Living Resources Committee **ICES CM 2002/G:01**

REPORT OF THE

STUDY GROUP ON THE ESTIMATION OF SPAWNING STOCK BIOMASS OF SARDINE AND ANCHOVY

Lisbon, Portugal
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International Council for the Exploration of the Sea

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1 INTRODUCTION

1.1 Terms of Reference

At the ICES Annual Conference in Brugge, Belgium in October 2000 (88th Statutory meeting) it was decided (C. Res. 2000/2G10) that a Study Group on Estimation of Spawning Stock Biomass of Sardine and Anchovy (SGSBSA) would be established (Chair: Yorgos Stratoudakis) and would meet for the first time in Lisbon from 22-25 October 2001 to:

a) design the sardine and anchovy DEPM surveys for spring 2002;

b) standardise all methodologies within DEPM, with particular attention on the estimation of spawning fraction;

c) decide the most appropriate adult sampling design in cases of anticipated large regional differences in abundance and fish reproductive properties;

d) analyse the feasibility of using the continuous underway fish egg sampler (CUFES) to improve DEPM estimates.

1.2 Participants

The Study Group met in Lisbon (Portugal) from 22-25 October 2001 with the following participation:

Bernal, Miguel	Spain
Carrera, Pablo	Spain
Cunha, Emilia	Portugal
Franco, Concha	Spain
Gaughan, Daniel (observer)	Australia
Hunter, John (observer)	USA
Ibaibarriaga, Leire	Spain
Jimenes, Mari Paz	Spain
Lago de Lanzós, Ana	Spain
Lonergan, Mike (observer)	UK
Lopes, Placida	Portugal
Martin, Inmaculada (observer)	Spain
Pérez, José Ramón	Spain
Quintanilla, Luis	Spain
Santos, Maria	Spain
Silva, Alexandra	Portugal
Soares, Eduardo	Portugal
Stratoudakis, Yorgos (Chair)	Portugal
Uriarte, Andres	Spain
Zwolinski, Juan (observer)	Portugal

1.3 Report on progress with respect to recommendations of WKSBS

Of the 7 recommendations related to sardine DEPM that resulted from the Vigo Workshop (ICES 2000a), 5 have advanced considerably before or during the Study Group (SG) meeting. The timing of the first SG meeting was set in order to serve as the planning group for the 2002 DEPM surveys (recommendation 1 of WKSBS). Ongoing work in Portugal is comparing the macroscopic and microscopic maturation scales for sardine (recommendation 2). Reference collection of sardine and anchovy egg stages and post-ovulatory follicles (POFs) were created during the meeting, while an inter-calibration exercise among experienced egg and histology readers was performed (recommendations 3 and 5). Finally, creation of a common database with all DEPM information for each species is underway as part of a EU (Study 99/080) research project (recommendation 6).

The main issues that have not been addressed to date are the laboratory experiments for egg incubation (recommendation 4) and validation of POF ageing criteria (recommendation 5) and the revision of available biological data in order to provide a definite decision on the most appropriate DEPM survey timing for sardine (recommendation 7). New work on stage-age modelling has been presented during the SG and a series of important issues related to egg

incubation experiments were highlighted. Finally, the retrieval and analysis of historical biological information is underway, but results were not available to permit final decision on sardine DEPM survey timing. It was accepted that the timing of the 2002 surveys will be the same as that of 1999 and that during the next SG meeting a final decision on this issue will be reached.

2 DEPM SURVEYS IN EUROPEAN WATERS

2.1 Sardine (*Sardina pilchardus*)

2.1.1 Atlantic waters (Iberian peninsula)

The DEPM was first used to estimate the spawning biomass of Atlanto-Iberian sardine during the spawning season of 1988 (Cunha *et al*. 1992; Garcia *et al.* 1992). Since the beginning of the studies it was recognised that the surveys should cover both the Portuguese and Spanish areas of sardine distribution because it was believed the stock was common. However, only during the 1997 and 1999 surveys the entire continental shelf of the Atlantic-waters of the Iberian Peninsula was surveyed (Figure 2.1.1.1). In 1990 there was no survey in the Portuguese coast and in 1988 the Bay of Cadiz was not sampled. A detailed review of the design, sampling and estimation aspects of the 1999 DEPM surveys are provided in the report of the Vigo Workshop (ICES 2000a). Table 2.1.1.1 summarises the main characteristics of the surveys. The parameter estimates necessary to calculate spawning biomass by DEPM are presented for each survey in Table 2.1.1.2. Detailed information on the values obtained to estimate daily egg production and daily specific fecundity for different geographical areas around the Atlantic waters of the Iberian Peninsula during the 1988 are presented in Tables 2.1.1.3 and 2.1.1.4, while Tables 2.1.1.5 and 2.1.1.6 show the same information for 1999. It should be noted however that post-stratification has only been applied in the early Spanish surveys.

2.1.2 Mediterranean waters

The DEPM was recently applied to estimate the spawning biomass of sardine in the central Aegean and Ionian Seas (Somarakis *et al.* 2001 – WD SGSBSA). This was the first reported application of the DEPM to Mediterranean sardine, and presented particular interest and difficulties due to the peculiar topography of the survey area (many small-sized semi-enclosed gulfs), the biological heterogeneity and the small size of the sardine populations. Table 2.1.2.1 shows the spawning biomass and DEPM parameters estimates. Since the stocks of sardine in the central Aegean and Ionian Seas exhibited different spawning peaks, the survey area was geographically stratified.

Daily egg production was estimated using both eggs and yolk-sac larvae in order to improve the precision of the estimate. Batch fecundity was measured in hydrated, tertiary-yolk globule and migratory nucleus females because in Mediterranean sardine exist a well-defined hiatus between the advanced batch and the stock of smaller oocytes in the tertiary yolk-globule stage. The histological examination and comparative analysis of sardine follicles revealed 3 classes of POFs: day-0 (0-9.5 hrs), day-1 (24-33.5 hrs) and day-2 (48-57.5 hrs). Despite differences in season and temperature regimes, batch fecundity and spawning fraction estimates from the Aegean and Ionian Sea were similar. Compared to existing values for Atlantic sardine stocks (Table 2.1.2.2), the estimates of spawning fraction and relative fecundity were slightly lower in the Mediterranean, despite considerable differences in mean female weight between Mediterranean and Atlantic populations.

2.2 Anchovy (*Engraulis encrasicolus*)

2.2.1 Atlantic Waters (Bay of Biscay)

The DEPM has been regularly applied to the Bay of Biscay anchovy to estimate its biomass and population at age, in numbers (Santiago *et al.* 1991, Motos *et al.* 1995, Uriarte *et al.* 2000). The series of DEPM estimates spans the period 1987-2001 (with a single gap in 1993) and is routinely used by ICES for the assessment of the Bay of Biscay anchovy stock (WGMHSA). These surveys have been undertaken by AZTI in cooperation with the Spanish (IEO) and French (IFREMER) institutes of marine research.

In order to obtain estimates of daily egg production and specific fecundity, two surveys (an egg and an adult cruise) have usually been carried out at peak spawning time (May/June) over the expected spawning area of the Bay of Biscay anchovy population. This area extends over the Southeast area of the Bay of Biscay, with limits at 5ºW in the Iberian Coast and at 47ºN in the French Coast (see Figure 2.2.1.1). Adult sampling during the survey is usually complemented with samples taken opportunistically on board the Spanish and French commercial fishing fleets of purse seiners and pelagic trawlers. In 1987, 1988, 1996, 1999 and 2000 no adult surveys took place and in those cases the opportunistic sampling obtained through the commercial fleet were the only source for adult information: in the first two years,

commercial samples were used to derive the daily fecundity estimates of the population, whereas in the latter three years those samples were not used at all and a regression method assuming constant daily fecundity were used instead (see below). The total set of DEPM estimates is presented in Tables 2.2.1.1 and 2.2.1.2.

Egg sampling is based on the CalVET net (Smith *et al.* 1985) and follows a systematic central sampling scheme. Eggs from both CalVET samplers are used in the analysis (Uriarte and Motos 1998), often giving rise to the term PAIROVET to distinguish from applications where only one CalVET sampler is used. Samples are taken 3 miles apart from each other along radials perpendicular to shore spaced 15 miles (45 square miles sampling units). Offshore edge of the spawning area is considered to be reached by three consecutive samples with 0 anchovy eggs. All eggs are sorted and a sub-sample is staged according to Moser and Alhstrom (1985). Traditional stage to age conversion (Lo 1985) is used, with parameters from an incubation experiment of eggs of the Bay of Biscay anchovy (Motos 1994). The daily egg production over the positive spawning area is estimated following the exponential mortality model and procedures described in Piquelle and Stauffer (1985).

From the whole set of adult samples gathered during the adult survey, a subset is chosen for final processing with the criterion of the capture date being within ±5 days of the egg sampling in the same area. The opportunistic adult samples from the fleet permit to expand the area of sampling coverage. In general, a broad spatial structure is evident in the adult population, with smaller fish tending to be closer to the shore. This leads generally to post-stratification of the DEPM estimation procedure (for P0 and adult parameters), with two or three spatial strata being defined according to depth. Adult parameters are unweighted averages of the strata. An extensive review and description of DEPM adult parameter estimation was presented in the EU Project 96/034 (Uriarte *et al.* 1999). Sex ratio is assumed 1:1 in numbers since 1994 with satisfactory checking during recent years. Standard regression estimate of batch fecundity is applied (Hunter *et al.* 1985, Santz and Uriarte 1992). Spawning fraction of mature females was calculated by the standard procedure (Hunter and Macewicz 1985, Motos 1996) till 1992 based on day 1 Post-Ovulatory Follicles (POFs). Since 1994 the adapted methodology of Motos and Uriarte (2001 – WD SGSBSA) based both on Day 1 and 2 POFs has been used instead. Finally, the DEPM formulation has been extended to provide spawning stock population at age (SSPa) estimates with variances inferred from the delta method (Uriarte 2001). Sensitivity analyses on the influence of the stratification and weighting factors are routinely made. Regression methods for the estimation of SSB in the absence of adult sampling have been applied since 1996 (Uriarte et al, 1999) based on the relationships between spawning area, daily egg production per unit surface and the biomass obtained in years where complete DEPM is applied.

2.2.2 Mediterranean waters

The DEPM has been used to evaluate the anchovy spawning biomass of the Catalan Sea in 1990 (Palomera and Pertierra 1993), Catalan Sea-Gulf of Lions in 1993 and 1994 (García and Palomera 1996), Ligurian-North Tyrrhenian seas in 1993 (García and Palomera 1996), Aegean sea in 1993 (Tsimenides *et al.* 1995) and 1999 (Somarakis *et al.* 2001), Ionian Sea in 1999 (Somarakis *et al.* 2001), south-western Adriatic sea in 1994 (Casavol, 1998), and Sicilian Channel in 1998, 1999 (Quintanilla and García 2001) and 2000 (Quintanilla, pers. comm.).

Spawning biomass and DEPM parameters estimates in the Mediterranean show high variability both within (inter-seasonal and inter-annual variations) and between regions (Table 2.2.2.1). Different methodologies can partially explain these variations. In the Aegean Sea, where exceptionally high egg production estimates were found in 1993, oblique Bongo tows were used instead of the vertical CalVET tows and the spawning area was not entirely covered. Also, different temperatures were used to assign ages to eggs in different regions (sub-surface temperature in the Aegean Sea, mean temperature of the first 10 m or 20 m in the Sicilian Channel and Catalan Sea respectively). Sampling with purse seiners instead of pelagic trawls, and commercial instead of research vessels in the Aegean and Catalan Seas, could restrict the sampling to commercial fishing grounds and explain some adult parameter differences. The use of the methodology of Laroche and Richardson (1980) instead of the hydrated oocyte method (Hunter *et al.*, 1985) may explain the high relative fecundity in the Catalan Sea. Differences in spawning fraction estimation method could also explain some inter-regional differences in this parameter.

Overall, the parameters with the highest variance are the Daily Egg Production (*P*) and the Spawning Fraction (*S*), while the large variation in egg mortality rates should also be noted. The temperature range during the peak spawning period may vary from 16° to 25°C in some Mediterranean areas. Egg development duration and post-ovulatory follicle degeneration can present great differences within this temperature range, thus affecting these parameter estimates.

2.3 DEPM estimates in assessment

The spawning biomass estimated by DEPM for the Atlanto-Iberian stock of sardine was used for the first time in 1998 as an absolute index of abundance to tune sardine assessment (WGMHSA). In the following year, the WG explored further the sensitivity of the assessment model (ICA) to the inclusion of this index and to the assumptions of a relative

or an absolute relation with the stock biomass (ICES 2000b). A similar effect in model fit and estimates was obtained either excluding the index or using it as a relative estimator of abundance, while the historical perception of the stock biomass (the balance in SSB between the 80s and the 90s) was more consistent with other sources of information (acoustic surveys and commercial catches) when the DEPM index was used as an absolute estimator. Sensitivity analysis carried out more recently provided similar results and the use of SSB from DEPM as an absolute estimator has been adopted. However, the assessment WG recognises several arguments against the current use of DEPM estimates in the assessment model (few years with estimates, large variance associated with the estimates, differences in area or time coverage between surveys and uncertainties in the sampling design and estimation for adult parameters) and the assumption of an absolute relation with the stock biomass. A thorough revision of the current procedure will be carried out when the 2002 DEPM estimates are made available and the revision of past DEPM estimates is completed.

Anchovy is a short living species for which traditional assessment methods such as VPA are not recommended. The assessment of the status of anchovy-like stocks relies heavily on the knowledge of the absolute level of the stock by direct methods. Since 1988 ICES is required by the European Commission to provide advise on the status of the Bay of Biscay anchovy and on catch options within safe biological limits for the following years. Since then, the assessment performed at ICES has been improving by the provision of the daily egg production and acoustic estimates of biomass by France and Spain. The DEPM biomass and population numbers at age estimates have been the basis of the analytical assessments carried out in the ICES assessment working group during the last years because of the longer set of data (1987-2000) and the absolute values provided by the DEPM method, compared with the acoustic series. In 1999 the decrease of anchovy biomass predicted for year 2000 led the European Council to halve the TAC for this species in the Bay of Biscay and provisions were made for a review of the management rule according to new scientific estimates of the biomass levels during the spawning time. The estimations produced independently by the DEPM and the acoustic survey in 2000, both suggested higher biomass levels than expected, allowing an upward revision of the TAC (in agreement with the estimates from the surveys).

Table 2.1.1.1: Summary of the sardine DEPM surveys off the Atlantic waters of the Iberian peninsula since 1988.

Date of survey	March 1988	April 1988	April 1990	March 1997	April 1997	January 1999	April 1999
Area of survey	Portugal	Spain	Spain	Portugal + Gulf of Cadiz	Spain	Portugal + Gulf of Cadiz	Spain
Type of haul	Vertical	Vertical	Vertical	Vertical	Vertical	Vertical	Vertical
Maximum sampling depth	100	100	100	150	100	150	100
Sampler	CalVET	CalVET	CalVET	CalVET	CalVET	CalVET	CalVET
Mesh size	200	100	100	150	100	150	100
Sampling grid (miles)	7x7	6x6	6x6	7x7.5	7.5x3	6x6	6x3
Number of plankton stations	309	524	475	373	462	417	290
Number of vessels involved	1	1	2	2	2	2	2
Number of positive fishing stations	17	44		24	30	40	32

Table 2.1.1.2: Parameter estimates (coefficient of variation in brackets) for the three Portuguese and four Spanish DEPM surveys. Parameter estimates for 1988 from Cunha *et al* (1992), egg production for 1997 from Cunha *et al.* (1997) and adult parameters from Gordo *et al.* (1999). Parameter estimates for 1988 from Garcia *et al.* (1992), for 1990 from Garcia *et al.* (1991), from 1997 from Lago de Lanzós *et al.* (1998), egg production from 1990 from Lago de Lanzós *et al.* (1999).

Portugal

	March 1988*	March 1997	January 1999
Egg production (eggs10^{-12})	2.87 (22)	4.41 (49)	5.24 (35)
Female weight (g)	40.94 (6)	41.28 (5)	44.42 (5)
Sex ratio	0.52 (11)		0.61 (22)
Batch fecundity	15 581 (8)	17 914 (3)	18 416 (5)
Spawning fraction	0.13 (18)	0.13 (19)	0.10 (15)
Spawning biomass (Kt)	115.1 (34)	127.2 (57)	205.1 (39)

Spain

	April 1988	April 1990	April 1997**	April 1999**
Egg production (eggs10^{-12})		1.78 (58)	0.72 (82)	0.34 (44)
Female weight (g)		-	70.05 (6)	66.03 (41)
Sex ratio			0.52 (11)	0.55 (45)
Batch fecundity			26 563 (5)	21 800 (12)
Spawning fraction			0.18 (15)	-
Spawning biomass (Kt)	180.2 (50)	77.7 (50)	20.7 (84)	10.4 (77)***

* Estimates do not include the Gulf of Cadiz
** Adult parameters correspond to the values obtained in Region III
*** Spawning biomass calculated using the spawning fraction obtained in 1997

Table 2.1.1.3: Parameters for estimation of daily egg production for the 1988 DEPM surveys by region. See Cunha *et al.* 1992 and Garcia *et al.* 1992 for limit of regions.

	A_0 (km^2) [n]	A_1 (km^2) [n]	Z (se)	P_{01} (eggs/0.05 m^2) (se)	P_0 (se)	P_0A (%CV)
Portugal S/SW	17 401 [107]	10 571 [65]	0.20 (0.19)	8.06 (2.21)	3.05 (1.36)	$1.952x10^{12}$ (87)
Portugal NW	9 160 [60]	11 755 [77]		5.49 (0.80)	3.09 (0.60)	$1.291x10^{12}$ (25)
Galicia	15 188 [123]	6 915 (56)	0.55 (0.33)	11.52 (3.78)	3.57 (2.10)	$1.578x10^{12}$ (59)
Cantabrian W	6 668 [81]	13 829 (186)	0.06 (0.14)	4.29 (0.86)	2.87 (0.70)	$1.176x10^{12}$ (25)
Cantabrian E	3 457 [37]	5 680 (41)	0.38 (0.38)	7.08 (3.02)	4.25 (2.34)	$2.149x10^{11}$ (55)

Table 2.1.1.4: Adult parameters estimates during DEPM 1988 surveys by region.(W – average female weight; F – batch fecundity; R – sex ratio; f – spawning fraction; (se) – standard error; n – number of stations). See Cunha *et al.* 1992 and Garcia *et al.* 1992 for limit of regions.

	n	W (g) (se)	F (eggs/batch) (se)	R (se)	f (se) [n]
Portugal S/SW	10	43.25 (2.821)	16 640 (1 439)	0.52 (0.075)	0.12 (0.091) [2]
Portugal NW	7	35.48 (4.334)	13 621 (1 797)	0.53 (0.079)	0.13 (0.036) [7]
Galicia	44	64.93 (0.06)	27 275 (0.06)	0.35 (0.12)	0.8 (0.20) [ ]
Cantabrian W		79.34 (0.08)	33 802 (0.09)	0.65 (0.10)	0.13 (0.11) [ ]
Cantabrian E		86.31 (0.03)	33 911 (0.03)	0.66 (0.08)	0.21 (0.13) [ ]

Table 2.1.1.5: Parameters for estimation of daily egg production for the 1999 DEPM surveys by region.

	A_0 (km^2) [n]	A_1 (km^2) [n]	Z (se)	P_{01} (eggs/0.05 m^2) (se)	P_0 (se)	P_0A (%CV)
Cadiz +Portugal S	15 007 [47]	7 967 [63]	-0.35 (0.165)	22.40 (5.64)	7.76 (3.32)	$3.568x10^{12}$ (43)
Portugal W	20 837 [134]	8 217 [66]	-0.06 (0.091)	11.32 (3.49)	3.20 (1.86)	$1.860x10^{12}$ (58)
Galicia	12 579 [120}	694 [9]	0.01 (0.01)	1.82 (1.09)	0.09 (0.24)	$2.526x10^{10}$ [267]
Cantabrian E	5 402 [70]	2 161 [28]	0.01 (0.01)	2.45 (0.71)	0.70 (0.37)	$1.059x10^{11}$ [53]
Cantabrian W	1 852 [24]	3 010 [39]	0.002 (0.003)	5.14 (1.21)	3.18 (0.96)	$3.094x10^{11}$ [30]

*- an extremely high value of 1305 eggs with an age of 28 hours found at just one station was considered an outlier and was not included in the regression

Table 2.1.1.6: Adult parameters for the 1999 DEPM surveys by region. (W – average female weight; F – batch fecundity; R – sex ratio; f – spawning fraction; (se) – standard error; n – number of stations)

	n	W (g) (se)	F (eggs/batch) (se)	R (se)	f (se) [n]
Cadiz +Portugal S	10	43.25 (2.821)	16 640 (1 439)	0.52 (0.075)	0.12 (0.091) [2]
Portugal W	7	35.48 (4.334)	13 621 (1 797)	0.53 (0.079)	0.13 (0.036) [7]
Galicia		64.93 (0.06)	27 275 (0.06)	0.35 (0.12)	0.8 (0.20) [ ]
Cantabrian W		79.34 (0.08)	33 802 (0.09)	0.65 (0.10)	0.13 (0.11) [ ]
Cantabrian E		86.31 (0.03)	33 911 (0.03)	0.66 (0.08)	0.21 (0.13) [ ]

Table 2.1.2.1: DEPM parameter and spawning biomass estimates for Mediterranean sardine in the central Aegean and Ionian seas (CVs in parentheses). Data from Somarakis et al. 2001 (WD for SGSBSA).

