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Universidad del País Vasco
Euskal Herriko Unibertsitatea

TESIS DOCTORAL

Quantification of key biological processes determining the dynamics and the assessment of the anchovy population in the Bay of Biscay: growth, reproduction, demography and natural mortality.

Cuantificación de procesos biológicos determinantes de la dinámica y evaluación de la población de anchoa del golfo de Vizcaya: Crecimiento, reproducción, demografía y mortalidad natural

By/Por

Andrés Uriarte Seminario

Trabajo presentado para la obtención del Doctorado en la
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Noviembre 2015

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azti tecnalia

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Andrés Uriarte Seminario

Dirigida por: **Dr. Lorenzo Motos (AZTI)**
Dr. José Ignacio Saiz Salinas (UPV-EHU)

Noviembre 2015

a mis Aitas

a Pepa y Ane

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El estudio de la composición por edad de la anchoa a través del método de producción diaria de huevos (MPDH) ha sido un trabajo exigente a nivel personal y del equipo de AZTI. Desde el comienzo comprendimos la importancia de incorporar este objetivo entre los del MPDH y por ello extendimos el muestreo a todos los parámetros involucrados en su estimación. Ante todo he de agradecer a las personas que han llevado las campañas durante todos estos años, Lorenzo Motos y a María Santos, y a todo el equipo que ha participado en las mismas tanto en el Mar como en el laboratorio de una manera continuada: Guillermo Boyra, Inma Martín, Iñaki Rico, Udane Martinez, Carlota Pérez, Betriz Beldarráin, Irene Gomez, Naiara Serrano, Deniz Kukul, Maite Cuesta, Goretti García, Mariví Luzero, Xiker Salaberria, Gaizka Bidegáin, entre otros analistas y becarios. Una vez más la lectura de otolitos por parte de Iñaki ha sido indispensable para efectuar este trabajo. En estas campañas también participaron en algunos años Unai Cotano, Paula Alvarez, Yolanda Sagarminaga, Naroa Aldanondo, Pablo Cermeño y un gran número de personas a las que sería aquí imposible mencionar. Finalmente quisiera mencionar de nuevo a Leire por su aportación decisiva a la modernización de los métodos de procesado a posteriori del MPDH, con quién he discutido muchos de los aspectos abarcados en este estudio de la demografía.

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Contents

List of figures

List of tables

List of papers

The following publications are part of this thesis:

Validation of age determination using otoliths of the European anchovy (*Engraulis encrasicolus* L.) in the Bay of Biscay and description of its annual growth. (Submitted to Marine and Freshwater Research). **(Chapter 2)**

A re-evaluation of the spawning fraction estimation procedures for Bay of Biscay anchovy, a species with short interpawning intervals. (Published in Fishery Research in 2012) **(Chapter 3)**

Anchovy Population at age estimates and variances from the application of the Daily Egg Production Method. (in preparation) **(Chapter 4)**

Assessing natural mortality of Bay of Biscay anchovy from survey population and biomass estimates. (in press in the Canadian Journal of Fisheries and Aquatic Sciences) **(Chapter 5)**

Summary

Growth, reproduction, demography and natural mortality are key biological characteristics determining dynamics and affecting the assessment of fish populations. In this thesis studies on these key biological characteristics of the anchovy in the Bay of Biscay, *Engraulis encrasicolus* (Linnaeus 1758), are presented along with a review of their estimation methods.

Regarding growth, the validation of the age determination procedure using otoliths of European anchovy in the Bay of Biscay is presented. This was achieved by monitoring very strong year classes in successive catches and surveys, and also studying the seasonal occurrence of edge types. In addition we provide an assessment of the historical performance of the ageing method since 1987. Summary annual growth (Yearly annuli) consists of a hyaline zone (either single or composite) and a wide opaque zone, disrupted occasionally by some typical checks (mainly at age-0 and at age-1 at peak spawning time). Age determination, given a date of capture, requires knowledge of the typical annual growth pattern of otoliths, their seasonal edge formation by ages and the most typical checks. Typically, otolith growth at age-1 and age-2 diminishes to about a half and a third of that at previous age, whilst at older ages growth is more sustained. Most opaque growth occurs in summer and is minimal (translucent) in winter. The older the fish the later opaque otolith growth resumes during the first half of the year. About 88% of the total asymptotic length is reached by anchovy at the age of two.

The study of reproduction is restricted to the spawning fraction (S), i.e. the fraction of mature females spawning per day. This is a key parameter to scale the Egg production estimates to spawning biomass for the surveys implementing the Daily Egg Production method. The estimation procedures of the spawning fraction were revised for anchovy using samples from 14 DEPM surveys. First the procedure to assign mature females to daily spawning classes was improved by incorporating all the available knowledge on final oocyte maturation and degeneration of post-ovulatory follicles (POFs) in an automated (matrix) allocation system of these histological indicators to pre- and post-spawning daily classes according to the time of capture. Subsequently several S estimators and their biases were evaluated: The mean proportion of day_0 and day_1 was selected as the best estimator of S due to its statistical properties. S was about 0.4 (CV=18%). In addition some oversampling of day_0 females was observed around peak spawning time (at 23:00 hours). The new estimates were rather invariant in the study period and independent of the fishing gear or sampling time. Female size had a small but significant influence on S. The new S estimates revised upward the former estimates and implied lower biomass estimates from the DEPM surveys.

To study of the anchovy demography is based on the surveys implementing the Daily Egg Production Method (DEPM) which has been traditionally applied to estimate only the spawning biomass of small pelagic fishes, like sardines and anchovies. The DEPM has been extended to produce population at age estimates and variances through cluster sampling of ages (or using age-length keys). This method has been applied to the DEPM surveys on the Bay of Biscay anchovy since 1990, including estimates of biological features (as mean length and weight at age). A detailed example of its application in a particular year is provided along with a discussion on the role that stratification of the survey and weighting of individual samples play in getting unbiased estimates. The series of population at age estimates revealed the dominant role of the 1-year-old recruits in sustaining the anchovy population. Old age classes (ages 2 and 3+) were usually a minor component. Finally the extension of the method to estimate total population at age over the surveyed area when mature and immature fishes coexist is also outlined.

Finally, the study of the natural mortality affecting this anchovy population in the Bay of Biscay is essayed from the joint analysis of the population at age estimates produced by the Acoustic and DEPM surveys in the region, which are applied since 1987 to assess this anchovy. The closure of the anchovy fishery between 2005 and 2010 due to low biomass levels provided a unique opportunity to estimate natural mortality using data from these surveys, without the interference of the fishery. Assuming that natural mortality (M) is constant over time and that catchability in both surveys is equal for all ages, natural mortality could be estimated using log-linear models on the series of surveys of population numbers at age, and seasonal integrated stock assessments. The analysis suggests M values of around 0.9 assuming it is equal for all ages. However, we found firm evidence that natural mortality at ages 2 and older (M2+) is markedly higher than at age 1 (M1), which suggests senescent

mortality, a possibility suggested a long time ago for this type of short-lived species. The increased mortality might be due to spawning stress. This pattern of increasing natural mortality at age might be applicable to other Engraulidae too.

The biological methods and parameters covered in this study have all implied changes in the perception of the dynamics and assessment of this anchovy population. This confirms the relevance of precise and accurate estimation of biological parameters for a correct assessment of populations and their management, and corroborates the thesis hereby defended.

Resumen

El crecimiento, la reproducción, demografía y mortalidad natural son características biológicas que determinan la dinámica y afectan a las evaluaciones de las poblaciones de peces. En esta tesis se presentan estudios de estos aspectos biológicos para la anchoa del golfo de Vizcaya, *Engraulis encrasicolus* (Linneo, 1758), junto con una revisión de sus métodos de estimación.

Del crecimiento, se presenta la validación de la determinación de la edad de la anchoa mediante el examen de los otolitos. Esto se consiguió mediante el seguimiento de clases de edad muy fuertes en las capturas y en campañas y con el estudio de la aparición estacional de los distintos tipos de bordes del otolito. Además se muestra una evaluación del funcionamiento histórico, desde 1987, de este método de asignación de edades. El crecimiento anual típico del otolito consta de una zona hialina (sencilla o múltiple) y de una banda ancha opaca, interrumpida ocasionalmente por algunos anillos falsos (principalmente a la edad 0 y edad 1 -- en el momento de su máximo reproductivo). Dada una fecha de captura, la determinación de la edad requiere del conocimiento del patrón típico de crecimiento anual del otolito, de la formación estacional del borde en función de la edad y de los anillos falsos más típicos. Por lo general, el crecimiento del otolito de los ejemplares de uno y dos años de edad disminuye a la mitad y a un tercio del realizado al año anterior respectivamente, mientras que para edades posteriores los incrementos son más sostenidos. La mayor parte del crecimiento opaco del otolito ocurre en verano y es mínimo en invierno cuando se vuelve traslúcido. Cuanto más viejas son las anchoas más tarde retoman el crecimiento opaco durante la primera mitad del año. Para la edad 2 las anchoas alcanzan ya un 88% de su crecimiento asintótico máximo.

El estudio de su reproducción se restringe al de la fracción de puesta (S), o fracción de las hembras maduras que ponen cada día. Este es un parámetro fundamental para escalar la producción de huevos a la biomasa de puesta en las campañas del Método de Producción Diaria de Huevos (MPDH). Los procedimientos de estimación de S fueron revisados para la anchoa usando datos de 14 campañas del MPDH. Primero se mejoró el procedimiento de asignación de las hembras maduras a las cohortes de puesta diaria mediante la incorporación del conocimiento de la maduración final de

los ovocitos, antes de la puesta, y de la degeneración de los folículos post-ovulatorios en un sistema automático de asignación matricial de las hembras a las cohortes de pre y post puesta, condicionado a la hora de captura. A continuación se evaluaron diversos estimadores de S y sus sesgos. La incidencia de las hembras en puesta día_0 y día_1 fue seleccionada como mejor estimador de S por sus propiedades estadísticas. S se estimó en torno a 0.4 (CV=18%). Además se observó algo de sobre-muestreo de las hembras día_0 alrededor del pico de puestas (entorno a las 23:00 horas). Las nuevas estimas de S fueron bastante constantes durante el periodo de estudio e independientes del arte y la hora de pesca. La nueva S revisa al alza las estimas anteriores de este parámetro e implican menores estimas de biomasa a partir de las campañas MPDH.

El estudio de la demografía se ha basado en las campañas del Método de Producción Diaria de Huevos (MPDH) que tradicionalmente se han aplicado únicamente para estimar la biomasa de pequeños peces pelágicos, como la sardina o la anchoa. Se ha extendido el MPDH para producir estimas de la población en números por edad, con sus varianzas, mediante el muestreo por conglomerados de edades (o usando claves talla edad). Este método se ha aplicado a la serie de campañas MPDH desde 1990 para la anchoa del golfo de Vizcaya, incluyendo características biológicas como las tallas medias y pesos medios por edad. Las estimas de la población por edad evidenciaron el papel dominante que juega el reclutamiento de anchoas de 1 año de edad en el sostenimiento de la población cada año. Las clases de edad más viejas (de 2 o 3 años de edad) suponen normalmente un parte menor. Finalmente se presenta una extensión del método a estimas de la población total por edad para el caso en que convivan peces maduros e inmaduros en el área prospectada.

Finalmente, se estudió la mortalidad natural de esta población de anchoa en el golfo de Vizcaya mediante el análisis conjunto de las estimas de la población en número por edad de la serie de campañas acústicas y del MPDH desde 1987. El cierre de la pesquería entre el año 2005 y 2010, por los bajos niveles de biomasa, supusieron una oportunidad única para estimar la mortalidad natural (M) a partir de las campañas, sin la interferencia de la pesca. Asumiendo que M es invariante en el tiempo y que las capturabilidades en las campañas no cambian por edad, la mortalidad natural puede ser estimada mediante modelos log-lineales aplicados a la series de estimas por edad de las campañas y también mediante modelos integrales de evaluación. El análisis sugiere M en torno a 0.9 si se asume constante para todas las edades. Pero hay firmes

evidencias de que la M de las edades 2 en adelante es bastante superior que a la edad 1, lo que sugiere mortalidad por senescencia, una posibilidad ya sugerida hace mucho tiempo para estas poblaciones de vida corta. Esta mortalidad añadida podría deberse a estrés reproductivo. Este patrón de mortalidad natural creciente con la edad podría estar presente también en otras poblaciones de Engráulidos.

Los métodos y parámetros revisados han supuesto cambios en la percepción de la dinámica y en la evaluación de la población de anchoa. Esto confirma la relevancia de obtener estimas precisas y exactas de los parámetros biológicos para lograr una correcta evaluación y gestión de estas poblaciones de peces, lo que corrobora la tesis defendida en este trabajo.

1 General introduction

1.1 Assessment of fish stocks and importance of biological parameters

Fish stock assessment is basically an evaluation of catches compared to the productivity of a stock (Gulland 1983; Hilborn and Walters 1992). Productivity of fish stocks is determined basically by recruitment, growth and natural mortality. If the effect of mortality induced by a fishery exceeds the natural productivity of the stock, the stock would decline and could eventually collapse. Otherwise the stock would stabilize at a level where the productivity is on average about the level of removals (catches).

There are several types of models of stock productivity. Global models, or surplus production models, describe the dynamics of the stock in terms of mass in time steps (usually annually) with few parameters (Gulland 1983; Hilborn and Walters 1992). Alternatively, other dynamic models describe separately the key processes such as recruitment, growth and natural and fishing mortality. Among them there are the full age disaggregated assessment models either deterministic (like the Virtual Population Analysis, Gulland 1965; Pope 1972) or stochastic (as the Integrated Catch at age Analysis Fournier, and Archibald 1982; Deriso *et al.* 1985) which are typically used for long lived temperate demersal species, but also for medium or short-lived stocks as clupeids and Engraulidae (Megrey 1989; Quin and Deriso 1999). There are some dynamic models of intermediate complexity, usually dealing with only two stages of the population (such as recruits and remaining adult fraction of the population) called delay-difference Models (Deriso 1980; Schnute 1985; Hilborn and Walters 1992).

Assessment consists on estimating the parameters defining the past stock dynamics (and its productivity) which best fit the observations (basically catches and abundance indices). In most of the assessments several sub-models are fitted sequentially (or occasionally simultaneously). First the series of past levels of population biomasses, recruitments and (natural and fishing) mortality levels are assessed from the observations on past catches (at age) and indices of stock abundance, through an integrated assessment. Next, the recruitment dynamics in the past is modelled as a function of the past stock size (including or not other ecological covariates). The latter is made in order to assess the stock productivity and to project the stock in the future under different exploitation strategies, so that sustainability of medium and long term fishing strategies can be assessed given the productivity of the stock (Punt *et al.* 2001).

When stock-recruitment relationships cannot be fitted, stock productivity remains not well understood. However, some reference points for the sustainable exploitation of stocks can be approximated from the Yield per recruit analysis (Beverton and Holt 1957; Mace and Sissenwine, 1993; Caddy and Mahon 1995). But cautious use of the results is recommended given the inherent underlying assumptions on recruitment of this approach (Pereiro 1992).

Typically deterministic age structured stock assessment models based only on catches at age (Megrey 1989; Quinn and Deriso 2009) are over parametrized and subject to indeterminacy (Shepherd and Nicholson 1986, Pope and Stokes 1989). Therefore, the fit requires additional assumptions about key parameters of the stock dynamics (such as natural mortality or terminal F on the oldest age group), reduction on the number of parameters by using separable fishing mortality models (Pope and Shepherd 1982, Shepherd and Nicholson 1986) and, more importantly, incorporation of auxiliary information to reduce the uncertainties of the assessment outcomes. Direct surveying of stocks and tagging are among the most useful auxiliary information (Deriso et al 1985; Shepherd 1999; Quinn and Deriso 2009).

Natural mortality is a key parameter of any dynamic assessment model. It has always been admitted than an incorrect election of natural mortality (denoted usually by M) affects directly the estimates of past levels of fish stocks (Ulltang 1977; Mesnil 1980; Gislason *et al.* 2010). This parameter is very difficult to be assessed because it can be confounded with fishing mortality and/or with catchability of surveys (Vetter 1988; Cotter *et al.* 2004; Wang *et al.* 2009). For this reason, the common approach is to apply a constant value of M across ages and along time (Hilborn and Walters 1992; Gislason *et al.* 2010). The M value is most often guessed according to the life span of the species. On other occasions, it is based on published empirical relationships between M and life history parameters valid for comparable groups of species and environments (Pauly 1980; Hewit & Hoening 2004). Sometimes it broadens to include size or age-dependent natural mortality as well (Caddy 1991; Lorenzen 1996; Gislason *et al.* 2008; 2010). Nevertheless several authors defend that M should be included among the parameters of the integrated assessment whenever sufficient auxiliary information is available (Lee *et al.* 2011).

Incorporating indexes of stock abundance from direct surveys of fish resources do benefit the assessment of all type fish stocks (Francis 2011; Maunder and Punt 2012): For demersal species bottom trawl surveys are typically applied to assess fish density over surveyed areas, which can be expressed as numbers at age of a given species per bottom surface (Gunderson 1992). These surveys greatly increase the reliability of the assessment of these demersal stocks (Shepherd 1999), because they can increase the accuracy of fishing mortality assumptions of the terminal year.

