



Pelagic survey series for sardine and anchovy in ICES subareas 8 and 9 – Towards an ecosystem approach

ICES COOPERATIVE RESEARCH REPORT

RAPPORT DES RECHERCHES COLLECTIVES



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Pelagic survey series for sardine and anchovy in ICES subareas 8 and 9 – Towards an ecosystem approach

Prepared by the Working Group on Acoustic and Egg Surveys for Sardine and Anchovy in ICES Subareas 8 and 9

Editors

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1 Introduction

Jacques Massé, Andrés Uriarte, Maria Manuel Angélico, and Pablo Carrera

1.1 Historical background on the coordination of acoustic and egg surveys in the southwestern waters of Europe

The history of active hydroacoustics for marine target detection began almost at the end of World War I when Paul Langevin, a French physicist, patented the first piezoelectric submarine detector. This device was soon improved, and in 1934, after some trials on board the MS "Glen Kidston", the first fish shoal was recorded on echogram paper. Since then, the fish-finder or echosounder became popular among the different fishing fleets and, for scientific applications, technological improvements made during the 1960s allowed the estimation of pelagic fish abundance using the echo-intregration method (Dragesund and Olsen, 1965; Fernandes et al., 2002). In the Bay of Biscay, the first scientific explorations targeting coastal pelagic fish (sardine [Sardina pilchardus], anchovy [Engraulis encrasicolus], and sprat [Sprattus sprattus]) were conducted in 1975 (Diner et al., 1976) whilst in the Iberian Peninsula, Portugal started acoustic survey trials in the late 1970s (Moura and dos Santos, 1982). Acoustic methods for fish detection and abundance estimation were first introduced by a cooperative development project coordinated by FAO and Norway (Marchal, 1982) and then in a series of working groups supported by the General Fisheries Council for the Mediterranean (FAO, 1980, 1983) in which France was also involved. As a result of the cooperation between Norway, Portugal, and Spain, the first intership calibration between RV "Fridtjof Nansen", RV "Noruega", and RV "Cornide de Saavedra" was done before the first joint acoustic survey on sardine in ICES divisions 8.c and 9.a took place in 1982 (Dias et al., 1983). Acoustic surveys have been regularly reported at meetings of the ICES Working Group on Fisheries Acoustics Science and Technology (WGFAST) since the 1980s where various questions or issues are discussed. Protocols consequently take into account all advice stemming from this working group.

On the other hand, and almost at the same time as the acoustic development in the Iberian Peninsula, the Intergovernmental Oceanographic Commission (IOC) launched an ambitious programme called the International Recruitment Experiment (IREX), aimed at assessing the present understanding of the mechanisms through which variability in the physical-chemical marine environment affects biological productivity of the ocean and the abundance and distribution of living marine resources. Through this programme, several projects were carried out, such as the Sardine and Anchovy Recruitment Process (SARP) project, focusing, among other things, on biological variables such as fecundity, egg production, and egg survival (Barber et al., 1982). At the same time, daily egg production, which had been developed and applied in California for northern anchovy (Engraulis mordax) (Hunter and Goldberg, 1980; Parker, 1980; Lasker, 1985), was being disseminated all over the world as a powerful, direct-estimation method for indeterminate spawning fish with pelagic eggs, characteristic of most small pelagic fish species. In this way, research in these fields in both Spain and Portugal during the 1980s allowed the application of the daily egg production method (DEPM), in 1987 for anchovy (Anon., 1987a; Santiago and Sanz, 1992a, 1992b) and in 1988 for sardine stocks (Anon., 1987b; Cunha et al., 1992; García et al., 1992).

While biological and catch information was routinely provided to the ICES assessment working groups¹, coordination between countries within the framework of ICES began in 1986 for acoustics (ICES, 1986a), first between Portugal and Spain and then, since 1998, including France. At that time, taking into account the preliminary results of the CLUSTER project (aggregation patterns of commercial fish species under different stock situations and their impact on exploitation and assessment; FAIR-CT-96.1799) and previous behaviour observations, the working group recommended that the acoustic track should be conducted only during daylight. The Working Group on the Assessment of Pelagic Stocks in Divisions VIIIc and IXa and Horse Mackerel also made a series of recommendations in order to (i) improve the hydrological characterization of the surveyed area; (ii) store the echograms on a digital basis for further post-processing analysis; (iii) increase the number of fishing stations, when possible, to characterize the whole pelagic community; and (iv) propose a common list of target strength/length relationships for the main fish species (ICES, 1987).

Regarding the egg surveys, after the first applications in the late 1980s, the surveys during the 1990s were implemented on an *ad hoc* coordinated basis between IEO and IPIMAR (presently IPMA) for sardines around the Iberian Peninsula and by AZTI for anchovies in the Bay of Biscay (ICES, 2004). Actual formal coordination within ICES between institutes was first framed at a workshop held in 2000 for the estimation of sardine spawning-stock biomass through the DEPM (ICES, 2000a). It demonstrated the convenience of having a common and more permanent forum for planning and discussing the implementation of the daily egg production method on both anchovy and sardine. As such, the ICES Study Group on the Estimation of Spawning Biomass for Sardine and Anchovy (SGSBSA) was launched in 2001 (ICES, 2002a) to design sardine and anchovy DEPM surveys in the following years, standardizing all methodologies of the DEPM, and generally discussing and evaluating improvements to the methodologies required for implementation of the DEPM on both adult and egg production parameters. It was also considered to analyze the feasibility of using the continuous underway fish egg sampler (CUFES) to improve DEPM estimates.

The ICES SGSBSA existed for five years, until 2005 (ICES, 2005a), and was a major step forward through standardization and modernization of the DEPM surveys, incorporating many improvements to the methods involved in the DEPM applications, particularly in the estimation of egg production parameters as well as adult parameters. The study group was supported by Portuguese and Spanish scientists from IPIMAR (presently IPMA), IEO, and AZTI; though the main focus was the application of the DEPM in southwestern European waters, connections with its application in other areas (e.g. the Mediterranean) were also achieved. Two major products of the group were the ICES CRR report on the surveys and methods applied for the applications on sardine and anchovies around the Iberian Peninsula (ICES, 2004) and a review paper on the applications and challenges of the DEPM around the world (Stratoudakis *et al.*, 2006).

¹Groupe de Travail pour l'Evaluation des Stocks de Reproducteurs de Sardines et Clupéidés au Sud des Îles Britaniques first met in 1979 (ICES, 1979). In 1980, the group changed its name to Working Group on the Assessment of Sardine Stocks in Divisions VIIIc and IXa (ICES, 1980, 1981). In subsequent years (1982–1886) the official name was Working Group on the Appraisal of Sardine Stocks in Divisions VIIIc and IXa (ICES, 1982a, 1986b); in 1987–1991, it was renamed the Working Group on the Assessment of Pelagic Stocks in Divisions VIIIc and IXa and Horse Mackerel (ICES, 1987); and in 1992–2008, it was the Working Group on the Assessment of Mackerel, Horse Mackerel, Sardine and Anchovy (ICES, 1992).

In parallel, at the beginning of the 21st century (2000–2002), the EU project PELASSES (Direct abundance estimation and distribution of pelagic fish species in Northeast Atlantic waters) was developed to improve acoustic and daily egg production methods for sardine and anchovy (DGXIV no. 99.010). The project was carried out by IEO, IFREMER, AZTI, IPIMAR, RUWPA (Mathematical Institute, University of St. Andrews), and MBA (Marine Biological Association, Plymouth) in order to update knowledge, standardize methods, and combine results from acoustic and egg surveys. The objectives of this project were to:

- (i) standardize methods, including age reading, acoustic post-processing analysis, and egg stage allocation from CUFES samples;
- (ii) evaluate the use of CUFES as a quantitative estimator of anchovy and sardine egg production;
- (iii) carry out synoptic coverage from the Gulf of Cádiz to the Celtic Sea to assess the abundance of sardine and anchovy and other pelagic fish species through the use of the echo-integration method;
- (iv) map the distribution of the main pelagic fish species at spawning time;
- (v) map egg distribution at 5 m depth with CUFES;
- (vi) study the feasibility of using a single research vessel to obtain abundance and biomass estimates by echo-integration acoustic and daily egg production methods;
- (vii) collect biological information obtained at fishing stations; and
- (viii) map climatic, hydrographic, and planktonic parameters that potentially influence the spatial distribution of pelagic fish species.

Following the PELASSES project, and with SGSBSA having achieved most of its objectives, the expediency of creating a common working group for acoustic and DEPM surveys on sardine and anchovy in southwestern European waters was recognized in order to facilitate the planning of coordinated surveys and the discussion of methods and improvements. This suggestion resulted in the ICES Working Group on Acoustic and Egg Surveys for Sardine and Anchovy in ICES Subareas 8 and 9 (WGACEGG), which first met 24–28 October 2005 in Vigo, Spain (ICES, 2006a).

The scope of the working group was formulated through the definition of the following terms of reference (ICES, 2009a):

- plan, coordinate, and review acoustic and egg surveys in ICES subareas 8 and 9 and standardize analysis procedures;
- 2) update innovations on sampling and estimation methods for DEPM and acoustics;
- 3) develop a framework to cross-validate and integrate egg production and acoustic methods for the estimation of spawning-stock biomass and its distribution;
- 4) produce an annual synoptic overview of distribution, abundance, and population structure of sardine and anchovy in relation to the pelagic ecosystem for ICES Subarea 8 and Division 9.a;
- 5) integrate biological/environmental information from surveys and additional sources to improve the understanding of the spatial distribution and dynamics of sardine and anchovy in relation to the pelagic ecosystem in ICES Subarea 8 and Division 9.a (Figure 1.1).



Figure 1.1. ICES divisions and subdivisions in southern Europe.

In relation to the last general term of reference, the working group soon realized that, in order to produce a synoptic presentation of results of DEPM and acoustic surveys in the region, a major output could be the development of a common database and maps on common spatial-grid cells. The initial idea was to produce synoptic maps of anchovy and sardine distributions (adults and their eggs) at a regional scale, also including concurrent environmental and biological covariates. Section 3 of the current publication details the atlas produced from all these surveys (2003–2012), based on those common synoptic maps.

More recently, UK and Irish participants joined the group to provide their own acoustic and biological data on sardine, anchovy, and other pelagic fish species from acoustic surveys in ICES Subarea 7. In addition, the WG has extended collaboration with other technical working groups through joint workshops such as the Joint AcousMed project/ICES WGACEGG Workshop on Geostatistics (WKACUGEO; ICES, 2011a). In addition, the design and results of the surveys were reported and discussed within WGFAST (the ICES Working Group on Fisheries Acoustics, Science and Technology).

In recent years, the working group has faced challenges related to the new orientation of the Common Fisheries Policy (CFP), which has gradually moved towards the ecosystem-based approach to fisheries management, and the implementation of the Marine Strategy Framework Directive (MSFD). All of this requires better indicators of both species status and ecosystem status. Accordingly, the working group is making an effort towards the production of deliverables useful for the implementation of these European policies (see Section 5).

1.2 Overview of the surveys carried out on small pelagic species in southwestern European waters

Continuous field research studies targeting the two main species, sardine and anchovy, based on surveys at sea have been carried for more than 30 years (Table 1.1) by France, Spain, and Portugal. At the same time, ancillary variables were gradually added in order to promote a better understanding of the complexity of the pelagic ecosystem related to those species.

France, Spain, and Portugal have traditionally participated through four institutions (IFREMER, AZTI, IEO, and IPIMAR [presently IPMA]) in carrying out the surveys, in several cases cosponsored through different EU projects and eventually (since 2002) within the Community framework for the collection and management of data needed to conduct the Common Fisheries Policy. Recently (2011), the UK and Ireland launched their own new surveys north of the Bay of Biscay (Celtic Sea, western English Channel, Irish Sea) and began to participate in WGACEGG.

Year Season IPMA IEO AZTI FREMER Year IPMA IEO AZTI IFREMER Year IPMA IEO AZTI IFREMER Galice Galice Gulf of Galice Gulf of Portugal Biscav Biscay Portugal Biscay Biscay Portugal Biscay Biscay Biscay Biscay Biscav Cadiz Cadiz • 1993 2003 1983 . umme utum 2004 1984 1994 summe . • autum vint . 1985 995 2005 summe . • autum . 1986 1996 2006 summe . . autui inte • 2007 1987 1997 • • umm autum • • 1988 998 2008 • ստա • autu • . 1989 1990 2009 summe • autum . 2000 2010 1990 umme . autun . . 200 2011 199[,] umme autum 2012 1992 2002 . .

Table 1.1. Seasonal survey effort on pelagic fish stocks made by IEO, IPMA, AZTI, and IFREMER institutes since the 1980s by regions in southwestern European Atlantic waters between Gibraltar and the Celtic Sea.

In order to allow for joint analyses of maps and data in this publication, the surveys operating exclusively in ICES divisions 8.a–c and 9.a since 2003–2012 are reported. Furthermore, these surveys occurred rather regularly in a coordinated and standardized form. Since the method used for Atlanto-Iberian sardine DEPM surveys is applied on a triennial basis, a decision was made to include the entire series (1988–2011). The surveys reviewed and coordinated by WGACEGG (or previous working groups) since 2003 are presented in Table 1.2. A summary of the surveys reported in

this document (acronyms, summary description, and usual period of implementation) is provided in Table 1.3.

Table 1.2. Surveys taken into account for the establishment of the common database and maps in this report. Orange refers to acoustic surveys and blue indicates DEPM surveys; sardine DEPM surveys in 1988, 1990, 1997, 1999, and 2002 are also reported.



Surveys	Country	Acronym	Month	Target species	ICES divisions and subareas
Spring DEPM surveys	Portugal	PT-DEPM- PIL	2	Sardine	9.a Central N., 9.a Central S., 9.a South
	Spain	SAREVA	4	Sardine	9.a North, 8.c, 8.b
Spring acoustic	France	PELGAS	5	Sardine and anchovy	8.a , 8.b, 8.c (East)
surveys	Spain	PELACUS	4	Sardine and anchovy	9.a North, 8.c
	Portugal	PELAGO	4	Sardine and anchovy	9.a Central N., 9.a Central S., 9.a South
Spring DEPM surveys	Spain	BIOMAN	5	Anchovy	8.a, 8.b, 8.c (East)
Summer acoustic survey in Division 9.a South	Spain	ECOCADIZ	6–7	Anchovy	9.a South
Summer DEPM survey in Division 9.a South	Spain	BOCADEVA	6–7	Anchovy	9.a South
Autumn acoustic surveys	Spain	JUVENA	9	Anchovy recruits	Subarea 8
Autumn acoustic surveys	Portugal	SAR	10–11	Sardine recruits	Subarea 8

Table 1.3. Acronyms, objectives, and the usual period of implementation of each survey series reported in this report.

1.3 Objectives and structure of the report

The purpose of this report is to present the results of the systematic surveys for monitoring the coastal pelagic fish species (mainly sardine and anchovy) in the southwestern Atlantic European waters, carried out between 2003 and 2012 by France, Spain, and Portugal and coordinated within ICES working groups. This is basically achieved as described below.

A description of the monitoring system used in the acoustic and DEPM surveys carried out in these years is given in Section 2 on a survey-by-survey time-series basis (Tables 1.2 and 1.3). This includes a synopsis of the background of each survey and its objectives, the global approach for the method applied (e.g. calendar, parameters collected, sampling procedures, standardization of methods throughout the series). In addition, a summary of the main results (overall maps of species distributions, series of biomass, and other indicators) is followed by a summary discussion.

The more technical procedures of each survey are further shown in annexes at the end of this report.

A synoptic series of spatial distributions of small pelagics and the environmental indicators obtained from the different surveys is presented in a standard format in Section 3. This is a novel contribution of WGACEGG to the scientific community in the form of an atlas of sardine and anchovy distribution in the region along with different covariates and indicators provided by these surveys, based on common spatial-grid cells. Individual maps are available for download from the ICES website (http://ices.dk/community/groups/Documents/Forms/AllItems.aspx?RootFolder=%2F community%2Fgroups%2FDocuments%2FWGACEGG%2FCRR%20332%20Supplem entary%20online%20material&View=%7B49A2EFDE-3932-4900-A03D-

<u>70258239F39E%7D</u>). Among the several indicators included are those on backscattering energy by species (Nautical area scattering coefficient [NASC], m² nautical mile⁻²; MacLennan *et al.*, 2002), sardine and anchovy egg abundance from vertical tows at fixed stations (PairoVET/CalVET) or continuous subsurface samples (continuous underway fish egg sampler, CUFES), sea surface temperature and salinity, cetaceans, or birds. These are provided on common scales and produced for each survey through the years, together with the mean distribution and its spatial variance across each survey time-series.

This contribution responds to terms of reference 4) and 5) of WGACEGG (Section 1.1). The final product is a joint common database and an atlas summarizing the trends and the mean situation of pelagic fish and its environment for over a decade. Section 4 aims at analyzing the interannual variability and spatial patterns of the indicators presented in Section 3, as well as the relationships among the parameters collected concomitantly during these surveys, making use of the common database assembled from these surveys. Section 4 presents a first essay for such crossed analysis, e.g. making use of the spring coordinated acoustic series (PELAGO, PELACUS, PELGAS) where parameters on environment, ichthyoplankton, fish, and top predators are collected simultaneously and cover the entire area from Cádiz to Brest. Characterizations of habitats and the spatial covariation of the indicators are also provided.

Section 5 briefly presents the challenges faced by the monitoring surveys on small pelagics, in addition to reporting on the abundance of the target species, and also contributing to the ecosystem monitoring required by the new CFP and MSFD.

Clearly, this report constitutes a major deliverable by the ICES WGACEGG and the surveys implemented for the monitoring of the small pelagics in the southwestern European Atlantic waters to the marine scientific community in Europe. It is expected that the description of the surveys used in the monitoring programmes, as well as the provision of the synoptic overview of the status and distribution of the small pelagic fish resources in this region during the first decade of this century will serve as a benchmark reference point on the small pelagics and/or ecosystem overviews of this region in any future studies.

2 Description of surveys on pelagic species in ICES subareas 8 and 9

2.1 Sardine DEPM surveys in Atlantic Iberian waters

Maria Manuel Angélico, Miguel Bernal, Paz Díaz, Ana Lago de Lanzós, Cristina Nunes, José Ramón Pérez, and Alexandra Silva

2.1.1 Introduction

The DEPM methodology was first applied for spawning-stock biomass (SSB) estimation in 1988 for the Atlanto-Iberian sardine (*Sardina pilchardus*) by Portugal (presently IPMA [used hereafter in the text], but previously under other designations: INIP, IPIMAR, INIAP, and INRB) and by IEO in Spain (Miranda *et al.*, 1990; Cunha *et al.*, 1992; García *et al.*, 1992). During the 1990s, through informal contacts, both countries organized surveys in 1997 and 1999; in 1990, only Spain carried out a survey that covered part of the area (García *et al.*, 1991, 1993; Cunha *et al.*, 1997; Lago de Lanzós *et al.*, 1998; Bernal *et al.*, 2000; ICES, 2000a; Stratoudakis *et al.*, 2000).

Since 2000, the surveys have been planned and conducted within the framework of ICES, with cofinancing from the national states and the EU, on a triennial basis. Coordinated surveys between IPMA and IEO were conducted in 2002, 2005, 2008, and 2011 (ICES, 2004, 2009a, 2010a, 2011a, 2012a; Stratoudakis *et al.*, 2006). Improvements in methods and standardization were possible due to methodological developments and effective coordination undertaken first within ICES SGSBSA (2002–2004) and later within WGACEGG.

The DEPM surveys targeting the Atlanto-Iberian sardine cover the area from the inner Gulf of Cádiz to the inner part of the Bay of Biscay (Atlanto-Iberian sardine stock). The region from the Gulf of Cádiz to the northern Portugal/Spain border (River Minho) is surveyed by IPMA in January–February, while IEO covers the northwestern and northern Iberian Peninsula and part of the Bay of Biscay (to 45°N) in March–April.

To obtain SSB estimation, the DEPM surveys are directed at egg abundance and spawning area definition for daily egg production determination and at adult sampling for daily fecundity calculation. Ichthyoplankton samples, simultaneous conductivity, temperature, depth and Fluorescence (CTDF) casts, and fishing hauls are undertaken over the entire spawning region. In recent years, data collection has been enhanced and diversified to not only promote improvement in the method (e.g. otoliths for population age structure), but also to take advantage of the comprehensive coverage of the pelagic environment and further the understanding of the ecosystem (hydrodynamics, zooplankton assemblages, fish diets, bird and marine mammal census, etc.).

2.1.2 Methodology: past and present

Since the DEPM was first applied in the late 1980s, it has evolved due to several methodological developments related to survey coverage, intensity of sampling, laboratory processing of samples, data analysis, and estimation of parameters. Moreover, there has been a systematic effort over the years in coordinating the surveys and standardizing methodologies for surveying, laboratory work, and data analysis. This was first due to the joint work between IEO and IPMA within the framework of national/international projects, and then under the auspices of ICES working groups, especially since 2002.

2.1.2.1 Surveying and sampling

Traditional DEPM is a survey-based estimation, requiring the entire potential spawning area to be covered with an adequate sampling effort, i.e. samples representative of the population – adults and eggs – and a sufficient number of observations for the required precision (Smith and Hewitt, 1985). On the other hand, Stratoudakis and Fryer (2000) suggested adopting a stratified random design in Iberian waters with an allocation proportional to local fish densities. This was done to obtain reliable estimates of spawning biomass when there are spatial differences in abundance and in the DEPM adult parameters. External information on fish abundance (acoustic density) and abundance of eggs observed during DEPM surveys are used as indicators of fish density, but there is no guarantee that the regional allocation of sampling effort carried out during the surveys has accurately reflected the relative abundance of the sampled population. Accordingly, the surveyed areas were divided into three geographical strata: Division 9.a South (Algarve and the Gulf of Cádiz); Division 9.a West (from Cape San Vicente to the Minho River at the northern Portuguese/Spanish border); and divisions 9.a North and 8.c (northern Spanish waters).

Regarding the Atlanto-Iberian sardine stock, the Gulf of Cádiz was not sampled in the first DEPM survey undertaken in 1988, and in 1990, only the north stratum was covered (there was no survey in Portugal). The survey carried out in 1997 was the first to include the entire distribution area of the sardine stock; since then, this same area has been surveyed jointly by IEO and IPMA.

The number of samples collected during the surveys also varied throughout the timeseries. In 2002, there was a substantial improvement in terms of sampling effort for both eggs and adults, resulting in sufficiently intense sampling that guaranteed good spatial coverage. This allowed, for the first time, an accurate spatial analysis of the data (see ICES, 2004 and below), and potentially increased the precision of the biomass estimates. Since then, this effort has been sustained (see Tables 2.1.1 and 2.1.2).

The grid of transects along which the fixed stations of ichthyoplankton (PairoVET) sampling are located has suffered some minor changes over the years (see Table 2.1.2). Since 2002, the fixed grid has remained unchanged, and an adaptive design has also been applied with the aid of the auxiliary CUFES, the use of which helps in delimiting sardine spawning areas and adapting the sampling intensity and the offshore limit of PairoVET sampling (as described in detail in the annexed protocol, Annex 1).

In DEPM, the most appropriate timing for surveying is the peak spawning period of the targeted species. Accordingly, for Atlanto-Iberian sardines, surveys are carried out in January/February in Portuguese waters (with the exception of the 1988 and 1997 surveys which took place simultaneously with the March acoustic survey) and in March/April in northern Spanish waters and the southern Bay of Biscay (up to 45°N). Furthermore, IEO surveys are carried out closely in time to the acoustic surveys, which also provides biological samples. On the contrary, since 1999, IPMA acoustic and DEPM surveys have become separated in time, and biological samples collected during the acoustic survey are no longer available. Since then, adult samples provided by the commercial fleet.

16/03

Transects PairoVET and grid Temp. Max. eggs Max. Strata Survey Positive (°C) **CUFES** Research nautical stations Eggs Eggs m-2 (ICES eggs m⁻³ Year Dates area area vessel miles (% with PairoVET stations CUFES Mindivisions) (km²) (km²) CUFES **PairoVET** (transects eggs) max × stations) 1988 9.a South 28/03-RV "Noruega" 15 (7×7) 55(25.5) 344 1 680 14.5-9 0 37 2 1 4 4 30/03 17.2 42 (7×7) 249(35.7) 944 1 360 12.8-39 073 14 889 9.a West 01/03 -08/03 16.1 and 21/03-28/03 9.a North + 31/03-RV "Cornide 68 (6×6–3) 516(51.7) 3 922 2 758.3 10.6-55 492 26 6 4 4 8.c 05/05 de Saavedra" 15.5 Iberian 125 820(45.1) 5 2 1 0 2 758.3 10.6-103 602 43 676 Peninsula 17.2 1990 9.a South 9.a West 9.a North + 18/04-RV 1 494 2 063.4 12.8-64 185 30 555 475(36.6) 8.c 10/05 "Investigador" 18.5 Iberian Peninsula 1997 9.a South 18/03-RV "Noruega" 29 (7×7) 135(43.0) 868 5 593.8 16-19.3 19 951 8 745 25/03 9.a West 01/03-6 6 9 6 39 (7x7) 238(16.0) 586 2 012.3 14-16.9 37 757

Table 2.1.1. Summary of plankton sampling for sardine DEPM surveys (Division 9.a South and West surveyed by IPMA, and divisions 9.a North and 8.c surveyed by IEO). In 1990, only areas in divisions 9.a North and 8.c were sampled. Division 8.b was covered by IEO from 1997 to 2011.

Year	Strata (ICES divisions)	Dates	Research vessel	Transects and grid nautical miles (transects × stations)	PairoVET stations (% with eggs)	Eggs PairoVET	Max. eggs m ⁻² PairoVET	Temp. (°C) Min– max	Survey area (km²)	Positive area (km²)	CUFES stations	Eggs CUFES	Max. eggs m ⁻³ CUFES
	9.a North + 8.c	05/03– 29/03	RV "Cornide de Saavedra"	44 (15 GAL,7.5 CANT ×3)	515(16.7)	1 465	5 381	13.2– 15.9	55 870	10 275			
	Iberian Peninsula			112	888(20.5)	2 919	5 593.8	13.2– 19.3	113 577	25 716			
	8.b	27/03– 02/04		12	89(63.6)	1 123	1 669.6	12.8– 15.3	20 149	12 755			
1999	9.a South	10/01– 19/01	RV "Noruega"	77 (6×6)	147(36.7)	3 184	13 431	14–17.1	20 633	7 451			
	9.a West	19/01– 03/02		(6×6)	272(23.2)	1 926	6 060	12.6– 16.3	36 919	9 829			
	9.a North + 8.c	17/03– 03/04	RV "Cornide de Saavedra"	50 (15 GAL,7.5 CANT ×3)	290(25.9)	900	1 196.6	12.2– 13.8	30 316	7 174			
	Iberian Peninsula				707(27.2)	6 010	13 431	12.2– 17.1	87 868	24 454			
	8.b	03/04– 05/04		11	37(77.1)	586	1 185.4	12.5– 13.3	6 793	5 724			
2002	9.a South	27/01– 02/02	RV "Noruega"	53 (8x3–6)	152(32.2)	530	1 733.4	14.5– 16.9	16 504	7 702	168	2 955	29.4
	9.a West	08/01– 27/01		(8x3–6)	332(41.9)	2 077	8 328.2	12.1– 16.8	34 442	18 711	375	8 774	131.0

Year	Strata (ICES divisions)	Dates	Research vessel	Transects and grid nautical miles (transects × stations)	PairoVET stations (% with eggs)	Eggs PairoVET	Max. eggs m ⁻² PairoVET	Temp. (°C) Min– max	Survey area (km²)	Positive area (km²)	CUFES stations	Eggs CUFES	Max. eggs m ⁻³ CUFES
	9.a Nort + 8.c	20/03– 16/04	RV "Cornide de Saavedra"	36 (8×3–6)	220(58.6)	1 939	1 896.1	10.9– 17.5	25 476	15 202	441	9 669	40.6
	Iberian Peninsula				704(45)	4 546	8 328.2	10.9– 17.5	76 422	41 615	984	21 398	131.0
_	8.b	06/04– 12/04	RV "Cornide de Saavedra"	10	55(73.3)	1 090	1 220.1	12.1– 13.9	11 888	9 154	103	5 096	98.3
2005	9.a South	13/02– 22/02	RV "Capricornio"	(8×3–6)	159(41.5)	1 733	4 825.6	13.1– 15.4	17 321	7 201	186	4 991	30.4
	9.a West	29/01– 12/02	_	(8×3–6)	249(32.9)	1 942	8 020	11.6– 14.8	26 808	10 723	312	4 278	55.6
	9.a North + 8.c	13/04– 01/05	RV "Cornide de Saavedra"	56 (8×3)	371(32.3)	3 216	3 231	12.4–16	38 476	12 307	323	9 748	85.0
	Iberian Peninsula				779(34.4)	6 891	8 020	11.6–16	82 605	30 231	821	19 017	85.0
2008	9.a South	20/01– 27/01	RV "Noruega"	22 (8×3)	174(56.3)	5 727	9 842.5	14.8– 17.1	18 164	9 692	181	10 710	124.9
	9.a West	28/01– 15/02		36 (8×3)	288(51.7)	7 895	8 142.4	13.3– 16.7	30 318	19 296	315	19 632	140.0
	9.a North + 8.c	02/04– 27/04	RV "Cornide de Saavedra"	56 (8×3)	426(54.2)	3 788	8 354.2	11.9– 15.2	42 381	242 64	416	17 225	162.7
	Iberian Peninsula			114	888(53.8)	17 410	9 842.5	11.9– 17.1	90 863	53 252	912	47 567	162.7

Year	Strata (ICES divisions)	Dates	Research vessel	Transects and grid nautical miles (transects × stations)	PairoVET stations (% with eggs)	Eggs PairoVET	Max. eggs m ⁻² PairoVET	Temp. (°C) Min– max	Survey area (km²)	Positive area (km²)	CUFES stations	Eggs CUFES	Max. eggs m⁻³ CUFES
	8.b	20/04– 24/04	RV "Cornide de Saavedra"	8	74(76.3)	1 104	2 332.1	12.6– 13.9	10 187	8 167	93	13 733	215.5
2011	9.a South	10/02– 20/02	RV "Noruega"	21 (8×3)	170(31.8)	2 208	4 950	14.6– 16.9	17 578	6 523	184	4 607	81.7
	9.a West	20/02– 08/03		36 (8×3)	309(12.9)	833	2 970	13.5– 16.1	32 098	4 817	308	479	6.0
	9.a North + 8.c	25/03– 10/04	RV "Cornide de Saavedra"	56 (8×3)	337(38.6)	1 794	1 537	12.5– 14.6	33 832	12 405	291	19 828	97.3
	Iberian Peninsula			113	816(27.5)	4 835	4 950	12.5– 16.9	83 508	23 745	783	24 914	97.3
	8.b	09– 15/04 (IEO)	RV "Cornide de Saavedra"	10 (8×3 IEO)	114(85.1)	2 764	2 322	13–14.7	14 091	12 400	129	36 978	92.1

Year	Strata	Vessel	Fishing hauls (% positive)	Total sardines sampled	Males	Females	Females for histology	Hydrated females	Mature females (%)
1997	9.a South	RV "Noruega"	12(83.3)	537	232	305	131	24	304(99.7)
	9.a West		28(57.1)	804	298	506	142	6	506(100)
	9.a North + 8.c	RV "Thalassa"	9(77.8)	402	142	260	255	113	259(99.6)
	Iberian Peninsula		49(67.3)	1 743	672	1 071	528	143	1 069(99.8)
	8.b	RV "Thalassa"	4(4)	239	104	135	68	42	135(100)
1999	9.a South	RV "Noruega"/	12(100)	1 208	536	672	151	19	624(92.9)
	9.a West	Commercial	28(100)	2 732	1 125	1 580	283	86	1 479(93.6)
	9.a North + 8.c	RV "Thalassa"	19(57.9)	997	532	463	100	19	422(91.1)
	Iberian Peninsula		59(86.4)	4 937	2 193	2 715	534	124	2 525(93)
	8.b	RV "Thalassa"	6(6)	516	241	271	50	12	266(98)
2002	9.a South	RV "Noruega"/	31(96.8)	2 416	934	1 478	499	47	1 462(98.9)
	9.a West	Commercial	43(93.0)	2 811	1 104	1 472	576	66	1 217(82.7)
	9.a North + 8.c	RV "Thalassa"	28(100)	2 058	1 019	1 039	470	69	1 038(99.9)
	Iberian Peninsula		102(96.1)	7 285	3 057	3 989	1 545	182	3 717(93.2)
	8.b	RV "Thalassa"	4(4)	199	106	93	20		93(100)

Table 2.1.2. Summary of adult sampling in the Iberian Peninsula (Division 9.a South, Division 9.a West, and divisions 9.a North + 8.c) and French coast (Division 8.b) sardine DEPM surveys. In 2005, Division 8.b was not sampled due to bad weather.

Year	Strata	Vessel	Fishing hauls (% positive)	Total sardines sampled	Males	Females	Females for histology	Hydrated females	Mature females (%)
2005	9.a South	RV "Capricornio"/	24(91.7)	1 652	759	891	510	52	851(95.5)
	9.a West	Commercial	42(97.6)	2 915	1 323	1 533	983	1	1 366(89.1)
	9.a North + 8.c	RV "Thalassa"	76(46.1)	1 625	721	897	562	115	755(84.2)
	Iberian Peninsula		142(69)	6 192	2 803	3 321	2 055	168	2 972(89.5)
2008	9.a South	RV "Noruega"/	27(92.6)	1 745	838	906	643	103	842(92.9)
	9.a West	Commercial	58(87.9)	3 195	1 352	1 839	1 371	76	1 554(84.5)
	9.a North + 8.c	RV "Thalassa"	41(87.8)	2 392	1 157	1 235	594	183	1 235(100)
	Iberian Peninsula		126(88.9)	7 332	3 347	3 980	2 608	362	3 631(91.2)
	8.b	RV "Thalassa"/RV "Cornide de Saavedra"	5(5)	503	280	223	164	3	223(100)
2011	9.a South	RV "Noruega"/	18(88.9)	975	480	495	397	11	495(100)
	9.a West	Commercial	40(80)	2 069	1 028	1 037	827	25	954(92)
	9.a North + 8.c	RV "Thalassa"	53(18.9)	718	334	384	230	31	380(99)
	Iberian Peninsula		111(52.3)	3 762	1 842	1 916	1 454	67	1 829(95.5)
	8.b	RV "Cornide de Saavedra"	11(3)	302	150	152	127	14	152(100)

2.1.2.2 Additional information collected

Hydrological information has been collected over the years. In early surveys, temperature data were obtained with probes (e.g. Minilogs) attached to the CalVET frame. Since 2005, the incorporation of the CTD to the CalVET structure allowed the regular collection of conductivity (salinity)/temperature/depth profiles simultaneously with each ichthyoplankton haul (IPMA and IEO). Additionally, at alternate stations and at the end of each transect, a second, more precise CTD was used during IEO surveys.

Catches from fishing hauls provide opportunistic information on the distribution of organisms composing the pelagic (and sometimes demersal) community as well as some demographical data (length distribution) mostly for the pelagic fish species, even though fishing hauls are directed at sardine.

DEPM surveys (since 2005 for IPMA) also include seabird and marine mammal observers who record all sightings of these animals along transects, contributing to the monitoring of their populations in Iberian waters.

2.1.2.3 Sampling and processing issues

Regarding egg sampling, the nets of the ichthyoplankton sampler (CalVET/PairoVET) have remained the same for all surveys (150- μ m mesh), whereas the overall structure has suffered some modifications over time, although it has remained unchanged since 2005 when the CTD became coupled to the net structure. As for the CUFES (the auxiliary system), it was equipped with a 500- μ m mesh net until 2005 when the mesh size was changed to 335 μ m, following a working group decision after initial stages of anchovy eggs were suggested to be potentially lost with the previous net.

Classification of eggs by development stages (11-stage scale) follows the general descriptions presented by Gamulin and Hure (1955). However, over the years, small adaptations have been introduced during discussions undertaken at workshops or working group meetings (e.g. ICES, 2002a; SGSBSA).

In DEPM, all adult estimates refer to mature fish (including those that are inactive during the spawning season) defined as those sardines containing gonads classified macroscopically as stage 2 and above. Until 2002, only stage 2 ovaries were sampled for histology. However, indication that misclassifications between stages 1 (immature/resting) and 2 (mature/developing) occurred (Afonso-Dias *et al.*, 2008), it was decided in 2005 to collect all ovaries irrespective of their maturity stage, the latter being confirmed microscopically for all fish sampled for histology.

Since 2005, histological slides have been analysed following a standardized procedure. For revision of the historical series, histological material prior to 2005 was reanalyzed following this same standardized procedure; oocytes are classified according to an 8-stage scale agreed within the working group for sardine and anchovy (adapted from Ganias *et al.*, 2003; Alday *et al.*, 2004). Regarding postovulatory follicles (POFs), these are assigned to daily cohorts using histological information according to both morphological and metric criteria (Ganias *et al.*, 2007). However, these daily cohorts are still delimited in time differently by IEO and IPIMAR/IPMA; each POF daily cohort begins at midnight (GMT) for the former and at sardine daily peak spawning time (21:00 GMT) for the latter.

Estimation of mean batch fecundity has always been limited by the number of hydrated female samples available in the fishing hauls (see Table 2.1.2). As in Atlantic sardine ovaries, separation in size of the spawning batch occurs much earlier than the

onset of hydration (at the transition between the primary and the secondary yolk stage; Ganias *et al.*, 2010). It is possible to use non-hydrated females having ovaries with oocytes at the above (or later) stages to obtain individual batch fecundity when hydrated females are scarce or even absent. Moreover, Ganias *et al.* (2010) developed an automated procedure (using Image J) by means of images of ovarian whole mounts to count the oocytes of the spawning batch.

2.1.2.4 Data analysis

The general common methodology currently used in the data analysis for the estimation of areas (surveyed and spawning areas), egg parameters (Z, P_0 , and P_{tot}), and adult parameters (sex ratio, mean female weight, spawning fraction, and bath fecundity) is explained in detail in the annexed common protocol (Annex 1).

All analyses are carried out using packages and routines developed in R software (<u>www.r-project.org</u>), which have already been updated since the initial versions. Several internal workshops took place, which helped standardize the use of these analytical tools between institutes.

2.1.2.5 Egg data

All calculations for area delimitation, egg ageing, and model fitting for egg production (*P*₀) estimation are based on the DEPM general theory and common methodology detailed in the annexed protocol (Annex 1). They are carried out using the R packages available within the open source project ichthyoanalysis (http://sourceforge.net/projects/ichthyoanalysis).

To avoid overestimation of areas at the borders of the survey limits, the value of the area represented by each station was forced to the minimum and maximum of 25 and 175 km², respectively. The range 25–175 was selected as a mean interval suitable for all surveys according to the distance between transect and stations. Distance varied in the initial years, but since 2002, it became fixed at 8 nautical miles between transects and 3 or 6 nautical miles between stations along transects.

The positive area (area with eggs) was obtained over the years using different calculation methods, but from 2008 onwards, the estimation of this parameter became automatic, and the 2012 revision considered this automatic procedure to recalculate estimates of all past surveys. Both the limits of the survey area (sampled) and the positive area (offshore and coastal) are estimated using the geofun library software, which mainly uses the spatial analysis functionality provided by the R-package spatstat. Both the survey and total areas are later corrected to avoid extrapolation to the coast by computing the intercept between the areas estimated, as above, and the area delimited by the coastline.

The model of egg development with temperature was derived from the incubation experiment data available within the egg R library (sardine incubation data from the Gulf of Cádiz, described in Bernal *et al.*, 2008).

Egg ageing was achieved by a multinomial Bayesian approach described by Bernal *et al.* (2008) and using *in situ* sea surface temperature (SST) data. Distribution of the daily spawning cycle was assumed to be a normal (Gaussian) distribution, with a peak at 21:00 GMT and a standard deviation of 3 h (spawning period from 21:00–6 h to 21:00+6 h). The upper age cutting limit was determined using a maximum age for the stratum considered, and it is not dependent on the individual stations (upper age = *F*). Older cohorts are dropped if their mean age plus 2 s.d. h is over the critical age at which 5% or more of the eggs are expected to have already hatched (how.complete = 95%).

The lower age cutting limit excluded the first cohort of stations in which sampling time is included within the daily spawning period (lower.age = T).

The approach to fitting egg data to estimate mortality (*Z*) and egg production (P_0) varied over the years (ICES, 2004; Stratoudakis and Bernal, 2006). Egg mortality estimates were revised by Bernal *et al.* (2011a), with results showing that single surveys are often unreliable, and that mean estimates obtained after aggregating data from various years are statistically more robust. Bernal *et al.* (2011b) also presented a revision of the estimation methods which included both generalized linear modelling (GLM) and generalized additive models (GAM)-based spatial analysis. For the 2012 revision, several GLM models were considered to test different stratification combinations for P_0 and *Z* (see detailed discussion in ICES, 2011a, 2012a), applying a standardized procedure. The exponential mortality model is fitted as a GLM with binomial negative distribution and with log link and weights proportional to the relative area represented by each station. The results presented here were stratified for P_0 , based on a single mortality estimate for the whole area.

2.1.2.6 Adult data

In DEPM, four parameters are estimated based on information collected from adult fish samples: (i) mean female weight (W), (ii) sex ratio (R), (iii) mean female batch fecundity (F), and (iv) daily spawning fraction (S).

Depending on the years, *W* was obtained either excluding the hydrated females from the calculations or by adjusting all sampled female weights to correct for the temporary bias due to ovary hydration. For the 2012 revision, only hydrated females had their weight corrected, whereas the observed weights were considered for all other females.

The sex ratio in weight per haul is obtained as the quotient between the weight of females and the combined weight of males and females. Ephemeral spawning aggregations around the daily spawning peak in sardine (Ganias and Nunes, 2011) may introduce bias and less precision in the estimation of R, unless samples around this time are avoided.

Batch fecundity is estimated based on the method described in Hunter et al. (1985) of modelling the individual batch fecundity observed in the sampled hydrated females and their gonad-free weight, and subsequently applying this model to all mature females. Depending on the year, the number of hydrated females collected varied (see Table 2.1.2). In 1988, hydrated females did not cover the entire range of female weights sampled off the Portuguese coast, and a unique model was fitted for all areas surveyed (three strata; see Cunha et al., 1992). In 1997, the same regression model was used to estimate mean batch fecundity (Cunha et al., 1997). In 1999, a weighted linear model (using weighted least squares, with weights equal to the inverse of W_{nov}) was fitted to the hydrated females samples (Stratoudakis et al., 2000). In 2002, several models were fitted to the Portuguese and Spanish data, and F was finally obtained by applying GLMs to the former, whereas estimation followed the standard weighted linear regression model (batch fecundity as a function of gonad-free weight $[W^*]$, weighted by the inverse of W^*) for the latter. Since 2005, batch fecundity has been modeled for the three strata with GLMs; several models are tested for each survey, the most appropriate being selected based on both biological and statistical significance.

Spawning fraction is the adult parameter estimated with less precision and more potential bias because it is a sample-based estimate and thus is considerably dependent on sampling design and number of samples. For sardine, the estimator of spawning fraction has always been the average proportion of female fish with day 1 and day 2

POFs in the sample of mature females collected per haul and examined by histology. Until 2005, POFs were classified based on descriptions by Hunter and Macewicz (1985) and Pérez et al. (1992a) on the time of capture and a peak spawning time of 19:00 (GMT). From 2005 onwards, POFs have been assigned to daily cohorts using both histomorphological and metrical (POF cross-sectional area) criteria (Ganias et al., 2007) and peak spawning time at 21:00 (GMT). In the first surveys carried out in Iberian waters, uncertainties in POF determination and ageing (limited experience in histological preparation and analysis) prevented the provision of reliable estimates. For the 2012 revision, all histological material was reanalysed following the same methodology, and the spawning fraction estimates revised. With the exception of 2011, the estimated S for the Portuguese survey in 2002 was the lowest of the series (and probably the lowest ever reported for a sardine species during peak spawning, see Table 2.1.3). Precision of the S estimate consequently remained low despite the considerable increase in the number of samples collected that year compared to previous years. In 1999, no S estimates could be provided in the north and along the French coasts, and in 2011, estimates obtained were considered unrealistic for the western and southern strata; a non-parametric bootstrap approach was applied to estimate S for those years and strata (see Section 2.1.3 – Results, and the protocol in Annex 1). Despite the improvements, several potential sources of bias still remain in S estimation: (i) impact of sampling gear and time of sampling (biased estimates of S can be obtained due to behaviour of sardines around spawning time), (ii) spatial heterogeneity (if there is spatial structure in S due to different population demography and environmental conditions), and (iii) correct assignment of POFs to daily cohorts depending on temperature.

2.1.2.7 Post-stratification and spatial analysis

A major assumption in DEPM estimation is that all parameters are constant over the duration and the area covered by the survey. When this assumption is violated, Picquelle and Stauffer (1985) recommend post-stratification. Results from several studies on the spawning areas (Bernal *et al.*, 2007), spawning seasonality (Stratoudakis *et al.*, 2007), sardine first maturation (Silva *et al.*, 2006), and population dynamics (Silva *et al.*, 2009) indicate geographical heterogeneity in spawning activity as well as life history traits among the northern, western, and southern Iberian coasts, suggesting DEPM analysis should consider the decision to post-stratify in these three areas.

Post-stratification was not used for adults in the western and southern areas until 2002 because there was insufficient information (number of fish samples) to stratify in 1988 and 1997, and post-stratification was not used in 1999 for comparability with the previous two surveys. Following recommendations by SGSBSA, post-stratified estimates for western and southern areas were provided later for these years, which led to an overall improvement in precision of the estimates (ICES, 2004; Stratoudakis and Bernal, 2006).

Additionally, spatial analysis was also carried out applying generalized additive models (GAM) fitted to egg and adult parameters for the first time in the 1999 and 2002 surveys (ICES, 2004). The results were in agreement with the post-stratification method, and GAM-based estimations reduced *CVs* for sardine (ICES, 2004).

During the 2012 revision, estimations were always obtained with post-stratification (for the southern, western, and northern strata) for adult parameters and P_0 . For mortality, different options were tested and discussed; the results presented here consider a single mortality for the whole area. As for GAM-based estimations, no further work has been carried out in recent years for adult parameters. For P_0 , spatially

explicit estimates were published by Bernal *et al.* (2011b), providing a flexible estimator of egg production and increasing the precision of the time-series estimates.

2.1.3 Results

This section describes the results obtained after the revision of the complete dataseries undertaken in 2012 for the ICES benchmark assessment of the Atlanto-Iberian stock, using the standardized analytical procedures and options described in detail in ICES (2012a). For this revision, the data used were revised, recompiled, and standardized by IPIMAR/IPMA and IEO (ICES, 2011a, 2012a). The revision of estimations included all data available for eggs (1988–2011); however, for adults, it was not yet possible to guarantee the correct interpretation of the raw data for the 1988 survey; therefore, these data were not included. This section also includes estimations from egg and adult data, collected by IEO in Division 8.b since 1997.

2.1.3.1 Environmental setting

Sea surface temperature (SST) and sea surface salinity (SSS) distributions observed during the DEPM survey series are presented in Figure 2.1.1. Water temperature is the only variable applied in the method for egg ageing. Therefore, at the beginning of the survey series, only temperature probes were used; regular CTD casts were implemented at a later point.

The SST and SSS maps show the general distribution features for the Atlanto-Iberian coastal region, with temperature and salinity decreasing from south to north. When the DEPM surveys take place in winter or early spring, the water column is usually still thermally mixed. However, haline stratification may occur outside the major river mouths (Guadalquivir and Guadiana on the southern coast; Tejo, Douro, and the Galician Rias in the west). In the inner corner of the Bay of Biscay, water stratification begins during spring. Shelf hydrology is subject to the influence of water originating from the Azores Current and the Gulf of Cádiz, mainly in the south and southern region and from the North Atlantic Current in the north. The geographic orientation of the shore of the Iberian Peninsula confers different oceanographic characteristics to the southern and northern zonal coasts and to the meridionally orientated west coast. The west coast, aligned with prevailing winds, is subject to upwelling events during summer (occasionally even off season) and, hence, has more productive colder waters. A poleward flow runs off the shelf edge, being more evident from Nazaré Canyon northward during winter. Off the southern and northern shores, local winds drive highly variable transient coastal currents. All year-round, the coastal Atlanto-Iberian waters are populated with mesoscale oceanographic features that promote patchy distributions of plankton and other components of the system (Mason et al., 2006).

Throughout the series, coastal temperature and primary production have fluctuated considerably (information not shown here). At the beginning of the series, typical average water temperatures were observed during the surveys. Winter 2005 was cold, particularly along the southern and western coasts and was preceded by a rather warm summer–autumn period in all regions. The winters of 2008 and 2011 were quite mild everywhere, and these years also had high primary production.

2.1.3.2 Spawning area

A summary of the plankton sampling for the sardine DEPM surveys is presented in Table 2.1.1. Modifications in the survey design were introduced during the first years of the DEPM application; from 2002 onwards, the surveys undertaken by IPMA and



IEO have followed a regular grid of transects, perpendicular to the coast and spaced eight nautical miles apart.

Figure 2.1.1. Sea surface temperature (SST) (top two rows of panels) and sea surface salinity (SSS) (bottom panels) distributions observed during the DEPM surveys for the Atlanto-Iberian sardine stock. SSS data is only available since 2002. In 2011, SSS data were not reliable and hence are not included. Discontinuity in the distributions indicates that surveying was not sequential.

Sardine egg distributions obtained from the PairoVET (1988–2011) and CUFES systems (since 2002), for the whole area are presented in Figures 2.1.2a and 2.1.2b. The egg distribution pattern derived from the observations from the two samplers is similar.

Sardine spawning grounds occupied all of the surveyed shelf, and in some years, eggs

were observed offshore (likely due to advection); however, egg distribution (hot spots or egg voids) varied over years. It is also important to note that the timing of the surveys also varied over years (due to logistic constraints), particularly in the southern and western regions where the DEPM survey was conducted simultaneously with the acoustics survey during March at the beginning of the series, when the spawning season is already advanced for the southern and western populations. During 1997 and 1999, a discontinuity in egg distribution was observed between the northern and western areas. The gap in egg presence was evident in Galician waters and at the northern part of the western area, although sardine eggs have been found in these areas since 2002. Recurrent areas of high egg abundance are found in the inner Gulf of Cádiz, over the northwestern shelf, and in the corner of the Bay of Biscay. In some years, either because of survey timing, environmental conditions, or population structure, egg distribution was very patchy. 2005, for example, was the coldest winter of the series where the percentage of young sardines (first-year spawners) in the northwestern region was very high, while egg abundance was low and very patchy. In 2008, when weather conditions were quite good and the population included a high percentage of middle-older age individuals, egg abundance (and egg production) was the highest of the series (over 50% of the samples contained eggs). Regarding egg distribution along the French coast, eggs were always observed to encompass the shelf extension to the 200-m isobaths.



Figure 2.1.2a. Egg distribution during the DEPM surveys for the Atlanto-Iberian sardine stock derived from PairoVET (eggs m⁻²). In 1990, only the northern Spanish coast was sampled.



Figure 2.1.2b. Egg distribution (eggs m⁻³) during the DEPM surveys for the Atlanto-Iberian sardine stock derived from CUFES sampling.

Figure 2.1.3 shows egg densities (sum of all stations) derived from PairoVET and CUFES sampling (see number of stations and maximum values by area and year in Table 2.1.1). An increase in the number of eggs was observed from 2002 to 2008 when high egg densities were obtained for all areas. After 2008, a generalized drop in abundance was observed, particularly in the south and west. Some very high values were observed in the north (1988) and in the south (1999).



