iberian population and question the northern

(Cantabrian Sea- southern France) and southern stock limits (Gulf of Cadiz-northern Morocco).

The analysis of temporal and geographic variability of sardine maturity at length in the northeastern Atlantic and the western Mediterranean (Chapter 4; study area in Figure 4.1) indicated:

- a decline of 3-4 cm in the length of first maturity (L_{50}) from north to south within the northeast Atlantic;
- lower maturity-at-length in the Gulf of Cadiz than in the remaining Iberian areas; comparable L_{50} between the Gulf of Cadiz, western Mediterranean and northern Morocco;
- highest L_{50} towards the northern (north France), southern (Mauritania) and western (Azores) limits of the species' range;
- a decline of L_{50} and extension of the spawning period off western Iberia during the past two decades, associated with increases in fish condition and fat reserves.

The above results do not support any of the current limits of the Atlanto-Iberian sardine stock. Sardine from the Gulf of Cadiz are significantly separated from those of the remaining Iberian areas.

The analysis of geographic variability of sardine growth across the northeastern Atlantic and the Mediterranean Sea (Chapter 5; study area in Figure 5.1) indicated:

- sardine maximum length, length-at-age and growth performance decline across the northeast Atlantic between the English Channel and northern Morocco;
- sardine growth is substantially higher in Mauritania than in northern Morocco; literature data suggests clinal variation along the northwest African waters;
- growth performance is generally lower in the Mediterranean Sea but with comparable growth patterns were observed in the Gulf of Cadiz, northern Morocco and southwestern Mediterranean;
- temporally persistent differences in sardine growth patterns exist between northern, western and southern areas of the Atlanto-Iberian stock; length-at-age declines from north to south in intermediate and older age classes; differences are mainly set within the first year of life;
- marked seasonality in growth is observed in the Iberian waters; main growing period is in spring/summer, when allocation of energy for reproduction ceases and temperature and plankton production are high;
- no obvious temporal changes in growth within Iberian waters since the mid-1980s.

As observed for L_{50}, growth patterns do not support any of the current limits of the Atlanto-Iberian sardine stock. Persistent growth differences suggest some separation between sardine from northern, western and southern regions of the stock;

length-at-age differences are more conspicuous between the northern and western regions.

The analysis of area-based sardine dynamics (Chapter 6, study area in Figure 6.1) indicated:

- sardine is distributed heterogeneously across the Iberia-Biscay region; biomass concentrates in the French Gulf of Biscay, western Cantabrian Sea, western Portugal and Gulf of Cadiz;
- average exploitation rates are substantially lower in the Gulf of Biscay and in the Gulf of Cadiz and are comparable among the remaining areas;
- main recruitment areas are located off northern Portugal, inner Gulf of Cadiz and probably the southern Gulf of Biscay;
- year-class variability is better correlated between neighbouring areas than between distant areas of the Iberian-Biscay region;
- age-related migrations are more plausible from the Gulf of Cadiz to southern Portugal and from northern Portugal to Galicia;
- immigration into the Cantabrian Sea is strongly supported; the origin of sardine (western Iberia vs French Gulf of Biscay) may change over time;
- failure of the strong 1991/1992 year-class to reach the Cantabrian Sea may have contributed to the more pronounced decline of sardine abundance in Spain than in Portugal during the 1990s.

The validity of the northern limit of the Atlanto-Iberian stock is challenged by indications of sardine migration from the Gulf of Biscay to the Cantabrian Sea. Synchronicity in year-class strength and abundance variability indicate a closer association between neighbouring areas than between distant areas; in particular, association is weaker between northern and southern Iberia than between these areas and western Iberia.