Assessment (and management) of pelagic resources relies even more on the availability of auxiliary information from direct surveys (Freón *et al.*, 2005; Barange *et al.* 2009). This is due to their biological features. Firstly, their short life makes their abundance highly dependent on incoming recruitment, and therefore they have little inertia (i.e., the past status of the stock says little about its status next year). Secondly the assessment of past levels of the stock cannot benefit much from the convergence properties of the sequential analysis of past catches at age (Pope 1972), as it depends on the amount of cumulated fishing mortality across its short life (usually not surpassing F/Z>50%). Finally their aggregative character makes Catch per Unit Effort (CPUE) not a good indicator of stock abundance, because catchability may increase inversely to the level of the Stock Biomass (SSB) (Csirke, 1988; Pitcher, 1995). Therefore CPUE cannot be used as auxiliary indicator of biomass.

The most typical surveying methods of pelagic fish species are the Acoustics (Gunderson 1992; Simmonds and MacLennan, 2005) and the Daily Egg Production Method (DEPM; Parker 1980; Lasker 1985; Stratoudakis *et al.* 2006).

Acoustics surveys prospect the area of distribution of target fish stocks with acoustic devices which continuously emit sound waves to estimate the density of fish shoals from the amount backscattering energy received from schools (by echo-integration) (Simmonds and MacLennan, 2005). Energy needs to be split by species according to either the shape or acoustic characteristics of the detected fish schools and/or on the fish species composition from fishing hauls carried during the survey (Petitgas *et al.* 2003). The total amount of Energy by species is converted to fish biomass according to the length dependent specific Target strength (TS). Target strength is therefore the conversion factor to biomass and is of critical importance in fisheries acoustics. The species specific TS are hard to be measured and require special conditions of scattered pure layers of fishes or in special tanks for measurements. When not available, this parameter is borrowed from the most similar species with TS measurement. For this reason usually acoustic indices are taken as relative indices of biomass, particularly suitable to measure the relative changes between years.

Egg production surveys prospect the area of distribution of target fish stocks with discrete plankton sampling to assess the density of eggs released by the spawning population (Saville 1964; Bernal *et al.* 2012). The method is most suited at peak spawning time and converts egg densities into biomass by measuring the fecundity of the stock by means of some parallel adult sampling. For the Daily Egg Production Method (DEPM), the Spawning Stock Biomass (SSB) estimate is derived according to Parker (1980) and Stauffer and Picquelle (1980) from the ratio between daily production of eggs in the spawning area and the daily specific fecundity of the adult population:

$$SSB = \frac{Daily\_Egg\_production}{PopulationDailyFecundity\,(DF'_t\,)} = k\ \frac{A \cdot E0}{R' \cdot S \cdot (F / W_f)} \qquad \text{Equation 1.1}$$

where

SSB = <u>S</u>pawning <u>S</u>tock <u>B</u>iomass in metric tons

E0 = Daily **<u>Egg</u>** production per surface unit in the sampled area

A = **Spawning <u>A</u>rea**, in sampling units

DF = **<u>D</u>aily specific <u>F</u>ecundity** (egg per gram of mature stock). DF= $R' \cdot S \cdot (F / W_f)$

Wf = **Mean <u>W</u>eight** of mature females in grams,

R = **Sex <u>R</u>atio**, fraction of population that are mature females, by weight.

F = **Batch <u>F</u>ecundity**, numbers of eggs spawned per mature females per batch

S = Fraction of mature females spawning per day (or simply **<u>S</u>pawning fraction**)

k = Conversion factor from gram to metric tons.

Certainly the accuracy of the SSB estimates depends on the accuracy of the egg production and of the different reproductive parameters defining the daily fecundity. Obtaining unbiased estimation of the Daily Egg Production requires good sampling and coverage of the spawning area and good understanding of the egg development in time (according to sea temperature) (Lo 1985; Stauffer and Picquelle 1985; Bernal *et al.* 2011; Geffen and Nash 2012). Similarly, obtaining unbiased estimation of the reproductive parameters require a) managing a good adult sampling in space to cope with potential spatial variability of the reproductive parameters and b) a good knowledge of gonad reproductive dynamics particularly for the estimation of Spawning fraction. Among the different adult parameters spawning fraction is the most difficult reproductive parameter to be estimated and accounts for a big portion of the overall uncertainty in most of DEPM applications (Stratoudakis *et al.*, 2006; Ganias 2012).

The DEPM can directly estimate all parameters concerned in the estimation of SSB and in principle it provides absolute estimates of biomass in tons. However its use as absolute estimator of biomass is subject to debate

Beyond the considerations on whether the acoustic and DEPM survey indices are absolute or relative (i.e. catchability parameter fixed to 1 or estimated), additional catchability effects may appear in both surveys due to partial coverage of the total area (missing offshore or too coastal areas) or unequal catching efficiency of different fish sizes of the target species (or of different species in fishing hauls). If these effects are not assessed externally and corrected, the indices would be biased and the corresponding catchability coefficients should be estimated within the assessment models with the rest model parameters (Maunder and Punt 2013).

Finally, it should be remarked that age structured models depends on the accuracy of the age disaggregated observations of catches and population estimates from surveys. Furthermore estimates of natural mortality depend upon availability of age disaggregated abundance indices. Therefore the age determination of fishes is fundamental for the study of their growth and population dynamics, as well as for understanding the exploitation pattern of the fishery (Gulland 1983; Hilborn and Walters 1992; Quin and Deriso 1999). Age composition of catches or of population estimates from surveys depends, among several issues, on unbiased age determination procedures. In order to assess the accuracy of age determination validation techniques need to be applied (Beamish and McFarlane 1983; Campana 2001).

In this thesis studies on the key biological parameters of anchovy in the Bay of Biscay are presented along with their estimation methods: Growth (and age determination), Spawning fraction (and their estimators), population age structure (or demography) (and their estimation procedures from DEPM surveys) and natural mortality (and its estimation procedures). They will be shown to have significant relevance and impact on the inputs and for the assessment of the anchovy in the Bay of Biscay.

1.2 The anchovy in the Bay of Biscay (*Engraulis encrasicolus* L. 1758).

1.2.1 Genera Engraulis

Anchovy belongs to the genus Engraulis of the family Engraulidae. In this family there are 17 genera and 144 species. They are found in all the oceans and the Mediterranean and Black sea.

The family Engraulidae is part of the Order of the Clupeiformes (with herring and sardines etc.) (Within the Phylum: Chordata // Class: Actinopterygii).

Engraulidae are iteroparous short-lived species living in salt-waters but capable of inhabiting river mouth entering some miles within estuaries. These are forage species eating on small plankton which from an ecosystem perspective make the link between planktonic production and higher trophic levels (Shannon *et al.* 2009). Most clupeids have aggregative distribution patterns and form dense fish schools which make them vulnerable to fishing (Csirke 1988; Pitcher 1995). Because of their low trophic level they are typically affected by oceanographic environmental factors (Csirke 1988; Alheit *et al.* 2009)

1.2.2 Ecology and biology of the anchovy in the Bay of Biscay

The following summary is not exhaustive. More comprehensive reviews of early works on the Bay of Biscay anchovy can be found in Uriarte *et al.* 1996, Petitgas *et al.* 2010 and the ICES stock annex for anchovy in Subarea VIII (ICES 2013).

Genetically the Bay of Biscay anchovy population pertains to a rather homogeneous group from the Bay of Biscay to the North sea, which seems to be more closely related with the NW Mediterranean anchovy populations than with another group inhabiting the west coast of the Iberian peninsula and Cádiz and Alboran Sea (Zarraonaindia *et al.* 2012). Anchovies in the English Chanel and North Sea seem to be increasing in the last decades, but are supposed to form independent populations from the one inhabiting the Bay of Biscay, showing occasional remarkable recruitments generated by small populations inhabiting those areas (Petitgas *et al.* 2012).

Within the Bay of Biscay, morfometrics and meristic studies suggest some heterogeneity (Prouzet and Metuzals, 1994; Junquera and Pérez-Gandaras, 1993). Recently, Borrell *et al.* (2012) have pointed out that there is some genetic isolation of anchovies in the mid-west side of division VIIIc from the eastern one. In addition, some genetic heterogeneity, based on proteins allocime loci, have been found between the Garonne spawning regions and southern regions in the Bay of Biscay (Adour and Cantabrian shores) (Sanz *et al.*, 2008). Nevertheless there are ample evidences that the major part of the population inhabits the Eastern and northern parts of the Bay of Biscay and show rather homogenous recruitment pulses and have a rather well understood common spatial dynamics throughout the year (Uriarte *et al.*, 1996).

Anchovies in the Bay of Biscay show a closed life cycle from spawning, to larvae and juvenile phases till arriving at maturity at its first year of life, when new spawning close the cycle by the first time. Adults may live up to a maximum age of about 4 years (Petitgas *et al.* 2012). Spawning locations are known to happen in the southeastern parts of the Bay of Biscay (Motos *et al.* 1996; Bellier *et al.* 2007). Eggs and larvae experience a southwestern drift from the spawning grounds occupying most of the mid-south of the Bay of Biscay and most of the entire Cantabrian shores (north of Spain) (Uriarte *et al.* 2001; Cotano *et al.*, 2008; Irigoien *et al.* 2007; 2008; Aldanondo *et al.*, 2010; Boyra *et al.* 2013). During the autumn they seem to recruit towards Eastern coastal areas all along the French coast were most of them will pass the winter preferable close to the areas of influences of large rivers (like Adour and Garonne) where they are predominantly found next spring (Motos *et al.* 1996; Vaz *et al.* 2002; Lethuta *et al.* 2010; Santos *et al.* 2015 – in press).

A large amount of works have been published on the role that the physical environment can play in determining the survivals of eggs and larvae. Borja *et al.* (1996; 1998) established the positive role that weak upwelling conditions from March to July may play in favoring the recruitments of anchovy in the Bay. In addition Allain *et al.* (2001) pointed out that, in addition to upwelling, a stratification breakdown was useful in predicting some negative effects (due to gales and storms at the beginning of summer time) for the survival of larvae. Whether the southwestern drift of larvae is beneficial or detrimental to the survival of larvae is controversial (Allain *et al.*, 2007; Irigoien, 2007; 2008; Cotano *et al.*, 2008 , Aldanondo *et al.*, 2010, Bachiller *et al.*, 2013).

Recent research for identifying and monitoring limiting factors of anchovy recruitment in the Bay of Biscay was made by Petitgas (2011). Indices of physical features were estimated (river plumes, gyres, stratification, fronts) as well as indices of larval dispersal, primary production and temperature. Indices of spawning aggregations derived from fisheries survey data were also estimated. Results showed that late spring and early summer is the period when survival is determined, confirming this is a critical period, something previously stated by Aldanondo (2010) analyzing juvenile survival. The limiting factors changed across the series, confirming the multifactor nature of the recruitment regulation. Fernandes *et al.* (2010) presents an alternative to attempt to relate environmental indices with recruitment by means of linear models. They use machine-learning techniques to obtain the probability of having a recruitment discretized into low, medium and high classes depending on environmental variables. Andonegui *et al.* (2011) incorporate an improved version of the former approach to forecast the evolution of the anchovy population under different fishing pressures and environmental regimes. More recently Fernandes (2013) used a multi-dimensional Bayesian network classifiers to the simultaneous forecasting of three fish species (anchovy, sardine and hake) getting some improvements over the single species approach. Environmental variables seem to explain a significant part of the observed variability of the small pelagics but not more than 50% of it (at least from the available indicators). The significance and reliability of all these indices is considered still insufficient for their consideration alone in the provision of management advice (De Oliveira *et al.* 2005). In fact, the parental stock does also play a minor but significant role in determining recruitment (Taboada and Anadón in press).

Field research on the distribution, growth and survival of larvae and juveniles has been carried out by Uriarte *et al.* (2001), Carrera *et al.* (2006); Allain *et al.* (2003; 2007), Cotano, *et al.*, (2008), Irigoien *et al.* (2008) and Aldanondo et al (2010; 2011), the latter following the validation of growth increments in larvae and juvenile anchovy carried out by Aldanondo *et al.* (2008) and Cermeño *et al.* (2003; 2008).

One year old anchovies become fully mature every year by May (Cort et al, 1976, Motos *et al.* 1991, Lucio & Uriarte 1990, Motos 1996). The reproductive biology of anchovy started with the study of the maturity (Furnestin 1945; Andreu 1950; Cendrero *et al.* 1981; Lucio and Uriarte 1990, Sanz and Uriarte 1990) and the spawning areas and the seasonality of the spawning (Arbault and Lacroix 1977; Solá 1990). These studies showed that the maturation of gonads goes rather parallel to the warming process in spring, when the water goes from 12°C, at the end of winter, up to 20°C at the beginning of summer. Maturity reaches its maximum in May-June, at peak spawning time and subsequently diminishes gradually during summer (Lucio and Uriarte 1990, Sanz and Uriarte 1990).

The implementation of the DEPM led to a major improvement in the understanding of the reproductive biology of the anchovy (Sanz *et al.* 1992; Motos 1994). Relative fecundity of females per spawning batch ranges between 350 and 700 eggs per gram and spawning frequency goes from spawning every 5 days to every other day (so spawning fraction ranges between 0.2 and 0.5) (Motos 1996; Uriarte *et al.* 2012 and Santos *et al.* --in press).

All ages mix during spawning and later on, in early summer, they tend to migrate towards the northern part of the Bay of Biscay, though some westward migration along the Cantabrian shore seems to occur as well (Uriarte *et al.* 1996; Petitgas *et al.* 2011). During autumn and early winter, anchovy inhabits the northern part of the Bay and later on, during winter, they move to the central and southern part of the Bay where spawning will take place during next spring.

Anchovy usually does not live more than 5 years, which is the age of the oldest fishes ever recorded. Growth is intense during the first two years of life (up to the age of 2) when it reaches most of its asymptotic growth (Uriarte and Astudillo 1987; Vaz *et al.* Hernandez *et al.* 2009). The sharp decay of the abundance of the oldest age groups suggests a high natural mortality at those ages.

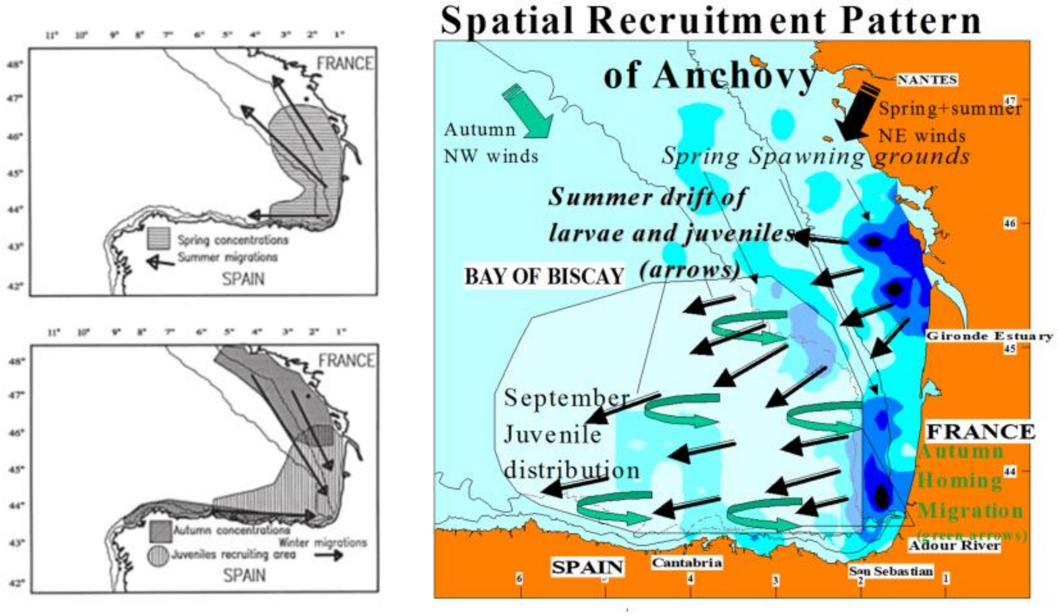


Figure 1.1 Summary of adult anchovy seasonal Migrations (upper left graph, from Uriarte *et al.* 1991), and summary of typical spawning grounds the spatial pattern of recruitment (bottom graph Uriarte *et al.* 2001).

Modelling of growth, reproduction and even migration with bioenergetics models is an area of recent research (Pecquerie *et al.* 2009; Politikos and Huret 2015).

In the Bay of Biscay, anchovy, among other small pelagic fishes, play an important role as prey for piscivorous species such as hake, megrim, sea bass, tunnidae or cetaceans and birds, etc (Preciado *et al.* 2008; Goñi *et al.* 2011; Lassalle *et al.* 2011; López-López *et al.* 2012). Much of predation is made upon the juvenile phases of the anchovy (López-López *et al.* 2012). In recent years major attention is being paid to the role that intraguild predation may have in affecting the survival of early life stages

(Irigoien and Ross, 2011). Specifically, the potential influence of sardine, as well as zooplankton, predating on anchovy eggs has been evidenced (Albaina *et al.* 2015; Bachiller *et al.* 2015a, b).

1.2.3 The Fishery Historical perspective; current fleets and its management

The fisheries on small pelagics (anchovy and sardine and horse mackerel) goes back several centuries along the coasts of Basque Country and all along the Northern coasts of Spain (Maiz 1993). Complete statistics of catches are available since 1940 (GAUR 1970; Cort *et al.* 1976) (Figure 2) and analysis of these catches, and the spatio temporal changes along the Spanish coasts was provided by Junquera (1986; 1988). Since 1989 the fishery is being monitored and assessed by ICES (ANON 1989).