Figure 2.1.3. Egg density from DEPM surveys in the Atlanto-Iberian sardine stock. Upper panel: number of eggs m⁻² from PairoVET sampling; lower panel: number of eggs m⁻³ from CUFES sampling. In 1990, only the northern Spanish coast was sampled.

The Iberian Peninsula surveys covered a total mean area of ca. 91 000 km², of which the northern stratum represented 47% of the surveyed area, the western shores 37%, and the southern coast 19%. The mean surveyed area for the entire series on the French coast (up to 45°N) is 12 600 km².

The spawning area estimates (Table 2.1.1 and Figure 2.1.4) for the southern area remained stable along the survey series, with values ranging between 9.6 and 6.5×10^3 km². In 1988, only the Algarve was surveyed (2.1×10^3 km²). For the western area, the spawning grounds increased gradually from 1997 to 2002 (6.7 to 18.7×10^3 km², respectively) and dropped to 10.7×10^3 km² in 2005. In 2008, the highest value of the series was attained (19.3×10^3 km²), and a sharp decrease was observed during 2011 (4.8×10^3 km²). The highest spawning area values for the northern stratum were estimated for the 1988 and 1990 surveys (26.6 and 30.5×10^3 km², respectively) when sardine eggs spread offshore. From 1997 onwards, the trend for the northern spawning area estimates is quite similar to the observations in the western area, increasing and decreasing in alternate years with a maximum in 2008 (24.3×10^3 km²) and a minimum in 2011 (12.4×10^3 km²). On the French coast, the spawning area estimates for all strata were lower in 2011 than in 2008, except in the French area, where an increase was observed.



Figure 2.1.4. Spawning area (km² × 10³) estimate in the DEPM surveys for the Atlanto-Iberian sardine stock. In 1990, only the northern Spanish coast was sampled.

2.1.3.3 Egg production

The exponential mortality model selected to estimate mortality (z) and egg production (P_0) for the three Iberian strata (south, west, and north) has three strata for P_0 and a common slope (single mortality estimate).

For the 2002 survey, a model without mortality was applied because an estimate for mortality led to a non-coherent (positive) mortality. The stratum farthest north (Division 8.b) was considered separately. For the 1999 coverage of Division 8.b, it was not possible to estimate mortality; therefore, a model without mortality was used.

Total egg production estimates (Figure 2.1.5 and Table 2.1.3) showed marked differences between areas and years. High concurrent variability, although without a perceivable trend, was observed for the southern and western strata. Total egg production in the south ranged from 3.3×10^{12} eggs d⁻¹ in 2002 to the extreme value of 5.96×10^{12} eggs d⁻¹ in 1999, while in the western stratum, estimates varied between 0.84×10^{12} eggs d⁻¹ in 2011, the lowest of the series, to 3.93×10^{12} eggs d⁻¹ in 2008, when estimates for the southern and western areas were very close.

For the northern region (divisions 9.a North and 8.c), a clear trend was observed with a decrease from the start of the series $(4.30 \times 10^{12} \text{ eggs d}^{-1} \text{ in 1988})$ until 1999–2002 ($0.85 \times 10^{12} \text{ eggs d}^{-1} \text{ in 2002}$), and then an increase, more accentuated between 2002 and 2005; the last two estimates were close in value and not far from the estimation undertaken at the beginning of the series. In 2011, the northern area egg production was higher than in 2008, but southern and especially western area egg production values were much lower than in 2008. The main differences in total egg production between 2011 and 2008 were related to spawning area differences, which were reduced in all regions.

On the French coast, similar values of total egg production were estimated for 1997, 2002, and 2008 (in 2005, due to bad weather, it was not possible to sample the Division 8.b area), and high total egg production was obtained in 2011 (2.72×10^{12} eggs d⁻¹); the lowest value of the series was obtained in 1999 (0.45×10^{12} eggs d⁻¹).



Figure 2.1.5. Egg production for the three spatial strata for all surveys off the Iberian Peninsula. Dots and lines indicate the estimates of egg production and their confidence intervals. The *y*-scale is the same for all strata.

Table 2.1.3. DEPM surveys for the Atlanto-Iberian sardine stock. Summary of the results for eggs, adults, and SSB estimates. Final egg production model for the Iberian Peninsula includes individual egg production estimates for each stratum (divisions 9.a South, 9.a West, and 9.a North–8.c) and a common mortality for the whole area. Egg production estimates and mortality values are also shown for the Division 8.b area. Significant mortality values (h⁻¹) are shown. * Significance at p < 0.05, ** significance at p < 0.01, and *** significance at p < 0.01. Mean females weight (*W*) (in g), Batch fecundity (*F*) (number eggs female⁻¹), sex ratio (*R*), spawning fraction (*S*), and spawning-stock biomass (SSB) (in t). *CV* corresponds to the coefficient of variation for each parameter estimated.

N	Strata	Mortali	ty	$P_{ m tot}$		W		R		F		S		SSB	
Year	(divisions)	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV
1988	9.a South			0.85	0.31										
	9.a West	-0.019***	0.20	1.84	0.17										
	9.a North + 8.c			4.3	0.15										
	Total Iberian Peninsula			6.99	0.11										
1990	9.a North + 8.c	-0.034***	0.24	3.56	0.26										
1997	9.a South	-0.032***	0.23	1.55	0.27	43.1	0.07	0.557	0.05	19 062	0.12	0.104	0.13	60 556	0.33
	9.a West	_		2.09	0.29	48.5	0.07	0.637	0.04	22 569	0.13	0.049	0.18	144 012	0.37
	9.a North + 8.c	_		2.91	0.27	72.2	0.05	0.493	0.14	28 544	0.07	0.144	0.10	103 611	0.33
	Total Iberian Peninsula			6.55	0.16									308 178	0.22
	8.b	-0.012*	0.41	1.74	0.20	74.5	0.12	0.508	0.08	32 269	0.17	0.131	0.10	60 332	0.310
1999	9.a South	-0.023**	0.34	5.96	0.33	42.1	0.05	0.531	0.03	22 436	0.11	0.074	0.22	284 749	0.42
	9.a West			3.59	0.30	44.9	0.06	0.639	0.05	24 086	0.09	0.142	0.05	73 672	0.33
N	Strata	Mortali	ty	Ptot		W		R		F		S		SSB	
------	-------------------------------	-----------	------	----------	------	----------	------	----------	------	----------	------	----------	------	----------	------
Year	(divisions)	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV
	9.a North + 8.c			0.95	0.33	65.9	0.09	0.514	0.04	34 137	0.10	0.09	0.09	41 963	0.37
	Total Iberian Peninsula			10.5	0.22									400 385	0.30
	8.b			0.45	0.13	63.6	0.13	0.535	0.11	32 704		0.131	0.10	13 200	0.52
2002	9.a South			0.33	0.19	38.8	0.05	0.621	0.05	12 881	0.06	0.035	0.19	45 781	0.29
	9.a West			1.38	0.12	43.3	0.05	0.619	0.03	15 212	0.07	0.061	0.18	103 982	0.24
	9.a North + 8.c			0.85	0.11	75.6	0.05	0.505	0.08	29 623	0.06	0.09	0.11	47 747	0.20
	Total Iberian Peninsula			2.56	0.08									197 511	0.15
	8.b	-0.022***	0.18	1.67	0.19	62.9	0.06	0.492	0.23	24 577		0.143		60 720	
2005	9.a South	-0.011*	0.4	1.38	0.23	45.4	0.07	0.574	0.11	13 169	0.08	0.135	0.13	61 328	0.30
	9.a West	_		1.87	0.21	46.2	0.06	0.556	0.06	15 304	0.44	0.063	0.21	160 988	0.54
	9.a North + 8.c	_		2.7	0.21	80.7	0.04	0.51	0.07	34 147	0.04	0.078	0.17	160 346	0.28
	Total Iberian Peninsula			5.95	0.13									382 662	0.26
2008	9.a South	-0.024***	0.18	4.04	0.21	56.3	0.06	0.489	0.07	20 956	0.06	0.088	0.08	252 405	0.25
	9.a West			3.93	0.18	59.3	0.03	0.593	0.03	26 424	0.04	0.078	0.10	190 549	0.22

Veen	Strata	Mortali	ty	$P_{ m tot}$		W	W R		F		S		SSB		
Year	(divisions)	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV
	9.a North + 8.c			3.79	0.17	83.9	0.04	0.482	0.06	35 139	0.04	0.09	0.13	208 604	0.23
	Total Iberian Peninsula			11.76	0.11									651 558	0.14
	8.b	-0.019***	0.26	1.4	0.23	55.4	0.11	0.483	0.09	15 849	0.29	0.137	0.24	73 942	0.47
2011	9.a South	-0.047***	0.13	2.86	0.27	54.3	0.07	0.498	0.09	17 157	0.11	0.081	0.09	223 745	0.33
	9.a West			0.84	0.29	50.1	0.06	0.496	0.04	11 838	0.09	0.066	0.08	108 154	0.32
	9.a North + 8.c			4.04	0.24	85.9	0.03	0.487	0.12	40 844	0.05	0.114	0.26	152 954	0.38
	Total Iberian Peninsula			7.74	0.16									484 852	0.21
	8.b	-0.018***	0.22	2.72	0.16	61.3	0.01	0.51	0.2	30 383	0.04	0.066	0.49	162 930	0.55

2.1.3.4 Mean female weight

The percentage of larger (older) individuals is greater for the population occupying the northern region than for populations in the other regions (Figure 2.1.6 and Section 2.2). As a consequence, mean female weight (*W*) is always higher in the northern stratum, where it has shown an increasing trend since 1999 (Figure 2.1.7). Conversely, the western (NW) and southern (SE) regions are loci for sardine recruitment, which is reflected in the structure of the population sampled during the DEPM series. Mean weights obtained for Division 8.b varied greatly, depending on the number of hauls considered and their locations. During the more recent surveys, the population observed in this region appears closer in weight composition to the more southern regions (proximity to the Bay of Biscay recruitment locus).

Minimum mean weight values were observed off northern Portugal and in the Gulf of Cádiz (Figure 2.1.7). Mean female weight was similar for the entire Portuguese and Cádiz coasts, and considerably higher for the northern Spanish coast. On the French coast, mean female weight was lower than in the northern stratum, particularly evident in the last two years (2008 and 2011). During the last survey in 2011, mean female weights obtained for all strata were similar to the ones estimated in 2008, the values calculated for the northern stratum being quite a lot higher than for the western and southern strata.



Figure 2.1.6. Sardine length (mm; top panel) and age distributions (bottom panel) per stratum for DEPM sardine surveys. Only values from females sampled randomly (absolute values), obtained according to the DEPM sardine sampling protocol (see Annex 1), are shown. No otoliths for age reading were available in 1997 in the divisions 9.a South/West and 8.b.



Figure 2.1.7. Mean female weight (*W*; in g), batch fecundity (*F*; number of eggs female⁻¹), sex ratio (*R*), and spawning fraction (*S*) estimates for the four strata (Division 9.a South in blue, Division 9.a West in green, divisions 9.a North and 8.c in blue, and Division 8.b in black). Vertical lines indicate the approximate 95% confidence intervals (i.e. ± 2 s.d.).

2.1.3.5 Batch fecundity

To estimate mean batch fecundity (*F*), several models were tested for each survey in the historical series. The model finally selected for all years was the one considering the same intercept, but different slopes (although in 2008 and 2011, the option was to consider the models with null intercept). In 1997 and 2005, very few hydrated females were collected off the west coast (n = 6 and 1, respectively, which means 1.2% and 0.1% of the total females sampled). For those years, the model considered the western and southern strata together, but *F* estimates were, nevertheless, calculated for the three strata separately. Percentages of hydrated females from the total females sampled by stratum ranged between 2.2% and 11.4% (southern), 0.1% and 5.4% (western), and 4.1% and 43.5% (northern). For the French coast, considering that few hydrated females (n = 3) were collected in 2008 and no hydrated females were available in 1999 and 2002, the adult data from those three years were pooled with data from the northern Atlantic Spanish coast for modelling the batch fecundity.

Results showed that *F* usually follows a similar trend to that of mean female weight, the exceptions being the 1999 estimate in the north (which is one of the highest, while mean female weight is the lowest of the series) and the 2011 estimates in the south and west (Figure 2.1.7). In the former case, data from hydrated females used to model batch fecundity were obtained only in Galician waters (one haul), although the model was then applied to all samples from Galician and Cantabrian waters (11 hauls). Spatially related differences in relative fecundity (the slope of the regression curve) between the two areas may have resulted in diverging weight and fecundity estimates. In the latter case, relative fecundity was apparently lower for those strata in 2011.

Mean batch fecundity estimates in 2011 were considerably lower (one-third) off the Portuguese and Cádiz coasts than off the northern Spanish coast. The latter presented the highest estimate of the historical series, although similar to the ones obtained for the 2005 and 2008 surveys. On the contrary, mean batch fecundity estimates from the Portuguese and Cádiz areas were among the lowest of the time-series, only comparable to the batch fecundities obtained for the 2002 survey from the western stratum and for the 1997 survey from the southern stratum. In particular, for the western stratum, although mean female weight was similar to that obtained during the 2008 survey, the batch fecundity estimate in 2011 was reduced to less than one-half when compared to the value obtained in 2008.

2.1.3.6 Spawning fraction

Spawning fraction (*S*) estimates show noticeable geographical and temporal differences (Figure 2.1.7). Spawning fraction is usually higher in the north and much higher on the French coast than in the remaining areas, except for the 2005 southern and 1999 western estimates. In the north, the highest spawning fraction value was observed in 1997, but it remained relatively stable in the 2000s at lower values, closer to the estimates of the western stratum. On the contrary, spawning fraction fluctuates more in the south.

The 2011 estimate for the northern Spanish coast was higher than that obtained during the 2005 and 2008 surveys (0.114 vs. 0.078 and 0.090, respectively). For the southern and western strata, the spawning fraction estimates obtained in 2011 were very low (0.015 and 0.019, respectively) and likely unrealistic taking into account female mean weight and egg production in those two strata. Those two spawning fraction values were not included in the estimation of the SSB, and a non-parametric bootstrap approach was applied (using mean *S* values per haul for the whole series, between

1997 and 2008, as described in the annexed protocol) to obtain alternative estimates of *S* for the southern and western strata (0.081 and 0.066, respectively). In 1999, no histology samples were available to estimate the spawning fraction in the north and along the French coast, and the bootstrap approach was also performed using mean spawning fraction by each haul obtained during the entire series in these areas (*S* = 0.09).

Despite the fact that the revised histological data are now more reliable, spawning fraction is still one of the most difficult and less precise estimates of the method. Interannual and geographical variability of this parameter needs to be studied in more detail in relation to population demography (spawning fraction is reported to be size-dependent in other clupeids, e.g. Claramunt *et al.*, 2007), seasonal dynamics of reproductive activity, and environmental conditions. Discussions are underway in order to define the validity of the estimator used to obtain this parameter for each survey separately (e.g. in case of over/under sampling of recent – day 0 POFs – spawners).

2.1.3.7 Sex ratio

In the north, the sex ratio is more or less constant (ca. 0.5) and similar to values obtained on the French coast, whereas the sex ratio in the west and south is usually higher and shows variations with no apparent explanation (Figure 2.1.7). This issue should be further explored in relation to sampling (time, depth, and fishing gear) and sardine behaviour (e.g. actively spawning sardines are known to separate from the main schools and form ephemeral spawning aggregations; Ganias and Nunes, 2011).

2.1.3.8 Spawning-stock biomass

Table 2.1.3 provides the DEPM parameter estimates, including spawning-stock biomass (SSB), for the three strata (south, west, and north) and the estimate for the entire Iberian Peninsula stock. SSB estimates for the French coast (Division 8.b area) are also shown.

The spawning-stock biomass for the Iberian Peninsula stock was estimated, taking into consideration the options discussed and provided in ICES (2012a). For assessment purposes, the following were selected:

- 1. The model for mortality and egg production, considering three strata for egg production and a common mortality for the whole area, was selected for all years, except 2002. For the 2002 survey, a model without mortality was applied because an estimate for mortality resulted in non-coherent (positive) mortality.
- 2. Spawning fraction estimates were obtained using a non-parametric bootstrap for the southern and western coasts in 2011 and for the north in 1999.
- 3. Batch fecundity for the south and west in 2011 was attained using the original dataset of hydrated females from that year.

For the French coast, spawning-stock biomass was estimated applying:

- 1. a non-parametric bootstrap in 1999 for the spawning fraction;
- 2. models of batch fecundity in 1999, 2002, and 2008 with data from the northern Atlantic Spanish coast;
- 3. a model without mortality in 1999, since an estimate for mortality led to non-coherent (positive) mortality.

Considering the entire Atlanto-Iberian Peninsula (Figure 2.1.8), SSB estimates showed higher values for the last three surveys of the series than in the first half. The lowest value was observed in 2002, while the highest estimation occurred in 2008. These variations may be partially related to population demography. Following an important recruitment in 2000, fish sampled in 2002 were mostly < 2–3 years, whereas the 2008 survey sampled older individuals.

Regarding each stratum separately, SSB estimates (Figure 2.1.7 and Table 2.1.3) showed noticeable temporal fluctuations in the south, with a particularly high estimate in 1999 (but with a very large confidence interval). Within the western and northern strata, SSB estimates also differed throughout the years of the series, but a common trend seems to come out with values that decreased during the 1990s and then increased to a peak in 2008 (observed also in the south) before subsequently falling. The SSB estimates decreased from 2008 to 2011 for all strata in the Iberian Peninsula, but this pattern was more perceptible for the western area. The SSB estimate for the French coast increased substantially in 2011 because of an increase in the area surveyed.



Figure 2.1.8. Estimates of spawning-stock biomass (SSB) for the Atlanto-Iberian sardine stock during 1997–2011 from the DEPM surveys (divisions 9.a and 8.c). Vertical lines indicate approximate 95% confidence intervals (i.e. ±2 s.d.).

Though the revised SSB estimates presented (ICES, 2012a) take a standardized analytical methodology into consideration, it is important to note that interannual natural biological variability as well as eventual mathematical constraints for observations in particular years may introduce specific biological or mathematical issues that could be approached in different ways; hence, more than one (correct) estimate may be produced using the dataset available. For example, different stratification options for P_0 or mortality or different approaches for fecundity modelling may lead to differences in SSB estimations. These aspects were discussed in ICES (2011a, 2012a).

2.1.4 Present and future challenges

Egg production methods (EPMs) are used worldwide to evaluate the spawning-stock biomass of a variety of fish species (Stratoudakis *et al.*, 2006), especially small pelagic species, but also demersal fish (Armstrong *et al.*, 2001; Jackson and Cheng, 2001). For small pelagic fish, EPMs together with acoustic estimates of biomass (Simmonds and MacLennan, 2005) are the fishery-independent methods most commonly used for biomass evaluation (Hampton, 1996; Barange *et al.*, 2009). EPMs assist in management by providing information that can be integrated into or used to inform the stock assessment process. The DEPM was adopted for Atlanto-Iberian sardines in the late 1980s (Miranda *et al.*, 1990; Cunha *et al.*, 1992; García *et al.*, 1992) and has been used for assessment ever since (ICES, 2012b). Methodological and analytical developments have reduced bias and improved the precision of the estimates (which are now being used in assessment modelling). The *CVs* for SSB calculated for the dataseries presented (revised in 2012) were mainly below or around 30%, within the error range for EPMs (Jacobson *et al.*, 1994; Armstrong *et al.*, 2001).

The Atlanto-Iberian sardine DEPM series is still short and because it is conducted triennially, it is more difficult to fully understand intersurvey fluctuations that could eventually have an effect during assessment model fitting. Moreover, when discrepancies between biomass estimation from acoustics surveying and DEPM are apparent, the stability of the assessment model may be affected. Non-concurrent estimation by both methods has precluded thorough discussion on the potential causes behind the differences in the estimates.

Future developments for the Atlanto-Iberian sardine DEPM will consider improvements in the estimations of mortality, e.g. external modelling (Bernal *et al.*, 2011b) and spawning fraction (stageing/ageing POFs, alternative estimators), assessment of the validity of single-survey estimations for *S*, *R*, and *F* vs. mean values or modeled estimates, calculation of SSB estimates at age, spatially explicit P_0 modelling, and the introduction of models with Bayesian approach and data assimilation to include yearly observations. Furthermore, interannual and between-strata variability of the DEPM parameters will be addressed in an integrated approach encompassing detailed environmental fluctuations, seasonal dynamics of reproductive activity, and population demography.

The monospecific nature of each EPM application has been criticized in the context of an ecosystem approach. However, EPM surveys are already providing, or have the potential to deliver, a range of products (e.g. biological indices, oceanographic data, foodweb indicators) to marine environment managers, particularly because of the obligation to comply with the Marine Strategy Framework Directive (MSFD), and a wealth of information for several scientific subjects (e.g. realized habitats, mortality, connectivity, reproductive potential, population fluctuations, and ecosystem modelling). There are good examples of the importance of egg production time-series in providing basic scientific data; the CalCOFI programme, for example, established in 1949, has promoted some of the most cited works in various aspects of marine pelagic ecosystems.

2.1.5 Acknowledgements

We acknowledge the work carried out at the research institutes IEO and IPMA as well as the crews of the research vessels used to collect the data. We are grateful to those who contributed to Atlantic sardine biological sampling in the laboratory, egg staging, histological processing of gonads tissues, otolith preparation, and age determination. Early surveys reported in this section were funded by each institute and national research funding bodies, but most of the recent surveys were funded through the EU Data Collection Framework (national programmes). Data collection coordination was carried out under the auspices of the ICES study and working groups SGSBSA and WGACEGG and the PELASSES research projects. The EU project SARDYN also introduced considerable developments to DEPM estimation results. We also thank the observers from SPEA who joined IPMA's surveys since 2005, adding value with the monitoring of birds and marine mammals. A special thanks to Miguel Bernal from IEO and Yorgos Stratoudakis from IPMA for their long contribution to the development of the sardine DEPM.

2.2 Spring acoustic surveys (PELAGO, PELACUS, PELGAS)

2.2.1 Introduction

Three acoustic surveys (PELGAS, PELACUS, and PELAGO) have been carried out from Brest to Gibraltar in the northern East Atlantic since the 1980s. Coordinated by WGACEGG since 2002, a great effort has been made to standardize the surveys so as to allow the merging of their results. As such, global maps of species distributions are provided for each year. In addition, from a specific assessment objective, despite the different target species by surveys (sardine for Portugal and Spain or anchovy for France), the institutes moved rapidly towards a pelagic community objective by fishing to identify echoes (whatever the species was suspected to be) instead of fishing only to obtain biological parameters when the target species was evident.

Three countries have been involved in the spring acoustic surveys:

- France (IFREMER): PELGAS surveys
- Spain (IEO): PELACUS surveys
- Portugal (IPMA): PELAGO surveys

The global transect network can be seen in Figure 2.2.1 based on the 2010 surveys. Transects are perpendicular to the coast and cover the entire shelf, which is wider in the Bay of Biscay than along the Portuguese and Spanish coasts. Protocols of these surveys are found in Annex 2.

The pelagic trawl fishing operations carried out during the three surveys (Figure 2.2.2) show, from one year to another, the heterogeneity of species along the shelf, from Brittany to Gibraltar.

The particularities or evolution of these acoustic spring surveys are described in the following sections.



Figure 2.2.1. Global transect network of PELGAS, PELACUS, and PELAGO acoustic surveys (2010 surveys shown as an example).



Figure 2.2.2. Species compositions by pelagic trawling during PELGAS, PELACUS, and PELAGO surveys from 2003 to 2012. Colour coding: light purple = boarfish; green = anchovy; dark purple = blue whiting; blue = sardine; red = mackerel; black = sprat; yellow = horse mackerel; grey = herring.

2.2.2 PELGAS surveys

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2.2.2.1 Introduction

IFREMER has conducted acoustic surveys in the Bay of Biscay since the early 1980s. The intent was to develop the method and also describe the distribution of pelagic species.

Acoustic surveys are carried out every spring in the Bay of Biscay on board the RV "Thalassa". Te objective of the PELGAS surveys has been to study the abundance and distribution of pelagic fish in the Bay of Biscay. The main target species are anchovy and sardine, but they are considered in a multispecies context and within an ecosystem approach as these species are located in the centre of a pelagic ecosystem.

These surveys are connected with IFREMER programmes on data collection for monitoring and management of fisheries and the ecosystem approach for fisheries. This task is formally included in the first priorities defined by Council Regulation (EC) No. 199/2008 of 6 November 2008 (EC, 2008), establishing the minimum and extended Community programmes for the collection of data in the fisheries sector and laying down detailed rules for the application of Council Regulation (EC) No. 1543/2000 (EC, 2000). These surveys must be considered in the framework of the IFREMER fisheries ecology action" resources variability", which is the French contribution to the international GLOBEC programme.

Historically, IFREMER has been involved in acoustic surveys since the 1970s and developed a numerical interface and software (INES-MOVIES) in 1988 in order to save echotraces and energies from a SIMRAD EK400 echosounder. A series of surveys was then developed in the southern Bay of Biscay (1989–1994), mainly in order to produce anchovy abundance indices for stock management considerations. In 1996, a new research vessel (also called RV "Thalassa") was available with considerable possibilities for the collection of data on a large group of parameters: acoustics, biology, behaviour, environmental factors. Therefore, aiming to discover the factors influencing anchovy recruitment, two surveys were carried out (1997 and 1998) in different seasons to analyse both the possibilities of this new research platform and factors of interest.

2.2.2.2 Survey methodology

In 2000, the first PELGAS survey was carried out, assembling IFREMER scientists as well as national and international collaborations in an acoustic survey designed to obtain abundance indices for the main pelagic species from the Spanish coast to the western point of Brittany, and also to collect relevant oceanographic data aimed at deriving ecosystem descriptors. Because of the common vertical migration and scattering behaviour of fish (mainly anchovy) close to the surface at night, it is difficult to separate fish from plankton inside a dense layer of plankton. Furthermore, being located in the surface acoustic blind zone, an unknown quantity of fish became inaccessible to the acoustic equipment. Therefore, priority was given to acoustic exploration and fishing operations by day. All parameters collected along transects during surveying were acceptable, and night-time was free for other operations even at stations (mainly physical, chemical, and plankton observations). Therefore, in order to produce an optimum horizontal and vertical description of the area, surveys have been carried out following the same transects and station network since 2000 (Figure

2.2.3) in order to characterize the pelagic ecosystem at each trophic level. This was achieved through two types of action:

- 1. Daylight: continuous acquisition along transects of acoustic data, seawater characteristics, and number of fish eggs by pumping under the surface using a CUFES system (continuous underway fish egg sampler). Concurrently, visual counting and identification of cetaceans (from the vessel) and birds (by plane) is carried out in order to characterize the higher-level predators of the pelagic ecosystem.
- 2. Night-time: discrete sampling at stations (by trawls, plankton nets, CTD) of the whole water column.



Figure 2.2.3. Acoustics, surface hydrology, and CUFES are operated along transects (in red), and vertical profiles are implemented at stations (blue stars) during PELGAS surveys on board RV "Thalassa".

Satellite imagery (sea surface temperature and chlorophyll concentration derived from MODIS sea colour) and modelling are also used before and during the cruise to recognize the main physical and biological structures and to improve the sampling strategy.

As specified previously, acoustic data are only collected during daylight in systematic parallel transects perpendicular to the French coast at ten knots. The length of the ESDU (elementary sampling distance unit) is one mile, while the transects are uniformly spaced by twelve nautical miles and cover the continental shelf from 20 m to the shelf break. Surveying is never interrupted except when echoes appear to be different and/or abundant enough to require an identification haul by pelagic trawling.

Acoustic energies corresponding to fish (NASC) are sorted on board and classified in echotrace categories in order to associate types of echoes with the species (or group of species) observed in trawl catches (see protocol and Table 2.2.1). These categories are defined according to school characterization and level in the water column. Over the years, school behaviour has partly changed; for example, anchovies appearing as small schools 20–30 m above the seabed during the 1990s (Massé *et al.*, 1996) had almost disappeared by the early 2000s when fish were more scattered and not even accessible to purse-seiners. After seven years of poor recruitment, including the closure of the fishery in 2005–2010, the recovered population displayed new behaviour wherein anchovies appeared both as small schools as in the 1990s and increasingly as small dense schools at the surface.

Since 2007, commercial pairtrawlers have been accompanying RV "Thalassa" to conduct additional identification pelagic hauls. Indeed, their experience and particular way of fishing with two boats make identification of echoes more efficient, particularly when fish are close to the surface. This does not influence the quantity of echoes measured (NASC), but increases the quality of identification (Figure 2.2.4). Moreover, working together has significantly enhanced mutual understanding and the acceptance by commercial fishers of future scientific advice. Seven years of cooperative work has considerably increased the suitability of acoustic abundance indices and dialog with stakeholders.

Year	Nautical miles	Area surveyed (nautical miles)	Sardine area >0 (nautical miles)	Anchovy area >0 (nautical miles)	Total hauls	RV "Thalassa" hauls	Commercial vessel hauls
2003	1 734	19 950	3 500	5 700	59	59	0
2004	1 818	20 900	5 100	2 900	51	51	0
2005	1 381	15 900	6 200	1 450	41	41	0
2006	1 355	15 600	4 650	2 800	46	46	0
2007	1 447	16 650	4 500	4 900	144	48	96
2008	1 695	19 500	7 800	3 800	111	57	54
2009	1 877	21 600	7 500	3 550	116	63	53
2010	2 074	23 850	5 350	2 850	104	66	38
2011	2 073	23 850	2 550	11 000	127	67	60
2012	1 724	19 800	3 000	5 200	96	53	43

Table 2.2.1. Number of operations and areas covered during PELGAS surveys.

Three modes of acoustic observations are stored during PELGAS surveys:

- six split-beam vertical echosounders (EK60) with frequencies of 18, 38, 70, 120, and 200 kHz (adding 333 kHz since 2009);
- one horizontal echosounder on the starboard side for surface echotraces (120 kHz);
- one SIMRAD ME70 multibeam echosounder (32 × 2°beams, 70–120 kHz), used essentially for visualization to observe the behaviour and aggregation pattern of fish schools during the survey; only echoes stored from the vertical echosounder (38 kHz) were used for abundance index calculation.



Figure 2.2.4. Fishing operations and catch composition during PELGAS12 by RV "Thalassa" alone (left) and with pairtrawlers (right).

Energies and samples provided by all sounders are simultaneously visualized and stored using the MOVIES+ and MOVIES3D software packages and stored at the same standard hydroacoustic data format (HAC). Sea surface temperature, as well as surface salinity and fluorometry are also stored simultaneously.

NASC are allocated by species according to echotype classification and catches, applying TS–length relationships from the literature (see Table A2.1).

The calibration method was the same every year and performed at anchorage, usually in the Concarneau bay, in the South of Brittany, in optimum meteorological conditions at the end of the survey.

Since 2003, top predators have also been observed because of the collaboration of PELAGIS (La Rochelle University, previously called CRMM), during both PELGAS and PELACUS surveys. Therefore, marine mammals, birds, and litters are simultaneously located, identified, and counted (see Section 2.2.5).

During night-time stations (Figure 2.2.3), vertical profiles are carried out with associated CTD and laser optical plankton counter (LOPC) followed by vertical fishing with a $315-\mu$ WP2 plankton net. Samples are filtered and preserved for further

investigation. Although about 40 parameters are presently collected, the following 18 have been systematically collected since 2003 for primary and secondary production characterization:

- bottom temperature
- vertical integrated chlorophyll
- potential energy deficit
- number of anchovy eggs (per 10 m³)
- number of sardine eggs (per 10 m³)
- maximum chlorophyll (< 3 μ)
- maximum chlorophyll (3 μ < n < 20 μ)
- maximum chlorophyll (>20 μ)
- maximum chlorophyll (total)
- depth of maximum chlorophyll
- depth of pycnocline
- surface chlorophyll (>20 μ)
- surface chlorophyll (< 20 μ)
- surface chlorophyll (3 μ < n < 20 μ)
- maximum chlorophyll (total)
- surface salinity
- surface temperature
- weight of dry mesozooplankton

2.2.2.3 Results

Following the completion of surveys, available abundance indices for the main species (Table 2.2.2) are provided to ICES working groups (successively WGMHSA, WGWIDE, WGHANSA) in charge of stock assessments (Figure 2.2.5). Distribution maps are provided for all collected parameters (adults and eggs of sardine, anchovy, and other species, surface temperature, salinity, fluorometry, marine mammals, birds). Population age structure is also provided, based on age determinations made on board from examination of otoliths collected for the target species (anchovy and sardine).



Figure 2.2.5. Abundance indices for the main pelagic species during PELGAS surveys.

Whilst the relative abundance indices provided for anchovy are obtained for nearly the entire distribution area (Figure 2.2.6), the indices of sardine should be regarded as

partial since the potential sardine distribution area at the time of the surveys can extend to more northern areas (Figure 2.2.7). Sardine distribution would, therefore, be representative of only that part of the stock present in the area covered at the time of the survey.

Table 2.2.2. Acoustic biomass indices (t) and coefficients of variation provided for the main pelagic fish species during the PELGAS surveys.

Species	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Anchovy	30 632	45 965	14 643	30 877	40 876	37 574	34 855	86 354	142 601	186 865
CV	0.13	0.17	0.17	0.14	0.10	0.16	0.11	0.15	0.08	0.05
Sardine	111 234	496 371	435 287	234 128	126 237	460 727	479 684	457 081	338 468	205 627
CV	0.24	0.12	0.14	0.12	0.16	0.14	0.10	0.09	0.07	0.08
Sprat	23 994	15 807	72 684	30 009	17 312	50 092	112 497	67 046	34 726	6 417
CV	0.20	0.18	0.23	0.16	0.13	0.27	0.11	0.11	NA	NA
Horse mackerel	198 528	186 046	181 448	156 300	45 098	100 406	56 593	11 662	61 237	7 435
CV	0.14	0.29	0.16	0.32	0.07	0.46	0.09	0.19	NA	NA
Blue whiting	1 953	12 267	26 099	1 766	3 545	576	4 333	48 141	11 823	68 533
CV	0.13	0.20	0.59	0.21	0.15	0.25	0.22	0.07	NA	NA

Nevertheless, for both species (anchovy and sardine), cohorts can be followed over the years as shown in Figure 2.2.8. For sardine, cohorts can be traced for up to five years, even if the abundance index is not accurate. This means that, despite the partial coverage of the spatial distribution by PELGAS, the index provides relevant information on the sardine stock dynamics, particularly with respect to the level and contribution of the recruitment to the total stock over years.



Figure 2.2.6. Distribution of anchovy (NASC) during PELGAS acoustic surveys.



Figure 2.2.7. Distribution of sardine (NASC) during PELGAS acoustic surveys.



Figure 2.2.8. Age distribution of anchovy (left) and sardine (right) as observed during PELGAS surveys.

Since the beginning of the series (2000), anchovy and sardine eggs were counted during daylight (Figure 2.2.9) using a continuous underway fish egg sampler (CUFES) (Checkley *et al.*, 1997). This system was acquired as a result of special funding in the framework of the PELASSES European project. It was first attached with a stick at 3.5 m depth along the hull and, since 2003, has been attached directly to the hull by an internal adaptation at 5 m depth. The mesh in the CUFES collector was initially 500 μ , but, following a suggestion by AZTI, two parallel CUFES were used (500 and 315 μ) from 2003 until 2006, when a 315- μ collector was finally adopted. Samples were collected every 3 nautical miles along transects and preserved in 4% formaldehyde for further processing.



Figure 2.2.9. Cumulative number of eggs 10 m⁻³ counted during PELGAS surveys for anchovy (green on the right) and for sardine (blue on the left). The sampling unit was 3 nautical miles.

2.2.2.4 Acknowledgements

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2.2.3 PELACUS surveys

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2.2.3.1 From SARACUS to PELACUS: Spanish acoustic surveys

Although echosounders for fish detection have been used by the Spanish commercial fleets since the 1950s, their use for scientific purposes only began in the 1980s. At the beginning of this decade, the Instituto Español de Oceanografía (IEO), together with its Portuguese counterpart Instituto Nacional de Investigação das Pescas (INIP, then IPIMAR, and now IPMA) started a series of projects targeting sardine, with the main

goal of improving biological knowledge and providing a fishery-independent index for its assessment (ICES, 1980).

In this context and, given the behaviour of the sardine shoals, scientific echosounders coupled with digital echointegrators were used following the methodology proposed by Dragesund and Olsen (1965) to achieve an assessment based on echointegration estimates (Midttun and Nakken, 1968; Nakken and Dommasnes, 1977).

The first challenge was to determine the relationship between target strength (TS) and length for sardine. *In situ* TS measurements and cage trials were carried out, although given both the physiological and biological proximity between sardine and herring (*Clupea harengus*) and also the similarity in results, the TS–length relationship estimated by Degnbol *et al.* (1985) for herring was finally adopted (ICES, 1988).

The first joint acoustic survey took place in August 1982 (Table 2.2.3) on RV "Noruega" (Portugal) and RV "Cornide de Saavedra" (Spain). This first survey covered only the Atlantic waters of the Iberian Peninsula, i.e. Galician (northwestern Spain) and Portuguese waters, targeting mainly juvenile sardines to achieve an estimate of recruitment strength. Both vessels were equipped with Simrad EK-400 scientific echosounders working at 38 kHz (in addition, RV "Cornide de Saavedra" had a second echosounder working at 120 kHz) coupled with a Simrad QD+QX digital echo integrator.

In order to standardize methods and analyse data, the Planning Group for Acoustic Surveys in ICES Subareas VIII and IX met for the first time in 1986 (ICES, 1986a). The group adopted a value of b₂₀ = –72.6 dB for sardine and, in addition, biomass estimates were established at fixed strata (this methodology had already been in use in previous years). The surveyed area was divided into sectors of ca. 10–30 nautical miles width, depending on geographical and oceanographic characteristics. Each sector was divided into depth strata (20–50, 50–100, 100–200, and 200–500 m). Within each sector, at least one track perpendicular to the coast (i.e. crossing all depth strata) was included. For practical reasons, rather than random allocation, a systematic survey design, either in zigzag or parallel, was adopted. Biomass was then estimated for each sector and depth stratum according to Nakken and Dommasnes (1977), using the mean (either arithmetic or geometric) integrated value, the area expressed in square nautical miles, and the length distribution provided from the neighbouring fishing stations. In cases when fishing stations were not available, length distributions from commercial purse-seine fleets were used.

The first acoustic survey covering the waters off the northern and northwestern Spanish coast (Atlantic and Cantabrian waters) was carried out in August 1983. This survey targeted young of the year sardine and was the starting point of the SARACUS (SARdine ACoUStic in Spanish waters) survey series. From 1983 to 1985, the surveys were conducted in August. In 1986, a new strategy was adopted to increase survey effort. Two SARACUS surveys took place: the first one in spring to obtain an estimate of sardine spawning biomass and the second in November to obtain an estimate of recruitment strength. While Portugal performed both surveys for several years, IEO focused its efforts only in spring after 1987. All surveys from 1983 to 1990 were carried out on RV "Cornide de Saavedra", except in 1990 when RV "Ignat Pavliuchenvok" was used due to the refitting of the Spanish vessel. For the same reason, there was no survey in 1989.

In 1991, IEO purchased the new Simrad EK-500, a fully compact digital echosounderecho integrator. This equipment, with better dynamic range and control settings, allowed the surveyed area to be extended to a depth of 1000 m. Although this improvement gave access to the main distribution area of new commercial target species such as horse mackerel (Trachurus trachurus), Atlantic mackerel (Scomber scombrus), blue whiting (Micromesistius poutassou), and anchovy, only anchovy and blue whiting, together with sardine, were routinely assessed. Accordingly, the timeseries changed its name to PELACUS (PELagic ACoUStic in northwestern Spanish waters). Survey durations increased to deal with these new objectives. The number of fishing stations increased as well, with new pelagic gear being used, CTD being deployed, and, as in previous years, acoustic data were collected both during day and night. In 1993, the assessment methodology was changed and, instead of estimates been calculated for fixed strata, estimates were based on the true distribution area. However, for comparison purposes, results were given by ICES division (divisions 9.a North, 8.c West, 8.c East [western part], and 8.c East [eastern part]). During 1994–1996, the survey design and objectives were again changed and, although SEFOS (Shelf Edge Fisheries and Oceanography Studies) in 1994 and 1996 took place in spring, both surveys focused on blue whiting distribution. This objective meant that the area surveyed covered mainly the shelf-break area from the Spanish–Portuguese border to Brittany. The acoustic estimate of sardine abundance and biomass was again the objective of the survey carried out in 1995 (IBERSAR 95) that took place on RV "Noruega".

From 1997 to 2012, PELACUS was carried out on RV "Thalassa", a new French-Spanish vessel. This modern ship presented advantages over the older vessels (silent diesel-electric engine and fixed-blade propeller) together with improved fishing facilities that resulted in a notable increase in the number of survey objectives. This vessel was also used for the French acoustic survey (PELGAS). Since 1998, the Planning Group for Pelagic Acoustic Surveys in ICES Subareas VIII and IX, attended until then only by Spanish and Portuguese scientists, incorporated French scientists (ICES, 1999). As a first joint recommendation, the Planning Group agreed that acoustic data would only be recorded during daylight, leaving the night-time available for physical, chemical, and plankton characterization of the water column. This recommendation was implemented in 1998. In 2000, under the framework of the DG FISH PELASSES project, the spring acoustic surveys incorporated the CUFES together with the routine collection of other systematic measurements (e.g. SSS, SST, fluorometry, CTD rosette casts, plankton hauls to determine primary production, or dry weight at different sizes among other biological descriptors of the water column). In addition, 120-kHz frequency began to be used to help discriminate between different fish species. During this period, acoustic estimates were also provided for non-commercial species.

In 2005, RV "Thalassa" was equipped with the new EK60 together with a series of new transducers (18, 70, and 200 kHz).

In 2007, a new team used the survey as a platform to obtain data on presence, abundance, and behaviour of top predators (marine mammals and seabirds). Since 2007, data have also been routinely collected on floating litter (type, number, and position) and on other human pressures such as fishing (e.g. number of boats, type, activity).

_				Target		Survey	Depth
Survey	Year	Month	RV	species	Equipment	design	(m)
PILCHARD	1982	August	CS	PIL	EK-400	Parallel	30–500
SARACUS	1983	August	CS	PIL	EK-400	Parallel	30-500
SARACUS	1984	August	CS	PIL	EK-400	Parallel	30–500
SARACUS	1985	August	CS	PIL	EK-400	Parallel	30–500
SARACUS	1986	March	CS	PIL	EK-400	Parallel	30-500
SARACUS	1986	September	CS	PIL	EK-400	Parallel	30–500
SARACUS	1987	February	CS	PIL	EK-400	Parallel	30–500
SARACUS	1988	April	CS	PIL	EK-400	Parallel	30-500
IBERSAR ¹	1989	November	Ν	PIL	EK-400	Parallel	30-500
SARACUS	1990	April–May	IP	PIL	EK-400	Parallel	30-500
PELACUS	1991	March	CS	PIL, WHB	EK-500	Zigzag	30-1 000
IBERSAR ²	1991	November	Ν	PIL	EK-400	Parallel	30-500
PELACUS	1992	April–May	CS	PIL, WHB	EK-500	Zigzag	30–1 000
PELACUS	1993	April–May	CS	PIL, WHB	EK-500	Zigzag	30-1 000
SEFOS	1994	March–April	CS	WHB	EK-500	Zigzag	150-1 000
IBERSAR	1995	May	Ν	PIL	EK-500	Parallel	30–500
SEFOS	1996	March–April	CS	PIL, WHB	EK-500	Parallel	30-1 000
PELACUS	1997	March	Т	All	EK-500	Parallel	30-1 000
PELACUS	1998	March	Т	All	EK-500	Parallel	30-1 000
PELACUS	1999	March	Т	All	EK-500	Parallel	30-1 000
PELACUS	2000	March	Т	All	EK-500	Parallel	30-1 000
PELACUS	2001	April	Т	All	EK-500	Parallel	30-1 000
PELACUS	2002	March	Т	All	EK-500	Parallel	30-1 000

Table 2.2.3. Acoustic surveys undertaken before 2003 in northwestern Spanish waters.

¹Suspended due to bad weather conditions. ²Only divisions 8.c West and 9.a North. Research vessels: "Cornide de Saavedra" (CS), "Noruega" (N), "Ignat Pavliuchenkov" (IP), "Miguel Oliver" (MO). Pelagic species: Sardina pilchardus (PIL), Micromesistius poutassou (WHB), pelagic community (AII).

Since the beginning of the time-series (1982), biological data (length, weight, sex, maturity, etc.) and samples obtained from individual fish caught in hauls have been used to construct length–weight and age–length relationships needed for the assessment of sardines and all other target species. Fish stomachs have also been routinely examined to quantify the trophic relationships between species, and isotope analysis of sardine and anchovy muscle have been also carried out to study their trophic position.

The evolution of the survey design and objectives has transformed this survey series into an essential platform for integrated data collection following the requirements posed by the ecosystem approach to fisheries management, the Marine Strategy Framework Directive (2008/56/CE), and the revised CFP.

Acoustic data presented in this report include estimates of abundance, distribution, and mean size for the eleven main pelagic species found in northern and northwestern Spanish waters.

2.2.3.2 Methods

The survey design consists of a grid of parallel transects, eight nautical miles apart and perpendicular to the coastline, and covering the continental shelf up to a depth of 200 m. The starting point of each transect is located close to the coast (1–1.5 nautical miles from the shoreline), although the exact location can be modified due to adverse weather conditions or the presence of shallows. The end point of each transect can be also extended if shoals are detected in deeper waters. Table 2.2.4 and Figure 2.2.10 show the main features of the survey series.

Table 2.2.4. Acoustic surveys undertaken during 2003–2012 in northwestern Spanish waters.

Survey	Year	Month	RV	Target species	Equipment	Survey design	Depth (m)
PELACUS	2003	March– April	Т	All	EK-500	Parallel	30–200
PELACUS	2004	April	Т	All	EK-500	Parallel	30-200
PELACUS	2005	April	Т	All	EK-60	Parallel	30–200
PELACUS	2006	April	Т	All	EK-60	Parallel	30-200
PELACUS	2007	April	Т	All	EK-60	Parallel	30–200
PELACUS	2008	April	Т	All	EK-60	Parallel	30–200
PELACUS	2009	April	Т	All	EK-60	Parallel	30–200
PELACUS	2010	April	Т	All	EK-60	Parallel	30–200
PELACUS	2011	April	Т	All	EK-60	Parallel	30–200
PELACUS	2012	April	Т	All	EK-60	Parallel	30–200



Figure 2.2.10. Spanish acoustic transects (in black) and ICES divisions (9.a N; 8.c W; 8.c EW [western part]; 8.c EE [eastern part]) considered for abundance estimation (in red).

Acoustic records were obtained during daylight together with egg samples from a CUFES, with an internal water intake located at 5 m depth. In addition, pelagic trawl hauls were performed opportunistically to verify the acoustic data.

2.2.3.2.1 Acoustic equipment

Acoustic equipment consisted initially of a Simrad EK-500 and then a EK-60 scientific echosounder, first operating at 38 and 120 kHz, and then increasing the number of frequencies used since 2005 (including 18, 70, and 200 kHz). All frequencies were calibrated according to standard procedures. The elementary sampling distance unit (ESDU) was fixed at 1 nautical mile. Acoustic data were obtained only during daylight at a survey speed of 8–10 knots. Data were stored in raw format and post-processed using Sonar Data Echoview software (Myriax Ltd.). Fish abundance was calculated with the 38 kHz frequency as recommended by the Planning Group on Aerial and 00Acoustic Surveys for Mackerel (ICES, 2002b), although echograms from 18, 120, and 200 kHz frequencies were used to visually discriminate between fish and other scatterproducing objects such as plankton or bubbles, and to distinguish different fish according to the strength of their echo at each frequency. The 38, 70, and 120 kHz frequencies were also used to create a mask, allowing better discrimination among fish species and plankton. The threshold used to scrutinize the echograms was -70 dB. The integration values were expressed as nautical area scattering coefficient (NASC) units or acoustic area backscattering coefficient (s_A) values (m² nautical mile⁻²) (MacLennan et al., 2002).

2.2.3.2.2 NASC allocation

Several pelagic gears were used during the survey series, both to identify species and size classes responsible for the acoustic energy detected and to provide samples. The choice of net was also dependent on the availability of rather unobstructed ground over which the net could be deployed and recovered, allowing for effective fishing. Haul duration was variable and ultimately depended on the number of fish entering the net and the conditions under which the fishing took place, with attempts being made to always achieve a minimum of 20 min. fishing. The quality of the hauls for verifying acoustic data was classified according to catch composition in numbers and length distribution of the fish caught.

Hauls considered to best represent the fish community for a specific area were used to allocate NASC of each ESDU within this area. This process involved the method of Nakken and Dommasnes (1977) for multiple species, but instead of using the mean backscattering cross section, the full length class distribution (0.5 cm length classes) were used as follows:

$$s_{A_i} = s_A \frac{w_{li} \cdot \sigma_{bs}}{\sum_{li} w_{li} \cdot \sigma_{bs}} \tag{2.2.1}$$

where w_{B} is the proportion in number of the length class *l* and species *i* in the hauls, and σ_{bs} is the corresponding proportion of backscattering cross section. This is related to the target strength (TS) as follows:

$$\sigma_{bs} = 10^{TS/10} (\text{in dB}) \tag{2.2.2}$$

This is computed from the formula $TS = 20 \log L_T + b_{20}$ (Simmonds and MacLennan, 2005), where L_T is the length class (0.5 cm). The b₂₀ values for the most important species present in the area are shown in Table A2.2.

2.2.3.2.3 Echo integration estimates

With the backscattering energy allocated to fish species, the spatial distribution for each species was analysed, taking into account both the NASC values and the length frequency distributions (LFD) to provide homogeneous assessment polygons. These were calculated as follows: an empty track determines the along-coast limit of the polygon, whilst three consecutive empty ESDUs determine a gap or the across-coast limit. Within each polygon, LFD is analysed.

LFD were obtained for all positive hauls for a particular species (either from the total catch or from a representative random sample of 100–200 fish). For the purpose of acoustic assessment, only those LFD which were based on a minimum of 30 individuals and/or a normal distribution were considered. Differences in probability density functions (PDF) were tested using the Kolmogorov–Smirnoff (K–S) test. PDF distributions without significant differences were joined, providing homogeneous PDF strata. Spatial distribution was then analysed within each stratum and, finally, the mean sA value and the surface (nautical miles²) were calculated using the GIS systembased ArcGis 9.3. These values, together with the length distributions, were used to calculate fish abundance in numbers, as described in Nakken and Dommasnes (1977). Numbers were converted into biomass using the length-weight relationship. Biomass estimation was carried out for each stratum (polygon) using the arithmetic mean of the backscattering energy (NASC, sA) attributed to each fish species and the surface expressed in nautical miles². For purposes of comparison, results are given by ICES divisions (9.a North, 8.c West, 8.c East [western part], 8.c East [eastern part], and 8.b; see Figure 2.2.10).

Otoliths were taken from anchovy, sardine, horse mackerel, blue whiting, hake (*Merluccius merluccius*), and Atlantic mackerel in order to determine age and to obtain the age–length key for each species and area.

Other biological variables such as maturity, stomach repletion, content and colour, and fat content are routinely registered for sardine, Atlantic mackerel, horse mackerel, hake, blue whiting, and other pelagic species.