7.2 Synthesis of results and implications for sardine stock assessment

Recent genetic studies indicate that sardine populations distributed within the Atlanto-Iberian stock area and to the north and south of its present limits are genetically homogeneous (Kasapidis et al., 2004; Laurent et al., 2007). Breaks to genetic flow were observed around 30°N (northern Morocco) and within the southwestern Mediterranean Sea; in both cases these genetic breaks seem to be associated with oceanographic features, the Cape Ghir filament off northern Morocco and the Almeria-Oran front in the Alboran Sea (Ramon and Castro, 1997; Kasapidis et al., 2004; Chlaida et al., 2005; Attarouch et al., 2006, 2007; Laurent et al., 2007). Clinal variation in some allozymic markers across the northeastern Atlantic from the North Sea to northern Morocco supports some level of population structuring across the area (Laurent et al., 2007). The whole region shows diverse topographic and oceanographic conditions; some permeable boundaries were associated with oceanographic fronts at Cape Breton Canyon (Biscay corner) and Galicia and with the Mediterranean outflow (Mason et al., 2006; Anon., 2006). However, none of these boundaries was assumed to prevent the exchange of sardine early life-stages between neighbouring areas (Anon., 2006). A study simulating particle advection from combined effects of wind and currents supported the hypothesis of mixing, although at a small rate ($<$5%), between adjacent shelf areas from the Cantabrian Sea to southern Portugal (Oliveira and Stratoudakis, in press). The same study also indicated that up 50% of particles released in the southern Iberian peninsula may reach the northern Moroccan shelf within a month, providing high potential for advection of sardine eggs and larvae in this area. Sardine spawning biology does not promote reproductive isolation across the area from northern France to the Gulf of Cadiz; spawning areas are almost continuous and the spawning season is broad with several months' overlap between adjacent regions (Coombs et al., 2006; Stratoudakis et al., 2007; Bernal et al., 2007). Thus, mixing among sardine populations within the Atlanto-Iberian stock and adjacent areas is unquestionable but its extent is uncertain.

The results from the present study indicated substantial geographic variation of sardine population traits within and around the Atlanto-Iberian stock area (Figure 7.1). Not unexpectedly, sardine from neighbouring areas showed higher phenotypic similarity and more synchronous population dynamics than sardine from