Parameter	Aegean Sea		Ionian Sea	
Daily egg production, P_1 (day^{-1}/m^2)	27.52	(0.518)	7.81	(0.258)
Survey area, A (km^2)	8702		8724	
Average weight of mature females, W (g)	19.01	(0.034)	15.87	(0.038)
Sex ratio, R	0.458	(0.095)	0.661	(0.053)
Batch fecundity, F (mean number of eggs per mature female)	6469	(0.051)	5149	(0.041)
Spawning fraction, S	0.095	(0.048)	0.087	(0.116)
Spawning stock biomass, B (MT)	16174	(0.521)	3652	(0.282)

Table 2.1.2.2: DEPM adult parameters for *Sardina pilchardus* in the Atlantic and the Mediterranean.

	Country		**Year**	**Month**	***S***	***F***	***W***	***R***	***F/W***
Iberian sardine	Portugal		1988[1]	Mar	0.126	15581	40.94	0.52	381
			1997[2]	Mar		17914	41.28	0.61	434
			1999[3]	Jan-Feb	0.101	18416	44.42	0.61	415
	Spain	E. Cantabrian	1988[4]	Apr-May	0.210	33911	86.31	0.66	393
		W. Cantabrian		Apr-May	0.130	33802	79.34	0.65	426
		Galicia		Apr-May	0.080	27275	64.93	0.35	420
		E. Cantabrian	1990[5]		0.230				
		W. Cantabrian			0.090				
		Galicia			0.120				
		E. Cantabrian	1997[6]	Mar	0.180	26563	70.05	0.52	379
		Total	1999[3]	Apr		21800	66.03	0.55	330
Mediterranean sardine	Hellas	C. Aegean	1999[7]	Nov-Dec	0.092				
			2000[8]	Dec	0.093	6469	19.01	0.46	340
		C. Ionian	2001[8]	Jan-Feb	0.087	5149	15.87	0.66	324

[1]Cunha et al., 1992; [2]Cunha et al., 1997; [3]ICES, 2000; [4]Garcia et al., 1992; [5]Garcia et al., 1991 (cited in Lago de Lanzos et al., 1998); [6]Lago de Lanzos et al., 1998; [7,] Somarakis, unpublished data, [8] Somarakis, 2001.

Table 2.2.1.1: Acronyms, description of parameters and units for the values presented in Table 2.2.1.2.

Acronyms	Estimates of...	Units
Po	Daily Egg Production per surface unit	Eggs/0.05 m²/day
Z	Daly mortality of eggs	
SA	Positive Spawning Area	Km²
Ptot	Total Daily Egg Production of the Population	Eggs/day *10E-12
SST	Sea Surface Temperature	ºC
SSB	SPAWNING STOCK BIOMASS	tonnes
DF	Daily Fecundity of the Population	eggs/gramme
ABtot	Total Egg Abundace in the area surveyed	eggs *10E-12
AB mean	Average Egg abundance per surface unit	Eggs/0.1 m²

Table 2.2.1.2: DEPM estimates of SSB and associated parameters available for the Bay of Biscay anchovy (see acronyms in Table 2.2.1.1). Sources: Santiago and Sanz, 1992, Sanz *et al.* 1992, Motos and Santiago, 1990, Motos and Uriarte 1991, 1992 and 1993, Motos *et al.* 1995, Motos *et al.* 1998 and from Uriarte *et al.* 1999. For the establishment of the lineal regression between Biomass (SSB) and Spawning area (SA) (equation 1) the data of June 89 and 90 were deleted.

YEAR	SURVEY DATES	SSB	P tot	Po	Z	SA	DF	Ab tot	Ab mean	SST
1987	2 -7 June	29,365	2.199	4.61	0.26	23,850	81.3	3.411	14.3035	16.4
1988	21- 28 May	63,500	5.01	5.52	0.18	45,384	81.4	10.41	22.9302	16.5
1989	10 - 21 May	11,861	0.73	2.08	0.18	17,546	62.3	0.896	5.10858	16.6
1989	14 - 24 June	10,058	0.826	1.5	0.94	27,917	54.8	0.79	2.825	20.8
1990	4 -15 May	97,237	4.518	3.78	0.34	59,757	52.2	7.842	13.1238	16.9
1990	29 May-15 June	77,254	7.239	5.21	0.62	69,471	90.1	8.052	11.5901	17.7
1991	6 May-7 June	19,276	1.238	2.55	0.22	24,264	67.5	3.179	13.101	15.6
1992	16 May-13 June	90,720	5.789	4.27	0.22	67,796	71.6	13.09	19.3072	17.7
1994	7 May-3 June	60,062	3.829	3.93	0.11	48,735	62.9	11.33	23.246	15.8
1995	11-25 May	54,701	3.094	4.96	0.19	31,189	56.7	8.751	28.0579	14.5
1996	18-30 May		2.771	4.87	0.31	28,448	-	5.953	20.9244	15.2
1997	9-21 May	51,176	2.697	2.69	0.19	50,133	53.2	7.123	14.2084	15.3
1998	18 May-8 June	101,976	5.595	3.83	0.28	73,131	56.5	11.96	16.3487	15.9
1999	22 May-5 Jun		3.593	3.52	0.12	51,019	-	9.061	17.7214	16.8
1999	Area-radial added		3.865	3.42	0.12	55,946	-	9.745	17.2126	16.8
2000	2-20 May		2.612	3.45	0.18	37,883	-	7.949	20.983	16.7

Table 2.2.2.1: Spawning Biomass and DEPM parameters estimates for anchovy in the Mediterranean. (CVs in parentheses)

			Egg Parameters						Adult Parameters							
		T^a	*A*	A_1	P_1	*P*	*Z*	P_t	*F*	*S*	*W*	*R*	*RF*	*DSF*	*SF*	*B*
SICILIAN CHANNEL	Jun-Jul 1998	18.5 22.5	13295	5329	65.55 (0.21)	26.27 (0.33)	1.63 (0.33)	0.14 (0.33)	4835 (0.16)	0.14 (0.12)	15.18 (0.07)	0.59 (0.12)	319	26	7	13224 (0.22)
	Jun 1999	18.4 22.7	5878	2692	45.86 (0.22)	21.00 (0.32)	1.25 (0.33)	0.05 (0.32)	5871 (0.11)	0.17 (0.10)	14.08 (0.08)	0.55 (0.10)	417	39	6	3138 (0.31)
	Jun-Jul 2000	16.3 25.8	11812	4505	34.98 (0.15)	13.34 (0.24)	2.07 (0.20)	0.06 (0.24)	8379 (0.06)	0.20 (0.28)	18.90 (0.04)	0.62 (0.08)	443	55	5	2850 (0.46)
CATALAN SEA	May 1990	17.6 19.6	17081	8095	120.61 (0.15)	57.16 (0.29)	0.56 (0.44)	0.46 (0.22)	8006 (0.02)	0.36 (0.10)	14.25 (0.04)	0.54 (0.09)	562	110	3	4199 (0.26)
	Jul 1990								7283 (0.12)	0.31 (0.16)	12.79 (0.10)	0.56 (0.10)	569	99	3	
CATALAN SEA & GULF OF LIONS	July 1993	13.3 22.5	44554	33012	86.67 (0.15)	64.22 (0.17)	1.09 (0.26)	2.12 (0.17)	4958 (0.11)	0.31 (0.13)	14.31 (0.07)	0.64 (0.05)	346	69	3	30849 (0.30)
	May-Jun 1994	15 22.0	42085	31692	81.71 (0.18)	61.53 (0.21)	0.47 (0.26)	1.95 (0.21)	7039 (0.02)	0.21 (0.20)	22.92 (0.06)	0.59 (0.19)	307	38	5	52557 (0.36)
LIGURIAN & TYRRHENIAN	July 1993	18.9 22.5	15424	8221	93.57 (0.28)	49.87 (0.32)	0.86 (0.34)	0.41 (0.32)	4894 (0.10)	0.32 (0.11)	14.17 (0.07)	0.63 (0.05)	345	70	3	5829 (0.36)
AEGEAN SEA	Jun 1993	16.7 25.0	17396	17396	259.49 (0.32)	259.49 (0.32)	1.04 (0.46)	4.51 (0.32)	11542 (0.04)	0.28 (0.15)	22.73 (0.02)	0.55 (0.04)	508	78	4	58988 (0.35)
	Jun 1999	18.0 25.0	8604	8604	13.29 (0.39)	13.29 (0.39)	0.53 (0.48)	0.11 (0.39)	4725 (0.05)	0.13 (0.21)	15.77 (0.03)	0.47 (0.09)	300	18	8	6273 (0.43)
IONIAN SEA	May-Jun 1999	18.0 25.0	12362	12362	8.88 (0.24)	8.88 (0.24)	0.52 (0.36)	0.10 (0.24)	9428 (0.08)	0.06 (0.26)	15.60 (0.05)	0.53 (0.07)	604	19	17	5588 (0.33)
SW ADRIATIC	Jul-Aug 1994		14790	9244	50.11 (0.16)	31.32 (0.10)	0.55 (0.12)	0.29 (0.10)	11866 (0.03)	0.16 (0.08)	18.57 (0.03)	0.55 (0.05)	639	56	6	8129 (0.24)

T^a Temperature range (ºC)
A Total survey area (km^2)
A_1 Positive stratum area (km^2)
P_1 Daily egg production per m^2 registered in the positive stratum
P Daily egg production per m^2 registered in the whole sampled area
Z Daily rate of instantaneous mortality
P_T Daily egg production for the whole sampled area (eggs/day $*10^{-12}$)

F Batch fecundity
S Spawning fraction
W Mean females weight
R Sex ratio
RF Relative fecundity, ratio between *F* and the *W*
SF Spawning frequency
DSF Daily specific fecundity (DSF = FSR/W)
B Spawning biomass

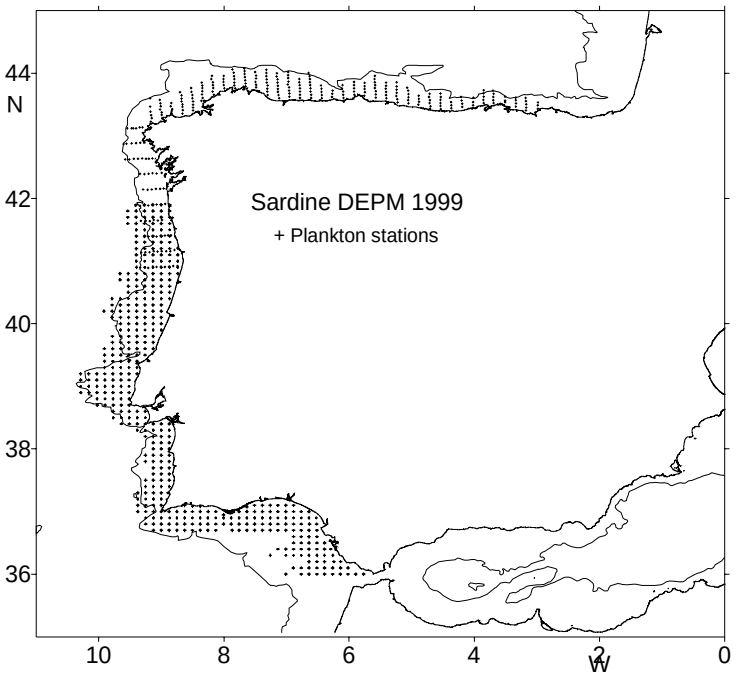


Figure 2.1.1.1: Locations of plankton stations at the Atlantic waters of the Iberian Peninsula during 1999 DEPM surveys.

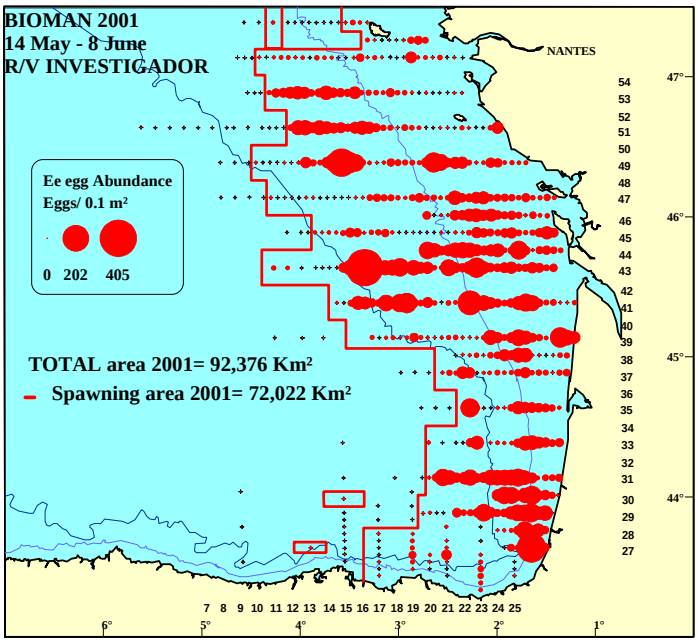


Figure 2.2.1.1: CALVET sampling and Anchovy egg abundance (eggs/0.1m²) distribution found during BIOMAN 2001 (Uriarte *et al.* 2001, the final estimate is not yet available). Solid line encloses the positive spawning area.

3 EGG SURVEYS

3.1 Practical aspects

3.1.1 Inter-calibration and reference collection of eggs stages

During the SG meeting, a random sample of 90 sardine eggs and 51 anchovy eggs collected from the Bay of Biscay during May 2001 was used to perform an inter-calibration exercise between egg stage readers and to create a reference collection of egg stages. Sardine eggs were independently staged by 8 observers (2 from IEO, 1 from AZTI and 5 from IPIMAR) following the staging criteria of Ahlstrom (1943). The results for sardine are shown in Table 3.1.1.1. Anchovy eggs were staged by 5 observers (1 from AZTI and 4 from IPIMAR) following the staging criteria of Moser and Ahlstrom (1985). The results for anchovy are shown in Table 3.1.1.2. After the conclusion of the calibration exercise, a reference collection was created for sardine (Annex I) and anchovy (Annex II) egg stages to facilitate and standardise future work on egg stage identification.

The results of the inter-calibration clearly demonstrate that the problem of consistency in sardine and anchovy egg staging has been previously underestimated, particularly within IPIMAR (where a mix of experienced and inexperienced readers currently participate in the staging of sardine and anchovy eggs). Table 3.1.1.1 also provides a possible explanation for the disproportionately large fraction of destroyed sardine eggs in CalVET found by IPIMAR (ICES 2000a). Overall, the SG demonstrated the urgent need for the establishment of common criteria for stage identification and the need for a joint session between the readers of the 2002 egg samples, preferably before the onset of the laboratory work for the 2002 surveys.

3.1.2 Ageing destroyed eggs

The main problem with destroyed eggs is that they cannot be used in stage-age models and have to be either randomly or proportionally allocated to daily cohorts. An alternative is to exclude them from the analysis but this introduces negative bias in the estimation of egg production. However, apart from IPIMAR, where destroyed eggs in past surveys have reached 10-20%, CalVET samples at AZTI and IEO generally have very few destroyed eggs (less than 5%). Judging from the results of the inter-calibration exercise (Tables 3.1.1.1 and 3.1.1.2) it is possible that many of the eggs classified currently as destroyed by IPIMAR are eggs that can be classified within the 11 stages of development. For the few eggs that exact staging is impossible, a useful approach would be to follow the principles used for destroyed eggs in CUFES samples (see below) and allocate a broad stage category that would provide some information when allocating them to daily cohorts. Finally, another unresolved issue that was highlighted during the meeting is the appropriate procedure of egg counting when embryos appear separated from their capsules.

In relation to CUFES, analysis with the samples from the PELASSES surveys (DGXIV OJC122 99/010), indicate that more than half of the eggs cannot be classifying to the 11 stages scale due to the mechanical damage suffered. Similar experiences have been presented from other parts of the world, and in California attempts to stage CUFES eggs are now abandoned. In cases where it is of interest to know the egg stages in CUFES samples (as in comparisons with vertical samplers), but it is impossible to perform the standard classification, an alternative would be to classify the eggs in broader groups based on features easier to distinguish. Within PELASSES, the following groupings have been used, providing useful information on the broad features of egg development (see section 3.4.1):

- No embryo: stages I, II, III and unfertilised/dead eggs
- Early embryo: stages IV, V and VI
- Late embryo: stages VII, VIII, IX, X and XI

3.1.3 Use of flowmeters in CalVET nets

Correct estimation of sampled volume is important in plankton surveys. Flowmeters are needed to estimate sampled volume in oblique and horizontal tows. Smith *et al.* (1985) suggested that a flowmeter is not required to ascertain volume in CalVET nets, but it is useful for checking the quality of the tow. In vertical tows, filtered volume can be affected by several factors: clogging of the net, undersea currents, ship drift and rolling. According to Smith *et al.* (1985), sequential clogging of the net can be detected using flowmeter readings. Flowmeter rotations of >10% below what is expected indicate that clogging is occurring. This potential problem can be alleviated by carefully washing the net after every tow. Flowmeter readings also exhibit variations not related to net clogging. Thus, even when the net is towed vertically, with angles lower than 5º, variations away from expected occur (Fig. 3.1.3.1).

Increasing overestimation of flowmeter readings (relative to the wire) occurred at increasing angles, being for instance significantly higher above 30º for the case of one Portuguese survey (Table 3.1.3.1). Because of these relatively large deviations an effort must be made to keep the wire as close to vertical as possible, preferably repeating the tow whenever the angle exceeds 20º. To this point may also help the application of larger weights to the sampler, since heavier weight will help to minimize variation in angle (weights up to 45 Kg have been successfully used). If conditions are such that a substantial number of tows have angles greater than 20º and it becomes apparent that all cannot be repeated, for those tows a correction will have to be applied.

3.1.4 Egg incubation experiments, stage-age models and ageing methods

The WKSBS report identified the process of assigning ages to staged eggs as a component of the DEPM that is worth closer examination and suggested the adoption of the method described in Bernal *et al.* (1999; Bernal *et al.* 2001) for future DEPM applications to Atlanto-Iberian sardine. Previously, Lo's (1985) method had been used in sardine and anchovy DEPM applications in European waters. Following the WKSBS recommendation, Bernal's ageing method was applied to the DEPM calculations for the 1999 Spanish sardine surveys. The WKSBS also recommended that incubation experiments should be carried out and data on the deil pattern in spawning should be collected to provide more information for use in the new ageing method. These experiments have not yet been carried out, though both IEO and plan to carry out incubation experiments during the 2001/2002 spawning season. For this purpose, IPIMAR has recently acquired a new incubator that can operate under 5 temperatures and permits 3 replicates within each temperature (Cunha *et al.* 2001 – WD SGSBSA). Early experiments with fertilised sea bass (*Sparus aurata*) eggs from a nearby aquaculture installation demonstrated promising results and the incubator will soon be tested onboard research vessels.

Despite the lack of new incubation experiments, re-analysis of existing data (anchovy incubation experiment from the Bay of Biscay, Motos 1994) has recently provided new insights into stage to age modelling and has alerted towards practical and biological issues that need to be considered during future incubation experiments (Ibaibarriaga, 2001). In this work, an innovative stage-age model was fit following a Bayesian approach that considers the likelihood of an egg being at different stages given age. This innovative approach improves on traditional stage-age models that inaccurately regard ages as the output of the experiment, with stages being the independent variable. The new stage-age model was obtained by fitting a proportional odds model on the multinomial distribution of stages within age for given temperatures (Agresti, 1990). Apart from the statistical innovations, this work also highlighted the potential impact of parental effects on egg development rates (a significant site effect was found when the results from two replicates of the experiment were compared) and demonstrated the need for more frequent observations (at 6 hourly observation intervals short stages at high temperatures can be by-passed altogether) and more complete registration of biological events (eg. egg mortality and hatching).

The stage-age modelling of Ibaibarriaga (2001) was also extended to provide an alternative ageing method to that of Bernal *et al.* (2001). One important methodological difference between the methods is the application of an exponential mortality curve to scale the priors used in the Bayesian method. This means that the mortality rate is applied twice in the full Bayesian method, which introduces complications that need further investigation. Figure 3.1.4.3 shows a comparison between the results obtained from both methods described here. It compares the distribution of the back-calculated spawning times from the final ages obtained from both models. The spawning time probability density function (pdf) represents the distribution of spawning times assumed in the ageing method accepted by WKSB (i.e. without including mortality) and is plotted for comparative purposes. The effect of the exponential decay is visible in the heights of the peaks of the Bayesian distribution. While the estimates of egg production and mortality produced by the two methods lie comfortably within each other's confidence intervals it should be noted that the two estimates of egg production differ by approximately 50% despite the similarity between some of their assumptions and the fact that the ages produced are subsequently collapsed into daily cohorts to compute egg production, concealing some of the actual differences between the ages they assign. Certainly, the new method requires further development and testing before being accepted for routine implementation.