2.2.3.2.4 CUFES counts

Samples from CUFES were collected every three nautical miles during acoustic surveys of the transects. After the sample was taken, it was fixed in a buffered 4% formaldehyde solution. Anchovy and sardine eggs were sorted, counted, and preserved in the same solution. The remaining ichthyoplankton (other eggs and larvae) were also preserved in the same way.

2.2.3.2.5 Plankton and hydrological characterization

Continuous records of SSS, SST, and fluorometry were taken using a SeaBird Thermosalinograph coupled with a Turner fluorometer. Plankton and CTD + bottle rosette for water sample casts were performed at night. Five stations of the acoustic survey were placed over the transects, but were extended onto open waters until the 1000–2000 m isobaths. The stations were evenly distributed over the surveyed area at a distance of 16–24 nautical miles.

Plankton was sampled using several nets (Bongo, WP2, and CalVet). Fractionated dried biomass was calculated at 53–200, 200–500, 500–1000, and >2000 μ m fractions, together with species composition and taxonomic groups at fixed strata from samples collected at the CTD + bottle rosette carrousel (pico and nanoplankton, microplankton,

and mesozooplankton). For this purpose, FlowCAM, LOPC, and Zoo-Image techniques were used.

Water samples were stored at -20°C for further analysis of dissolved nutrients (NO₃, NO₂, P, NH₄⁺, SiO₄).

Top predator observations: three observers, working in turns of two, are placed above the bridge of the vessel at a height of 16 m above sea level, surveying an area of 180° (each observer covers a field of 90°). Observations are carried out by sight, although binoculars are used (7 × 50) to confirm species identification and determine predator behaviour. Observations are carried out during daylight while the vessel surveys the transects and covers the distance between transects at an average speed of 10 knots. Observers record species, number of individuals, behaviour, distance to the vessel, angle to the trackline, and observation conditions (windspeed and direction, sea state, visibility, etc.). Observers also record presence, number, and type of boats and type, size, and number of floating litter. The same methodology is used on the PELGAS surveys, and both observer teams shared a common database.

2.2.3.3 Results and discussion

The pelagic fish community in northwestern Spanish waters, as derived from PELACUS (2003–2012), is mainly characterized by sardine, Atlantic mackerel, and horse mackerel, together with bogue, blue whiting, and boarfish (*Capros aper*), whilst anchovy, Mediterranean horse mackerel, chub mackerel, and blue jack mackerel have only appeared occasionally. Besides, since the 1990s, the number of direct echotrace allocations to species from the echogram scrutiny has decreased, mainly due to a change in the aggregation pattern. This change, together with the change in survey objectives to assess the main pelagic fish species, has led to an increase in the number of fishing stations for echotrace identification (shown in Table 2.2.5 and Figures 2.2.11 and 2.2.12), aimed at increasing precision in the echotrace analysis.

Sardine abundance estimated in PELACUS in ICES divisions 9.a N and 8.c is used as a relative fishery-independent index for assessment purposes. This index shows a decreasing trend during the time-series in both abundance and distribution area (Figures 2.2.13–2.2.17). The increase in 2008 was mainly due to the strength of the 2004 year class (Figure 2.2.18).



Figure 2.2.11. Species composition by pelagic trawl station in PELACUS (2003–2008).



Figure 2.2.12. Species composition by pelagic trawl station in PELACUS (2009–2012). Se Table 2.2.6 for species coding.

Year	Survey start	Survey end	Target species	Echosounder	Fishing operations	Sardine- positive fishing operations
2003	20 March	10 April	Pelagic community	EK-500	30	13
2004	30 March	23 April	Pelagic community	EK-500	31	21
2005	6 April	28 April	Pelagic community	EK-60	45	20
2005	3 April	26 April	Pelagic community	EK-60	52	22
2007	29 March	22 April	Pelagic community	EK-60	49	16
2008	27 March	22 April	Pelagic community	EK-60	56	35
2009	28 March	20 April	Pelagic community	EK-60	59	25
2010	27 March	20 April	Pelagic community	EK-60	62	25
2011	27 March	20 April	Pelagic community	EK-60	51	10
2012	29 March	20 April	Pelagic community	EK-60	54	6

Table 2.2.5. Number of fishing hauls carried out in PELACUS (2003–2012).

Year	MAC	PIL	НОМ	BOC	BOG	BWH	MAS	ANE	JAA	HMM	TOTAL
2003	368 894	123 297	32 188	36 163	10 326	12 213	2 546	0	0	1 039	586 667
2004	620 726	114 638	32 297	51 770	10 612	3 263	395	102	0	1 927	835 730
2005	541 659	77 191	43 075	53 516	17 008	9 465	803	0	0	1 101	743 817
2006	110 214	70 617	16 910	54 612	18 353	2 161	588	0	342	0	273 797
2007	152 575	97 895	31 289	30 576	15 409	4 832	7 154	2 791	2 297	0	344 819
2008	333 685	139 076	36 497	15 345	28 982	4 624	3 617	3 225	8 416	0	573 468
2009	286 362	42 254	23 214	25 130	27 306	6 583	4 552	102	5 298	1 317	422 118
2010	732 203	39 964	51 420	17 045	21 038	10 419	3 606	225	775	3 388	880 082
2011	354 068	11 629	9 450	27 187	26 984	4 833	12 757	2 702	464	8 547	458 622
2012	244 809	17 377	18 264	33 238	15 640	7 164	0	52	0	253	336 798

Table 2.2.6. Biomass (tonnes) estimated for the pelagic species of the pelagic community. Coding of the species: PIL = Sardina pilchardus, ANE = Engraulis encrasicolus, MAC = Scomber scombrus, HOM = Trachurus trachurus, MAS = Scomber colias, SPR = Sprattus sprattus, BOC = Capros aper, BOG = Boops boops, HMM = Trachurus mediterraneus, JAA = Trachurus picturatus, BWH = Micromesistius poutassou.



Figure 2.2.13. Sardine biomass in the Spanish area during the spring survey series (2003–2012).



Figure 2.2.14. Sardine abundance (no. individuals in millions) in the Spanish area during the spring survey series (2003–2012).



Figure 2.2.15. Sardine area distribution in the Spanish area during the spring survey series (2003–2012).



Figure 2.2.16. Sardine density areas in the Spanish area during the spring survey series (PELACUS 2003–2007).



Figure 2.2.17. Sardine density areas in the Spanish area during the spring survey series (PELACUS 2008–2012).



Figure 2.2.18. Sardine age distribution during the PELACUS survey series (2003-2012).

Abundance by age for sardine is reflected in Figure 2.2.18, where abundance of the 2004 cohort can be clearly followed year after year until 2008, when high abundance of sardines at age 4 produced high biomass for this species (Figure 2.2.13).

Younger sardines (ages 1 and 2) are mainly located in Atlantic waters (Division 9.a North), whereas the older ages used to be found in the inner part of the Bay of Biscay, as shown in Figure 2.2.19. Nevertheless, and contrary to what was observed in the 1990s, the proportion of younger sardines found in some years in the inner part of the Bay of Biscay was also noticeable. Whether these sardines came from ICES Division 8.b (French waters) or were hatched in Division 8.c is still unknown.

Atlantic mackerel, representing about 69% of the total biomass, is generally the most abundant fish species estimated in PELACUS surveys (Table 2.9 and Figures 2.2.20 and 2.2.21). As in the case of sardine, younger mackerel are mainly located in Division 9.a North.



Figure 2.2.19. Sardine age distribution by ICES subdivision during the PELACUS survey series (2003–2012).



Figure 2.2.20. Biomass (tonnes) estimated for Atlantic mackerel (MAC) and for the rest of the pelagic fish community (Other Pelagic fish) during the 2003–2012 pelagic surveys.


Figure 2.2.21. Mackerel spatial distribution during PELACUS (top, 2008–2012 cruises; bottom, 2003–2007 cruises). Polygon colours indicate the mean backscattering energy range (sA values, m² nmi⁻², 1–10; 11–30; 31–50; 51–100; and >100).

Since 2007, there has been some increase in perciforms, such as bogue, Mediterranean horse mackerel, and others (Table 2.2.6; Figure 2.2.22), although this was not sustained in 2012.

Anchovy distribution in the Bay of Biscay is mainly outside the PELACUS coverage in Division 8.c. Only in the years when the southern part of ICES Division 8.b was surveyed was anchovy found in relative high densities, as shown in Figure 2.2.23. The PELACUS survey demonstrates that the presence of anchovy is scarce and in low relative density off the northern Spanish coast.



Figure 2.2.22. Biomass (tonnes) estimated for nine pelagic species during the 2003–2012 pelagic surveys.



Figure 2.2.23. Anchovy distribution area and relative density (NASC) during PELACUS (2004–2012).

Concerning the rest of the pelagic species detected during the PELACUS surveys, a potential bias exists for horse mackerel, which is a bentho-pelagic species. Parts of the schools are potentially undetected because some horse mackerel may be present within the bottom acoustic blind zone. However, in general, most of the schools are expected to be accessible to the echosounders. Younger fish are found in shallower waters, whilst the adults are mainly located close to the slope extending over the shelf. No clear distribution pattern along the surveyed area is found, as shown in Figure 2.2.24.

Blue whiting is mainly located around the shelf break, but contrary to the spatial pattern shown in northern European areas, rather than forming pelagic layers from the slope towards more oceanic waters at depths of 200–500 m, the fish extends its distribution area in Spanish waters towards the continental shelf (Carrera *et al.*, 2001). Therefore, although PELACUS only surveys the continental shelf (from 30 to 200–250 m), it covers part of its potential distribution area. In addition, mean size estimated during PELACUS is ca. 18–20 cm, which is similar to that found in the surveys which target this species (Carrera *et al.*, 2001), and 8–10 cm less than the size of adult fish. As shown in Figure 2.2.25, the main distribution area is located in the northwestern corner (ICES Division 7.c West).



Figure 2.2.24. Horse mackerel distribution area and relative density (NASC) during PELACUS (2008–2012).



Figure 2.2.25. Blue whiting distribution area and relative density (NASC) during PELACUS (2003–2012).

Although acoustic surveys in northwestern Spain were primarily targeted on sardine, improvements in survey strategies, equipment (including new research vessels such as RV "Thalassa"), and post-processing analysis made PELACUS an ideal platform for collecting multiple types of oceanographic data, both at fixed stations and through continuous recordings. These data were used in the beginning to improve the precision of fish abundance estimates by echo integration, but also allowed detailed integrated monitoring of pelagic communities and ecosystems. Data on abundance, distribution, and behaviour of pelagic species can be collected systematically and geo-referenced at the same temporal and spatial scales as environmental data. Future work, which will deal with the Marine Strategy Framework Directive (MSFD) challenges, will include the comparison between pelagic communities of different areas and their evolution

over time as well as the development of indices and coupled models to analyse the state of the pelagic ecosystem.

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2.2.4 PELAGO surveys

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2.2.4.1 Historical perspective and objectives of the Portuguese acoustic surveys

The Portuguese acoustic surveys began in August 1982. The first survey was a joint exploratory survey, together with IEO, covering the Iberian sardine stock and carried out with RV "Noruega" and RV "Cornide Saavedra". During 1984–1988, the Portuguese surveys were systematically performed in spring and autumn/winter each year. After no surveys in 1989–1991, two sporadic surveys were carried out in 1992 and 1995, the latter, with a Portuguese and Spanish scientific team, covering all Iberian Atlantic waters. The acoustic surveys resumed with a systematic periodicity effective in 1996. The Portuguese spring acoustic surveys have always been funded by the Portuguese Ministry of Fisheries; between 2000 and 2002, they also benefited from an EU Project (PELASSES). Since 2002, they have been cofunded by the EU Data Collection Framework (DCF).

Surveys were carried out mainly on RV "Noruega", except in July 1996, August 1999, and June 2004 when they were performed with RV "Capricórnio".

The survey area has been extended to the Spanish part of the Gulf of Cádiz since 1992, but was not covered in 2004.

The main objective of the Portuguese spring acoustic surveys has been the abundance estimate for the part of the Atlanto-Iberian sardine stock inhabiting the Portuguese shelf. Additionally, the biological data collected provides information on the population demography (numbers and biomass per length and age).

Since 1999, anchovy abundance has also been obtained from these surveys. This species is mainly distributed off the southern coast from Cape Santa Maria to Gibraltar (Division 9.a South).

2.2.4.2 Material and methods

2.2.4.2.1 Changes in methodology and equipment during the time-series

The acoustic equipment used before 1995 was a Simrad EK38S echosounder with 38and 120-kHz frequencies and a Simrad QM analogic echo-integrator. Since 1995, a Simrad EK 500 has been used. Since 2000, the IFREMER MOVIES+ (Weill *et al.*, 1993) software has been used to acquire and process the acoustic data. Previously, the echograms were registered on paper, and the acoustic energies were extracted visually, for each nautical mile. The integration deflection line on the echogram was read with a ruler.

Before 1996, a large pelagic trawl net (20-m vertical opening) was used. This net was replaced by a smaller net (10-m vertical opening) to allow fishing operations in shallow waters.

Acoustic coverage became systematic only after 1997, following a parallel grid perpendicular to the coastline with a separation of eight nautical miles between transects, where a "zig-zag" grid had previously been applied. In addition, before 1997, the acoustic coverage was done continuously during daylight and night-time, whereas it has subsequently been done only by day to allow distinguishing between pelagic schools in the echograms. In all the surveys, fishing trawl hauls have been done during daylight, whereas night-time has often been used to perform hydrology and plankton stations.

Since 2000, a CUFES system (Checkley *et al.*, 1997) has been used in the acoustic surveys for underway surface (ca. 3 m) plankton sampling (every three nautical miles) with the main objective to describe sardine and anchovy spawning grounds. A SeaBird thermosalinographer and a SeaPoint fluorometer are coupled to the CUFES system allowing continuous recording of SST, SSS, and SSF (fluorescence, ~chlorophyll). Information from the TCF probes is compiled, together with GPS data, by dedicated software (EDAS). High-resolution data acquisition on egg abundance, temperature, salinity, and fluorescence allow the production of detailed distribution maps that assist in environmental characterization and spawning area definition.

Regarding the TS for sardine, the relationship $TS = 21.7 \log L - 75.5 dB$ (Haldorsson and Reynisson, 1983) was used until 1986; later, the relationship $TS = 20 \log L - 72.6 dB$ (Degnbol *et al.*, 1985) was used for sardine, following the recommendations of the Planning Group for Acoustic Surveys in ICES Subareas 8 and 9 (ICES, 1986a). The TS relationships for the other main pelagic species were also recommended by this Planning Group. The TS relationship for anchovy has changed from $TS = 20 \log L - 71.2 dB$ to $TS = 20 \log L - 72.6 dB$, becoming similar to that for sardine, in the 2007 and subsequent surveys. After discussing TS values at the WGACEGG 2007 meeting, it was decided that the anchovy TS could not be higher than the sardine TS. The relationship used before for anchovy ($TS = 20 \log L - 71.2 dB$) was the same as used for North Sea herring and was an average of several TS measurements. As such, the reported series for anchovy make use of two different TS.

The following target strength b_{20} (20 logL – b_{20}) relationships are currently used in the Portuguese acoustic surveys:

Sardina pilchardus (PIL): 72.6 dB Scomber colias (MAS): 68.7 dB Scomber scombrus (MAC): 82 dB Trachurus trachurus (HOM): 68.7 dB Trachurus picturatus (JAA): 68.7 dB Boops boops (BOG): 67.0 dB Engraulis encrasicholus (ANE): 72.6 dB Micromesistius poutassou (WHB): 80 dB Macroramphosus spp. (SNS): 80 dB

The spring surveys referred to in this section (covering the period 2003–2012, though the Portuguese spring survey did not take place in 2012 due to technical problems) were performed in the consolidated phase, when the methodology was already well established. Although the main objective of these spring surveys is to estimate the abundance of sardine and anchovy, there has been an increasing concern in light of the ecosystem approach, and fishing trawls aim at catching different fish school aggregations, not just sardine and anchovy. As a result, the abundance and distribution of other pelagic species have been estimated whenever possible. Since 2005, the observation and monitoring of marine birds and mammals has also been carried out during the acoustic surveys (within the framework of projects in collaboration with SPEA and SPVS). The night-time (when acoustic surveying ceases) is used for hydrology and zooplankton surveying.

2.2.4.2.2 Equipment used in the PELAGO series

A Simrad EK 500 – 38 kHz echosounder, with a split-beam transducer $8^{\circ} \times 7^{\circ}$ (equivalent beam angle: $10 \log \psi = -20.2 \text{ dB}$; pulse duration = 1 ms) calibrated prior to the survey, is used. For data storage and post-processing, the software Movies+ (© IFREMER) is used.

Calibration of the acoustic instruments, using standard procedures, is performed before each acoustic survey (Foote *et al.*, 1987).

A pelagic trawlnet (10-m vertical opening) and a bottom trawlnet are used in the fishing stations to collect information on species distribution and biological data. The trawl data are used to help identify the echoes and to split the acoustic energy NASC into species and length classes. A Scanmar Netsounder (trawl-eye and depth sensor) is used in the fishing operations.

2.2.4.2.3 Sample design in the PELAGO series

The total surveyed area is divided into four zones to allow between-year comparison (Figure 2.2.26): Occidental North – OCN (Caminha to Nazaré), Occidental South – OCS (Nazaré to Cape St. Vicente), Algarve – ALG (St. Vicente to Vila Real Santo António), and Cádiz – CAD (Vila Real Santo António to Cape Trafalgar).

The surveyed area is covered using a parallel systematic grid, with transect separation of eight nautical miles on the west coast and six nautical miles in Algarve; in Cádiz, the transects are perpendicular to the coastline, making them not perfectly parallel, and divided by around eight nautical miles in the middle of the radials (Figure 2.2.26). The acoustic survey is done only during daylight. The ESDU used for NASC collection is one nautical mile. The survey vessel speed is 9–10 knots.

2.2.4.2.4 Abundance estimates

The RV "Noruega" has a draft of four metres. The echo integration is made using a surface blind depth (from transducer) of 3 m, but depending on weather conditions, it can increase up to 10 m. The bottom blind zone height is fixed at 0.2 m. When needed, the bottom contour in the echograms is manually corrected to avoid bottom integration.

The acoustic energy (NASC) is split into species according to the proportion of species (in numbers) in the fishing trawls, taking into account the species TS when direct energy allocation is not possible (for details, see the acoustics protocol in Annex 2).



Figure 2.2.26. Portuguese acoustic transects and subareas used for abundance estimation.

There is post-stratification in coherent areas (i.e. areas with similar species length composition). Area density is calculated using the arithmetic mean.

Abundance estimate is calculated in numbers of individuals by length class in each coherent area. Hauls are combined in this area, usually without weighting each haul differently.

Biomass estimation is calculated using weight–length relationships obtained from fishing samples.

Estimated abundance by age groups is calculated using age–length (total length) keys produced from the species otoliths obtained from the biological sampling on board.

2.2.4.3 Results and discussion

Until 2007, sardine was the predominant species on the Portuguese continental shelf north of Lisbon and the other pelagic species were distributed more southerly, mainly in the Algarve and Cádiz areas. Since 2007, the diversity and mixture of species extended to the entire Portuguese coast (Figure 2.2.27). At the same time, the decrease in sardine abundance has been noticeable (Table 2.2.7 and Figure 2.2.28) in recent years. The sardine distribution area has also decreased during this time (Figure 2.2.29). Figure 2.2.30 shows the change in sardine acoustic density and geographic distribution.



Figure 2.2.27. Species trawl composition in the PELAGO survey series (2007–2011). Species FAO key code.



Figure 2.2.28. Sardine biomass (thousand tonnes) in the surveyed area during the spring survey series. Cádiz area was not covered in the 2004 survey.



Figure 2.2.29. Sardine distribution area (nautical miles²) during the spring survey series. Cádiz area was not covered in the 2004 survey.



Figure 2.2.30. Sardine NASC distribution (2003–2011). Circle sizes are proportional to the acoustic energy.



Figure 2.2.30 (continued). Sardine NASC distribution (2003–2011). Circle sizes are proportional to the acoustic energy.



Figure 2.2.30 (continued). Sardine NASC distribution (2003–2011). Circle sizes are proportional to the acoustic energy.

The sardine stock biomass is mainly sustained by recruitment strength (Figure 2.2.31). During 2003–2011, the strongest recruitment took place in 2004, which resulted in a biomass increase in 2005 and 2006 (Table 2.2.7 and Figure 2.2.32).



Figure 2.2.31. Sardine age distribution during the PELAGO survey series.

Survey	OCN	OCS	Algarve	Cádiz	Portugal	Total
						aica
SAR03FEV	153	145	60	73	359	432
SAR04JUN	240	60	39	-	339	-
SAR05ABR	286	199	62	40	547	587
SAR06ABR	370	138	40	89	548	637
PELAGO07	215	89	40	107	344	452
PELAGO08	170	13	26	35	209	244
PELAGO09	112	84	14	84	210	294
PELAGO10	125	43	11	26	179	205
PELAGO11	90	15	20	2	125	127

Table 2.2.7. Sardine biomass (thousand tonnes) in each zone, Portugal, and total surveyed area during the spring acoustic surveys in 2003–2011.

The main anchovy distribution area was in the Gulf of Cádiz from Cape Santa Maria to Cape Trafalgar, with some spots off the west coast near Figueira da Foz and Lisbon. In 2011, there was exceptional recruitment of anchovy in the north, and the traditional distribution pattern changed for this species as a result of very few anchovies found in the south (Table 2.2.8).

Table 2.2.8. Anchovy estimated biomass (tonnes) for the West coast (Division 9.a OCN and Division 9.a OCS), South coast (Division 9.a South), and total surveyed area.

Survey	West	South	Total
February 2003	112	24 677	24 789
April 2005	1 062	14 041	15 103
April 2006	0	24 082	24 082
April 2007	1 945	38 020	39 965
April 2008	5 500	34 200	39 700
April 2009	2 000	24 800	26 800
April 2010	1 188	7 395	8 583
April 2011	27 050	0	27 050

2.2.4.3.1 Sardine and anchovy egg distributions

Sardine and anchovy egg density spatial distributions and total abundance by area (from CUFES sampling) during 2003–2011 are presented in Figures 2.2.32–2.2.34. Sardine eggs, in agreement with fish biomass distribution, are usually more abundant on the west coast (larger area with higher number of samples), with hot spots appearing on the northwestern shelf and off Promontório da Estremadura (just north of Lisbon); in the south, higher numbers generally occur in the inner Bay of Cádiz. On the contrary, anchovy eggs are more common on the southern coast, particularly towards the east, where a regular population is maintained. The peak spawning period for sardine is centred in winter, whereas southern anchovy is a summer spawner, which is reflected in the low abundance of anchovy eggs observed during the

Portuguese acoustic survey that usually takes place in early to mid-spring. The 2003 survey was conducted earlier in the season, starting in February, while the 2004 campaign, due to logistic constraints, was conducted in June after the normal acoustics season.

Sardine total egg abundance followed a similar pattern for the western and southern areas, except for 2005–2006. During this period, high adult (predominantly young adults) biomass was observed, particularly in the west. The trends in adult biomass and egg densities over time are not in close agreement. High adult sardine biomass was observed until 2007. High egg densities were observed in 2003, but then decreased in 2005–2006, before a very pronounced increase in 2008. The peak in high egg abundance in 2008 over the entire surveyed area was not observed in adult acoustic abundance, but was highlighted in the DEPM egg production (and fecundity) of that year and was also observed off the Cantabrian shores (Section 2.1). 2008 had a particularly mild winter–spring season, with high primary productivity (data not shown). After 2008, the trend in egg abundance and adult fish is more comparable. However, egg abundance during the last reported survey showed a small increase that was not observed in adult sardines, but egg occurrence was very localized (particularly in the northern region).

During the reported period, sardine egg spatial distribution varied substantially, with years of very patchy distribution, particularly on the west coast. Egg abundance fluctuations are very likely related not only to the abundance of adult fish, but also to the demography of the population, its reproductive dynamics, and environmental conditions. Sea surface temperature is higher in the south (ca. 14–19°C) than on the west coast (ca. 13–16°C; see maps in sections 3 and 4) and suffers less fluctuations. During the period reported, the more recent years were slightly warmer, particularly in the south (also with high winter/spring river runoff); in 2003 and 2009, sea surface temperatures were lower than during the remaining years.

During IPMA acoustic surveys in spring, egg densities for anchovy are lower than for sardine, not only because the sardine and anchovy populations have very distinct levels of abundance, but also because the survey generally occurs early in the anchovy spawning season. Despite this fact, in 2010 and 2011, egg abundance was higher for anchovy than for sardine. Furthermore, egg observations have shown that echosounding detection of anchovy in the Bay of Cádiz was not consistent with egg densities every year because the highest egg abundance was found in 2010 and 2011 when the acoustic anchovy estimates were minimal. In addition, it has been shown that egg production estimates in the region from the DEPM increased in recent surveys (Section 2.5). During the years of high anchovy biomass, egg densities (generally higher in the south) are also noticeable on the west coast in areas other than the regular patch off Lisbon.



Figure 2.2.32. Sardine egg density (eggs m⁻³) distribution during the acoustic surveys (2003–2011) derived from the CUFES sampler (equipped with a 500- μ m mesh net in 2003–2004 and a 335- μ m mesh net in 2005–present).



Figure 2.2.33. Anchovy egg density (eggs m⁻³) distribution during the acoustic surveys (2003–2011) derived from the CUFES sampler (equipped with a 500- μ m mesh net in 2003–2004 and a 335- μ m mesh net in 2005–present).





Number of CUFES stations												
Area/Year 200		2004 2005		2006 2007		2008	2009	2010	2011			
West	269	272	321	331	313	349	324	330	315			
South	159	108	150	146	159	151	167	160	159			

Figure 2.2.34. Sardine (top panel) and anchovy (bottom panel) egg densities (sum of egg m⁻³) per stratum (West, green; South, blue) from CUFES sampling along the acoustics transects during 2003–2011 and number of samples (unit: 3 nautical miles) collected per survey and area. The 2004 survey was not conducted in the usual season (in June, and only covered the Portuguese shores). CUFES was equipped with a 500-µm mesh net in 2003–2004 and a 335-µm mesh net in 2005–present).

2.2.4.4 Acknowledgements

We acknowledge all of the IPIMAR/IPMA staff who contributed to the work presented here, as well as the crew of the research vessels used to collect data. Early surveys were funded by the national research budget and since 2002 were cosponsored by the EU Data Collection Framework. Special thanks to Carlos Afonso Dias, the real pioneer of the fisheries acoustic surveys in Portugal.

2.2.5 Seabirds and marine mammals

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Seabirds and marine mammals are upper trophic level predators. As such, they are thought to be reliable indicators of ecosystem health and functioning, assuming bottom–up forcing. Any impact of lower trophic levels may reverberate up the foodweb and affect top predators. These charismatic species can be monitored at sea (cetaceans and seabirds) or on land during the breeding season (seabirds and pinnipeds). However, the highly mobile species are difficult to monitor across large, but ecologically meaningful, areas. This report illustrates a rare case of fruitful collaboration across several countries to map the distribution and relative abundance of marine mammals and seabirds over several successive years.

Marine mammals and seabirds were recorded along transects performed during the three spring surveys PELAGO, PELACUS, and PELGAS. However, monitoring protocols were slightly different between PELAGO and the two other surveys.

For PELAGO, the methodology is that of the European Seabirds at Sea (ESAS), with more emphasis put on monitoring seabirds. Two observers carried out a visual search for cetaceans and seabirds within an angle of 180° ahead of the ship's bow. Upon detection, species identification is made with the aid of binoculars. Snapshot censuses of seabirds and marine mammal presence and activities are made every 5 min within a 300-m transect parallel to the ship's direction.

For PELGAS and PELACUS, the methodology used is that of continuous recording of seabirds and marine mammals from a single platform line transect survey. Two observers searched with the naked eye for cetaceans and seabirds within an angle of 180° ahead of the ship's bow. Upon detection, species identification is made with the aid of binoculars. For birds and cetaceans, distance and angle from the ship's main route are recorded based on distance sampling methodologies. Any seabird detected within a 1000-m transect (or less depending on observation conditions) parallel to the ship's direction and spanning an angle of 180° ahead of the ship's bow is recorded.

To account for the slightly different methodology between PELAGO and PELACUS/PELGAS, the numbers of detected marine mammals and seabirds are standardized by transect length (in nautical miles). Data are a linear density of seabirds and marine mammals.

Additionally, crude taxonomic groupings were made (see top predators protocol in Annex), except for two easily identified and abundant species (northern gannets [*Morus bassanus*] and common dolphins [*Delphinus* spp.], labelled "Bird_Gannet" and "Mam_CommonDolphin", respectively). These crude groupings are all seabirds (including rare and vagrant species labelled "Birds") and all marine mammal species (excluding sperm whales [*Physeter macrocephalus*], labelled "Mam"). Within seabirds, a further functional distinction was made between diving (labelled "Bird_Div") and surface-feeding (labelled "Bird_Surf") species. Within these two groups, rare and vagrant species were excluded. Within marine mammals, balenopterids (labelled "Mam_BaleenWhales") and delphinids (labelled "Mam_Dolphins") were further distinguished. Compared to the larger marine mammal grouping, these two subgroups exclude beaked whales. The pooled observations from the three surveys were gridded and are presented in sections 3 and 4.

2.3 Anchovy DEPM surveys 2003–2012 in the Bay of Biscay (Subarea 8): BIOMAN survey series

María Santos, Andrés Uriarte, Guillermo Boyra, and Leire Ibaibarriaga

2.3.1 DEPM BIOMAN survey series

The BIOMAN survey series began in 1987, aiming to improve the direct monitoring and assessment of the Bay of Biscay anchovy population, for which no internationally agreed assessment had been made until then (Santiago and Sanz, 1992a; Motos, 1994; Uriarte et al., 1996). The main objective was the estimation of the spawning-stock biomass of the Bay of Biscay anchovy by applying the daily egg production method (DEPM; Lasker, 1985). In addition, the survey aimed to improve the knowledge on the spawning environment (Motos et al., 1996) and reproductive biology of anchovy (Sanz and Uriarte, 1989; Sanz et al., 1992; Motos, 1996; Alday et al., 2008, 2010). The survey soon collaborated with other ichthyoplankton national and international surveys for the estimation of spawning stocks of other pelagic species such as sardine (García et al., 1992), Atlantic mackerel, and horse mackerel (Ibaibarriaga et al., 2007a). Today, BIOMAN collaborates with the triennial egg surveys for these species concerning coverage of the Bay of Biscay (ICES, 2011a). Currently, the principal objective of the survey is the same, but other objectives have been added to try to revalue the survey within the European Marine Strategy Framework Directive as hydrographic characterization, acoustic estimates, stomach content of fish species, zooplankton distribution, genetics of fish, microchemistry of otoliths.

The survey has always been funded by the Department of Fisheries of the Basque Government within Spain. Moreover, since 1989, most of the surveys have been directly supported by EU projects funded by the European Commission. In 2002, the survey was included in the list of EC-funded research surveys at sea within the Community framework for the collection and management of data needed to support the Common Fisheries Policy and has since been carried out in that context [Council Regulations (EC) No. 1543/2000 and No. 199/2008]. The survey is also being supported by the Spanish General Secretariat of Sea providing the RV "Emma Bardán" since 2003.

The first two surveys in 1987 and 1988 were made autonomously by AZTI (Santiago and Sanz, 1992a; Sanz *et al.*, 1992; Motos and Uriarte, 1992); since then, most of the surveys have been in the context of international projects and in collaboration with other institutes, particularly IFREMER and IEO. The annual spawning-stock biomass (SSB) estimates obtained applying the DEPM have been referred to the successive ICES working groups through working documents (ICES, 2004, 2006a, 2006b, 2007, 2008; Santos *et al.*, 2009, 2010, 2011a, 2012, 2013a) and were summarized in contributions to conferences (Ibaibarriaga *et al.*, 2005; Motos *et al.*, 2005), publications (Santos *et al.*, 2011b), or collective publications on the application of the method to anchovies in European areas (Somarakis *et al.*, 2004).

In this section of the report, we discuss the application of the DEPM to the Bay of Biscay anchovy during 2003–2012, which is only part of the entire historical series (1987–2014). In this period, the anchovy population passed from a period of very low biomass (2005–2009), associated with a fishery closure for five years, to a recovery up to its historical maximum in 2011 (Santos *et al.*, 2013a).

2.3.2 Material and methods

2.3.2.1 DEPM and application to the Bay of Biscay anchovy

For the DEPM, the anchovy spawning-stock biomass estimate is derived according to Parker (1980) and Picquelle and Stauffer (1985) from the ratio between daily production of eggs in the sea and the daily specific fecundity of the adult population:

$$SSB = \frac{P_{tot}}{DF} = \frac{P_0 \cdot A + k \cdot R \cdot F \cdot S / W_f}{k \cdot R \cdot F \cdot S / W_f}$$
(2.3.1)

where *SSB* is spawning-stock biomass in tonnes, P_{tot} is the total daily egg production in the sampled area, P_0 is the daily egg production per surface unit in the sampled area, A+ is the spawning area in sampling units, and *DF* is the daily specific fecundity:

$$DF = \frac{k \cdot R \cdot F \cdot S}{W_f} \tag{2.3.2}$$

Where W_f is the average weight of mature females in grams, R is the sex ratio by weight, F is the batch fecundity or numbers of eggs spawned per mature females per batch, S is the fraction of mature females spawning per day, and k is the conversion factor from grammes to tonnes (10⁶).

An estimate of an approximate variance and bias for this biomass estimator is derived by the delta method (Seber, 1982; Picquelle and Stauffer, 1985).

For the Bay of Biscay, the method is extended to produce spawning population (in numbers) at age estimates (SSP_a) , as follows (Uriarte, 2001):

$$SSP_a = SSP \cdot P_a = (SSB/W_t) \cdot P_a \tag{2.3.3}$$

where *SSP* is the total spawning-stock estimate in numbers (= *SSB/W*_t), W_t is the mean weight of anchovies in the population, and P_a is the relative frequency (proportion in numbers) of age *a* in the population.

Variance estimate of the anchovy stock in numbers-at-age and total is derived applying the delta method (Uriarte, 2001).

Obtaining the estimate of the different parameters is achieved first through egg and adult sampling at spawning time in May and second through laboratory analysis of those samples and analysis of the results of samples to produce joint estimates of the parameters and final SSB. For this population, as all anchovies are fully mature at the spawning peak (May), SSB equals total population biomass.

2.3.2.2 Surveys 2003–2012

All surveys were carried out in May, covering the anchovy spawning area in the Bay of Biscay. This area extends over the southeastern area of the Bay, with typical limits at 5°W along the Iberian coast and at 48°N along the French coast (Figure 2.3.1). In order to get the estimates of the daily egg production and daily specific fecundity, egg and adult sampling took place simultaneously on board different vessels. In addition, the cooperation of the commercial fishing fleet provided opportunistic adult samples in several years (Table 2.3.1).



Figure 2.3.1. Predetermined stations of the vertical hauls (PairoVET) that could be performed during the surveys.

				PV	CUFES	Total area	Positive	Adult	Fishing	Selected	
Year	Name	Dates	Egg vessel	no.	no.	(km²)	area (km²)	vessel	hauls	no.	Reference
2003	BIOMAN 2003	22 May– 9 June	RV "Investigator"	503	1 122	70 041	42 915	OPT, CPS	36	36	Santos <i>et al.</i> (2004)
2004	BIOMAN 2004	2–17 May	RV "Vizconde de Eza"	352	656	53 285	20 365	OPT, CPS	47	30	Santos <i>et al.</i> (2004)
2005	BIOMAN 2005	8–28 May	RV "Vizconde de Eza"	414	900	61 619	27 863	OPT	30	20	Santos <i>et al.</i> (2005)
2006	BIOMAN 2006	4–24 May	RV "Vizconde de Eza"	404	798	53 110	24 614	OPT	43	37	Santos <i>et al</i> . (2006)
2007	BIOMAN 2007	3–23 May	RV "Investigator"	420	775	56 079	34 449	OPT	44	34	Santos <i>et al.</i> (2007)
2008	BIOMAN 2008	6–26 May	RV "Investigator"	544	1 175	69 150	33 502	OPT	39	29	Santos <i>et al</i> . (2008)
2009	BIOMAN 2009	5–25 May	RV "Investigator"	409	949	60 733	28 214	OPT	38	31	Santos <i>et al</i> . (2009)
2010	BIOMAN 2010	5–20 May	RV "Investigator"	484	1 035	61 940	37 633	OPT, CPS	34	30	Santos <i>et al</i> . (2010)
2011	BIOMAN 2011	6–28 May	RV "Investigator"	699	1 200	98 405	69 094	OPT, CPS	52	40	Santos <i>et al.</i> (2011a)
2012	BIOMAN 2012	10–29 May	RV "Ramón Margalef"	529	1 156	80 381	38 974	OPY	42	24	Santos <i>et al</i> . (2012)

Table 2.3.1. Some characteristics of DEPM surveys BIOMAN during 2003–2012. More details in references ICES WGACEGG. OPT (oceanographic pelagic trawler), CPS (commercial purse-seiner).

Egg sampling was done in vertical plankton tows, deploying PairoVET nets (Smith et al., 1985) every three nautical miles along transects perpendicular to the coast and separated by fifteen nautical miles. Although in areas of high egg abundance or where historically high egg production is found, additional transects separated by 7.5 nautical miles were completed. Typical areas of high egg abundance usually appear around the Gironde area and the southeastern corner of the Bay of Biscay, near the inflow of the Adour river. In addition, station distance along transects was switched from three to six miles when eggs were absent, particularly when moving towards offshore areas at the edge of spawning areas. This adaptive sampling along transects, moving offshore to delimit the bounds of spawning, was helped by the use of the concurrent CUFES sampling (Checkley et al., 1997; details in Annex 2). This adaptive sampling strategy allows, on one hand, adequate definition of the spawning area limits and, on the other, increasing sampling intensity in areas where heavy spawning occurs during the cruise. This leads to a gain in precision regarding egg counts at the cost of a slight downward bias (Petitgas, 1997). CUFES sampling was introduced in this survey in 1998 and has since been routinely applied all along vessel tracks every 1.5 miles at a depth of 3 m aside the deck. Although no quantitative use is made, CUFES has been kept for the implementation of the adaptive sampling scheme. Initially, its plankton collector had a 500- μ m net, but this was changed to 335 μ m in 2004 after realizing that some anchovy eggs were being lost through the former mesh size.

A typical survey deployed a mean of 475 PairoVET (range 352–699) and a mean of 977 CUFES (range 656–1200) nets over a mean sampling area of 68 000 km² (range of 50 000–100 000 km²; Table 2.3.1). Preservation of PairoVET samples and sorting of eggs from the plankton followed the standards that are detailed in Annex 2.

<u>Adult samples</u> were obtained by pelagic trawling on board research vessels all over the spawning area. Opportunistic samples were also obtained by the commercial purse-seine fleet. Samples were collected at any time throughout the day, parallel to the egg sampling, in areas with relevant egg densities and school detections. This "judgment sampling" usually tries to sample proportional to abundance, but assuring a sampling all over the spawning area. This usually does not achieve a balanced sampling proportional to abundance. Hence, some posterior checking of the proportionality of the adult sampling to spawning biomass in space may be required, especially when spatial heterogeneity in adult parameters is found. In this way the need of some spatial stratification or the application of differential sampling weighting factors can be addressed to get unbiased estimates (Santos *et al.*, 2011b). Between 30 and 52 fishing hauls were carried out in a typical survey; along with the opportunistic samples, this gave a selection of between 20 and 40 (average of 30) anchovy samples per survey for the estimation of the adult parameters (Table 2.3.1).

2.3.2.3 Egg production estimates

Eggs are staged according to the 11 stages of Moser and Ahlstrom (1985), and aged taking into account the sampling time and presumed incubation temperature of the eggs (taken as the temperature at a depth of 10 m from the CTD at each station). At the beginning of the series, ageing was done with the Lo (1985) development model using the *ad hoc* incubation experiments for this anchovy (Motos, 1994). However, the series presented here is fully updated and follows the Bayesian ageing method described in Bernal *et al.* (2011a), making use of the anchovy temperature-dependent egg development multinomial model fitted to the anchovy incubation experiments by Ibaibarriaga *et al.* (2007b). Afterwards, given the egg abundance by daily cohorts per sample, the daily egg production per unit area (P_0) is estimated together with the daily

egg mortality rate (*Z*) from the general exponential decay mortality model. This model is fitted using a generalized linear model with negative binomial distribution and logarithmic link (Stratoudakis *et al.*, 2006) and following the iterative procedure described in Bernal *et al.* (2011a). The P_0 estimates have been jointly produced for the entire positive spawning area without any post-stratification of the area.

When the bulk of spawning for the day is not over at the time of sampling, or when the cohort is so old that its constituent eggs had started to hatch in substantial numbers, these incomplete cohorts are removed from the model fit in order to avoid any possible bias. At each station, the youngest cohorts are dropped if they are sampled before twice the spawning peak width; after the spawning peak, the oldest cohorts are dropped if their mean age plus twice the spawning peak width is over the critical age at which < 99% of the eggs are expected to be still unhatched. In addition, the oldest cohorts at survey level are excluded; for that, the upper limit is set at the age in which 99% of the eggs are unhatched, having developed at the 50th quantile of the survey incubation temperature (weighted by egg abundance at each station; Santos *et al.*, 2013b).

After the final model estimates are obtained, the coefficient of variation of P_0 is given by the standard error of the model intercept ($log(P_0)$; Seber, 1982) and the coefficient of variation of *Z* is obtained directly from the model estimates. The analysis is conducted in R (<u>www.r-project.org</u>). The two libraries are used for different purposes, the "MASS" library for fitting the GLM with negative binomial distribution and the "egg" library (<u>http://sourceforge.net/projects/ichthyoanalysis/</u>) for the ageing and the iterative algorithm.

A typical fitted model corresponding to the 2012 survey is presented in Figure 2.3.2.



Figure 2.3.2. Exponential mortality model adjusted in 2012, applying a GLM to data obtained in the age estimation process following the Bayesian method (spawning peak 23:00 GMT). The red line is the adjusted line. Data in log scale.

2.3.2.4 Adult parameters and daily fecundity estimates

Mean weight of mature females (W_f) and sex ratio (R) per sample were obtained following standard procedures. As R in numbers is assumed to be 0.5, R in weight per sample is estimated as the ratio between the average female weight and the sum of the

average female and male weights of the anchovies in each of the samples. This is because, in the past, it was found that *R* in numbers was not significantly different from 50%.

For the batch fecundity (*F*), the hydrated oocyte method has always been followed (Hunter *et al.*, 1985). A model between the number of hydrated oocytes and the female gonad free weight is fitted to the available collection of hydrated females, using a generalized linear model with Gamma distribution and "identity" link. The model is subsequently applied to the mean female gonad-free weight of each sample to get the sample estimate of batch fecundity. Routine checking of potential spatial differences in the batch fecundity is made for all surveys, with particular attention to differences between the major nursery area, the Gironde area, where 1-year-old anchovies predominate, and the remaining areas. However, during 2003–2012, no spatial significant differences in the batch fecundity relationship was found, so a single relationship was obtained for the whole sampled area in each year. A typical fitting is shown in Figure 2.3.3, for the year 2009.



Figure 2.3.3. Generalized linear model between weight gonad-free and hydrated oocytes fitted to hydrated females in year 2009.

The estimation process of the spawning frequency (*S*) has been recently revised (Uriarte *et al.*, 2012), and the current work reports on the estimates resulting from this revision. The revision was based on the validation of post-ovulatory follicles (POF) degeneration stages with time, made for this population by Alday *et al.* (2008, 2010). It indicates a faster degeneration process of POFs than previously thought (Motos, 1996). Application of this knowledge to age estimate oocytes and POFs led to selection of an estimator of *S* per sample, based on the joint occurrence of Day 0 and Day 1 daily spawning cohorts of females (Uriarte *et al.*, 2012). The current estimates of *S* have an average of 0.4, which is about 60% higher than previous estimates reported for this population (Motos, 1996; Somarakis *et al.*, 2004; ICES, 2006b, 2007). This led to a downscaling by 38% of the series of SSB produced by this survey. For 2006, the analysis of the histological slides has not yet been done; hence, the long-term average spawning fraction was taken as the value of reference for *S* in this year.

Routine checking of potential spatial differences of the *S* estimates is made in all surveys, with particular attention to differences between the major nursery areas vs. the remaining areas. In several years, some differences were observed between the estimates of *S* in the Gironde area, where 1-year-old anchovies predominated over the remaining areas with lower *S* values in the former area, although the difference was

not statistically significant. Nevertheless, such spatial differences were taken into account, not to stratify the estimates, but to give differential individual weighting factors to the samples from each of the different areas in order to get unbiased estimates of the adult parameters. This happened in 2004, 2005, and 2009–2012.

In order to get individual weighting factors for the adult samples, egg abundance over large regions was taken as a proxy of spawning biomass, together with the number of samples by region (Santos *et al.*, 2011b). For the purposes of producing population-at-age estimates, a collection of about 60 otoliths per sample was obtained from most of the samples. If any sample did not have such a collection, the age–length key constructed from the rest of samples was applied to its length distribution to infer the age composition of the sample. Estimates of anchovy mean weights- and proportions-at-age in the population were the average of proportions-at-age in the samples, weighted by values proportional to the population each sample represents (Uriarte, 2001; Santos *et al.*, 2011b).

2.3.3 Results and discussion

On average, the surveys covered an area of around 67 000 km² (range of 50 000–100 000 km²), of which about 50% (range 40–70%) corresponded with the actual spawning area of anchovy, as evidenced by the occurrence of anchovy eggs. Thanks to the adaptive sampling scheme, the density of egg samples across the spawning area of anchovy was about one sample per 35 nautical miles², instead of the *a priori* standard density of one sample per 45 nautical miles².

The survey showed some regular areas with relevant spawning activities (a) around the coastal areas in front of the Gironde (all years) and Adour river mouths and the surrounding French coastal areas, as clearly seen in 2004, 2006–2008, and 2012, and (b) along the shelf, particularly in the southernmost part of the Bay, as seen in 2006, 2009, 2010, and 2012. The latter areas often excede the shelf edge, which, in some years, is separated from the more coastal areas, as in 2007, 2008, and 2011. These are the typical spawning areas, as previously described (Motos *et al.*, 1996, 2005), situated mainly in coastal regions and around the river mouths, that correspond to nursery areas with 1-year-old fish. Older fish, on the other hand, are more frequently found over the shelf and shelf-edge regions.



Figure 2.3.4. Series of biomass estimates (tonnes) obtained since 1987 with DEPM for anchovy in the Bay of Biscay. The 2013 value is taken from ICES (2013).

Anchovy egg abundance in 2003–2012 was low in comparison with historical series and reflected the poor levels of biomass of this species during the past decade (Figure 2.3.4). Maximum egg abundance occurred in 2011 when the spawning area also expanded offshore and north, making it the maximum positive area of the series (Figures 2.3.5 and 2.3.6).

Sardine spawning occurred typically all along the coastal continental shelf from the south to north of the Bay, in some years concentrating more at the northern areas, as in 2005, 2007, and 2011, and in others showing higher concentrations to the south, as in 2006 and 2009 (Figure 2.3.7). The overall tendencies of the sardine egg abundance series are parallel to the acoustic estimates of biomass from the PELGAS survey. Accordingly, the sardine egg abundance series has been considered an auxiliary source of information for the assessment of the tendencies of this population in the Bay of Biscay (ICES, 2013).

Egg spatial distributions obtained with CUFES at a depth of 3 m were similar to those produced by the vertical plankton sampler PairoVET (Figure 2.3.8).

Analyzing the whole historical series (1987–2013), it was found that the spawning area increased linearly with the spawning biomass estimates obtained through the DEPM (Figure 2.3.9). The model explains 69% of the total variability (p = 0). As mentioned by Somarakis *et al.* (2004), this is partly due to the area being part of the DEPM formulation, but the condition for it to be true is P_0 (daily egg production per surface unit) and daily fecundity (DF) being rather invariant parameters (Figure 2.3.5, Table 2.3.2).

Year	Dates	SSB	CV	DF	CV	F	CV	S	CV	R	CV	$W_{ m f}$	CV	P_{tot}	CV
2003	22 May–9 June	16 109	0.17	137.04	0.04	14 591	0.07	41.43	0.03	54	0.01	23.65	0.07	2.2E+12	0.17
2004	2–17 May	11 496	0.12	75.46	0.07	8 871	0.13	37.99	0.04	54	0.01	24.13	0.08	8.6E+11	0.09
2005	8–28 May	4 832	0.20	93.68	0.10	12 094	0.11	42.75	0.03	55	0.01	30.40	0.06	4.5E+11	0.18
2006	4–24 May	14 872	0.19	77.19	0.06	9 046	0.12	40.41	NA	54	0.01	25.46	0.08	1.1E+12	0.21
2007	3–23 May	13 060	0.18	95.95	0.05	11 897	0.08	39.93	0.04	54	0.01	26.56	0.07	1.2E+12	0.17
2008	6–26 May	12 898	0.20	135.22	0.04	14 692	0.09	49.62	0.02	54	0.01	29.15	0.08	1.7E+12	0.20
2009	5–25 May	12 832	0.14	119.99	0.05	14 097	0.09	42.82	0.02	57	0.04	28.45	0.08	1.5E+12	0.13
2010	5–20 May	31 277	0.16	71.57	0.10	8 353	0.09	34.21	0.05	53	0.00	21.21	0.05	2.2E+12	0.12
2011	6–28 May	135 732	0.16	71.00	0.09	6 990	0.08	34.62	0.06	55	0.02	18.86	0.06	9.6E+12	0.13
2012	10–29 May	26 663	0.20	79.16	0.08	8 901	0.09	35.10	0.08	53	0.01	20.92	0.07	2.1E+12	0.18

Table 2.3.2. SSB (spawning-stock biomass in tonnes), DF (daily fecundity, number of eggs per gramme of biomass), F (batch fecundity, number of eggs spawned per mature females per batch), S (spawning frequency, fraction of mature females spawning per day), R (sex ratio, fraction of population that is mature females by weight), W_f (female mean weight [g]), and P_{tot} (total daily egg production, eggs per day), actual estimates from 2003 to 2012 and their CV.

Regarding the adult reproductive parameters (Figure 2.3.5, Table 2.3.2), the sex ratio (R) in mass is basically a constant around its long-term mean of ca. 0.54, given our own assumption of an invariant sex ratio 1:1 in numbers. Female mean weight (W_f) peaks in years with poor levels of 1-year-old anchovies when the proportion of the 2+ age group becomes relevant, as in 2005, 2008, and 2009. An exception happened in 2012 when, despite the high occurrence of 2-year-old anchovies (50%), the mean weight did not increase markedly. This was due to abnormal low mean weights-at-ages (Figure 2.3.10).



Figure 2.3.5. Series of parameters needed to apply the DEPM from anchovy surveys in the Bay of Biscay: spawning area (km²), daily egg production (eggs m⁻²), total daily egg production (eggs d⁻¹), batch fecundity (number of eggs spawned per mature females per batch), sex ratio (fraction of population that are mature females by weight), females mean weight (g), spawning frequency (fraction of mature females spawning per day), total mean weight (g), daily fecundity (number of egg per gramme of biomass), and biomass in tonnes.



Figure 2.3.6. Anchovy egg abundance (egg m⁻²) distribution obtained with PairoVET during DEPM surveys in 2003–2012 in the Bay of Biscay.



Figure 2.3.7. Sardine egg abundance (egg m⁻²) distribution obtained with PairoVET during DEPM.