Historically most of the catches on anchovy were produced by Spanish purse seiners during spring in the so called “Costera de la anchoa”. The fishery on anchovy in the Bay of Biscay achieved maximum landings in the sixties of the past century (with peak catches around 84 000 t). Subsequently, catches diminished progressively up to the eighties (Figure 2); this was followed by a similar decline of the Spanish purse seine fishery, which until then was the major component of the fleet (Uriarte *et al.* 1996). Since the end of the eighties, French pelagic trawlers entered the fishery and, in a few years their catches equaled those of the Spanish purse seiners. During the nineties, international catches reached a maximum of approximately 40 000 t. However, during the present century, catches declined to a historical minima until the collapse of the fishery in 2005 and subsequent closure periods, due to low stock sizes (ICES 2008). The Spanish fishing fleet operating on anchovy had dropped from about 600 vessels in the sixties to about 196 boats in 2006 (Uriarte *et al.* 1996; 2008; Villamor *et al.* 2008). In 2013 the Spanish fleet (of approximately 175 purse seines) operates as usual mainly in spring within the southeastern corner of the Bay of Biscay, covering an area between half or two thirds of that of the total spawning stock, and getting 80 % of the Spanish annual catches (ICES 2014).

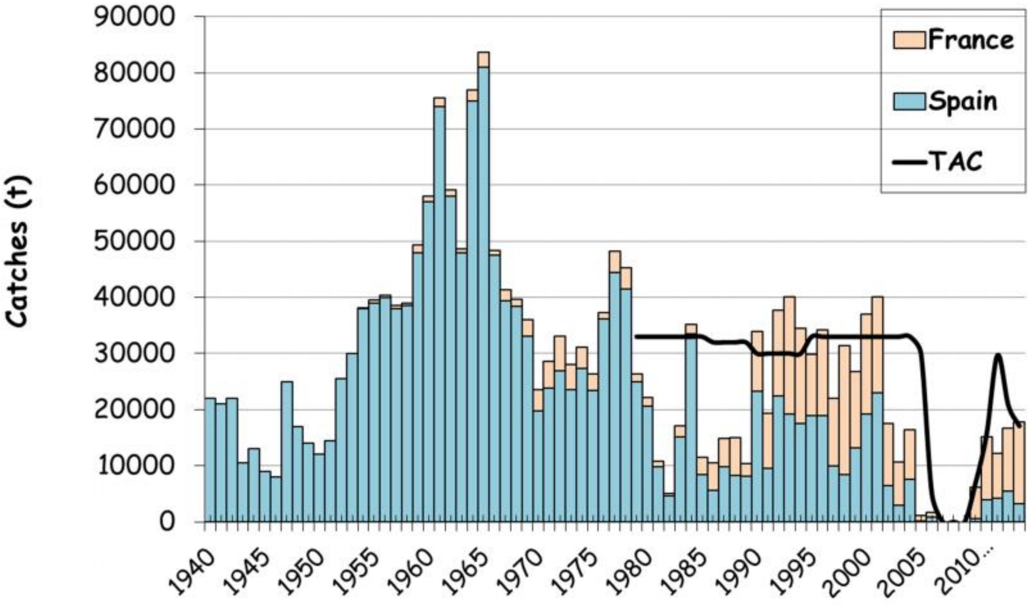


Figure 1.2 Historical Catches on anchovy in the Bay of Biscay and agreed TAC (between 2010 and 2014 the TAC applied to the catches between July to June next year.

The French fleet in 2013 was composed of about 27 purse seines (the exact number is not fixed) most of them from Britanny. In addition about 10 pair pelagic trawlers (20 vessels) operate for fishing anchovy (ICES 2014). The main French anchovy catches were taken by the pair trawlers which used to operate, prior to the closure of the fishery, in the north of Subdivision VIIIb in the first quarter of the year, and in Subdivision VIIIa during the second half of the year (ICES 2014; Vermard *et al.*, 2008). After the closure the French fishery does not operate any more in the first quarter of the year, so catches are produced during the second half of the year mainly in VIIIa.

Until the seventies the fishery had developed unregulated. In 1978 a precautionary Total Allowable Catch (TAC) for anchovy was established within EEC waters (Divisions VIIIa,b) at the level of 32,000 tonnes, which was about the average level of the catches of those recent years and about the level of Maximum Sustainable Yield (MSY) deduced from the application of a surplus production model (Schaefer model) to this fishery (Cort *et al.*, 1976). In 1986, with the inclusion of Spain in the EEC, the

TAC for anchovy was applied to the whole Bay of Biscay (Subarea VIII) which remained basically constant around 30 or 32 000 t till the collapse of the fishery. Hence, anchovy TAC was therefore set regardless of scientific advice and catches have often exceeded or not reached it (Figure 1), supposing no major conditioning to the actual fishing activities until 2006. Fishing though relevant was not considered the major driver of stock decline, but the repeated failures of recruitment led to the low stock sizes (ICES 2008). On those failures environmental factors did also play a major role (Petitgas 2011; Fernandes *et al.* 2010).

After the closure the European Commission promote the development of a Long Term Management Plan in collaboration with Member states, stake holders and scientists (STECF 2008a,b; COM2009). The plan was adopted in 2009 to be operative since the reopening of the fishery which happened in 2010. Since then TACs are set according to the Harvest control rule of the LTMP which define the Total allowable level of catches for the next fishery based on the most recent information from surveys (COM 2009). In 2014 the LTMP was reviewed according to the improvements in the assessment carried out by ICES (2013) (STECF 2013).

1.2.4 Monitoring and assessment of the anchovy population in the Bay of Biscay

The catches of anchovy in the Bay of Biscay have been monitored since the early forties of the past century fifties (Figure 1.1.X) (Cort *et al.* 1976; GAUR 1970; Igelmo *et al.* 1984; Junquera 1986; Uriarte *et al.* 1996). Sporadic biological sampling of catches started in the fifties (Navaz and Lozano-Cabo 1966; Lozano *et al*. 2000) aiming at obtaining biological information on length distribution, growth and maturity of the anchovy. But comprehensive sampling of the Spanish catches for estimation of the catches by length and at age started in the seventies by the IEO (Cort *et al.* 1976; Cendrero *et al.* 1981). Through this monitoring catches by length and at age were reported between 1974 and 1987 by Cendrero *et al.* (1980; 1981; 1983), Astudillo (1986) and Uriarte and Astudillo (1987). Since late eighties, both the Spanish and French landings are monitored for length and age composition by the research institutes, AZTI and IEO (Spain) and by IFREMER (France), with the support since

2000 of the European Commission through the National sampling programs (Uriarte *et al.* 1996; ICES 2014).

During the eighties series of acoustic (Simmonds and MacLennan, 2005) and Daily Egg production method (DEPM; Parker, 1980; Lasker, 1985; Stratoudakis *et al.*, 2006) started. The Acoustics are carried out by IFREMER France since 1983 (Masse 1988) though coverage of the whole anchovy spatial distributions was only systematically carried out from 1989 onwards (Massé 1996; Petitgas *et al.* 2003; Massé *et al.* 2015 –in press). On the other hand the DEPM on anchovy started in 1987 (Santiago and Sanz, 1992) and since then it has been continuously applied every year, with a sole gap in 1993 (Motos 1994, Somarakis *et al.* 2004; Motos *et al.* 2005; Santos *et al. in press*). Both surveys provide stock biomass and population in numbers at age, which are supplied routinely to ICES (the International Council for the Exploration of the Sea) for stock assessment and management advice purposes.

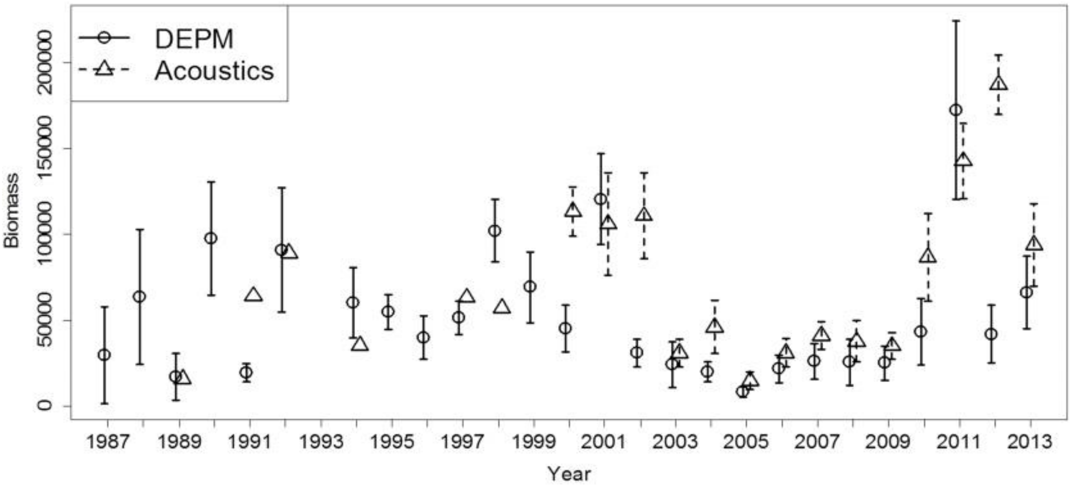


Figure 1.3 Historical series of spawning stock biomass estimates and the corresponding confidence intervals from DEPM (solid line and circles) and acoustics (dashed line and triangles), as used by ICES up to 2013.

Since 2003 an acoustic survey (JUVENA) is applied in autumn to assess the abundance of anchovy juveniles in the Bay of Biscay (Boyra *et al.* 2012). The index was adopted in 2013 as a valid index capable of predicting next year recruitment at age 1 to the population and the fishery (ICES 2013) and since then it is included as an auxiliary observation for the assessment.

The direct estimates of biomass from these research surveys have been analyzed by ICES since 1988 to monitor the stock status and to provide management advice to managers (Anon. 1988). Since 1995 the assessment was based on an Integrated analysis of catches at age and population estimates (both in mass and in numbers by age) (Anon. 1996), analyzing all information available since 1987. The analysis used the ICA package (Patterson and Melvin 1996–Integrated Catch at Age Analysis) which assumes a separable model of fishing mortality and it was run assuming a constant natural mortality of 1.2 (at the average level of the previous analysis, Uriarte *et al.* 1996). The assessment moved in 2009 to a Bayesian two-stage biomass dynamic model (BBM) (Ibaibarriaga *et al.* 2008; ICES 2009), using age 1 and age 2+ (ages 2 and older) as the two age groups sufficient to track the dynamics of the stock. In this assessment model commercial catches were subtracted from the stock on half year basis without modelling the fishing process.

Since 2014 an improved version of the BBM is applied for the assessment of the anchovy population in ICES (Ibaibarriaga *et al.* 2011; ICES 2013). This model allows incorporating into the assessment the recruitment index provided by JUVENA surveys and deals with the catches at age as a result of a separable model of fishing mortality (with year and age effects).

All assessments between 1995 and 2013 were carried out under the assumption that DEPM survey index provided an absolute level of spawning biomass (in tons) at peak spawning time in May. For the acoustic survey, however, it was assumed that it supplied a relative index of biomass, for which catchability had to be estimated, though a priori it was considered unbiased. Since 2014, the default setting of the assessment is that both indices supply relative indices of biomass, a priori unbiased, but which need estimation of their catchability (or proportionality factor between the survey index and the actual stock size in mass).

1.3 The Biology behind some key inputs for the assessment of Bay of Biscay anchovy.

A correct assessment and management depends on the accuracy of the biological parameters. For the Bay of Biscay anchovy population, some of the key biological parameters that deserve further consideration and will be dealt in this Thesis are: the age determination and growth, the reproductive parameters from the DEPM, the age structure from the DEPM and the natural mortality. This subsection describes briefly the state of the art of each of them and identifies potential improvements to be addressed.

Age determination and growth:

Early studies on the growth of anchovy in the Bay of Biscay had been carried out by direct examination of catch by length distributions and biological sampling (Navaz and Cabo 1966; Guerault and Avrilla 1973). Soon after, otolith examination was preferred because hyaline marks, presumably related to winter growth detention, were well marked on them (Guerault and Avrilla 1974). Since then and up to the mid-nineties, multiple scientists and at least 4 institutes worked on the age determination of anchovy in the Bay of Biscay (Guerault and Avrilla, 1978; Cendrero *et al.* 1981; Astudillo, 1986; Junquera, 1986; Uriarte and Astudillo 1987; Martín and Lucio 1988; Prouzet *et al.* 1995; Uriarte *et al.* 1996). The age determination procedure applied since the mid-eighties in Uriarte and Astudillo (1987) and all subsequent works resulted in a discontinuity in the age composition of the Spanish anchovy catches prior to and after 1983, whereby 1 year-old catches became far more relevant (Table 1) (Anon. 1993).

Table 1.1. Matrix of catches at age reported for the period 1974-1992 in (Anon. 1993 from ICES)

Anchoa	**1974**	**1975**	**1976**	**1977**	**1978**	**1979**	**1980**	**1981**	**1982**	**1983**	**1984**	**1985**	**1986**	**1987**	**1988**	**1989**	**1990**	**1991**	**1992**
Edad 0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	150	180	17	87	38
Edad 1	776	0	156	31	0	1	14	3	0	388	161	53	52	339	508	180	1365	440	1442
Edad 2	602	861	1322	1687	1307	405	688	0	25	166	813	105	80	171	106	134	135	323	225
Edad 3	0	77	262	435	574	535	267	330	133	69	309	177	63	33	11	20	13	29	17
Edad 4	0	0	0	0	7	7	0	0	0	10	46	4	54	15	1	0	0	0	0
Edad 5	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0
Total	1378	938	1740	2153	1888	948	969	333	158	633	1329	339	249	605	777	514	1531	879	1722
Capt.	31117	26302	37261	48191	45219	26349	22102	10815	4991	14153	35179	7923	14762	15308	15581	10614	34272	19635	37885

Certainly the implications of such change in terms of assessment could be relevant because in case the age composition of catches was younger than formerly perceived there would not be any buffer (unexploited) biomass at age 1 before recruitment to the fishery occurs (in other words, the fishery would exploit anchovy as soon it reaches age 1).

In addition former estimates of the parameters of the Von Bertalanffy growth function differed with the new ones. The Linf and K were respectively reported at 24.3 cm and 0.7 in Orestes *et al.* (1981) or at 22 cm and 0.54 in Guerault and Avrila (1974), whilst from the mean sizes at age in Uriarte and Astudillo (1987) they were at 18.3 cm and 0.79 respectively (Anon. 1988b). Furthermore mean length at age 2 in Orestes *et al.* (1981) was closer to mean length at age 1 than at age 2 from Uriarte and Astudillo (1987).

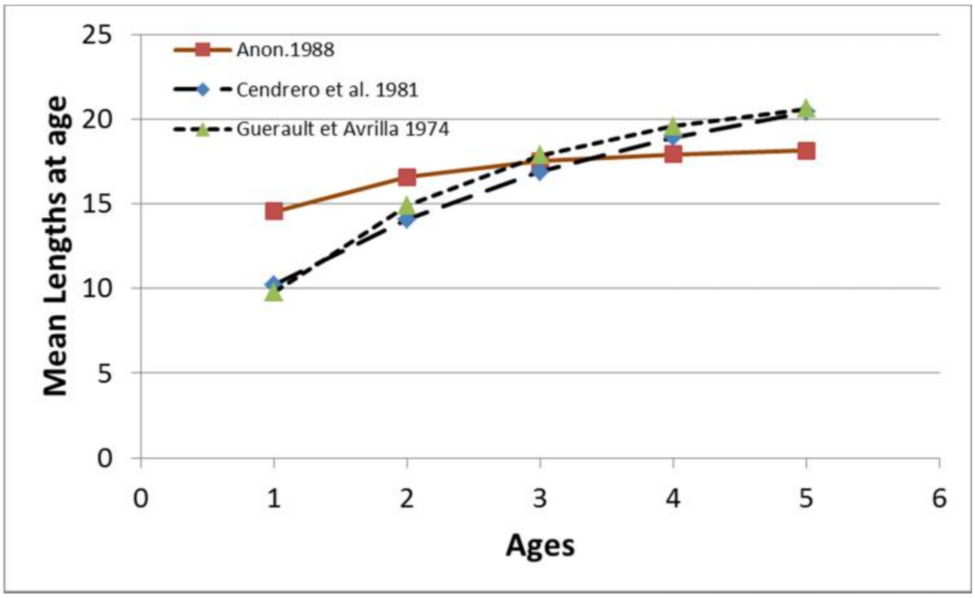


Figure 1.4. Published Von Bertalanffy Growth curves until 1988

Such changes in the age composition of catches and pattern of growth evidenced the need for a proper validation of the ageing methodology. Such work was carried out in the late eighties and early nineties (Uriarte 2002). Chapter 2 includes such work and an updated historical analysis of the performance of the age determination. It includes: a) the study of the annual and seasonal growth of the otolith which served to validate the age determination from otoliths for the anchovy in the Bay of Biscay; completed by b) a quantitative description of the annual growth increments in the otoliths; c) a historical corroboration of the age determination method up to the present day and d) a summary of the annual growth pattern (in length) of this anchovy resulting from it.

The age determination detailed in Chapter 2 was shared and improved through discussion during several exchanges and workshops on anchovy age reading in southeast Europe carried out since 1990 (see complete references in ICES 2009). During these exchanges of otoliths major difficulties in the interpretation of checks on anchovy otoliths were highlighted which required a proper study and validation. Much attention was paid to the basis for a correct interpretation of those checks.

Spawning fraction.