3.2 Survey design

3.2.1 Timing of surveys

Since the Vigo meeting on sardine DEPM (ICES 2000a,) data review and new research has started at IPIMAR aiming to describe adequately the spatio-temporal distribution of sardine spawning off Portugal. Biological data from market sampling and research surveys (acoustic, demersal and DEPM) from the 1980s are in the process of being compiled and reviewed, while intensive sampling during the 2001/2002 spawning season will follow the evolution of sardine reproduction, separately in northern and southern Portugal. Unfortunately, results from this work are not available in time for this SG meeting to take final decisions for the future timing of sardine DEPM surveys in Iberian waters.

However, this work is expected to have been completed by the next SG meeting, when decisions on the most appropriate survey timing can be taken.

3.2.2 Adaptive allocation of sampling effort

Problems with non-coherent egg data, whereby application of standard DEPM techniques produce positive or non-significant mortality rates, is seen to arise from inadequate sampling. The inadequate sampling is predominantly a result of the patchy/clumped distribution of eggs, particularly the earlier stages. A requirement in processing egg-count data is to weight densities of eggs of particular stages in samples by the aerial polygon applicable to individual samples. In a systematic survey (evenly spaced stations along evenly spaced transects) the area of the polygon around each station will be similar in size. A problem with this approach is that such a polygon may be 45 nm^2, but a particularly high egg-count associated with the polygon will in fact be related to a patch of considerably smaller size, possibly <1 nmi^2. Although application of geostatistical techniques to egg-density data provides an improved estimate of the mean egg density and elucidates spatial structure such as patch size it has yet to address the issue of possible over-representation of Day 2 eggs in a survey. Therefore, geostatistics do not provide a direct improvement in estimating P_0, but help to detect deficiencies in sampling design, which can be improved upon in subsequent surveys.

The recommended approach to alleviate the impact of clumped distributions of eggs is to use an adaptive allocation of sampling effort (Thompson and Seber 1996) to obtain more realistic estimates of mean density of eggs of any stage. With respect to the particular problem of patchy distribution of eggs, in simple terms, adaptive allocation means if a patch is encountered (a count above some threshold level) during the systematic survey, then take more samples close around the station so as to obtain a better estimate of the polygon applicable (aerial weighting) to the egg stage(s) encountered (Jackson and Cheng, 2001). Because these extra samples cannot be considered to be independent of those previously taken, the statistical analysis has to be modified to avoid biasing the results and producing an underestimate of total numbers. Lo *et al.* (1997) and Jackson and Cheng (2001, and references therein) provide examples of how to apply the adaptive sampling procedure in a way that increases the precision of the estimated mean egg density. Logistical problems, such as defining the threshold (what egg-count constitutes a patch), how to enumerate eggs in real time at sea and the spatial structure and number of adaptive samples to take must be considered on a case by case basis with the expected information gain balanced against cost. Note that Thompson and Seber's (1996) form of adaptive sampling dictates that extra adaptive samples continue to be taken until no more eggs are found and is thus different from the approach of Jackson & Cheng (2001); the original approach would be appropriate to use in egg production surveys if estimation of spawning area was the parameter at issue. However, CUFES appears a much better approach for estimating this parameter.

Finally, it should be stressed that adaptive sampling is not a trivial exercise and if applied it is imperative that adequate time is allocated to this task during the survey, otherwise every adaptive effort allocation will be at the expense of part of the systematic survey. An adaptive survey also needs to be adopted to utilise the effort dedicated to the "adaptive" sampling efficiency and ensure the whole survey is covered as effectively as possible. If trading off a portion of the systematic survey against a better estimate of P_0 is not an option, then at least an extra 20% of sea time above that required for the systematic survey must be allocated.

3.2.3 Spatial correlation

The statistical independence of ichthyoplankton observations was already addressed in ICES (2000a). Preliminary analysis of the spatial autocorrelation using the 1988 Portuguese DEPM survey data suggested a spatial correlation of the sardine eggs ranging up to 26 nmi in northern Portugal, although experimental variograms showed anisotropy across and along shore directions.

Sardine egg data from both CalVET and CUFES obtained in different surveys and years were used during the SG in a new attempt to understand the spatial structure of sardine egg distribution. The stationary spatial statistical model was used. The model assumes that the spatial distribution to be surveyed is a random function $Z(z(x1),\ldots z(xn),\ldots)$. The survey provides one realisation of Z. Each variable $Z(x)$ is a random variable. Each sampled value $z(x)$ provides an outcome of a random variable with mean m and variance σ^2. Mean and variance are considered constant at each point x of the estimation area (statistical stationarity), which implicitly assumes no trends in the data. Autocorrelation between variables $z(x)$ and $z(x+h)$ spatially separated by vector h is given by the variogram function $\gamma(h)$ or the covariance $C(h)$ as follows:

$$E[Z(x) - Z(x+h)] = 0\,; \quad Var[Z(x) - Z(x+h)] = 2\gamma(h) = 2\sigma^2 - 2C(h)$$

The variogram has 3 parameters: the correlation range, the nugget and the sill. The range is related to the average dimension of patches of high values as well as the average distance between patches. The nugget effect quantifies spatial discontinuity and is related to the sharp spatial transitions when passing from lows to highs values. The sill is related to the maximum variance level between samples. In the case of autocorrelation (i.e., structured variogram), the variogram will increase and stabilise around the sill beyond the range.

CalVET observations from the Spanish DEPM surveys of 1988 (524 stations), 1997 (655 stations) and 1999 (376 stations) were analysed to explore further spatial autocorrelation in DEPM egg samples. Survey design consisted in all years of 3 x 15 nmi grid nodes, intensified to 3 x 7.5 nmi in areas of high egg density. Offshore stations were not sampled when the outer limit of the egg distribution was reached. Data from 1988 and 1997 refer to egg density per m^2 whilst those form 1999 refer to egg density per 0.05 m^2. The surveyed area in northern Spain shows two main orientations. In the western part (South Galicia) North-South direction is predominant whilst in the northern part (Cantabrian Sea) coastline is West-East oriented. The continental shelf is narrow (10 nmi mean width) and only in the NW corner it extends further, reaching up to 35 nmi. In addition, coast line is rather abrupt and the bottom is rough close to the coast, reaching more than 50 m depth just 1 nmi away from the coast. These features have an influence on sardine distribution and on sardine egg occurrence. Eggs mainly occurred in the Cantabrian Sea in all years, thus spatial analysis was restricted to that area. For this area, latitude is related with depth (i.e. across coast) while longitude is related with the geographic area (i.e. along coast). Egg distribution area was surrounded by a regular polygon in order to calculate the spawning area, and experimental variograms were only calculated inside these polygons.

Spawning area shows a sharp decrease from 1988 to 1997 (from 7500 nmi^2 to 2500 nmi^2) while from 1997 to 1999 the decrease was lower (from 2500 nmi^2 to 1700 nmi^2). This shrinking process occurred towards both coastal waters and the inner part of the Bay of Biscay as suggested by the coefficient of correlation between eggs and latitude and longitude for each year (Table 3.2.3.1). Experimental variograms were calculated for both raw and log transformed data for two main directions (0^o and 90^o). Spatial structures are shown in Figure 3.2.3.1. Structural analysis was previously done on log-transformed data since the experimental variogram on raw showed large fluctuations. The logarithmic scale stabilises the variance, being more appropriate for investigating spatial correlation. In addition, fitted variogram models were much clearer and easier to infer. Variograms for raw data, whose spatial structures were derived from logarithmic ones, are given in Table 3.2.3.2.

This analysis was restricted to the main egg distribution area (empty areas or isolated patches were excluded). Clearly, the range of the fitted variogram is related to the size of the spawning area. In 1988, when the largest spawning area was observed, the range of the autocorrelation was much higher than those observed in 1997 and 1999 and their spatial analysis match better with a nested structure. The range observed for the 1988 DEPM Portuguese data was found at 30 nmi (ICES 2000a), being similar to that observed in the French area for sardine eggs over a wide spawning area using CUFES (Petitgas, Annex to the First Interim Report of PELASSES –DGXIV 99/010 project.). Spatial structure of the egg distribution from 1988 also showed a hole at around 15-30 nautical miles, between the ranges of the first and second model. Both the nested structure and the hole could be related with a second order of variability found over two well differentiated types of egg distribution. This phenomenon was observed in both 0° and 90°. In the case of the 90° direction it seems to be related with two major spawning areas located close to the coast and close to the slope. In addition, spawning aggregations with different egg density located along the spawning area might have contributed to the hole effects and nested structures observed at 0° direction.

Spatial structure of adult sardine was also analysed using the backscattering energy allocated to sardine provided during the acoustic surveys. Ranges of the spatial correlation are lower than those found for the eggs either in the Portuguese area (between 8 to 10 nmi, ICES CM 1997/H:1, Report of the Planning Group for sardine acoustic survey in ICES Sub-Areas VIII and IX) , the Spanish area (between 5 to 7 nmi, Carrera pers. comm) or in the French plateau (around 8 nmi, Petitgas, op. cit.), and this range does not seem to be affected by the size of the adult distribution area. These ranges are close to that found for the egg distribution in 1997 and 1999.

Another structural analysis was performed by truncating the transects in one dimension (1-D analysis). Covariograms for 1988 and 1999 were calculated over the same area used in the previous analysis in two dimensions. 53 and 29 transects were analysed respectively as shown in Table 3.2.3.3. Figure 3.2.3.2 shows the cumulated sardine egg per transect for each year. In 1988 most eggs occurred in the central part whilst in 1999 the bulk of the egg distribution was found at the inner part of the Bay of Biscay. Figure 3.2.3.3 shows the experimental covariogram and the fitted models for both 1988 and 1999 egg surveys. Adjusted models are given in Table 3.2.3.4. The range of the first models are similar to those observed in the variograms. From this analysis, the range of the spatial autocorrelation of the egg distribution as derived from CalVET tows seems to be higher than 8 nmi and depends on the size of the egg distribution area. The higher distribution area the higher range of the spatial autocorrelation is expected.

During the acoustic surveys in spring 2000 and 2001, CUFES was installed on each research vessel. The surveys covered the entire European southern coastal Atlantic waters and were co-ordinated under the project PELASSES (DG XIV 99/010). Survey design and strategies were described in Anon (2000; PELASSES First interim Report). During daytime samples from CUFES were collected every 3 nmi over the acoustic track. Transects were 8 nmi apart in Portuguese and Spanish areas, while in the French area the inter-transect distance was set at 10 nmi. For spatial analysis, the middle geographical position of each station was used and a mean depth was allocated to these geographical positions based on the acoustic records. Contrary to the Spanish plateau topography, the continental shelf off northern Portugal is wide (30 nmi), with mainly sandy bottom and shallower waters (i.e. less than 50 m depth for more than 5 nmi). On the other hand, the southwestern coast of Portugal is characterised by a narrow continental shelf which becomes again wider in the southern part, especially in the Gulf of Cadiz.

In this exercise, the 2000 Portuguese data and both 2000 and 2001 Spanish data were analysed. In 2000, eggs were mostly found in the inner part of the surveyed area (i.e. eastern part of the Cantabrian Sea and off South Portugal and in the Gulf of Cadiz). No eggs were found off south Galicia while eggs in north Portugal were scarce. In 2001, sardine egg distribution in the Spanish area was wider than that observed in 2000, although in south Galicia the presence of sardine eggs was again scarce. In some transects, especially those located in the south Atlantic waters and in the eastern part of the Cantabrian Sea, the acoustic track seems to be shorter than the egg distribution and therefore the outer border of the egg distribution remained unknown. This is much clearer in the Spanish survey (Figure 3.2.3.4). In addition, it seems that in the Cantabrian Sea eggs occur in two main locations, close to the coast and close to the slope (Figure 3.2.3.5), whereas in Portugal most of the eggs are located near shore, in shallower waters. In all cases, as in the previous set of data, area distribution of sardine eggs was surrounded by a polygon and the spatial analysis was only done inside that area. Experimental variograms were calculated for both raw data and log transformed data. Variograms are shown in Figure 3.2.3.6a-c and the parameters of the fitted variogram models are given in Table 3.2.3.5.

In the Spanish data, the lack of sufficient number of pairs makes difficult to establish the behaviour of the spatial structure near of the origin. Different lag sizes, from 1 nautical mile to 6, were used to fit the most reliable variogram model. At lag sizes between 5 to 8 nmi, the number of pair data used to construct the variograms gave similar results at distances higher than 30 nmi although little information on the spatial structure near the origin is available. Nugget effect is an important feature, especially in the Spanish area. Portuguese data from the 2000 survey did not show N-S or W-E anisotropies in both raw and log-transformed data. In the Spanish 2001 data set, spatial structure calculated with the log data did not show anisotropies whilst the spatial structure derived from the raw data revealed different behaviour for N-S and W-E directions. Both variogram increased until 15 nmi, then the W-E variogram decreased rapidly. On the hand, spatial structure for the Spanish data from 2000 was difficult to establish in both log and raw data. In this case, spatial autocorrelation was derived on account of the variograms observed in this area during the 1997 and 1999 DEPM surveys. Nevertheless, in all cases, the structural analysis revealed a hole effect between the ranges of the fitted nested structures.

Although the egg distribution was not fully covered by CUFES during the PELASSES surveys, the range of autocorrelation seems to be reached at around 25 nmi (although the Spanish data from 2001 showed a second model with range at 60 nmi). Currently, inter-transect distance is fixed at either 7.5 or 15 nmi. According to this analysis, unless the spawning area is extremely small, transects could be correlated. Also, as it was already observed, stations are spatially correlated. Nevertheless, it should be noted that the eggs might be related either with the coast shore or the slope and therefore the analysis of the spatial correlation using variogram assuming stationarity could be biased on account such trends in the spatial distribution of eggs. Nugget effect is an important feature, especially in the Spanish area. Although the whole egg distribution was not fully covered, the range of the autocorrelation seems to be reached at around 20 nmi, with no clear anisotropies N-S or W-E. As it was already pointed out, the range of the spatial autocorrelation seems to increase as the egg distribution area becomes higher. A simple explanation for this fact could be the occurrence of spawning at two main grounds, near shore and close to the slope, as suggest the Spanish data, whilst at low egg distribution area, possibly related with a lower spawning biomass, spawning is more concentrated on coastal waters.

An approach to solve this problem could be to generalise the linear modelling methodology to account for correlated data. With such an approach, the trend would first be modelled using either linear (LMs) or generalised linear models (GLMs). If spatial autocorrelation would be detected on the residuals, then an iterative procedure could be applied in which the variance-covariance matrix estimated by variograms is included in the fitting procedure. The GLM or LM models would allow the egg distribution to be characterised while the variogram on the residuals would provide a more precise idea on the spatial auto-correlation of eggs. Once the range of the spatial autocorrelation is estimated, inter-transect distance should be fixed in order to satisfy the requirement of transect independence. Then, transects might be used as basic sampling units as suggested in ICES (2000a).

3.3 Estimation of egg production

3.3.1 Generalised linear models for traditional egg production estimation

Generalised linear models (GLMs, McCullagh and Nelder, 1989) provide a way to fit the mortality curve traditionally used to estimate egg production that is as accurate, but much less complex than the more widely used alternatives. The egg production estimate is traditionally based on classifying sampled eggs into daily or half-daily cohorts and fitting a mortality curve (Lasker, 1985; Gunderson 1993). The usual model fitted to data from the Iberian sardine and anchovy assumes constant exponential mortality through ages, i.e.

$$D_t = D_0 \, e^{(-zt)} \qquad (1)$$

where D_t is egg density of eggs of a given cohort, with mean age t, z is the mortality parameter and D_0 is the egg production parameter. Both D_0 and z are usually estimated by non-linear least squares fitting, with additional weights used to account for uneven sampling.

Model (1) can be re-parameterised as a Generalised Linear Model (GLM), using a log link:

$$\log\left(E[D_t]\right) = \log\left(D_0\right) - zt \qquad (2)$$

where $E[D_t]$ refers to the expected value of the egg density by cohort, and the error structure can be chosen from the distributions in the exponential family (which include poisson, binomial and negative binomial along with gaussian), or it can be described directly in terms of the relationship between the mean and the variance of the data.

The main advantage of using GLMs instead of fitting formula (1) using a least-squares procedure is in reducing the difficulty of estimating the correct confidence intervals. Variance estimates for the mortality curve (no matter how it is fitted) are usually obtained from the information matrix, and confidence intervals for each parameter can be computed in various ways, depending on the assumed distribution of the observations. Usually the variability of the age distribution of eggs increases with density, and this has a particularly strong effect on the confidence intervals for the egg production parameter, whose estimation is the main objective of this model. It is therefore important that neither the gaussian error distribution nor tests based on it (such as the student's t test) are used, if meaningful results are to be obtained. Instead, one of three other strategies needs to be adopted. One option is to transform the data and to stabilise the variance, and thereby permit the use of models relying on the normal distribution, before back-transforming them and applying a bias correction to the confidence intervals. Another option is to use computer intensive methods like bootstrap or jack-knife procedures to estimate confidence intervals directly or correct bias (e.g. as in Motos 1994). Finally, if GLMs are used, and an appropriate statistical distribution that describes the variability of the data can be found, then the estimates of variance and the confidence intervals of the model parameters can be extracted directly from the information matrix using the appropriate transformation. This reduces the problem of estimating the appropriate confidence intervals for the egg production estimate to become largely one of finding an appropriate statistical distribution that can describe the data. One such distribution that can apply to a wide range of cases is the negative binomial distribution, in which the relation between the mean and variance can be set with an additional parameter called the "dispersion parameter". Nowadays, software that can estimate iteratively both the mean and the dispersion parameter of the negative binomial in the context of the GLMs is available (Venables and Ripley 1999), so fitting model (2) using GLMs with negative binomial errors and the dispersion parameter estimated directly from the data is straightforward, and provides a reliable way to obtain acceptable estimates of egg production with appropriate confidence intervals.

3.3.2 GAM-based estimation of egg production

In order to make any estimates of the uncertainty in their predictions, all estimation techniques have to assume that the basic model they use is able to capture the most important aspects of reality. One simple example of this occurs in simple linear regression, where confidence and prediction intervals can easily be produced along with the straight line that 'best' fits the underlying relationship between the observed data, but these assessments of uncertainty have very little meaning unless the true function is actually linear. Mathematically the DEP methodology is largely based on regression, and the problem is compounded by the small amount of data available for estimating some of the parameters. A further complication is produced by the post-stratification applied to both species. The effects of uncertainty in the location of the boundaries between strata is often ignored in the calculation of the precision of final estimates from such models, even though it may actually be relatively large.

Generalised Additive Models (Hastie & Tibshirani 1990) provide a means of sidestepping some of the problems of model mis-specification. Within the DEPM process this has two advantages, the estimates of overall biomass produced are more accurate and the narrower confidence intervals around them are actually more reliable than the traditional ones. GAMs gain their advantage by avoiding the necessity of specifying the exact nature of the relationship between the variables. Instead of declaring that the function is linear, it is only necessary to assert that it is smooth, where smoothness implies continuity along with certain conditions on the continuity of the function's derivatives. Given any continuous function, a smooth function can be found that lies as close as we like to it throughout its length. The major difficulty in using GAMs, which can be thought of as just regression with smooths, is their extreme flexibility. This makes them vulnerable to overfitting, though recent developments have greatly reduced this problem (Wood 2000).

GAMs are particularly appropriate to situations where the underlying reality is complex and difficult to capture analytically. Biological systems are therefore an obvious candidate for their use, and they have been applied to several fisheries stock assessment problems (Borchers *et al.* 1997, Augustin *et al.* 1998). The major limitation on their use has been their perceived novelty, difficulty and obscurity.

The European Union is currently funding a research project (EU Study 99/080), involving AZTI, IPIMAR and IEO along with the University of St Andrews to develop and apply GAMs to the DEPM for sardine and anchovy. As part of this, multidimensional GAMs are being implemented in an R library called mgcv (R is a computer program that provides a statistics and graphics environment and is available free from http://www.cran.r-project.org/ along with mgcv). The project will finish in April 2003, by which time staff at all the institutes involved should have applied the methodology to the DEPM for the two species.

The currently available version of mgcv implements only one-dimensional smooths, while the software for multidimensional ones are currently being tested by members of the project and should be available soon. The library uses penalised regression and generalised cross-validation to select between smooths and provides both numerical and graphical output (Figure 3.3.2.1). It can predict values at given locations and supply confidence intervals around them. Its use is straightforward and reasonably intuitive and allows a range of different options and error distributions to be selected.

As with all statistical tools the major uncontrollable difficulty is misuse. The exact location and detailed shapes the prediction surfaces produced by the methodology should not be over-interpreted. This same problem applies to polynomial regression, though, in that case it is generally ignored.