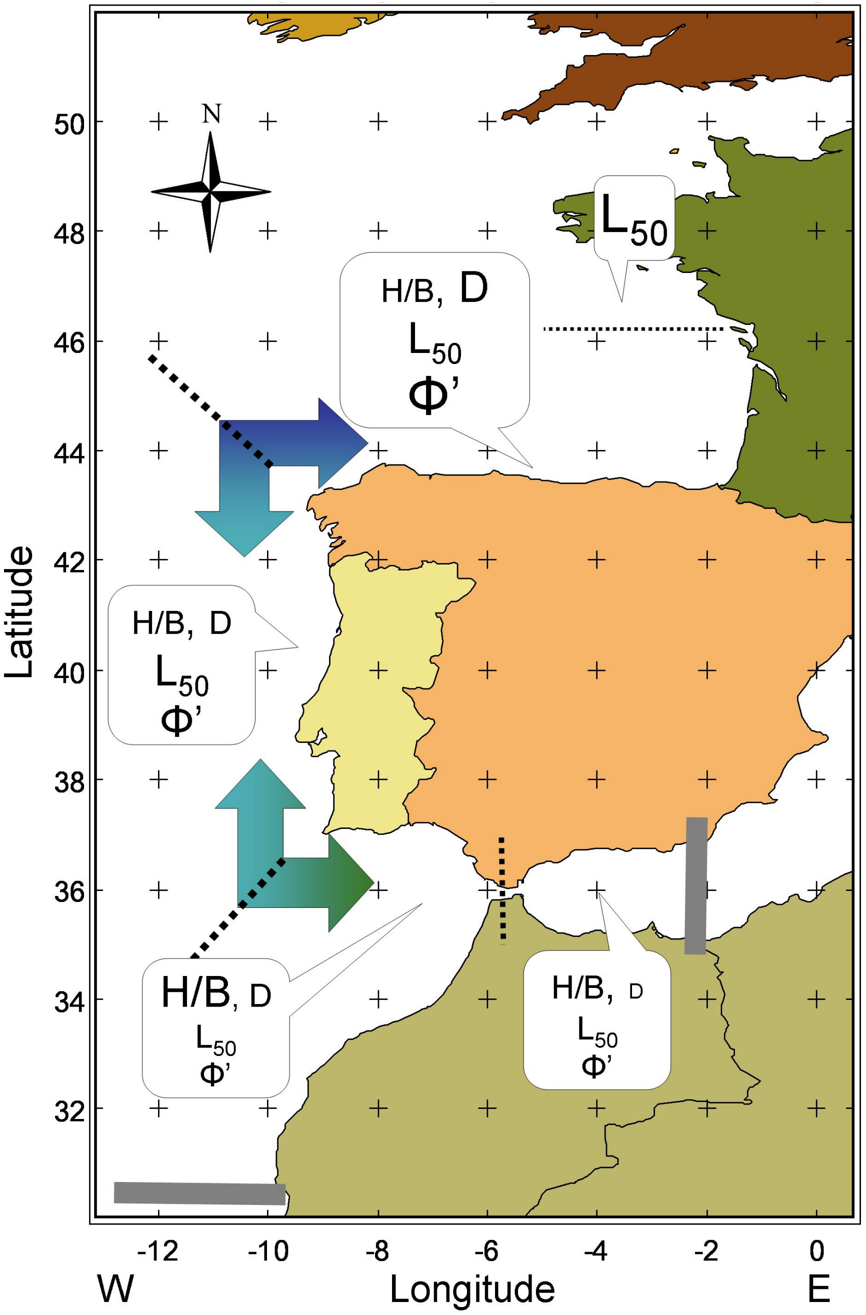

Figure 7.1: Synthesis of geographic variation in sardine phenotypic traits within the Atlanto-Iberian stock and adjacent areas. H/B: head to body size ratio; D: distance from snout to dorsal fin; L_{50}: length of first maturity; ϕ': index of growth performance. Larger letter/symbols indicate higher values of the characteristics. Dashed lines show approximate limits of regions with homogeneous characteristics while arrows show transition areas. Solid thick lines show genetic breaks between populations.

distant areas. Within the stock area, sardine distributed within the northern (east Cantabrian Sea to northern Galicia), western (southern Galicia to southwestern Portugal) and southern (southern Portugal and the Gulf of Cadiz) regions are generally homogeneous with respect to the characteristics analysed in the present study. Furthermore, none of the phenotypic characteristics showed significant changes across the northern (eastern Cantabrian Sea to southern France) and the southern (Gulf of Cadiz to northern Morocco) boundary areas of the stock. On the other hand, a discontinuity was observed in sardine body shape at the southeastern stock boundary (Gulf of Cadiz to southwestern Mediterranean Sea), while growth and maturity-at-length patterns were homogeneous across this border.

Sardine from the northern and western Iberian regions showed persistent differences in growth and occasional asynchrony in year-class strength. Larger maximum length and length-at-age in the northern region may reflect size/age-related immigration from the western region, with larger/older fish migrating farther as observed for related species (e.g. Nöttestad et al., 1999). Evidence from area-based assessment models (Anon., 2006; ICES 2006a), from otolith elemental composition (Castro, 2007) and from synchronicity in year-class strength obtained in this study provided some support to this hypothesis. However, immigration of 1-year old sardine from the French Gulf of Biscay to the eastern Cantabrian Sea was shown to be a likely hypothesis too (ICES, 2006a). In the present study, some strong year-classes appearing in the Cantabrian Sea could not be traced off western Iberia and may have alternatively originated in the Gulf of Biscay. It is also possible that migration patterns change over time, as previously suggested (Anon., 2006) and this could explain occasional asynchrony in year-class strength between the northern and the western Iberian regions. Overall, the current perspective of sardine migration between these regions is strongly influenced by the assumption of an impermeable boundary in the Biscay corner. Firm conclusions about the relationship between sardine populations from western Iberia, northern Iberia and the Gulf of Biscay cannot be drawn from the data presently available.