The implementation of the DEPM greatly improved the knowledge on reproductive dynamics of anchovy (Sanz *et al.* 1992; Motos 1994; 1996).

In the DEPM, spawning fraction (S), the fraction of mature females spawning per day is estimated histologically by the postovulatory follicle (POF) method (Hunter and Macewicz, 1985; Somarakis *et al.* 2004; Ganias 2012). This method was developed Hunter and Goldberg (1980) following upon the original finding of Moser (1967) that postovulatory follicles can be seen and used to track and determine time of past after spawning in rockfish. Based on that Hunter and Goldberg (1980) and Hunter and Macewicz (1985) stablished criteria for ageing POFs in *Engraulis mordax* Girard 1854. By doing so, females could be assigned to daily spawning cohorts and S could be calculated from the incidence of the female past spawning cohorts (Picquelle and Stauffer, 1985).

Small pelagic fishes tend to aggregate in spawning schools, few hours before and after peak spawning time, where actively spawning females go along with a large amount of males pharming ephemeral spawning aggregations (Alheit *et al.* 1984; Ganias 2008). For the estimation of spawning fraction such behaviour is a matter of concern as there is a tendency to oversampling actively spawning females around spawning time from the catches done on those fished schools (Picquelle and Stauffer 1985, Ganias 2008). For this reason, the fraction of Day 0 females (those spawning in the night or day of sampling) are not generally used for spawning frequency estimates.

During the first applications of the method to the anchovy in the Bay of Biscay, over-sampling of day_0 spawners at night (between 20:00 h and 04:00 hours) was noticed (Santiago and Sanz 1992; Motos 1996). And sampling at the beginning of the series used to take place mostly at night, day_0 cohort was discarded from the estimator of S. Examination of the degeneration state of POFs and their ageing was made directly in a single step by an expert, following the descriptions of Hunter and Macewicz (1985). S averaged 25% during the implementation of the DEPM surveys based on day-1 and day-2 past spawning females (Motos 1996; Somarakis *et al.* 2004; Santos 2011).

In order to obtain unbiased estimates the effect of the oversampling of day-0 spawning anchovy must be corrected (Stauffer ad Picquelle, 1980, Picquelle and Stauffer 1985, Santander *et al.* 1984). Hence, according to literature and until 2005, the day-0 spawners (when observed) had been replaced by the average of day-1 and day-2 spawners, i.e. by the expected number of females to be actively spawning in the sample in the absence of oversampling (Somarakis *et al.* 2004). The formula for estimating spawning frequency in the presence of day 0 per sample, based on the incidence of females which had spawned 1 and 2 days before sampling, was:

$$S(1+2)_i = \frac{(n_{1i}+n_{2i})/2}{[n'_{0i}]+n_{1i}+n_{2i}+n3_i^+} = \frac{(n_{1i}+n_{2i})/2}{(n_{1i}+n_{2i})/2+n_{1i}+n_{2i}+n3_i^+} \qquad \text{Equation 1.2}$$

With,

$S(1+2)_i$ = proportion of daily spawning females in sample ***i*,** according to the observed amount of females which had spawning 1 or 2 days ago.

$[n'_{0i}]$ = Substitute of the number of females of day-0 (which have spawned or are to spawn the day of capture) in sample ***i***, which is calculated as $(n_{1i}+n_{2i})/2$,

n_{1i} = number of females of day-1 in sample ***i***, i.e. females which spawn a day ago (the night before sampling)

n_{2i} = number of females of day-2 in sample ***i***, i.e. females which spawn two days ago (two nights before sampling)

$n3_i^+$ = number of females older that day-2 in sample ***i***, which are not classified as active spawners (day 0), i.e. females which spawn more than two days ago (three or more nights before sampling) and will not spawn the day of capture.

In absence of day-0 females the element in squared brackets $[n'_{0i}]$disappears from the denominator.

The substitutions were adopted to overcome the potential oversampling of actively spawning females aligned with the recommendations in literature, even though there was an indication that this might not be sufficiently reliable. There were increasing evidences that oversampling of pre-spawning females were not limited to the hours just prior to spawning but affected all day around (Motos and Uriarte *et al.* 1999). It was found that the estimates of S from those pre-spawning females for 1997 and 1998 were about the double of what was being expected by the usual day1 and 2 estimators. This was taken as indication of oversampling of these pre-spawning females.

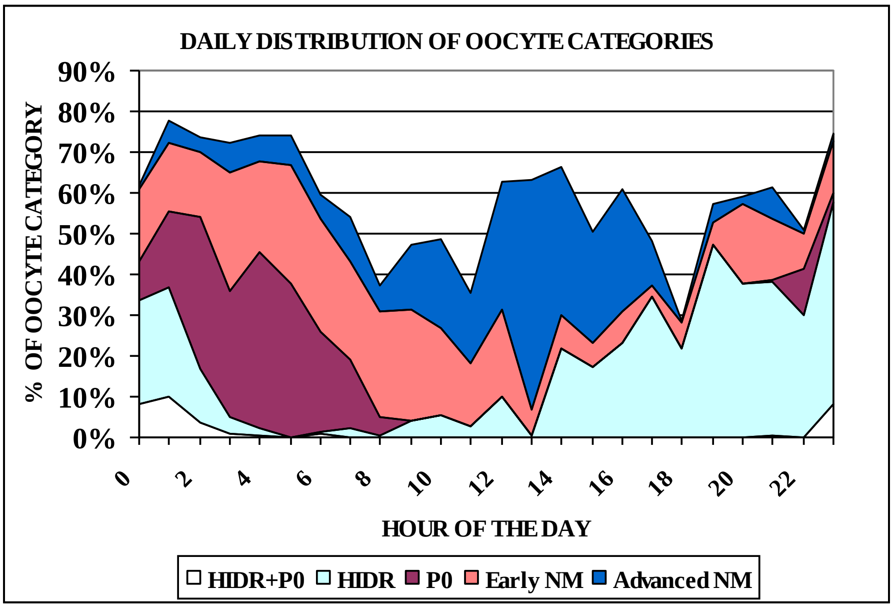


Figure 1.5. Incidence of actively spawning and immediately pre-spawning females along the day (from Motos and Uriarte 1999).

Application of the post-ovulatory follicle method for the estimation of S requires a good understanding of the degeneration process of POFs over time. For anchovy the major reference was the original study of the aging of POFs for northern anchovy *Engraulis mordax* (Hunter and Goldberg 1980 and Hunter and Macewicz 1985, which guided most of the applications of the DEPM on anchovy.

A proper study on the degeneration of POFs was actually lacking for the Bay of Biscay anchovy. Some tank experiments carried out in 1990 (Motos 1994) were reviewed by Alday *et al.* (2008) and resulted in a validation of the degeneration of POFs over time for this anchovy, which indicates faster degeneration process of POFs for *E. encrasicolus* than for *E. mordax*, for the range of sea surface temperatures between 13 and 21ºC. The study showed that full resorption of POFs was achieved usually in about 56-60 hours for this anchovy, a bit faster than perceived earlier. This would imply increasing the amount of females which should be allocated to day-1 past spawners at the expenses of reducing some of the previously females allocated to day-2. As such S could be higher than previously estimated. This finding along with the oversampling of pre-spawning females outlined above required a revision of our understanding the reproductive dynamics of females and a review of the basis of our estimation procedures of the spawning fraction for this anchovy.

Chapter 3 present the review of the basis for the estimation of S made for the Bay of Biscay anchovy on the basis of the study of Alday *et al.* (2008) and provide revised estimates for the past surveys of S. To this aim, the basis for the determination of spawning cohorts, from the histological examination of the gonads, were reviewed according to the prior knowledge on the maturation of oocytes (Motos 1996 and updated) and the study of Alday *et al.* (2008) on the degeneration of POFs.

Demography of Anchovy population.

Estimates of indices of population at age are among the most valuable auxiliary information to estimate past trajectories of the stock biomass and population at age through integrated assessments (Francis 2011). In addition they are capital for the studies on growth and mortality.

The DEPM was designed to estimate Spawning Biomass not population or biomass by ages (Parker, 1980 and Stauffer and Picquelle, 1980; Lasker 1985). However the adult sampling required for the estimation of the reproductive parameters should allow getting population at age estimates too, provided the sampling is good enough as to describe the spatial length and age structuring of the population. The only application of the DEPM being extended to produce population at ages estimates is the one we applied to the Bay of Biscay anchovy (Motos *et al.* 2005; Santos *et al.* 2015-in press). In fact, soon after the beginning of the implementation of the DEPM on anchovy in the Bay of Biscay, the survey included among its objectives that of obtaining population at age estimates, in addition to biomass, as a way to enhance its contribution to the assessment of the stock (Motos *et al.* 1991; Motos and Uriarte 1991).

There are several features of the bay of Biscay anchovy that makes it particularly suitable for essaying the extension of the DEPM to produce Population at age estimates:

1- All anchovy become mature when reaching its first year of life and those first spawners overlap with the spawning season of adults during May and June (Cort et al, 1976, Motos *et al.* 1991, Lucio & Uriarte 1990, Motos 1996). This implies that a single survey in these months serve to estimate the total entire spawning population (SSB) and this value equals then to the total population since all age classes are fully mature. Hence there is no need of maturity ogive estimates to infer the total population at age estimates.

2- In May, when the egg survey has been traditionally applied, daily fecundity of the population (eggs/gram) is rather invariant in space and across ages (Motos & Uriarte, 1991; Motos 1996; Uriarte *et al.* 2012). This confers a strong robustness to the DEPM estimator of SSB (small sensitivity to the age composition of the population or to the goodness of the spatial sampling). At the same time, it makes eggs/area proportional to biomass/area. The latter feature will be used in the procedure described in the study presented in chapter 3 to infer weighting factors for the individual samples within homogenous regions of the surveys.

This anchovy, like most other small pelagics, form schools which are clusters of fishes of rather similar sizes (Picquelle and Stauffer, 1985; Fréon and Misund 1999). So adult sampling is cluster sampling and this affects to the formulae of the estimations associated to the DEPM and to the procedure adopted for its extension to population at age estimates.

Chapter 4 describes the methodology followed during these years to expand to the DEPM to obtain population at age estimates. It provides, as an example the application to the anchovy in the 2009 DEPM survey and summarizes the application of the method to the series of DEPM surveys since 1990, along with the estimates of the population at age and variance. Finally a general discussion on the robustness and weakness of this procedure is provided.

Natural Mortality.

Predation is usually perceived to be the major driver of natural mortality. As mentioned earlier, anchovy is a relevant prey in the Bay of Biscay for the piscivorous species (Preciado *et al.* 2008; Goñi *et al.* 2011; Lassalle *et al.* 2011; López-López *et al.* 2012). However estimates of natural mortality are not available from multispecies models, except for an analysis of carried out by Sanchez and Olaso (2004) using Ecopath model which obtained an M around 1.5-1.6. Much of the predation on anchovy studied so far seems to focus much on juveniles rather than on adults (Guichet 1995; Preciado *et al.* 2008; Lezama-Ochoa *et al.* 2010; Goñi *et al.* 2011; 2012; López-López *et al.* 2012) and the actual mortality on adults remained uncertain.

Making use of the first initial estimates of the spawning population provided by the DEPM in the period 1987-1992, and assuming equal mortality for all ages, Uriarte *et al.* (1996) and Prouzet *et al.* (1999), reported rough estimates of natural mortality about 1.2 per year at all ages, but seemingly highly variable as well. The value was inferred assuming that DEPM population estimates were absolute and unbiased. As such a constant value for M at 1.2 was adopted for the integrated assessment of the population since 1995 onwards, until 2013.

Until 2004, ICA (Integrated Catch at age Analysis, Patterson and Melvin 1996) was used (ICES 2005), under the assumption of natural mortality constant at 1.2 per year at all ages. While the Bayesian models used later on assume constant catchability at age of surveys, ICA calculated catchabilities at age which were 50% higher for age 2 than for ages 1 or 3 (ICES 2005) in both surveys. This result was not regarded as realistic, taking into account the standard designs of the DEPM and acoustic surveys, with non-selective fishing gears for adults (retaining sizes well below the minimum sizes of anchovy in Spring), and sufficient spatial coverage of the anchovy distribution (ICES 2013) . Certainly, an alternative explanation of that result could be that natural mortality was not constant over ages.

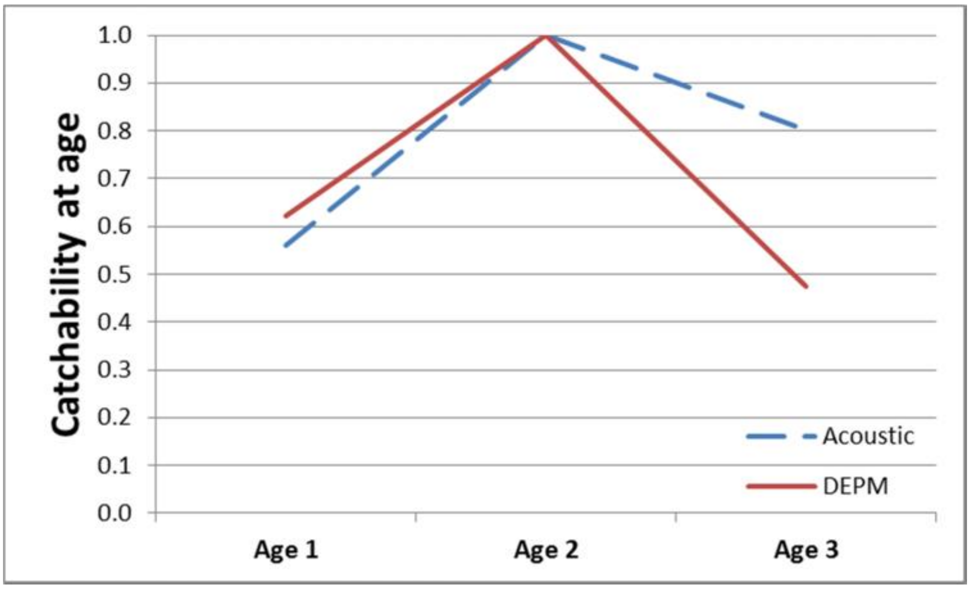


Figure 1.6. Relative catchability at age of surveys from a Seasonal Integrated Catch at Age analysis up to 2013.

Due to recruitment failures since 2001 and subsequent low biomass (ICES 2013), the anchovy fishery in the Bay of Biscay was closed between 2005 and 2010. With scientific surveys still ongoing during the closure, this provided a unique opportunity to estimate the actual level of natural mortality and possible patterns in natural mortality at age. Likewise, the closure of the fishery allows comparisons to be made between the total mortality levels during the closed period and the former period of exploitation, in order to get estimates of natural and fishing mortalities, under the assumption that no major changes in M occur between both periods (Gulland 1983, Vetter 1988; Cook 1994).

Chapter 5 presents the analysis of the Acoustic and DEPM survey series of population at age estimates, applying several methods to estimate the natural mortality of anchovy in the Bay of Biscay. The methods go from simple ANOVA analysis of Z estimates between surveys and for the closure and fishing periods, passing through linear models, and ending with integrated analysis of the fishery and survey series. The chapter aims at estimating a single natural mortality as well a likely pattern of natural mortality at age

1.4 Thesis, Objectives and structure of the report

In this Thesis we defend the relevance of accurate estimation of biological parameters for a correct assessment of populations and their management. This is applied to the anchovy in the Bay of Biscay and we focus on four key biological processes (and associated methods) which condition relevant inputs for the assessment. These are: growth (and age determination), reproduction (spawning frequency), demography and natural mortality. As the two later depend on the two first parameters, the thesis is:

“The accuracy and precision of the parametrization of growth and reproduction determine the perception of population dynamics and abundance estimates of the anchovy in the Bay of Biscay”

Partial Thesis and objectives by chapters

Four studies are addressed in this Thesis with the following partial thesis and objectives:

a) Study on Growth (Chapter 2): Validation of age determination using otoliths of the European anchovy *Engraulis encrasicolus* (L. 1758) in the Bay of Biscay.

Thesis: Anchovy otoliths show a regular pattern of checks and opaque bands deposition which can be used to determine the age of fishes, allowing the subsequent studies on growth and demography of catches or surveys’ population estimates, on which the integrated assessment of the population depends.

Chapter 2 addresses this study on Growth with the following objectives:

i) to present the study of the annual and seasonal growth of the otolith which validated the age determination from otoliths for the anchovy in the Bay of Biscay
ii) to provide a quantitative description of the annual growth increments in the otoliths;
iii) to assess the historical performance of the age determination method up to nowadays

iv) To summarize the annual growth pattern (in length) of this anchovy resulting from this ageing method.

Chapter 2 will present therefore the validation of the ageing methodology of anchovies from otoliths, describes the growth of otoliths, includes an updated historical analysis of its performance and summarizes the growth pattern of anchovies in length.

b) Study on Reproduction (Chapter 3): A re-evaluation of the spawning fraction estimation procedures for Bay of Biscay anchovy, a species with short inter-spawning intervals.

Thesis: The understanding of the late maturity of oocytes just prior to spawning and the degeneration of the post-ovulatory follicles in time allow checking several spawning fraction estimators and produce revised estimates of this parameter for the anchovy in the Bay of Biscay, which is capital for the DEPM biomass estimates.

Objectives:

i) To review the methodology used to estimate the spawning fraction (S) of the Bay of Biscay anchovy as an example of a species with indeterminate fecundity and short inter-spawning intervals.
ii) To evaluate alternative S estimators regarding their precision and robustness to the potential bias induced by the oversampling of active spawning females and their aggregation pattern
iii) To provide reviewed series of S estimates of past DEPM surveys since 1990, along with an examination of the role that temperature or female size may play in determining the levels of S.