Figure 2.3.8. Some year examples of egg distribution obtained with CUFES, for sardine in years 2004, 2005, and 2006 and for anchovy in 2008–2012.



Figure 2.3.9. Relationship between spawning area (km²) and anchovy biomass estimates (tonnes) by the DEPM. Data from the whole historical series (1987–2013).



Figure 2.3.10. Series of (top) percentage-at-age and (bottom) weight-at-age (g) for anchovy in the Bay of Biscay from DEPM surveys BIOMAN.

As expected, batch fecundity (*F*) was closely related to the mean weight of females. Spawning frequency (*S*) tended to be around the historical mean of about 0.4, except in 2005 and for the last three years. In 2005, *S* peaked at 0.5, coinciding with a high mean weight of females as a result of the relevant occurrence of 2-year-old anchovies in the population. However, for the last three years, *S* stayed at about 0.34, below average, coinciding with the lower mean weights of the females during 2003–2012.

The relationship between *S* and *W_f* was perceived to be significant, but of little intensity (Uriarte *et al.*, 2012). However, for these last years, the intensity of this relationship seems to be greater than perceived in the past series. According to all these results, daily specific fecundity (*DF*) has varied during 2003–2012 between 70 and 140 in a parallel trend to the mean weight of females (correlation coefficient of 0.56 and *p* of 0.073 due to being random), peaking in 2003, 2008, and 2009 and being at minima for the last three years (2010–2012).

The *DF* in the Gironde area, where the smallest anchovies are found, has been slightly below that in the remaining areas in several years (2004, 2005, and 2009–2012).

Accordingly, in these years, the weighting factors per adult sample were differentiated according to the egg abundance in the area where they were obtained and to the number of adult samples by area. Even though the results of the *DF* did not vary much, depending or not on the applications of those weighting factors, they were prefered to avoid potential bias in the results. These results suggest that at the time of the survey, smaller anchovies around the Gironde area tended to have slightly smaller *DF* than the remaining bigger anchovies.

Mean weight and mean lengths by age showed no clear trend, although in 2012, the weights of the 1- and 2-year-old anchovies were the lowest in this series. At this stage, no study on potential ecological reasons for this shift in mean weights and *DF* in recent years has been done.

Biomass series, weight-at-age, and population in numbers-at-age are presented in Figures 2.3.5 and 2.3.10. Between 2003 and 2010, DEPM SSB estimates were below 20 000 t. During this period, the fishery had difficulties obtaining normal levels of catch. In 2003, the Spanish fishery was in deep crisis (STECF, 2003) and later, in 2005 and 2006, it collapsed completely and was unable to get any significant catch. This led to the repeated closure of the fishery, first in June 2005 and another closure in June 2006 that lasted until January 2010. The DEPM estimated a recovery of the population in 2010 (up to ca. 31 300 t, *CV* = 16%) and peaked in 2011, with about 135 000 t (*CV* = 16%). In these two years, the recovery was due to strong recruitment, as reflected in the high percentage of 1-year-old anchovies in the population (>85%). In 2012, however, a sharp drop in total egg production was recorded, which led to an estimate of ca. 27 000 t (CV =20%) by the DEPM. The latter estimate contrasted with the results of the acoustic survey which pointed to a further increase in population size to ca. 186 000 t. The percentage-at-age 1 fish in the DEPM dropped to ca. 50%, which indirectly supported, for an assumed level of natural mortality, some decrease in the population in 2012 relative to the high peak of 2011.

The consistency between the acoustic and DEPM estimates has been proven for a long period of parallel surveys in May in the Bay of Biscay. However, in some years, there are disagreements, as in 2012. The latest assessment of anchovy made by ICES in 2013 suggests that both surveys provided noisy results in 2012 in terms of biomass, with opposite signs of their errors. ICES (2013) concluded that an intermediate biomass estimate for that year was the one best accommodating all different information collected up to 2013.

2.3.4 Concluding remarks

Overall, the DEPM has proven to be a very helpful monitoring system of the biomass of anchovy in the Bay of Biscay, providing inputs for the assessment both in terms of biomass and population (percentages) at age. The general consistency of the DEPM estimates with those of the acoustic surveys are high, even though ICES assessments reveal that there are overall catchability phenomena associated with both methods; therefore, the series are not compatible in absolute, but in relative terms.

In this survey, CUFES is essentially used for the implementation of the adaptive sampling scheme. There have been several studies trying to convert egg sampling at the surface to the integrated water column egg abundance (Boyra *et al.*, 2003; Petitgas *et al.*, 2006), but the method is still not sufficiently developed to be used.

The utility of the DEPM survey on anchovy also applies to sardine in the region, because by the time of the survey, sardine spawning still takes place in an intense manner, even though peak spawning is known to take place earlier in this area (Carrera
and Porteiro, 2003). The overall tendencies of PairoVET sardine egg abundance is parallel to the acoustic estimates of biomass and these egg abundance estimates have been considered an auxiliary source of information for the assessment of this sardine population in the Bay of Biscay (ICES, 2013).

Beyond its use as input for assessment, the DEPM has contributed largely to a better understanding of the reproductive biology of anchovy and of its spawning dynamics. The first studies were undertaken in the past century on batch fecundity (Sanz and Uriarte, 1989) and on spawning frequency and the dynamics of spawning females (Sanz et al., 1992; Motos, 1994, 1996). Afterward, the study of adult parameters, especially spawning frequency, was revisited, leading to a review of the classification of POFs and oocytes (Alday et al., 2008, 2010) and, subsequently, the estimation procedures of the spawning fraction (Uriarte et al., 2012). Moreover, a review of the model to age anchovy eggs was realized (Ibaibarriaga et al., 2007b) and a review on the procedures for estimating egg production (Bernal et al., 2001; Ibaibarriaga et al., 2007b) and studies on egg mortality rates (Peña et al., 2010) were conducted. The review of the whole series with these improvements on spawning frequency and egg production was presented in ICES WKPELA (Santos et al., 2013b). Other studies were performed related to the spatial distribution of anchovy eggs and their interaction with the environment (Motos et al., 1996; Irigoien et al., 2007), sampling errors (Uriarte and Motos, 1998), vertical distribution and modeling of anchovy eggs (Motos and Coombs, 2000; Boyra et al., 2003; Coombs et al., 2004), or in relation to recruitment (Irigoien et al., 2008, 2009). During these surveys, zooplankton samples, phytoplankton samples, other fish species, gut content of fish, fish larvae, and hydrographic parameters have also been collected. These data have led to several PhD theses that have provided ecosystem information of the Bay of Biscay (Albaina, 2007; Zarauz, 2007; Díaz, 2008; Aldanondo, 2010; Bachiller, 2012).

2.3.5 Acknowledgements

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2.4 Summer acoustic surveys in the Gulf of Cádiz: ECOCADIZ survey series (2004–2010)

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2.4.1 Introduction of the survey

A purse-seine fishery on anchovy and sardine operates traditionally in the Spanish waters of the Gulf of Cádiz (ICES Division 9.a South). Other species like chub mackerel and, to a lesser extent, horse mackerel stand out as secondary species (Millán, 1992; Ramos and Millán, 2004). The IEO started the studies on both this fishery and anchovy biology in the late 1980s, with such studies being successively subordinated to different IEO structural research projects.

The series of Spanish acoustic surveys in ICES Division 9.a South started in its current form in 2004, although the IEO had already conducted two previous surveys in 1993 and 2002 with the RV "Cornide de Saavedra", surveying only the Spanish waters of the Gulf of Cádiz. The ECOCADIZ 0693 survey, carried out in June 1993, was aimed at the acoustic assessment of anchovy (Baro *et al.*, 1993). In February 2002, the SIGNOISE survey was carried out, which was an acoustic intercalibration exercise between the RV "Cornide de Saavedra" and the RV "Vizconde de Eza". This survey also obtained the noise signature of both vessels (Carrera, 2003).

The IEO accepted the formal commitment to initiate regular assessments of the Gulf of Cádiz anchovy by direct methods in 2004, proposing to conduct the BOCADEVA 0604 survey series. This survey was one of the research activities comprised within the IEO research project PELCOSAT, a project mainly focused on the study of the Gulf of Cádiz anchovy fisheries, biology, and population dynamics. BOCADEVA 0604 was considered the starting point of two newly proposed survey series that IEO expected to develop structurally in the study area: the triennial Gulf of Cádiz anchovy DEPM survey (the BOCADEVA series, see Section 2.5) and the annual acoustic survey from the ECOCADIZ series. The season for both survey series (late spring–early summer) was initially chosen to obtain either an acoustic or a DEPM-based estimate of the anchovy SSB in the study area. Furthermore, the geographical coverage of these survey series was extended to both Spanish and Portuguese waters of the Gulf of Cádiz in order to obtain synoptic coverage of the distribution of pelagic resources all over ICES Division 9.a South.

BOCADEVA 0604 was planned as a pilot combined acoustic–DEPM survey. There were several reasons why this survey was considered exploratory. First, as noted above, the only preceding acoustic survey conducted during the same season as the present one in the study area dated back to June 1993 (ECOCADIZ 0693). Second, the same sampling area surveyed in BOCADEVA 0604 was also acoustically assessed in a routine way by the Portuguese IPMA (former IPIMAR) since 1996 (SAR survey series in those years), but the timing (February–March and November) was not very suitable for assessing the anchovy SSB. Third, the distribution pattern of abundance of the main pelagic species in early summer in the study area was unknown from direct surveying. Hence, those problems related to both the acoustic sampling and coverage, and the fishing hauls used for echotrace identification during the survey season had to be evaluated in order to adequately plan and design the next acoustic surveys. Additionally, the survey also included a series of exploratory activities (ichthyoplankton and adult DEPM-based sampling) aimed at elucidating certain aspects of the Gulf of Cádiz anchovy spawning ecology and reproductive strategy.

Results from this survey provided the clues for the subsequent design and planning of the next DEPM surveys, the first one in 2005.

Regarding the acoustic surveying, the ECOCADIZ series actually started in 2004 with the BOCADEVA 0604 survey, with the next surveys in the series already termed as ECOCADIZ surveys since 2006.

The main objective of the ECOCADIZ surveys since 2004 was the acoustic assessment (by echo integration) and mapping of the abundance and biomass of the populations of the main neritic pelagic fish species in the Gulf of Cádiz over the continental shelf (depths of 20–200 m), including both Portuguese and Spanish waters (i.e. the entire ICES Division 9.a South).

The Gulf of Cádiz is a mixed-species ecosystem where gregarious pelagic fish species form numerous small schools. Main pelagic species assessed in the ECOCADIZ surveys include: anchovy, sardine, round sardinella (*Sardinella aurita*), Atlantic mackerel, chub mackerel, Atlantic horse mackerel, Mediterranean horse mackerel, blue jack mackerel, and bogue. However, round sardinella and Atlantic mackerel usually show an incidental occurrence in the surveyed area. Under the sampling scheme of a "pelagic ecosystem survey", the above overall objective is routinely complemented with the following ones:

- biological characterization of the assessed species in relation to their main habitats (i.e. feeding, spawning, nursery, or recruitment habitat, according to the case), especially from their size composition and/or age structure, maturity, repletion, and condition status;
- characterization and (rough) delimitation of the anchovy spawning habitat in the surveyed area from the spatial distribution of both the adults (by acoustics and pelagic hauls sampling) and eggs (by CUFES sampling);
- distribution and abundance of the top predators of the pelagic community (cetaceans, sea turtles, and seabirds);
- oceanographic and environmental characterization of the surveyed area, namely the thermo-haline properties of the shelf waters through continuous (subsuperficial layer) and discrete (vertical profiles) sampling, as well as the recording of climatic variables.

Throughout the recent series, ECOCADIZ surveys have included other objectives such as the realization of the first trials of *in situ* measurements of anchovy target strength in the study area (2006 and 2007), the collection of biological material for anchovy DEPM-based studies (2004, 2006, 2007, and 2009), studies of the summer feeding behavior of the main pelagic species in the surveyed area (2009), and direct studies of the importance and use of discards by scavenger seabirds (2009 and 2010).

2.4.2 Material and methods

The surveys in this new series were planned to be routinely performed on a yearly basis. However, the series had some gaps in those years (2005, 2008, and 2011) coinciding with the conduct of the anchovy DEPM survey (the true BOCADEVA series; see Section 2.5 below) because of available ship time (Table 2.4.1). Given the above constraints, the ECOCADIZ series was conducted in 2004, 2006, 2007, 2009, 2010, and 2013 (this last survey is not analysed in the present report).

Survey acronym	BOCADEVA0604	ECOCADIZ0606	ECOCADIZ0707	ECOCADIZ0609	ECOCADIZ0710
ICES areas	9.a South (Portuguese and Spanish waters)	9.a South (Spanish waters only)			
Dates	06-13/06/04	18/06-01/07/06	03-12/07/07	26/06-06/07/09	25/07-01/08/10
Quarter	2	3	3	3	3
Depth range (m)	30–200	20–200	20–200	20–200	20–200
Acoustic sampling scheme	Systematic parallel	Systematic parallel	Systematic parallel	Systematic parallel	Systematic parallel
No. sampled acoustic transects	21(21)	21(21)	21(21)	21(21)	14(21)
Research vessel	"Cornidede Saavedra"	"Cornidede Saavedra"	"Cornidede Saavedra"	"Cornidede Saavedra"	"Cornidede Saavedra"
Other vessels	Ν	Ν	Ν	Ν	Ν
Echosounder	SimradEK500	SimradEK60	SimradEK60	SimradEK60	SimradEK60
Echosounder working freq. (kHz)	38	18, 38, 70,120, 200	18, 38, 70,120, 200	18, 38, 70,120, 200	18, 38, 70,120, 200
Nautical miles sampled	262	307	301	316	229
Fishing gear/vertical opening (m)	Pelagic trawl/20	Pelagic trawl/20	Pelagic trawl/10	Pelagic trawl/20	Pelagic trawl/20
Fishing gear monitoring	Simrad FR500	Simrad FS20/25	Simrad FS20/25	Simrad FS20/25	Simrad FS20/25
Number of valid fishing hauls					
No. pelagic trawls	13(20)	37(39)	32(33)	28(30)	17(17)
No. purse-seines	Ν	Ν	Ν	Ν	Ν
No. bottom trawls	Ν	Ν	Ν	Ν	Ν
No. CUFES stations	151	134	157	104	102
No. plankton hauls	26	Ν	Ν	Ν	10
No. hydrographic stations	37	23	23	60	42
Thermo-salfluor. (Y/N)	Y	Υ	Y	Υ	Υ
Top predators (Y/N)	Y	Y	Y	Y	Y

Table 2.4.1. ECOCADIZ survey series. Summary of the main characteristics of the analysed surveys in this report.

Unfortunately, the current economic crisis also affected the original planning and degree of accomplishment of the objectives of the last ECOCADIZ surveys, a series that is so far financed with IEO's own funds (and with no Data Collection Resolution [DCR] partial funding). Thus, recent surveys experienced a gradual reduction in the number of survey days in relation to the 14 days originally requested (e.g. 10 days in 2009 and 7 days in 2010). The drastic reduction in ship time available for the 2010 survey resulted in the surveying of only those waters located east of Cape Santa Maria. Because of these budgetary problems, no standard summer ECOCADIZ survey was carried out in 2012.

As noted above, late spring (i.e. early-mid June) was initially considered the most suitable time for conducting the ECOCADIZ surveys as this coincides with the peak spawning of anchovy in the Gulf of Cádiz (Millán, 1992) and is, therefore, the best season to acoustically sample and estimate the anchovy SSB in the area. However, the start of these surveys was also progressively delayed to mid-summer as a consequence of both a reduction in ship time and the priority given to other DCR-funded IEO surveys within the IEO research vessels' timetables.

All the surveys in the series have been carried out with the IEO's RV "Cornide de Saavedra", a 66.7-m stern trawler (Table 2.4.2). Research vessels of smaller dimensions and lower draughts, such as the IEO's RV "Francisco de Paula Navarro" and the General Secretariat of Maritime Fisheries' (SGPM) RV "Emma Bardán", were occasionally used in some experiments of acoustic surveying in the shallowest waters of the Gulf of Cádiz (see below). The RV "Cornide de Saavedra" went out of service after the ECOCADIZ 0813 survey conducted in August 2013. The SGPM's RV "Miguel Oliver" will be the new candidate to conduct the next ECOCADIZ surveys beginning in 2014.

Research vessel	Length (m)	Width (m)	Draught (m)	GRT	HP	
"Cornide de Saavedra"	66.7	11.3	4.65	1 113	2 250	
"Francisco de Paula	30.5	7.4	4.26	178	750	-
Navarro"						
"Emma Bardán"	29.0	7.5	3.90	200	900	

Table 2.4.2. ECOCADIZ surveys series. Technical characteristics of the research vessels used in the IEO standard surveys and pilot experiments (in the shallowest waters) of acoustic surveying of the Gulf of Cádiz.

As mentioned in Section 2.2, the IEO acoustic surveys, including the ECOCADIZ series, experienced a continuous and very dynamic standardization process during the second half of the past decade. This involved not only improvement of the design and objectives of the surveys (trying to satisfy the implementation of the ecosystem approach), but also the development of techniques of data post-processing and analysis (use of specific software, algorithms, masks, and virtual echograms) as a logical consequence of the incorporation of multifrequency acoustic instrumentation (see ICES, 2006a,b and Annex 2 for details). These and other related research activities in fisheries bioacoustics were then and still are currently developed by IEO within the framework of the consecutive proposals of its structural research project DETAC. Both research project and standardization process benifitted from the progress and expertise achieved in previous EU projects (e.g. PELASSES, CLUSTER, SIMFAMI) as well as from recommendations from different ICES expert groups on fisheries acoustics (Working Group on Fisheries Acoustics Science and Technology [WGFAST]; former Planning Group for Acoustic Surveys in ICES Subareas 8 and 9 [PGPAS]; Working

Group on Acoustic and Egg Surveys for Sardine and Anchovy in ICES Subareas 8 and 9 [WGACEGG]), assessment of pelagic stocks (former working groups on the Assessment of Mackerel, Horse Mackerel, Sardine and Anchovy [WGMHSA]; on Anchovy and Sardine [WGANSA]; and on Anchovy [WGANC], currently merged as working groups on Widely Distributed Stocks [WGWIDE] and on Southern Horse Mackerel, Anchovy and Sardine [WGHANSA]), and small pelagic fish ecology (former Study Group on Regional Scale Ecology of Small Pelagic Fish [SGRESP]; ICES/GLOBEC Working Group on Life Cycle and Ecology of Small Pelagic Fish [WGLESP]).

In parallel, the RV "Cornide de Saavedra", as the observational platform of the Gulf of Cádiz pelagic ecosystem during the ECOCADIZ surveys, was equipped during a short time-period with the required scientific-technical equipment for accomplishing the above-mentioned recommendations (Table 2.4.1). Thus, since the beginning of the ECOCADIZ series, the research vessel was equipped with samplers of the biological and hydrological environment, namely a continuous underway fish egg sampler (CUFES; Checkley et al., 1997), a thermo-salinometer and fluorometer, a meteorological station (since 2009), and the discrete sampling of vertical profiles of physical variables in the water column with a CTD. Regarding acoustic sampling, the replacement in 2006 of the old Simrad[™] EK500 echosounder, working at 38 kHz, by the Simrad[™] EK60 scientific echosounder, working in a multifrequency fashion, satisfied the ICES recommendations on the use, as a minimum, of two working frequencies, 38 and 120 kHz. Following this line of improvements, the RV "Cornide de Saavedra" also incorporated a new system of pelagic trawl gear monitoring consisting of a SimradTM Mesotech FS20/25 net sonar, allowing the net geometry and vertical positioning of the fishing gears as samplers of pelagic fish assemblages to be monitored.

Notwithstanding the above, there are still steps to be taken to obtain a better and more complete characterization of the Gulf of Cádiz pelagic ecosystem (e.g. the routine sampling of the mesozooplankton species composition and biomass and the collection of direct measures of primary production and nutrients in the water column are not yet available). However, it is expected that, in the medium term, ECOCADIZ surveys may achieve the same level as other pelagic ecosystem surveys that are conducted on board more modern research vessels and considered as references, such as the PELACUS and PELGAS surveys on board RV "Thalassa".

On the other hand, the acoustic sampling scheme adopted in the ECOCADIZ surveys has also experienced some changes and attempts to improve the spatial coverage through its relatively short history. The standard surveyed area comprises the Gulf of Cádiz waters, both Portuguese (Algarve) and Spanish waters, with an acoustic sampling grid consisting of a systematic parallel grid of 21 transects, equally spaced by 8 nautical miles and perpendicular to the shoreline (Figure 2.4.1). This scheme allows the entire study area to be sampled in about 12–14 days.



Figure 2.4.1. ECOCADIZ survey series: the acoustic sampling grid. The ECOCADIZ-COSTA 0709 survey surveyed the coastal waters between the R05 and R11 transects at depths between 50 and 6–9 m.

Whereas throughout the survey series, the deepest limit for acoustic surveying is established at 200 m, the shallowest depth limit of the surveyed area in the first survey in the series, BOCADEVA 0604, was established at 30 m as a very restrictive security measure for vessel navigation in that year. This resulted in part of the coastal zone between the Guadalquivir and Guadiana river mouths not being acoustically sampled. In the next ECOCADIZ surveys, the shallowest depth limit corresponded to the standard one of 20 m (Figure 2.4.1). Even so, a relatively large coastal area between the Guadalquivir and Guadiana rivers (where the inner shelf exhibits a greater widening) was still not sampled, not only by the ECOCADIZ surveys, but also by the Portuguese surveys (i.e. the former SAR and new PELAGO series) in the same waters.

It is well known that these coastal areas play an important role in the marine ecosystem, particularly for the small pelagic fish populations. Previous studies (e.g. Guillard and Lebourges, 1998; Guennégan *et al.*, 2004; Brehmer *et al.*, 2006) have shown the potential of these areas in quantitative and qualitative terms of biomass and the possibility of a rather important underestimation of stock biomass if such areas are not acoustically assessed. In fact, results from previous surveys have shown that the bulk of the Gulf of Cádiz anchovy and sardine populations usually occur in relatively shallow waters of the surveyed area. However, the importance of waters shallower than 30 or 20 m was not taken into account in the acoustic assessments.

The problem of acoustic (under-)sampling of waters shallower than 20 m was first addressed in July and October 2008 by two pilot experiments surveying these waters (PACAS surveys; ICES, 2008) with two small-draught research vessels (RV "Francisco de Paula Navarro" and RV "Emma Bardán"; Table 2.4.2). Such experiments were useful for testing the vessels' draught, autonomy, and facilities, as well as the capabilities and performance of their respective acoustic equipment (both for scientific vertical echosounding and trawl monitoring) and fishing gears used as samplers. Thus,

in 2009, the standard ECOCADIZ 0609 survey was complemented with the ECOCADIZ-COSTA 0709 survey. This last survey was conducted with the IEO's RV "Francisco de Paula Navarro", almost synchronously to the conventional one, in waters shallower than 20 m off the central part of the study area. However, because the acoustic equipment used in the coastal survey was not properly calibrated, the resulting estimates were considered only indicative, although they demonstrated that coastal shallow waters not covered by conventional surveys (either Spanish or Portuguese) may hold a relatively important biomass (Ramos et al., 2010). Tentative estimates for the combination of standard and coastal surveys indicated that these central shallow waters yielded about 14% and 12% of the acoustically assessed overall fish abundance and biomass,. Obviously, the relative importance of these coastal waters was greater for coastal species; anchovy abundance and biomass accounted for 18% and 14% of the total estimates, respectively, whereas the corresponding abundance and biomass estimates for sardine were 16% and 17%. Coastal relative contributions for bogue and Mediterranean horse mackerel were even higher (ca. 36% for both variables in the former species and ca. 50% for the latter). Unfortunately, sampling of the shallowest coastal waters by a survey complementary to the standard one did not continue in subsequent years.

Species identification, size classes, and other biological characteristics comprising fish echotraces heavily depend on identification via trawl hauls. Fishing stations are opportunistic, according to the echogram information, and are performed in daylight as often as possible according to the echogram information. In any case, trawl catches do not allow for the identification of single schools, but rather for an ensemble of schools over several nautical miles, resulting in identifying groups of schools to species assemblages. These ground-truth trawl hauls have been carried out throughout the ECOCADIZ series using the different pelagic trawl gears of the RV "Cornide de Saavedra", depending on their availability during the survey. The usual research vessel's set of pelagic trawl gears comprises three different gears with vertical openings of ca. 12-13 m (Pedreira), 16-18 m (Tuneado), and 20-22 m (Gran Hermano), with the last one being the preferred option because of its greater sampling coverage, but mainly used in waters deeper than 30–40 m. Acoustic transects are adaptively interrupted to perform the trawl hauls and subsequently resumed. Trawls are carried out at a mean speed of four knots. In the ECOCADIZ surveys, duration of the fishing hauls will depend on the success in capturing the situation to be identified, although the effective fishing time (as monitored by netsonde or sonar) usually lasts between 30 and 45 min.

Ground-truth fishing in the shallowest waters (usually between the 20- and 40-m isobaths) in the central and western parts of the sampled area is not always possible because of the presence of artisanal fixed fishing gears. Coastal fishing in Spanish waters is additionally complicated by the presence of artificial reefs and of a fishing reserve in the coastal waters close to the mouth of the Guadalquivir River (Figure 2.4.1). Some locations in this coastal area, unfortunately, have recorded high backscattering values, indicating relevant fish abundance. As an alternative to this serious drawback, fishing hauls in these situations are usually attempted by fishing over an isobath as close as possible to the depths where the fishing situation of interest is detected over the acoustic track. It was also found that fishing over an isobath, instead of along the acoustic track, was preferable in various other situations to avoid mixing different size compositions (i.e. bi- or multimodality of length frequency distributions). This mixing is more apt to occur close to nursery or recruitment areas and in regions with a very narrow continental shelf. Given that all of these situations are quite common in the

sampled area, these kinds of fishing hauls usually represent a relatively high percentage of the total in each survey. In any case, because the echotraces occur close to the seabed during daylight (especially those echotraces attributed to anchovy), most of the pelagic hauls are carried out very close to the seabed.

As for the acoustic assessment, a detailed description of the adopted species-specific TS values and methods for NASC allocation to species and computation of echo integration estimates (including the definition of coherent post-strata) are given in Section 2.2.2.

The sampling of oceanographic variables also experienced some improvement in recent years regarding coverage with CTD casts. Thus, the CTD sampling grid has been progressively intensified to include a greater number of stations than those initially planned in the first surveys in the series (Table 2.4.1, Figure 2.4.2).

A census of top predators (seabirds, sea turtles, and cetaceans) along all of the acoustic transects and intertransects has also been carried out since the beginning of the series by a bow observer, using standardized strip-transect techniques (Tasker *et al.*, 1984) adapted to match the specific conditions of the surveyed area. A 600-m strip-width (i.e. 180°) transect band is normally used, with the observer surveying both sides of the vessel. The width of the band is checked periodically using a range-finder (Heinemann, 1981). Snapshot counts are used to carry out a census of flying birds (Tasker *et al.*, 1984). Only those observations recorded between the depths of 20 and 200 m are considered.



Figure 2.4.2. ECOCADIZ surveys series. Recent CTD sampling grid.

2.4.3 Results and discussion

The number of valid groundtruth trawl hauls from those surveys that sampled the whole study area oscillated between 13 and 37 hauls. This range even increases up to 28–37 trawl hauls if the 2004 survey is not considered because of its experimental nature (Table 2.4.1, Figure 2.4.3). Although they were performed in an opportunistic way, it is assumed that, with the above coverage, such pelagic hauls provide a relatively realistic picture of the pelagic fish species assemblage in the study area in

summer. Thus, the percentages of occurrence of fish species in the surveys' valid hauls indicate that chub mackerel, anchovy, and sardine, in that order, are the most frequent species, with average presence indices at ca. or >70% (Table 2.4.3). These species are followed by Atlantic mackerel, hake, and bogue, with mean presence indices at ca. 60%, and Atlantic and Mediterranean horse mackerel and blue jack mackerel, with average indices for the surveys series < 50%.

Some inferences on species distribution may also be made from the results from the pelagic trawls (Figure 2.4.3 shows species composition in the catches by haul and survey in terms of percentages in weight) and from the acoustic energy attributed to species (as a proxy for species density in the surveyed area; Figures 2.4.4 and 2.4.5). Visual inspection of all these figures suggests that anchovy exhibits some preference for the central and eastern waters of the Gulf, mainly where the shelf widens, a zone that corresponds to the Guadalquivir River estuary. Sardine shows a more variable pattern depending on the year, but generally with a more coastal distribution than anchovy, and sardine also seems to avoid, to some extent, the preferred waters of anchovy. Chub mackerel, although widely distributed, seems to show some preference for the westernmost mid- and outer shelf waters of the Gulf, with a secondary nucleus of occurrence in the easternmost extreme of the surveyed area. Conversely, Atlantic mackerel seems to be restricted to the central-eastern waters of the Gulf. Regarding representatives of the genus *Trachurus*, Atlantic horse mackerel is widely distributed throughout the surveyed area, whereas blue jack mackerel prefers the mid- and outer shelf waters of the western sector, while Mediterranean horse mackerel is mainly distributed in the coastal waters of the easternmost sector, closer to the vicinity of the Strait of Gibraltar.



Figure 2.4.3. ECOCADIZ surveys series. Species composition (% in weight) in valid groundtruth pelagic trawls. BOCADEVA 0604 (2004), ECOCADIZ 0606 (2006), ECOCADIZ 0707 (2007), and ECOCADIZ 0609 (2009) and 0710 (2010) surveys.

Tables 2.4.4 and 2.4.5 show the overall and regional (i.e. Portuguese and Spanish) acoustic estimates of abundance and biomass of assessed species in each of the ECOCADIZ surveys. The contribution of the regional estimates to the total surveyed area also provides some information on the species' distribution patterns and corroborates the previously described ones derived from pelagic trawls. Thus, the bulk of the estimated populations of anchovy and sardine are usually recorded in Spanish waters, whereas the opposite occurs with chub mackerel, a typical Portuguese species. The remaining species show a lower number of point estimates, based on their very low densities that makes it difficult to assess them acoustically during the surveys. Nevertheless, the available acoustic estimates seem to confirm again the preference shown by Mediterranean horse mackerel and blue jack mackerel for Spanish and Portuguese waters, respectively.



Figure 2.4.4. ECOCADIZ survey series. Distribution of the backscattering energy (nautical area scattering coefficient, NASC, in m² nautical mile⁻²) attributed to anchovy (*Engraulis encrasicolus*). BOCADEVA 0604 (2004), ECOCADIZ 0606 (2006), ECOCADIZ 0707 (2007), and ECOCADIZ 0609 (2009) and 0710 (2010) surveys.

Regarding the target species in the present report, Figure 2.4.6 shows the trends in the estimated biomass of Gulf of Cádiz anchovy and sardine populations through the ECOCADIZ series. In order to achieve better visualization of the trend, anchovy biomass estimates by acoustics are complemented with the DEPM-based estimates obtained during the BOCADEVA surveys (see Section 2.5). The Gulf of Cádiz anchovy biomass estimated by acoustics for the period under study is about 26 kt on average (a value computed for the whole series, except for the 2010 partial estimate). Nevertheless, the period starts with relatively low biomass levels in 2004–2005, well below the mean in 2006, which shows a remarkable increase to 36 kt. Since 2007, the biomass shows a slight decreasing trend, but with point estimates relatively close to the average. During the period under analysis, the size composition of the estimated population ranged between the 7- and 18.5-cm size classes, with modal classes mainly located at 13 or 14 cm (Figure 2.4.7). Larger (older) anchovies are usually recorded in Portuguese waters. A smaller mode at 9.5 cm (and composed of age 0 anchovies) was also recorded in 2010 as a probable consequence of the delay in the start of the survey to early August, a time that is usually coincident with the first main events of anchovy recruitment in the Gulf. In any case, age-structured estimates of anchovy abundance show that the population is supported mainly by age 1 anchovies, with their annual fluctuations being responsible for the fluctuations observed for the whole population (Figure 2.4.7).



Figure 2.4.5. ECOCADIZ survey series. Distribution of the backscattering energy (nautical area scattering coefficient, NASC, in m² nautical mile⁻²) attributed to sardine (*Sardina pilchardus*). BOCADEVA 0604 (2004), ECOCADIZ 0606 (2006), ECOCADIZ 0707 (2007), and ECOCADIZ 0609 (2009) and 0710 (2010) surveys.

The sardine biomass estimated for the period under analysis averaged ca. 69 kt. The interannual evolution of the sardine biomass estimates seems to run parallel to that described above for anchovy, with a very low estimate in 2004, a peak in 2006, and a decreasing trend since 2007, somewhat more pronounced than observed in anchovy, although suggesting a certain recovery up to average levels in 2010 (Figure 2.4.6). Unfortunately, there are no sardine age-structured estimates available. The size composition of the estimated population is rather constant throughout the series and is characterized by a size range usually oscillating between 9 and 21.5–22.5 cm and with two clear modes. The smallest mode is composed of recruits (and occurs more frequently in coastal Spanish waters) at ca. 10.5–11.5 cm, and the second mode encompasses the major part of the population at ca. 18–18.5 cm (Figure 2.4.8).

Regarding the assessment of the sardine and anchovy stocks in ICES divisions 8.c and 9.a (sardine), or in Division 9.a only (anchovy), indices provided by the ECOCADIZ survey series have become a relevant data source to verify the actual trends in at least the southern components of these stocks, although more specifically in the case of the Gulf of Cádiz anchovy because of the survey dates (ICES, 2013).

For the time being, no analytical assessment model has been successfully applied to anchovy in Division 9.a. The provision of advice on anchovy in Division 9.a currently relies almost exclusively on a qualitative analysis of survey trends (those corresponding to the PELAGO, ECOCADIZ, and BOCADEVA surveys; see sections 2.2, 2.4, and 2.5). Recent results on genetics and independent trends observed in the fisheries and in the population abundance estimates have confirmed an independent stock of anchovy in the northwestern part of Division 9.a (western Iberian waters) separate from the anchovy in Division 9.a South. In this context, ICES has considered each of the above survey series (including the PELACUS results for the northernmost area in Division 9.a) as an essential tool for the direct assessment of the population in their respective survey areas (subdivisions) and has recommended their continuity, mainly in those series that have suffered interruptions in their recent history, as in the case of ECOCADIZ. Regarding this last series, ICES has emphasized that this survey could trigger the revision in autumn of the advice for anchovy in Division 9.a South provided in June, particularly in case of contradicting tendencies observed by the (spring) PELAGO surveys in this area. ICES also recognizes that these sources of information might become the knowledge base for the implementation of future alternative management regimes based on survey information and analytic assessments.

Survey		BOCADEVA 0604	ECOCADIZ 0606	ECOCADIZ 0707	ECOCADIZ 0609	ECOCADIZ 0710
Number of valid hauls		13	34	32	28	17
Family	Species					
Alopiidae	Alopias superciliosus	8				
Xiphiidae	Xiphias gladius					12
Molidae	Mola mola	46	9	6	4	
Belonidae	Belone belone			9		
Scombridae	Scomber colias	92	79	88	82	76
	Scomber scombrus	77	53	66	57	65
Carangidae	Caranx rhonchus			3		
	Trachinotus ovatus		6			
	Trachurus trachurus	8	38	28	61	59
	Trachurus picturatus	8	62	34	75	59
	Trachurus mediterraneus	8	15	53	29	29
Stromateidae	Stromateus fiatola	8				
Echeneidae	Remora brachyptera					6
Mugilidae	Liza aurata			3		12
	Mugil cephalus	8				6
Cupleidae	Alosa alosa					6
	Alosa fallax					6
	Sardina pilchardus	62	82	56	54	76
	Sardinella aurita			3		
Engraulididae	Engraulis encrasicolus	77	68	59	82	88
Caproidae	Capros aper	8		3	4	
Macrorhamphosidae	Macroramphosus scolopax		3		7	

Table 2.4.3. ECOCADIZ surveys series. Percentage of occurrence of fish species in valid groundtruth pelagic trawl hauls.

Curron		BOCADEVA	ECOCADIZ	ECOCADIZ	ECOCADIZ	ECOCADIZ
Survey		0604	0606	0707	0609	0710
Zeidae	Zeus faber			3		
Centracanthidae	Centracanthus cirrus				4	
	Spicara flexuosa	8	6	16	21	18
Haemulidae	Pomadasys incisus					6
Sparidae	Boops boops	38	71	66	54	65
	Dentex maroccanus	8				6
	Diplodus annularis	23	24	16	21	18
	Diplodus bellotti	15	9	19	14	35
	Diplodus cervinus		3			
	Diplodus sargus				4	
	Diplodus vulgaris		6	16	29	18
	Pagellus acarne	8	9	13	21	18
	Pagellus bellotti	8	3	6	4	12
	Pagellus erythrinus	23	15	19	21	35
	Pagrus pagrus				4	
	Spondyliosoma cantharus	8	35	22	43	59
Congridae	Conger conger	8		3		
Cepolidae	Cepola macrophthalma		3	3		
Gadidae	Micromesistius poutassou				4	
Merlucciidae	Merluccius merluccius	54	50	38	71	100
Phycidae	Phycis phycis				4	
Trichiuridae	Lepidopus caudatus	8	26	16	29	18
Labridae	Symphodus spp.				4	

Surrow		BOCADEVA	ECOCADIZ	ECOCADIZ	ECOCADIZ	ECOCADIZ
Survey		0604	0606	0707	0609	0710
Serranidae	Anthias anthias				7	
	Callantias ruber				4	
	Serranus cabrilla		3			
	Serranus hepatus		3	6	11	
Scorpaenidae	Scorpaena notata		3	3	4	
Triglidae	Aspitrigla cuculus		3			
	Chelidonichthys lastoviza				4	
	Chelidonichthys lucerna				7	18
	Chelidonichthys obscurus					6
	Lepidotrigla cavillone	8			7	
	Lepidotrigla dieuzeidei			3	7	
	Trigla lucerna	8				
Trachinidae	Trachinus draco	8	3	3	11	29
Mullidae	Mullus barbatus		3	3	14	
	Mullus surmuletus				4	
Gobiidae	Aphia minuta	31			4	18
	Lesueurigobius sanzoi			3		
Batrachoididae	Halobatrachus didactylus			3		
Tetraodontidae	Sphoeroides pachygaster				7	
Citharidae	Citharus linguatula	8				6
Soleidae	Microchirus boscanion				4	

Total

Spain

Total

Portugal

Boops boops

Abundance (millions)/sj geographical stratum/ye	pecies/ ar	2004	2006	2007	2009	2010
Engraulis encrasicolus	Portugal	125	363	558	35	?
	Spain	1 109	2 801	1 232	1 102	954
	Total	1 235	3 163	1 790	1 137	?
Sardina pilchardus	Portugal	56	1 446	483	62	?
	Spain	881	1 428	1 216	588	2 068
	Total	937	2 874	1 699	649	?
Sardinella aurita	Portugal	-	-	-	-	?
	Spain	-	-	0,2	-	-
	Total	-	-	0,2	-	?
Scomber colias	Portugal	332	476	542	365	?
	Spain	39	48	247	264	43
	Total	371	524	788	629	?
Scomber scombrus	Portugal	-	-	0,9	-	?
	Spain	-	-	3	-	-
	Total	-	-	3	-	?
Trachurus trachurus	Portugal	-	-	11	52	?
	Spain	-	-	36	13	13
	Total	-	-	47	66	?
Trachurus	Portugal	-	-	4	0	?
mediterraneus	Spain	-	-	19	28	28
	Total	-	-	24	28	?
Trachurus picturatus	Portugal	-	-	104	92	?
·	Spain	-	-	9	13	65

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Table 2.4.4. ECOCADIZ survey series. Overall and regional acoustic estimates of abundance (million fish) of the assessed species. The 2010 estimates are only available for the waters located east of Cape Santa Maria that, for simplicity, are considered Spanish waters.

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6

?

104

16

27

42

113

28

12

41

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Biomass (t)/species/		2004	2006	2007	2000	2010
geographical stratum/y	ear	2004	2000	2007	2009	2010
Engraulis encrasicolus	Portugal	2 474	6 477	11 639	1 075	?
	Spain	15 703	30 043	17 243	20 506	12 339
	Total	18 177	36 521	28 882	21 580	?
Sardina pilchardus	Portugal	2 310	64 187	31 525	3 880	?
	Spain	24 258	59 662	55 252	33 140	66 964
	Total	26 568	123 849	86 777	37 020	?
Sardinella aurita	Portugal	-	-	-	-	?
	Spain	-	-	49	-	-
	Total	-	-	49	-	?
Scomber colias	Portugal	30 077	29 995	39 206	28 935	?
	Spain	3 181	5 585	22 324	27 341	2 861
	Total	33 258	35 580	61 530	56 276	?
Scomber scombrus	Portugal	-	-	218	-	?
	Spain	-	-	272	-	-
	Total	-	-	381	-	?
Trachurus trachurus	Portugal	-	-	578	4 330	?
	Spain	-	-	1 847	790	739
	Total	-	-	2 425	5 121	?
Trachurus	Portugal	-	-	314	0	?
mediterraneus	Spain	-	-	1 548	2 705	3 732
	Total	-	-	1 862	2 705	?
Trachurus picturatus	Portugal	-	-	3 429	6 904	?
	Spain	-	-	424	344	4 126
	Total	-	-	3 853	7 248	?
Boops boops	Portugal	-	-	1 932	1 088	?
	Spain	-	-	903	2 324	551
	Total	-	-	2 836	3 412	?

Table 2.4.5. ECOCADIZ survey series. Overall and regional acoustic estimates of biomass (t) of the assessed species. The 2010 estimates are only available for the waters located east of Cape Santa Maria that, for simplicity, are considered Spanish waters.





Sardina pilchardus

Figure 2.4.6. ECOCADIZ survey series. Recent trends in biomass estimates (in tonnes) for anchovy (top) and sardine (bottom). Gaps for the 2005 and 2008 anchovy acoustic estimates are filled with the BOCADEVA DEPM survey estimates (see Section 2.5.1). Note that the 2010 survey only partially covered the study area.



ECOCADIZ series Anchovy length-based estimates





Figure 2.4.7. ECOCADIZ survey series. Anchovy size- (top) and age-structured (bottom) abundance estimates. Note that the 2010 survey only partially covered the study area.



Figure 2.4.8. ECOCADIZ survey series. Sardine length frequency distributions of the estimated populations. Note that the 2010 survey only partially covered the study area.

2.4.4 Acknowledgements

The authors express their sincere gratitude to the different captains and crews of the RV "Cornide de Saavedra" during the ECOCADIZ surveys for their help, collaboration, and professionalism. They are also very grateful to the IEO scientific and technical teams involved in these surveys. Of special mention is Milagros Millán (now retired) who provided continuous support from the beginning. Finally, the authors express their thanks to the IEO scientific directorate for promoting the financial support given to the ECOCADIZ series with IEO funds.

2.5 Anchovy DEPM surveys in Cádiz (Division 9.a South) BOCADEVA 2005/2008/2011

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2.5.1 Introduction: DEPM BOCADEVA survey series

The BOCADEVA series was launched by IEO in 2004 in order to provide absolute estimates of the anchovy in Division 9.a (Algarve + Gulf of Cádiz anchovy) by means of the daily egg production method. This was decided after several attempts of various ICES assessment working groups (the former Working Group on the Assessment of Mackerel, Horse Mackerel, Sardine, and Anchovy [WGMHSA] and the Working Group on Anchovy and Sardine [WGANC]) to produce analytical assessments of this anchovy stock between 2001 and 2008 (Ramos *et al.*, 2001; ICES, 2009b). However, all of those attempts were considered by ICES as mere data exploratory analyses since it was impossible to reliably assess the stock without having information provided by direct methods, particularly without the scaling role of direct absolute estimates (i.e. SSB estimates from DEPM surveys). This is why ICES in those years repeatedly recommended initiation of a historical series of anchovy biomass indices in the study area (especially by DEPM, but also by acoustics).

The first BOCADEVA survey (BOCADEVA 0604) was experimental in nature and included the following DEPM-based objectives: (1) delimitation of the extension of the anchovy spawning area in ICES Division 9.a South; (2) non-intensive collection of anchovy adult samples for a preliminary (exploratory) analysis of DEPM adult parameters; and (3) evaluation of CUFES sampling as a quantitative method to estimate anchovy egg abundance in the study area.

Since 2005, the IEO has conducted a DEPM survey every three years to estimate the Gulf of Cádiz anchovy SSB. The BOCADEVA 0605 survey was the first standard anchovy DEPM survey in the series and aimed to be the beginning of a historical series carried out by IEO in this area. BOCADEVA 0608 and BOCADEVA 0711 were the second and third surveys in this series, respectively. All the surveys are included as research activities developed within the framework of the IEO project ICTIOEVA for the different years.

In order to collect the required parameters for the estimation of the Gulf of Cádiz anchovy SSB by the DEPM (main objective), the surveys undertake:

- ichthyoplankton sampling along a grid of parallel transects perpendicular to the coast in order to obtain the spatial distribution of anchovy eggs, the delimitation and calculation of the extension of the anchovy spawning area, and the estimation of the Gulf of Cádiz anchovy daily egg production (*P*₀) and total egg production (*P*_{tot});
- fishing hauls to obtain the spatial distribution of adults as well as samples for the estimation of DEPM-based adult parameters: sex ratio (*R*), mean female weight (*W*), batch fecundity (*F*), and spawning fraction (*S*) within the mature component of the population.

The survey objectives also include estimating the size composition and biological characterization of other important commercial pelagic fish species in the area as well as characterizing oceanographic and meteorological conditions in the study area during the survey.

2.5.2 Material and methods

The Gulf of Cádiz anchovy DEPM surveys (BOCADEVA series) have been carried out on board RV "Cornide de Saavedra" (IEO), the same vessel used for the ECOCADIZ surveys (see Section 2.4). The surveyed area extends from the Strait of Gibraltar (Spain) to Cape San Vicente (Portugal), from 36°18′N to 36°75′N and 6°22′W to 8°92′W. This area includes the continental shelf waters between the 20- and 200-m isobaths, comprising both Spanish and Portuguese waters of the Gulf of Cádiz. The survey dates are determined by the reproductive cycle of the species in the study area and should coincide with peak spawning (Table 2.5.1).

Survey acronym	Year	Dates	
BOCADEVA 0605	2005	10–23 June	
BOCADEVA 0608	2008	21 June–3 July	
BOCADEVA 0711	2011	22 July–2 August	

Table 2.5.1. Dates of the BOCADEVA survey series.

The sampling scheme was established following the standards set by the Study Group on Spawning Biomass of Sardine and Anchovy (ICES, 2003). Also, several workshops have been developed to standardize and improve the methodologies:

- Anchovy and Sardine Egg Staging Workshop. AZTI, Pasajes (Spain), January 2002. Standardization of anchovy and sardine eggs staging criteria.
- Anchovy and Sardine DEPM workshop. IEO Cádiz (Spain), June 2006. Standardization of methodologies and estimation of anchovy and sardine SSB by DEPM.
- Workshop on Anchovy and Sardine DEPM using R. IEO, Cádiz (Spain), October 2007. Standardization of methodologies and estimation of anchovy and sardine SSB by DEPM using R. Gulf of Cádiz anchovy eggs development modelling. A summary of the process (incubation experiment and modelling) is shown below.
- Workshop on Anchovy and Sardine DEPM using R (WKRESTIM). IEO, Madrid (Spain), June 2009. Standardization of methodologies and estimation of anchovy and sardine SSB by DEPM using R.

2.5.2.1 Incubation model summary

Assigning ages to eggs sorted from plankton samples represents some degree of uncertainty related to variability at spawning time, development temperature, stage duration, and ontogenetic variability, allowing possible systematic errors in the laboratory identification of stages in multiple spawning pelagic species (Ahlstrom, 1943 in Motos, 1994). Therefore, the development of incubation experiments where eggs are reared from fertilization to hatching under controlled conditions improved a more precise estimation for assigned ages, validating the daily egg production method (DEPM) (Miranda *et al.*, 1990; Motos, 1994; Stratoudakis *et al.*, 2006).

A temperature-dependent embryonic development model for *Engraulis encrasicolus* in the Gulf of Cádiz was obtained (Duarte *et al.*, 2007). The data used came from an incubation experiment carried out during July 2007. The samples of adults were obtained on board a commercial purse-seine vessel. Forty-four (44) hydrated females and 15 ripe males were used, and the fertilization of eggs was carried out on board.

Fertilized eggs were removed to the laboratory and reared in an incubator composed of five aquariums which were maintained at five different controlled temperatures (10, 14, 18, 22, and 26°C). The incubation experiment occurred inside a controlled temperature room at 18°C. Routine sampling took place about once every hour throughout the experiment, and the assignment of stages was based on Moser and Ahlstrom (1985) morphological keys.

The number of eggs in each stage follows a multinomial distribution, which has a certain probability of being at any given stage. Ibaibarriaga *et al.* (2007b) proposed a multinomial model that can be used to analyze data from an incubation experiment by modelling the probability of being at a certain stage as a function of age and other variables that may affect egg development (like temperature):

 $(n_1, n_2, ..., n_k) \sim [Multi(n, \pi_1, \pi_2, ..., \pi_k)]$

where $\pi_i = P(\text{stage} = i)$ represents the individual probability of being at a certain stage *i*. The age referred to above is defined as the elapsed time from the beginning of the incubation experiment until the sampling event. The final model included stage, age, and temperature as covariates and was fitted with age and temperature interaction.

The transition from one stage to the next is well defined at all temperatures and shows a certain increasing degree of overlap between stages when temperature increases (Figure 2.5.1). The model shows there is highest probability of finding eggs at stage 2, followed by stage 6 at all temperatures. Apparently also these stages had the largest stage duration, visualized as the width of the curves. Stages 10 and 11 have a very low probability of being found for a given age and temperature, and especially the transition to stage 11 is not clearly marked. Stage 11 has the lowest probability of being found for a given age and the stage with the lowest number of sampling observations for all temperatures. The fact that this stage could be characterized by a very fast development and also energetic movements of the tail as the moment of hatching approaches (Miranda *et al.*, 1990), may make the counting and stage identification difficult. A comparison with development rates in the Bay of Biscay has been done in Bernal *et al.* (2011c).



Figure 2.5.1. Multinomial model fitted to the anchovy incubation experiment data. Each panel represents one of the three temperature incubations taken into account for the analysis. The numbers within each plot represent the stage number (stages 1–11) at each sampling time. Each line represents the evolution of the modeled probability of eggs being at a given stage over age and temperature (Bernal *et al.*, 2011c).

The sampling methodologies and the data analysis for estimation of areas (surveyed and spawning areas); egg parameters (Z, P_0 , and P_{tot}) and adult parameters (sex ratio, mean female weight, spawning fraction, and bath fecundity) are explained in Annex 1 (DEPM surveys protocol). Table 2.5.2 summarizes a brief description of the methodology used to obtain samples of eggs and adults during the surveys. Only particular considerations about the BOCADEVA series are explained in this section.

2.5.2.2 Egg sampling and processing

The sampling grid is established over the continental shelf following a systematic sampling scheme, with transects perpendicular to the coast and equally spaced eight nautical miles apart, in the same way as in the ECOCADIZ acoustic surveys (Figure 2.4.1). A total of 21 transects are established, 11 in Spanish waters and 10 in Portuguese waters. The surveys are carried out from east to west, starting in transect 1 located close to the Strait of Gibraltar. The egg sampling strategy is identical in all surveys. Adaptive sampling is carried out using the PairoVET net at discrete stations as the main sampler and a continuous record with CUFES as secondary sampler (Annex 1).