3.3.3 Egg mortality

At present the DEP methodology assumes that egg mortality per unit time is constant across the whole study. This assumption does not appear unreasonable for sardine, given the similarity in the proportions of eggs of each stage observed in the samples collected in the north and south of the study area (Figure 3.3.3.1), though the strong pattern caused by the differences in the duration of the various stages makes detailed comparison difficult. Combining the assumption of constant mortality rates with the faster egg development observed at higher temperatures and the stability in age structure assumed by the DEPM implies that samples collected at higher temperatures should tend to contain a higher proportion of eggs in the later stages than those collected at lower temperatures. A similar mortality function is assumed for the anchovy assessment. Figure 3.3.3.2 seems to contradict the assumption of constant mortality rates for sardine eggs in the wild. Although the evidence is difficult to interpret, it seems to suggest that the effect of increase in mortality with temperature (or some factor correlated with it within this dataset) may be greater than the changes in development speed.

Accurate estimates of the average proportion of development time spent in each stage may provide a means of testing the assumption of constant mortality rates over time. If necessary more sophisticated mortality functions might then be investigated, using the data already collected (from surveys and laboratory experiments). At the very least, such a study should enable an assessment to be made of the risk and potential effects of model mis-specification on this component of the methodology and the actual uncertainty in the mortality estimates.

Evaluation of the steady-state mortality assumptions underlying estimation of daily egg production is an important scientific issue. Unfortunately a relatively large amount of data is required to investigate this, and in many DEPM studies egg abundance is far too under-sampled to allow any meaningful conclusions. In some cases, sample size may be insufficient to produce a significant slope to the egg mortality function. Under these conditions it may be preferable to include yolk-sac larvae as an additional point in the mortality curve, even through the mortality rate of yolk-sac larvae may not be the same as the eggs. An alternative approach under these under-sampled conditions would be to use the mean egg abundance to measure P_0.

3.4 The Continuous Underway Fish Egg Sampler (CUFES)

3.4.1 Comparison of CUFES and CalVET

CUFES provides a continuous near-surface index of egg abundance while the CalVET provides a quantitative estimate of the abundance of eggs over the full water column based on discrete samples. Comparison of sardine and anchovy egg presence/absence data between CalVET and CUFES are presented in Table 3.4.1.1 (Portuguese survey of March 2000). The chief benefits of CUFES are that it provides a high spatial resolution of spawning area and egg patches and egg abundance can be monitored rapidly in quasi real time. In addition, CUFES can be used with other survey devices (acoustics or trawling) and in different weather conditions, which gives a wide range or potential applications as a source of auxiliary information for other type of surveys. The major disadvantage of CUFES are: some eggs are damaged during the collecting process, which may bias estimates of daily production because younger eggs may be more vulnerable than older eggs (Table 3.4.1.2 and 3.4.1.3); all egg stages may not be fully vulnerable to the pump and the samples are not independent leading to complicated variance formulations. Thus for CUFES to be used in an EPM a way must be found to take advantage of the high spatial resolution and rapid monitoring of characteristics of the instrument without giving up either precision or incorporating new biases.

3.4.2 Using CUFES in a DEPM survey

Three possible ways for incorporating CUFES into a DEPM were considered:

I. Building a CUFES to CalVET conversion coefficient into the survey design, and use CUFES as the primary sampler, with CalVET only being used to convert the CUFES egg density to a full water column value.

II. Develop a mixing model with environmental covariates to convert CUFES counts to full water counts, and use CUFES as the basic egg sampler while monitoring the environmental parameter that input the mixing model; a minimal CalVET sampling for validation purposes would only take place.

III. Use CalVET as the primary sampler and use CUFES to map the spawning area and to schedule the sampling of CalVET samples.

Each of these options was discussed as follows.

Direct Conversion (ad hoc calibration coefficients per survey): Direct conversion of CUFES to a full water column tow would be expected to be imprecise since the conversion factor would be a function of the specific gravity of the egg stage and the extent of vertical mixing which may be highly variable within and between surveys. Computation of such conversion coefficients, largely support this view with R^2 as low as 50% in some cases. Thus using the direct conversion method is likely to diminish any gains in precision that a CUFES-based DEPM might afford.

Direct Conversion with a mixing model:

In theory, if the extent of mixing and specific gravity of the eggs were known, one may be able to convert the abundance of eggs taken in CUFES to a full water column tow with reasonable precision and low bias. If this were possible, one would be able to carry out an entire DEPM egg survey without stopping the vessel, although a minimal CalVET sampling would always be desirable for ad-hoc validation purposes. No such model has been developed and tested within the context of the DEPM, although general egg mixing models exist. Thus this option is in the realm of research direction, but it cannot be adopted already with existing knowledge.

Within EU project PELASSES (DGXIV OJC122 99/010) work is being carried out with the aim of modelling the vertical distribution of eggs with depth according to the environmental conditions in the areas steamed by the cruise. A vertical distribution model for anchovy and sardine eggs (based on Sunbay 1983; 1991) using ancillary variables is being developed. For this issue CALVET, CUFES and LHPR sampling were implemented during 2000 and 2001 under different environment conditions. Results are expected to appear during 2002, upon which time further evaluation of this issue will be made.

Using CUFES to schedule CalVET sampling and boundaries of the spawning habitat: Improvement in the design of CalVET-based plankton surveys can be obtained with CUFES. For example, in the Bay of Biscay anchovy DEPM surveys presence and absence of eggs in CUFES at the outer edges of the expected spawning distribution is being used to decide whether to abandon or not the coverage of radial tracks, while the CUFES abundance serves to identify areas where sampling could be intensified. A more thorough implementation of CUFES in the design and estimation of

CalVET-based DEPM surveys for sardine is found in California (Lo *et al.* 2001). A rule is established to start CalVET sampling along a radial transect conditioned on egg densities in CUFES being above a threshold of 2 eggs per minute. This leads to a spatial stratification of the sample according to abundance, those of low abundance being only covered by CUFES whereas in areas of high egg abundance both CUFES and CalVET are hauled. Daily egg production is primarily calculated for the later area and the total area estimation is based on a raising factor according to the CUFES egg density ratio in the two strata.

Table 3.1.1.1: Number of sardine eggs allocated to each development stage by each of the 8 observers that participated in the inter-calibration exercise during the SG meeting. DIS refers to eggs considered disintegrated.

Observer	**Development stage of sardine**												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	DIS	TOTAL
1 (Spain)		20	1		35		5	14	12	1	2		90
2 (Spain)		20	1		35		5	14	12	1	2		90
3 (Spain)		20	1		35		5	13	14	2			90
4 (Portugal)		20	1		34		7		13	12	2		89
5 (Portugal)	5	12			35	1	3	3	26	1	1	3	90
6 (Portugal)	6				29		4	15	10	4	2	12	82
7 (Portugal)	5		1	1	4	30	5	10	17	2		15	90
8 (Portugal)	3	14			28	3	3	3	5	17	1	4	81

Table 3.1.1.2: Number of anchovy eggs allocated to each development stage by each of the 5 observers that participated in the inter-calibration exercise during the SG meeting. DIS refers to eggs considered disintegrated.

Observer	**Development stage of anchovy**												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	DIS	TOTAL
1 (Spain)	5	18		10	8		6	1	2	1			51
2 (Portugal)	5	18		10	6			7	1	1		1	49
3 (Portugal)		14	9		15			8			1	2	49
4 (Potugal)		22		9	7		7		1	1		3	50
5 (Potugal)	14	9		1	13			7	1		1	2	48

Table 3.1.3.1: Effects of stray angle (measured by angulometer, 0 indicating vertical tow) on flowmeter deviations (difference between length of cable and distance estimated by flowmeter) during the November 1999 Portuguese survey.

Angle	Mean deviation (m) (s.e.)	p-value
0	5.877 (4.558)	0.2
5	4.406 (3.103)	0.16
10	3.973 (0.679)	0.5
15	-4.283 (4.683)	0.36
20	-9.460 (8.110)	0.25
25	-12.807 (7.024)	0.07
30	-36.333 (8.110)	<0.01
40	-54.051 (14.048)	<0.01

Table 3.2.3.1: Coefficient of correlation between egg number and latitude and longitude

Year	Latitude	Longitude
1988	0.08112824	0.0416881
1997	0.06548379	-0.26611226
1999	0.36465939	-0.53565045

Table 3.2.3.2: Fitted variogram models for sardine egg distribution (CalVET samples from DEPM surveys). Range is expressed in nautical miles.

		Spatial structure								
Year	Nugget	Model I	Sill	Range	Model II	Sill	Range	Model III	Sill	Range
1988	35000	Spherical	45000	15	Spherical	10000	30	Spherical	38000	60
1997	100000	Spherical	47000	10						
1999	122	Spherical	100	10						

Table 3.2.3.3: Number of transects in the positive area of sardine egg presence, average distance between transects (expressed in nautical miles), minimum, maximum, average and its variance of egg per transect and the inter-transect variance.

Year	No transect	Min	Aver dist	Max	Average	Variance	Intertran. Var
1988	53	1.00	6.0	246	72	3467	2719086
1999	29	1.00	7.4	142	30	980	396127

Table 3.2.3.4: Fitted variogram models for sardine egg distribution (CUFES samples from PELASSES surveys). Range is expressed in nautical miles. *French data area available in Anon (2000)

		Spatial structure-Transitive (1-D)								
Year	Nugget	Model I	Sill	Range	Model II	Sill	Range	Model III	Sill	Range
1988	0	Spherical	720000	15	Spherical	2000000	318			
1999	0	Spherical	115000	10	Spherical	150000	140	Spherical	130000	215

Table 3.2.3.5: Fitted variogram models for sardine egg distribution (CUFES samples from PELASSES surveys). Range is expressed in nautical miles. *French data area available in Anon (2000)

		Spatial structure								
Year	Nugget	Model I	Sill	Range	Model II	Sill	Range	Model III	Sill	Range
Pt-00	1700	Spherical	5000	8	Spherical	4800	25			
Sp-00	1700	Spherical	8700	8	Spherical	2000	25			
Sp-01	35000	Spherical	35000	15	Spherical	5084	60			
Fr-00	100	Spherical	240	33						

Table 3.4.1.1: Comparison of sardine and anchovy egg presence/absence data among CALVET and CUFES (at, before and after CALVET stations) samples during the Portuguese 2000 PELASSES survey. Symbols: PP = Presence-Presence. PA = Presence Absence. AP =Absence Presence AA = Absence Absence.

Species	Comparison	**P-P**	**P-A**	**A-P**	**A-A**	**% Agreement**
Sardine	CALVET/CUFES (At)	39	17	7	55	79.7
	CALVET/CUFES (Before)	46	10	22	40	72.9
	CALVET/CUFES (After)	42	14	18	44	72.9
	CUFES (At)/CUFES (Before)	43	3	25	47	76.3
	CUFES (At)/CUFES (After)	41	5	19	53	79.7
	CUFES (Before)/CUFES (After)	53	15	7	43	81.4
Anchovy	CALVET/CUFES (At)	6	12	0	100	89.8
	CALVET/CUFES (Before)	9	9	2	98	90.7
	CALVET/CUFES (After)	9	9	0	100	92.4
	CUFES (At)/CUFES (Before)	4	2	7	105	92.4
	CUFES (At)/CUFES (After)	6	0	4	108	96.6
	CUFES (Before)/CUFES (After)	6	5	4	103	92.4

Table 3.4.1.2: Intact and damaged anchovy eggs sampled by CUFES during the THALASSA cruise in the Bay of Biscay as a function of crude groupings in development. No embryo refers to stages I-III or unfertilised/dead eggs. Early embryo refers to stages IV-VI and late embryo refers to stages VII-XI.

	NO EMBRYO	**EARLY EMBRYO**	**LATE EMBRYO**
Not damaged	78	124	163
Damaged	665	254	296
Increase %	753%	105%	82%

Table 3.4.1.3: Intact and damaged sardine eggs sampled by CUFES during the THALASSA cruise in the Bay of Biscay as a function of crude groupings in development. No embryo refers to stages I-III or unfertilised/dead eggs. Early embryo refers to stages IV-VI and late embryo refers to stages VII-XI.

	NO EMBRYO	**EARLY EMBRYO**	**LATE EMBRYO**
Not damaged	78	424	1340
Damaged	1637	478	1360
Increase %	1999%	13%	1%

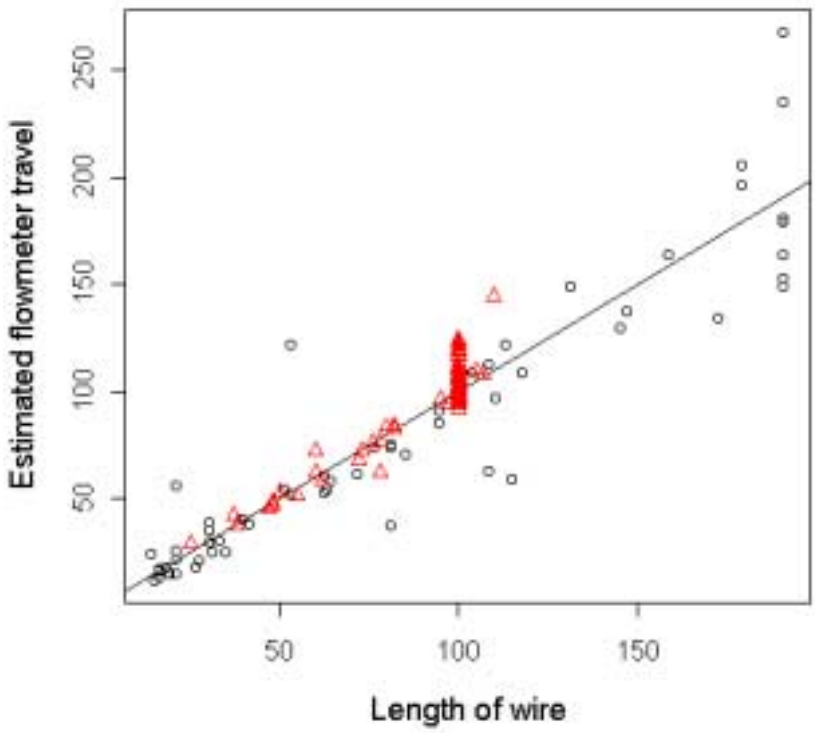


Figure 3.1.3.1- Estimated travel of the net by the flowmeter vs. length of wire. Black circles represent IPIMAR readings during a single cruise at angles lower than 5º. Triangles are AZTI data at angles lower than 10º.

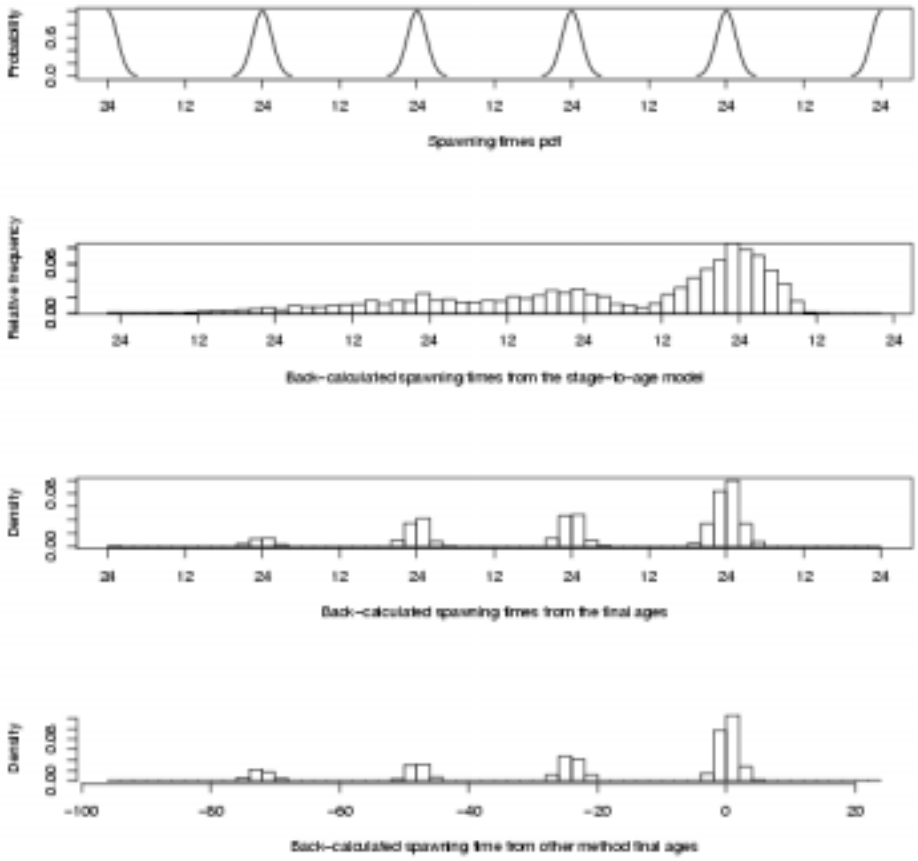


Figure 3.1.4.1: Comparison between assumed distribution of spawning times (top) and estimated spawning times from modified Lo model (second from top), the standard ageing method (third from top) and the Bayesian ageing method (bottom). Note that the assumed distribution of spawning times represent the assumption from the standard ageing method, while in the Bayesian method, the height of each peak is scaled with an exponential decay.

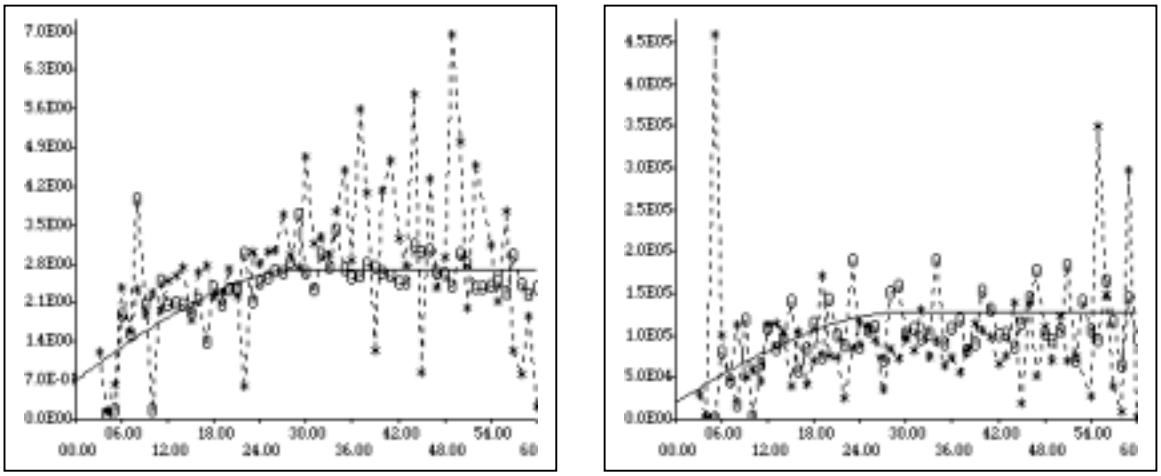


Figure 3.2.3.1a: Experimental and fitted variograms for 0º and 90º direction for sardine eggs from 1988 DEPM data. Left panel logarithmic scale and right raw data

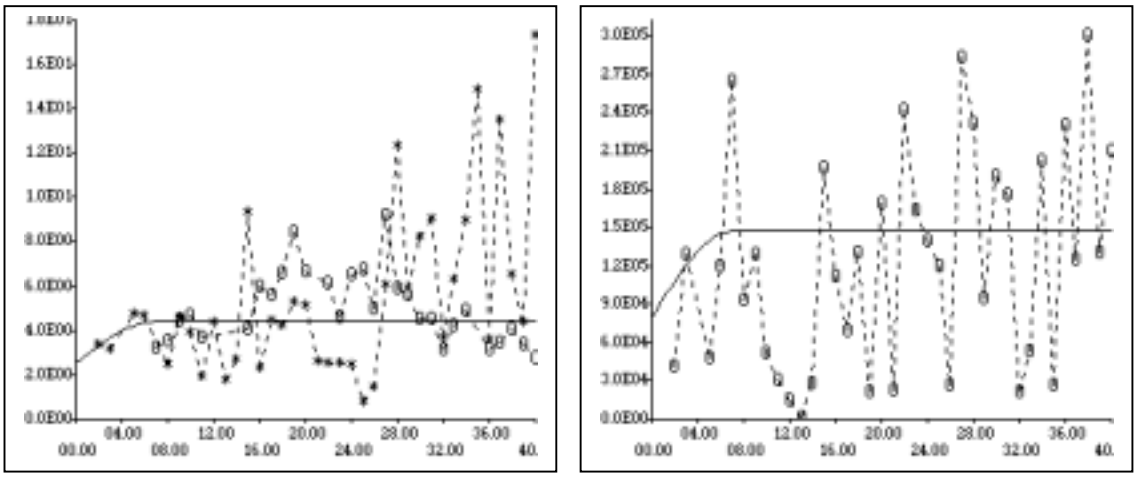


Figure 3.2.3.1b: Experimental and fitted variograms for 0º and 90º direction for sardine eggs from 1997 DEPM data. Left panel logarithmic scale and right raw data