Interestingly, the northwestern corner of the Iberian Peninsula represents an important boundary for other small pelagic fish populations. A genetic break was observed in this area in a recent study of mitochondrial DNA in European anchovy (*Engraulis encrasicolus*) (Magoulas et al., 2006). This break corroborated earlier indications of significant morphometric differences between anchovy from the Gulf of Biscay and northwestern Iberia (Caneco et al., 2004). A barrier to genetic flow in northwestern Iberia was also indicated by single-strand DNA analysis of horse mackerel populations (although not by mitochondrial and microsatellite DNA) (Abaúnza et al., 2003). Horse-mackerel populations from the northern and western areas were also shown to have distinct body shape, otolith shape, and parasite infestation (Murta, 2003). Evidence from these studies (compiled in Abaunza et al., 2003) was used to re-define horse mackerel stocks for assessment purposes (ICES, 2004); the previous single Iberian stock was divided in a northern stock extending along the shelf from Norway to Cape Finisterra and a southern stock from here to the Gulf of Cadiz.

Sardine from the western and southern Iberian regions were differentiated by all the analyses presented in this study (Figure 7.1). L_{50} was clearly lower in the Gulf of Cadiz while southern Portugal seems an intermediate area. Growth differences were clear and may have increased over time, but were less pronounced than between the northern and western regions. On the other hand, the morphometric division of western and southern sardine populations seems feeble. The separation suggested by the 1999-2000 data analysis (Chapter 3) was not corroborated in a subsequent study using samples collected in 2003/2004, where clinal variation was observed but no clear separations were found across the northeast Atlantic (Anon., 2006). However, strong recruitments occurred between the two sampling periods, off northern Portugal (2000) and in the Gulf of Cadiz (2000/2001); spread of these year-classes to neighbouring areas may have smoothed morphometric differences (Anon., 2006), a hypothesis deserving further investigation. Increased mixing between western and southern sardine populations over age was suggested by the analysis of otolith elemental composition (Castro, 2007) while area-based assessment models further indicated net immigration to the southern region (Anon., 2006; ICES, 2006a). Despite evidence of movements between areas, recruitment centres are located in both regions, suggesting that populations from each region are potentially self-seeded, unlike off the northern Iberian region.

None of the analyses supported the currently defined northern and southern limits of the Atlanto-Iberian sardine stock. Morphometry, growth and maturity-at-length were homogeneous across these limits. The distribution of spawning areas is also consistent with a stock extending across these limits (Bernal et al., 2007). The northern limit was further questioned by evidence that some strong year-classes in the Cantabrian Sea immigrated from recruitment areas in the Gulf of Biscay, corroborating earlier indications from area-based assessment modelling (ICES, 2006a). The northern extent of homogeneous populations is still unclear. Maturity-at-length seems to decline substantially in northern France and growth might increase in the English Channel. Young sardine is not usually observed in this area (although juveniles have been recently sampled in the North Sea), suggesting that older (2+) spawning individuals from the English Channel possibly originate in the French coast. The Biscay corner does not represent a barrier for other small pelagic fish populations either; as horse mackerel (see above), anchovy and mackerel stocks are also considered to distribute across the Cantabrian Sea and Gulf of Biscay. A recent tagging study indicated that adult mackerel cross this area in their southward spawning migration (Uriarte et al., 2001). No other barriers were evidenced within the French waters for any of these species.