To achieve these objectives, Chapter 3 will incorporate all the available knowledge on final oocyte maturation (Motos 1996, updated here) and POF degeneration (Alday *et al.* 2008) over time in an improved system of allocating mature females to daily spawning classes. This will allow checking the performance of five estimators of S: four estimators based on the proportions of daily spawning classes and a fifth one based on the individual spawning frequencies of females.

c) Study on Demography (Chapter 4): Anchovy Population at age estimates and variances from the application of the Daily Egg Production Method

Thesis: The adult sampling required for the implementation of the Daily Egg Production Method (DEPM) should allow expanding the spawning biomass estimates to spawning population at age estimates with variances, as to serve as an age disaggregated abundance index for the integrated assessment of the anchovy in the Bay of Biscay (or any other target species).

Objectives:

i) To describe procedures to extend the DEPM to estimate spawning population at age and corresponding variances.
ii) To provide generalized procedures to obtain total population at age estimates from the DEPM for cases of populations having ages with partial maturity (i.e. when not all individuals have reached maturity).
iii) To present the implementation of the method to the series of the DEPM on anchovy in the Bay of Biscay

Chapter 4 will present therefore the methodology followed during these years to expand to the DEPM to obtain population at age estimates with some generalization for the cases of ages with partial maturity. It provides, as an example the application to the anchovy in the 2009 DEPM survey and summarizes the application of the method to the series of DEPM surveys since 1990, along with the estimates of the population at age and variance.

It should be noticed that Population at age estimates from the DEPM depends upon the age determination method, described in chapter 2, and upon the spawning biomass which is determined by the spawning fraction, described in Chapter 3. Hence the accuracy and precision of the outcomes depends upon the reliability of the two first methods described so far in the Thesis.

d) Study on Natural Mortality (Chapter 5): Assessing natural mortality of Bay of Biscay anchovy from surveys population and biomass estimates

Thesis: The age structured population estimates from surveys (acoustic and DEPM) and the closure of the fishery between 2005 and 2010, should allow inferring the average level of natural mortality for the anchovy in the Bay of Biscay, as well as any potential pattern by ages of this parameter which is capital to scale the abundance estimates obtained from integrated assessments.

Objectives:

i) To estimate the average level of natural mortality of anchovy from the past series of surveys’ population at age estimates.
ii) To estimate potential patterns of natural mortality by ages.

Chapter 5 therefore will present the analysis of the Acoustic and DEPM survey series of population at age estimates, applying several methods, to estimate the natural mortality of anchovy in the Bay of Biscay, as well a likely pattern of natural mortality at age.

It should be noticed that natural mortality estimates depend directly upon the reliability of the population at age composition of surveys both in relative terms by ages as well as on the relative changes between years. The relative composition by ages will depend on the reliability of the age determination (chapter 2), on a good spatial sampling during DEPM and on the reliability of the estimation procedures (Chapter 4), whilst the relative changes between years will depend on the absolute levels of biomass (and hence from the S estimates of Chapter 3). Therefore the accuracy and precision of the outcomes on natural mortality will depend upon the reliability of the methods described in the three previous chapters of the Thesis.

2 Growth: Validation of age determination using otoliths of the European anchovy (*Engraulis encrasicolus* L.) in the Bay of Biscay and description of its annual growth

2.1 Introduction

Age determination for short-lived species by examination of otoliths can be difficult as it implies dealing with a large amount of young fishes (0, 1 or 2 year-old fishes) which typically may show some false rings (checks) as a result of the great sensibility of the youngest of otoliths to environmental changes, which can induce incorrect allocation of ages (Thomas 1983; Melo 1984; Waldron 1994; Waldron and Kerstan 2001; Panfili *et al.* 2002). In addition, some of these species inhabit tropical or subtropical regions with occasional, less marked seasonal cycles of temperature which may weaken the formation of typical annual growth patterns and also cause difficulties in ageing otoliths, although in other cases environmental seasonality in those latitudes is strong enough to lay down a neat seasonal growth pattern in otoliths (Morales-Nin and Panfili 2005). These difficulties and/or the lack of collection of otoliths have often led to the assessments of growth and demography of short-lived species in temperate and tropical latitudes to be purely based on length-based methods (Palomares *et al.* 1987; Bellido *et al.* 2001; Cubillos *et al.* 2001). However, insights into the growth dynamics and actual demography gained from the use of otoliths for age determination lead many groups working with short-lived pelagic species to adopt such procedures.

Age validation (Campana 2001; Panfili *et al.* 2002) for short lived species has usually been achieved through a combination of length frequency analysis with macro-increments in otoliths (Morales-Nin and Pertierra 1990) or with daily growth increments in the otoliths, particularly useful for the very first age groups (Thomas 1985; Morales-Nin 1989a and b; Hoedt 1992); or combining this with studies of marginal edge formation and back-calculation from otoliths of the length and time of ring formation (Thomas 1983; Melo 1984), etc. Overall, various patterns of growth have been reported for small pelagics, as reflected in different K parameters of the von Bertalanffy models (ranging from about 0.3 to 2) or in the ϕ' growth index of Pauly and Munro (1984) (Basilone *et al.* 2004). In addition, the potential of routinely or occasionally laying more than a single hyaline zone per year (particularly for the youngest age groups) has also been reported in some of these populations (Aguayo 1976; Melo 1984; Thomas 1983; 1984). Unsurprisingly, there are still problems with age determination of sardines and anchovies in several areas of the Mediterranean and Atlantic waters (ICES 2009; 2011).

The Bay of Biscay is located at middle latitudes (between 42º-48º N) with sea surface temperatures (SST) ranging from 11ºC to about 22ºC. Anchovy spawning takes place mainly from April to July during the warming up of SST between 13ºC and 19ºC (Figure 2.1) (Motos *et al.* 1996; Petitgas *et al.* 2010). There is a traditional Spanish fishery taking place in spring based on purse seining, and a French fishery occurring partly in winter but mainly during the second half of the year, based mostly on pelagic trawling (Figure 2.1) (Uriarte *et al.* 1996; ICES 2014). The fishery peaked in the sixties and declined irregularly, going through some peaks and troughs until the first decade of this century when successive recruitment failures crashed the fishery and forced a ban between 2005 and 2009 (ICES 2014). Assessment relies on direct monitoring by surveys. In May, two independent surveys are carried out: an acoustic survey (Pelgas series –Ifremer- Massé *et al.* 1996; in press) and a Daily Egg Production Method survey (DEPM Bioman series –AZTI- Motos *et al.* 2005; Santos *et al.* in press). These series started in the late eighties and both supply biomass and population at age estimates. In autumn, an acoustic survey on juveniles is carried out since 2003 (JUVENA series - Boyra *et al.* 2013). The three surveys and the catches at age are the input data for the integrated assessment carried out by ICES (ICES 2014), which applies a Bayesian Biomass based model (Ibaibarriaga *et al.* 2011) structured on two age groups: one and two or more years older age classes (referred to as age-1 and age-2+).

Early studies on the growth of anchovy in the Bay of Biscay had been carried out by direct examination of catch by length distributions (Navaz and Cabo 1966; Guerault and Avrilla 1973). Soon after, otolith examination was preferred because hyaline marks, presumably related to winter growth detention, were well marked on them (Guerault and Avrilla 1974). Since then and up to the mid-nineties, multiple scientists and at least 4 institutes worked on the age determination of anchovy in the Bay of Biscay (Guerault and Avrilla, 1978; Cendrero *et al.* 1981; Astudillo, 1986; Junquera, 1986; Uriarte and Astudillo 1987; Martín and Lucio 1988; Prouzet *et al.* 1995; Uriarte *et al.* 1996). The age determination procedure applied since the mid-eighties in Uriarte and Astudillo (1987) and all subsequent works resulted in a discontinuity in the age composition of the Spanish anchovy catches prior to and after 1983, whereby 1 year-old catches became far more relevant (Anon. 1993). This evidenced the need for a proper validation of that ageing methodology.

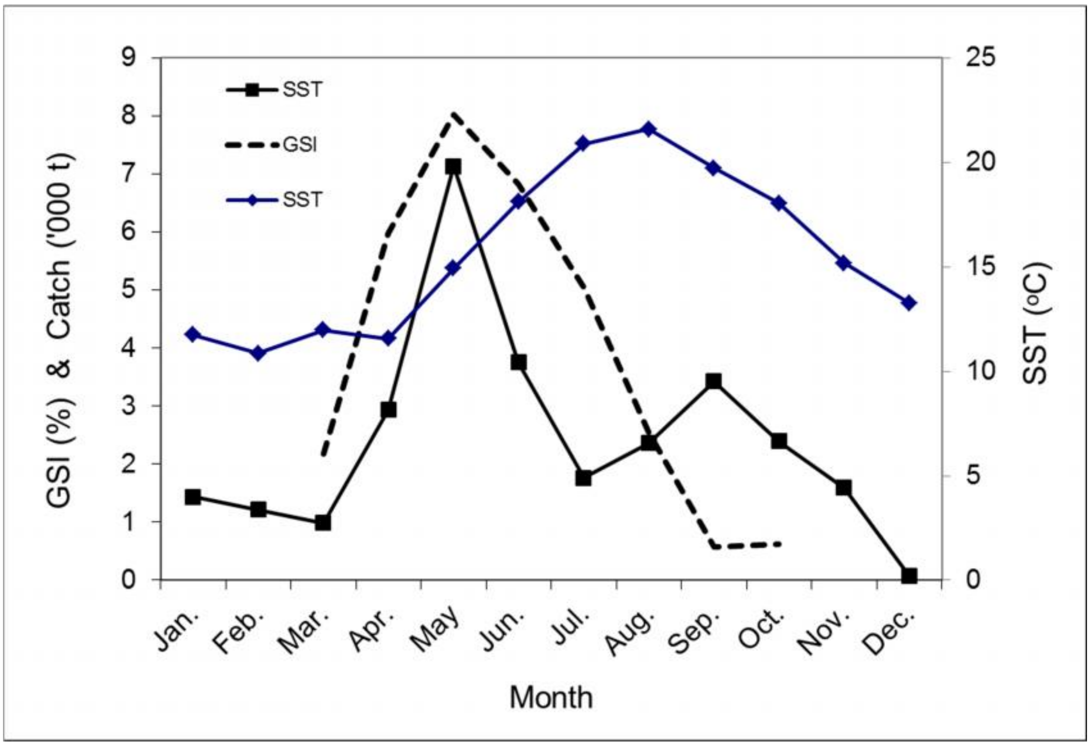


Figure 2.1: Monthly gonadosomatic index of the anchovy in the Bay of Biscay (in percentages, averaged from Sanz and Uriarte 1989 and Lucio and Uriarte 1990), mean monthly landings in the period 1992-2004 (thousands of tons) and evolution of the sea surface water temperature throughout the year (taken from the San Sebastián Aquarium).

The purpose of this article is to present a) the study of the annual and seasonal growth of the otolith which served to validate age determination from otoliths for the anchovy in the Bay of Biscay (originally carried out in the eighties and early nineties but never published); completed by b) a quantitative description of the annual growth increments in the otoliths; c) a historical corroboration of the age determination method up to the present day and d) a summary of the annual growth pattern (in length) of this anchovy resulting from it.

The method of age determination detailed in this paper was improved through discussions during several workshops on anchovy age reading carried out since 1990 (reports available in the repository of the ICES PGCCDBS (http://www.ices.dk/community/Pages/PGCCDBS-doc-repository.aspx; see complete references in ICES 2009). The method has served to deduce the demography of the catches and of the population estimates from surveys since 1987. It provided the foundations for several studies on spatial distribution of adults at spawning time (Motos *et al.* 1996; Vaz *et al.* 2002; Ibaibarriaga *et al.* 2013) and on the modelling of otolith shape (Gonzalez-Salas 2007) and growth (Hernandez *et al.* 2009, Pecquerie 2009; 2012). Beyond this, recent studies on otolith microincrement formation of this anchovy have enabled the daily rhythm deposition to be established in larvae (Aldanondo *et al.* 2008) and in juveniles and adults (Cermeño *et al.* 2003), as well as the method for otolith daily increment examination (Cermeño *et al.* 2008). A validation of the formation of the first annulus for this anchovy is also presented in the current special volume by daily increment examination of reared juveniles (Aldanondo *et al.* submitted).

2.2 Material and methods

2.2.1 Sampling

The monthly collections of samples (by years) and of otoliths (by ages) used for the original validation are detailed in Table 2.1A and B respectively. They come from the Spanish (national and regional) catch sampling programs between 1984 and 1992, taken at random up to 1986 and stratified to size categories from 1987 onwards. Some additional samples came from acoustic surveys (SARACUS 1985, IEO, Spain and EIGAS 1985 & 1986, IFREMER, France). Length distribution and age composition of the Spanish spring fishery between 1983 and 1987 appeared in Astudillo (1986), Uriarte and Astudillo (1987), and between 1988 and 1992 in ICES reports (Anon. 1989; 1991; 1992; 1993). Although no otoliths were available for 1983, for the purposes of this presentation we borrowed the rough inference from lengths made by Uriarte and Astudillo (1987). Finally for 1984 a new Age Length Key (ALK) was prepared based on the current age determination criteria and applied to the length distribution in Astudillo (1986).

Biological samples (Table 2.1A) consisted of a minimum of 40 fishes, taken randomly from the landings, for which total length (mm), weight (g) and sexual maturity was recorded and sagittal otoliths extracted. Otoliths were washed with water and dried for 24 hours before setting the entire otolith in transparent resin inside holes made over black plastic slide containers. Both otoliths were laid in parallel with the sulcus facing down.

Table 2.1: A - List of samples (by years and months) and B - List of otoliths (by age classes and months) available for the analysis of occurrence of edge types throughout the year in the period 1984 to 1992; C - List of anchovies used for the quantitative analysis of the size of opaque growth zones in otoliths from May in several years.

A: Samples

	Months												
Year	1	2	3	4	5	6	7	8	9	10	11	12	Total
1984	0	0	2	1	5	4	4	5	0	0	0	0	21
1985	0	0	1	3	3	4	4	1	1	0	0	0	17
1986	0	0	2	2	5	1	0	0	0	0	0	0	10
1987	0	0	4	14	11	9	1	1	0	4	2	0	46
1988	0	0	0	10	9	6	3	0	1	0	0	0	29
1989	0	0	3	1	9	5	1	3	1	3	8	0	34
1990	1	0	10	6	14	6	4	1	8	0	2	2	54
1991	0	0	0	3	8	5	0	0	1	2	0	1	20
1992*	2	0	0	0	0	0	0	0	0	0	0	0	2
Total	3	0	22	40	64	40	17	11	12	9	12	3	233

* Including in 1992 a sample from January 1993

B: Otoliths

	Months												
Ages	1	2	3	4	5	6	7	8	9	10	11	12	Total
Age 0	0	0	0	0	0	0	0	1	95	259	327	78	760
Age 1	87	0	435	541	1048	908	308	177	242	6	16	6	3774
Age 2	6	0	205	483	805	375	132	61	25	5	11	0	2108
Age 3+	0	0	149	271	266	116	69	1	17	0	2	0	891
Total	93	0	789	1295	2119	1399	509	240	379	270	356	84	7533

C: Otolith measurements.

	Years						
Ages	2004	2005	2006	2007	2008	2009	Total
Age 1	166	129	232	250	148	198	1123
Age 2	23	164	131	203	187	152	860
Age 3	12	12	45	37	82	95	283
Age 4	7	1	0	0	3	24	35
Total	208	306	408	490	420	469	2301

2.2.2 Age determination procedures

Age was estimated by interpreting and counting annuli on the otoliths. The otoliths were observed under binocular microscope at 20x, applying incident double oblique light (from both sides) as recommended in ICES 2009. The basic information required for age determination is the date of capture. Knowledge of the individual fish length is not required. Very doubtful age determinations were discarded from posterior processing if not agreed after a second examination by two readers. For this anchovy, conventional birthdate is set at 1st January.

Age determination is based on knowledge of the annual growth pattern of the anchovy otoliths, seasonal growth of the edge (by ages) and of the most typical checks.

Two ageing criteria were agreed in the 2002 workshop (Uriarte *et al.* 2002; ICES 2009), following the original validation. The first one is basically common to the age determination of many fish species based on otoliths, while the second one is more appropriate for short-lived species. These criteria are:

a) Criteria of complete annual growth zones (annuli) contained in the otolith (annulus) in conformity with the typical annual growth pattern of this anchovy, so that assigned age equals the number of complete opaque growth zones corresponding to the expected annual growth pattern of the otoliths, excluding the marginal edge development of the year. The latter arise from the fact that hyaline zones are usually formed in winter time during the first months of the year but are not necessarily present from the beginning of the year. Typically successive annual opaque growth zones are expected to be of decreasing length (Morales-Nin and Panfili 2002), but the expected growth pattern for this anchovy, which the readers should bear in mind when interpreting the otoliths, is fully described in the results section. In case the number of opaque zones does not correspond to the typical expected annual growth pattern for the presumed age, the existence of some false hyaline increments (checks) can be suspected and evaluated.

b) The criteria of conformity of the marginal edge development with the expected type of edge at the month (season) of capture which does change by ages (particularly for age-1). The actual formation of the otolith edge throughout the year by age classes is described in the results section; age readers should know this dynamic pattern of the edge formation to assess conformity with current criteria. If the edge of the otolith does not correspond with the expected edge type of the assigned age at the date of capture (as deduced from criteria a), then alternative interpretations could be considered, including the potential occurrence of checks.