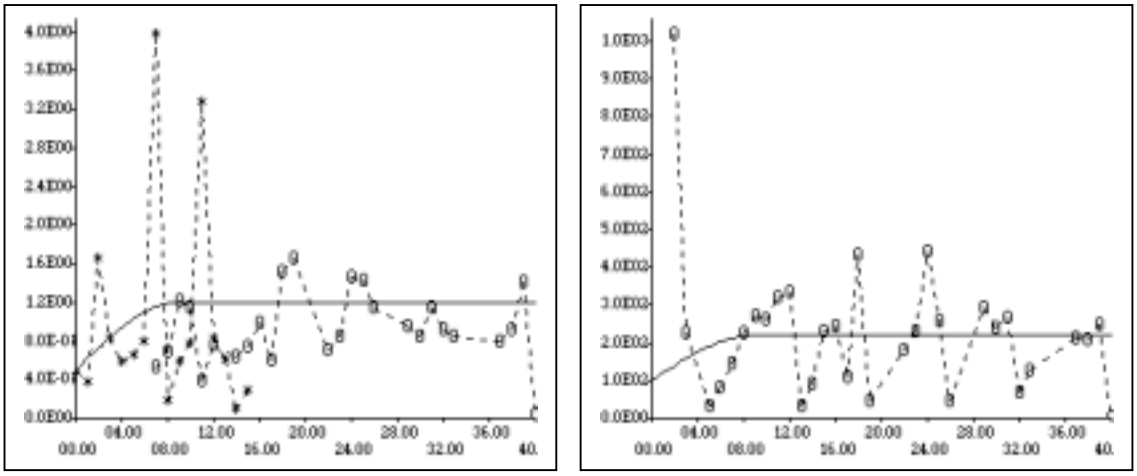


Figure 3.2.3.1c: Experimental and fitted variograms for 0º and 90º direction for sardine eggs from 1997 DEPM data. Left panel logarithmic scale and right raw data

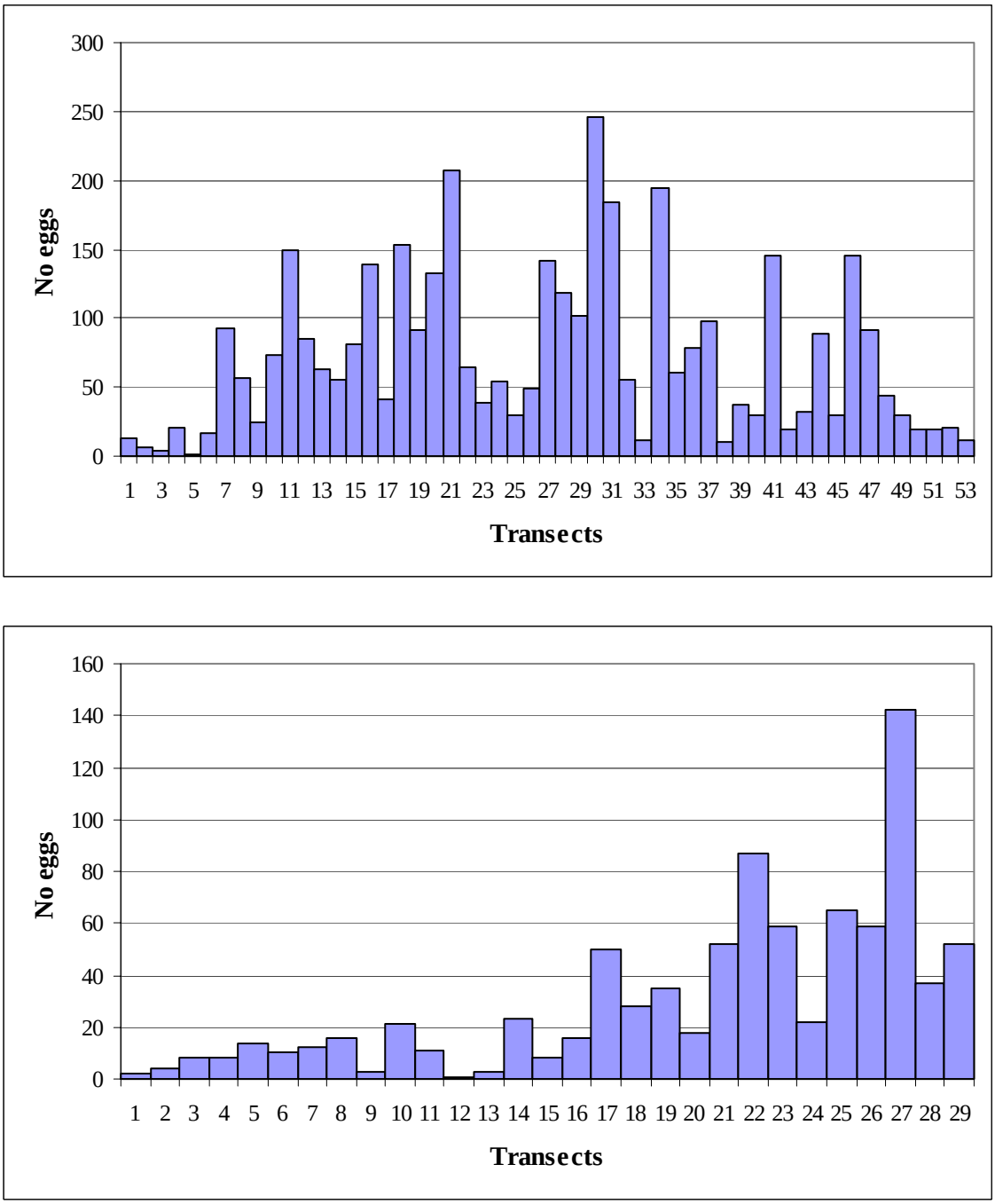


Figure 3.2.3.2: Number of sardine eggs per transect during 1988 (above) and 1999(below) DEPM surveys.

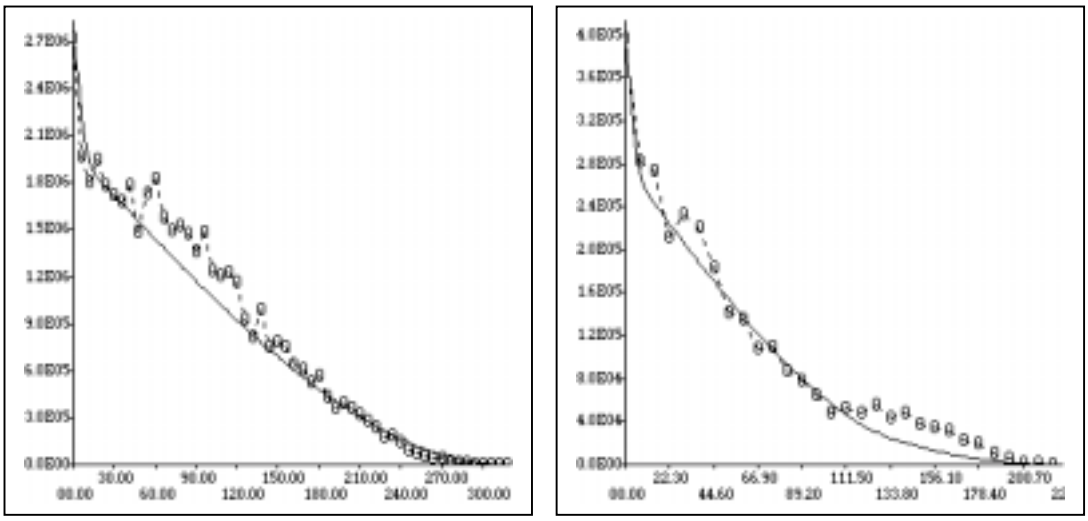
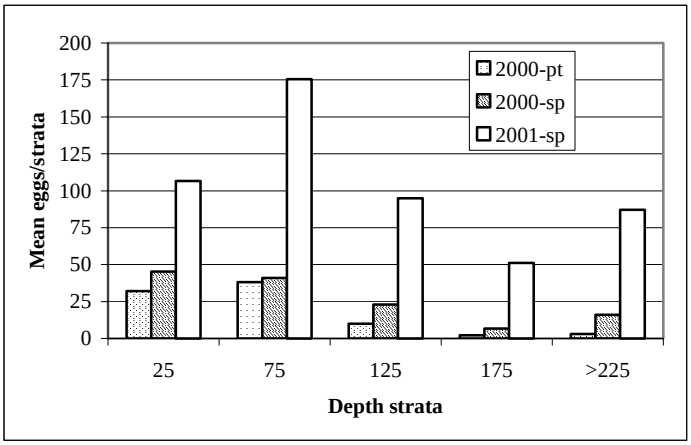


Figure 3.2.3.3: Covariogram and fitted models for 1988 DEPM survey (left panel) and 1999 DEPM survey (right panel)



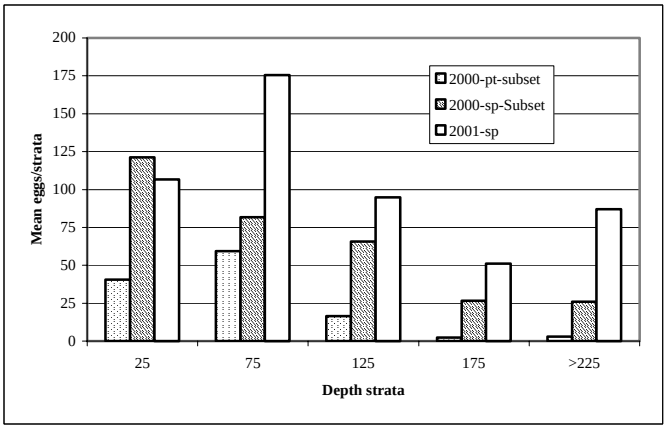
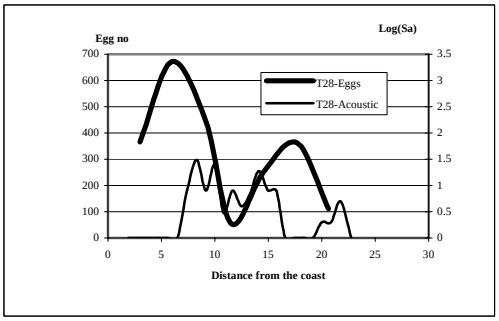


Figure 3.2.3.4: Mean sardine eggs per depth strata from the CUFES samples (depth strata 10-50 m; 50-100 m; 100-150 m ; 150-200 m and more than 200 m). Above panel using the whole data set for the 2000 surveys and below using only the southern part of the Portuguese data and the eastern area of the Spanish data for the 2000 surveys.



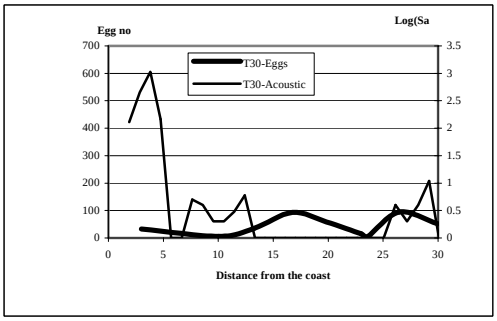


Figure 3.2.3.5: Sardine egg abundance (total number of eggs from CUFES samples integrated each 3 nmi) and acoustic back-scattering energy allocated to sardine (Sa values in logarithmic scale) across the shore in two transects located in NW Spain during spring 2001.

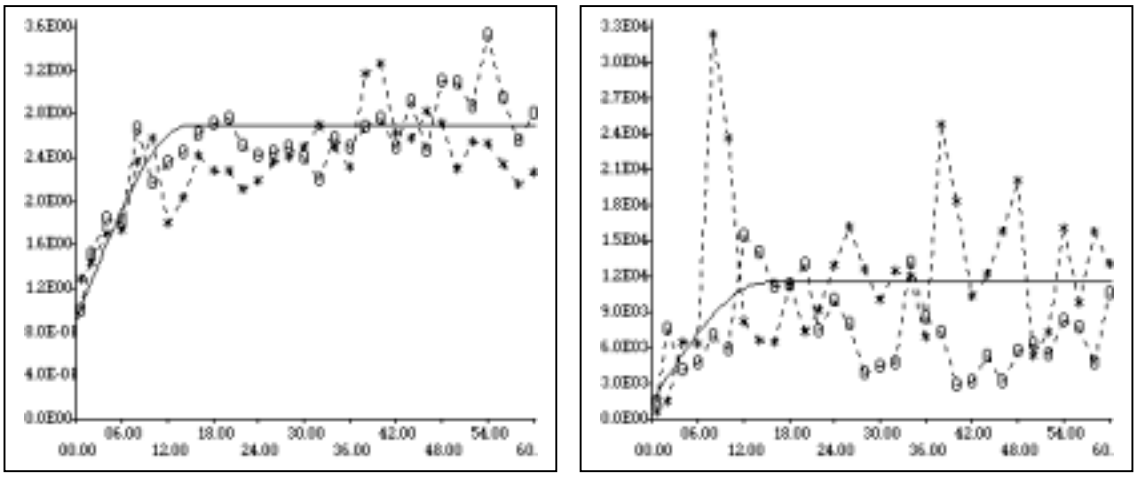


Figure 3.2.3.6a: Experimental and fitted variograms for 0º and 90º direction for sardine eggs from the Portuguese 2000 CUFES data. Left panel logarithmic scale and right raw data

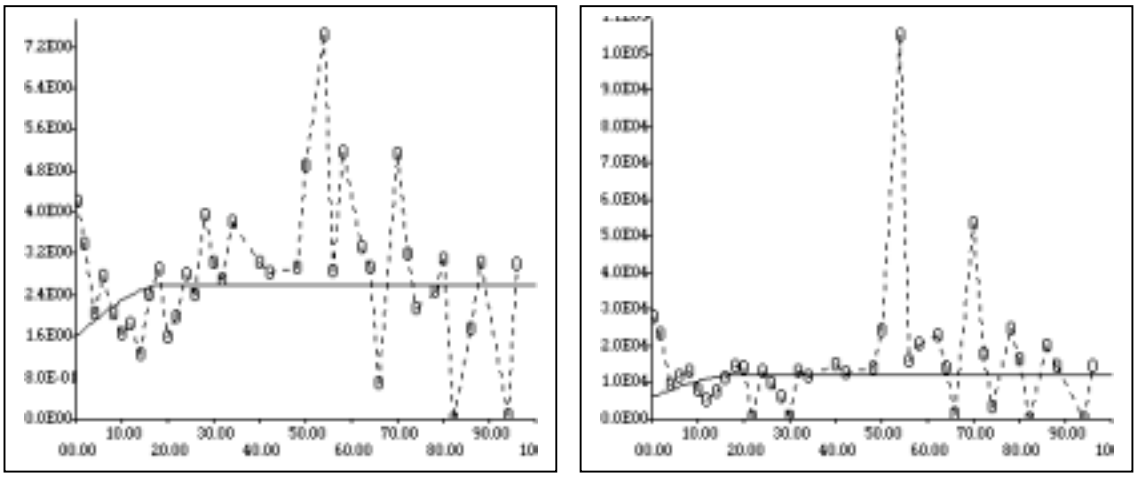


Figure 3.2.3.6b: Experimental and fitted variograms for 0º and 90º direction for sardine eggs from the Spanish 2000 CUFES data. Left panel logarithmic scale and right raw data

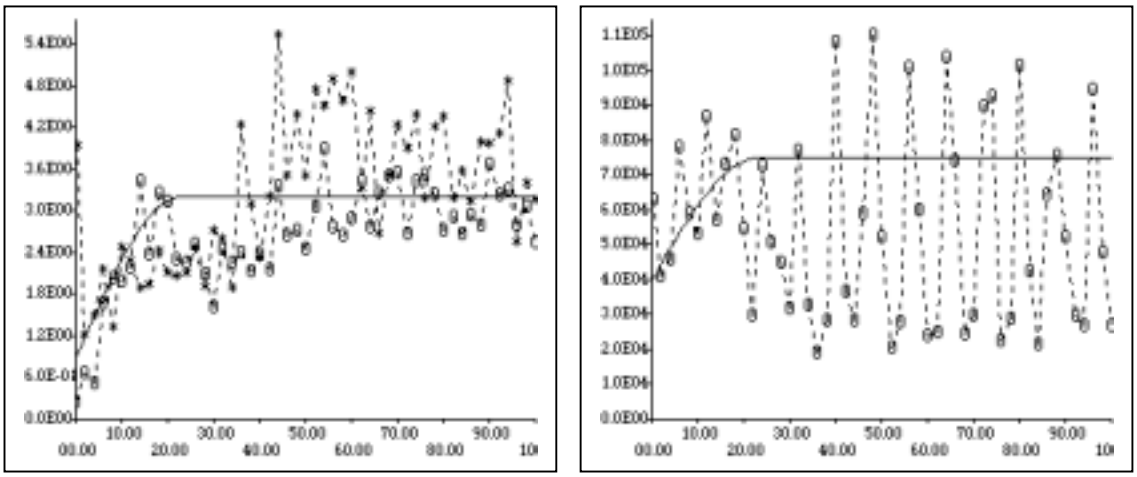
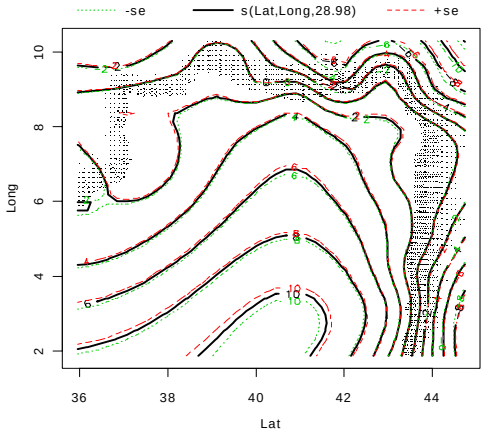


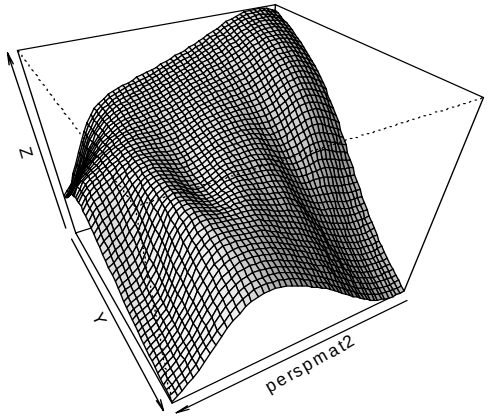
Figure 3.2.3.6c: Experimental and fitted variograms for 0º and 90º direction for sardine eggs from the Spanish 2001 CUFES data. Left panel logarithmic scale and right raw data

Figure 3.3.2.1: Overall geographic pattern of densities of sardine eggs around Iberia produced by fitting a Generalised Additive Model to all the samples collected during the surveys carried out within the DEPM stock assessment exercises of the 1980s and 1990s. This uses a two-dimensional smooth of latitude and longitude with poisson errors.

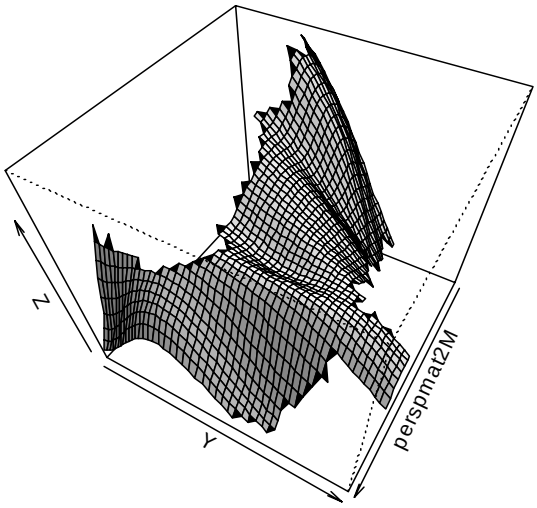
a)



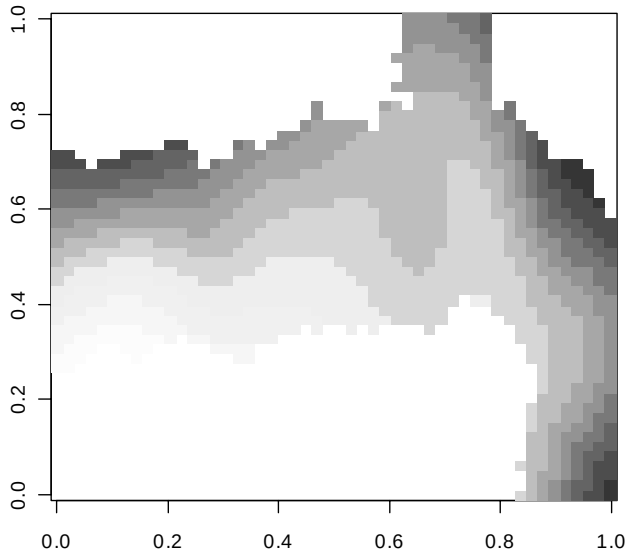
b)



c)



d)



a) is a contour plot of the smooth and its standard errors for the whole area between 2° and 14° West and 36° and 46° North.. The dots indicate the locations of the samples used.

b) shows a perspective plot of the same area.

The vertical scales are arbitrary, but the high values produced for inland central Spain are obvious, this is due to the high densities of eggs observed along the inshore edge of the study area and could be prevented by adding artificial (structural) zero data points along the coastline, though this assumes there is a gradual decline in density rather than a discontinuity.

c) is the same as b, but with a mask applied to remove the areas outside the available data.

d) contains the same information as c, but with density shown as a variation in colour/intensity.