Leakage of sardine eggs and larvae across the southern stock limit seems plausible (Oliveira and Stratoudakis, in press). Fish migrations have not been investigated but are less plausible due to the large area of nutrient-poor oceanic water that separates the southern Iberian coast from northern Morocco. Sardine populations in northern Morocco seem to have low abundance and no major spawning or recruitment areas are recognised (FAO, 2003). Therefore, mixing across the southern limit may be mainly mediated by early life stages while major impact from northern Morocco to southern Iberia populations is not anticipated. The southward extent of eventual mixing between Iberian and Moroccan sardine populations is uncertain. Sardine off northern Morocco was clearly distinguished from sardine from Mauritania in all the analyses presented in this study, in agreement with results from genetic markers (e.g. Kasapidis et al., 2004; Laurent et al, 2007). However, samples were not available between the two areas and significant differences may reflect sampling of distant locations across a continuum. Genetic studies (Chlaida et al., 2005; Attarouch et al., 2006, 2007) providing a comprehensive coverage of the northwest African waters suggested a genetic break around Safi ($\approx 32°$N), in northern Morocco, which may also define the southern extent of mixing between sardine from Iberia and Morocco.

In summary, the analyses presented in this study, considered concurrently with other evidence on sardine population structure emphasize the need to explore alternative scenarios of stock structure and to account for geographical variation in biological traits in the assessment of sardine fisheries. The relationship between sardine dynamics within the Atlanto-Iberian stock area and sardine dynamics in neighbouring waters deserves further investigation, particularly at the northern stock limit. Evidence of large sardine abundance in the French Gulf of Biscay and continuity of adult and egg distributions suggests that demographic influence on the stock area can be strong. The relationship between sardine across the southern stock limit is less clear but the influence of populations from northern Morocco on sardine dynamics within the stock area is possibly lower, since smaller populations are distributed in this area. Nevertheless, leakage of early life-stages to the Moroccan shelf and potential impact on sardine dynamics within the stock area deserve further investigation. Concerns about the southeastern stock limit seem comparatively smaller since Atlantic and Mediterranean populations seem to be reproductively isolated. Some mixing between sardine from the Gulf of Cadiz and southwestern Mediterranean possibly occurs, but the low sardine abundance in the latter area does not anticipate a major influence on the stock dynamics.

7.3 Conceptual model of sardine population structure and dynamics

Sardine population structure fits within the conceptual framework for pelagic fish life cycle dynamics developed recently (ICES, 2005b). According to this framework, young sardine initiate their spawning activity close to their recruitment (localised) centres, thus closing their life cycle as early spawners locally. Older sardine are able to undertake relatively large migrations and can reproduce in grounds with little geographic affinity to their original recruitment area. Local life-cycle closure warranted by young individuals promotes spatial structuring while the large-scale movements of older fish counteract population structuring. This conceptual life cycle dynamics is combined with evidence of spatial variation in biological traits and population dynamics discussed in the present study to construct Figure 7.2. The perspective provided in Figure 7.2 is consistent with the observation of persistent geographical differences in phenotypic traits, superimposed on a background of clinal variation in both genetic and phenotypic characters. It is also plausible that sardine forms a metapopulation comprising three local populations recruiting in the Gulf of Biscay, northern Portugal and the Gulf of Cadiz respectively. However, care should be taken in the use of this term (Smedbol et al., 2002). The metapopulation concept implies that each local population within a metapopulation "determines its own internal dynamics to a large extent, but with a degree of identifiable and nontrivial demographic influence from other local populations through dispersal of individuals." (Kritzer and Sale, 2004). At present, both the links between sardine from each putative local population and the extent of their mutual influence are inadequately known.

As sardine, other small pelagic species have typically complex population structure, marked by variability in life-history traits and migrations (Barange et al., in press). Most species, even those distributed across a narrow latitude range, encompass several stocks. Basis for stock definition are variable, ranging from purely genetic evidence (e.g. Chilean common sardine, *Strangomera bentincki*) to holistic