Parameter	Anchovy DEPM survey BOCADEVA0711
Surveyed area	36–37°N 6–8°5′W
Research vessel	"Cornide de Saavedra" (IEO)
Eggs	
Transects (Sampling grid)	21 (8 × 3)
Sampler	PairoVET net (150 μm)
Sampling maximum depth	100 m
Hydrographical sensor	CTD SBE25 and SBE37
Flowmeter	Yes
Continuous sampler	CUFES (335 μm)
Environmental data	Temperature and salinity
Adults	
Gear	Pelagic trawl
Trawling time	Ca. 07:00–20:00 GMT
Biological sampling	On fresh material, on board the RV
Sample size	Individual samples of at least 60 and up to 120 randomly picked individuals, adding batches of 10 randomly picked anchovies until a minimum of 30 mature females is found for spawning fraction estimation. At survey level a minimum of 150 hydrated females is required for batch fecundity estimation.
Fixation	4% phosphate-buffered formaldehyde
Preservation	4% phosphate-buffered formaldehyde

Table 2.5.2. BOCADEVA survey series. General sampling.

Egg samples by PairoVET are taken every 3 nautical miles in the inner shelf up to a maximum depth of 100 m, or 5 m above the seabed at shallower depths, at a speed of ca. 1 m s⁻¹ (ICES, 2003). The inshore limit of transects is determined by the bottom depth (as close to the shore as possible), while the offshore extension is decided adaptively depending on the results of the CUFES samples. Vertical hauls of plankton are carried out with a PairoVET sampler equipped with nets of 150-µm mesh size. Sampling depth and temperature of the water column are recorded initially with a minilog sensor. Since 2008, a CTD SBE 37 (or SBE 19) fitted onto the tow wire has been used. Flowmeters are used to calculate the volume of water filtered during each haul.

To estimate P_0 , only samples from one codend were used in 2005 and 2008, while samples from both codends were used in 2011. However, an analysis to test differences of using one or two samples was carried out. From this, it was concluded that no differences exist (Jiménez and González, 2009).

During the CUFES sampling (Checkley *et al.*, 1997), the volume of filtered water (ca. 600 l min⁻¹) is also integrated every 3 nautical miles at a fixed depth of 5 m. The CUFES collector was fixed with a 335-µm net.

Samples from both PairoVET and CUFES are preserved in a 4% buffered formaldehyde solution. Samples are preliminarily sorted on board, and a preliminary analysis is conducted in order to obtain results of anchovy egg abundance in real-time. Later in the laboratory, anchovy eggs from the PairoVET samples are resorted and classified into 11 developmental stages (Moser and Ahlstrom, 1985), while the CUFES samples are resorted and classified into three categories (no embryo, early embryo, and late embryo; ICES, 2003).

2.5.2.3 Adult sampling and processing

Adult anchovy samples for DEPM purposes are obtained from pelagic trawl hauls. Two types of pelagic trawl were used throughout the period: the Pedreira (vertical opening of 12–13 m, 20-mm codend mesh) and the Tuneado (vertical opening of 17–18 m). The performance and geometry of both trawls as well as the entrance of fish into the net were monitored by a Simrad® Mesotech FS20/25 net sonar (120–200 kHz). The location of the fishing hauls was opportunistic, according to echogram information on expected anchovy presence (by visual scrutiny based on expertise). Acoustic data were first recorded (in 2005) with a Simrad® EK 500 echosounder working at 38 kHz and subsequently with a Simrad® EK60 echosounder working in a multifrequency fashion (18, 38, 70, 120, and 200 kHz).

Except when searching for anchovy females with hydrated gonads, fishing hauls were mostly conducted during daylight (ranges for fishing stations were 06:08–20:23 GMT) and were carried out over the isobaths after echo traces presumably belonging to anchovy were detected by the echosounder. Additionally, in 2005, adult anchovy samples were also collected with a commercial purse-seiner at night between 21:55 and 05:30 GMT.

Maturity in anchovy females was established by a 6-stage macroscopic maturity scale (Pinto and Andreu, 1957): virgin or resting (stage I), developing (stage II), prespawning (stage III), spawning (stage IV), partial post-spawning (stage V), and ultimate post-spawning (stage VI).

Spawning fraction (*S*) was determined in 2005 and 2008 by histological analysis of the post-ovulatory follicles (POFs) (Hunter and Macewicz, 1985). Ovaries were initially classified according to oocyte type (i.e. developmental stage) following the classification key proposed by Alday *et al.* (2004) (see also Millán *et al.*, 2005; Jiménez *et al.*, 2009). In 2011, POFs were assigned to stages according to Alday *et al.* (2010), and the correspondence in days according to Hunter and Macewicz (1985): Day 0 POFs (stages 1 and 2, new post-ovulatory follicles [0–6 h]); Day 1 POFs (stages 3 and 4, 7–30 h post-ovulatory follicles); Day 2 POFs (stages 5 and 6, 31–54 h post-ovulatory follicles); Day 3+ POFs (stage 7, >54 h post-ovulatory follicles; Solla, 2012), considering the specific peak spawning for the species in the study area (22:00 GMT).

2.5.3 Results and discussion

This section shows the main results obtained from Gulf of Cádiz anchovy DEPM surveys (BOCADEVA series): surveyed and positive areas, eggs, adult parameters, and anchovy spawning-stock biomass estimates. There are no results for sardine because of the very low, practically negligible, presence of sardine eggs in the samples in summer when the surveys are conducted.

Some results in the study area suggest a persistently increasing gradient in the anchovy mean weight from east to west. Heavier fish usually occur in the western limit of their distribution, whereas a recruitment area featuring the presence of smaller anchovies is detected in the shallower waters close to the mouth of the Guadalquivir River. The distribution of the daily egg production estimated in the 2005 and 2008 surveys also shows a spatial structure. So, the anchovy spawning (= positive) area is separated into two regions by a spatial discontinuity placed just west of the mouth of the Guadalana River (Jiménez *et al.*, 2005, 2009). Taking into account all evidence, post-stratification was adopted to derive stratified estimates of both egg and adult parameters in 2005 and only of egg production in 2008, establishing the meridian corresponding to $7^{\circ}30'W$ (Figure 2.5.2) as the geographic limit of the two strata.

2.5.3.1 Anchovy egg distribution surveyed and positive area

In order to show both the spatial distribution of anchovy egg abundance and the surveyed and positive areas, data collected by CUFES are also presented because they form a longer dataseries. Such data come from acoustic (2004, 2006, 2007, 2009, and 2010) and DEPM surveys (2005, 2008, and 2011), all of which were carried out by the same vessel, with the same equipment, and during a similar survey season (summer).

2.5.3.2 CUFES

The number of CUFES stations ranged between 104 in 2009 and 157 in 2007 (only 11 radials were sampled in 2010, see Section 2.4). Positive stations for anchovy eggs averaged 66.5% of the total. A total of 122 102 anchovy eggs were collected throughout the sampled period (mean = 15 263). The maximum number of anchovy eggs captured by CUFES occurred in 2011 (Table 2.5.3). The highest densities (no. m⁻³) were obtained in 2004, 2007, and 2011. Until 2009, the spatial distribution of anchovy egg abundance, as sampled by CUFES, shows a clear zone of maximum abundance in the coastal area between Huelva and Cádiz (Figure 2.5.2), where the areas with maximum egg concentrations have been oscillating depending on the year. During this period (2004–2009) most eggs were captured in Spanish waters (86.1% on average). However, since 2010, higher abundance has been observed in the Portuguese part (63.5% in the most recent year).

 Table 2.5.3. Number of stations and anchovy eggs collected (number and maximum egg density)

 by CUFES in the Gulf of Cádiz.

Survey	2004 ¹	2005 ²	2006 ¹	2007 ¹	2008 ²	2009 ¹	20101*	2011 ²
No. stations	151	107	134	157	121	104	102	114
No. positive stations	82	48	93	114	85	85	74	76
No. anchovy eggs	21 109	2 955	5 723	26 770	8 213	9 885	9 615	37 832
Maximum egg density (no. m ⁻³)	265.1	38.5	49.3	244.0	42.7	94.8	226.1	274.1

¹ Acoustic surveys.

² DEPM surveys.

*Surveyed from Strait of Gibraltar to Cape Santa Maria only.







Figure 2.5.2. Spatial distribution of the anchovy egg abundance (no. m⁻³) by CUFES in the Gulf of Cádiz (2004–2011).

2.5.3.3 PairoVET

The number of PairoVET stations was similar in all DEPM surveys, and the positive stations were the same in 2008 and 2011, but lower in 2005 (Table 2.5.4). Regarding egg densities, the highest maximum density, ca. 4500 eggs m⁻², was recorded in 2005. This value was largely attributed to a single station which accounted for 35% of all anchovy eggs collected at all stations that year (a total of 204 anchovy eggs were caught at this station located at the mouth of the Guadalquivir River).

Table 2.5.4. BOCADEVA survey series. Number of stations and anchovy eggs collected (number and maximum egg density) by PairoVET.

Survey	2005	2008	2011
No. stations	119	127	124
No. positive stations	46	70	71
No. anchovy eggs	583*	1373	2387
Maximum egg density (no. m ⁻²)	4359.2	1228.7	2194.9

*One codend only.



Figure 2.5.3. BOCADEVA survey series. Spatial distribution of anchovy egg abundance (no. m⁻²) by PairoVET.

As described above for the CUFES sampling, the spatial distribution of anchovy egg abundance in 2005 and 2008 was also higher in the coastal area between Huelva and Cádiz where 92 and 84% of the eggs, respectively, were caught in this zone. In 2011, the scenario was different, with more than half of the total eggs collected in Portuguese waters (59% in areas close to the Guadiana River and Cape Santa Maria (Figure 2.5.3).

2.5.3.4 Surveyed and positive area

The series of surveyed areas by CUFES ranged between 14 150 km² in 2007 and 10 846 km² in 2005 (only Spanish waters were surveyed in 2010). The largest positive area was estimated in 2009 and the smallest in the two first years in the series (Figure 2.5.4). Except for some interannual variations, no trend is detected throughout the series. Regarding PairoVET data and taking into account post-stratification, the Portuguese positive area comprised 23–25% of the total in both 2005 and 2008 (Table 2.5.5). The total surveyed and spawning areas for the Gulf of Cádiz estimated in the three DEPM surveys (BOCADEVA) were very similar.



Figure 2.5.4. Surveyed and positive areas by CUFES: acoustic (2004, 2006, 2007, 2009, and 2010) and DEPM (2005, 2008, and 2011) surveys. In 2010, the survey was only from Strait of Gibraltar to Cape Santa Maria.

Table 2.5.5 BOCADEVA survey series	. Surveyed and	positive areas sam	pled by	PairoVET.
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Area	Stratum	2005	2008	2011
Surveyed area	Total Gulf of Cádiz	11 982	13 029	13 107
	Portuguese waters	1 409	1 690	-
Positive area	Spanish waters	4 729	5 172	-
	Total Gulf of Cádiz	6 138	6 862	6 770

2.5.3.5 Egg parameters

2.5.3.5.1 Daily and total egg production (P₀ and P_{tot})

As described above, both daily egg production (P_0) and total egg production (P_{tot}) in 2005 and 2008 were calculated for two strata: Portuguese and Spanish waters. Figure 2.5.5 shows the results obtained for these parameters in each stratum. In both cases, the regional estimates obtained from the Portuguese area in 2005 and 2008 were lower than those obtained in the Spanish area: 18 and 35% for daily egg production per surface unit, 6 and 14% for total production, respectively. In general, we can observe an increasing soft trend in P_{tot} , with the highest value in the middle of the series (1.87 × 10¹² eggs d⁻¹).



Figure 2.5.5. Daily egg production per surface unit (left) and total egg production (right) for anchovy in the Gulf of Cádiz, estimated by the DEPM (BOCADEVA surveys) (*CV* in bars).

2.5.3.6 Adult parameters

2.5.3.6.1 Sampling data

The number of hauls was similar in the three surveys, ranging between 24 in 2011 and 27 in 2005. The percentage of occurrence for anchovy was very high, >85% in all cases. Other frequent species were mackerel, chub mackerel, sardine, or hake (Table 2.5.6 shows the species with catches of >10 kg in each survey). The abundance of anchovy, both in numbers and weight, was highest in 2011, with a clear increasing trend in the yield of this species during the period: 46 kg h⁻¹ in 2005, 70 kg h⁻¹ in 2008, and 311 kg h⁻¹ in 2011.

Figure 2.5.6 shows the species composition in the catches by haul and survey in terms of percentages in weight. In 2005, anchovy was widely distributed, mainly in front of the Guadiana and Guadalquivir rivers and along the Portuguese coastal area. In 2008, the species showed some preference for the central and eastern waters of the study area, mainly where the shelf widens, a zone that corresponds to the environment of the Guadalquivir River estuary. In 2011, anchovy distribution was more reduced, mainly occurring in the area between Huelva and the Guadiana River, and in the mouth of the Guadalquivir River. Sardine exhibited a more variable spatial pattern, depending on the year. In general, the species had a more coastal distribution than anchovy. In 2011, sardine was the most important species in some hauls, especially in Spanish coastal waters. Chub mackerel was more abundant in the Spanish part in 2005, but was mainly distributed in the western part of the Gulf in 2008 and 2001.

Year	Species	Weight (kg)	Number	Occurrence (%)
2005	Engraulis encrasicolus	1 153.1	75 459	94
	Scomber colias	717.5	5 011	71
	Lepidopus caudatus	285.6	8 076	29
	Sardina pilchardus	197.0	5 174	61
	Mola mola	72.0	6	16
	Scomber scombrus	34.0	177	74
	Boops boops	26.0	296	52
	Merluccius merluccius	23.3	219	81

Table 2.5.6. BOCADEVA survey series. List of the main species caught (only for species with catches >10 kg).

Year	Species	Weight (kg)	Number	Occurrence (%)
	Trachurus trachurus	22.2	1 586	87
	Diplodus bellottii	19.8	338	35
	Spondyliosoma cantharus	18.9	104	32
	Pagellus acarne	15.0	73	13
	Pagellus erythrinus	13.0	68	16
	Serranus hepatus	10.5	308	39
	Trachinotus ovatus	10.1	30	16
	Scomber colias	1 687.9	16 726	100
	Engraulis encrasicolus	839.8	45 966	88
	Trachurus picturatus	449.5	7 194	62
	Sardina pilchardus	236.9	5 707	50
	Scomber scombrus	107.0	939	96
2008	Trachurus mediterraneus	44.0	405	42
	Trachurus trachurus	34.8	459	50
	Boops boops	33.6	408	65
	Merluccius merluccius	32.7	220	81
	Pagellus erythrinus	20.1	121	31
	Mola mola	19.8	5	12
2011	Scomber colias	3 957.8	82 041	91
	Sardina pilchardus	3 018.3	111 702	68
	Engraulis encrasicolus	2 760.3	234 958	95
	Scomber scombrus	1 444.4	11 245	86
	Trachurus mediterraneus	347.8	2 155	23
	Boops boops	77.7	840	77
	Pagellus acarne	61.2	333	18
	Merluccius merluccius	53.5	575	100
	Pagellus erythrinus	46.2	309	32
	Diplodus bellottii	35.2	751	55
	Mola mola	19.3	1	5
	Alopias vulpinus	18.8	2	9
	Spondyliosoma cantharus	18.5	150	36


Figure 2.5.6. BOCADEVA survey series for the years 2005, 2008, and 2011. Species composition (% in weight) in valid fishing hauls.

The number of samples from both size and biological sampling are shown in Table 2.5.7. The results from 2005 showed some spatial structure, clearly evidenced by visual inspection of the mature female mean weight and batch fecundity. In agreement with the spatial distribution of daily egg production in that year, data post-stratification in two geographic strata was also considered and tested for all adult parameters. The limit of separation of these two different strata was established at 7°30′W, which, to some extent, splits the whole study area in Spanish and Portuguese waters.

Year	2005	2008	2011
Size sampling	3 328	2 538	2 528
Non-hydrated	643	565	707
Hydrated	317	208	303

Table 2.5.7. BOCADEVA survey series. Number of anchovies sampled.

The values obtained for the sex-ratio have been very similar in the three years analyzed, very close to the 1:1 ratio (Figure 2.5.7). The female mean weight estimated in the Portuguese area in 2005 was higher than in Spanish waters (8.5 g less). In 2008, female mean weight was 23.7 g, whereas it decreased significantly in 2011 (15.2 g). Relative to batch fecundity, the results follow a very similar trend to that observed for female mean weight, recording the lowest value in 2011 (ca. 8000 eggs per female). The values obtained for the spawning fraction have been similar, ranging from 0.22 in 2005 to 0.28 in 2011. The value obtained for the Spanish area was slightly higher than in



Portuguese waters in 2005, with a difference of 0.05% (Figure 2.5.7). In all cases, the estimated *CV*s have been low.

Figure 2.5.7. Sex ratio (in weight) of the mature population (*R*), mean female weight (*W*), batch fecundity (*F*), and spawning fraction (*S*) estimated for anchovy in the Gulf of Cádiz (BOCADEVA survey series).

2.5.3.7 Anchovy spawning-stock biomass

Gulf of Cádiz anchovy spawning-stock biomass estimated by DEPM for 2005, 2008, and 2011 averaged ca. 26 kt. In the first year, a relatively low biomass level (14 kt) was estimated. In 2008, a remarkable increase up to 31.5 kt was estimated (Figure 2.5.8, Table 2.5.8). This value is very similar to that obtained in 2011. The size composition of the estimated population ranged between the 7.5- and 19.0-cm size classes, with modal classes mainly located at 11.5–13.5 cm (Figure 2.5.9). In 2011, a high number of anchovies measuring 11–11.5 cm was registered.



Figure 2.5.8. Gulf of Cádiz anchovy spawning-stock biomass estimates by the DEPM (BOCADEVA series). The bars indicate the *CV* (not available for 2005).



Figure 2.5.9. BOCADEVA survey series. Anchovy length frequency distributions of the estimated population.

Table 2.5.8. BOCADEVA survey series. Summary table of the parameters obtained during the series.

Parameter	200	2005		2008	
Area	Portuguese waters	Spanish waters	Portuguese waters	Spanish waters	Total Gulf of Cádiz
Surveyed area (km²)	11 98	32	13 0	20	13 107
Positive area (km²)	1 409.4	4 729.1	1 690.4	5 172.2	6 770
Eggs					
P_0 (eggs m ⁻² d ⁻¹) (<i>CV</i>)	51 (0.80)	225 (0.69)	184 (0.44)	348 (0.35)	276 (0.32)
P _{tot} (eggs d ⁻¹ × 10 ¹²) (<i>CV</i>)	0.07 (0.76)	1.06 (0.65)	0.31 (0.44)	1.80 (0.35)	-0.29 (1.14)
Z d ⁻¹ (CV)	-0.039 (0.75)	-1.43 (0).29)	1.87 (0.36)

Parameter	20	2005 2008		08	2011
Area	Portuguese waters	Spanish waters	Portuguese waters	Spanish waters	Total Gulf of Cádiz
Adults					
Female weight (g) (<i>CV</i>)	25.2 (0.03)	16.7 (0.04)		23.67 (0.06)	15.2 (0.11)
Batch fecundity (<i>CV</i>)	13 820 (0.05)	11 160 (0.05)		13 778 (0.07)	7 486 (0.12)
Sex ratio (CV)	0.53 (0.01)	0.54 (0.01)		0.53 (0.005)	0.53 (0.007)
Spawning fraction (<i>CV</i>)	0.26 (0.07)	0.21 (0.07)		0.22 (0.065)	0.27 (0.036)
Spawning biomass (t) (<i>CV</i>)	14 65	73	31 527	(0.32)	32 757 (0.40)

2.5.4 Acknowledgments

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2.6 Autumn acoustic surveys on juvenile anchovy in Subarea 8: JUVENA 2003– 2012

Guillermo Boyra and Udane Martínez

2.6.1 Introduction

In the Bay of Biscay, the management of the anchovy fishery relies on an annual estimate of abundance, based on the information provided by the spring acoustic and DEPM surveys plus the annual catches of the fishing fleet (ICES, 2011a). This approach has been applied since the late 1980s and has produced a well-established and robust time-series. However, this annual estimate of abundance is provided at the end of the spawning season when a significant part of the annual anchovy fishery has already ended, thus limiting the potential effectiveness of the management measures resulting from the scientific advice.

A succession of low recruitments since 2001 that eventually caused the closure of the fishery in 2005–2009, highlighted the need for an early index of recruitment strength in order to improve management (Barange *et al.*, 2009). With this purpose in mind, an acoustic survey (JUVENA) for estimating the abundance of juvenile anchovy in early autumn was set up (Boyra *et al.*, 2013), following methodologies derived from the European project JUVESU (Uriarte, 2002; Carrera *et al.*, 2006). The objective of the survey was to use the juvenile abundance estimate as an indicator of the abundance of recruits that would enter the adult stock in the following year.

2.6.2 Material and methods

The JUVENA surveys were carried out annually during 2003–2012 between September and October in the Bay of Biscay (Table 2.6.1) following an acoustic methodology specifically designed to sample juvenile anchovy (Boyra *et al.*, 2013). An adaptive sampling strategy was chosen, using a combination of two small vessels (instead of the more commonly used single larger vessel) to allow more flexible coverage of the potentially variable and near-surface sampling area (see Annex 2 [Section A2.5] and Boyra *et al.*, 2013 for details). Also, the biological sampling and fish species identification was based on commercial purse-seining (highly efficient for the surficial distribution of juvenile anchovy, but of limited capacity for deeper detections) in the first years of the survey, and incorporated pelagic trawling since year 2006 (see Table A2.4 for vessel characteristics and equipment employed in each survey).

Since the addition of the second vessel, intercalibration exercises were carried out each year. The intercalibration process consists of comparing the echo-integrated acoustic backscattering of the water column and the bottom echo in areas with a smoothly variable seabed. A minimum distance of 30 nautical miles was covered simultaneously by the two vessels for these exercises. The NASC values (MacLennan *et al.*, 2002) obtained by the layer echo integration of both the water column and bottom echoes obtained by the two vessels were compared to detect recording biases or other potential problems.

Year	Start of survey	End of survey	No. vessels	Effective survey time (d)	Fishing operations	Anchovy- positive fishing operations	Inter- transect distance (nautical miles)	Sampled area (nautical miles²)	Positive area (nautical miles²)
2003	18/09	14/10	1	20	47	36	17.5	16 829	3 476
2004	19/09	19/10	1	18	21	9	17.5	12 736	1 907
2005	12/09	07/10	2	20	47	45	17.5	25 176	7 790
2006	13/09	14/10	2	42	80	52	15	27 125	7 063
2007	04/09	30/09	2	37	70	40	15	23 116	5 677
2008	26/08	25/09	2	33	85	46	15	23 325	6 895
2009	26/08	25/09	2	39	67	42	15	34 585	12 984
2010	1/09	30/09	2	40	79	60	15	40 500	21 110
2011	1/09	5/10	2	40	77	64	15	37 500	21 025
2012	2/09	5/10	2	40	67	51	15	31 500	14 271

Table 2.6.1. Summary of sampling effort in the JUVENA surveys in 2003–2012.

In general terms only, the 38-kHz data were echo integrated using the TS-length relationships agreed in ICES WGACEGG for the main species (ICES, 2006b). Acoustic data were converted to biomass using information from the hauls and following standard procedures (see Annex 2 and Boyra et al., 2013 for details). Each fishing haul was classified by species. A random sample of each species was measured to determine the length frequency distribution of the different species. Complete biological sampling of anchovy was performed to analyze age, size, and the size-weight relationship. The hauls were grouped by strata of homogeneous species and size composition. The species and size composition of each homogeneous stratum were obtained by averaging the composition (in numbers) of the individual hauls contained in the stratum, weighted to the acoustic density in the vicinity (±2 nautical miles around the fishing haul). This species and size composition of each stratum was used to obtain the mixed species echo integrator conversion factor (Simmonds and MacLennan, 2005) for converting the NASC values of each ESDU into numbers of each species. However, although the methodology involved estimating multiple species, the survey strategy was focused strongly on juvenile anchovy, and only the positive areas for anchovy were processed. Therefore, estimates were produced only for this species.

Anchovy juveniles (age 0) and adults (age \geq 1) were separated and treated as different species. To separate juveniles from adults, the length frequency distribution of anchovy by haul was multiplied by a corresponding age–length key. The key was determined every year for three broad areas: the pure juvenile area, the mixed juvenile area (a mixture of juveniles and adults), and the Garonne River area (located at the coastal area around 45°40'N; also a mixed area, but here, adult anchovy were usually smaller than in the other areas). Finally, the abundance in numbers of juveniles and adults was multiplied by the mean weight to obtain the biomass per age and length class for each ESDU.

Juvenile anchovy showed a near-surface distribution during the JUVENA survey period (Uriarte *et al.*, 2001). In order to achieve the best possible coverage of the vertical distribution of this species in the JUVENA surveys, the echo integration was started at

a range of 5 m from the transducer face (i.e. at a depth of 7.5 m from the surface) rather than the 10.5 m (13-m depth) recommended to avoid integrating the near-field of the 38-kHz transducer (Simmonds and MacLennan, 2005). Instead of ignoring these first layers, the bias produced by the near-field in the 7.5–15 m echo integration channel was estimated and corrected for in the biomass estimates. The details of this analysis are explained in Boyra *et al.* (2003).

Yearly maps of anchovy NASC values by ESDU along the survey transects were produced, including SST based on the CTD casts. Also, in order to estimate the recruitment predictive capability, the annual biomass estimates for anchovy juveniles were compared with the estimates of anchovy recruitment the following year. The recruitment is the biomass of age 1 anchovy in January of the following year, which is estimated using a Bayesian-based assessment model (ICES, 2013). This model takes information from spring acoustic and DEPM surveys into account, but not the juvenile index from JUVENA (hence being independent from the JUVENA estimates). The biomass estimates were fitted by linear, log-linear, and rank regressions between variables using the R software version 2.14.0 (R Development Core Team, 2011).

2.6.3 Results and discussion

The sampling coverage over the surveys (Figure 2.6.1) reflects the adaptive sampling scheme followed and shows the methodological changes made to improve the coverage, particularly since 2005. For example, the northern coverage along the French coast was considerably enlarged. The northern limit changed from 46°N in the first two years to beyond 47°N in subsequent years. The number of fishing operations increased from 40 in 2003 to >60 in the years since 2005. The incorporation of the pelagic trawler in 2006 improved fishing flexibility, making it possible to fish near the seabed. This is shown in the fishing maps (Figure 2.6.1) by the increase in species diversity (due to the addition of the near-bottom species).

Every year, the juvenile anchovy population could be divided into two clearly distinguishable groups. One part of the population consisted of nearly 100% juvenile anchovy (not mixed with adults), and the other part was mixed with, or in close proximity to, adults. The predominantly juvenile group was located in the southwestern part of the Bay of Biscay, normally off the shelf or in the outer part (Figure 2.6.2), and the mixed group was located to the northeast in shelf waters at depths < 130 m. In each group, juveniles were found in a different species composition context and bathymetrical distribution pattern. In the largely juvenile region, anchovy was generally the only or the predominant species (the percentage of anchovy individuals in these strata was always >90%), mixed sometimes with smaller proportions of salp, jellyfish, and juvenile horse mackerel (Figure 2.6.1). In the mixed group, juvenile anchovy was found mixed in variable proportions with adult anchovy (the adult proportion increasing towards the northeast, Figure 2.6.2) and other pelagic species, mainly sardine, horse mackerel, mackerel, and sprat (Figure 2.6.1).





Figure 2.6.1. Sampling design and species composition of the hauls of JUVENA surveys in 2003–2012.



Figure 2.6.2. Age composition of anchovy for the ten years of the survey. The pie charts represent the mean percentage of juveniles (white) and adults (black) in the fishing hauls at each fishing site.

For the years that had the highest juvenile biomass estimates, the largest biomass contributions were invariably observed in the largely juvenile areas (Figure 2.6.3). This region contained, on average throughout the series, ca. 58% of the total biomass. This average increased to 75%, when the least abundant years (2004, 2007, and 2008) were excluded, and reached 95% of the total biomass in 2010, when the strongest recruitment in the JUVENA series was recorded. The contribution of the mixed-juvenile region to the biomass was quite constant across the series, and in the years with the highest total juvenile abundance, the contribution was relatively weak. The occupation of the off-shelf waters by predominantly juvenile anchovy is consistent throughout the series and agrees with previous work on juvenile anchovy in the Bay of Biscay (Uriarte *et al.*, 1996, 2001; Carrera *et al.*, 2006; Lezama-Ochoa *et al.*, 2010). Uriarte *et al.* (2001) described a large juvenile abundance in the French outer-shelf waters (as in 2009 and 2010) in the 1999 JUVESU survey, which was followed by strong recruitment in 2000 (ICES, 2010a).



Figure 2.6.3. Positive area of presence of anchovy and total acoustic energy echo integrated (from all the species) for JUVENA 2003–2012. The area delimited by the dashed line is the minimum or standard area used for interannual comparison.

A wide range of acoustic estimates of juvenile anchovy biomass was assessed over the JUVENA series (Table 2.6.2, Figure 2.6.4). The estimates showed differences of more than two orders of magnitude between the lowest value in 2004 and the highest in 2010. The series of juvenile anchovy biomass estimates showed good parallelism to the independent estimates of anchovy biomass at age 1 recruitment at the beginning of the following year (Figure 2.6.4). Both quantitative linear (Pearson) and non-parametric rank (Spearman) correlations showed highly significant values (the coefficients of determination were 0.93 and 0.86, respectively, with probabilities of being random < 0.0001 and 0.01, respectively). These significant relationships were not dependent on the extreme values of the series; both correlations continued to be significant (at an alpha of 1%) even when the highest or lowest juvenile index from the JUVENA series was excluded from the analysis. Several quantitative linear or log-linear models can be fitted to series of recruitment based on the juvenile abundance indexes from JUVENA. The best fitting was achieved by the log-linear model, i.e. a potential model in linear scale, with coefficient of determination of 0.95 and p-value = 5×10^{-5} (Figure 2.6.4). Concerning the main observed groups, the biomass of the purely juvenile group was significantly correlated with recruitment, explaining 87% of the recruitment variability, while the biomass of the mixed juvenile and adult group showed no significant relationship and explained less than 20% of the recruitment variability.

Table 2.6.2. Synthesis of the abundance estimation (acoustic index of biomass) for the ten years of JUVENA surveys.

Year	Sampled area (nautical miles²)	Positive area (nautical miles²)	Juvenile length (cm)	Juvenile biomass (year y)	Recruits age 1 (year <i>y</i> +1)
2003	16 829	3 476	7.9	98 601	46 500
2004	12 736	1 907	10.6	2 406	6 648
2005	25 176	7 790	6.7	134 131	29 530
2006	27 125	7 063	8.1	78 298	36 350
2007	23 116	5 677	5.4	13 121	12 960
2008	23 325	6 895	7.5	20 879	13 010
2009	34 585	12 984	9.1	178 028	61 755
2010	40 500	21 110	8.3	599 990	128 400
2011	37 500	21 063	6.0	207 625	37 650
2012	31 724	14 271	6.4	142 083	32 860



Figure 2.6.4. Temporal series of juvenile (J) against recruitment (R) indices and fitting of different regression models between them: linear, log-linear, and and by order of rank (Spearman).

The main aim of these surveys was to test whether the abundance of juveniles in autumn constitutes a reliable indicator of the strength of recruitment to the adult stock each year in the Bay of Biscay. The hypothesis has been confirmed by the significant positive correlations between the biomass estimates of juveniles and the age 1 recruits in the following year (Figure 2.6.4). Boyra et al. (2013) showed that a significant correlation exists between juvenile and recruitment indices, thus supporting the reliability of the JUVENA index to anticipate abundance trends in the Bay of Biscay anchovy. In the present work, after the addition of two more survey estimates corresponding to medium and high recruitment levels, the correlations between juvenile and recruitment are still highly significant, further supporting the validity of the JUVENA index. The study period has shown a broad range of recruitment levels, from an extremely low level in 2004, which caused the collapse of the fishery, to one of the highest recruitment levels of the entire time-series in 2011. The relationship between the juvenile and recruitment indices is not too dependent on any particular point of the series, neither the maximum nor the minimum. The relationship was also independent of the model chosen. Although linear and log-linear models were shown to be good candidates, the definition of the best quantitative model for prediction is left to the ICES assessment working group to decide.

The results of the JUVENA acoustic series reveal the spatial pattern of anchovy recruitment in the Bay of Biscay, in which the southwestern drift of larvae towards southern and offshore areas, surpassing the shelf and occupying oceanic waters within the bay until becoming juveniles, is not detrimental to the success of recruitment, but constitutes a natural phase before juveniles reach a sufficient size to migrate back towards more coastal areas during autumn. This was already postulated by Uriarte *et al.* (2001) and Irigoien *et al.* (2008) and finally supported by the finding of JUVENA that the biomass of the purely juvenile group, which mostly occupies the southwestern waters in the Bay, is significantly correlated with the next recruitment at age 1, explaining 87% of its variability (already pointed out in Boyra *et al.*, 2013).

2.6.4 Acknowledgements

This project was funded by the Basque Government "Viceconsejería de Agricultura, Pesca y Politicas Alimentarias – Departamento de Desarrollo Económico y Competitividad" – Gobierno Vasco" and the Spanish Government "Secretaría General de Pesca, Ministerio de Agricultura, Alimentación y Medio Ambiente – Gobierno Español". We acknowledge the skippers and crew of the vessels that participated in the survey: FV "Divino Jesús de Praga", FV "Nuevo Erreinezubi", FV "Mater Bi", FV "Gure Aita Joxe", FV "Itsas Lagunak", RV "Emma Bardán", and RV "Ramón Margalef".

2.7 Autumn acoustic surveys off the Portuguese continental shelf and Gulf of Cádiz (ICES Division 9.a)

Vitor Marques, Maria Manuel Angélico, Eduardo Soares, Alexandra Silva, and Cristina Nunes

2.7.1 Introduction

The Portuguese autumn acoustic surveys were mainly designed to estimate the abundance of young sardines recruiting to the fishery as age group 0. Sardine peak spawning is in winter, and young-of-the-year sardines are more visible to the fisheries in autumn. In 1984–1987, the Portuguese surveys were systematically performed each year in autumn/winter. Only a sporadic attempt took place in 1992, after which the surveys resumed with a systematic periodicity in 1997–2008. During the last decade, four acoustic autumn surveys were performed with RV "Noruega", identified with the acronym SAR: SAR05NOV, SAR06NOV, SAR07NOV, and SAR08OUT. The surveys presented in this report were carried out during the last decade, using a standardized methodology and covering the majority of the area.

2.7.2 Material and methods

The acoustic methodology was the echo integration of the acoustic targets and fish schools, surveyed along 60 transects perpendicular to the coast line and spaced ca. 8 nautical miles apart. For acoustic data collection, a Simrad EK500 echosounder with 38-kHz frequency was used. The acoustic data were stored and processed using the IFREMER software MOVIES+ (Weill *at al.*, 1993). For species identification and biological sampling, trawl hauls were performed (for details, see the Portuguese acoustic survey protocols in Annex 2).

2.7.3 Results

2.7.3.1 Abundance estimates

The main results of these surveys are presented in the tables and figures below (Table 2.7.1 and Figures 2.7.1–3). The results of each survey are then described separately.

Table 2.7.1. Autumn survey main information: trawl hauls, sardine biomass (thousand tonnes), and juvenile percentage for each zone.

Survey	SAR05NOV	SAR06NOV	SAR07NOV	SAR08OUT
Number of trawl hauls	29	27	33	31
Number of hauls with sardine	21	16	24	20
OCN biomass	458	257	258	121
OCS biomass	34	69	114	35
Algarve biomass	12	27	11	0.6
Cádiz biomass	-	57	133	149
Total biomass	504	410	516	306
% juv_OCN	75	7	41	76
% juv_OCS	43	29	32	10
% juv_Alg	63	41	17	11
% juv_Cádiz	-	3	34	46



Figure 2.7.1. Trawl haul geographic location and proportion of the main pelagic species in the trawl catches. Species FAO key code.



Figure 2.7.2. Acoustic energy (NASC) attributed to sardine along the transects. Sardine abundance and length composition in each zone. The Portuguese southwestern coast and the western Algarve coast were not covered during the 2007 survey.



Figure 2.7.3. SAR05NOV and SAR06NOV surveys: proportion of sardine juveniles (white colour) in each trawl. AP – pelagic trawl, AF – bottom trawl, AD – purse-seine (during the 2005 survey, one sample collected from the commercial fleet was also used).

2.7.3.2 SAR05NOV

2.7.3.2.1 Sardine distribution and abundance

Sardine was the dominant species in the Occidental North area (OCN; Figure 2.7.2). Off the remaining Portuguese shelf, other pelagic species were observed (horse mackerel, chub mackerel, and bogue), usually more abundant than sardine in this survey. In the Occidental South (OCS) and Algarve areas, the acoustic energy attributed to sardine represented only 7 and 11%, respectively, of the total fish acoustic energy.

2.7.3.2.2 Recruitment strength and sardine length structure

The sardine population presented significant differences between areas (Figures 2.7.2 and 2.7.3). In the OCN area, the population was clearly dominated by sardine juveniles (total length <16 cm), with a modal length distribution of 15.5 cm. In numbers, juveniles represented 75% of the total estimated number for this area.

In the OCS area, the sardine length distribution was bimodal, with one mode at 10.5 cm and another at 20.5 cm. In this area, the juveniles were mainly distributed off Lisbon, near Cabo da Roca, and represented 43% of the abundance for this area.

In Algarve, the length structure was also mixed, with juveniles dominating the eastern part of the area, representing 63% of the individuals.

2.7.3.3 SAR06NOV

2.7.3.3.1 Sardine distribution and abundance

Sardines were mainly distributed off the western shelf, north of Lisbon (Figure 2.7.2). There was an increase in other pelagic species in all areas (Figure 2.7.1). Anchovy was mainly distributed in the Bay of Cádiz, as usual.

Off the southwestern part of the coast, south of Lisbon, sardine was almost absent.

2.7.3.3.2 Sardine recruitment

The sardine length structure was clearly dominated by adults in the whole area, which suggested low recruitment in 2006 (Figures 2.7.2 and 2.7.3). Only 7% of the sardine in the OCN zone were juveniles. The OCS zone showed a bimodal distribution, with 9- and 19-cm modes. The juveniles, corresponding to 29% of the abundance in this area, were located near Lisbon. Algarve had also a mixed length structure, with juveniles representing 41% of the abundance for this area. Cádiz was dominated by adults, with only 3% juveniles. For all areas surveyed, juveniles represented only 13% of the estimated sardine abundance.

2.7.3.4 SAR07NOV

The area was not completely covered in autumn 2007 due to lack of time (the southern coast and the Algarve west coast were not covered), but the main juvenile distribution area was surveyed, fulfilling the main objective of these surveys.

In the eastern part of Algarve and Cádiz, an increased fishing effort during the survey was aimed at collecting more information on the pelagic community. As a result, it became possible to estimate the abundance and distribution of anchovy and chub mackerel, in addition to sardine (Figures 2.7.4 and 2.7.5).



Figure 2.7.4. SAR07NOV: acoustic energy (NASC) attributed to anchovy (*Engraulis encrasicholus*) along the transects, with anchovy abundance and length composition shown for each zone. The Portuguese southwestern and western Algarve coasts were not covered.



Figure 2.7.5. SAR07NOV and SAR08OUT: acoustic energy (NASC) attributed to chub mackerel (*Scomber colias*) along the transects, with chub mackerel abundance and length composition shown for each zone. The Portuguese southwestern and western Algarve coasts were not covered in the 2007 survey.

2.7.3.4.1 Sardine distribution and abundance

Sardine was distributed along the continental shelf, but was abundant on the west coast between Figueira da Foz and Peniche (Figure 2.7.2). Sardine abundance for the OCN and OCS areas was 258 and 114 thousand tonnes, respectively. These amounts are average within the historical series. In Algarve, only the east part was covered, and the estimated abundance was 11 000 tonnes. In the Cádiz area, sardine was abundant, with the biomass being estimated at 133 000 tonnes.

2.7.3.4.2 Sardine recruitment and length structure

The sardine length distribution was bimodal in all areas (Figure 2.7.2), the small mode corresponding to juveniles. This confirms the existence of sardine recruitment in the whole distribution area. In the OCN area, 41% of the individuals were juveniles. In the OCS zone, 32% were juveniles and were found off Lisbon, as is usually the case. In Algarve, only 17% were juveniles, while in Cádiz, juveniles constituted 34% of the abundance in this area. These numbers suggest that the average level of recruitment for sardine corresponded to that in 2007.

2.7.3.4.3 Anchovy distribution and abundance

As usual, anchovy was caught mainly in the Gulf of Cádiz and in minor quantities off Lisbon (Figure 2.7.3). In the Cádiz area, anchovy was relatively abundant, the biomass estimated at 16 000 tonnes. Anchovy was also found in the eastern part of Algarve, with the biomass for this area estimated at 7600 tonnes. The anchovy "spot" found off Lisbon represented only 1100 tonnes (Figure 2.7.4).

2.7.3.4.4 Chub mackerel distribution and abundance

It was the first time that an attempt was made to estimate the distribution and abundance of chub mackerel off the Portuguese continental shelf (Figure 2.7.5). In the surveyed west coast (southwestern coast not covered), a biomass of 159 000 tonnes was estimated. In the east part of Algarve, 18 500 tonnes were estimated, with 61 000 tonnes of chub mackerel estimated at Cádiz. The mode of the length distribution for the whole area covered was ca. 22 cm.

2.7.3.5 SAR08OUT

2.7.3.5.1 Sardine abundance and distribution

Sardine was mainly distributed off the west coast, north of Peniche (Figure 2.7.2). In the OCN zone, the sardine biomass was estimated at 121 000 tonnes. On the west coast, south of Lisbon, and in Algarve, sardine was not abundant. In these areas, chub mackerel was the dominant species. The estimated sardine biomass in the OCS zone was only 35 000 tonnes, and sardine were almost absent (601 tonnes) in Algarve. The abundance estimate for Cádiz (149 000 tonnes) was within the average for this area.

2.7.3.5.2 Sardine length structure

The sardine length structure was bimodal for the OCS and Cádiz areas and trimodal for the OCN area.

2.7.3.5.3 Chub mackerel abundance and distribution

Chub mackerel was abundant in the OCS and Algarve areas and less so in the Cádiz zone (Figure 2.7.5). The length structure was bimodal in all areas.

2.7.3.6 Environmental setting and sardine egg distribution

During the first half of the decade, weather conditions in the region in late autumn were very unsettled, and the survey results were incomplete and discontinuous. For that reason, it was decided to include only the surveying period from 2005 to 2008 in this report.

During the October–November period in southern and western Iberian waters, the structure of the water column is typically still stratified and water temperatures fairly warm. Normally, it is only later in the season that wind events and the continuous drop in atmospheric temperature lead to the breakdown of thermal stratification and the onset of winter conditions. Once rainfall episodes become frequent, river plumes become evident in the surface temperature and/or salinity data and haline stratification may occur in some inshore areas; often associated with these conditions, river runoff promotes local plankton bursts.

The oceanographic setting during the Portuguese autumn surveys in 2005–2008 was diverse (Figures 2.7.6–2.7.9). Water temperatures were lower during the 2005 and 2008 surveys, milder across the entire region in 2006, and in-between in 2007. While water temperatures were still mild in November 2006, the occurrence of rainfall promoted the onset of extended river plumes off Lisbon and over the northwestern shelf.

Sardine egg distributions shown in Figure 2.7.9 indicate that the spawning season was underway at the time of all the surveys, but also highlight the differences encountered between years. During the 2006 survey, egg abundance was higher than in the other years. In 2005, the low number of eggs and their distribution was quite likely associated with the population structure dominated by young individuals less fecund and with a

shorter (often delayed) spawning season. For the region, surveying in October– November generally coincides with the beginning of the spawning season, which reaches its peak in winter, but egg abundance is usually still quite high during the spring survey (Section 2.2).



Figure 2.7.6. Sea surface temperature distribution contour derived from the CUFES CT sensor.



Figure 2.7.7. Sea surface salinity distribution contour derived from the CUFES CT sensor.



Figure 2.7.8. Sea surface fluorescence distribution contour derived from the CUFES system.



Figure 2.7.9. Sardine egg abundance distribution during the acoustic survey, derived from the CUFES sampler.

2.7.4 Acknowledgements

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3 Spatial distributions of small pelagics and environmental indicators from surveys

3.1 Method of common database and gridding

Pierre Petitgas and Matthieu Authier

To achieve the combination at a regional scale of the data from the DEPM and acoustic surveys, the data for each period were block-averaged on a common spatial grid. On this grid it is possible to represent all variables (environmental parameters, egg and fish concentrations, top predators, plankton, etc.) and to structure a common database.

The spatial resolution of the grid (i.e. mesh size) depends on the spatial configuration of the surveyed areas and sample locations. Data averaging over tiny blocks has the disadvantage that either there are no data in the blocks or the average depends on a single high value. Also, the position of the origin of the grid influences the block averaging. To mitigate these effects, the origin of the grid is randomized in a larger block that comprises four smaller blocks, as shown in Figure 3.1.1. This will have the effect of local smoothing and deconditioning the averaging from the origin of the grid. The procedure applied is the following: (i) 200 grids are generated, each with a different origin; (ii) block averaging is performed for each; (iii) all grids are then superposed; and (iv) the mean in each cell is calculated by averaging the cell means of all grids. The grid mesh is $0.25 \times 0.25^\circ$, and the lower left corner of the grid is positioned at 10.2° W 35.8°N.



Figure 3.1.1. Schematic of the standard grid (black: $0.25 \times 0.25^{\circ}$), the large block (dashed red line) in which the grid origin is randomized. The cross (blue) shows the position where the origin of the grid is positioned to present the final results.

The above blocking procedure was applied to the reported surveys in this report for CalVET hauls, CUFES samples, and acoustic area backscatterinog cefficient (sA) data. For CalVET or CUFES data, the cells containing less than three samples were not retained because of insufficient observations. For acoustic data, the cells containing less than five samples were not retained. The number of observations per cell is 3–18 for CUFES, 3–12 for CALVET, and 5–32 for acoustic data (using 2008 as an example).

3.2 Grid database

A series of gridded data files was created by blocking the raw data from each survey, institution, and available parameter (Table 3.3.1). Parameters considered were concentration of eggs, juveniles and adults of anchovy and sardine, physical parameters, and top predators, all collected simultaneously and continuously along the ship's track.

As a whole, 73 surveys were taken into consideration, and 39 blocked data files were created. Some of the surveys were annual, some were triennial, and some were irregular over the years and seasons. Nevertheless, each survey was carried out between 2003 and 2012; the methods were acoustics, CUFES, or DEPM; and the main

objectives of the surveys were to provide abundance indices of eggs, juveniles, or adults of anchovy and sardine.

3.3 Grid maps

Jacques Massé, Andrés Uriarte, Maria Manuel Angelico, Pablo Carrera, Mathieu Doray, and all WGACEGG members

A total of 382 maps were provided and are available for download from the ICES website via the <u>WGACEGG page</u> or using the following link:

http://ices.dk/community/groups/Documents/Forms/AllItems.aspx?RootFolder=%2Fc ommunity%2Fgroups%2FDocuments%2FWGACEGG%2FCRR%20332%20Suppleme ntary%20online%20material&View=%7B49A2EFDE-3932-4900-A03D-70258239F39E%7D

Each folder contains maps for the survey in question along with a file providing detailed explanations. Annual maps are produced for individual survey series, with each series concluded by two maps, an average one and one with standard deviation.

Table 3.3.1. List of surveys for which blocked data files were produced.

Survey type	File	Parameter	Years
Summer acoustic survey in Cádiz; ECOCADIZ	AC_ECOCADIZ_GC_SSS	Sea surface salinity	2004, 2006, 2007, 2009, 2010
	AC_ECOCADIZ_GC_SST	Sea surface temperature	2004, 2006, 2007, 2009, 2010
	AC_ECOCADIZ_GC_SA_SAR	Anchovy acoustic energies (NASC)	2004, 2006, 2007, 2009, 2010
	AC_ECOCADIZ_GC_SA_ANE	Sardine acoustic energies (NASC)	2004, 2006, 2007, 2009, 2010
Autumn acoustic survey in Bay of Biscay; JUVENA	AC_JUVENA_BB_SA	Total anchovy acoustic energies (NASC)	2003–2012
	AC_JUVENA_BB_SA ANE_age0	Anchovy age 0 acoustic energies (NASC)	2003–2012
	AC_JUVENA_BB_SA ANE_age1+	Anchovy adults acoustic energies (NASC)	2003–2012

Survey type	File	Parameter	Years
Summer– autumn	AC_SAR_AS_SA_SAR	Sardine acoustic energies (NASC)	2005–2008
acoustic survey in Division 9.a;	AC_SAR_AS_SSS_SAR_q_95	Sea surface salinity	2005–2008
SAR05/06/07NO V; SAR08out	AC_SAR_AS_SST_SAR_q_95	Sea surface temperature	2005–2008
_	AC_SAR_AS_CUFES_ANE_q_99	Number of anchovy eggs by CUFES	2005–2008
	AC_SAR_AS_CUFES_SAR_q_95	Number of sardine eggs by CUFES	2005–2008
Annual spring acoustic survey	AC_SPRING_IBBB_SSS_q_98	Sea surface salinity	2003–2012
from Cádiz to Brittany; Division 0 a cod	AC_SPRING_IBBB_SST_q_98	Sea surface temperature	2003–2012
Division 9.a and Subarea 8; PELGAS/PELAC US/ PELAGO -	AC_SPRING_IBBB_CUFES_ANE_ q_98	Number of anchovy eggs by CUFES	2003–2012
	AC_SPRING_IBBB_CUFES_SAR_ q_98	Number of sardine eggs by CUFES	2003–2012
	AC_SPRNG_IBBB_SA_ANE	Anchovy acoustic energies (NASC)	2003–2012
	AC_SPRNG_IBBB_SA_SAR	Sardine acoustic energies (NASC)	2003–2012
	AC_SPRING_IBBB_Bird	Number of birds observed	2003–2012
	AC_SPRING_IBBB_Bird_Div	Number of diving birds observed	2003–2012
	AC_SPRING_IBBB_Bird_Gannet	Number of gannets observed	2003–2012
-	AC_SPRING_IBBB_Bird_Surf	Number of surface-feeding birds observed	2003–2012
	AC_SPRING_IBBB_Mam	Number of mammals observed	2003–2012
	AC_SPRING_IBBB_Mam_BaleenW hales	Number of baleen whales	2003–2012
	AC_SPRING_IBBB_Mam_Common Dolphins	Number of common dolphins	2003–2012
	AC_SPRING_IBBB_Mam_Dolphins	Number of dolphins	2003–2012

Survey type	File	Parameter	Years
Spring–summer egg survey; DEPM survey on	DEPM_ANE_BBGC_CUFES_ANE _q_98	Number of anchovy eggs by CUFES	2003–2012
anchovy; BIOMAN/ BOCADEVA;	DEPM_ANE_BBGC_CUFES_SAR _q_98	Number of sardine eggs by CUFES	2003–2012
Subarea 8 and Division 9.a South	DEPM_ANE_BBGC_PV_SAR_q_9 5	Number of anchovy eggs by PairoVET	2003–2012
	DEPM_ANE_BBGC_PV_ANE_q_9 8	Number of sardine eggs by PairoVET	2003–2012
	DEPM_ANE_BBGC_SSS_q_98	Sea surface salinity	2003–2012
	DEPM_ANE_BBGC_SST_q_98	Sea surface temperature	2003–2012
Triennial spring egg survey;	DEPM_SAR_IBBB_SSS_q_98	Sea surface salinity	2002–2011
DEPM survey on sardine; PT- DEPM-PIL/ SAREVA; Iberian coasts; divisions 9.a and 8.b–c	DEPM_SAR_IBBB_SST_q_98	Sea surface temperature	2002–2011
	DEPM_SAR_IBBB_CUFES_ANE_ q_98	Number of anchovy eggs by CUFES	2002–2011
	DEPM_SAR_IBBB_CUFES_SAR_q _98	Number of sardine eggs by CUFES	2002–2011
	DEPM_SAR_IBBB_PV_ANE_q_98	Number of anchovy eggs by PairoVET	2002–2011
	DEPM_SAR_IBBB_PV_SAR_q_98	Number of sardine eggs by PairoVET	1988–2011

4 Variability and habitats in spring

Pierre Petitgas, Martin Huret, Mathieu Doray, Jacques Massé, Matthieu Autier, Maria Manuel Angélico, Vitor Marques, Joana Andrade, Isabel Riveiro, Begoña Santos, Pablo Carrera, and Maria Santos, in collaboration with all WGACEGG members

All parameters averaged at the same spatial resolution on a common grid (Section 3) are used to analyze the interannual spatial variability as well as the relationships among parameters. The procedures were applied to the spring acoustic surveys (PELAGO, PELACUS, and PELGAS) during which environmental information as well as data on ichthyoplankton, fish, and top predators are collected, covering the entire area from Cádiz to Brest.