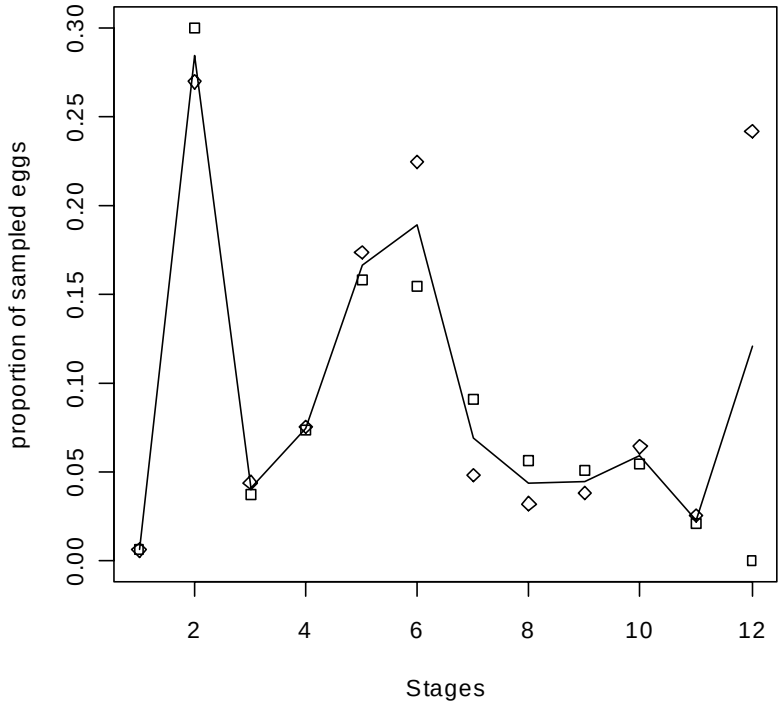


Figure 3.3.3.1 Proportions of 15742 eggs of each stage in samples collected as part of the sardine DEPM. The line refers to all the data, diamonds to that collected by Portuguese boats in the southern half of the study area and squares to that collected in the north by the Spanish. “Stage 12” is destroyed eggs, those that could not be assigned to a stage.

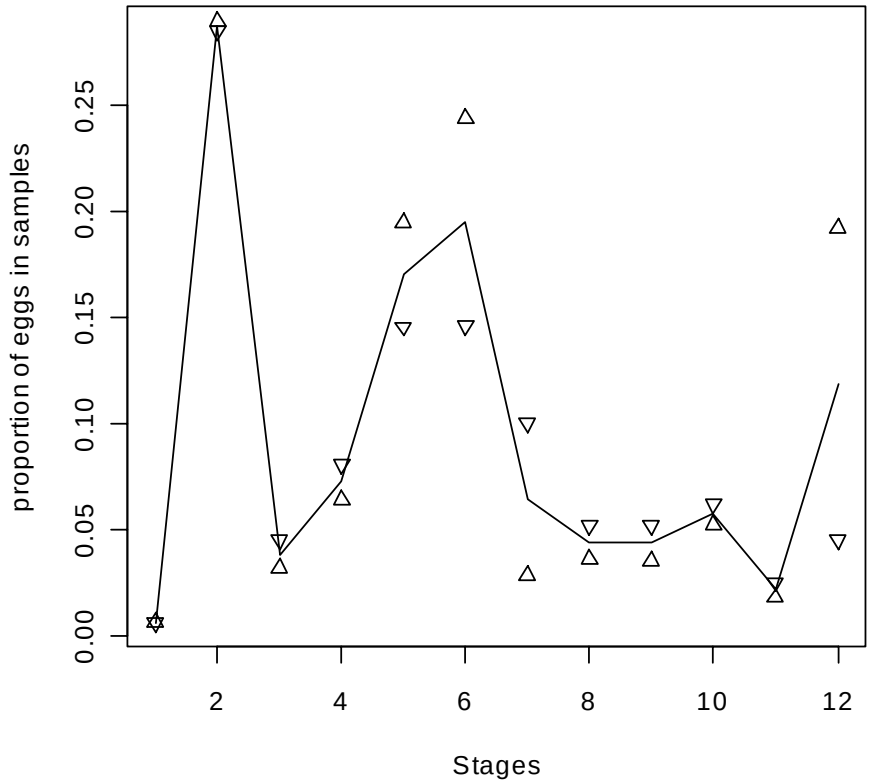


Figure 3.3.3.2: Proportions of eggs in different stages observed in samples taken below (downward pointing triangle), and above (upward pointing triangle) 15^0C. The line combines all the samples. The data is the same as that used in figure 3.3.3.1, and it must be noted that the similarity in the pattern of differences between the two temperatures and the two regions is at least partly due to the higher temperatures at which the more southerly samples were collected.

4 ADULT SURVEYS

4.1 Practical aspects

4.1.1 Fish maturation

Different criteria for the definition of mature sardine are currently used for DEPM purposes (fish in macroscopic stage 2 and above) and for the estimation of the maturity ogive used in stock assessment (fish in macroscopic stage 3 and above). While in DEPM the identification of fish contributing to the SSB is confirmed microscopically, data used for the estimation of maturity at age rely only on macroscopic criteria and the correspondence between the two sets of criteria is not clear. To investigate whether all stage 2 fish should be included in the mature population, IPIMAR is carrying out an histological calibration of the macroscopic maturity scale (using samples stratified by sex and maturity stage, 5 fish per strata) and the estimation of a microscopic maturity ogive (using sex and length class stratified samples, 5 fish per strata) based on samples collected in the Portuguese acoustic surveys (November 1999 and March 2000). Analysis of a subset of the November sample suggested that in general, fish classified in macroscopic stage 2 will spawn during the ongoing spawning season, while some of them showed signs of atresia (not quantified yet). Atresia may indicate that these gonads will not finish development but may also be a sign of recent spawning, being very difficult to distinguish between the two situations, therefore it was considered not worthwhile to pursue a more detailed study of atresia. The concept of mature fish was discussed and both the use of the gonadosomatic index and the establishment of a minimum oocyte size (based on microscopic observable macroscopically) were considered to be useful tools to help the macroscopic definition of mature fish.

4.1.2 Study of post-ovulatory follicles

The pattern of post-ovulatory follicle (POF) degeneration with time is the basic knowledge required for the estimation of the spawning fraction of multiple spawning fishes. It was first for the Californian anchovy that the ability to asses and to age the degree of degeneration of POFs was first established and used to determine the time elapsed since the last spawning (Hunter and Goldberg 1980, Hunter and Macewicz 1985). Since then this pattern of POF degeneration has been studied for many pelagics under DEPM applications (see review in Alheit 1993). For the Bay of Biscay anchovy, the pattern and rate of POF degeneration was described by Motos (1996) and for sardine it has been described by Perez *et al.* (1992). For each stock, once the rate of POF degeneration with time is established, criteria for the allocation of POFs to spawning cohorts (POFs of day 0, day1, day 2 or day 2+) have to be defined, depending on the level of degeneration, the time of capture and the spawning time (see section 4.1.4). Whenever possible the background supporting that knowledge has to be established based on field or experimental data for each population.

Classifying POFs is a matter of qualifying ovaries and then assigning ages (in days) to them according to the convention described above. This is very similar to the stage to age procedures used for fish eggs, but with both steps collapsed in one and having day instead of hours as the age unit. In parallel to the stage to age conversion, temperature may affect the degeneration rate of POFs with time, although this has never been properly evaluated and it is something that deserves further research. In any case, the range of temperatures for spawning and those experiencing the spawners after spawning is probably badly represented by surface temperature and its knowledge may require a very detailed understanding of the occupation of the space with time of those fishes. Experimental approaches to that problem may require changing temperatures along the day time for the captive spawners and should be carefully designed in order to emulate the actual environment of degeneration of ovaries for wild females.

In analogy with the problems in otolith age determination, for the identification and age determination of POFs there should be cross validation between readers. Sets of standard photos of well classified POFs should be available for each population subject to DEPM estimations. These sets of photos would serve as training sets for new readers and for tracing changes with time of the way POFs are interpreted and understood. Regular exchange of POF material and workshops on POF age determination can be devised when several readers are working on the same stock as a way to evaluate and improve accuracy and consistency among readers.

Minimal number of female gonads to examine per adult sample may be around 20 (25 or 30 is preferable) but as usual is a matter of balance between cost and accuracy desired for the expected level of spawning fraction. Specific evaluation of sampling requirements should be established for each stock being studied with the DEPM, after some years of application have allowed sufficient data to be gathered. Finally, it is worth noting the following:

- The time of capture should be taken into account for the dating of POFs.

- It is worth observing the ovary at different levels of augmentations at the microscope and examine several POFs of the same ovary (the most common ones). There may be small differences in the level of degeneration among POFs of the same ovary (if the spawning extended 1 - 2 hours around the peak spawning time), although those differences should be minor and should not affect the final age determination.

- The area occupied by POFs often provides a crude indication of the degree of degeneration, with bigger areas within the slide occupied by POFs usually being associated with younger follicles. With time they tend to reduce in volume and area and the probability of finding one will be smaller, so it may be helpful cutting the gonad section in a perpendicular direction or getting several slides from different portions of the ovary.

- There is information within each sample that may help in the age determination of the POFs of every female in the sample. The comparison of the POFs found in different females of the same sample can give indications for the identification of the different cohorts present among the females of the sample.

4.1.3 Gonad preparation for histological analysis

After extraction ovaries must be immediately stored and preserved. Ovaries are usually preserved in modified Bodian's AAF (Perez *et al.* 1992) or in 4% buffered formaldehyde solution (Hunter *et al.* 1985). In the laboratory, a tissue sample is taken from one lobe of each gonad, weighted, dehydrated with alcohol and embedded in paraffin or resin. The resulting blocks are sectioned (sections around 3-6μ thick), mounted in slides and stained by Harris' Hematoxylin and Eosin procedure. In hydrated females the other gonad lobe is used for the counting of hydrated oocytes (for the estimation of batch fecundity), if postovulatory follicles are not histologically detected.

During the ICES Workshop on sardine DEPM (ICES 2000a), differences in the quality of the histological slides derived from the Portuguese and Spanish surveys of 1999 were observed. Portuguese slides (gonads fixed in AFA and mounted in resin) had less clearly defined follicle structures than Spanish slides (gonads fixed in buffered formaldehyde and mounted in paraffin). Two possible explanations were proposed: i) the difference of the fixative or ii) the longer time interval between fish collection and gonad fixation (many portuguese samples were collected by commercial purse-seiners). Given the importance of the quality of histological slides in the identification and dating of POFs, and thus the estimation of spawning fraction, an experiment was set-up to test the above hypotheses (Stratoudakis *et al.* 2001 – WD SGSBSA).

In 7 samples of 20 female sardines, the 2 lobes from the gonad of each fish were separated. In the first group of 10 fish (experiment 1), one lobe of the gonad was stored in AFA and the other in buffered formaldehyde. In the second group (experiment 2), one lobe was immediately immersed in AFA, while the second was left within the fish body cavity for 5 hours before immersion in AFA. All histological slides, were inspected and the following data were recorded: microscopical maturation stage, presence of atresia, presence of POFs, macroscopic aspect of slide, microscopic aspect of slide. Macroscopic and microscopic aspects of slide refer to the readability of the slide and the quality of the gonad preparation in a scale from 1 (very bad) to 5 (very good). Table 4.1.3.1 shows the results of the paired t-tests for the macroscopic and microscopic evaluation of the two gonad lobes in each experiment. The comparisons show a significant difference in microscopic and macroscopic aspect of the two treatments in experiment 1 (AFA vs formalin), while no differences are found in experiment 2 (immediate vs 5 hours delayed immersion in AFA). POFs were only identified in 4 fish and in one case only in one lobe were clearly visible. Atresia was evident in many fish and agreement between lobes was generally good. Overall these results do not permit to identify the reasons for the differences in quality between Portuguese and Spanish histological slides in the 1999 DEPM survey. The significantly higher quality of lobes preserved in AFA (confirming previous tests in 1988) leaves few doubts that AFA is a better means of fixation for sardine gonads. On the other hand, the 5 hours delay in gonad preservation did not introduce any significant deterioration in the general aspect of the gonad. This suggests that samples from commercial purse seiners preserved soon after fishing can be used for DEPM purposes, provided that fish were also stored in low temperatures to avoid POF deterioration.

For future applications it is recommended that, despite its better performance, AFA is not used due to its higher toxicity, while it is preferable that commercial vessels that collecting samples for DEPM do so in specifically furnished containers fill-in with formaldehyde solution.

4.1.4 Inter-calibration and reference collection of POFs

Experienced observers of histological slides from sardine and anchovy gonads performed a small inter-calibration exercise during the SG, discussed the staging of POFs and agreed on a reference collection of follicle images for future guidelines. The inter-calibration exercise for sardine and anchovy POF dating involved 6 observers and 12 slides with POFs, 6 for sardine and 6 for anchovy. From the 6 observers, 3 were experienced in sardine (observer 1, 2 and 3) and 2

in anchovy (observer 5 and 6). Observer 4 has experience with *Sardinops sagax*. Observers 2 and 4 observed *Sardina pilchardus* POFs during this exercise.for the first time. The inter-calibration results are shown in Table 4.1.4.1. These results show that there was a better agreement between observers experienced in the same species.

The staging of POFs is based on the follicle structure, time of spawning and of fish capture. It was agreed to classify the POFs in four stages, Day-0, Day-1, Day-2 and Day-3, based in Hunter and Macewicz (1985), Motos (1994) and Perez *et al.* (1992), while the difference in the time intervals between anchovy and sardine ageing conventions relates to the fact that traditionally only anchovy samples have been obtained throughout the deil circle:

- Day-0 (first row of Annex III for sardine and first photo of Annex IV for anchovy) involves females with recent POFs, (0-6 hours old in sardine and anchovy), with or without hydrated oocytes), cord-like cell layers forming loose folds or loops, well evident lumen, a theca layer of cells with well organised nucleus, distinguishable from the granulosa and showing no signs of follicle degeneration.

- Day-1 (second row of Annex III for sardine and second photo of Annex IV for anchovy) involves gonads with POFs (7-30 hours old in anchovy; 18-30 hours in sardine) showing signs of initial degeneration, shrunk with fewer folds and loops, a less evident lumen and less organised theca and granulosa.

- Day-2 POFs (31-54 hours old in anchovy; 42-54 hours in sardine) are more degenerated and shrunk, theca and granulosa are hardly distinguishable, cellular organisation not evident and lumen much reduced.

- Day-3 POFs (more than 54 hours in anchovy; more than 60 hours in sardine) are characterised by a major reduction in their structure and occurrence. In general they present a triangular shape and the theca layer is totally undistinguishable from the granulosa layer. The lumen is very reduced or absent.

4.2 Adult survey design

4.2.1 Effort allocation in the presence of spatial structure in adult parameters

Currently, the adult parameters used in sardine and anchovy DEPM estimates assume that the adults used are randomly sampled from a uniform and homogeneous population. There are two difficulties with this in practice, since the assumption ignores both the possibility of spatial variation in the animals' characteristics and the non-random nature of the sampling methodology. As with most other adult survey designs used for in DEPM applications (Piquelle and Stauffer 1985), the adult sardine and anchovy are selected by judgement sampling, based on real time acoustic or egg density observations. Unfortunately, as is often the case, 'judgement sampling' (which does not represent a statistically identifiable survey design) is used as a euphemism for the subjective allocation of sampling effort taking some consideration of the relative (fish or egg abundance) in the close vicinity of the surveyed area at any given period (claims of probability proportional to size approximation under judgement sampling clearly do not hold when the "size" information is simultaneously collected during the survey).

The case of anchovy in South Africa (Armstrong *et al.* 1988; Shelton *et al.* 1993) provides a good model of the alternative, where a stratified survey design (based on results from previous surveys) has been applied, rather than some arbitrary post-stratification imposed on the results of a less structured methodology. In general, the small number of adult samples available for most DEPM studies has limited the calculation of adult parameters to simple overall mean values for the whole of each survey. This however can lead to considerable bias in cases where abundance and population parameters are not homogenously distributed across a survey area (Shelton *et al.* 1993, ICES 2000a). After the 2000 WKSBS, the 1999 sardine DEPM data from Portugal were reviewed with this in mind (Stratoudakis and Fryer 2000), and simulations used to identify the impact of design and estimation under various population and sampling scenarios. A series of populations consisting of two strata were constructed, with fish abundance and mean spawning fraction in each stratum allowed to vary widely, and egg production, sex ratio and batch fecundity assumed known without error. Each population was then sampled using simple random sampling and various forms of stratified random sampling (with allocation proportional to either survey area or fish abundance, or allocated optimally). Ignoring spatial structure in spawning fraction led to very biased and imprecise estimates of fish abundance (Figure 4.2.1.1). In the population scenario that appeared to most closely resemble the 1999 Portuguese DEPM survey, the bias was –25%, suggesting that un-stratified estimation can seriously underestimates the true SSB if a significant difference in adult abundance and mean spawning fraction actually occurs across the Portuguese survey area. Stratified random sampling with allocation proportional to population density and optimal allocation both outperformed allocation proportional to area, and were robust to moderate levels of misallocation (Figure 4.2.1.2).

4.2.2 Acoustic and DEPM surveys from a single vessel

Up to now, acoustic and DEPM surveys for sardine and anchovy in Atlantic European waters have been conducted separately. However, both covered the same area, and in most cases at practically the same time. In such cases, the fishing stations for the acoustic survey also provides the adult samples for the DEPM. The main reasons for using two vessels instead of one were:

- Limited vessel space did not allow different teams to operate on a 24 hours basis.
- For acoustic purposes the vessels have to steam without large changes in vessel speed. Otherwise, air bubbles will appear in the resonant surface of the transducer making the echogram noisier and unreadable.
- The survey time would increase considerably if the combined survey accounted for the same survey design as stated in the previous DEPM surveys.

Since 1998, a new survey strategy for the acoustic surveys in ICES Sub-Areas VIII and IX was adopted (ICES CM 1999/G:06). Both acoustic records and fishing stations are collected only during light hours. Night is used to perform CTD casts and additional samples to characterise the surveyed area (i.e. plankton tows). Since 2000, under the frame of the PELASSES project acoustic surveys routinely undertake sampling using CUFES, but also some CalVET tows are obtained in order to test CUFES as a quantitative device for the estimation of egg production.

Acoustic surveys for Atlanto-Iberian sardine cover the continental shelf with transects 8 nmi apart. Inter-transect distance has been calculated taking into account the spatial distribution of sardine, which seems to be correlated up to 6-7 nmi on average. In the Portuguese area, transects reach out to 150-200 m depth whilst in the Spanish case transects are extended further to cover the whole pelagic fish distribution, including blue whiting and horse mackerel (i.e. between 500 and 1000 m isobath). In total, the Portuguese survey has 65-70 transects giving a total of around 1000 nmi steamed. In the Spanish area, the number of transects are around 55 with a total of 950 nmi. On account of the inter-transects distance, the whole grid has 1525 nmi in Portugal and 1353 nmi in Spain. Assuming a vessel speed of 10 knots and 12 hours of work each day, then 13 and 11 days are needed to cover the survey area. On the other hand, about 40 to 50 fishing station are also performed during each survey. Assuming a mean of 2 hours for the total fishing operation, a standard acoustic survey should take around 20 days in each area. If calibration of the acoustic equipment and general checking are also included, total effective survey time would be 23 days. However, experience has shown that bad weather (not uncommon during the Iberian winter/spring) and other unpredicted events (vessel or equipment failures) can extend the overall survey time up to 28-30 days.

In the case of the Atlanto-Iberian sardine, the following procedure could be adopted to combine both surveys in one boat: the acoustic track would remain as stated, but extending further the end of the tracks in order to guarantee that the egg distribution is fully covered. For this purpose, historical egg distribution data could be revised to adapt the length of transects to the expected maximum egg distribution. Also complementary methods using satellite images to delineate high productive areas where fish might be expected to occur, could be used. Assuming a 5 nmi lineal increase in each acoustic transect the survey track would become 1325 nmi instead of 1000 nmi in the Portuguese area and 1200 instead 950 nmi in the Spanish area. The inter-transects not change, representing only 2 more days of effective survey time. CUFES samples would be taken at the same time of the acoustic records to delimitate the egg distribution. Nevertheless, in order to achieve a more reliable delimitation, samples should be examined fixed (around 30-60 min later). Once an area is covered (i.e. each ICES Sub-Division), it will revisited to perform CalVET stations. Sampling intensity could be modulated to account for the spatial egg distribution derived from the CUFES samples. Table 4.2.2.1 summarises the features of the proposed survey grid from the acoustic needs. Both survey track and fishing station are done during daytime and working days allowing 23 nights to be used for other purposes. Table 4.2.2.2 also summarises the total time needed to cover the survey track making CalVET stations each 3 nmi and assuming 10 minutes to perform each station and 5 knots to steam within transects and 10 knots to steam between transects. The first two rows present a scenario assuming 16 nmi between tracks and the last two assuming 8 nmi as inter-transect distance. In the former case CalVET sampling could be performed without any significant increase in survey days whilst in the later, around 8 extra days could be needed.

The advantages of using this procedure are:

- Reduce ship time from around 55-60 days to 30-35, including some extra days lost for bad weather conditions.