2.2.3 Identification of Checks or false (non annual) hyaline zones.

As this anchovy can lay down several typical checks (mainly during age-0 and age-1), being familiar with them is relevant to the correct application of the former two criteria. The presence of checks becomes evident when looking at old fishes for which annual growth patterns are usually well established in the otoliths and their age determination is easy. They are usually faint hyaline zones; well visible but of less intensity than true winter hyaline zones placed within the typical annual opaque growth of otoliths and may occasionally not be well followed all around the otolith. Their aspect and position make them evident in older fishes, so that if taken as annual winter zones they would contradict the typical expected annual growth of otoliths. Checks have been typified and named with a C plus two digits according to the age of the fishes when formed (first digit) and to the approximate relative position (one decimal) over the expected annual growth of the otolith at that age (second digit). For instance, the most typical checks formed during their first year of life (age-0) are named C05 or C08 because they are formed approximately at about 50% or 80% of the expected annual growth of the otolith at age-0. Other typical checks are C12, C15 or C18, which correspond to checks formed at age-1 at about 20%, 50% or 80% of the expected annual growth of the otolith for that age. For older ages, checks become very rare. Not all fishes lay down checks in their otoliths and when present there can be only some of the typical checks but not others. The incidence of the most typical checks C05, C08 and C12 or C15 was recorded for the collection of otoliths in May of the years 1984-1991. In addition, the formation of check C12/15 was studied along with the study of edge formation for age-1, noting the time of the year when those checks were already recognizable. Checks C12 /15 refer to checks C12 and C15 together due to the difficulty of discerning one from the other when annual opaque growth is not complete and because they define a continuous range of relative positions.

The age compositions of catches are obtained by applying the respective ALK to the length distribution, usually on half year basis (but occasionally quarterly). Usually, more than a thousand age determination supports every ALK, along with a great sampling for length distribution (ICES 2014), well above the minimum standard sampling requirement established in the EU Data Collection Regulation. For the surveys, spatial explicit ALKs are applied to length distribution of the population estimates of the respective spatial strata (Massé *et al.* in press; Santos *et al.* in press; Boyra *et al.* 2013).

2.2.4 Age Validations

The age determination method and otolith growth pattern was established through three complementary validation studies.

First, the annual growth pattern of otoliths (or annuli identification) was achieved by establishing the correspondence between the types of otoliths and ages in spring through the following two indirect methods (sensu Panfili *et al.* 2002):

a) Monitoring of the progression of strong year classes (yc) in the catches over several years (correspondence of successive modal lengths with modal otolith types), which is a well suited indirect method for short-lived species and also for the first age groups of other species (Holden and Rait 1974; Campana 2001). This was applied to the progression of the 1982 yc in the Spanish spring purse seine fishery between 1983 and 1986 (up to the age of four, which was also seen as age five in 1987) and for the 1989 yc in the Spanish fishery throughout the year (between 1989 and 1991 up to the age of two).
b) Verifying the consistency between sharp spawning biomass fluctuations recorded in the spring DEPM surveys 1987-1992 and fluctuations in the biomass of the (presumed) 1 year-old recruits, which validates absolute correspondence between the Type I otoliths and the age-1 group. This relies on the fact that for a fish fully mature at age.1 (like this anchovy. Motos 1996), if survey’s observations are rather precise, any sharp increase in the spawning biomass has to be due to a major increase in the 1 year-old recruits.

Second, the seasonal growth pattern of otoliths was established by following the seasonal formation of the otolith margin edge throughout the year using the following semi-direct qualitative validation method (Panfili *et al.* 2002):

c) Monitoring of the occurrence of edge types throughout the year by age classes (as established above), for the collection of otoliths in Table 2.1. Basically, this validates the yearly rhythm of annuli formation, improves the understanding of the growth pattern of otoliths throughout the year and completes the age determination criteria in terms of the expected seasonal otolith edge type by ages. We basically followed the nomenclature for the edge types in otoliths adopted by ICSEAF for hake (1983 – which originated from Jensen 1965) by naming HN, HW, ON and OW to the narrow (N) and wide (W) Hyaline (H) or Opaque (O) edges respectively. To increase our precision we then established a border for the opaque edge between narrow and wide types in 33% of the expected opaque growth zone of the otolith by age, as measured with a micrometer. The expected size of the annual mean opaque growth zone for ages 1, 2 and 3 were measured in a subset of otoliths resulting in 479 µm for age-1 (N=121, CV=7.3%), 124 µm for age-2 (N=62, CV=7.5%) and 56 µm for age-3 (N=14, CV=15.7%). Additional edge types, OH and HO were defined for the transition forms from opaque to hyaline and from hyaline to opaque respectively; these correspond to an opaque or hyaline edge starting to be formed which is not yet visible all around the margin of the otolith respectively.

2.2.5 Annual growth increments

In addition, for a better quantitative comprehension of the annual growth of otoliths, additional measurements of the annual otolith increments (between annual hyaline zones) were carried out on a subset of the otoliths corresponding to the years 2004-2009 in the context of a recent project (AFISA EU Project no. 044132) (Table 2.1C). Measurements were taken on the radius from the core (or primordium) to successive annuli along the posterior axis of the otoliths (in microns) using a light microscope (at x20 magnification) coupled with an image analyzer (Visilog, TNPC Software, v.3.2, Ifremer, France).

2.2.6 Historical corroboration of the age determination method

Finally a set of corroborative indirect validation methods was applied to all available historical age composition of catches and population estimates from surveys (between 1987 and 2013), as reported to ICES (2014) by the Spanish and French Fishery Institutes (applying the current age determination procedures):

i) Checking correlation between the abundance of successive age groups of the same year classes either in catches or in population estimates from surveys. Significant correlations are demonstrative of coherent correlative age estimations (Panfili *et al.* 2012).

ii) Checking the consistency between the yearly biomass fluctuations in surveys and changes in percentages of the 1year-old recruits in the respective population estimates, which is a generalization of the validation method ***b*** explained above. This was tested by applying the following model which relates the ratio of biomasses in two consecutive years of a survey series to the ratio of age-1 proportions over older fishes in the second year (the odds ratio for age-1) (Uriarte 2014):

$$\frac{P_{1,y+1}}{(1-P_{1,y+1})} = \frac{B_{1+,y+1}}{B_{1+,y}} \cdot e^{-g_{1+}} - 1 \qquad \text{Eq. 2.1}$$

As such, this is a linear model with an intercept of -1 (offset) and a slope (parameter) equal to the inverse of the average survival in biomass of a population from year to year ($\exp(-g_{1+})$), where g_{1+} is the instantaneous rate of biomass decay/increase of all ages pooled together ($g_{1+} = G_{1+} - M_{1+} - F_{1+}$, with G, F and M corresponding to the rates of individual growth in mass, natural mortality and fishing mortality respectively). Since G, M and F usually vary across ages and may change along the time series, the slope cannot properly be considered a constant, but is subject to structural and process error. Although if such variability turns out to be of little magnitude, finding a significant fitting to such relationship should be indicative of an overall satisfactory performance of the age determination as well as of the biomass estimation procedures of the surveys. This is a suitable model for anchovy as the population mostly consists of two age groups; the 1 and 2+ year old fishes (Ibaibarriaga, 2008).

iii) Checking the correlation between the juvenile (age-0) estimates in the autumn acoustic survey series (JUVENA- Boyra *et al.* 2013) with the age-1 recruits estimates in the Spring surveys (both DEPM and acoustic) of the following year. This served to verify that juveniles (0 group in autumn) and recruits at age-1 (1 year-old in spring) are correctly identified from otoliths.

2.2.7 Verification

We verified the consistency of the anchovy age interpretation, i.e. the repeatability and/or precision of the estimation of ages (Panfili *et al.* 2002) by international otolith exchanges and workshops for this anchovy in the Bay of Biscay (see review in ICES 2009). For the current paper we will refer to the results of the 2002 and 2006 and 2009 exercises (Uriarte *et al.* 2002; 2007; ICES2009) in terms of agreement (%), precision (CV, Chang 1982) and relative accuracy (average relative Bias) relative to modal age between age readers of the teams carrying out the readings for the monitoring of this fishery.

2.2.8 Annual Growth pattern in length and growth parameters

Annual growth in length has been studied using the mean length at ages in the Spanish spring fishery 1985-2013, and also from the mean lengths at ages in the population obtained from the spring DEPM survey (1990-2013). The mean length at age class was integrated into the von Bertalanffy growth equation:

$$L_t = L_{inf} \cdot (1 - \mathrm{e}^{(-K\cdot(t-t_0)}) \qquad \text{Eq. 2.2}$$

In this equation, L_t is the mean fish length at age *t*; L_{inf}, *K* and t_0 the parameters that determine the shape of the growth curve: L_{inf} is defined as the asymptotic mean length; *K* the rate at which the curve approaches the asymptote and *t0* the age at which mean length is zero (Ricker, 1975). The Von Bertalanffy growth function (VBGF) has been fitted by direct minimisation of squared residuals to the former mean lengths in an Excel spread sheet between ages 1 to 5 (the latter from a single year). Comparison with the growth of other Engraulidae was based on the mean length at ages 1 and 2, on the Von Bertalanffy parameters and their combination in Pauly and Munro’s growth performance index (ϕ') (1984):

$$\phi' = \log(K) + 2 \cdot \log(L_{inf}) \qquad \text{Eq. 2.3}$$

where *K* and L_{inf} refer to the VBGF parameters.

2.3 Results

2.3.1 Annual growth pattern (annuli)

Figure 2.2 shows the series of typical otoliths in spring, ordered by an increasing number of opaque growth zones, labelled as otolith types I, II, III, IV and V (though the latter was only seen in 1987). The length distribution of the spring Spanish catches between 1983 and 1986 (Figure 2.3), showed that there was an increasing modal length during those years, while catches peaked in 1984 and later decreased. This was indicative of a very strong year class (the 1982) passing through the fishery being followed by weak year classes so that the progression in length of the cohort could be tracked year after year. Although in 1983 no collection of otoliths was available, the modal incidence of otolith types II and III in 1984 and 1985 catches and the maximum incidence of otolith type IV in the series occurring in 1986 led us to conclude that those otoliths labelled as type I, II, III and IV corresponded to ages 1 to 4 respectively. Finally, in 1987, a new group of otoliths showing an additional opaque growth band (compared to age-4) appeared for the first time in a rather remarkable frequency which obviously corresponded to the age of 5 of that 1982 cohort. The progression of modal lengths in the Spanish catches between the autumn of 1989 to the spring of 1991 (Figure 2.4) also revealed the passing of a big year class (the 1989 yc) and corroborated the former correspondence of otoliths type I and II with ages 1 and 2.

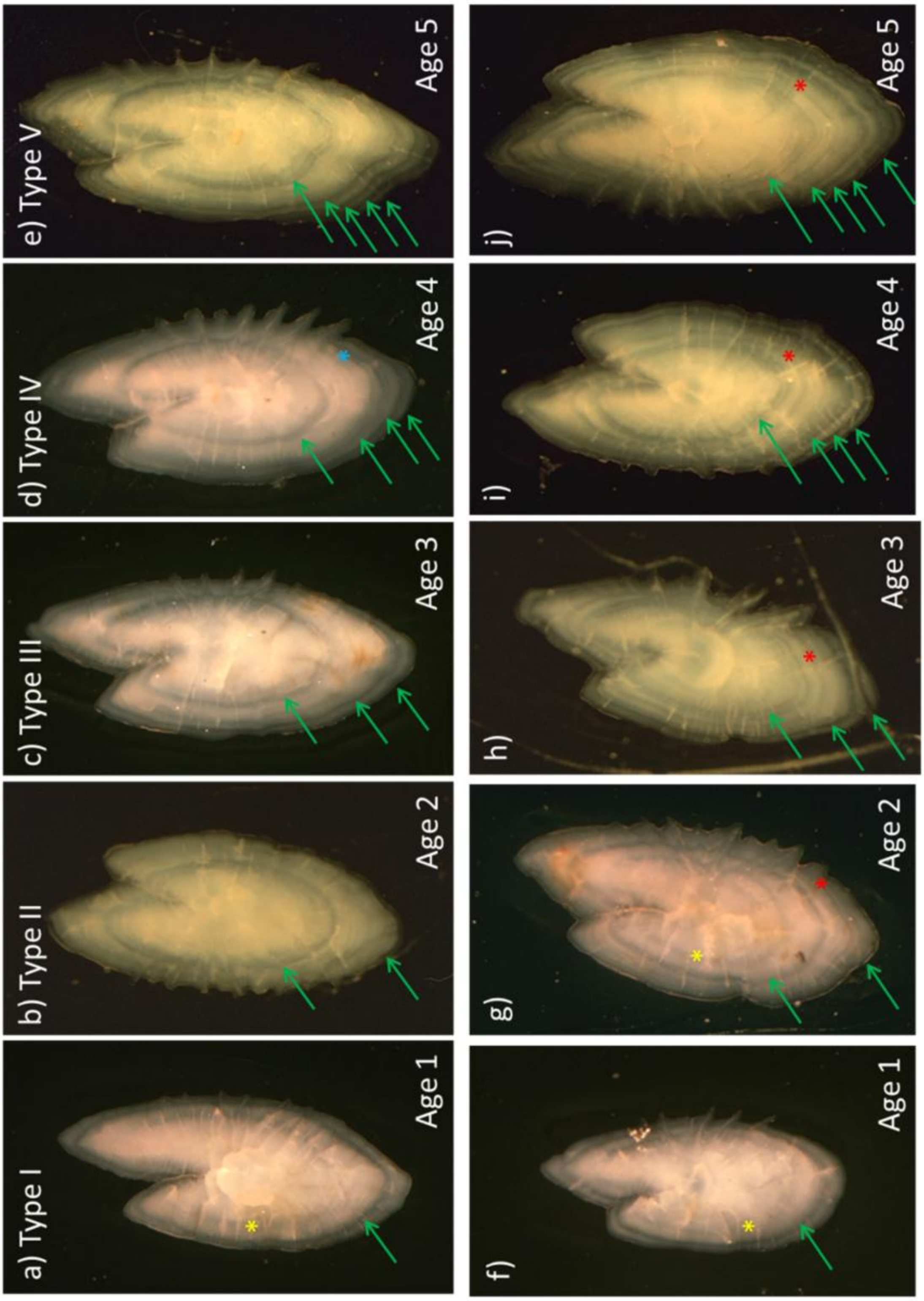
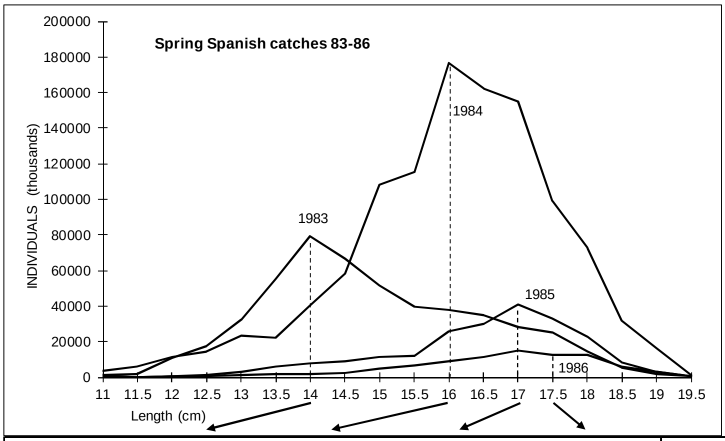


Figure 2.2: Pictures of anchovy otoliths. Upper panels: Typical otoliths found at spring time (May) by increasing number of white opaque growth zones: a) otolith type I (Age-1: 29/05/1990); b) Type II (Age-2: 31/05/1985); c) Type III (Age-3: 23/05/1985); d) Type IV (Age-4: 15/05/1986) and e) Type V (Age-5: 06/05/1987). Bottom panels: Set of pictures of otoliths showing most typical checks. Pictures from left to right: f) Age-1 showing check C08 (05/07/1990); g) Age-2 showing C08 and C12/15 (22/05/1991); h) Age-3 showing double first hyaline zone (split ring) and C12/15 (11/04/1985); i) Age-

4 showing double first hyaline zone (split ring) and C12/15 (22/05/1986) and j) Age-5 showing C12/15 (22/04/87). Symbols: green arrows point to the successive winter hyaline zones, yellow * point the C08; red * point the C12/15 and blue * point a C18 on picture d. Scale: pictures images contain an area of 2447µ * 4344µ.



Otoliths	Type I	Type II	Type III	Type IV	Total
1983 (*)	*64.4%*	*29.7%*	*5.9%*	*(*)*	*100%*
1984	12.8%	78.0%	8.8%	0.4%	100%
1985	11.8%	32.0%	54.9%	1.3%	100%
1986	17.6%	32.3%	26.3%	23.8%	100%
Conclusion	Age 1	Age 2	Age 3	Age 4	0

(*) IN 1983 There was no biological sampling and proportions by ages were inferred from length distributions (Uriarte & Astudillo 1987) with a 3+

Figure 2.3: Length distribution of the spring Spanish catches from 1983 to 1986 and respective percentages of the different otolith types I, II, III and IV in the catches, corresponding to the age groups 1 to 4 respectively, as pointed out in the bottom line (adapted from Uriarte and Astudillo, 1987; Uriarte *et al.* 1996).