4.1 Interannual variability

Interannual variability was first characterized globally by computing a coefficient of variation for each cell as follows : CV(x) = sd(x)/m(x), where m(x) is the arithmetic mean and *sd* is the standard deviation over time for each cell (*x*). Further, to characterize differences between years more precisely and visually, spatial patches (Woillez *et al.*, 2009) were estimated, with an *R* script provided as supplementary material to Woillez *et al.* (2009). The *R* script identifies patches by aggregating to a large value the closest values in its surroundings. The procedure starts with the highest value and the next patch is formed around the next highest value that is not included in the previously formed patch. The patches retained were those that each contained at least 10% of the total abundance over the entire area.

4.2 Surface temperature and salinity

The coldest surface waters are observed around Galicia and on the northern coast of Spain; these areas show the lowest variability (Figure 4.2.1). The coastal waters north of 46°N are also generally cold and show high variability. The warmest waters are located south of 40°N, with intermediate variability. The French shelf is where the greatest variability is observed across years in spring, depending on the seasonal evolution of temperature stratification.



Figure 4.2.1. Spring sea surface temperature: time average (left) and CV (right).

The waters with highest salinity are located south of 40°N (Figure 4.2.2). River plumes are easily recognized in the Bay of Cádiz, off the rias (estuaries) in Galicia, off northern

Portugal, and off Gironde and Loire on the French shelf. The areas of river plume extent also show the highest interannual variability. The northern Portugal shelf and French mid-shelf are also areas of high interannual variability in sea surface salinity.



Figure 4.2.2. Spring sea surface salinity: time average (left) and CV (right).

4.3 Anchovy and sardine adults

For anchovy, the areas of highest concentration are located on the French shelf south of 47° N, close to the Gironde estuary (45° N) and in the Bay of Cádiz (Figure 4.3.1). These are recurrent high concentration areas ("core habitats") as revealed by their low *CVs*. In two coastal areas, north of Biscay (47° N) and north of Portugal (41° N), the *CV* is high, with mean abundance at medium–low values, meaning that anchovy in these areas do not always occur ("secondary habitats"). Northern Spain and northwestern Biscay are areas of very low mean abundance and high interannual variability. Occasional patches, only for particular years, occur in the secondary habitats (defined by medium–low mean and high *CV*), while gravity centres of patches are recurrent in all years in the core habitats (defined by high mean and low *CV*) as shown in Figure 4.3.2. Particularly in the Bay of Biscay, two patches (one farther north than the other) are observed in almost all years. The locations of gravity centres of spatial patches are more variable in this area than in Cádiz (Figure 4.3.2).



Figure 4.3.1. Spring distribution of anchovy (s_A): time average (left) and *CV* (right); gravity centres of spatial patches in each year (bottom right).



Figure 4.3.2. Spring distribution of anchovy (sA): gravity centres of spatial patches in each year.

For sardine, core and secondary habitats are also observed (Figure 4.3.3). Four core areas show high mean abundance and low *CV*: coastal waters in northern Biscay (47°N); southern shelf Biscay (44°N off Landes); central Portuguese shelf (37–41°N), where mean abundance is highest; and the Gulf of Cádiz. The two major core areas are the central Portuguese area and the coastal waters of northern Biscay. It is noteworthy that these sardine core areas show very little overlap with the anchovy core areas. The coastal waters off the Galician rias (42–43°N) show medium values for the mean abundance and *CV* and could thus be considered as a secondary habitat. In contrast, the northern coast of Spain and northwestern Biscay are areas of very low abundance and high variability. The gravity centres of spatial patches complement this picture with interannual variability (Figure 4.3.4). In the Bay of Biscay, two patches are observed each year corresponding to the two major core areas, with little variation in location (good spatial recurrence), which is also observed in the core central Portuguese area (39–41°N). Patches are observed for particular years in the secondary habitats in the western part of the Bay of Cádiz and west of Galicia.



Figure 4.3.3. Spring distribution of sardine (sA): time average (left) and CV (right).



Figure 4.3.4. Spring distribution of sardine (s_A): gravity centres of spatial patches in each year.

4.4 Anchovy and sardine eggs

The distributions of anchovy and sardine eggs show overall distribution patterns similar to those observed for spawning adults, with similar regions of core and secondary habitats (Figures 4.4.1 and 4.4.2), although the egg distribution seems to be shifted from that of spawning adults (see Section 4.3).

Also for sardine, eggs are observed with medium abundance levels and medium CV in areas where adults were recorded in very low abundance. These areas are located in the shelfedge north of 45°N in Biscay, the northern coast of Spain, and the northern coast of Portugal. It is possible that the adults in these areas are less accessible to the acoustic surveys than the eggs (e.g. very coastal adult distribution, schools too close to surface).



Figure 4.4.1. Spring spatial distribution of anchovy eggs (CUFES): time average (left) and CV (right).



Figure 4.4.2. Spring spatial distribution of sardine eggs (CUFES): time average (left) and CV (right).

4.5 Common dolphins

The average distribution of common dolphins (Figure 4.5.1) shows the highest abundance off Portugal and western Galicia (40–44°N) as well as offshelf in the southern part of the Bay of Biscay. The lower abundance is located on the mid-outer shelf in the Bay of Biscay (north of 45° N). The recurrent core habitats (high mean abundance and low *CV*) are located off Portugal and in the mid-inner shelf in the Bay of Biscay (central and northern Biscay). Western Galicia and southern offshore Biscay areas are less recurrent, with higher *CVs*. In contrast, the Gulf of Cádiz and the shelfedge of Biscay (45–47°N) show very low mean abundance and high *CV*.



Figure 4.5.1. Spring spatial distribution of common dolphins: time average (left) and CV (right).

4.6 Birds

Two groups of birds are distinguished, depending on their feeding strategy: those that feed at the surface (only in the first few meters of the water column) and those that feed by diving (see Section 2). They have similar overall distribution patterns but also subregional differences (Figures 4.6.1 and 4.6.2). Birds are more abundant south of 42°N, along the Portuguese coast and in the Gulf of Cádiz. In contrast to diving birds, surface birds show more concentrated aggregations in the northern part of Biscay, the northwestern coast of Spain, and in the Gulf of Cádiz.

Recurrent core areas for surface birds (high mean abundance and low *CV*) are located in southern Brittany, northern Portugal, and in the Gulf of Cádiz. In contrast, recurrent core areas for diving birds are located in northern Portuguese waters (north of 39°N) and in northern Biscay (Brittany).

The most variable areas (high *CV* and low mean abundance) for surface birds are the coastal central Biscay, the northern coast of Spain, and off the central Portuguese coast (39–41°N). The similar areas for diving birds are the northern coast of Spain and the central part of Biscay.



Figure 4.6.1. Spring spatial distribution of surface birds: time average (left) and CV (right).


Figure 4.6.2. Spring spatial distribution of diving birds: time average (left) and CV (right).

4.7 Habitats

Perry and Smith (1994) used cumulative distribution functions (CDF) to identify how fish concentration depend on environmental parameters. Röckmann *et al.* (2011) proposed a method to estimate the observed fish distribution in classes of an ancillary parameter, where the estimated frequencies do not depend on the number of observations in the different classes. With this method, it was possible to estimate the CDF of anchovy and sardine eggs and adult concentrations as a function of surface temperature and salinity. The departure from a straight line characterizes the affinity or repulsion of the fish for particular classes of temperature or salinity. All grid values from all years were pooled in the analysis.

4.8 Anchovy and sardine eggs

The distribution of anchovy eggs is limited to warm waters only (>14°C), while sardine eggs do not seem to be affected by surface temperature >12°C (Figure 4.8.1). Temperatures between 12 and 14°C are encountered in coastal waters on the French shelf and in upwelling areas along the Iberian coast and thus may represent key areas. There is no influence of salinity on the distribution of anchovy and sardine eggs (Figure 4.8.1) above 31 psu. Below that value, hardly any eggs are observed. Similar results are obtained using DEPM survey data, but with more erratic curves (not shown) because the survey series contains less samples.



Figure 4.8.1. Cumulative distribution of anchovy (plain line) and sardine (dashed line) eggs (CUFES) in spring as a function of sea surface temperature (left) and salinity (right).

4.9 Anchovy and sardine adults

The distributions of anchovy and sardine do not seem to be influenced by surface temperature >12°C or by surface salinity >30 psu (Figure 4.9.1). Therefore, these parameters cannot be used as proxies to describe their habitats.



Figure 4.9.1. Cumulative distribution of anchovy and sardine (s_A) in spring as a function of sea surface temperature (left) and salinity (right).

4.10 Spatial overlaps and differences

To characterize differences in the spatial distribution of spawning adults, as observed by acoustics and that of their eggs as observed by CUFES, spatial patches (Woillez *et al.*, 2009) were estimated on the average maps and their gravity centres plotted. Only those patches that totalled at least 10% of the global mean were considered as the main patches.

Spatial overlaps between high concentrations in predators and fish were mapped to identify the main interactions of top predators with anchovy and sardine. There were grid cells where predator and fish concentrations were both higher than their overall mean in each year. By counting the number of years when such overlap occurred throughout the series it was possible to map the occurrence frequency of such overlaps. Distinction was made between diving and surface birds, and the most abundant

marine mammal, the common dolphin (*Delphinus* sp.), was also taken into consideration. Distribution maps of demersal predatory fish (e.g. hake) were unavailable during the spring surveys. Core habitats explained largely the spatial pattern observed over the years in the frequency of overlaps.

4.10.1 Differences between spawning adults and egg distributions

The egg distributions observed with CUFES are not exactly centred on the observed distribution of the spawning adults (Figure 4.10.1), although the egg phase duration is < 4 d (5 d at 13°C). This is observed for anchovy and sardine in the Bay of Biscay where eggs are shifted westward in comparison to the adults, whereas sardine eggs in the Gulf of Cádiz are shifted eastwards. For sardine along the Portuguese coast, the dense patches of spawning adults were not associated with those of eggs.



Figure 4.10.1. Spatial patches of eggs (CUFES) and spawning adults (s_A) in spring: anchovy (left) and sardine (right). Spatial patches are estimated on the time average distributions.

4.10.2 Overlap between top predators and anchovy and sardine

At first is discussed how each predator overlaps with prey, and then how each prey overlaps with the predators. Common dolphin and anchovy distributions overlapped (Figure 4.10.2) with high frequency on the mid-shelf in the Bay of Biscay (45–46°N), corresponding to a portion of the core habitat of the anchovy. Similarly, common dolphin and sardine overlapped with high frequency in one of the core areas of sardine off central Portugal (39–41°N). Overlaps also occurred in the Bay of Biscay and the Gulf of Cádiz, but with lower frequency in sardine habitats less frequently occupied.

Surface and diving birds overlap with anchovy with highest frequency in similar areas (Figures 4.10.3 and 4.10.4) such as core anchovy habitat in the Gulf of Cádiz, Bay of Biscay, and also along the Portuguese coast. A similar pattern is seen for sardine where the highest overlap frequencies are along the Brittany coast, Portugal, and the Gulf of Cádiz, corresponding to portions of core sardine habitats.

The anchovy core habitat in the Bay of Biscay overlapped mainly with common dolphins, while its core habitat in the Gulf of Cádiz overlapped mostly with birds. The

core habitats of sardine off central Portugal overlapped with common dolphins as well as with the birds. On the French shelf in the Bay of Biscay, sardine overlapped with common dolphins over a large area, while the overlap with birds was more located at hot spots.



Figure 4.10.2. Occurrence frequency in the yearly overlap between common dolphins and anchovy (left) and sardine (right).



Figure 4.10.3. Occurrence frequency in the yearly overlap between surface feeding birds and anchovy (left) and sardine (right).



Figure 4.10.4. Occurrence frequency in the yearly overlap between diving birds and anchovy (left) and sardine (right).

5 Discussion: towards pelagic ecosystem monitoring

5.1 Ecosystem indicators from regular surveying

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5.1.1 Indicator categories, spatial/temporal and taxonomic scales

WGACEGG participants explored a list of potential indicators of the (good) environmental status of the pelagic ecosystem in ICES divisions 7.e–h and in subareas 8 and 9 that could be provided by the group in the mid-term (ICES, 2011a), within the framework of the Marine Strategy Framework Directive (MSFD). These indicators could be provided, at both the survey and regional scale, for time-periods including spring, summer, autumn, and winter/spring, depending on surveys. The regional scale was defined as the area encompassing divisions 7.e–h and subareas 8 and 9. The survey scale was defined as the areas covered by each survey.

The WGACEGG list of potential indicators describes changes in both the biodiversity and commercial species of the regional pelagic ecosystem. Indicators also describe marine litter.

Biodiversity indicators are defined at the species and community of species taxonomic levels. Species for which indicators are available at the regional scale are European anchovy and sardine at the adult and egg stages, as well as adult marine mammals and seabirds. Indicators of anchovy and sardine larvae are available for certain surveys. Indicators of juvenile anchovy are also available in the Bay of Biscay in autumn (JUVENA survey). Species community indicators describe the pelagic fish community at the adult and egg stages, as well as the zooplankton community. Commercial fish indicators describe variations in anchovy and sardine stocks.

5.1.2 WGACEGG MFSD indicators list

The WGACEGG MSFD indicators are summarized in Table 5.1.1.

Table 5.1.1. List of WGACEGG Marine Strategy Framework Directive indicators. The indicators describing the species distributional patterns are described in Woillez *et al.* (2007). Indicators in bold are already provided by WGACEGG. Indicators not in bold could be provided every year in the near future.

Descriptor	Attribute	Criteria	Indicators
Bio-	Species	Population size	DEPM and
diversity			acoustic total
			biomass and
			abundance
			estimates,
			along with
			estimation
			error
		Population condition	Acoustic
			biomass and
			abundance
			estimates per
			size/age

Descriptor	Attribute	Criteria		Indicators
				DEPM biomass and abundance estimate per size/age (Biscay anchovy)
		Species distribution	Distribu- tional range	Area of potential distribution
			Distribu- tional pattern	Centre of gravity Spatial
			(survey scale)	patches
				Inertia Isotropy
				Positive area
				Spreading area
				Equivalent area
				Gini index
				Coefficient of variation of strictly positive densities
				Microstructure
				Global index of collocation
				Mean biomass
				Percentage of total area occupied
			Distribu- tional pattern (region- al scale)	Regional synthesis of spatial indicators at the survey scale
	Community	Community condition		Total pelagic fish NASC
				LOPC zooplankton abundance at length (PELGAS, PELACUS)
				Species composition

Descriptor	Attribute	Criteria		Indicators
				Relative population abundance
		Habitats H	labitat ondi	Surface temperature
		ti	on	Surface salinity
			_	Nutrient (PELGAS, PELTIC)
Commercial fish	Reproduc- tive capacity		_	DEPM spawning- stock biomass (SSB) estimate
			_	Acoustic SSB estimate
				DEPM fecundity
			_	DEPM spawning frequency
	Age and size distribution			95th percentile of the population length distribution
				Proportion of fish >L50 (only relevant for sardine, as all age 1 anchovies are mature)
Litter in the marine environ-	Amount, compo the water colum	osition, and source of litter floating at an, and on the seabed	sea, in	Floating litter (PELGAS, PELACUS)
ment			_	Litter in trawl catches (PELGAS, PELTIC)
			_	Microlitter in zooplankton samples (PELTIC)

5.2 Forward look: expansion of survey coverage north of the Bay of Biscay Jeroen van der Kooij and Ciaran O'Donnell

5.2.1 Introduction

It has long been known that the northern limit of anchovy and sardine distribution expands beyond the Bay of Biscay. Plankton surveys in the Celtic Sea have reported large numbers of sardine eggs (Wallace and Pleasants, 1972) comparable to those in the Bay of Biscay, demonstrating the importance of these northern regions for spawning. Locally important ringnet fisheries operate in the region and specifically target sardine. In the case of anchovy, landings in the English Channel and Dutch estuaries were reported in the late 19th century, but anecdotal information goes back much farther. Both anchovy and sardine abundance in the north has fluctuated, with periods of high abundance following periods of near absence (Aurich, 1953). There is some evidence that the boreal herring dominates this transition region during colder periods, and sardine during warmer periods (Southward et al., 1988). Since the mid-1990s, both anchovy and sardine have apparently increased in the north, a feature that has been linked to climate variability (Beare et al., 2004; Alheit et al., 2012; Petitgas et al., 2012). In the case of anchovy, evidence has emerged that the northern anchovy is, in fact, a separate population from that in the Bay of Biscay (Zarraonaindia et al., 2009, 2012; Petitgas et al., 2012), yet little is known about it.

Most of the above studies have been based on anecdotal information and indirect data because of the absence of suitable survey data targeting these species in this area. Recently, however, concerted efforts have been made to improve on this. Endorsed by the WGACEGG forum, the UK conducted a one-off, 23-day multidisciplinary pelagic survey in summer 2011 which covered the western English Channel and the Celtic Sea, extending pelagic coverage in summer beyond 48°N. In 2012, a new five-year pelagic survey programme commenced that sampled the pelagic component of the coastal ecosystem around the southwestern part of the UK in autumn. Additionally, the Marine Institute (Ireland) has conducted an autumn pelagic survey in the northern Celtic Sea and southwestern Ireland since 1989, targeting autumn- and winterspawning components of the herring stock in both coastal and offshore waters. In summer 2011, a new survey programme was initiated, targeting spawning aggregations of boarfish along the shelf edge and shelf seas from north of Scotland to northern Biscay. This survey offers many opportunities as a newly established large-scale summer survey in western waters.

5.2.2 Celtic Sea herring survey

The Celtic Sea herring acoustic survey is carried out annually in October over 21 days on board the RV "Celtic Explorer". The survey is carried out to acoustically assess the size of the autumn- and winter-spawning components of the herring stock in the Celtic Sea and southwest of Ireland (ICES divisions 7.a, g, and j; Figure 5.2.1). This survey was first established in 1989 and has been carried out in its current form since 2004. Area coverage is broken into broad-scale (offshore) and spawning (inshore) strata. Broad-scale strata are important transit routes for herring migrating to inshore spawning areas from offshore summer feeding grounds. A parallel transect design is used with transects running perpendicular to the coastline following standard survey methods. Transect resolution is set at 2–4 nautical miles for the broad-scale strata, increased to 1 nautical mile for the spawning strata. Bay and inlets are surveyed using zigzag transects. Transect starting points within strata are randomized each year within established baseline strata bounds. Further details of survey methods are provided in the annual cruise report (O'Donnell *et al.*, 2013).

Calibrated acoustic data are collected at four frequencies (18, 38, 120, and 200 kHz) from transducers mounted within a drop-keel system. Biological sampling is carried out using a single pelagic midwater trawl, and biological data are collected from all species encountered.



Figure 5.2.1. Pelagic survey coverage north of 48°N in ICES Subarea 7 during autumn (top) by the Celtic Sea herring survey (CSH, red transects) and PELTIC survey (blue transects), and during summer (bottom) during the boarfish survey.

In addition to herring, other small pelagic fish species are routinely encountered and sampled, including sprat, mackerel, horse mackerel, anchovy, and sardine. Acoustic

abundance estimates are produced for herring and sprat from survey data. At present, no abundance estimates are calculated for the other species.

In addition to the acoustic component of the survey, comprehensive hydrographic sampling is undertaken as well as coordinated sighting surveys for marine mammals, seabirds, and marine litter. Marine mammal and seabird sighting surveys now form an increasingly important component of this multidisciplinary survey.

5.2.3 Pelagic ecosystem survey in the English Channel and eastern Celtic Sea: PELTIC

In 2011, a one-off, 23-day pelagic survey was conducted in the Celtic Sea and western English Channel in early summer (May–June). The primary aim was to study the distribution and abundance of the small pelagic fish community in the area, with particular focus on anchovy and sardine. Other than information on the small pelagic fish species, this survey collected data across the foodweb, from physical oceanography to top predators such as birds and marine mammals.

The findings of the above-mentioned summer survey led to the design and implementation of a new five-year pelagic autumn survey series which commenced in 2012 on the RV "Cefas Endeavour". The survey covers the coastal waters of the western English Channel, Isles of Scilly, and Bristol Channel, following a series of parallel transects spaced 10 nautical miles apart and perpendicular to the coast (Figure 5.2.1). Along these transects, calibrated acoustic data are collected at 38, 120, and 200 kHz from drop-keel mounted transducers, which, combined with pelagic trawl operations, provide information on the small pelagic fish community.

However, during this survey, other components of the pelagic ecosystem are also sampled: (i) continuous data on surface oceanography and apex predators in conjunction with the fisheries acoustics; (ii) two ringnets are deployed at 70 fixed stations with a mini CTD, collecting zooplankton and ichthyoplankton data; and (iii) a Rosette with CTD is deployed at ca. half of the fixed stations to provide high-resolution information on physical oceanography, nutrients, and production in the water column.

5.2.4 Contributions of northern surveys to understanding pelagic fish in the Iberian-Biscay-Celtic Atlantic ecoregion

The above-mentioned surveys have contributed towards an improved understanding of the pelagic component of the western shelf seas ecosystems of the Northeast Atlantic. The established Celtic Sea herring survey has collected a valuable time-series on the target species and now also provides information on other pelagic fish species, such as anchovy and sardine, as well as other levels of the foodweb. For example, fin (Balaenoptera physalus) and humpback (Megaptera novaeangliae) whales arrive in the northern Celtic Sea to feed on sprat and herring aggregations in coastal waters, and peak abundance has been shown to be aligned with the timing of the herring survey (Ryan et al., 2013). Although not yet a significant time-series, the PELTIC survey series has started to contribute important spatial and quantitative data which is further improving understanding on the small pelagic community and its ecosystem. For example, it appears that in autumn, sardine spawning is widespread in the western English Channel, and according to other fixed-station time-series data, is increasing (Coombs et al., 2010). Sprat is the most abundant small pelagic species in coastal waters of the southern Celtic Sea and western English Channel, and in some years, good numbers of the "northern" anchovy (Petitgas et al., 2012) are found in the English Channel, boosting incomes of local fishers. Herring is generally found in low numbers (<1% of the pelagic fish biomass) in the southern Celtic Sea, but it is the most important clupeoid in the northern Celtic Sea. Both Celtic Sea autumn surveys also encounter large quantities of mackerel, some years into the thousands of tonnes. Mackerel is abundant in the Celtic Sea throughout the year, occurring both as immature and mature fish. Part of the area has been closed for mackerel trawling to protect what is thought to be an important nursery area for the juvenile fish which have traditionally moved there after hatching at the shelf edge. As mentioned above, the southern Celtic Sea and western English Channel areas mark the southern and northern boundaries of boreal and Lusitanian species, respectively. One example of a warm-water species which has extended further north in recent years in greater abundance is the boarfish. Since the early 1970s, its abundance in the Northeast Atlantic has increased dramatically after successive years of good recruitment, attributed to an increase in water temperature during the spawning season (Blanchard and Vandermeirsch, 2005). Knowledge on this species has developed quickly since the first dedicated summer acoustic survey was carried out in 2011 (O'Donnell *et al.*, 2012), particularly in areas such as spawning conditions (Farrell *et al.*, 2012), boarfish specific TS–length relationship (Fässler *et al.*, 2013), and age determination (Hüssy *et al.*, 2012a, b).

As stand-alone units, these surveys have already provided valuable contributions to fisheries management, as well as to an improved understanding of some of the ecological mechanisms in the area. However, their scientific contribution will be more significant when combined with other existing surveys in the area. Coordinated temporal and geographical coverage of core and peripheral species distribution areas will help to increase the precision of, and reduce uncertainty in, acoustically derived abundance indices for transboundary species. Other than the primary target species of the WGACEGG, sardine and anchovy, it also includes other widely dispersed stocks such as mackerel. The need for a coordinated acoustic survey approach is becoming increasingly important to further complement existing survey methods. The ability of multidisciplinary research cruises to fill gaps in knowledge, not only for traditional target fish species, but also for wider-scale research purposes, is not just extremely beneficial, but also increasingly an EU data requirement.

5.3 Conclusion

Jacques Massé, Andrés Uriarte, Maria Manuel Angelico, and Pablo Carrera

This report culminates more than 30 years of collaboration among Portugal, Spain, and France. From unofficial collaboration to the annual meetings of the WGACEGG, each country and institution made progress and efforts to move toward better monitoring of the pelagic species and their ecosystems. Each partner progressed in methodology and enlarged the range of parameters collected. Through European projects, work on standardization of methods and sampling strategies was done in parallel with several intercalibration exercises among vessels. Despite some disagreements and open debates on methods or results, these three countries succeeded in working together in a constructive way to assemble information into a common database to allow combining or cross-analysing several parameters. The results today appear in this report as a synthesis of the last ten years of pelagic surveys (2003–2012) across southwestern European waters.

In the framework of WGACEGG, it is planned to supply, year after year, the agreed parameters in an agreed format whatever the season or area covered by the survey in order to achieve together a better understanding of the pelagic ecosystem in southwestern European Atlantic waters. In this objective, northern countries are now joining this group with complementary surveys, and the geographical coverage will certainly result in a better delimitation of the northern limits of sardine and anchovy, as well as a better comprehension of the southern distribution of more northern pelagic resources like herring or sprat.

Nevertheless, the members of WGACEGG still have to work further to solve some of their original challenges, such as achieving a combined estimate from acoustics and egg surveying, or including egg production estimates from (or with the aid of) CUFES, improving species recognition from multifrequency analysis of echograms, and so on. Although some progress has been made along these lines (Petitgas *et al.*, 2009; Lezama-Ochoa *et al.*, 2011), there is still much room for improvement. In addition, there might be new ways of approaching old challenges, such as making use of modelling of parameters to improve survey estimates. For instance, survey observations of DEPM parameters could be modelled in space (potentially including environmental or biological covariates) to lead to improved estimates of either adult or egg abundance estimates (see Bernal *et al.* [2011b] for such an approach to estimating sardine egg mortality). A parallel study could be devised to apply acoustic TS corrected by the vertical location of schools. This can be used also to infer combined acoustic and egg biomass estimates. Bayesian inference could certainly be a suitable framework in which to address these challenges.

Furthermore, the surveys should gradually evolve to provide more suitable outputs for the integrated monitoring of pelagic habitats and ecosystems where the target populations develop and interact as forage species. The main challenge addressed by the Intergovernmental Oceanographic Commission (IOC) during the 1970s (i.e. to assess the present understanding of the mechanisms through which variability in the physical–chemical marine environment affects the biological productivity of the ocean and the abundance and distribution of living marine resources) was the most important trigger to implement monitoring programmes aiming to assess the pelagic coastal stocks along the Iberian Peninsula and in the Bay of Biscay. From the exploratory phase in the 1980s and 1990s, for both acoustics and egg production surveys, to the consolidated phase of the surveys included in this report, in terms of technology, survey design, and data analysis, there has been great improvement in knowledge about this pelagic ecosystem. While sampling design and the main assessment objectives of these surveys will remain nearly unchanged for the next few years, new vessel-borne tools and techniques, together with an improvement in the precision of the estimates, will offer an opportunity for monitoring biotic and abiotic parameters so as to contribute to the monitoring of the ecosystem and environmental status related to the Marine Strategy Framework Directive and the Common Fisheries Policy. This will of necessity mean a close relationship among the different teams involved in the surveys (planktologists, acousticians, biologists, physical and chemical oceanographers, and others, including fishers) in order to integrate the results of each discipline. This integration should certainly lead to an improved series of ecosystem indicators.

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Annex 1: DEPM protocols for surveying and data processing

A1.1 Introduction

The daily egg production method (DEPM) was developed in the late 1970s (Parker, 1980) as a direct method for the assessment of northern anchovy (*Engraulis mordax*). DEPM only applies to indeterminate fecundity spawners with pelagic eggs. It requires a single survey during peak spawning period (when the proportion of fish that are reproductively active is highest; Stratoudakis *et al.*, 2006) to obtain an instantaneous estimate of the total number of eggs produced daily over the spawning area and of the adults' daily fecundity. Spawning biomass is then estimated directly by the quotient of daily egg production over daily fecundity, based on the following equation (Parker, 1985):

$$SSB = \frac{A \cdot P \cdot W_f}{R \cdot S \cdot F} \tag{A1.1}$$

where *P* is the daily egg production (number of eggs produced per day per unit area), *A* is the spawning surveyed area, *W* is the average body mass of mature females, *R* is the fraction of the mature population that is female (by mass), *F* is the batch fecundity (number of eggs spawned per mature female per batch), and *S* is the spawning fraction (fraction of mature females spawning per day).

The daily egg production method is being used to estimate the population spawning biomass of both Atlantic sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) by the Spanish (IEO, AZTI) and the Portuguese (IPIMAR/IPMA) fisheries institutions. The sardine DEPM was started in 1988 by IEO and IPIMAR (Cunha *et al.*, 1992; García *et al.*, 1992), was repeated in 1990 only by IEO (García *et al.*, 1993), and jointly again in 1997 and subsequently triennially since 1999 by IEO and IPIMAR, covering the Atlantic waters of the Iberian Peninsula. The northern limit of the sardine DEPM survey was extended to the inner part of the Bay of Biscay (up to 45°N) since 1997 by IEO. Since 2011, AZTI has also collaborated in collecting data and samples for sardine from 45°N to 48°N in the Bay of Biscay, done within the framework of the survey dedicated to the application of the DEPM to anchovy in the Bay of Biscay. The current Bay of Biscay anchovy DEPM surveys have been carried out annually since 1987 by AZTI (Santiago and Sanz 1992; Motos 1992), while the Gulf of Cádiz anchovy DEPM surveys have been carried out triennially since 2005.

Lasker's manual (1985) contains a very thorough description of the mathematical principles of the method, the parameters, the field sampling and data collection, laboratory methodology, and data analysis developed for the northern anchovy in southwestern America. The sampling design and methodology of the DEPM surveys targeting European anchovy and Atlantic sardine, carried out from Cape Trafalgar to the Atlantic French coast, have been standardized since 2002 by the ICES SGSBSA and WGACEGG to coordinate both surveying and common analyses of the data collected. The present protocol aims at compiling the methodology applied currently for these two species in the southwestern Atlantic area.

A1.2 Surveying

A1.2.1 Area sampled and timing of the survey

The DEPM survey targeting the Atlantic Iberian sardine covers the area from the Gulf of Cádiz to the inner part of the Bay of Biscay (sardine Atlanto-Iberian stock). The region in the Gulf of Cádiz to the northern Portugal/Spain border (River Minho) is surveyed by IPMA (previously IPIMAR) in January–February, while IEO covers the northwestern and northern Iberian Peninsula and part of the Bay of Biscay (up to 45°N) in April (Figure A1.1). For 2008, extra sampling was considered by inclusion of information for sardine eggs collected during AZTI's anchovy DEPM survey in the northern part of Subarea 8. Since 2011, the triennial sardine DEPM survey of the entire Subarea 8 has been carried out in collaboration between IEO (up to 45°N) and AZTI (up to 48°N, within the annual anchovy survey).

The DEPM surveys targeting anchovy in the Bay of Biscay (ICES divisions 8.a–d) and the Gulf of Cádiz (ICES Division 9.a South) are performed by AZTI in May, covering the Spanish eastern Cantabrian Sea and the Atlantic French coast (43–48°N, and from the French coast to 5°W; Figure A1.1), and by IEO in July–August, covering the Gulf of Cádiz continental shelf (from Cape Trafalgar to Cape São Vicente). These surveys cover the main spawning grounds of the Bay of Biscay and Gulf of Cádiz anchovy stocks.



Figure A1.1. Area covered by the DEPM sardine surveys (left) and DEPM anchovy surveys (right).

A1.3 Sampling

A1.3.1 Eggs

The main ichthyoplankton sampler is the PairoVET (double CalVET – Smith *et al.*, 1985) net, with the auxiliary use of the continuous underway fish egg sampler (CUFES; Checkley *et al.*, 1997) for adaptive decisions in order to delimit the spawning area and to modify adaptively the intensity of the PairoVET sampling. The DEPM surveys perform ichthyoplankton sampling on fixed stations with a PairoVET, using nets with 150-µm mesh and fitted with flowmeters, operating vertically (1 m s⁻¹) to the surface from 5 m above the seabed, with a maximum sampling depth of 100 m (or 150 m in the IPMA survey). The CUFES, equipped with a 335-µm mesh net, operates underway (between PairoVET stations) filtering water pumped 3–5 m from the surface. Both samplers are used on a survey grid consisting of fixed transects perpendicular to the coast and spaced 8 nautical miles apart (in the case of the AZTI transects, they are spaced 15 or 7.5 nautical miles apart in areas of high egg abundance). The inshore limit of the transects is determined by bottom depth (as close to the shore as possible), while

the offshore extension is decided adaptively (based on presence/absence of eggs), but always covering the extension of the platform to the 200-m isobath. The sampling protocol agreed by the ICES Working Group on Acoustic and Eggs (WGACEGG) can be summarized as follows:

- CUFES samples (ongoing) are taken every 3 nautical miles throughout the transects (AZTI, every 1.5 nautical miles).
- PairoVET samples are always taken every 3 nautical miles on the inner shelf (down to a towing depth of 100 m for IEO and AZTI, or 150 m for IPMA).
- PairoVET samples are taken every 3 nautical miles or 6 nautical miles beyond the inner shelf, depending on the results of the most recent CUFES sample, collected every 3 nautical miles (1.5 nautical miles in the case of the AZTI survey). When an ongoing CUFES is negative for sardine or anchovy egg presence, the following PairoVET, at 3 nautical miles, is skipped.
- The outer limit of a transect is reached when two consecutive CUFES samples, spaced 3 nautical miles apart (for AZTI this is six consecutive samples spaced at 1.5 nautical miles) are negative beyond the 200-m isobath.
- When finishing a transect offshore, the vessel should proceed to the next transect and carry out CUFES sampling on the intertransect distance to check for egg presence. When eggs are found, sampling should be extended offshore in the next transect. If no eggs are found, sampling starts (always) with a PairoVET at a point at the same latitude or longitude or equal distance from the isobath, depending on transect orientation, and then continues from there towards shore using the sampling criteria defined above.
- Whenever a towing angle deviates from the vertical more than 30°, the sample should be discarded and the haul repeated.

After hauling, nets are washed from the outside with seawater under pressure, and plankton samples are preserved in buffered formaldehyde at 4% (sodium tetraborate or sodium acetate) in appropriately labelled containers. After 6 h in the preservation solution, IEO and AZTI sort the samples and count sardine and anchovy eggs at sea. Some of the samples are rechecked on land to assess the quality of the sorting made at sea. IPMA processes all the samples in the laboratory on land.

Date, time, position (GPS), sampling and bottom depth data, cable released and its angle, and flowmeter readings are registered.

A1.3.2 Hydrology data collection

Concurrently with the plankton sampling, profiles of temperature and salinity (and fluorescence) are recorded at each PairoVET station. The institutes use specific ways for coupling the CTD(F) probes to the plankton nets. Surface temperature, salinity, and fluorescence are also registered underway by probes associated with the surface water pumped by the CUFES system or dedicated pumps. Other environmental variables such as wind and current may be recorded and used for general environmental characterization.

Table A1.1 summarizes the main characteristics of the ichtyoplankton and hydrology surveying carried out during the sardine and anchovy DEPM surveys.

	Sardine		Anchovy	
DEPM	Portugal (IPMA)	Spain (IEO)	Spain (AZTI)	Spain (IEO)
Survey area	Portugal and Gulf of Cádiz (36–42°N to 6–10°W)	NW and N Spain and Bay of Biscay (42–45°N to 1–10°W)	Eastern Cantabrian Sea and Bay of Biscay (43–48°N to 5–1°W)	Gulf of Cádiz (36–37.2°N to 6–9°W)
Sampling grid (nautical miles)	8 × 3	8×3	15 × 3 (7.5 × 3)	8×3
PairoVET nets	2	1	2	2
Sampling maximum depth (m)	150	100	100	100
Hydrographic sensor	CTDF (RBR)	CTD (SBE25) CTD (SBE37)	CTD (RBR)	CTD (SBE25) CTD (SBE37)
Flowmeter	Y	Υ	Y	Y
Clinometers	Y	Y	Ν	N
CUFES, 335-µm mesh	3 nautical miles (sample unit)	3 nautical miles (sample unit)	1.5 nautical miles (sample unit)	3 nautical miles (sample unit)
Environmental data	Temperature, salinity, fluorescence	Temperature, salinity, fluorescence (surface only)	Temperature, salinity, fluorescence	Temperature, salinity, fluorescence (surface only)

Table A1.1. Common general sampling for icthyoplankton and hydrology.

A1.4 Adults

A1.4.1 Fishing

Fishing hauls are undertaken for the estimation of adult parameters (sex ratio, female weight, batch fecundity, and spawning fraction) within the mature component of the sardine or anchovy population. Surveying for adults takes place simultaneously with ichthyoplankton sampling. Fishing hauls should be distributed over the surveyed region according to fish abundance distribution. The number of samples and their spatial distribution is thus organized to ensure good and homogeneous coverage of the survey area and an adequate representation of population demography and distribution. Fishing hauls are conducted by pelagic or bottom trawling, following the detection of species schools by echosounder.

In order to increase sample size, samples collected by the RV can be complemented by samples obtained from the commercial purse-seine fleet at the main landing harbours, ideally within a week of the surveying by the RV in each area. This practice makes the adult sampling both opportunistic and directed.

A1.5 Fish sample processing

A1.5.1 General procedure

On board the RV, species caught by the trawl are identified and specimens are sorted by species. During IEO surveys, length frequency distributions are obtained for all fish species in the trawl (either from the total catch or from a representative random sample of 100–200 fish). For the main pelagic species, random samples (consisting of up to 100 fish from each haul) are collected, and the fish are measured and weighed to obtain a length-weight relationship. Moreover, biological information (sex, macroscopic maturity stage, gutted weight, stomach contents) is obtained for anchovy, sardine, horse mackerel, blue whiting, and mackerel, and otoliths are also extracted in order to estimate age and to obtain the age-length key (ALK) for each species. During AZTI surveys, 100 individuals of each species caught are taken at random from the haul and measured. During IPMA surveys, and for the pelagic species (sardine or anchovy, Trachurus spp., mackerel, chub mackerel, bogue, boarfish), a random sample of 100 specimens of each species is taken. In both AZTI and IPMA surveys, the sample weight is then determined and noted, and length frequency and weight per length class are determined (except for the DEPM survey targeted species – sardine or anchovy – that will follow the next procedure).

A1.5.2 Sardine or anchovy

For each haul and immediately after fishing, a minimum of 60 sardine or anchovy are randomly selected (this sample is not separated by length classes) and biologically sampled (length, total weight, gutted weight, sex, macroscopic maturity stage; Table A1.2). The objective is to preserve the gonads of 25–30 females (25–30 mature but non-hydrated females for AZTI; 30 females at all macroscopic maturity stages for IEO and IPMA). If the target of those 25–30 females for histology is not completed, ten more sardine or anchovy are taken at random from the same haul and processed in the same manner. Sampling is stopped when 120 (100 for IPMA and IEO) sardine or anchovy in the haul have been sampled or when the target of those 25–30 females for histology was achieved. Otoliths are extracted on board and analysed in the laboratory to obtain the age composition per sample. From the same haul, extra hydrated females for fecundity estimations can also be obtained.

The gonads of the 25–30 females are immediately collected and preserved in formaldehyde solution (4% in distilled or tap water, buffered with sodium phosphate) in individual containers properly tagged for identification (survey code, trawl station and observation numbers, FAO species code, and sexual maturation stage) for posterior weighing and histological processing at the laboratory.

The DEPM requires that samples contain a minimum of mature individuals for the estimation of its parameters. Therefore, in a given haul, if sardine are sized < 11 cm, i.e. most individuals likely are still immature, the fish from this haul will not be sampled and the ovaries not preserved. For anchovy, this does not occur because all the population is mature when the survey is taking place.

Biological sampling and ovary fixation are always carried out with fresh material, except for some of the commercial samples obtained from the Portuguese purse-seines if biological sampling is impractical to perform on fresh material immediately or within a few hours after fishing. In this case, immediately after the fish are landed, the abdomen of each fish sampled is slightly opened, the two lobes of the gonad are removed, and they are immediately preserved in formaldehyde solution for histology. The remaining body of the fish is frozen for posterior complete biological sampling in

the laboratory, with the correct total body weight of the fish recorded taking into account the weight of the removed lobes of the gonad. For AZTI, if some of the adult samples are from purse-seiners, a 2-kg sample is selected from the trawl and preserved in 4% formaldehyde. Afterwards, the samples are processed in the laboratory in the same manner as explained above. For sardines sampled during AZTI's anchovy surveys, the procedure is the same as for anchovy.

Table A1.2. Common general sampling for adults.

	Sardine			Anchovy	
DEPM	Portugal (IPMA)	Spain (IEO)	Spain (AZTI)	Spain (AZTI)	Spain (IEO)
Survey area	Portugal & Gulf of Cádiz (ca. 36–42°N)	NW and N Spain and Bay of Biscay (9.5°W to 42– 45°N)	Bay of Biscay (45–48°N)	Eastern Cantabrian Sea and Bay of Biscay (5°W to 43– 48°N)	Gulf of Cádiz (36-37°N to 6–9°W)
Gear	Pelagic and bottom trawl, purse- seiner	Pelagic trawl	Pelagic trawl	Pelagic trawl and purse- seiner	Pelagic trawl
Trawl time	During the whole day	During the whole day	During the whole day	During the whole day	During the daylight hours
Biological sampling	Fresh material used on board the RV, and frozen for commer- cials; gonads fresh	Fresh material used on board the RV	Fresh material used on board the RV	Fresh material used on board the RV, and in formalin for commercials	Fresh material used on board the RV
Sample size	60 fish randomly selected (minimum 30 female); extra if needed and when hydrated	60 fish randomly selected (minimum 30 mature female); extra if needed and when hydrated	60 fish randomly selected (minimum 30 mature female); extra if needed and when hydrated	60 fish randomly selected (minimum 25 non-hydrated mature females); extra if needed and when hydrated	60 fish randomly selected (minimum 30 mature female); extra if needed and when hydrated
Fixation	Buffered formalde- hyde 4% (distilled water)	Buffered formalde- hyde 4% (distilled water)	Buffered formalde- hyde 4% (distilled water)	Buffered formalde- hyde 4% (filtered tap water)	Buffered formalde- hyde 4% (filtered tap water)
Preservation	Formalin	Formalin	Formalin	Formalin	Formalin

A1.6 Census on seabirds and marine mammals

Data on seabirds and marine mammals are also collected during IPMA sardine surveys and IEO anchovy surveys in the Gulf of Cádiz, according to a modified version of the Tasker *et al.* (1984) methodology, as recommended by the European Seabirds At Sea (ESAS) group (Camphuysen and Garthe, 2004). Sightings are recorded as densities (number of individuals km⁻²) along the transects of the survey during observations each lasting 5 min. All birds in contact with the water or with marine mammals observed within the transect are counted. All birds in flight are recorded once (as regular snapshots) in order not to overestimate their density.

A1.7 Laboratory processing

A1.7.1 Eggs

At the laboratory, samples are sorted, and sardine and anchovy eggs are identified and counted (if not already done during the survey). Anchovy eggs are staged following an adapted version of the 11-stage development scale of Moser and Ahlstrom (1985), while sardine eggs are staged following an adapted version of the 11-stage scale of Ahlstrom (1943) and Gamulin and Hure (1955). For sardine IEO surveys, only samples from one codend are sorted; samples from the second codend are used for plankton biomass quantification. Table A1.3 summarizes the methodology used in egg analyses.

A1.7.2 Egg development stages

The following are the descriptions agreed during the Workshop on Anchovy and Sardine Egg Staging, undertaken at AZTI in 2002, and the Workshop of EU PELASSES project, Study Contract DG XIV 99/010.

Stage I	Cell division has not begun. The cytoplasm of the single cell appears as a clear hemisphere at one pole; the cytoplasm may be displaced to other locations around the periphery of the yolk mass. The unfertilized eggs are included in this stage.
Stage II	<u>Start</u> : Cell division starts (initially small bubble-like structures are often visible). The cytoplasm divides into 2, 4, 8, 16, 32 cells. After the cytoplasm has divided into 32 cells, it is very difficult to count the cells; the blastodisc has a mulberry-like appearance. <u>End</u> : Blastula: cells are very small, but it is still possible to distinguish them individually.
Stage III	<u>Start</u> : The egg now has the appearance of tissue rather than of a collection of individual cells. The segmentation cavity is visible. <u>End</u> : The blastodermal cap is ≤ $\frac{1}{3}$ down the yolk mass, and the bilateral nature of the primordial embryo is apparent.
Stage IV	<u>Start</u> : The blastodermal cap is >⅓ of the yolk mass. <u>End</u> : The blastodermal cap is ≤ ⅔ of the yolk mass.

Table A1.3. Anchovy egg staging.

Stage V	Start: The blastodermal cap is >⅔ of the yolk mass and grows until it completely encloses the yolk.
	<u>End</u> : Closure of the blastopore; the tail lies flat against the polar region of the yolk and continues the line of the yolk mass.
	There is a rapid differentiation at this stage, resulting in the formation of several myomeres in the mid-region of the embryonic axis.
Stage VI	Start: The blastopore is always closed, and the tail thickness is apparent.
	End: The tail remains against the yolk mass.
	The angle formed between the tail and the yolk is $\geq 90^{\circ}$.
	During this stage, the myomeres are apparent along the entire body axis (except at the caudal portion).
Stage VII	Start: The tip of the tail is free from the yolk and mostly rounded.
	End: The length of the free tail is $\leq \frac{1}{2}$ the length of the head.
	The angle formed between the tail and the yolk is $< 90^{\circ}$.
Stage VIII	Start: The length of the free tail is $>\frac{1}{2}$ of the head length.
	End: The tail length is $\leq \frac{1}{4}$ of the yolk-sac length.
	During this stage, the tail becomes pointed and begins to bend away from the axis of the body.
Stage IX	Start: The tail length is >¼ of the yolk-sac length.
	End: The tail length is $\leq \frac{1}{2}$ of the yolk-sac length.
	The curvature of the tail is evident at this stage.
	The gut is now apparent along the ventral surface of the tail, and the fin-fold is considerably wider than in the previous stage.
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From this stage onwards, the free portion of the tail is considered to extend from the body and not from the fin-fold. Furthermore, the end of the embryo is considered to be the end of the fin-fold.

Stage X	<u>Start</u> : The tail length is >½ of the yolk-sac length. <u>End</u> : The tail length is ≤ $\frac{3}{4}$ of the yolk-sac length.
Stage XI	Stage XI: <u>Start</u> : The tail length is >¾ of the yolk-sac length. <u>End</u> : Hatching.

DIS: Damaged eggs, for which it is impossible to identify morphological features, are assigned to the DIS (disintegrated) category.

No Embryo (NE) stage: Includes stages I-III.

Early Embryo (EE) stage: Includes stages IV-VI.

Late Embryo (LE) stage: Includes stages VII-XI.


Stage IV

Stage V





Stage VII

Stage VIII

Stage IX



Stage X





Figure A1.2. *Engraulis encrasicolus* (Bay of Biscay) egg stages (adapted from Moser and Ahlstrom, 1985).

Table A1.4. Sardine egg staging.

Stage I	Eggs are smaller than later stages because of the very small width of the perivitelline space. The yolk is the same size as the eggs undergoing development, but cleavage has not yet begun.					
Stage II	The cleavage begins. After several divisions, the blastodermal cap is shaped like a berry. This stage persists until the segmentation cavity is formed.					
Stage III	<u>Start</u> : The germinal ring with a little pick (formation of the embryonic shield) can be seen from the top. However, the outline of the embryo is not clearly defined. <u>End</u> : The germinal ring begins the development of the yolk with a cellular sheath until it encloses $\frac{1}{3}$ of the yolk mass. The germinal ring takes on a waist-like form ($\leq \frac{1}{3}$ of the yolk mass).					
Stage IV	Start: The germinal ring is $>\frac{1}{3}$ of the yolk mass. The outline of the embryo can be discerned in the median line of the embryonic shield. End: The germinal ring encloses $\leq \frac{4}{5}$ of the yolk mass.					
Stage V	Start: The outline of the embryo is seen around the yolk, covering > ⁴ / ₅ of the yolk mass. The cephalic region is more apparent than the caudal one, which is a narrow line, joined to the yolk. End: The germinal ring completes its evolvement around the yolk until total closure of the blastopore.					
Stage VI	Start: The optic vesicles are visible. The myomers are discerned in the medial region of the embryo, but not in the caudal region or in the cephalic region. End: The tail is swollen, but never separates from the yolk. The angle formed between the tail and the yolk is ≥90°. If in doubt, the oil globule is a little behind the tail.					
Stage VII	Start: The principal characteristic is that the tail begins to separate from the yolk. The angle formed between the tail and the yolk is < 90°. If in doubt, and if the fin-fold is visible, these characteristics are conclusive. End: The free portion of the tail is the same length as the head. The tail reaches the oil globule, but not beyond. If in doubt, for this stage, the myomeres can only be visible at the beginning of the free portion of the tail.					
From this stage onwards, the free portion of the tail is considered to extend from the body and not from the fin-fold. Furthermore, the end of the embryo is considered as the end of						

the fin-fold.

Stage VIII	<u>Start</u> : The free portion of the tail is bigger than the size of the head, and at this stage, the tail extends beyond the position of the oil globule.						
	<u>End</u> : The fin-fold is becoming very apparent and is half as wide as the embryo width ($\leq \frac{1}{2}$). $\frac{1}{3}$ of the body at the tail end is separated from the yolk ($\leq \frac{1}{3}$). If in doubt, myomeres are visible in the free portion of the tail until the anus.						
Stage IX	<u>Start</u> : $>\frac{1}{3}$ of the body is free. The width of the fin-folder is $>\frac{1}{2}$ the width of the body. It is wider in the ventral part of the body than in the dorsal part. The tail is often curved to one side.						
	<u>End</u> : Generally, the tail is curved to one side of the embryo. The free tail is $\leq \frac{2}{5}$ of the total body length. The body is straightened and fits less the shape of the yolk.						
Stage X	Start: > $\frac{1}{3}$ of the body is free.						
	<u>End</u> : The fin-fold of the free portion of the body does not exceed the base of the head. From the dorsal view, the tail is very twisted to one side of the yolk and appears as a U-shape. The dorsal fin-fold is as wide as the body and the ventral is wider.						
Stage XI	Start: The free portion of the body extends beyond the base of the head. End: The hatching of the egg. The fin-fold is wide, 1.5–twofold as wide as the body. In case there are doubts between two adjacent stages, a consensus of all the characteristics is taken.						
DIS: Damaged eg assigned to the D	ggs, for which it is impossible to identify morphological features, are DIS (disintegrated) category.						
No Embryo (NE)) stage: Includes stages I–III.						
Early Embryo (E	E) stage: Includes stages IV–VI.						
Late Embryo (LE) stage: Includes stages VII–XI.							