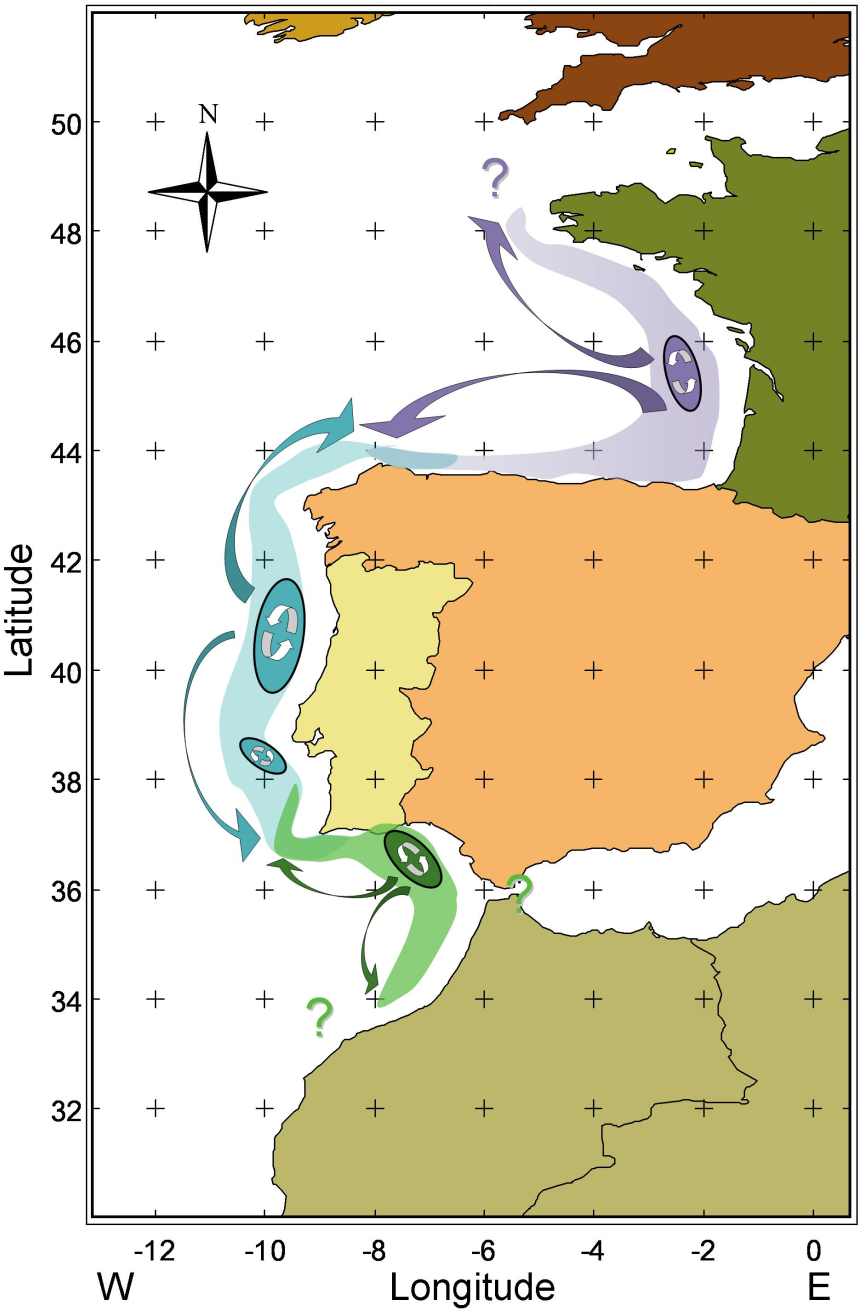

Figure 7.2: Schematic diagram of sardine population structure and life-cycle dynamics in and around the Atlanto-Iberian stock and adjacent areas. Ellipses represent sardine recruitment centres and shaded areas indicate regions of influence from each centre. Arrows inside each ellipse represent local life-cycle closure by reproduction of younger fishes. Arrows radiating from each ellipse indicate potential migration routes of older fish.