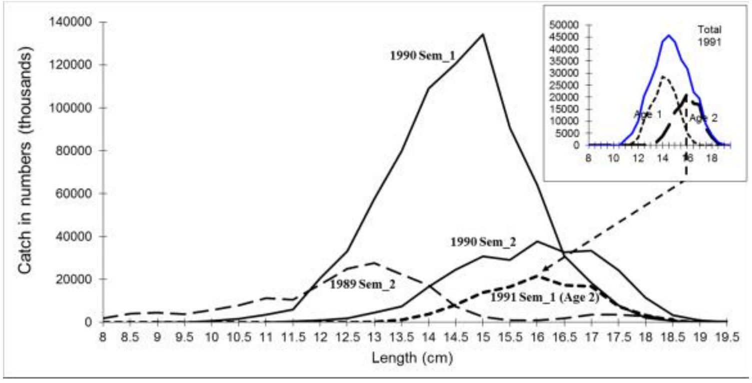


Figure 2.4: Progression of the length distribution of the 1989 cohort up to Age-2, as reflected in the Spanish catches from the second half of 1989 to the first half of 1991, on half- year basis. For the first half of 1991 only the length distribution corresponding to Age-2 is shown as subtracted from the total length distribution. The inner graph at the upper corner corresponds to the spring Spanish catches at

length and by age in 1991, from which the length at Age-2 distribution is included in the main graph, connected by the arrow.

The close correspondence between the ups and down of the population between 1987 and 1992, as estimated by the DEPM surveys, and the incidence of otoliths type I (age-1) in those surveys **(**Figure 2.5), proved that type I otoliths actually corresponded to age-1 and completed the former validation procedure.

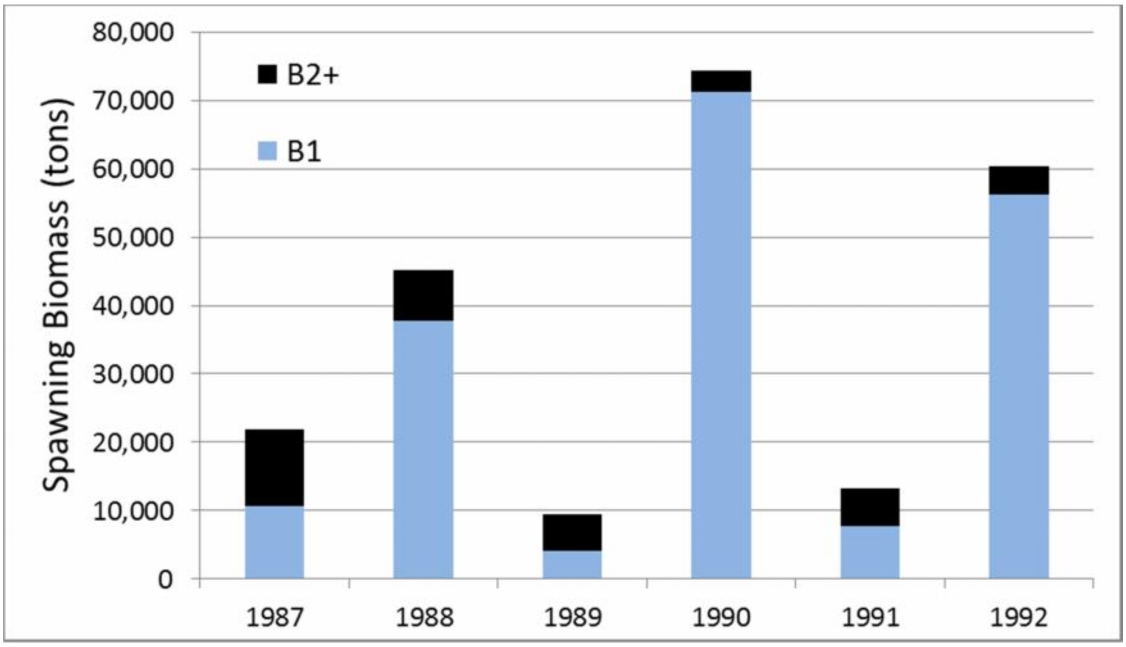


Figure 2.5: Spawning biomass fluctuations 1987-1992 (DEPM Surveys ICES 2014) and corresponding biomass by ages 1 and 2+ (B1 and B2+).

The quantitative analysis of otolith increments revealed that otolith growth is rather asymptotic due to the decreasing size of the annual increments (Figure 2.6a-b): Otolith growth during the first year of life (as age-0, until first winter hyaline zone) is vast and usually supposes the major part of the otoliths (even the oldest ones). During the second year of life (age-1), the opaque growth between first and second winter hyaline zones is still substantial though reduced to about 50% (CV=44%) compared to the growth achieved at age-0. And during the third year of life (age-2) the reduction of the opaque growth zone is very much pronounced, to about 29% (CV=33%) of that achieved at age-1. In subsequent ages, opaque growth still diminishes but to a lesser extent than the trend shown during the first three years of growth. As such, at age-3 opaque growth is still about 48% (CV=34%) of that produced at age-2, and at age-4 growth is probably larger than 50% of that achieved at the age of 3 as deduced from the few ages 5 of the 1982 cohort (Figure 2.2). In summary, the decreasing rate of annual growth with age (relative to the former age) is not constant but accelerates from ages 1 to 2 then slows down from age-3 onwards (Figure 2.6c), leading to a gradual (less intense) narrowing of increments subsequently. This typical pattern of otolith growth is clearly shown by the old ages of Figure 2.2.

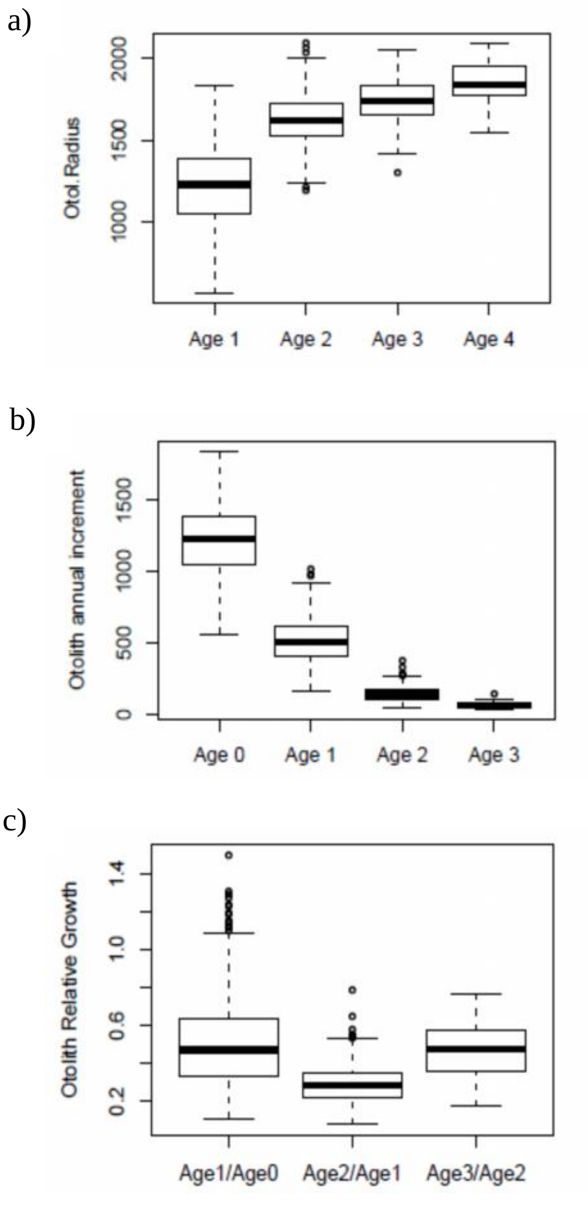


Figure 2.6: Box-and-whisker plots of otolith growth by age in absolute (microns) and relative terms: a) Upper panel - cumulative otolith growth along the post-rostrum axis; b) Middle panel - absolute growth increment by age and c) Bottom panel - growth increment by age relative to (/) former age growth increment.

Otolith increments during the second and third years of life (at ages 1 and 2) are inversely related to the growth achieved at age-0 (Figure 2.7a-b), so that the greater the growth achieved until its first winter, the smaller will be the growth increments during ages 1 and 2, resulting in a positive relationship between the growth increments of the latter two ages (Fig. 7c, bottom panel). The increment of otoliths at age-3 was not significantly related to the growth at age of any previous ages.

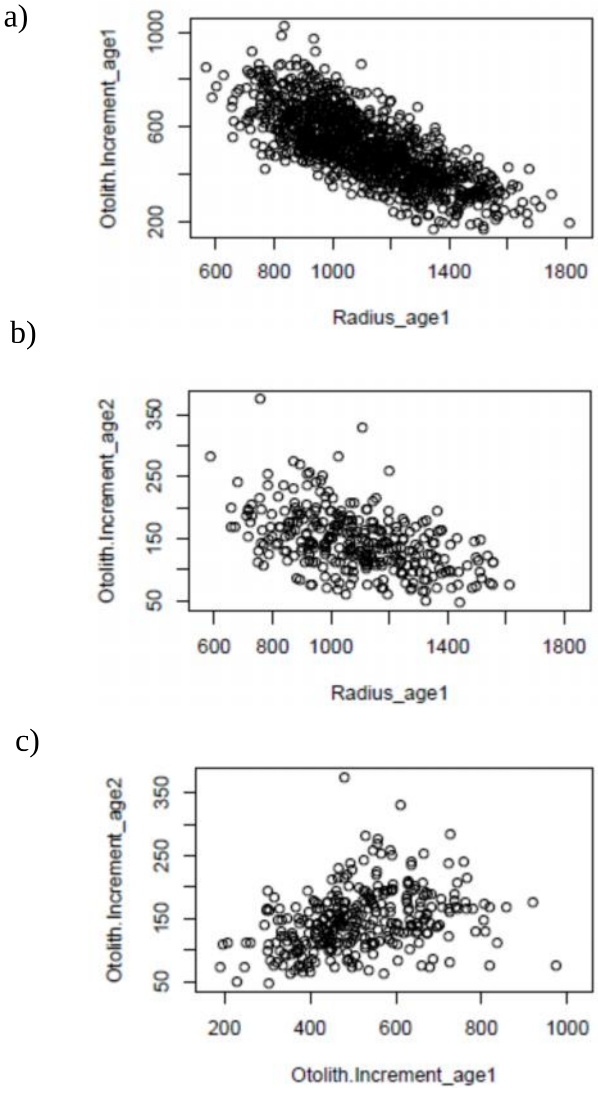


Figure 2.7: Covariation of anchovy otolith growth along ages 0, 1 and 2: a) Upper panel - Absolute otolith growth increment at age-1 versus growth achieved at age-0 (Radius.age_1); b) Middle panel - absolute otolith growth at age-2 versus growth achieved at age-0 (Radius.age_1) and c) Bottom panel - absolute otolith growth increment at age-2 versus growth increment at age-1. Age growth measurements correspond to the distance between successive winter hyaline zones (for age-0 this applies to the radius from the primordium to the first hyaline winter zone).

2.3.2 Seasonal growth pattern of otoliths by ages

Table 2.2 shows the occurrence of the different edge types of otoliths by age groups throughout the year for the whole set of samples between 1984-1992. A summary of the starting time of the formation of the marginal opaque growth of otoliths during the year by age classes is shown in Figure 2.8. And a set of pictures of typical otoliths by age groups throughout the year can be seen in **Appendix 1** along with a synthetic description of the otoliths by age group on a quarterly basis.

Age-0 of this species can be generally found in autumn with their otoliths completely opaque. Semi hyaline (OH) edges are occasionally seen in December. In January they will be by convention assigned to the 1 year-old group, though they will be about 6-8 months old.

Age-1: In January, some specimens will have entirely opaque otoliths, with many others laying down their first hyaline zone (OH or HN) and a few starting the formation of the marginal opaque edge (ON). By March, quite a few may still be showing a hyaline edge but about half of them will have already started the marginal opaque edge formation (ON) (Figure 2.8). In many cases, the first winter hyaline zone is in a composite of two or three close hyaline rings with very narrow opaque growth between them (split rings, Figure 2.2).

During spring, the formation of the marginal opaque growth in otoliths is continuous and fast, initially showing narrow opaque edges (ON) but by June the majority will have achieved a wide opaque growth (OW). However, by June/July some of them may show new partly hyaline edges after a substantial opaque growth (OH2).

By September, the marginal opaque growth of the age-1 group is perceived to be almost complete regarding the expected growth for this age group. In general, opaque edges predominate during the second half of the year until November, whilst new partial hyaline edges (OH2) or complete hyaline edges (HN2) appear in increasing abundance from October.

Table 2.2: Percentages of occurrence of the different otolith edge types by age throughout the year, for the whole set of samples between 1984 and 1992 (Sub-tables for ages 0, 1, 2 and 3+). The last rows of the tables, labelled "Total Otol.", refer to the total amount of otoliths examined per month. Edge types: OO refer to the otoliths entirely opaque until the edge, without bearing any hyaline zone inside them. Edge types HN, HW, ON and OW refer to the narrow (N) and wide (W) Hyaline (H) or Opaque (O) edges respectively. Edges OH and HO refer to the transition forms from opaque to hyaline and from hyaline to opaque respectively (i.e. not entirely visible all around the margin of the otolith). By OH2, HN2 and HW2, we mean new, partially, completely or intensely formed hyaline edges after having resumed (or completed) the annual marginal opaque growth.

Age 0						Month						
Edge Type	1	2	3	4	5	6	7	8	9	10	11	12
OO								100	99	100	100	87
OH									1			10
HN												
HW												
HO												
ON												
OW												
OH2												
HN2												3
HW2												
Total Otol.	0	0	0	0	0	0	0	1	95	259	327	78

Age 1						Month						
Edge Type	1	2	3	4	5	6	7	8	9	10	11	12
OO	36		1	0								
OH	55		7	1	0							
HN			29	1	0	1						
HW	2		14	1	0	1		1				
HO			1	0	0							
ON	7		47	95	61	27	2	1				
OW				1	24	51	68	83	73	33	69	17
OH2					13	20	30	16	21	50	31	
HN2									6	17		67
HW2									0			17
Total Otol.	87	0	435	541	1048	908	308	177	242	6	16	6

Age 2						Month						
Edge Type	1	2	3	4	5	6	7	8	9	10	11	12
OO												
OH	33		13	2	1	0						
HN	67		67	41	33	12	2					
HW			18	54	39	33	9					
HO			0	2	8	13	10					
ON			1	1	17	29	26	18				
OW					2	12	54	80	52	20	64	
OH2								2	36	80	36	
HN2									8			
HW2									4			
Total Otol.	6	0	205	483	805	375	132	61	25	5	11	0

Age 3+						Month						
Edge Type	1	2	3	4	5	6	7	8	9	10	11	12
OO												
OH			9	2	2		4					
HN			74	67	59	20	3					
HW			15	31	33	53	36					
HO					3	16	14					
ON			1	1	2	8	38		6			
OW						3	4	100	88		100	
OH2									6			
HN2												
HW2												
Total Otol.	0	0	149	271	266	116	69	1	17	0	2	0

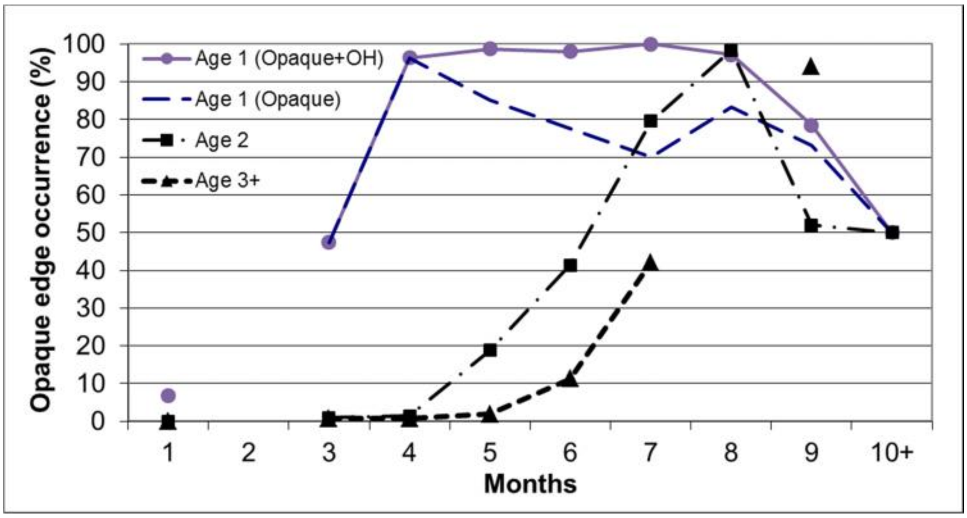


Figure 2.8: Occurrence of marginal opaque edges (adding up the opaque narrow and wide edges: ON + OW) by age class and month for the Bay of Biscay anchovy. Age-1 is shown either including the new semi-hyaline edges (opaque edges + OH2) which occur during summer time or excluding them (Opaque = ON+OW). New semi-hyaline edges (OH2) refer to the transition forms from opaque to hyaline not entirely visible all around the margin of the otolith which appear after having resumed (or completed) the annual marginal opaque growth.