Stage IX







Stage X

Stage XI



Figure A1.3. *Sardina pilchardus* (Cantabrian Sea) egg stages (adapted from Ahlstrom [1943] and Gamulin and Hure [1955]).

A1.7.3 Adults

The preserved ovaries are weighed in the laboratory (before eventual transfer to ethanol 70°), and the obtained weights are corrected by a conversion factor (between fresh and formaldehyde-fixed material) established previously.

These ovaries are then processed for histology. They are embedded in either resin (IEO, AZTI) or paraffin (IPMA), the histological sections (3–5 µm) are stained with haematoxylin and eosin, and the slides are examined and scored for their maturity stage (based on the most advanced batch of oocytes and atresia intensity; Hunter and Macewicz, 1985; Ganias *et al.*, 2004; Alday *et al.*, 2010), and for POF presence and POF age assignment to daily cohorts (Hunter and Macewicz, 1985; Pérez *et al.*, 1992a; Ganias *et al.*, 2007; Alday *et al.*, 2008; Uriarte *et al.*, 2012).

Prior to fecundity estimation, one lobe of each hydrated ovary is processed histologically in order to check for POF presence and thus avoid underestimating fecundity (Hunter *et al.*, 1985; Pérez *et al.*, 1992b). The individual batch fecundity is then measured by means of the gravimetric method applied to the hydrated oocytes on 1–3 whole mounted subsamples per ovary, with an average weight of 50–150 mg (± 0.1 mg; Hunter *et al.*, 1985). For sardine, if very few hydrated females are collected during a given survey or for a given stratum, an additional set of samples can be obtained following the methodology developed by Ganias *et al.* (2010), i.e. the gravimetric method is instead applied to non-hydrated females (at the oocyte migratory nucleus stage, MN).

A1.7.4 Analysis

Different geographical strata are considered for the sardine data in the analysis: <u>south</u> – from Gibraltar to Cabo de S.Vicente (Division 9.a South); <u>west</u> – Santa Vicente to the northern Portuguese–Spanish border (Division 9.a West); and <u>north</u> – Spanish waters from Galicia to the French border (divisions 9.a North and 8.b) and the French coast (Division 8.b). For assessment purposes, the Iberian Peninsula sardine stock databases for Division 9.a (South and West) and divisions 9.a North–8.c (IPIMAR/IPMA and IEO, respectively) are merged in a common standardized dataset. Data from Division 8.b is analysed using the same methodology as for the Iberian Peninsula dataset, but not merged together with data from divisions 9.a and 8.c.

For the analysis of the anchovy data in the Bay of Biscay and in the Gulf of Cádiz, there is no *a priori* stratification of the area covered by the survey, unless the spatial distribution of the estimates obtained justify it (see Section 2).

A1.7.5 Estimation of total daily egg production

Total daily egg production (P_{tot}) is calculated as the product of the daily egg production per area unit (P_0) and the spawning area (A):

 $P_{tot} = P_0 A \tag{A1.2}$

The spawning area is the fraction of the total area surveyed where spawning is perceived to take place, as revealed by the occurrence of eggs of the target species (positive stations). The spawning area (*A*) is delimited by stations with no eggs, but it may contain some embedded negative stations (no eggs). A continuous single spawning area can usually be obtained, but occasionally several separate spawning zones could be defined if several spawning nuclei are clearly distinguishable. The size of the spawning area is computed as the sum of the areas represented by the stations within the spawning area. In the case of sardine in the Iberian Peninsula and anchovy

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in the Gulf of Cádiz, the geographical limits of sampled areas are defined by an automated routine using neighbourhood distance (in km) between stations (minimum distance in ratio represented by each station). The area represented by each station within the survey limits is estimated by a Dirichlet tessellation of the survey stations (geofun, in the R library). For anchovy in the Bay of Biscay, both the survey limit and the area represented by each station are calculated with Arcview.

The daily egg production per area unit (P_0) is estimated together with the daily mortality rate (Z) from a general exponential decay mortality model, fitted over the spawning area, in the formula:

$$P_{i,j} = P_0 \exp(-Z a_{i,j})$$
(A1.3)

where $P_{i,j}$ denotes the number of eggs per unit area in cohort *j* of station *i* and $a_{i,j}$ denotes the corresponding mean age and density of eggs in cohort *j* of station *i*. $P_{i,j}$ is computed as the ratio between the number of eggs $N_{i,j}$ and the effective sea area sampled R_i (i.e. $P_{i,j} = N_{i,j}/R_i$). The model was written as a generalized linear model (GLM; McCullagh and Nelder, 1989; ICES, 2004) with a logarithmic link function:

$$\log(E[N_{i,j}]) = \log(R_i) + \log(P_0) - Z a_{i,j}, \qquad (A1.4)$$

where the daily number of eggs in cohort *j* of station *i* (N_{ij}) was assumed to follow a negative binomial distribution. The logarithm of the effective sea surface area sampled [log(R_i)] is an offset accounting for differences in the sea surface area sampled, while the logarithm of the daily egg production log(P_0) and the daily mortality *Z* rates are the parameters to be estimated.

In order to fit the model above, the eggs collected at sea and sorted by morphological stages have to be transformed into daily cohort frequencies and their mean age calculated. For that purpose, the Bayesian ageing method described in ICES (2004), Stratoudakis *et al.* (2006), and Bernal *et al.* (2011a) is used. This ageing method is based on the probability density function (pdf) of the age of an egg *f(age|stage, temp)*, which is constructed as:

$$f(age | stage, temp) \propto f(stage | age, temp) f(age)$$
 (A1.5)

The first term f(stage | age, temp) is the pdf of stages with given age and temperature. It represents the temperature-dependent egg development, which is obtained by fitting a multinomial model, such as extended continuation ratio models (Agresti, 1990), to data from temperature-dependent incubation experiments carried out for these populations of sardine and anchovy (see Bernal *et al.*, 2001, 2008, 2011a; Ibaibarriaga *et al.*, 2007b). The second term is the prior distribution of age, *a priori* the probability of an egg that was sampled at time τ of having a certain age is the product of the probability of an egg being spawned at time $\tau - age$ and the probability of that egg surviving since then [exp(-Z age)]:

$$f(age) \propto f(spawn = \tau - age) \exp(-Z age)$$
 (A1.6)

The probability density function of spawning time $f(spawn = \tau - age)$ allows refining the ageing process for species with spawning synchronicity that spawn at (approximately) defined times of the day (Lo, 1985; Bernal *et al.*, 2001). Spawning time is assumed to be normally distributed, with a mean at 21:00 GMT and a standard deviation of 3 h (spawning period: 15–3 h) for the Iberian Peninsula sardine. For the Gulf of Cádiz anchovy, peak spawning is assumed to occur at 22:00 GMT, with a standard deviation

of 2 h. For the Bay of Biscay anchovy, the pdf function representing spawning has a mean at 23:00 GMT and a standard deviation of 1.25 h (ICES, 2004). Peak spawning time is also used to define the age limits for each daily cohort (peak spawning time \pm 12 h). Details on how the number of eggs in each cohort and the corresponding mean age are computed from the PDF of age are given in Bernal *et al.* (2011a). The incubation temperature considered is obtained from CTD profiling; the strata used for mean temperature calculation may vary according to surveys and oceanographic conditions.

Given that this ageing process depends on the daily mortality rate, which is initially unknown, an iterative algorithm (ICES, 2004; Stratoudakis *et al.*, 2006; Bernal *et al.*, 2011a; R MASS Library) is used in which the ageing and the model fitting are repeated until convergence of the Z estimates. The procedure is as follows:

Step 1. Assume an initial mortality rate value.

Step 2. Using the current estimates of mortality, calculate the daily cohort frequencies and their mean age.

Step 3. Fit the GLM and estimate the daily egg production and mortality rates. Update the mortality rate estimate.

Step 4. Repeat steps 1–3 until the estimate of mortality converge (i.e. the difference between the old and updated mortality estimates is smaller than 0.0001).

Incomplete cohorts, either because the bulk of the day's spawning was not complete at the time of sampling, or because the cohort was so old that its constituent eggs had started to hatch in substantial numbers, are removed in order to avoid possible bias (the level of cutting is defined by the user).

Once the final model estimates are obtained, the coefficient of variation of P_0 is calculated from the standard error of the model intercept $[log(P_0)]$ (Seber, 1982), and the coefficient of variation of *Z* is obtained directly from the model estimates.

The analysis is conducted in R (<u>www.r-project.org</u>), using packages and routines available free at ichthyoanalysis (<u>http://sourceforge.net/projects/ichthyoanalysis</u>).

Table A1.3 summarizes the methodology adopted for the sardine and anchovy egg data.

A1.7.6 Estimation of adult parameters

The adult parameters estimated for each fishing haul consider only the mature fraction of the population (determined by the fish macroscopic maturity data). Estimation of the sex ratio, the mean female weight, and the mean female expected batch fecundity is based on the biological data collected from both survey and commercial samples, whereas the preserved gonads are used to measure individual batch fecundity, assess the mature/immature condition of females, and estimate the daily spawning fraction.

Before the estimation of the mean female weight per haul (W), the individual total weight of the hydrated females is corrected by a linear regression between the total weight of non-hydrated females and their corresponding gonad-free weight (W_{nov}).

		Sardine	Anchovy			
	Portugal (IPMA)	Spain (IEO)	Spain (AZTI)	Spain (AZTI)	Spain (IEO)	
Number of eggs staged	er of eggs All All Subsampling up to a max. of 75 eggs if necessary		Subsampling up to a max. of 75 eggs if necessary	Subsampling up to a max. of 75 eggs if necessary	All	
Classification of stages according to (adapted during dedicated workshops)	Gamulin and Hure (1955)	Gamulin and Hure (1955)	Gamulin and Hure (1955)	Moser and Ahlstrom (1985)	Moser and Ahlstrom (1985)	
Depth of reference for egg incubation temperature	Surface (continuous underway CTF)	10 m	10 m	10 m	5 m	
Peak spawning hour	21:00 GMT	21:00 GMT	21:00 GMT	23:00 GMT	22:00 GMT	
Egg ageing procedure	Bayesian (Ibaibarriaga <i>et al.,</i> 2007b; Bernal <i>et al.,</i> 2008)	Bayesian (Ibaibarria ga <i>et al.,</i> 2007b; Bernal <i>et</i> <i>al.,</i> 2008)	Bayesian (Ibaibarriaga <i>et al.,</i> 2007b; Bernal <i>et al.,</i> 2008)	Bayesian (Ibaibarriaga <i>et al.,</i> 2007b; Bernal <i>et al.,</i> 2008)	Bayesian (Ibaibarriaga <i>et al.,</i> 2007b; Bernal <i>et al.,</i> 2008)	
Egg production estimation	GLM	GLM	GLM	GLM	GLM	

Table A1.5. Processing and analyses for eggs.

The sex ratio (R) in weight per haul is obtained as the quotient between the total weight of females and the total weight of males and females. However, given the constancy of this parameter for anchovy in the Bay of Biscay since 1994, the proportion of mature females per sample is assumed equal to 1:1 in numbers. This implies the adoption of a mean value for R obtained as the ratio between the average female weight and the sum of the average female and male weights of the anchovies by samples.

The expected individual batch fecundity (F) for all mature females (hydrated and nonhydrated) is estimated by the method described by Hunter *et al.* (1985), i.e. by modelling the individual batch fecundity observed (F_{obs}) in the sample and their gonadfree weight (W_{nov}) by a GLM (with an identity link and a negative binomial error distribution for IEO and IPMA, and a Gamma error distribution for AZTI) and applying this subsequently to all mature females.

For sardine, fecundity models always consider geographical stratification. The GLMs always include a factor *Stratum*, as follows:

$$F_{obs} \sim W_{nov} + Stratum \tag{A1.7}$$

and mean batch fecundity (F) is estimated separately for the three areas. However, when none or only very few females are available for F_{obs} estimation in a given stratum (e.g. the 1997 and 2005 surveys off the Portuguese western coast), two strata can be

considered together in the model (e.g. West and South pooled), although *F* estimates are still calculated separately for each stratum. Several model options are tested for each year/survey (same or different intercepts and/or slopes, intercepts through the origin or not). The model considered as the most adequate is selected, taking into account both statistical (residual plots, lower AIC value, graphical representation of the regression curve) and biological significance (models with significant positive intercepts not accepted).

For anchovy in the Bay of Biscay, an analysis is conducted to verify if there are differences in batch fecundity between different strata. Similarly, for anchovy in the Gulf of Cádiz, post-stratification is undertaken when F estimates obtained are statistically different (see Section 2).

For sardine (as for anchovy in the past), the spawning fraction (*S*), the fraction of females spawning per day, is determined for each haul as the average number of females with day 1 or day 2 post-ovulatory follicles (POFs) divided by the total number of mature females. Hydrated females are not included due to possible over-sampling of active spawning females close to peak spawning time (Santiago and Sanz, 1992b; Bernal, 2007). In this case, the number of females with day 0 POF (of the mature females) is corrected by the average number of females with day 1 or day 2 POF (Picquelle and Stauffer, 1985; Pérez *et al.*, 1992a; Motos, 1994; Ganias *et al.*, 2007). For sardine, POFs are assigned to daily cohorts using both histomorphological and metrical (POF cross-sectional area) criteria (Pérez *et al.*, 1992a; Ganias *et al.*, 2007).

If no *S* estimation is possible for sardine or estimates are considered unrealistic, taking into account the dataseries and the other parameters (particular situation), a non-parametric bootstrap approach is performed using mean spawning fraction by each haul obtained during the series and considering a single haul as the basic sampling unit. Hauls are resampled, with replacement from the original dataset, leading to a new, artificial sample that is then used to estimate the spawning fraction. By repeating this procedure an adequate number of times (1000 in this application), an empirical probability distribution is obtained for the *S* parameter.

For anchovy in the Bay of Biscay, spawning frequency estimates are obtained by applying the new classification for oocyte and POF stages by Alday *et al.* (2008) and the procedures described in Uriarte *et al.* (2012).

The degeneration of POFs over time at different temperatures was studied for the Bay of Biscay anchovy by Alday *et al.* (2008) based on captivity experiments and field samples. For this purpose, a key of seven POF stages, solely defined on the basis of their histological degeneration characteristics, was applied (Alday *et al.*, 2008, 2010). The novelty of this procedure is that it separates staging of POFs from their ageing process. There was close agreement in the succession of POF stages after spawning between the experiment and the field samples, and, for the range of temperatures examined (13–19°C), little effect of temperature on the degeneration of POF was noticed.

The procedure to assign mature females to spawning classes is then improved by incorporating all the knowledge on oocyte maturation and degeneration of POFs in a matrix system which defines the probabilities of females with those histological indicators belonging to pre- or post-spawning cohort according to the time of capture (Uriarte *et al.*, 2012). The selected estimator for anchovy is the mean of *S* for day 0 and *S* for day 1.

Mean and variance of the adult parameters are estimated following equations for cluster sampling (as suggested by Picquelle and Stauffer, 1985):

$$Y = \frac{\sum_{i=1}^{n} M_{i} y_{i}}{\sum_{i=1}^{n} M_{i}}$$
(A1.8)

$$Var(Y) = \frac{\sum_{i=1}^{n} M_{i}^{2} (y_{i} - Y)^{2}}{\overline{M}^{2} n (n - 1)}$$
(A1.9)

where Y_i is an estimate of whatever adult parameter from sample *i* and M_i is the size of the cluster corresponding to sample *i*. A station occasionally produced a very small catch, resulting in a small subsample size.

In the case of IEO and IPMA, all estimations and statistical analysis are performed using the R software (http://www.R-project.org). For sardine, calculation of the parameters includes a minimum samples criterion (n = 30); hauls containing less than 30 fish sampled are excluded from the mean and variance calculations. In the case of AZTI, the calculations are made using an Excel workbook; to reflect the actual size of the station and its lower reliability, small samples are given less weight in the estimate. For the estimation of W, F, and S, a weighing factor is used, which equals 1 when the number of mature females in station i (M_i) is ≥ 20 and otherwise equals $M_i/20$. In the case of R when the total weight of the sample is < 800 g, the weighing factor is equal to total weight of the sample divided by 800 g; otherwise, it is set equal to 1. In summary, for the estimation of the parameters of anchovy daily fecundity, a threshold weighing factor (TWF) is used under the assumption of homogeneous fecundity parameters within each stratum.

A1.7.7 Spawning-stock biomass estimation

Spawning-stock biomass (SSB) mean estimates are calculated based on the following equation:

$$SSB = \frac{A \cdot P \cdot W_f}{R \cdot S \cdot F} \tag{A1.10}$$

For the calculation of the coefficient of variation, variance is approximately estimated using the Delta method (Seber, 1982, as shown by Parker, 1985 for the DEPM):

$$CV(B)^{2} = CV(P)^{2} + CV(W)^{2} + CV(R)^{2} + CV(F)^{2} + CV(S)^{2} + 2COVS$$
(A1.11)

(for sardine application the 2COVS term is omitted).

When estimates are stratified in several strata (*i*) then the variance of the total SSB estimated for the whole area is obtained as the addition of the variance of the stratified estimates:

$$Var(TotSSB) = \sum_{i} Var(SSB)_i$$
(A1.12)

In addition, population in numbers-at-age are produced for anchovy in the Bay of Biscay (see Uriarte, 2001) using the age readings available from otoliths from the adult samples collected during the survey. Estimates of anchovy mean weights and proportions-at-age in the adult population are computed as a weighed average of the mean weight and age composition per samples, where the weighing factors are proportional to the population (in numbers) in different substrata. These weighing factors are calculated according to the relative egg abundance and the amount of samples in the substrata defined with the purpose of estimating the numbers-at-age. These strata are defined each year depending on the distribution of the adult samples, i.e. size, weight, age, and the distribution of the anchovy eggs.

The mean and variance of the adult parameters for the population in numbers-at-age and the population length distribution (total weight, proportion by ages, and length distribution) are estimated following equations for cluster sampling from Picquelle and Stauffer (1985) (see Section 2).

Table A1.4 summarizes the methodology used in the processing and data analyses of sardine and anchovy adult samples.

	Sarc	line	Anchovy			
DEPM	Portugal (IPMA)	Spain (IEO & AZTI)	Spain (AZTI)	Spain (IEO)		
Histology: Embedding material and staining	Paraffin Haematoxilin Eosin	Resin Haematoxilin Eosin	Resin Haematoxilin Eosin	Resin Haematoxilin Eosin		
Analyses: W estimation	Weight of hydrated females corrected previous to estimation	Weight of hydrated females corrected previous to estimation	Weight of hydrated females corrected previous to estimation	Weight of hydrated females corrected previous to estimation		
R estimation	The observed weight fraction of the females	The observed weight fraction of the females	Theoretical expected values assumed to be equal to 1:1 in numbers checked with observed	The observed weight fraction of the females		
S estimation	Day 1 and day 2 POFs (according to Pérez <i>et al.,</i> 1992a and Ganias <i>et al.,</i> 2007)	Day 1 and day 2 POFs (according to Pérez <i>et al.,</i> 1992a and Ganias <i>et al.,</i> 2007)	The mean incidence of day 0 and day 1 spawners (Uriarte <i>et al.,</i> 2012)	Day 1 and day 2 POFs		
F estimation	On hydrated females (without POFs), according to Pérez <i>et al.</i> (1992b) and Ganias <i>et al.</i> (2010)	On hydrated females (without POFs), according to Pérez <i>et al.</i> (1992b)	On hydrated females (without POFs), according to Hunter <i>et al.</i> (1985)	On hydrated females (without POFs), according to Hunter <i>et al.</i> (1985)		

Table A1.4. Processing and analyses for adults.

Annex 2: Acoustic survey protocols and data processing

A2.1 Introduction

After more than 30 years of acoustic surveys carried out in southeastern European waters, and more than 20 years of meetings and working groups, the institutes involved in this endeavour have achieved considerable progress in survey strategy and methodology in order to standardize, to the extent possible, their actions and to ensure data compatibility. Nevertheless, several types of pecularities have made it impossible to establish a formal single protocol for surveying that would be applicable to all areas, seasons, and institutes. This can be attributed to the following:

- different geographic characteristics the continental shelf is very narrow along the Spanish coast, variable along the Portuguese coast and in the Cádiz area, and very large in the French waters of the Bay of Biscay;
- research vessels from the institutes have different size, performance (e.g. speed, scattered rays, noise level, fishing possibility), and implementation of systems;
- different fish behaviour and distribution (schooling characteristics, avoidance, distribution relative to the coast, to the surface, in rias, etc.);
- distinct relative occurrence of species, mixing of populations, biological stages, etc.

Because of these pecularities, it has been generally accepted that each team did its best to collect clean and compatible data, despite the possible constraints, and always keeping in mind the strict application of the basic principle of following a common work plan and general requirements.

A2.1.1 Acoustic surveying

- Each survey must be carried out following the same transects year after year and according to a network of preferable parallel transects.
- Vessel speed must be as constant as possible and up to 10 knots.
- Acoustic surveying for adult fish must be done only during the day to avoid missing echoes at night when fish are close to the surface and scattered in plankton layers.

A2.1.2 Identification fishing hauls

• Fishing hauls must be made each time notable echotraces are observed, whatever the echotraces look like, and as soon as possible after observation. This is to avoid over- or undersampling of particular species, considering that first, we are never sure of intuitive identification (possible change in behaviour by season or year) and secondly, because surveys must address all pelagic species, not just the targeted species.

A2.1.3 Echotyping

• Echoes must be sorted by echotypes, taking into account their location, shape, and density, according to expert knowledge, identification hauls made nearby, and/or similarity with known echotraces.

A2.1.4 Target strength (TS)

• No agreement was obtained, and each institute applies its usual TS relationships to save the validity of relative index series. Nevertheless, each

institute operates particular experiments in order to solve this problem. Recent experiments proved that variations could be important relative to the depth of individuals, their biological conditions, and different behavioural events.

A2.1.5 Biomass calculation

• Biomass calculations are done with standard methods using different software.

Protocols for each acoustic survey series are described in the following sections.

A2.2 PELGAS survey protocols (IFREMER – France)

A2.2.1 Sampling strategy

A2.2.1.1 Survey design

Acoustic data are collected along systematic parallel transects perpendicular to the French coast (Figure A2.1), from the north coast of France to Spain, over a total distance of ca. 6500 nautical miles (1 nautical mile = 1852 m). Transects are uniformly spaced every 12 nautical miles (22 km). The mean size of clusters of pelagic fish schools in the Bay of Biscay has been estimated to be 8 km (Petitgas, 2003). The intertransect distance results from a compromise between ship time and cluster mean size.



Figure A2.1. Bay of Biscay map and PELGAS survey design. Blue lines: acoustic transects; red dots: trawl haul locations; colored areas: post-stratification regions.

The survey design allows for the coverage of the entire Biscay continental shelf (ca. 23 000 nautical miles²) from a depth of 25 m to the shelf break (200 m). The nominal sailing speed is 10 knots (1 knot = 1852 m s⁻¹), the speed being reduced to an average of 2 knots during fishing operations. This speed permits sampling of the entire Biscay shelf in ca. 30 d.

A2.2.1.2 Hydrobiological environment sampling

During daylight, the hydrobiology team operates a continuous underway fish egg sampler (CUFES) fitted with a 315-µm mesh size collector. A CUFES sample is collected every 3 nautical miles along survey transects during acoustic sampling.

During night-time, 3–4 hydrobiological stations are performed on alternate transects. Hydrostations are ideally performed on a transect that was surveyed during the previous daylight period to synoptically characterize the bio-physical environment of the fish and to allow for the adjustment of station locations, according to the hull-mounted thermo-salinometer measurements, as well as to egg counts.

Supplementary hydrostations are generally performed in an adaptative manner during the last week of the PELGAS cruise close to the Gironde estuary. The PELGAS hydrobiological environment sampling scheme is summarized in Figure A2.2.



Figure A2.2. PELGAS hydrobiological environment sampling scheme. Blue lines: daylight CUFES transects; crosses: night-time CTD station locations; colored area: higher frequency sampling area.

A2.2.1.3 Acoustic sampling

The pulse length is set to 1.024 ms for all frequencies and echosounders. *In situ* on-axis calibration of the echosounders is performed before each cruise using standard methodology (Foote, 1987; Trenkel *et al.*, 2008).

Acoustic data are acquired with the Movies+ (Weill *et al.*, 1993) and Hermes software and archived in the international hydroacoustic data format (HAC; ICES, 2005b) at a 100 dB threshold.

A2.2.1.4 Species identification by trawling

The identification of species and size classes comprising fish echotraces (ICES, 2000b) depends heavily on identification via trawl hauls performed by RV "Thalassa" using a 2-door, 57-m headline, and 52-m footrope pelagic trawl. Echograms are scrutinized in real-time, and trawl hauls are performed as often as possible. The rationale for performing an identification haul includes:

• observation of numerous fish echotraces over several elementary sampling distance units (ESDUs) or of very dense fish echotraces in one ESDU;

- changes in echotrace characteristics (morphology, density, or position in the water column);
- observation of an echotrace type fished on previous transects, but never fished on the current transect.

Acoustic transects are adaptively interrupted to perform the trawl hauls and then subsequently resumed. During PELGAS, the trawl stations are conditioned on the positions of particular acoustic images that are considered to be representative of communities of echotraces during the survey (Petitgas, 2003).

Trawl catches do not allow for the identification of single schools, but rather to an ensemble of schools over several nautical miles, resulting in identifying groups of schools to species assemblages.

At least one commercial pair trawler has accompanied the RV "Thalassa" during the PELGAS cruise since 2007 to increase the effort devoted to echotrace identification.

A2.2.1.5 Marine mammals and birds sampling

Marine mammals, birds, macrolitter, and ship activity are spotted during daylight during PELGAS transects. Detailed protocols can be found in the dedicated annex in this volume.

A2.2.2 Hydrobiological data collection

A2.2.2.1 Workspace and equipment

Located on deck C of the research vessel, the hydrobiology workspace comprises three laboratories:

- Hydrobiology lab: sample handling, filtrations, and binocular observations.
- Control room: one computer connected to the conductivity-temperaturedepth probe (CTD) and to the vessel GPS (NMEA through local network since 2012) for real-time monitoring of CTD profiles, one computer for laser optical plankton counter (LOPC) data upload, and one computer to define net closing depths and retrieve MultiNet data.
- Chemistry lab: density column experiments.

The hydrobiological equipment routinely used during PELGAS surveys includes:

- three computers to operate the CTD, the MultiNet, and the LOPC;
- two binoculars;
- a filtering system;
- pillboxes, formalin for CUFES and MultiNet sample preservation, 90° alcohol for larvae and otolith preservation;
- a CUFES;
- a CTD probe fitted with conductivity-temperature-depth, fluorimeter, and turbidimeter sensors, a LOPC (with datalogger), and nine Niskin bottles;
- three WP2 nets (200- and 50-μ mesh);
- a 315-µ mesh "filet Carré" (Bourriau, 1991) for egg and larvae sampling;
- a 500-µ mesh MultiNet (Hydrobios) fitted with five nets for mesozooplankton sampling.

A2.2.2.2 Staff

The hydrobiology team consists of 6–7 people working 24 h, with 8-h shifts (two people per shift).

Although the geographic positions of the night-time hydrostations are predefined, the responsible hydrobiology lab person may have to adapt them according to hydrological conditions. This person needs to have a strong background in ichthyology as well as in biological and physical oceanography to adapt to the station positions.

The other hydrobiology team members need the following specific skills:

- anchovy and sardine egg and larvae taxonomic identification;
- Niskin bottle content filtering to extract phytoplankton, zooplankton, and suspended matter;
- sample preservation (nutrients, phytoplankton, zooplankton, and ichthyoplankton).

A2.2.2.3 Protocols

- <u>Daylight operations</u>. During daylight, the hydrobiology team operates the CUFES, fitted with a 315- μ mesh size collector. A CUFES sample is collected every 3 nautical miles along survey transects during acoustic sampling. Sardine and anchovy eggs are sorted, counted, and preserved with 4% formalin.
- <u>Night-time operations</u>. The CTD profiles are first performed from the surface to 1–2 m above the seabed at 2 m s⁻¹. Groups of three bottles are subsequently fired near the seabed, well below the pycnocline, in the chlorophyl maximum (generally near the pycnocline), and at the surface.

A vertical WP2 net tow is then performed at each station. The WP2 is shot to a maximum depth of 100 m, or at 5 m above the seabed if the depth is < 100 m. The first WP2 sample is preserved as a CUFES sample for further mesozooplankton taxonomic analysis. The second WP2 sample is filtered into four size classes (2000, 1000, 500, and 200 μ) and dried for dry biomass analysis. The third WP2 sample is devoted to other analyses, depending on the year.

In cases of special hydrographic or meteorological features, or if eggs and/or larvae are present in the CUFES samples, supplementary Filet carré and/or MultiNet tows are adaptively performed.

The Filet carré is shot to a maximum depth of 100 m, or at 5 m above the seabed if the depth is < 100 m. The net is then towed at 1.5–2 knots for 10 min (for eggs) to 15 min (for larvae), following an oblique tow profile, from the maximum depth to the surface. The eggs are transferred into the density column to measure their density and calibrate an egg vertical distribution model (Petitgas *et al.*, 2006).

The MultiNet is shot to a maximum depth of 100 m, or at 5 m above the seabed if the depth is < 100 m. The MultiNet is then towed at 1.5–2 knots for 15 minutes, following an oblique tow profile, from the maximum depth to the surface. The five nets are automatically closed, using the OceanLab software, when reaching the minimum depth limits that were scheduled.

• <u>Data preprocessing and storage</u>. The raw CTD data are smoothed, outliers are removed, and preprocessed data are stored in Ocean Data View format (one file per year).

Plankton net tow metadata are recorded in a spreadsheet.

A2.2.3 Pelagic trawling

A2.2.3.1 Basic equipment

Located on deck B, the sorting room (130 m²) is, by default, equipped with:

- a trunk where the catch is stored;
- an automated sorting system fitted with a conveyor belt and a balance, which allows weighing and delivering the fish to the fish sorters, recording the catch composition (PUPITRI software interfaced with the balance), and cleaning the plastic boxes where the sorted fish were stored;
- three tables to process fish subsamples (length measurements, stomach extraction, etc.);
- two balances (50 kg and 5 kg maximum weights);
- a biology laboratory equipped with a computer fitted with RAPTRI software to record catch data, a printer, and a small freezer.

A2.2.3.2 Staff

The person in charge of the sorting room (or deckmaster) is an experienced technician with good knowledge of the following:

- PELGAS survey protocol;
- sorting room functioning;
- PUPITRI and RAPTRI software;
- pelagic species taxonomy.

In addition to this person, the fish sorter team consists of 4–5 extra people, including preferably at least one technician with good experience in small pelagic fish otolith extraction and catch data checking.

In cases of large catches of mixed species, scientists from other teams are called to assist in sorting the fish.

A2.2.3.3 Setting up the sorting room

Additional equipment installed at the beginning of the PELGAS includes:

- binoculars;
- fume cupboard;
- poster with catch processing protocols affixed to the wall (including maturity scales);
- an additional light;
- 5-kg balance weight;
- dissection and otolith extraction tools;
- taxonomic reference books.

At the beginning of the cruise, the "RAPTRI" software and biological files are initialized. The species list is checked, as well as the availability of all fish processing protocols (genetic, stomacal content, energy).

A2.2.3.4 Protocols

The actions performed in the sorting room are summarized below.

• <u>Catch visual inspection, rescue of live robust species</u>. The deckmaster evaluates the volume and degree of mixing of the catch and eventually asks for extra manpower.

A check is made for live "robust" species (sunfish, marine mammals, pelagic sharks) that might survive if returned to the sea. If any such species are present, they are quickly identified, measured, weighed, photographed, and returned to the sea.

• <u>Choice of sampling scheme and conveyor start-up</u>. The deckmaster determines the sampling scheme, based on the catch amount and composition. Decision is made on (i) whether to sort the entire catch or only a subset, (ii) whether to use 40-kg subsamples or less if fish are very small, and (iii) which are the main and secondary species in the catch.

The deckmaster starts the PUPITRI software operating the conveyor.

- <u>Split, weigh, and deliver the catch</u>. The deckmaster operates the conveyor system. The catch is automatically split into subsamples which are weighed. Each subsample is then either sent to the sorting belt or thrown overboard.
- <u>Catch sorting</u>. (a) *Presorting of large species*. To precisely assess the contribution of large species (mackerel, hake, etc.) to the catch, the deckmaster, aided by a fish sorter if necessary, visually detects and sorts out large fish before they pass through the PUPITRI system. (b) *Catch sorting on conveyor belts*. A minimum of four people sort the catch around the conveyor belts. Fish of the main species are left on the conveyor, whereas secondary species are sorted into plastic boxes. If some species display a clear bimodal length distribution, they are sorted by size category to improve the precision of length distributions.

The deckmaster operates the PUPITRI workstation which records the amount of catch sorted and/or discarded.

If the sorting process is long, a second line of belts is opened.

Macroscopic litter and severely damaged fish are sorted into the same box.

• <u>Sorted catch data input</u>. The fish sorters around the conveyor belts send the boxes containing sorted species onto the weighing belt.

The deckmaster inputs the following in the PUPITRI software: (i) the scientific name of the species in each box passing on the weighing belt, and (ii) the type of box to enable removing the box weight from the subsample weight. The actual species weight is automatically recorded by PUPITRI.

Boxes with sorted species are then sent to the biological measurements area.

• <u>Biological measurements on subsamples</u>. The following biological measurements are conducted by 2–3 fish sorters on each species (and eventually size class). One or two fish sorters perform the measurements, while another records the size or weight distributions on a sheet of paper.

If the catch of one species is too abundant, measurements are conducted on a subsample. Either way, all fish are measured.

About 100 fish are usually measured to assess the length distributions of species with short size distribution ranges (*Capros aper* and *Sprattus sprattus*), and ca. 150–200 fish for other species.

(a) *Size measurements*. Size data are recorded in a table with one row per size class on a measurement sheet. Fish lengths are rounded to the nearest lower 0.5 cm for clupeids, or to the nearest lower centimeter for all other species.

Each time a fish length is recorded, a tick is drawn in the table row corresponding to its size class on the measurement sheet. This permits real-time visual control of the length distribution aspect.

Fish are sized until sample exhaustion or until the fish sorter decides that a robust length distribution appears graphically on the sheet.

(b) *Mean weight measurements.* The total weight of subsamples used for defining size distributions is measured and used to derive a global mean weight for the subsample.

• <u>Length-weight keys</u>. Based on the level of completion of the fish sampling scheme, the deckmaster determines when a length-weight key must be defined for *Engraulis encrasicolus*, *Sardina pilchardus*, *Scomber scombrus*, *Scomber colias*, *Trachurus trachurus*, *Capros aper*, and *Sprattus sprattus*.

A total of 1–3 length–weight equations are derived at each trawl station. Anchovy and sardine length–weight equations are derived each time the catch is sufficient. Length–weight equations of other species are adaptatively derived, time permitting, to ensure good spatial coverage.

To define a length–weight key, individual fish used to derive the length distribution are split by size class into small boxes. The box weight and the number of fish in each box are recorded on a sheet.

• <u>Biometric measurements and otolith extraction</u>. (a) *Fish selection*. Based on the level of completion of the fish sampling scheme, the deckmaster determines when specific biometrics and otolith extraction are to be conducted for anchovy and sardine only.

For biometric and otolith reading purposes, a total of ca. 40 (sardine) or 50 (anchovy) individuals are selected over the length classes of the subsample used to derive the length distribution and stored in small boxes in the laboratory.

(b) *Biometric measurements*. The following individual biological data are written on biometric cards by two fish sorters (one trained reader and one possibly novice writer) for each individual in every sardine and anchovy subsample: (i) weight (in g), (ii) sex (male/female/undetermined), and (iii) maturity stage.

The following extra data are recorded for sardine: (i) level of fatness, and (ii) level of infestation by *Anisakis* spp. parasite.

(c) *Otolith extraction*. Different types of trays, each containing 10 pairs of otoliths, are used for anchovy and sardine.

A trained fish sorter extracts pairs of otoliths from fish whose biometrical parameters have been previously recorded. Otoliths are cleaned with a wet sponge and dried for 24 h on a tray. Pictures are taken of the completed trays.

After 24 h of drying, the otoliths are placed on the right side, with the concave face looking downwards, and the point to the top of the tray. Eukitt resin is put into each of the tray holes with a plastic pipette in a fume cupboard. The resin dries for 1–2 d.

Otolith seasonal growth rings are read using a binocular by a technician certified for anchovy and sardine otolith reading.

Age of the fish is determined based on the number of growth rings and is expressed in years, assuming that the birthday is 1 January. The age and nature of the otolith edge are added to the fish biometric card and input to a standard spreadsheet.

On average, a total of 1200 anchovy and 1400 sardine otoliths are collected during a cruise.

Anchovy otolith readings are *a posteriori* double-checked by another certified technician, who also measures the growth ring patterns.

- <u>Subsamples and biometry data input</u>. The deckmaster and a fish sorter input subsamples and biometric data to the RAPTRI database and a spreadsheet, respectively.
- <u>Catch data check</u>. Subsamples and biometric data input are checked against the original values on paper sheets by two fish sorters familiar with the RAPTRI software. Eventual input errors are corrected in the databases.

A2.2.4 Pelagic fish biomass assessment by acoustic method

Biscay fish population biomass is assessed during a PELGAS cruise using "expert" methodology to combine acoustic and fishing data.

A2.2.4.1 Acoustic data preprocessing

Only 38-kHz backscatters are used for biomass assessment. However, echograms recorded at the 120-kHz frequency are also scrutinized to assist in isolating fish echotraces from sound scattering layers (SSLs).

Pelagic fish are frequently scattered close to the sea surface and within the surface acoustic blind zone (0–10 m depth) at night. SSLs are also denser during night-time than during daylight, making fish echotrace partitioning less reliable. Only daylight acoustic data are then used for stock assessment purposes.

At first echograms are corrected manually for bottom detection errors. Daylight 38kHz volume backscattering coefficients (Sv) higher than –60 dB (Petitgas *et al.*, 1998) and recorded from depths of 10–150 m along acoustic transects are then echo integrated in each beam over a standard depth channel of 10-m thickness and averaged over 1 nautical mile ESDUs. The resulting values of nautical area backscattering coefficients (NASC) are used in subsequent analysis.

A2.2.4.2 Classification of echo integrals

The rectified echogram is then used by experts to allocate the echo integrals (S_A) thought to correspond to fish targets for several echotrace categories in each ESDU, based on echotrace shape, density, and position. Echotrace categories correspond to species or group of species found in midwater identification trawls. At least four categories are generally considered during a survey:

- D1: diffuse shoals or layers close to the seabed or small "drops" extending up to 10 m above the seabed allocated to horse mackerel and gadoids;
- D2: schools displaying sharp edges and often high density, generally distributed up to 50 m above the seabed in coastal areas and sometimes offshore allocated to anchovy, sprat, sardine, and mackerel;
- D3: diffuse echotraces often observed offshore all along the shelf break allocated to a mixture of blue whiting and myctophids;

• D4: small, dense, and very superficial (0–30 m depth) schools – attributed to sardine, mackerel, or anchovy.

Other echotype categories are adaptively defined every year to accommodate new temporary aggregation patterns or species mixtures (e.g. when sardine forms either large schools very close to the coast or dense, small, superficial schools offshore).

When fish echotraces cannot be visually allocated to species, especially in the case of diffuse, multispecies layers, echo integrals are partitioned according to the catch composition in the area.

A2.2.4.3 Association of acoustic and fishing data

• <u>Selection of homogeneous regions</u>. On a large scale, acoustic ESDUs are allocated to homogeneous regions, defined visually based on trawl haul composition (species and size; Figure A2.1). Regions are further partitioned into two depth layers for depths >50 m. Fish backscatter classified into the D4 category is then allocated to the surface layer, whereas other categories are pooled in the bottom layer.

Regional averages of the trawl haul compositions are computed by weighting the species/size compositions of the hauls from one region against the mean fish backscatter recorded in a 10-nautical mile square centred around the haul position (Massé and Retière, 1995).

• <u>Reference hauls</u>. A "reference haul" is manually allocated to each ESDU, according to: (i) the haul depth – surface hauls are exclusively applied to D4 (surface echotraces) and bottom hauls to other echotrace categories (D1, D2, Dn...); and (ii) in the case of bottom hauls, the resemblance between echotraces observed in the specific ESDU and the echotraces observed in nearby trawled ESDUs.

Size composition distributions derived from reference-haul catches are generally used to compute biomass at length in the associated ESDU. Catches from another haul are alternatively used if the reference-haul sample size is too small.

A2.2.4.4 Pelagic fish biomass assessment

• <u>General methodology</u>. The methodology described below is adapted from Simmonds and MacLennan (2005).

A2.2.4.4.1 Partitioning of the total echo integrals between species

As two or more species are commonly found in mixed concentrations and their marks cannot be distinguished in the echogram of the PELGAS surveys, further partitioning to the species level is possible by including the composition of trawl catches (Nakken and Dommasnes, 1977). Echo integrals E_i are allocated to species *i* (Simmonds and MacLennan, 2005):

$$E_{i} = \frac{W_{i} \langle \boldsymbol{\sigma}_{i} \rangle}{\sum\limits_{j=1}^{N} W_{j} \langle \boldsymbol{\sigma}_{j} \rangle} E_{m}$$
(A2.1)

where w_i is expressed as the proportional number or weight of each species in the trawl catches (if need be weighted by total haul catches or mean acoustic backscatter in the vicinity of the haul[s]) and $\langle \sigma_i \rangle$ is the mean backscattering cross section of species *i*.

The mean backscattering cross section is derived from the mean target strength of one fish TS_1 as a function of its length *L* (usually expressed in cm):

$$TS_1 = b_i + m_i \log \left(L \right) \tag{A2.2}$$

where b_i and m_i are species-specific coefficients assumed to be known from experimental evidence. A formula for the mean backscattering cross section (expressed in m² of backscattering surface) is:

$$\langle \sigma_{bs-i} \rangle = 10^{\left[b_i + m_i \log\left(\langle L \rangle\right) \right] / 10} = \langle L \rangle^{m_i / 10} 10^{b_i / 10}$$
(A2.3)

where *<L>* is species *i* mean length while *b_i* and *m_i* are coefficients used for PELGAS surveys (Table A2.1).

Species	Frequency (kHz)	b20 in use at IFREMER	Closest b20 value in literature	Reference	Literature species
Engraulis encrasicolus	38	71.2	71.2	ICES (1982b)	Clupea harengus and Sprattus sprattus
Sardina pilchardus	38	71.2	71.2	ICES (1982b)	<i>Clupea harengus</i> and <i>Sprattus</i> <i>sprattus</i>
Scomber colias	38	70	70.9	Guttierez and MacLennan (1998)	Scomber japonicus
Scomber scombrus	38	86	86.4	Misund and Betelstad (1996)	Scomber scombrus
Sprattus sprattus	38	71.2	71.2	ICES (1982b)	Clupea harengus
Trachurus mediteranneus	38	68.7	68.9	Lillo et al. (1996)	Trachurus symmetricus
Trachurus trachurus	38	68.7	68.9	Lillo et al. (1996)	Trachurus symmetricus
Micromesistius poutassou	38	67	67.4	Foote (1987)	Physoclystii (gadoids)
Gadoids	38	67	67.4	Foote (1987)	Physoclystii (gadoids)

Table A2.1. TS coefficients used at IFREMER for acoustic fish biomass assessment.

If echo integrals E_i are expressed as nautical area-scattering coeffcients, S_A (in m² nautical mile⁻²), backscattering cross sections must be expressed in Equation (A2.1) as spherical backscattering cross sections: $\sigma_{sp-i} = 4\pi\sigma_{bs-i}$, to obtain fish density estimates (MacLennan *et al.*, 2002).

A2.2.4.4.2 Estimation of the density of targets of species *i*

The density of targets of species *i* can be estimated using the generic formula (Simmonds and MacLennan, 2005):

$$F_{i} = \frac{C_{E}}{\langle \sigma_{i} \rangle} E_{i}$$
(A2.4)

where F_i is the areal density of target of species *i*, E_i is the mean acoustic backscatter of species *i*, C_E is the equipment calibration factor which is the same for all species, and $\langle \sigma_i \rangle$ is the mean backscattering cross section of species *i*.

A2.2.4.4.3 Number-weight relationships

 F_i can be expressed in weight of fish per surface unit by multiplying F_i by an estimate of the overall mean weight of species *i*.

Alternatively, one can use a weight-based TS function, i.e. the target strength of 1 kg of fish to compute F_i . The mean relationship between the length L of a fish and its weight W is expressed as:

$$W = a_f L^{o_f} \tag{A2.5}$$

Since the number of individuals of mean weight *<W>* per unit weight of fish is *1/<W>*, the weight-based TS function (Simmonds and MacLennan, 2005) is:

$$TS_{w} = b_{w} + m_{w} \log(L) \tag{A2.6}$$

where:

$$b_w = b_i - 10\log(a_i)$$
 and $m_w = m_i - 10\log(b_i)$ (A2.7)

From Equation (A2.3), and assuming that $m_i = 20$, the weight-based spherical scattering cross section hence is:

$$\left\langle \sigma_{sp-w} \right\rangle = \frac{\left\langle \sigma_{sp-1} \right\rangle}{\left\langle W \right\rangle} = \frac{4\pi}{\left\langle W \right\rangle} \times \left\langle L^2 \right\rangle \times 10^{b_i/10} \tag{A2.8}$$

A2.2.4.4.4 Abundance estimation

Areal densities of target species *i* per ESDU must then be raised to the total surface of the surveyed area. This implies making some assumptions on the density of fish in areas that have not been sampled. Abundance is calculated independently for each species or category of target defined during echo partitioning.

In the case of PELGAS surveys, total abundance estimates in previously defined homogeneous regions are computed by multiplying the mean fish density per ESDU by the total surface of the region.

From equations (A2.1) and (A2.4), the total abundance in number Q_i of species i in a homogeneous region of surface A then is:

$$Q_{i} = F_{i} \times A = \frac{C_{E}}{\sigma_{i}} \frac{z_{i}\sigma_{i}}{\sum_{j} z_{j}\sigma_{j}} E_{m} \times A = C_{E} \frac{z_{i}}{\sum_{j} z_{j}\sigma_{j}} E_{m} \times A = Z_{i} \times E_{m} \times A$$
(A2.9)

Z_i is a region-specific weighting factor depending only on trawl catches and TS equations (Diner and Le Men, 1983).

In the same way, the total abundance in weight Q_{w-i} of species *i* in a homogeneous region of surface *A* is then:

$$Q_{w-i} = W_i \cdot F_i \cdot A = W_i \cdot C_E \cdot \left[Z_i / \sum_j (Z_j \cdot \sigma_j) \right] \cdot E_m \cdot A = X_i \cdot E_m \cdot A$$
(A2.10)

where $\langle W_i \rangle$ is the mean weight of species *i* in the region (kg) and X_i is a region-specific weighting factor depending only on trawl catches and TS equations (Diner and Le Men, 1983), expressed in kg m⁻².

Using the weight-based spherical scattering cross section, Equation (A2.8), X_{i-k} is expressed as:

$$X_{i-k} = C_E z_i / \left(\sum_j z_j \left\langle \sigma_{w-j} \right\rangle \right)$$
(A2.11)

where $\langle \sigma_{w_{-j}} \rangle$ is the weight-based mean spherical scattering cross section of species *j* in the region. To express the abundance in numbers of fish, the weighting factor X_{i-j} must be used:

$$X_{i-1} = X_{i-k} \langle W \rangle \tag{A2.12}$$

• <u>Abundance and biomass-at-size per species and ESDU</u>. Fish densities per species and size class are computed for each echotype category and ESDU based on: (i) fish backscatters allocated to the echotype category in ESDU *x*; and (ii) the species composition and size distribution in the reference haul associated with the ESDU.

Acoustic backscatter $E_{ild}(x)$ of species *i* of mean length *l* in echotype category *d* and ESDU *x*, associated with reference haul *r* is (Diner and Le Men, 1983):

$$E_{iid}(x) = \frac{q_{iid}(r)\sigma_{ii}(r)}{\sum\limits_{i=1}^{N} q_{jid}(r)\sigma_{ji}(r)} E_d(x)$$
(A2.13)

where $q_{ild}(r)$ is the ratio of the catches of species *i* of size *l* over the total catches of the *N* species of echotype *d* in reference haul *r*, $E_d(x)$ is the average fish backscatter allocated to echotype category *d* in ESDU *x*, expressed as a nautical area backscattering coefficient (NASC), and $\sigma_{il}(r)$ is the backscattering cross section of species *i* of size *l* in reference haul *r*.

Replacing $E_{ild}(x)$ in Equation (A2.4) by its expression in Equation (A2.13), the density of fish of size *l* and species *i* in echotype category *d* and ESDU *x* associated with reference haul *r* is:

$$F_{ild}(x) = \frac{C_{E}}{\sigma_{il}(r)} \frac{q_{ild}(r)\sigma_{il}(r)}{\sum_{j} q_{jld}(r)\sigma_{jl}(r)} E_{d}(x) = C_{E} \frac{q_{ild}(r)}{\sum_{j} q_{jld}(r)\sigma_{jl}(r)} E_{d}(x)$$
(A2.14)

Further, replacing $q_{ild}(r)$ by $c_{ild}(r)/c_d(r)$, where $c_{ild}(r)$ is the catch of species *i* of size *l* in reference haul *r*, and $c_d(r)$ the total catch of *N* species of echotype *d* found in haul *r*, one gets:

$$F_{ild}(x) = C_{E} \frac{\frac{c_{ild}(r)}{c_{d}(r)}}{\sum_{j} \frac{c_{jld}(r)}{c_{d}(r)} \sigma_{jl}(r)} E_{d}(x) = C_{E} \frac{c_{ild}(r)}{\sum_{j} c_{jld}(r) \sigma_{jl}(r)} E_{d}(x) = X_{E-ild}(r) E_{d}(x)$$
(A2.15)

where $X_{E-ild}(r)$ is a scaling factor depending only on trawl catches, echotype species composition, and TS equations.

The total density of targets of species *i* and size *l* for each ESDU is then computed as the sum of the fish densities at size *l* over all echotype categories comprising species *i*:

$$F_{il}(x) = \sum_{i} F_{ild}(x) \tag{A2.16}$$

Total abundance in number and weight of fish of species *i* and class size *l* per square nautical mile are actually computed for each ESDU using equations (A2.7) and (A2.8), with *A* equal to 1:

$$Q_{il}(x) = F_{il}(x) \text{ and } Q_{w-il}(x) = F_{il}(x) \times \langle W_{il} \rangle (r)$$
(A2.17)

where $\langle W_i \rangle \langle r \rangle$ is the mean weight of species *i* of size *l* in haul *r*.

 <u>Abundance and biomass-at-age per species and ESDU</u>. Size-age keys are derived from biological samples by otolith reading.