information from genetic, morphometric, demographic and migration studies (e.g. the Humboldt anchovy, *Engraulis ringens*). Population structure is still a matter of debate for California sardine (*Sardinops sagax caerulea*), a related species to the European sardine, distributed also in an eastern current upwelling system. Three sub-populations or stocks are generally accepted: a northern and a southern sub-population, distributed in neighbour coastal areas between Alaska and southern California and a sub-population distributed within the Gulf of California (Smith, 2005; Hill et al., 2006). The two coastal populations show differences in growth and mortality rates and their separation was recently supported by studies of temperature-at-catch and otolith morphometry (Hill et al., 2006). Seasonal migrations, with larger/older fish reaching farther areas, have been evidenced by tagging studies, and migration ranges are observed to overlap although the extent of mixing is uncertain; population sub-structure seems to be supported mainly by geographical and temporal separation of spawning (Smith, 2005). Overall, the California sardine seems to show higher local population discreteness than the European sardine, mainly due to spawning site fidelity and seasonal migration. Evidence of this type of dynamics has not been obtained for the European sardine. The northern and southern sub-populations are assumed as separate stocks for assessment and management purposes but alternative scenarios of stock structure will be explored in the future (Hill et al., 2006; Barange et al., in press).

The population structure and dynamics of *Sardinops sagax* off southwestern Australia have also been recently revisited (Gaughan et al., 2002). Sardine distributed across this area was considered to form three regional adult assemblages distinguished by gonadosomatic indices and independence of age composition dynamics. The inter-relationships between pre-recruit sardine across the three regions are still poorly understood and alternative mechanisms, such as natal homing and association to localized areas of higher habitat suitability, may explain their spatial persistence. Nevertheless, evidence of limited mixing among these assemblages and uncertainty about the origin and movement of pre-recruits was considered to increase the risk of local over-exploitation. The *Sardinops sagax* fisheries in this region are therefore managed taking into account both the individual regions and the whole distribution area of the species, using TACs and individual transferable quotas, adjusted separately for each adult assemblage region

7.4 Topics for future research

Geographical variation in biological traits (growth and maturity) has substantial impact on estimates of spawning stock biomass (ICES, 2006a). This variation has been recently accounted for in stock assessment, using stratified maturity ogives and stock weights for northern, western and southern areas since 1996 (ICES, 2006a). The revision of input parameters needs to be extended backwards in time, although in the earlier assessment years (1980s) it will be limited by lower spatial disaggregation of samples. In addition, the perception of recruitment variability obtained in the analysis of survey data (Chapter 6) is distinct from that provided in the ICES assessment (ICES, 2006a). Recruitment and biomass trends in the 1980s are strongly affected by catch data due to the lack of independent survey data for tuning the assessment. Conflicting signals provided by catch and survey data within the mid-1980s (in southern Galicia) increase concern about the reliability of the stock estimates in this period. The extent to which catchability differences between acoustic survey series and changes in time may influence this perspective is uncertain and deserves further investigation (this topic is one of the aims of the ICES Working Group on acoustic and egg surveys for sardine and anchovy in ICES areas VIII and IX, ICES, 2006b). Finally, the analyses presented in Chapter 6 indicate that sardine is exploited at lower rates in the Gulf of Cadiz than in the remaining areas of the Atlanto-Iberian stock. The influence of this difference in the assessment needs to be examined.

The exploration of alternative stock structure scenarios in the assessment of the Atlanto-Iberian sardine depends on additional information about the extent of migration and/or dispersal of early life-stages across sub-areas and limits. In particular, a longer time series of survey and catch-at-age data from the Gulf of Biscay is needed to clarify the origin of sardine spawning in the Cantabrian Sea and the destiny of eggs and larvae produced in this area. Area-disaggregated data for northern and southern parts of the Gulf of Biscay will improve understanding of the northern extent of the sardine stock and connections with sardine in the English Channel. The exploration of sardine relationships across the southern and southeastern stock limit can be improved combining survey and fisheries data from southwestern Mediterranean (GFCM 2006) and from northern Morocco (e.g. FAO 2001b, ICES, 2003a). The investigation of mechanisms promoting exchange of sardine early-life stages among areas and namely advection across the southern stock limit (e.g. Oliveira and Stratoudakis, in press) are worthwhile pursuing.