- Adult fish for DEPM purposes would be sampled taking into account not only the egg distribution but also the adult distribution gathered from the acoustic records.

- The number of CalVET stations would be reduced if sampling was adapted to the distribution obtained from the CUFES.

Main disadvantages are:

- More time would e saved if most of the CalVET samples are undertook during night time. This would imply a reduction in the spread of mean cohort ages, possibly affecting the quality of the egg production curve fit.

- Inter-transect distance of 16 nmi could satisfy the spatial independence assumption between transects for the DEPM methodology. However, the spatial resolution for mapping or 2-D modelling would be scarce.

- The adaptive sampling for CalVET on account of the CUFES egg distribution will be unbiased if stationarity for both adults and eggs is assumed. Weather conditions in spring may change suddenly and could break the spatial structure seen few days ago. Also changes on adult fish distribution might happen which could also change the egg distribution.

- The above calculation on time did not include CTD casts. Assuming CTD profiles only in the half of the total stations up to 250 m as maximum depth, practically two days more should be added.

- The survey could be feasible if different teams can work properly aboard, which depend on the size of the research vessel. At least five teams are needed (acoustic, CaLVET, CUFES, fish sampling and CTD). Therefore, the possibility for combining methods is heavily dependent on the size of the ship.

On account of these particularities, prior to merging both methods in a single ship, the possible problems in egg production estimation due to the allocation of the majority of the samples at a particular part of the day should be investigated. On the other hand, the adaptive strategy as suggested in this section was successfully applied to the Pacific sardine off California spawning biomass (Lo et al, 2001). Also some promising results were achieved during the surveys undertook within the frame of the PELASSES project. The use of CUFES in both the acoustic and the CalVET coverage also will help to check possible short-time changes in the egg spatial distribution. It is also suggested to conduct this combined survey in a small pilot area prior to extending it to the whole distribution. In summary, the issue should be further considered before proposing any implementation of a full-scale joint survey for DEPM purposes. Ongoing research should provide new information for evaluation during the next SG meeting.

4.3 Estimation of adult parameters and spawning biomass

4.3.1 Estimation of the spawning fraction

Spawning fraction is the proportion of adult females spawning per day. Hunter and Goldberg (1980) developed a histological method to determine spawning fraction by recording the percentages of females with post-ovulatory follicles (POFs) of a certain age remaining in the ovaries after the spawning (fraction of females from the same daily cohort of spawners in the sample, Hunter and Macewicz 1985). Since the females that are preparing to spawn are characterised by nuclear migration in the oocyte and those that are ready to spawn show hydrated oocytes (than can be noticed by the increase of the volume of the ovaries), in principle the incidence of those pre-spawning developmental conditions in the sample could also be used as an estimate of the spawning fraction. However, for anchovy the hydrated females are over-sampled around the spawning time in comparison to that of females with POFs day 1 or 2 (Piquelle and Stauffer 1985, Hunter and Macewicz 1985, Santiago and Sanz 1992). Over-sampling at the hydrated stage may also occur in other species. This feature of over-sampling may even appear several hours before hydration (during the nuclear migration phase in oocytes, Motos and Uriarte WD - SGSBSA). This bias complicates the use of the incidence of day 0 (or spawners during the day of sampling) to estimate the spawning fraction. Nevertheless the possible influence of over-sampling day 0 spawners must be checked for each population under DEPM application to determine if day 0 should be used.

In temperate environments the incidence of day 1 or day 1 and 2 POFs are usually considered for the estimation of the spawning fraction, provided that day 2 degenerating POFs can be distinguished from older POFs. The idea of using both cohorts of spawning fishes was implemented in order to reduce the large variance of the spawning fraction estimate. In both cases, in the presence of over-sampling for the day 0 females, a correction is made so that frequency of

day 0 is replaced by that of day 1 (Picquelle and Stauffer 1985) or by the average frequency of day 1 and 2 POFs (Alheit 1993) respectively. In some cases, over-sampling may appear even the night before spawning, resulting in two cohorts of spawners being simultaneously over-sampled: the ones that are spawning during that day and the ones which will spawn during the next day, as in the Bay of Biscay anchovy (Motos & Uriarte 2001, WD - SGSBSA). In this case a double substitution has been applied to obtain unbiased estimates during those hours. All these deviations of sampled females from expected frequencies are likely linked to the spawning behaviour of fishes (Hunter and Goldberg 1980, Alheit 1985) and the amount of bias they can generate in the estimation of the spawning fraction changes according to the time of sampling. In addition the extent of over-sampling may depend upon the fishing gear (Alheit 1985). Hence, understanding spawning dynamics and behaviour, gear selectivity, and the way they affect the estimation of spawning fraction is important for determining optimum periods of sampling that can reduce the potential estimation bias.

The expertise of ageing degenerating POFs is the base of this spawning fraction estimation and should also be carefully calibrated and tested for the correct implementation of the DEPM. Several approaches exist for calibrating the ovarian stages used to estimate spawning fraction. Induction of spawning in the laboratory serves to establish unequivocally the rates of POF degeneration (Hunter and Goldberg 1980, Hunter and Macewicz 1985), but this is biologically difficult for some species. The aquarium can also simply serve to sustain captive fishes just after spawning for several days in order to perceive the rates of POF degeneration through successive sampling. Observations can also be made at sea in boats with live bait tanks (Motos 1994). As temperature affects the rate of POF degeneration, it is desirable to consider its effect on the resorption rate of follicles, designing experiments with realistic temperature variations. Finally, to understand the spawning dynamics of small pelagics, the fish availability to gear and the exact duration of reproductive stages, repeated sampling at different times throughout the day over a spawning ground and with different gears is desirable.

Additional information regarding spawning activity can be obtained by examination of all reproductive stages that may exist in the ovary. For instance the number of females with POFs already showing nuclear migration would suggest higher spawning frequencies than in cases those females do not show it. Presence of multiple stages (hydrated, plus POFs of more than one age) are indicative of rapid spawning rate. Concomitant information among samples about non observations may be as useful as the observation made on the spawning conditions: For instance the amount of samples without day 1 or without day 2 or simply without any sign of POFs would have information on the overall level of S for the population. Care should be taken that one does not use the numbers of Pos at various ages as an indicator of the extent of past spawning, since smaller (older POFs) will have a lower frequency simply because of their size.

4.3.2 Bootstrap estimation of survey estimates

Nonparametric bootstrapping is conceptually attractive for use in DEPM estimates because the data collected for estimation of both adult parameters and egg production are based on relatively small samples; these data are thus inherently difficult to assess statistically because it is impossible to elucidate with any certainty the underlying distribution (Jackson and Cheng 2001). While this distribution-free method produces parameter estimates that may be much the same as traditional approaches, the confidence limits can be much wider than those estimated using the delta method (e.g. Fletcher *et al.*, 1996). However, this should not be seen as a problem with this approach, but rather as a more realistic assessment of the variability and inherent measurement limitations of the DEPM method. The wider confidence intervals may be indicative that incorrectly assuming some underlying statistical distribution can produce artificially low CVs. Furthermore, bootstrapping can also be used to assess the level of influence of individual "outliers". However, as outliers in DEPM are in fact real data, identifying an influence does not necessarily provide a solution of how to deal with rare samples of very high egg densities. Such cases are ideally identified in real time and be subjected to adaptive sampling.

4.3.3 Estimation in the presence of spatial structure in adult parameters

The environmental differences across the study areas make it hard to believe that all the adult parameters required for the DEPM remain entirely constant. Given appropriate sampling strategies, weighted means can be used to provide an unbiased estimate of the overall average values (Armstrong *et al.* 1988, Shelton et al. 1993), though it is much harder to then calculate the precision of the resulting biomass. Within both the assessments, post-stratification has been applied as a means to represent the obvious spatial structure present. This has provided a workable solution, though it is accepted that it is not ideal, particularly because no real statistical justification of the decisions used in the setting of the strata boundaries has been provided. Simulations carried out of the effects of stratification on the Portuguese sardine data from 1999 suggest that choice of stratification can have a very large effect on the final biomass estimates produced (Stratoudakis and Fryer 2000).

A more satisfactory and defensible strategy would be to either identify some strata by identifying differences in their character based on data outside that used to for the parameter estimation or by fitting some function to the data from the samples. Unfortunately both these approaches require additional data, and detailed information on the adult populations is in very short supply. Taking the 1999 Portuguese sardine case as an example, fitting a GLM (binomial errors with logit link) to the 35 adult samples suggests a significant decrease in the spawning fraction with distance from the northern border, but one extremely high value comes from the southern end of the study (Figure 4.3.3.1). A GAM fitted to the same data produces a very different pattern (figure 4.3.3.2) which,, along with the obvious patterning in the GLMs residuals and the wide confidence intervals around the GAM (which include zero almost everywhere), make it hard to justify its acceptance as a real phenomenon.

Overall it appears that a far larger number of adult samples would need to be taken for any reliable representation of spatial structure in the adult population to be included in the estimation process.

4.3.4 Requirements for estimating mature population in numbers by age

Traditionally, the DEPM has only been applied to estimate the spawning biomass of small pelagic fish such as anchovies and sardines (Alheit 1993). However, the level of adult sampling required for the estimation of the adult fecundity parameters may allow getting to population at age estimates when the spawning area of the population is sufficiently covered. For the Bay of Biscay anchovy (which is fully mature from its first year of life) the DEP estimator of biomass has been extended to produce population at age estimates, including derivation of variances by the delta method (Uriarte 2001). This is accomplished by dividing the biomass by the mean weight of fishes and by the average proportions at age in the population. The situation where an existing age length key has to be used is also considered. The method has been applied during the 1990s to the anchovy in the Bay of Biscay. The major requirement for this estimate is having sufficient sampling over all major spawning grounds and adequately weighting the parameters W (mean fish weight in the population) and Pa (proportion at age in the population) with weighting factors proportional to numbers (not to biomass) in the area they represent. The generalization of this approach to other species not fully mature at age 1, subject to maturity ogive, deserves further research since more parameters need to be included in the model.

Table 4.1.3.1: Mean difference in slide quality (macroscopic and microscopic, scale 1-5), paired t-values and probability of Ho (95% CI includes 0) in the two experiments testing fixative (experiment 1) and duration to fixation (experiment 2).

Case	Difference	t	p
Experiment 1 – Micro	0.62	3.80	**<0.01**
Experiment 1 - Macro	0.79	4.71	**<0.01**
Experiment 2 - Micro	0.17	1.23	0.22
Experiment 2 - Macro	0.16	0.35	0.73

Table 4.1.4.1: Inter-calibration of POF ageing (ranking from youngest to eldest) for 5 sardine and 5 anchovy histological preparations with post ovulatory follicles.

	OBSERVER		**1**	**2**	**3**	**4**	**5**	**6**
	SAMPLE	IdN	FTYPE	FTYPE	FTYPE	FTYPE	FTYPE	FTYPE
SARDINE	CAM1	3	D1	D1	D2	D0	D2	D1
	CAM1	6	D2	D1	D2	D0	D1	D1
	CAM1	51	D2	D1-D2	D2	D2	D1	D2
	CAM1	57	D1	ATRESIA	D1	D2	D2	D2
	AVE2	10	D3		D3	HYDRATED	D3	D3
	AVE2	44	D3	D3	D3	D3	D3	D3
ANCHOVY	522	28	D2		D2		D1	D2
	526	56	D2		D2		D2	D2
	526	14	D2		D3		D2	D3
	P7	5	D2		D2		D3	D3
	526	45	D1		D1		D0	D0
	527	49	D2		D1		D2	D1

Table 4.2.2.1: Summary properties of the proposed survey grid based on the acoustic needs.

	No of tracks	**Track***	**Intertrack**	**Total**	**Time (hs)-Days**	**Fishing st**	**Time (hs)-Days**	**M. Along dist**	**M. Accros Dist**
Portugal	65	1325	512	1837	183.7-15.31	45	90-8	520	20.38
Spain	53	1200	430	1630	163-13.58	50	100-8	524	22.64

Table 4.2.2.2: Summary of the total time needed to cover the survey track making calvet stations each 3 nmi and assuming 10 minutes to perform each station and 5 knots to steam between transects and 10 knots to steam between transects. First two rows present a scenario assuming 16 nmi between tracks and the last two assuming 8 nmi as inter-transect distance. In the former case calvets could be done without any significant increase in survey days whilst in the later, around 8 extra could be needed.

		No tran.	Distance	No st	Days	Steaming d	Total
16 nmi spaced	**Portugal**	33	673	224	1.56	7.81	9.36
	Spain	27	611	204	1.42	6.89	8.31
8 nmi spaced	**Portugal**	65	1325	442	3.07	13.21	16.28
	Spain	53	1200	400	2.78	11.77	14.54

Table 5.2.1: Brief summary of the 2002 DEPM surveys for sardine and anchovy in Atlantic European waters.

EGG SURVEY

Issue	**Sardine DEPM**		**Anchovy DEPM**
	Portugal	**Spain**	**Spain**
Survey direction	S-N	W-E	S-N
Survey period	January	March	May
Survey area	Cadiz+Portugal	Northern Spain	Bay of Biscay
Sampling unit	transect	transect	transect/station
Distance between (miles)	8	8	15
Distance within (miles)	3/6	3	3
No of transects	65-70	55-60	
Mesh size (CalVET)	150	150	150
Weight on sampler	20-30	35	45
Flowmeter	Y	Y	Y
Angulometer	Y	Y	Y
Max acceptable angle	20	20	20
Temperature sensor	Minilog	Minilog	Minilog
Depth to register T (m)	10	10	10

CTD	N	Y	Y
Sampling depth	150	100	100
Towing speed (m/sec)	1	1	1
Samplers used	1	1	2
Staged eggs	all	all	50/75/all
Use CUFES in design?	Y	Y	Y

ADULT SURVEY

Separate vessel?	N	Y	Y
No of hauls aimed	50	40	40
A priori effort allocation?	Y	N	N
Design			
Use commercial samples?	Y	Y	Y
No of fish per haul	100	100	60
Fish for histology	25-30	25-30	25 non-hydrated
Fixative	AFA/formalin	formalin	formalin

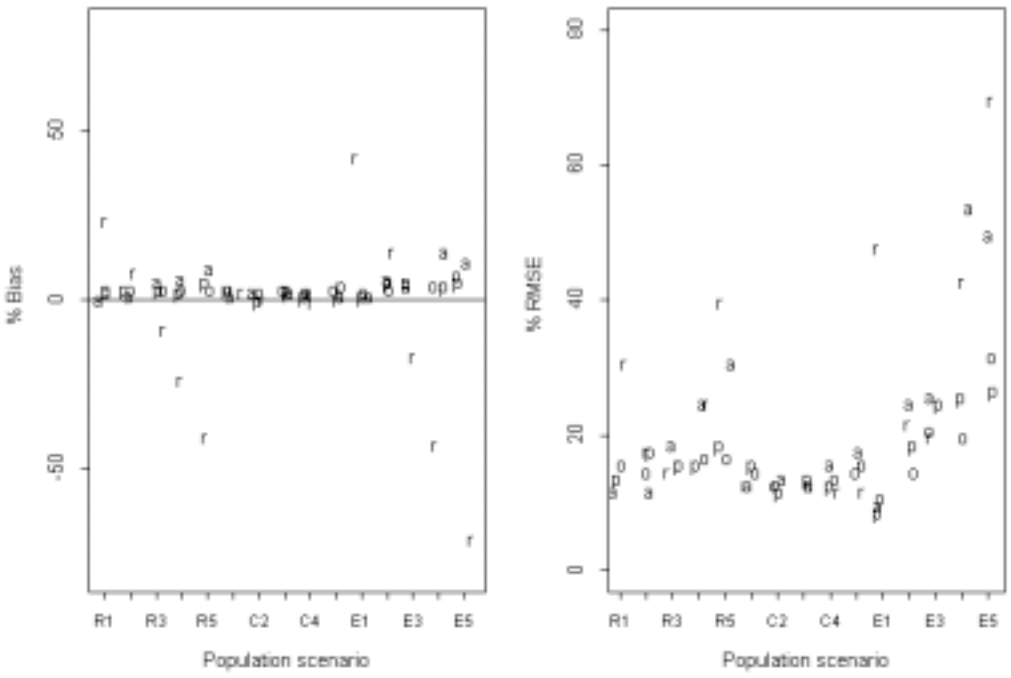


Figure 4.2.1.1: : Percent bias (left) and root mean square error (right) in estimated fish abundance using the 4 sampling designs (r – simple random, a – stratified random in proportion to area, p – stratified random in proportion to stratum abundance, o – stratified optimally to minimise estimated variance) in 15 population scenaria (five relative abundance scenaria (k = 0.1 to 0.9) for realistic (R), common (C) and extreme (E) spawning fraction scenaria).

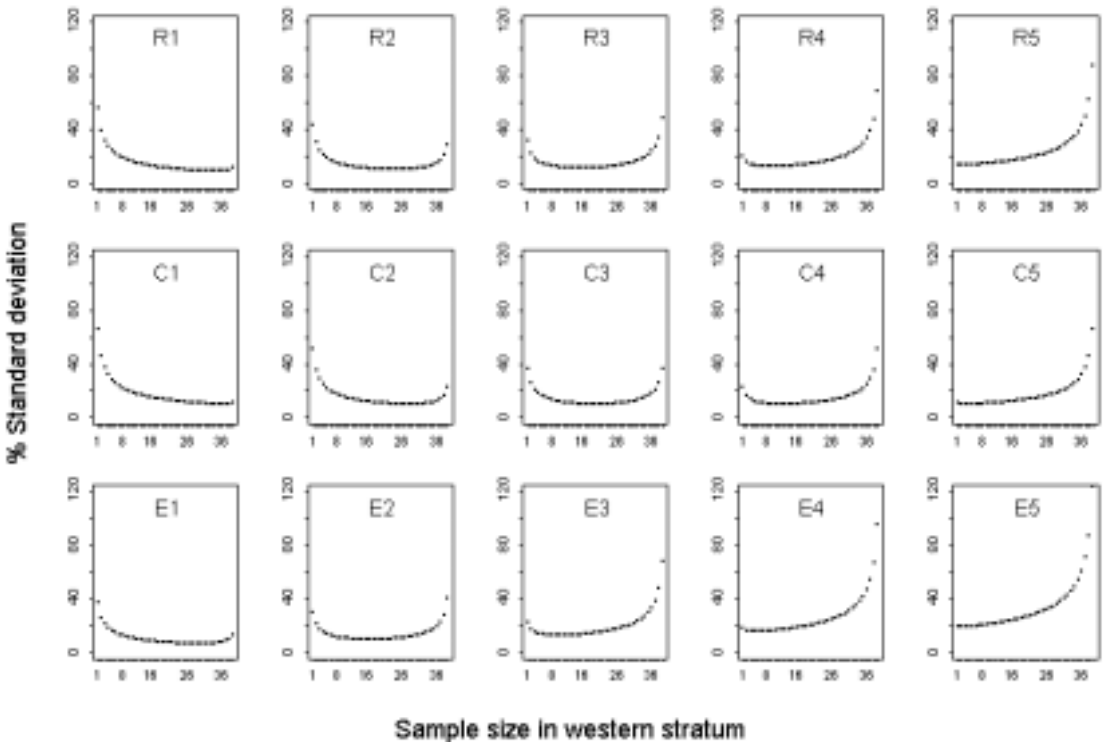


Figure 4.2.1.2: Profiles of theoretically estimated % standard deviation (standard deviation devided by abundance) of estimated fish abundance for each of the 15 population scenaria and with sampling allocation ranging from 1/39 to 39/1 for western/southern strata.

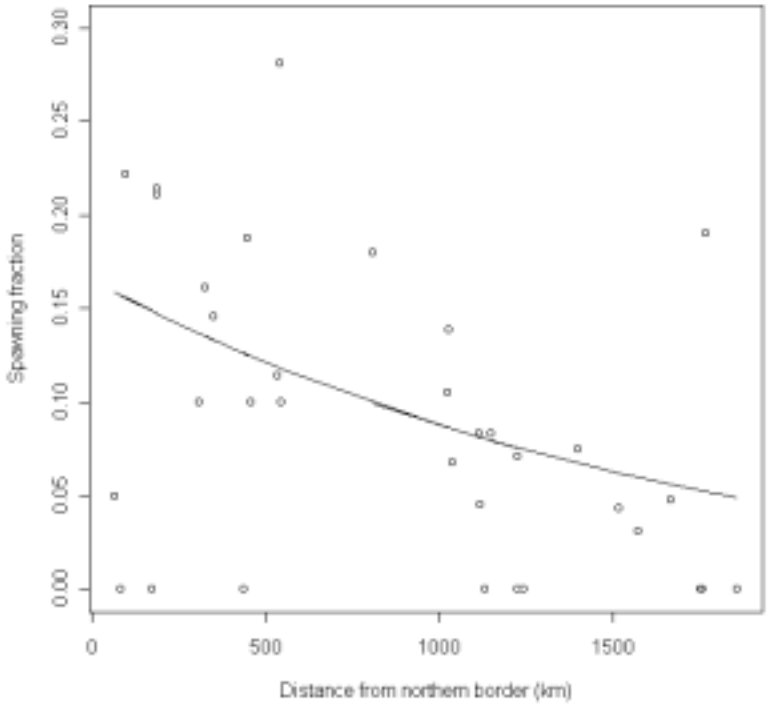


Figure 4.3.3.1: GLM (binomial errors with logit link) for spawning fraction as a linear function of the distance from northern Portuguese border during the 1999 Portuguese DEPM survey of sardine.