The density of fish of age *a* and species *i* in echotype category *d* and ESDU *x* associated with reference haul *r* is then:

$$F_{ila}(x) = q_{ila}F_{il}(x)$$
(A2.18)

where q_{ila} is the proportion of fish of species *i* and age *a* in size class *l*, according to the size–age key, and $F_{il}(x)$ is the density of fish of species *i* and size *l* in ESDU *x*.

The total density of fish of age *a* and species *i* in ESDU *x* is computed as the sum of $F_{ila}(x)$ over *l*.

Total abundance and biomass estimates per square nautical mile are actually computed in each ESDU for each species and age class using equations (A2.7) and (A2.8), with A equal to 1.

• <u>Biomass estimates per species and region</u>. Echo integrals allocated to each echotype category in each ESDU are averaged over each homogeneous region. Mean echo integrals are then partitioned to species level, relative to the species composition in the region's mean haul.

In each region, the estimated areal fish density *Fi*,*d* of species *i* in echotype category *d* and comprising *N* species is computed as (Diner and Le Men, 1983):

$$F_{id} = C_E \frac{W_{id}}{\sum\limits_{j=1}^{N} W_{jd} \left\langle \sigma_{sp-j} \right\rangle} E_d \tag{A2.19}$$

where C_E is an equipment calibration factor, E_d is the mean nautical area fish scattering coefficient (NASC) per ESDU for echotype category d in the region, w_{id} is the weight of species i in the computation of the mean species composition of echotype category d in the region (Diner and Le Men, 1983):

$$w_{id} = \frac{\sum_{k=1}^{M} E_{kd} q_{ik} / q_{dk}}{\sum_{k=1}^{M} E_{kd}},$$
 (A2.20)

and $\langle \sigma_{sp-i} \rangle = 4\pi \pi 1^{b_i + m_i \log(\overline{L_i})/10}$ is the mean backscattering cross section of species *i*, derived from the species mean length $\overline{L_i}$ in the region's mean haul and from coefficients b_i and m_i (Table A2.1), where q_{ik} is the catches of species *i* recorded in the *M* hauls *k* performed in the region, q_{dk} is the total catches of the species in echotype category *d* in

haul k, and E_{kd} is the average fish backscatter allocated to echotype d recorded in a 6nautical mile square centred around the position of haul k (Massé et al., 1996).

For each region, abundance Q_{id} and biomass Q_{w-id} of species *i* in echotype category *d* are computed as:

$$Q_{id} = F_{id} \times A \text{ and } Q_{w-id} = Q_{id} \times \overline{W}_i$$
 (A2.21)

where A is the region area and \overline{w}_i is the mean weight of species *i*, derived from biological samples.

Total density estimates for species *i* in the region are actually computed as the average of density estimates of species i in all echotype categories. In the same way, total abundance and biomass estimates for species i are computed as the sum of abundance/biomass estimates of species *i* in all echotype categories in the region.

Estimation error •

An estimation variance $\sigma^{2_{E-i}}$, taking into account the catches and acoustic backscatter variability, is computed for each species i in echotype d and region j, based on the product variance:

$$Var\left(\overline{s_{A}}\overline{X_{e}}\right) = var\left(\overline{s_{A}}\right)\overline{X_{e}}^{2} + var\left(\overline{X_{e}}\right)\overline{s_{A}}^{2}$$
(A2.22)

Assuming that $var(\overline{s_A}) = var(s_A)/N_{esdu} = var(s_A)w_{sA}$, with $w_{sA} = \frac{1}{N_{sA(d,j)}}$, and $var(\overline{X_e}) = var(X_e)w_{Xe} = var(X_e)\Sigma[s_A(nei_k)/\Sigma s_A(nei_k)]^2$, the estimation variance hence

is:

$$\sigma_{E-i}^{2} = A^{2} \times \sum_{d} \sum_{j} \left(w_{Aj} \cdot \left[\overline{s_{A-d,j}}^{2} var(X_{E-d,i,j}) w_{E} + \overline{X_{E-d,i,j}}^{2} var(S_{A-d,j}) w_{sA} \right] \right)$$
(A2.23)

where A is the surface of the estimation zone, $S_{A-d,j}$ and $var(s_{A-d,j})$ are the average and variance of acoustic backscatters allocated to echotype *d* in region *j*, respectively, $\overline{X_{r-di}}$ and $var(X_{E-di,j})$ are the average and variance of the XE scaling factors of species *i* in region *j* and echotype *d*, $w_{Aj} = \frac{A_j}{\Sigma A_j}$ is the weighting factor of region *j* of area *Aj*, and

 $w_{x_{E}} - d, i, j = \sum_{k} \left(\frac{s_{A-neigh, k, d, i}}{\sum_{k} s_{A-neigh, k, d, i}} \right)^{2}$ is the weight of the XE factor of species *i* in region *j* and

deviation *d*, computed over trawl haul *k*, as the mean fish S_A value $S_{A-neigh,k,d,i}$ around the hauls.

Software. The fish biomass acoustic computations described above are . performed at IFREMER, using the EchoR package (Doray, 2013).

A2.3 PELACUS survey protocol (IEO – Spain)

The IEO systematically undertakes acoustic surveys off the Spanish Atlantic coasts. In the northern area, from the Spanish–Portuguese border to the French–Spanish border, the PELACUS time-series takes place annually in spring (March–April). In the southern area (i.e. Gulf of Cádiz), the ECOCADIZ time-series covers the area between the Strait of Gibraltar and the Cape of San Vicente in Portugal.

The survey design consists of a grid with systematic parallel transects equally separated by 8 nautical miles and perpendicular to the coastline covering the continental shelf between the 20–30-m (or 1 nautical mile from the coast or shallower banks) and 200-m isobaths. The outer limit has been extended to 1000 m since 2013.

A2.3.1 Data acquisition

A2.3.1.1 Acoustic

Acoustic equipment consists of a Simrad EK-500/EK-60 scientific echosounder, first working at 38 and 120 kHz, and then increasing to 18, 70, and 200 kHz. All frequencies were calibrated according to standard procedures (Foote *et al.*, 1987). The elementary sampling distance unit (ESDU) was fixed at 1 nautical mile. Acoustic data were obtained only during daylight at a survey speed of 8–10 knots. Data were stored in a raw format and post-processed using SonarData & Myriax Echoview software (Myriax Ltd.).

A2.3.1.2 Egg sampling

Coupling with the acoustic track, samples from CUFES were gathered each 3 nautical miles with the intake located at a depth of 5 m on the starboard side. The collector mesh size was $350 \,\mu\text{m}$, samples were sorted on board, and sardine and anchovy eggs were counted before preserving them in a 4% buffered fomaldehyde solution.

A2.3.1.3 Fishing stations

Pelagic trawl hauls were performed in an opportunistic way to provide groundtruthing for acoustic data and for sampling purposes. Several pelagic gears were used throughout the different time-series: 12-, 16-, and 24-m vertical opening and 20-mm codend on RV "Cornide de Saavedra"; and a 76/70 pelagic trawl with 20-m vertical opening and 10-mm mesh codend or a 57/52 pelagic trawl with 10-m vertical opening and the same mesh size in the codend on RV "Thalassa". Effective towing duration is ca. 15–30 min, depending on fish abundance. All catch is weighed and sorted up to the species level.

A2.3.1.4 Length and biological sampling

Both length and biological samples (weight, age, sex, and maturity stage) are collected at fishing stations. All pelagic fish are measured (total length) by 0.5- or 1-cm classes. Subsampling is done when the catch has >100 specimens. This is used for raising the length structure to the entire catch.

Biological sampling consisting of individual weight, length, sex, gonad stage (from immature to resting stage), and otolith removal for further age group allocation is done for each target species (hake, mackerel, horse mackerel, anchovy, sardine, chub mackerel). For boar fish and bogue, only individual weight and length is collected.

Additional samples are collected for analysis of stomach contents and trophic position through stable isotopes, determination of batch fecundity and spawning fraction from

gonads (to apply the daily egg production method for spawning–stock biomass estimation), or genetic studies.

A2.3.1.5 Top predators

Monitoring of top predators was also done during daylight according to the general methodology described in Tasker *et al.* (1984). Three observers, placed above the bridge of the vessel at a height of 16 m above sea level, work in turns of two, searching an area of 180° (each observer covers a field of 90°). Observations are carried out with the naked eye, although binoculars are used (7×50) to confirm species identification and determine predator behaviour. Observations are carried out during daylight while the vessel surveys the transects and while it covers the distance between transects at an average speed of 10 knots. Observers record species, number of individuals, behaviour, distance to the vessel, angle to the trackline, and observation conditions (wind speed and direction, sea state, visibility, etc.). Observers also record presence, number, and type of boats, as well as type, size, and number of floating litter.

A2.3.1.6 Continuous meteorological hydrological characterization

Oceanographic data from surface (thermo-salinograph and flourimeter) and meteorological data (wind strength and direction, air temperature, humidity, radiation, atmospheric pressure) are automatically recorded and stored every minute together with position (GMT and latitude/longitude from a differential GPS).

In PELACUS, CTD casts and plankton and water samples are taken during night-time over the same grid steamed for acoustic purposes. Stations are located every 3–4 transects (i.e. 16–24 nautical miles apart), with five stations in each from the coast to the slope and with the last station located outside the continental shelf. Plankton is sampled using several nets (Bongo, WP2, and CalVet). Plankton samples are sorted using four size classes (20–200, 200–500, 500–1000, and >1000 μ m). Samples < 200 μ m are further sorted through an array of sieves of 20, 40, and 80 μ m and filtered on preweighed glass fibre filters (Millipore Type A). Plankton biomass is then expressed as dry weight. Zooplankton is classified up to species composition and taxonomic groups at fixed strata from samples collected at the CTD + bottle rosette carousel (pico-and nanoplankton, microplankton, and mesozooplankton). For this purpose, FlowCAM, LOPC, and ZooImage techniques are used.

Dissolved nutrients are collected using Niskin water bottle samples which are stored at –20°C for further dissolved nutrients analysis (NO₃, NO₂, P, NH₄₊, SiO₄).

A2.3.2 Data processing

A2.3.2.1 Acoustic assessment

Fish abundance is calculated at the 38-kHz frequency recommended by the ICES Planning Group on Arial and Acoustic Surveys for Mackerel (PGAAM; ICES, 2002b), although echograms at frequencies from 18, 70, 120, and 200 kHz are used to visually discriminate not only between fish and other scatter targets such as plankton or bubbles, but also among different fish according to the strength of their echo at each frequency (i.e. frequency response, or more generally fish with or without swimbladder). Also, frequencies of 38, 70, and 120 kHz have been used to create a mask (virtual echogram), allowing better discrimination.

A2.3.2.2 NASC allocation

Hauls considered to be the best representation of the fish community are used to allocate the backscattering energy in the surrounding area and with echotraces similar to those already fished. These hauls are chosen according to fishing performance, accounting for results in terms of species and length compositions. This criterion is used as a proxy for ground-truthing.

This is done by applying the Nakken and Dommasnes (1977) method for multiple species, but instead of using the mean backscattering cross section, the full length class distribution (0.5- or 1-cm length classes) are used, as follows:

$$s_{A_{i}} = s_{A} \frac{w_{li} \cdot \sigma_{bs}}{\sum_{li} w_{li} \cdot \sigma_{bs}}$$
(A2.24)

where w_{li} is the proportion in number of length class *l* and species *i* in the catches, and σ_{bs} is its corresponding proportion of backscattering cross section. This is related to the target strength (TS) as follows:

$$\sigma_{bs} = 10^{TS/10} (\text{in dB}) \tag{A2.25}$$

This is computed from the formula $TS = 20 \log LT + b_{20}$ (Simmonds and MacLennan, 2005), where LT is the length class (0.5 cm). The b_{20} values for the most important species present in the area are shown in Table A2.2.

Table A2.2. Target strength values for the pelagic species assessed in the PELACUS survey.

Species [*]	WHB	MAC	HOM	PIL	JAA	ANE	BOG	MAS	BOC	HMM	
b 20	-67.5	-84.9	-68.7	-72.6	-68.7	-72.6	-67.0	-68.7	-72.6	-68.7	

* WHB = blue whiting; MAC = mackerel; HOM = horse mackerel; PIL = sardine; JAA = blue jack mackerel; BOG = bogue; MAS = chub mackerel; BOC = boar fish; HMM = Mediterranean horse mackerel; and ANE = anchovy.

When possible, direct allocation is also done. For this purpose school descriptors are used, either morphometric (such as length, perimeter, height, or area), energetic (scaled volume backscattering coefficient [sv] and acoustic area backscattering coefficient [sA] at 38 kHz), or positional (position in the water column, distance to the coast, geographical area).

A2.3.2.3 Post-stratification

Once backscattering energy is allocated to fish species, the spatial distribution for each species is analysed, taking into account both the NASC values and the length frequency distributions (LFD) to provide homogeneous assessment polygons. These are calculated as follows: an empty track determines the along-coast limit of the polygon, whilst three consecutive empty ESDUs determine a gap or the across-coast limit. Within each polygon, LFD is analysed.

LFD are obtained for all positive hauls for a particular species (either from the total catch or from a representative random sample of 100–200 fish). For the purpose of acoustic assessment, only those LFD based on a minimum of 30 individuals and/or a normal distribution are considered. Differences in probability density functions (PDF) are tested using the Kolmogorov–Smirnoff (K–S) test. PDF distributions without significant differences are joined, providing homogeneous PDF strata. Spatial distribution is then analysed within each stratum and finally the mean s_A value and surface (nautical miles²) are calculated using system-based GIS (ArcGis 9.3 or QGIS). These values, together with the length distributions, are used to calculate fish

abundance in number, as described in Nakken and Dommasnes (1977). Numbers are converted into biomass using the length–weight relationship. Biomass estimation is carried out on each stratum (polygon) using the arithmetic mean of the backscattering energy (NASC, s_A) attributed to each fish species and with the surface expressed in nautical miles². For purposes of comparison, results are given by ICES divisions (9.a North, 8.c West, 8.c East [western part], 8.c East [eastern part], and 8.b). Numbers are converted into biomass through a length–weight relationship.

A2.4 PELAGO surveys (IPMA – Portugal)

A2.4.1 Equipment

- Simrad EK 500 38 kHz, split-beam transducer 8 × 7° (equivalent beam angle: 10logψ = -20.2 dB; pulse duration = 1 ms) calibrated prior to the survey. Data storage and post-processing software: Movies+.
- Netsounder: Scanmar trawl-eye and depth sensor.
- Pelagic trawl (10-m vertical opening) and bottom trawl (NTC) to identify echoes, split acoustic energy, and gather biological data. Opportunistic fishing hauls.

A2.4.2 Sample design

- Parallel systematic grid, 8 nautical miles apart (west coast), 6 nautical miles apart in Algarve; in Cádiz, not parallel, ca. 8 nautical miles apart in the middle of the radials (Figure A2.3). The acoustic survey is done only during daylight.
- ESDU: 1 nautical mile.
- Vessel speed: 9–10 knots.
- Vessel draft: 4 m.

A2.4.3 Abundance estimates

- Integration ESDU: 1 nautical mile.
- Surface threshold (from transducer): 3–10 m, according to the weather.
- Bottom threshold: 0.2 m.
- Survey area is divided in four zones: (i) OCN (Caminha to Nazaré), (ii) OCS (Nazaré to Cape Santa Vicente), (iii) ALG (Cape Santa Vicente to Vila Real de Sto António), and (iv) CAD (Vila Real de Sto António to Cape Trafalgar).
- The acoustic energy is split by trawl proportion (in number), taking into account the species TSs, if direct energy extraction is not possible.
- There is post-stratification in coherent (length composition, density) areas for each species.
- Area density is calculated by arithmetic mean.
- Abundance estimation is calculated in number of individuals by length class in each coherent area. The hauls are combined in this area, usually without weighting.
- Biomass estimation is calculated using the length–weight relationship.
- Estimated abundance by age groups is calculated using age–length keys extracted from the otholith readings.
- The number of fish (*N*) is obtained by dividing the total acoustic fish energy in the area by the scattering energy of a single fish, which is a function of the length (*L*) for each species: $N = \frac{S_A}{\langle \sigma \rangle}$ with $\langle \sigma \rangle$ being the mean backscattered

acoustic energy of a fish with length (*L*).

- The conversion constant (*C*) between acoustic energy *s*_A and the number of fish is: $C = \frac{1}{\langle \sigma \rangle}$
- < σ > is obtained by back transforming the TS of the species: < $\sigma >= \frac{10^{\frac{15}{10}}}{4\pi}$
- The number of fish of length class (*L*) in each sector is $N_L = C_i C_L < S_A > A$, where C_i is the calibration constant (unity for the calibrated EK500), C_L is the conversion constant from acoustic energy to number of fish of length (*L*), $<s_A >$

is the acoustic density in the sector (total acoustic integration divided by the number of miles surveyed in the sector), and *A* is the area of the sector.

• For sardine, the conversion constant is $C_L = 1448072L^{-2} \text{ m}^{-2}$ nautical mile⁻².

A2.4.4 Energy splitting between species and between length classes

If S_A is the total energy of the species mixture and N_i is the proportion in number of species *i* in the fishing sample, then the acoustic energy of species *i* (S_{Ai}) is:

$$S_{Ai} = S_A \times \frac{N_i < \sigma_i >}{\sum N_i < \sigma_i >}$$
(A2.26)

where $\langle \sigma_i \rangle$ is the mean acoustic section (TS in linear units) of species *i* in the sample.

For the split of acoustic energy between length classes, the methodology is similar:

$$S_{Aj} = S_A \times \frac{P_j \sigma_j}{\sum\limits_{i} P_j \sigma_j}$$
(A2.27)

where S_{Aj} is the acoustic energy attributed to class j, P_j is the proportion of length class j in the sample, and σ_j is the backscattering acoustic equivalent section (TS in linear units) for a fish of class j.

A2.4.5 Target strength b₂₀ used (20logL - b₂₀)

- Sardina pilchardus (PIL): 72.6 dB
- Scomber colias (MAS): 68.7 dB
- Scomber scombrus (MAC): 82 dB
- Trachurus trachurus (HOM): 68.7 dB
- Trachurus picturatus (JAA): 68.7 dB
- Boops boops (BOG): 67.0 dB
- Engraulis encrasicholus (ANE): 72.6 dB
- Micromesistius poutassou (WHB): 80 dB
- Macroramphosus spp. (SNS): 80 dB
- Capros aper (BOC): 80 dB



Figure A2.3. Portuguese acoustic transects and considered areas for abundance estimation.

A2.4.6 Sampling and biology

A2.4.6.1 Pelagic fish species sampling

General sampling procedure

Five (5) sampling boxes (ca. 30 l) randomly filled from the capture in each trawl station. Species identification.

Total weight of specimens and number of each species.

Random samples of 100 individuals of each species.

Total weight of sample and individual total lengths.

Distribution of individuals of each species by length classes:

- 0.5-cm length classes in fast growing species: sardine, anchovy, sardinellas, shad, twaite shad, boarfish, snipefish, thickback sole, flounders, gurnards, argentines, weevers, octopus, squids, cuttlefish:
- 1.0-cm length classes: horse mackerel, chub mackerel, bogue, and blue whiting.
- Total number and weight by length class.

Individual total length of the remaining sampled species of each haul and no distribution by length classes. **Biological sampling procedure**

Primary species:

Sardine, horse mackerel, anchovy, mackerel, and chub mackerel.

Procedure:

Ten (10) individuals by length class (sardine and anchovy: 0.5 cm; chub mackerel, mackerel, and horse mackerel: 1.0 cm). Extreme length classes completed with specimens taken from the sample box. Individual total length, total and gutted weights, gonad weight (whenever possible!), sex and sexual maturation stage, visceral fat stage, stomach filling stage, and colour.

Stomach collection:

Primary species (stomachs always collected) - sardine, anchovy, chub mackerel, and horse mackerel. Secondary species (stomachs collected if there is spare time and when >30 specimens are caught) mackerel, blue jack mackerel, Mediterranean horse mackerel, bogue, European squid, squid (Alloteuthis), crab (Polybius henslowi), boarfish, snipefish, and blue whiting; for squid (Alloteuthis) and crab (Polybius henslowi), 20 individuals are randomly taken from the sample and frozen.

For the remaining species, 10 stomachs with contents:

- 4 stomachs from the modal length class (most abundant class);
- 2 stomachs from the length class below and 2 from the one above the modal class;
- 1 stomach from the length class below and 1 from the one above the previous ones;
- if the length distribution has 2 modes, the above procedures must be followed for each one (this will result in a total of 20 collected stomachs for that haul/species);
- the individual total length of fish from which stomachs were extracted is measured and recorded as well as the total and gutted weights. Otoliths are not collected;
- evaginated or torn stomachs are not collected;
- stomachs individually collected to a plastic bag and identified with the observation number on a baking paper tag inside the bag. Bags with stomachs of each trawl haul/species are gathered into a large plastic bag properly identified (survey code, sampling station number, and species) and immediately frozen.

Otolith collection:

- A maximum number of otoliths by length class at the beginning of each area survey for a reasonable sampling in case of scarce sampling trawl stations. A homogeneous area coverage and an indicative number of otoliths in each of the following sampling stations must be reached and controlled in order to achieve the objectives for each area:
- Sardine 10 otolith pairs by length class (total length up to 13 cm); 16 pairs (between 13.5 and 17.5 cm); 20 pairs (18 cm and above);
- Anchovy 10 otolith pairs by length class;
- Mackerel, chub mackerel, and horse mackerel 15 otolith pairs by length class. For chub mackerel, if individuals with total length >27-28 cm (does not frequently occur) are caught in a significant amount at a sampling station, opportunity must be taken to collect 15 otoliths by length class.

Surveyed areas

OCN - "Occidental Norte" (West Northern coast) - ICES Division 9.a-Central North

OCS - "Occidental Sul" (West Southern coast) - ICES Division 9.a-Central South

ALG - "Algarve" - ICES Division 9.a-South

CAD - "Cádiz" (Gulf of Cádiz) - ICES Division 9.a-South

A2.4.7 Sardine otolith preparation procedures and age-reading criteria

The Iberian–Atlantic sardine stock is commonly surveyed by Portugal and Spain; therefore, its assessment is also jointly undertaken by both countries. Consequently, standardized age-reading methodologies and criteria had to be adopted. Otolith exchange and age-reading workshops have been regularly undertaken by the fisheries research institutes of both countries for this purpose. Based on the experience of agereading experts of both countries and on the results of these events, protocols with recommendations and age-reading criteria have been produced in order to improve data quality and, consequently, the precision of the stock assessments.

The otolith preparation procedures and age-reading criteria commonly applied by readers of both countries are those agreed during the last Workshop on Age Reading of European Atlantic Sardine (WKARAS) held in Lisbon, Portugal, 14–18 February 2011 (ICES, 2011b).

A2.4.8 Sardine and anchovy eggs and environmental conditions

Since 2000, a CUFES system (Checkley *et al.*, 1997) has been used in the acoustic surveys for underway surface (ca. 3 m) plankton sampling (every 3 nautical miles) with the main objective to describe sardine and anchovy spawning grounds. A SeaBird thermosalinographer and a SeaPoint fluorometer are coupled to the CUFES system allowing continuous recording of SST, SSS, and SSF (fluorescence, ~chlorophyll). The information from the TCF probes is compiled, together with GPS data, by dedicated software (EDAS). High-resolution data acquisition on egg abundance, temperature, salinity, and fluorescence allow the production of detailed distribution maps that assist in environmental characterization and definition of spawning areas.

A2.4.9 Seabird and marine mammal census

Data on seabirds and marine mammals are collected during IPMA surveys according to a modified version of the methodology of Tasker *et al.* (1984), as recommended by the European Seabirds At Sea (ESAS) group (Camphuysen and Garthe, 2004). Sightings are recorded as densities (number of individuals km⁻²) along the transects of the survey during observation periods each lasting 5 min. All birds in contact with the water or with marine mammals observed within the transect are counted. All birds in flight are recorded once (as regular snapshots) in order not to overestimate their density.
A2.5 JUVENA survey protocol (AZTI – Basque country, Spain)

A2.5.1 Sampling strategy

The JUVENA surveys are carried out annually between September and October in the Bay of Biscay (Table A2.3). In these months, the juveniles have grown to a size that makes them visible to the echosounders (allowing the tuna fishing fleet to target them as live bait) and normally occupy large outer- and off-shelf areas off the northern Spanish and western French coasts (Cort et al., 1976; Martín, 1989; Uriarte et al., 2001). Acoustic sampling is performed in daylight because, at this time of year, juveniles usually aggregate in schools in the upper layers of the water column during the day where they can be distinguished from plankton structures (Cort et al., 1976; Uriarte et al., 2001). Sampling is carried out following a regular grid formed by transects arranged perpendicular to the coast (Figure A2.4), spaced at 17.5 nautical miles (2003–2005) or 15 nautical miles (2006 onwards) to ensure their independence (Carrera et al., 2006). Sampling starts at the northern Spanish coast, going from west to east, and then moves to the north to cover the waters off the French coast of the Bay of Biscay. It is considered important to conduct the survey in the precise temporal window that extends from mid-August to mid-October, which is not too early for the juveniles to be detected and caught, and not so late that they have abandoned the offshore grounds.

Year	Start of survey	End of survey	No. of vessels	Effective survey time (d)	Fishing operations	Anchovy- positive fishing operations	Inter- transect distance (nautical miles)	Sampled area (nautical miles²)	Positive area (nautical miles²)
2003	18/09	14/10	1	20	47	36	17.5	16 829	3 476
2004	19/09	19/10	1	18	21	9	17.5	12 736	1 907
2005	12/09	07/10	2	20	47	45	17.5	25 176	7 790
2006	13/09	14/10	2	42	80	52	15	27 125	7 063
2007	04/09	30/09	2	37	70	40	15	23 116	5 677
2008	26/08	25/09	2	33	85	46	15	23 325	6 895
2009	26/08	25/09	2	39	67	42	15	34 585	12 984
2010	01/09	30/09	2	40	79	60	15	40 500	21 110
2011	01/09	05/10	2	40	77	64	15	37 500	21 025
2012	02/09	05/10	2	40	67	51	15	31 500	14 271

Table A2.3. Summary of sampling effort.

The survey is intended to cover the entire expected spatial distribution of juvenile anchovy in these months of the year in the Bay of Biscay (Figure A2.4), from offshore areas well beyond the continental shelf to very coastal waters, because the spatial process of anchovy juvenile recruitment occurs from offshore areas towards the coast during autumn (Uriarte *et al.*, 2001). This survey area can vary from year to year and is potentially large. Consequently, considerable effort is made to achieve the broadest possible coverage of the area by using an adaptive sampling strategy. In this strategy, the boundaries of the sampling area are defined according to the findings of each survey and to the parallel information obtained from the commercial fishing fleet, which uses juvenile anchovy as live bait for tuna fishing. Along the Spanish and French coastlines, the minimum limits of the sampling area are set at 5°W and 46°N, respectively. According to previous information on juvenile distribution, this area is expected to contain the vast majority of the juvenile anchovy stock (Cort et al., 1976; Uriarte et al., 2001; Carrera et al., 2006). This area is expanded according to the adaptive sampling strategy, but, for practical reasons, a maximum surveying area is set within the limits of 7°30'W and 48°N. Between these limits, the actual along-coastline boundaries were set each year at the points where there was a clear decrease in abundance or, if possible, a transect in which juvenile anchovy was not detected. The length of the transects extends from about the 20-m to at least the 1000-m isobaths, and, according to the adaptive scheme of the survey, if the detections continued, they were expanded offshore to 4 nautical miles beyond the last detection of an anchovy school. In addition, information from the commercial live-bait tuna fishery collected before and during each survey is taken into account in making decisions about survey sampling strategy. As a result of this sampling scheme, the years with higher abundance of anchovy typically require larger sampling coverage.



Figure A2.4. The current JUVENA default sampling design. The length of the transects and the actual western and northern boundaries of the sampling area change every year according to the adaptive sampling strategy.

In the period 2003–2004, the area was sampled with a single commercial purse-seiner subcontracted for the survey and equipped with scientific echosounders. In 2005, a second purse-seiner was added to the survey to provide extra fishing operations, and in 2006, a pelagic trawler with complete acoustic equipment, the RV "Emma Bardán", replaced the second purse-seiner (Table A2.4).

A2.5.2 Data acquisition

The acoustic equipment included Simrad EK60 split-beam echosounders (Kongsberg Simrad AS, Kongsberg, Norway) of 38 and 120 kHz from 2003 to 2006, plus a 200-kHz transducer from 2007 (Table A2.4). The transducers are installed looking vertically downwards, at a depth of ca. 2.5 m, at the end of a tube attached to the side of the

commercial fishing vessels and on the vessel hull of the research vessel. The transducers are calibrated using standard procedures (Foote, 1987).

The water column is sampled acoustically to a depth of 200 m. Catches from the fishing hauls and echotrace characteristics are used to identify fish species and determine the population size structure. The fishing stations are decided based on the typology on the detected echoes, trying to identify each change in the detected typologies. Purseseining was used to collect samples until 2005 and was then combined with pelagic trawls from 2006 onwards. To improve species identification in the first three surveys when only purse-seiners were available, additional night fishing operations were performed by focusing bright light on the water to attract fish from surrounding waters. In 2006, pelagic trawling was included in the surveys, which made it possible to fish at greater depths than the purse-seine range (50 m maximum). The purse-seiners generally cover the coastal areas and the waters off the shelf where juveniles occupy the surface waters and are accessible to the purse-seine fishing range. The pelagic trawler covers the intermediate shelf regions where it may be necessary to sample at all depths. In addition, when deep, anchovy-like aggregations are detected by the purse-seiners, the pelagic trawler may temporally leave its coverage area to carry out additional fishing operations in these areas.

Table A2.4. Vessels and equipment.

			Vessel 1	Vessel 2	
Vessel	Name Length (m) Side (m) Draft (m) Acoustic installation		Variable vessel*	"Emma Bardán"	
			30–35	27	
			8	7	
			3.5–4	3.5	
			Side perch	Hull	
Acoustic equipment	Transducer frequencies (kHz)		38, 120, (200)**	38, 120, 200	
	Power (for 38, 120, 200 kHz) (W)		1 200, 250, (210)**	1200, 250, 210	
	Pulse duration (10 ⁻ 6 s)		1 024	1 024 (except in 2006: 256)	
	Ping interval (s)		0.25–0.5		
Target strength (b20)***	Engraulis encrasicolus Sardina pilchardus Sprattus sprattus	–72.6 dB	Degnbol <i>et al.</i> (1985)		
	Trachurus trachurus Trachurus mediterraneus Scomber colias	–68.7 dB	ICES (2006b)		
Scomber scombrus		-88 dB	Clay and Castonguay (1996)		
	Jellyfish (mean TS)	–81.7 dB	Average TS for jellyfish species in Simmonds and MacLennan (2005)		

			Vessel 1	Vessel 2
Fishing gear	Pelagic trawl	No. of doors		2
		Vertical opening Mesh size (mm) Depth 75		15
				4
	Purse-seine			
		Perimeter	400	
		Mesh size (mm)	4	

* Purse-seiners: "Divino Jesus de Praga" (2003), "Nuevo Erreñezubi" (2004), "Mater Bi" (2005), "Gure Aita Joxe" (2005, 2008), "Itsas Lagunak" (2006, 2007, 2009, 2010, and 2011); pelagic trawler: RV "Ramón Margalef" (2012).

** The 200-kHz transducer has been available on board purse-seiners since 2007.

*** TS of the mean pelagic species. The TS is obtained according to the relationship $TS = b_{20} - 20\log(L)$, where L is the standard length of the fish (in cm).

Since the 2006 survey, when the acoustic sampling was split between two vessels, intercalibration exercises between the two vessels have been carried out routinely each year, based on the intercalibration methodology described by Simmonds and MacLennan (2005). The data are collected by two vessels advancing side by side ca. 100 m apart. The intercalibration process consists of comparing the echo integration of the bottom echo in areas of a smoothly variable seabed.

A minimum distance of 30 nautical miles is covered simultaneously by the two vessels for these exercises. The ratio of NASC (MacLennan *et al.*, 2002) values per ESDU between vessels is calculated. Initially, layer echo integration of both water column and bottom echo obtained by the two vessels were compared, but preliminary results indicated that, while both target types provided similar ratios, the bottom echo provided much less variability than the water column. Therefore, the bottom echo was chosen as the reference target and the rest of the analysis is based on bottom echo NASC values. The intercalibration factor F_{int} was estimated as the median of the NASC ratios R_i .

$$F_{int} = Median(R_i) \tag{A2.28}$$

The median was chosen as a robust alternative to the average, given the high number of outliers of the data (because the transducers did not point to the exact same targets).

As a robust standard deviation, the median absolute deviation (MAD; Hoaglin *et al.*, 1983) was calculated, based on the deviation of individual data points from the median of the population:

$$MAD_{int} = [Median(|R_i - F_{int}|)] \times 1.4826$$
(A2.29)

The value 1.4826 is a constant factor that adjusts the resulting robust value to the equivalent of a normal population distribution. Thus, for a normally distributed population, the s.d. and the MAD should be equal. In accordance to this, a robust *CV* was calculated as:

$$CV_{int} = MAD_{int}/F_{int}$$
 (A2.30)

A2.5.4 Acoustic abundance estimation

Echograms are examined visually with the aid of the species composition of catches to identify positive anchovy layers. Noise from bubbles, double echoes, and, when necessary, plankton are removed from the echograms. Acoustic data are processed in the positive strata by layer echo integration, using an ESDU of 0.1 nautical mile with the Movies+ software (IFREMER, France). Echoes are thresholded to –60 dB and integrated into six depth channels: 7.5–15, 15–25, 25–35, 35–45, 45–70, and 70–120 m (no anchovies have been found below 120 m).

Generally, only the 38-kHz data are echo integrated using the TS–length relationships agreed in the ICES WGACEGG for the main species (ICES, 2006b; Table A2.4). Each fishing haul is classified by species. A random sample of each species is measured to determine the length frequency distribution of the different species, in 0.5-cm classes for the smaller species (anchovy and sardine) and 1-cm classes for the rest. Complete biological sampling of anchovy is performed to analyse age, size, and the size–weight ratio. The hauls are grouped by strata of homogeneous species and size composition.

The species and size composition of each homogeneous stratum are obtained by averaging the composition (in numbers) of the individual hauls contained in the stratum weighted by the acoustic density in the vicinity (2-nautical-mile diameter). This species and size composition of each stratum is used to obtain the mixed species echo integrator conversion factor (Simmonds and MacLennan, 2005) for converting the NASC values of each ESDU into numbers of each species. However, although the methodology involves estimating multiple species, the survey strategy is strongly focused on juvenile anchovy, and only the positive areas for anchovy are processed. Therefore, only estimates of this species are considered reliable and thus produced.

Each fish species has a different acoustic response, defined by its scattering cross section that measures the amount of the acoustic energy incident to the target that is scattered backwards. This scattering cross section depends on species *i* and the size of the target *j*, according to:

$$\sigma_{ii} = 10^{TS_{ij}/10} = 10^{\{(a_i + b_i \log L_j)/10\}}$$
(A2.31)

Here, *TS* stands for "target strength", L_j represents the size class, and the constants a_i and b_i are determined empirically for each species. For anchovy, we have used the following *TS*–length relationship:

$$TS_i = -72.6 + 20 \log L_i$$
 (A2.32)

The composition by size and species of each homogeneous stratum is obtained by averaging the composition of the individual hauls contained in the stratum, with the contribution of each haul weighted to the acoustic energy found in its vicinity (2-nautical-mile diameter). Thus, given a homogeneous stratum with M hauls, if E_k is the mean echo-integrated acoustic backscattering in the vicinity of the haul k, w_i , the proportion of species i in the total capture of the stratum, is calculated as follows:

$$w_{i} = \sum_{j} w_{ij} = \sum_{j} \left(\frac{\sum_{k=1}^{M} \left(q_{ijk} \cdot E_{k} \right)}{\sum_{k=1}^{M} E_{k}} \right)$$
(A2.33)

where q_{ijk} is the quantity of species *i* and length *j* in haul *k*, and Q_k is the total quantity of any species and size in haul *k*.

In order to distinguish their own contribution, anchovy juveniles and adults are separated and treated as different species. To this end, the proportion of anchovy in the hauls of each stratum w_{ij} is multiplied by an age–length key to separate the proportion of adults and juveniles. Thus, individual w_i are obtained for each.

Inside each homogeneous stratum, a mean scattering cross section was calculated for each species by means of the size distribution of such species obtained in the hauls of the stratum:

$$\langle \sigma_i \rangle = \frac{\sum_{j} W_{ij} \sigma_{ij}}{W_i}$$
 (A2.34)

Let S_A be the calibration-corrected, echo-integrated energy by ESDU (0.1 nautical mile). The mean echo-integrated acoustic backscattering in each homogeneous stratum, $E_m = \langle s_A \rangle$, is divided in terms of the size-species composition of the haul of the stratum. Thus, the echo-integrated backscattering attributed to each species *i*, *E*_{*i*}, is calculated as:

$$E_{i} = \frac{w_{i} \langle \sigma_{i} \rangle E_{m}}{\left(\sum_{i} w_{i} \langle \sigma_{i} \rangle\right)}$$
(A2.35)

Here, the term inside the parenthesis sums over all the species in the stratum. Finally, the number of individuals *F*_i of each species is calculated as:

$$F_{i} = H \cdot l \frac{E_{i}}{\langle \sigma_{i} \rangle} \tag{A2.36}$$

where l is the length of the transect or semi-transect under the influence of the stratum and H is the distance between transects (ca. 15 nautical miles). To convert the number of juveniles to biomass, the size–length ratio obtained in each stratum is applied to obtain the average weight of the juveniles in the stratum:

$$\langle W_i \rangle = a \cdot \langle L_i \rangle^b$$
 (A2.37)

Thus, the biomass is obtained by multiplying F_i by $\langle W_i \rangle$.

Anchovy juveniles (age 0) and adults (age \geq 1) are separated and treated as different species. To separate juveniles from adults, the anchovy length frequency distribution by haul is multiplied by a corresponding age–length key. The key is determined every year for three broad areas: pure juvenile area, mixed juvenile area (with a mix of juveniles and adults), and Garonne River area (located at the coastal area around 45°40′N; also a mixed area, but adult anchovy here are usually smaller than in the other areas). Finally, the abundance in numbers of juveniles and adults is multiplied by the mean weight to obtain the biomass per age and length class for each ESDU.

A2.5.5 Near-field correction

Juvenile anchovies exhibit a near-surface distribution during the JUVENA survey period (Uriarte *et al.*, 2001; Boyra *et al.*, 2013). In order to achieve the best possible coverage of the vertical distribution of this species, and after comparing two different methods (Boyra *et al.*, 2013), a methodology was proposed to deal with the presence of juvenile anchovy aggregations inside the near-field range of the Simrad EK60 38-kHz transducers (10.5 m; Simmonds and MacLennan, 2005). The method consisted of measuring and correcting the bias produced when echo integrating fish schools inside the near-field (5–11 m range from the transducer face, i.e. ca. 7.5–13 m below the surface), instead of deleting the entire layer. The reason for occasionally integrating the 7.5–

13-m depth layer (and thus not following the standard procedure) is that it is considered important to retrieve as much information as possible about abundance of juvenile anchovy, a species that largely occupies the top metres of the water column.

To estimate the near-field bias, we selected a set of the cleanest possible 38-kHz echograms, i.e. with the least presence of plankton in the uppermost layers (7.5–13-m depth). From these areas relatively free of plankton, five acoustic records of ca. 5 min duration each were selected for each year and vessel (Figure A2.5). The idea was to try to characterize the "rainbow" pattern visible in the first layers of these echograms (visible because the echograms are displayed with a threshold of -80 dB), but not accounting for the noise produced by the plankton. For this purpose, the backscatter was echo integrated at -100 dB threshold by a single ping in the 7.5–13-m layer. As the records were not completely free of plankton, a filtering procedure was applied to

clean it as much as possible. The echo-integrated pings of each file were divided into four groups per minute (about 50 pings per group). Inside each group, the 25th percentile was computed for each depth layer to characterize the purest possible nearfield reverberation, free from plankton backscattering, for each group. The resulting echo-integration values per layer were further averaged using the mean value for all records. This estimation of "clean water" backscatter at 7.5–13 m was used as a proxy for the near-field and was subtracted from the fish NASC values of the uppermost echo integration channel (7.5–15 m). As the filtering procedure could not completely remove the plankton backscatter, some quantity was included in the near-field estimates and thus subtracted from the fish backscatter of the first layers. Thus, this correction procedure of the near-field is considered to be slightly conservative.

A2.5.6 Recruitment predictive capability

In order to estimate the recruitment predictive capability, the annual biomass estimates for anchovy juveniles are compared with the estimates of anchovy recruitment the following year. The recruitment is the biomass of age 1 anchovy in January of the following year, estimated according to the ICES assessment using a Bayesian model with inputs from catches and biomass estimates of two spring surveys: an acoustic one (PELGAS) conducted by IFREMER, and a survey based on DEPM (BIOMAN), conducted by AZTI (ICES, 2011a). The biomass estimates are fitted by linear, log-linear, and rank regressions between variables using the R software version 2.14.0 (R Development Core Team, 2011).



Figure A2.5. Sample echograms of the chosen low plankton areas for the near-field characterization. The threshold is set at -80 dB, the same as used during the visual inspection for the selection of these areas. The process tries to characterize the "rainbow" pattern at the top part of each echogram by filtering the overlapping "noise" produced by marine organisms, wave-induced bubbles, etc.

A2.6 Top predator sighting protocol (UMS PELAGIS)

A2.6.1 General method

Data on top predators (seabirds and cetaceans) are collected along transects over the continental shelf of the Bay of Biscay by ship-based surveys conducted between 2003 and 2012. These ship-based surveys involved the use of the RV "Thalassa" during the PELGAS, PELACUS, and EVHOE surveys. The general method used is a single-platform, line-transect survey.

A2.6.2 Method of data acquisition

Top predator sightings are recorded all day during the observation leg. An observation leg is a segment with the same sighting conditions, observers, bearing, and speed. If any one of these parameters changes, the observation leg changes. All sightings collected are linked to the observation leg concerned. During the observation leg, data on weather and sighting conditions are recorded, including sea state, windspeed and -direction, swell, glare severity, cloud cover, and an index of the subjective conditions (good, moderate, or poor) estimated to detect a small cetacean.

Two observers are placed 16 m above sea level (upper bridge of the ship, see Figure A2.6). Ship speed is maintained at 10–12 knots during the observation leg. Two observers search for cetaceans and seabirds within an angle of 180° ahead of the bow and are replaced every two hours.

They search with naked eye close to the ship (out to 1000 m and less if sighting conditions are moderate or poor). For each sighting, number, species, and time (UTC) are recorded, and the distance and angle are estimated by eye and with a stick and angleboard. Additional data collected from each detected group of cetaceans or birds include age for birds (adult, juvenile, immature), behaviour (attracting, flying, sitting, feeding – for birds; swimming, logging, attracting, feeding – for cetaceans). Data on litter (macro size more than 30 cm), large pelagic fishes (shark, sunfish, swordfish, tuna,...), turtles, and boats (fishing, sailing, and commercial) are collected during the observation leg.

The GPS positions are provided by CASINO and the link with the data location is performed each day at UTC time.

For every change in the observation leg or activity of the research vessel (trawling), the birds (scavengers) following the vessel are recorded (number and species composition).



Figure A2.6. Diagram showing the placement of two observers on the upper bridge of the vessel and their fields of vision while searching for seabirds and cetaceans on board the RV "Thalassa" during the PELGAS, PELACUS, and EVHOE surveys.

7 Abbreviations and acronyms

AcousMed	European project – Harmonization of the acoustic data in the Mediterranean 2002–2006				
AIC	Akaike's Information Criterion, used to compare statistical models				
ALK	Age-length key				
AZTI	Food and Fisheries Technological Institute / Instituto Tecnológico Pesquero y Alimentario				
CalCOFI	California Cooperative Oceanic Fisheries Investigation				
CalVET	The CalCOFI vertical egg tow net				
CDF	Cumulative distribution functions				
CFP	EU's Common Fisheries Policy				
CLUSTER	Aggregation patterns of commercial fish species under different stock situations and their impact on exploitation and assessment; FAIR-CT-96.1799				
CRMM	Centre de Recherche sur les Mammifères Marins (now PELAGIS)				
CTD	An oceanographic instrument of conductivity, temperature, and depth recording in vertical water tows				
CTDF	a CTD that includes a fluorescence recording device				
TF	An oceanographic instrument of conductivity, temperature, and fluorescence recording, usually coupled to CUFES				
CUFES	Continuous underway fish egg sampler				
CV	Coefficient of variation				
DCF	EU Data Collection Format				
DCR	EU Data Collection Resolution				
DEPM	Daily egg production method, applied in surveys to assess the SSB of pelagic fishes				
DETAC	A project on the development of acoustic techniques, run by the IEO				
DF	Daily fecundity				
EAFM	Ecosystem approach to fisheries management				
ECOCADIZ					
	Spanish acoustic survey series				
EDAS	Spanish acoustic survey series EGNOS Data Access Service, a terrestrial data service that can provide raw GPS data collected by the EGNOS monitoring reference network				
EDAS EPM	Spanish acoustic survey series EGNOS Data Access Service, a terrestrial data service that can provide raw GPS data collected by the EGNOS monitoring reference network Egg production method				
EDAS EPM ESAS	Spanish acoustic survey series EGNOS Data Access Service, a terrestrial data service that can provide raw GPS data collected by the EGNOS monitoring reference network Egg production method European Seabirds at Sea				
EDAS EPM ESAS ESDU	Spanish acoustic survey series EGNOS Data Access Service, a terrestrial data service that can provide raw GPS data collected by the EGNOS monitoring reference network Egg production method European Seabirds at Sea Elementary samling distance unit				
EDAS EPM ESAS ESDU FlowCAM	Spanish acoustic survey series EGNOS Data Access Service, a terrestrial data service that can provide raw GPS data collected by the EGNOS monitoring reference network Egg production method European Seabirds at Sea Elementary samling distance unit Brand of Fluid Imaging Technologies, Inc. for dynamic imaging particle analysis				

GLM	Generalized linear model						
GLOBEC	Global Ocean Ecosystem Dynamics Project						
HAC	Hydroacoustic data format						
ICTIOEVA	Egg Production Method Project						
IEO	Instituto Español de Oceanografía (Spanish Institute of Oceanography)						
IFREMER	Institut français de recherche pour l'exploitation de la mer						
INIAP,							
INIP, INRP,							
IPIMAR	These are old acronyms for the Portuguese national research institute that is now called IPMA						
IPMA	Instituto Português do Mar e da Atmosfera						
IOC	Intergovernmental Oceanographic Commission						
IREX	International Recruitment Experiment						
JUVENA	A series of autumn acoustic surveys in the Bay of Biscay						
LFD	Length frequency distributions						
LOPC	Laser Optical Plankton Counter						
MAD	Median absolute derivation						
MBA	Marine Biological Association, Plymouth, UK						
MODIS	Moderate resolution imaging spectroradiometer						
MSFD	EU's Marine Strategy Framework Directive						
NASC	Nautical area scattering coefficient						
OCN	Occidental North						
OCS	Occidental South						
P0	Daily egg production (number of eggs produced daily per sea surface unit)						
PACAS	Autumn acoustic survey (Spain)						
PairoVET	Paired (two-ring) vertical egg nets launched jointly (in parallel, usually twin CalVETs)						
PDF	Probability density functions						
PELACUS	Spring acoustic survey (Spain)						
PELAGAS	Spring acoustic survey (France)						
PELAGIS	Observatory systems for the conservation of marine mammals and birds (La Rochelle, France)						
PELAGO	Spring acoustic survey (Portugal)						
PELASSES	EU project: Direct abundance estimation and distribution of pelagic fish species in Northeast Atlantic waters. Improving acoustic and daily egg production						

PELCOSAT	Biology and fishing eco-ethology of pelagic fish species in the Gulf of Cádiz		
PELTIC	Pelagic ecosystem survey in the western English Channel and eastern Celtic Sea		
PGAAM	ICES Planning Group on Aerial and Acoustic Surveys for Mackerel		
PGPAS	ICES Planning Group for Acoustic Surveys		
POF	Post-ovulatory follicle		
Ptot	Total daily egg production (the integral of P ₀ over the sampling area)		
RUWPA	Mathematical Institute, University of St. Andrews, UK		
SA	Acoustic area backscattering coefficient		
SAR	Synthetic aperture radar		
SARACUS	Spanish sardine acoustic survey		
SARDYN	EU project: Sardine Dynamics and Stock Structure in the Northeastern Atlantic		
SARP	Sardine and Anchovy Recruitment Process		
SGPM	General Secretariat for Maritime Fisheries		
SGRESP	ICES Study Group on Regional Scale Ecology of Small Pelagic Fish		
SGSBSA	ICES Study Group on the Estimation of Sardine and Anchovy Spawning Biomass		
SPEA	Sociedade Portuguesa para o Estudo das Aves		
SPVS	Sociedade de Pesquisa em Vida Selvagem e Educação Ambiental		
SSB	Spawning-stock biomass		
SSF	Sea surface fluorescence		
SSL	Sound scattering layer		
SSS	Sea surface salinity		
SST	Sea surface temperature		
sV	Volume backscattering coefficient		
TCF	Temperature-corrected fluorescence provided from CTDs		
TOR	Terms of reference		
TS	Target strength		
WGFAST	ICES Working Group on Fisheries Acoustics Science and Technology		
WGACEGG	ICES Working Group on Acoustic and Egg Surveys for Sardine and Anchovy in ICES Subareas 7, 8, and 9		
WGANC	ICES Working Group on the Anchovy, existing in 2008		
WGANSA	ICES Working Group on Anchovy and Sardine, existing in 2009–2011		
WGHANSA	ICES Working Group on Southern Horse Mackerel, Anchovy and Sardine, existing since 2012		

- **WGMHSA** ICES/GLOBEC Working Group on the Assessment of Mackerel, Horse Mackerel, Sardine and Anchovy, existing until 2007
- WGLESP ICES Working Group on Life Cycle and Ecology of Small Pelagic Fish
- WGWIDE ICES Working Group on Widely Distributed Species
- WKACUGEO ICES Workshop on Geostatistics
- WKPELA ICES Benchmark Workshop on Pelagic Stocks
- **WKRESTIM** ICES Workshop on the Estimation of DEPM-based Spawning Stock Biomass of Sardine and Anchovy using R (established in 2009)

8 FAO 3-Alpha Species Codes (ASFIS) (for the most frequent species in this report)

The colour coding used in figures for the six most common species is shown.

Species scientific name	Common name	ASFIS code	Colour used in figures
Engraulis encrasicolus	anchovy	ANE	
Boops boops	bogue	BOG	
Capros aper	boar fish	BOC	
Trachurus picturatus	blue jack mackerel	JAA	
Trachurus trachurus	horse mackerel	НОМ	
Trachurus mediterraneus	Mediterranean horse mackerel	HMM	
Scomber scombrus	mackerel	MAC	
Scomber colias	chub mackerel	MAS*	
Sardina pilchardus	sardine	PIL	
Macroramphosus spp.	snipefishes	SNS	
Sprattus sprattus	sprat	SPR	
Micromesistius poutassou	blue whiting	WHB	
Merluccius merluccius	hake	НКЕ	
Scomberesox saurus	Atlantic saury	SAU	

* MAS actually corresponds to *Scomber japonicus*, not to *Scomber colias*. After 2010, *S. colias* and *S. japonicus* were accepted as being two different species, with VMA being the official FAO code of *Scomber colias*. However, in this report the old code name MAS has been retained because prior to 2010 *S. japonicus* and *S. colias* were considered to be the same species (and both species were thus labelled MAS).

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