Several topics for future research on sardine stock structure were listed in Anon. (2006). Among these, topics providing information about sardine migration are vital. A large-scale tagging study was recently conducted within the Atlanto-Iberian stock area (Anon., 2006). Despite successful results in early experiments in captivity, tag recovery was extremely low, mainly due to higher early mortality in the field than expected from laboratory experiments, high tag detachment rate and low reporting of recovered tags. Tagging studies are worthwhile pursuing but alternative indirect methods should be explored to obtain information about sardine migrations. A promising method is the analysis of otolith elemental composition. This method already provided useful information on sardine mixing within the Iberian-Biscay region (Castro, 2007). It could be further extended to trace the origin of migrants and estimate rates of mixing between fish from different recruitment areas. Extension along these lines could be particularly useful to clarify the relationship between the recruitment areas off northwestern Iberia and Gulf of Biscay to the spawning population in the Cantabrian Sea. Several case studies exemplifying this type of application of otolith elemental fingerprints are reported in Campana (2005). Additional trace elements to those used by Castro (2007) could be explored and fine control of age and year-class effects could be performed to facilitate the interpretation of connectivity/separation between areas (Campana, 2005). Samples used for elemental composition could also be analysed for differences in otolith shape (Cadrin and Friedland, 2005) and structure (Friedland and Cadrin, 2005). The observation of geographical differences in sardine otolith structure (Soares et al., 2004) anticipates their utility as natural stock markers.

The spatial structure of sardine populations also suggests that area-based assessment models need to be further explored. These models may help to solve the apparent inconsistency between regional indices of population abundance and overcome uncertainty due to catchability differences when they are combined in a single abundance index (e.g. ICES, 2006a). One additional advantage of area-based models is that assumptions about the population dynamics are more likely to be satisfied on an area-basis (Quinn and Deriso, 1999). This is the case of assumptions related to exploitation rates, survey catchability and fisheries selection over age for sardine, which were observed to vary among stock regions. Geographical variability in biological traits is also readily included in area-based models. Finally, area-based models provide a perception of local/regional population dynamics allowing management decisions to be made separately for different regions, if necessary (Quinn and Deriso, 1999). The need to understand sardine dynamics at a lower spatial scale than the whole stock area seems unquestionable, since recruitment and population trends vary among areas, as shown in the present study. A more local perspective will also approach the perception of analysts and managers to that of fishermen, facilitating dialogue and acceptance of management action.

The year-class curve models (YCC) used in this study provided useful insight on the spatio-temporal variation of sardine local dynamics. Future development of these models may consider the inclusion of catch-at-age data and indices of fishing effort to estimate fishing mortality (Cotter et al. 2004, 2007). Parameters not estimated in this study, such as total abundance and spawning biomass by area are easily obtained and model fitting can be further refined (Cotter et al., 2007). Abundance estimates are relative and assumptions on selectivity over age and mortality are rigid. Migration is not explicitly modelled but neither it can be in other models, unless independent estimates of migration are available (ICES, 2006). Nevertheless, YCCs have simple underlying statistics and lead generally to parsimonious models, characteristics gaining importance among stock assessment analysts (Hilborn, 2003; Cotter et al., 2004).

Area-based assessment models within a Bayesian framework offer some advantages, such as, the possibility to test alternative hypothesis of stock structure and migration and accommodation of model uncertainty. The Bayesian state-space model (BSSM) developed within project SARDYN (Anon., 2006) seems therefore a natural candidate for sardine stock assessment in the future. BSSM was extended recently to test the hypothesis of sardine migration between the Cantabrian Sea and the French Gulf of Biscay. In this test, 1-year old sardine were likely to migrate from French to Spanish waters while 2+ individuals were likely to migrate from Spanish to French waters at a lower rate. Estimation of migration rates may be possible with the BSSM but may prove difficult unless specific information on migration (i.e. from tagging data or otolith elemental composition) becomes available. Alternative models within the Bayesian state-space framework have been developed to describe the dynamics of animal metapopulations (e.g. Buckland et al., 2004), and are also worth exploring.

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