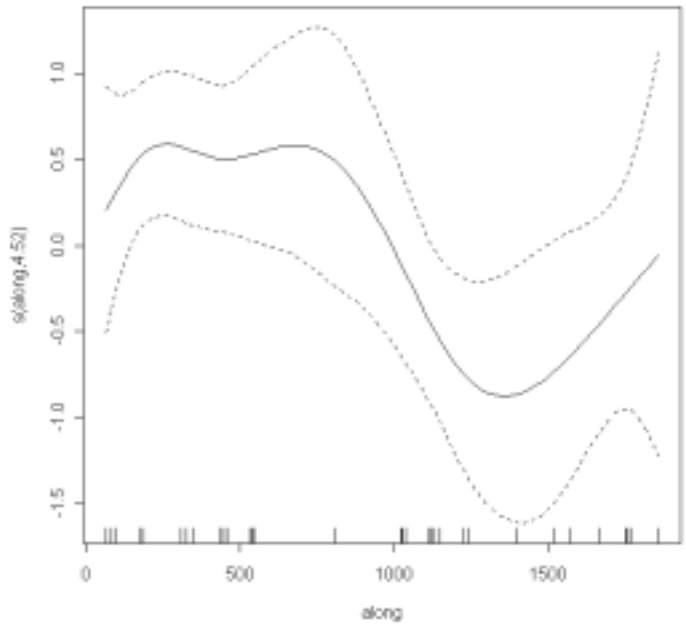


Figure 4.3.3.2: Partial effect of distance from northern Portuguese coast to sardine spawning fraction when a GAM (binomial errors with logit link) is fitted to the 1999 Portuguese DEPM data.

5 DESIGN OF 2002 SURVEYS

5.1 General issues

The egg surveys in 2002 will continue to be CalVET-based, with CUFES only providing auxiliary information for station spacing along transects or for the decision to define the oceanic limit of transects. It was decided that despite the obvious appeal of the CUFES application in the Californian sardine DEPM survey (Lo *et al.* 2001), neither sufficient information on the CUFES/CalVET relationship is currently available to establish the rule for distinguishing low from high-density areas, nor the need for fast shipping through large areas of very low density is as pronounced as in California. For this, CUFES will be used to define qualitatively areas requiring more intense CalVET sampling and might in some cases replace CalVET for the decision to identify the oceanic boundary of the transect. Arguably, this is a minimal use of CUFES but even this application can lead to some reduction in the number of CalVET tows and, equally important, can increase the number of vertical hauls with eggs, thus contributing to more data for the estimation of egg mortality and production (see section 3.3.3).

A total of 40-50 fish samples are aimed at each survey, possibly being partly supplied by commercial fishing vessels operating in the vicinity. Commercial samples need to be immediately stored in formaldehyde solution and collection date should never exceed 1 week from the egg survey at a given area. 50-100 randomly selected fish should be biologically sampled in each sample and 20-30 female gonads need to be stored for histological analysis. It is suggested that otoliths from the biologically sampled fish are also stored for eventual future use. Sampling of hydrated females will be opportunistic, aiming at 100 fish spread across the survey area.

5.2 Issues specific to the Iberian DEPM surveys

The Portuguese survey will take place in January/February and will cover the Gulf of Cadiz and the Portuguese coast. Because more than 1month will intervene between the Portuguese and the Spanish survey, an attempt will be made to cover rapidly the western Galician coast at the end of the Portuguese survey. The egg survey will be slightly modified from previous ones to follow the design of acoustic surveys and to be in closer accord with the Spanish egg survey. Thus, instead of the 6 x 6 nmi regular grid, transects will be spaced by 8 nmi, while CalVET stations along transect will be spaced every 3 or 6 nmi depending on local density (identified with the aid of CUFES). Although this modification possibly does not sufficiently address the issue of spatial independence between transects, it is in the right direction (increasing inter-transect distance) and contributes to the harmonisation with the Spanish DEPM and the acoustic surveys.

The Portuguese survey will be carried out with a single vessel (RV Noruega), aiming to perform all plankton and most of the adult sampling simultaneously. Additional fish samples will be requested from the commercial purse-seine fleet, aiming a total of 50 sardine samples distributed roughly in proportion to the regional sardine abundance that will result from the November 2001 acoustic survey. Difficulties are anticipated in the second leg of the survey (northern Portugal) when DEPM sampling will probably coincide with the period of closure to the purse-seine fishery, thus limiting the options for extra sampling via the commercial vessels. It should also be noted that during the Portuguese DEPM survey a first attempt to perform DEPM for anchovy in southern IXa sub-area (Algarve and Cadiz) will be made.

In the Portuguese egg survey, a double CalVET sampler with 150 μm mesh will be deployed to a maximum depth of 150m. The quality of the tow will be monitored on real time by an angulometer, adjusting or repeating the tow in cases that the stray angle exceeds 20^{o} from vertical. Flowmeters will be used to verify a posteriori the quality of tows. Vertical temperature profiles will be obtained from a Minilog recorder attached to the egg sampler and the temperature register at 10m depth will be used for the ageing of staged eggs. Towing speed will be 1m/sec and the samples from both samplers will be stored. CUFES samples (4 min) will be taken during CalVET and stored for future analysis.

The Spanish DEPM survey will take place in March/April and will cover the northern Spanish coast and part of the southern French coast. Because more than 1month will intervene between the Portuguese and the Spanish survey, an attempt will be made to cover rapidly the northern Portuguese coast at the start of the Spanish survey. Egg survey design will remain unaltered to previous, although CUFES will be used to explore offshore patches of sardine eggs that were possibly under-sampled in 1997 and 1999. The Spanish survey will use 2 vessels, adult samples being obtained by RV Thalassa during the spring acoustic survey and eggs being obtained by RV Cornide Saavedra.

5.3 Issues specific to the Bay of Biscay DEPM survey

The anchovy DEPM survey for the Bay of Biscay will take place in May/June 2002 and will cover the Spanish eastern Cantabrian Sea and the southern French coast covering the usual spawning grounds (ranging at least from 5ºW to

47ºN). A survey for egg sampling will be organized by AZTI, and adult sampling will be obtained through the French acoustic survey (if scheduled for the same dates as the egg cruise). If not, AZTI will try to charter a vessel capable of both sampling egg and adults of anchovy. In any case, adult sampling during the survey will be complemented with samples taken opportunistically on board the Spanish and French commercial fishing fleets of purse seines and pelagic trawlers.

Egg sampling will make use of the CalVET net (Smith *et al.* 1985) and will follow the historical systematic central sampling scheme. Double CalVET net or PAIROVET will be used. Samples will be taken 3 miles apart from each other, along radials perpendicular to shore spaced 15 miles (45 square miles sampling units). Flowmeters in both CalVET nets will be used; mesh size of 150µm; a 45 kg depressor; and a depth and temperature sensor coupled to the net. CTD casts will be set in pre-selected stations. Offshore edge of the spawning area is considered to be reached by three consecutive samples with 0 anchovy eggs or 6 nm of CUFES empty of eggs. CUFES will be continuously checked on board and will be use to intensify or relax (to space in 6 nm the PPAIROVET) sampling.

If no adult sampling would be achieved then, regression methods for the estimation of SSB will have to be applied (as done in other years) based on the relationships between spawning area, daily egg production per unit surface and the biomass obtained in years when complete DEPM is applied.

5.4 Anticipated results for WGMHMSA

With the next DEPM survey for Atlanto-Iberian sardine being planned for early, it is considered impossible to have the 2002 DEPM estimate ready for the September 2002 assessment working group (histological analysis alone is not expected to last less than 6 months). This, together with the revision work on course for the 1997 and 1999 surveys, suggests that a realistic aim is to provide the new SSB estimate of sardine and revised estimates for earlier DEPM surveys in time for the 2003 analytical assessment. In the case of anchovy, preliminary results based on spawning area and egg production are provided as a norm within the year of the survey. Nevertheless, the final 2002 anchovy estimate for the Bay of Biscay will be confirmed during the next SGSBSA meeting, in due time for the 2003 assessment working group.

6 CONCLUSIONS

In relation to the four main objectives set for the Lisbon SG meeting, the following points can be highlighted:

Design of 2002 surveys: The egg surveys in 2002 will continue to be CalVET-based, with CUFES only providing auxiliary information for station spacing along transects and for the decision to define oceanic limit of transects. The sardine DEPM survey will follow the design of acoustic surveys (transects 8 nmi apart) and stations along transects will be spaced from 3-6 nmi depending on CUFES indications. The sampling unit will be the transect, although transects might still be correlated at this distance. The anchovy survey in Biscay will follow a similar design, although in the latter case transects are 15 nmi apart and the sampling units will be stations or transects. A total of 40-50 fish samples are aimed at each survey, possibly being partly supplied by commercial fishing vessels operating in the vicinity. Commercial samples need to be immediately stored in formaldehyde solution and collection date should never exceed 1 week from the egg survey at a given fishing location. 50-100 randomly selected fish should be biologically sampled in each sample and 20-30 female gonads need to be stored for histological analysis. Sampling of hydrated females is opportunistic, aiming at 100 fish spread across the survey area. It should also be noted that during the Portuguese DEPM survey a first attempt to perform DEPM for anchovy in southern IXa sub-area (Algarve and Cadiz) will be made.

Standardisation of DEPM methodologies: Several practical, sampling and estimation aspects related both to egg and adult DEPM parameters were discussed and new work was presented. Short inter-calibration exercises during the SG meeting revealed some between-reader variation in POF cohort definition and (unexpectedly) in egg staging. Reference collections of sardine and anchovy egg stages and POF cohorts were created, but the need for further work in this area is evident (through a dedicated workshop). Interesting new work on incubation experiments set-up and analysis were presented and novel ideas on stage-age modelling are being tested to substitute traditional ageing procedures. The use of the angulometer was shown to be an inexpensive and efficient real-time indicator of inappropriate CalVET towing. GLMs were shown to be a more reliable way to fit the egg production curve, while substantial improvements in GAM fitting and model selection are underway. Work on the Bay of Biscay anchovy demonstrated that over-sampling of females near spawning can also occur at the migratory nucleus phase, thus necessitating additional adjustments to avoid bias in the estimation of spawning fraction.

Adult survey design and estimation: Small number of adult samples continues to plague several DEPM applications, not permitting reliable identification of post-stratification criteria or spatial modelling of adult parameters. Nevertheless,

inappropriate decisions in relation to sample weighting or stratification can lead to biased (in the case of undetected real structure in space) or unnecessarily imprecise (in the case of unjustified post-stratification) estimates of adult parameters. Sensitivity analyses to post-stratification and weighting decisions were recommended, while the use of bootstrap with balanced resampling seems a promising method for variance estimation with sparse data.

CUFES in DEPM: Existing data on the comparative performance of CUFES and CalVET demonstrate a relatively good agreement in egg presence/absence indication, weaker relations in egg abundance estimates and make problematic any comparison of staged eggs. Three ways of integrating CUFES in DEPM egg production design and estimation were considered during the SG. It was agreed that although the potential of CUFES as a primary egg sampler in egg surveys should be explored, current information does not permit advances in this direction. The most thorough application of CUFES to a routine DEPM survey was presented (Pacific sardine in California) and the experience of using CUFES to identify low/high egg abundance areas was discussed. CUFES was also considered a useful sampler for describing the spatial structure of fish eggs and identifying the appropriate distance between sampling units to guarantee statistical independence.

7 RECOMMENDATIONS

Next SGSBSA meeting: The SG recommends that the next meeting of the group will be held during March/April 2003 to permit enough time for all laboratory analysis and preliminary estimation for the 2002 DEPM surveys to be completed. Malaga (Spain) is a possible meeting place, although confirmation from the local IEO laboratory is required. It also recommends that the duration of the SG meeting is extended to 5 days. Finally, on the light of the overseas contributions during the Lisbon meeting, the SG strongly recommends that initiatives to attract scientists with DEPM experience from around the world continue and intensify. The main objectives proposed for the next SGSBSA meeting are:

- Provide final 2002 DEPM estimates for sardine and anchovy in Atlantic European waters.
- Complete the review of previous sardine DEPM estimates and provide a clear and synthetic description of the uncertainties associated with each estimate.
- Decide the most appropriate timing of future DEPM surveys for the Atlanto-Iberian stock of sardine.
- Consult recent developments in the use of CUFES and GAMs to consider their most appropriate application in DEPM surveys and estimation.
- Update work on egg stage-age models, POF dating and spawning fraction estimation.
- Revise the maturity ogive of sardine based on past and 2002 DEPM histological information, in order to clarify its appropriate use in analytical assessment.

Egg incubation experiments, data analysis and ageing methods: The SG recommends that new egg incubation experiments take place for sardine and anchovy, with particular attention on the experimental set-up (selection of appropriate time intervals between observations, recording of egg mortality and newly hatched larvae, replication within temperature and between spawner groups) and the data analysis (multinomial distribution of egg stages given age). It also recommends that comparative analyses between ageing methods are performed and the most appropriate method is selected and made available for routine DEPM purposes.

Estimation of egg production: The SG recommends the use of a generalised linear model (GLM) with a log link and some appropriate error distribution (in most cases negative binomial) for the traditional estimation of egg production. It also recommends that the comparisons between traditional and GAM-based estimation of egg production continue, taking into account very recent statistical developments in model fitting and selection procedures.

Estimation of the variance of the egg production estimates: The group recommends that more work should be undertaken to estimate the appropriate distance between sampling units to ensure no correlation between samples, and thus will improve the variance estimates of the egg production parameter. If necessary, this information can be used to revise the time series by collapsing the existing sampling units (so far sampling stations) into aggregated units (e.g. in transects), which are separated the adequate distance to ensure no correlation of the data.

CUFES applications: CalVET/CUFES comparisons in relation to pelagic fish egg presence and density in the two samplers should be continued. This will help to evaluate the reliability of CUFES as an ichthyoplankton sampler and test for variations in relation to environmental conditions, survey vessel, etc. Finally, the modelling of the vertical distribution of pelagic fish eggs in relation to environmental proxies for water mixing should be concluded in order to permit an evaluation of the potential of the method for routine DEPM surveys.

Intrinsic DEPM assumptions: The DEPM is based on strong assumptions about various parameters in the population remaining constant over space, time and the range of environmental conditions occurring within the study area. The group recommends that the accumulated data from past surveys are used to test some of these assumptions. Simulation studies should also be carried out to investigate the accuracy of the assessments if, as seems likely, reality is actually somewhat more complex than the model allows.

Adult sampling and estimation: It is recommended that the adult survey is always aiming to obtain more than 40-50 independent samples. Samples from fishing vessels can further increase the total number of samples thus permitting more reliable estimation and testing for spatial structures. It is also recommended that sensitivity analyses are carried out to evaluate decisions on weighting and post-stratification, while the use of bootstrap with balanced re-sampling is an interesting alternative for variance estimation.

Spawning fraction: It is recommended that additional work related to factors that affect spawning fraction estimation is performed, particularly in sardine. Laboratory experiments or around the clock adult sampling can provide valuable information for standardising and validating the identification of POF cohorts. Simultaneous analysis of historical data on migrating nucleus, hydrated, and POF samples can provide indirect evidence on the potential problems of over-sampling and estimation bias. Finally, histological and other biological data (gonadosomatic index, macroscopic maturation, etc.) should be re-analysed to test for time, space and age variations in population reproductive properties.

Workshop: The brief inter-calibration exercise on egg staging and POF dating during the SG demonstrated the need for future work in that area. It is suggested that a dedicated informal workshop takes place before the analysis of the 2002 DEPM samples (possibly in January/February 2002) to perform a thorough inter-calibration exercise and to provide a very detailed description of the morphological characteristics and criteria used for distinguishing egg stages and POF cohorts. Attention should be taken to consider (and include in the reference collection) samples with average/poor quality.

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9 WORKING DOCUMENTS

Bernal M., Stratoudakis Y., Borchers D. - Using presence-absence data to evaluate changes in spawning areas (oral presentation): In this presentation, a simulation exercise is shown in which the relation between probability of presence given different underlying distribution of eggs and surveyed area. Simulation results indicate that the probability of finding an egg depends on both the sampled area and the aggregation of the eggs, as expected, but the relation between sampling area and probability of presence is not linear under any of the simulated scenarios. Thus, it will be necessary to understand this relation under real scenarios in order to compare presence-absence data from different sampling gears.

Bernal M., Borchers D.L., Valdes L., Lago de Lanzos A., Buckland ST - Asigning ages to eggs of synchronous spawning fish (oral presentation). In this presentation, the main principles and results of the new ageing procedure adopted by the WKSBS were highlighted. The method is based on combining information about diel spawning pattern with the stage-age models obtained from incubation experiments, in order to obtain the most accurate ages for the sampled eggs. Main advantages are that the new method allows to introduce the assumptions about diel spawning

pattern and the stage-age model in more detailed ways as previous methods, and that the uncertainty of the ageing method, given the assumptions can be evaluated.

Cunha E, Pina S, Oliveira JM - Dependence of embryonic development of fish eggs with temperature – an experiement: In this working document we describe a system developed at IPIMAR that enable experiments with eggs fertilised *in vitro* under controlled temperature conditions on board the ship, present the results of the first experiment with S*parus aurata*, identified problems and propose possible solutions. This WD is meant to work as a basis for discussion during the Study Group meeting, in order to improve the performance of future experiments with sardine and anchovy.

Cunha, E., Stratoudakis, Y., Figueiredo, I., Soares, E., Gordo, L. -Sardine DEPM studies in Portugal: This working document introduces the studies carried on in Portugal on the application of the Daily Egg Production Method (DEPM) to sardine (*Sardina pilchardus*. Based on the information and experience already obtained we identified problems and propose possible solutions in order to improve the planning and performance of future DEPM surveys in Iberian waters.

Lonergan M - Why use Generalised Additive Models? (oral presentation): Model mis-specification is a component of the imprecision of most regression-based estimates that is generally ignored. The DEPM is one such method and therefore vulnerable to this problem. Generalised Additive Models (GAMs) largely sidestep this difficulty by widening the family of functions under consideration. The fundamentals of this approach, along with mgcv, an implemention of the methodology within an R library, are presented. Examples of the library's output are shown including some from a version currently under development, which implements multidimensional functions and interactions. The software used is available to download free from http://www.cran.r-project.org/.**Motos L, Uriarte A – Estimation of spawning frequency for the Bay of Biscay Anchovy (1997-1998)**: This document summarise the actual procedure followed to estimate the spawning frequency for the Bay of Biscay anchovy. This anchovy is a batch spawner of indeterminate fecundity, spawning around midnight during the spawning season, being routinely assessed by the DEPM since 1987. The spawning fraction of mature females was calculated by standard procedures till 1992 based on the incidence of day 1 Pofs, but since 1994 this is based on the incidence of day 1 & 2 Pofs. Active spawning anchovies caught between 20:00 and 7:00 (day-0), are consistently being over-sampled which implies the adoption of correction procedures for the estimation of the spawning frequency based on the POFs during this hours of sampling. During 1997 and 1998 (EU Project 96/034) the over-sampling phenomena of spawning and pre-spawning females has been more thoughtfully studied. The presence of oocytes with nuclear migration (early and advanced) allows for the study of over-sampling of pre-spawning females since up to 20:00 hours of the day before spawning. We found estimates of S from those pre-spawning females for 1997 and 1998 of about double of what would be expected by the usual day1 and 2 estimator. This was an indication of over sampling of these pre-spawning females. The WD describes the shape of over-sampling along the day time pooling up all the years with histological analysis of ovaries, and give adapted *ad hoc* estimator of the spawning frequency for cases with such over-sampling phenomena of pre-spawning females.

Stratoudakis, Y., Soares, E., Zwolinski, J., Mota, F., Garção, N. - The impact of differences in gonad fixing to the quality of histological slides used for the estimation of spawning fraction in DEPM: During the ICES Workshop on sardine DEPM (ICES 2000), differences in the quality of the histological slides derived from the Portuguese and Spanish surveys of 1999 were observed. Portuguese slides (gonads fixed in AFA and mounted in resin) had less clearly defined follicle structures than Spanish slides (gonads fixed in formalin and mounted in paraffin). Two possible explanations were proposed: i) the difference of the fixative (Portugal used AFA, Spain used formalin), ii) the longer time interval between fish collection and gonad fixation (many Portuguese samples were collected by commercial purse-seiners). Given the importance of the quality of histological slides in the identification and dating of Post-Ovulatory Follicles (POFs), and thus the estimation of spawning fraction, an experiment was set-up to test the above hypotheses (impact of fixative and time from sampling to fixation). Histological slides of gonad lobes preserved in AFA were found to have a significantly higher macroscopic and microscopic quality (confirming previous tests in 1988), leaving few doubts that, despite its toxicity, AFA is a better means of fixation for sardine gonads. On the other hand, 5 hours delay in gonad preservation did not introduce any significant deterioration in the general aspect of the gonad, although the particularly small number of gonads with POFs does not permit an evaluation on potential POF deterioration.