A neat verification of the growth of otoliths at age-1 throughout the year was achieved with the strong 1989 cohort, which accounted for more than 80% of the international catches in numbers in the period 2nd half 1989 to the end of 1990, and about 50% of the catches during the first half of 1991 – ICES 1998). The length distribution of the Spanish catches during the second half of 1989 and the two halves of 1990 (Figure 2.4) enabled to follow the growth of this year class from age-0 to age-1. By comparing the 1990 autumn length distribution with the subsequent length distribution of age-2 in 1991 (Figure 2.4), it was clear that most of the growth in length of the age-1 had been achieved by September 1990 which was the month of maximum catches in that period. Similarly, we found that this year class already showed a seemingly completed marginal opaque growth of otoliths by September 1990.

Age-2: During winter, age-2 fishes show partial or completely formed hyaline edges (OH and HN). In April and May, anchovies show complete hyaline edges (HN and HW), being increasingly more pronounced and wide (HW) until June (or occasionally until July). In contrast with the first winter hyaline zone, the second one generally results in a single and well pronounced hyaline zone, with cases where intermittent growth during winter results in double hyaline zone being rare and exceptional. Formation of the marginal opaque edges starts in May and June (with ON) and rapidly becomes wide opaque growth in the majority by July (OW). There are some yearly changes in the starting and ending time of opaque edge formation. For instance, age-2 in 1989 showed a rapid and vigorous edge development so that maximum incidences of ON and OW edges appeared about a month earlier (May-June) than in other years (June-July). In August, most of the opaque edges are very wide and by September the growth of the 2 year-old seems visually completed; this being indicative that most of the otolith growth increment of the two year-old fishes takes place during the summer months. By September, some of these otoliths show new partial hyaline edges (OH2) with increasing occurrence in October. However opaque edges can still be seen until November.

Age-3 (and older): Narrow hyaline edges (HN) predominate from March to May while wide hyaline edges predominate in June. The transition from hyaline to opaque edge occurs in July, while in summer opaque edges predominate. The two otoliths examined in November were still showing wide opaque edges (OW).

The timing of formation of the opaque edges by age is summarised in Figure 2.8: For age-1, growth resumes usually during March and by April the vast majority of them show marginal opaque growth. For the age-2 group however, only a few start laying down the marginal opaque growth by May, many do it during June and the majority of them have already resumed opaque growth by July. For age-3, it is only in June when some of them start showing the marginal opaque growth, while by July most of them will have resumed the otolith opaque growth and by August all of them show opaque edges. So the older the fish, the later the formation of the marginal opaque growth resumes. As such, for ages 2 and 3 (and older) spring time is a period when the hyaline zone, laid down in winter, intensifies and becomes more pronounced and wide (HW) (Figure 2.8 and Table 2.2), while for age-1 it is a period of intense growth and formation of opaque edge. This a major contrast in the edge type of otoliths which age readers should bear in mind.

In addition we have noticed that by September otoliths of all ages show such a wide marginal opaque growth that they seem to have culminated the expected total annual opaque growth.

2.3.3 Checks

The most typical checks are: the C05, C08, C12, C15 and C18 (Figure 2.2). Checks during the second year of life (such as checks C22 or C25) are very rare and are not further dealt within this work.

The incidence of Checks C05, C08 and C12/15 in otoliths sampled in May between 1984 and 1991 changes largely over these years (Table 2.3). Check C05 is the least frequent one with a yearly incidence ranging between 0% and about 20%, having an average incidence of about 9.6% on the 1 year-old fishes but being detected in a lesser extent at the age of 2 or older, probably due to some loosing of contrast as the otolith grows while aging. The incidence of Check C08 varies a lot across year classes, ranging between 0% and about 33%. On average, C08 is observed in about 15-20% of the otoliths. And Check C12/15, almost absent at age-1 in May, is the most frequent one at age-2 or older, ranging between 15% and 60% depending on year classes and ages, being detected on average in about 39% of the 2+ year-old fishes.

The presence of C12/15 in the otoliths of the 1 year old fishes increases from mid-year to autumn (from around 10% in June, to around 30-40% in July-September and to around 50% in autumn. This allows inferring that check C12/15 is laid down in the otolith on average about June-July. The semi-hyaline edges which are rather common for the age-1 between June and July (Figure 2.8) should correspond with the formation of this check C12/15.

Table 2.3: Incidence (percentage) of checks C05, C08, C12/15 by ages 1 and 2+ in the May samples between 1984 and 1991.

Age 1	Year							
Check	1984	1985	1986	1987	1988	1989	1990	1991	Mean
C05	18.2	0.0	4.7	12.7	19.4	5.1	4.9	12.1	9.6
C08	54.5	11.4	26.6	32.9	18.8	8.9	1.3	11.0	20.7
C12/15	0.0	8.6	8.3	2.5	2.6	0.0	0.0	0.0	2.8
N. otoliths	11	35	84	158	191	79	390	91	129.9

Age 2+	Year								
Check	1984	1985	1986	1987	1988	1989	1990	1991	Mean
C05	0.0	3.0	0.7	8.8	0.0	0.5	2.9	2.8	2.3
C08	11.0	19.5	25.5	27.5	14.3	11.2	8.8	6.2	15.5
C12/15	49.0	51.2	54.5	35.7	33.3	25.7	20.6	42.8	39.1
N. otoliths	100	162	145	182	105	187	34	145	132.5

2.3.4 Historical corroboration of the age determination method

Application of the current ageing method to infer the age composition of catches in the period 1987-2013, results in a significant correlation of the successive catches at age by year classes in the international fishery (Figure 2.9). This result indirectly validates the former methodology of age determination from the examination of otoliths as ages have to be determined rather precisely for it to be true. Parallel analysis of the National fisheries reveals that the French catches at age show significant relationships for all ages throughout the whole time series (with r= 0.829 p=1*10E-5 and r=0.555 p=0.011, for ages 1 to 2 and ages 2 to 3 respectively). For the Spanish spring fishery the correlations were also significant up to the closure in 2004 (with r= 0.489 p=0.046 and r=0.657 p=0.004, for ages 1 to 2 and ages 2 to 3 respectively), but the addition of the years after reopening the fishery (2010-2013) render the relationship between ages 1 and 2 not significant at p= 0.160, due to unusual large quantities of catches at age-2 over age-1 in comparison to those occurring in previous years, whilst the relationship between ages 2 and 3 remained significant (p=0.013).

The same analysis for surveys shows significant correlation of successive age groups by year classes of the populations at age estimates (at alpha of 10%, not always at 5%). For brevity, no graphs are shown but correlation between successive population estimates at ages 1 and 2 and ages 2 and 3 for the DEPM (N=20) were of 0.435 (p=0.055) and of 0.677 (p=0.001) respectively, whilst for the acoustics (N=14) these correlations were 0.819 (p=0.0003) and 0.509 (p=0.063) respectively.

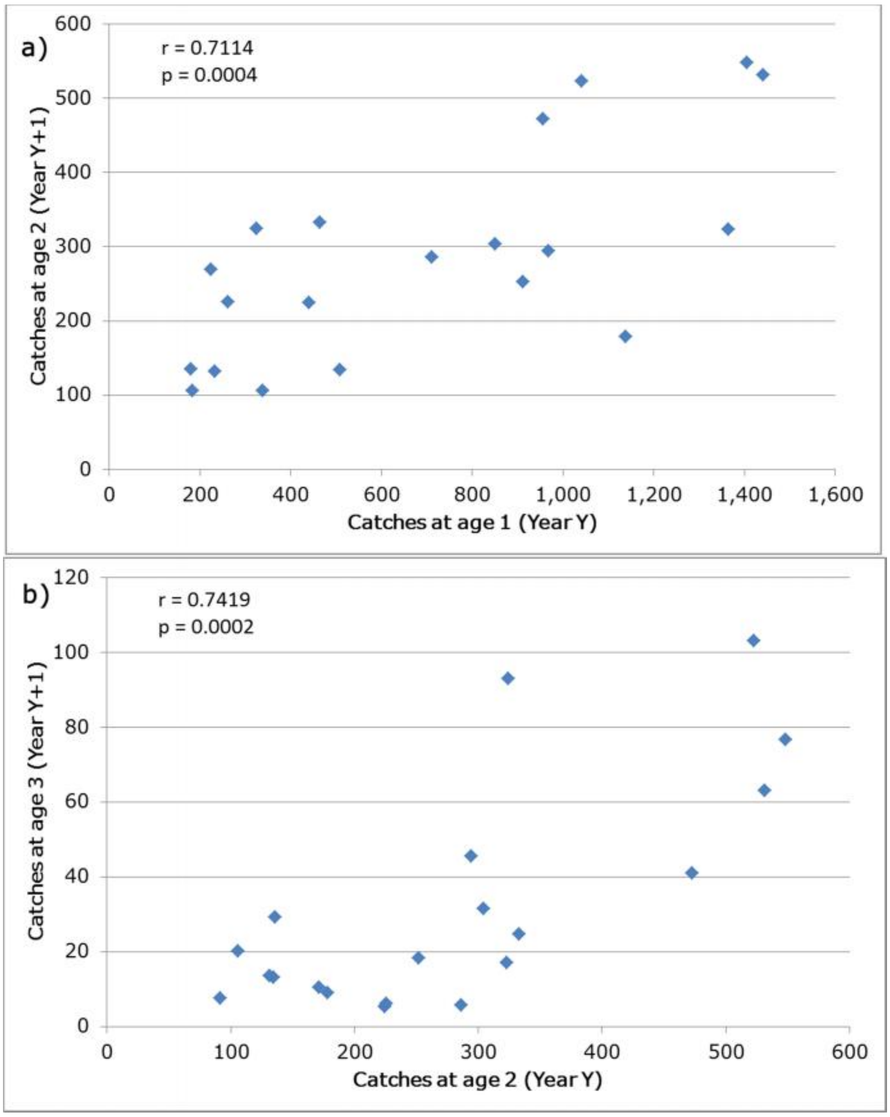


Figure 2.9: Covariation of successive age groups in the catches in numbers (millions) of the international fishery for a) Age-1 vs. Age-2 and b) Age-2 vs. Age-3.

Two additional indirect historical validations of the age determination were achieved with surveys: First, a significant linear relationship was found between the ratio of biomasses in two consecutive years of the spring surveys and the odds ratio for age-1 in the second year (r=0.722 p<0.0000, N=37) (Figure 2.10a). This implies that globally the surveys were properly tracking the biomass oscillations of the anchovy population and that the age readings of age-1 and age-2+ were globally accurate. Second, a significant linear relationship was found between the abundance of juvenile anchovies (age-0) estimated by the JUVENA acoustic survey series in autumn and the estimates of recruits at age-1 in either of the two spring surveys of the following year (For the DEPM r=0.955 and p<1.6*E-5 and the acoustic r=0.931 and p<9.1*E-5, for 10 observations each) (Figure 2.10b). This implied both that surveys were consistently estimating the biomass of recruits and that age-0 and age-1 groups were well identified from otoliths.

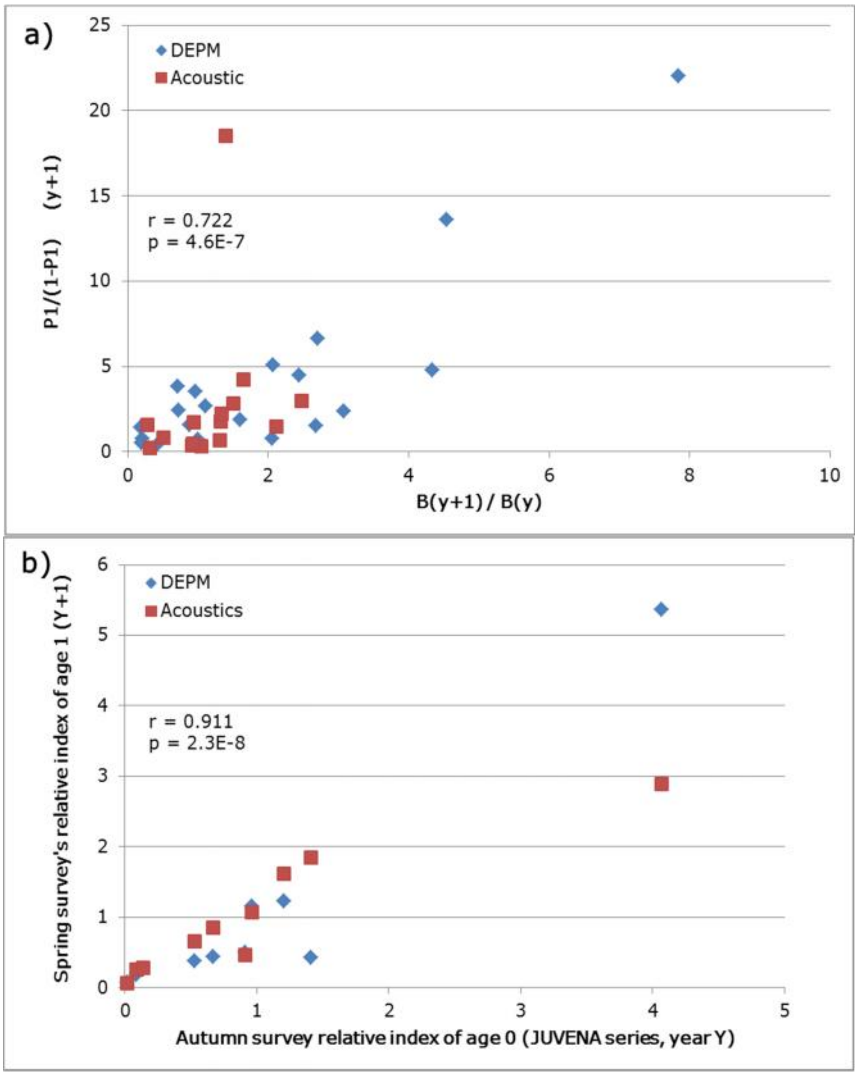


Figure 2.10: a) Covariation of the ratio of successive biomass estimates in the spring DEPM and acoustic survey series and the odds ratio of P1 in every second year of the respective survey series. b) Abundance of juvenile anchovies (Age-0) in the JUVENA acoustic survey series in autumn (in any year Y) versus the estimates of recruits at Age-1 in the spring of the following year (Y+1) in the DEPM and Acoustic surveys.

2.3.5 Verification

The agreement, CV and relative bias of the age readings globally improved in the workshops (WKs) held after the exchange programs (EXs). The average agreement in EXs was usually on average above 85%, but after workshops the agreements always increased to about 92% (Table 2.4). The higher agreements occur for the younger ages (0 and 1 year-old with agreements about 100% and 93% respectively) followed by slightly poorer agreements for the older fishes (ages 2 to 4, with agreements between 80 and 92%). After workshops, the CV ranged between 0% and 15% with an average mean across ages of about 9%, whereby CV usually increases with age up to the age-2 (ending at about 15%). In the exchanges prior to workshops, bias are small for young ages but tend to be slightly negative for older ages (age-2 and age-3), whilst after workshops, the relative bias to modal age is on average very low (about +/- 0.03), usually null at age-0, and low for age-1 ranging between 0.02-0.07, at age-2 ranges between 0.13 and -0.14 and being usually slightly negative at age-3 by about -0.14/-0.22 (omitting the info from the 2 otoliths examined in 2009).

Table 2.4: Results of anchovy age determinations achieved during the latest exchanges and workshops carried out by the Bay of Biscay expert age readers (just the subgroup contributing to the assessment) (in 2001, 2005/2006 and 2009), with indication of the number of otoliths examined by age and exercise, the level of agreement, coefficient of variation (CV) and relative bias by modal age. The number of readers appears at the heading of every sub-table. Sources: 2001/2002 exercices from Uriarte *et al.* 2002, 2005/2006 exercices from Uriarte *et al.* 2007 and 2009 exercices from ICES 2009.

a1) Exchange 2001 *Readers* 4

Ages	Otoliths	Agreement	CV	Rel.Bias
0	33	96.3%	21.0%	0.04
1	99	94.2%	11.8%	0.06
2	45	77.6%	22.9%	-0.18
3	22	72.7%	27.4%	-0.38
4	NA	NA	NA	NA
All	200	88.5%	17.5%	-0.05

a2) Workshop 2002 *Readers* 4

Ages	Otoliths	Agreement	CV	Rel.Bias
0	34	99.3%	5.9%	0.01
1	104	90.6%	13.6%	0.09
2	41	91.4%	6.6%	0.01
3	21	85.7%	9.8%	-0.14
4	NA	NA	NA	NA
All	200	91.7%	10.5%	0.05

b1) Exchange 2005 *Readers* 5

Ages	Otoliths	Agreement	CV	Rel.Bias
0	4	94.1%	55.9%	0.06
1	163	94.0%	9.2%	0.06
2	32	85.1%	13.4%	-0.04
3	18	93.7%	4.6%	-0.04
4	2	100.0%	0.0%	0.00
All	219	92.6%	10.2%	0.03

b2) Workshop 2006 *Readers* 5

Ages	Otoliths	Agreement	CV	Rel.Bias
0	4	100.0%	0.0%	0
1	165	95.3%	8.3%	0.02
2	29	86.4%	12.4%	-0.02
3	18	80.0%	14.1%	-0.22
4	2	88.9%	5.9%	0.11
All	219	92.7%	9.2%	-0.03

c1) Exchange 2009 *Readers* 3

Ages	Otoliths	Agreement	CV	Rel.Bias
0	12	100.0%	0.0%	0
1	18	83.0%	20.0%	0.19
2	8	91.0%	11.0%	0.13
3	2	63.0%	38.0%	-0.63
4	NA	NA	NA	NA
All	40	88.8%	12.9%	0.08

c2) Workshop 2009 *Readers* 3

Ages	Otoliths	Agreement	CV	Rel.Bias
0	12	100.0%	0.0%	0
1	14	92.9%	9.3%	0.07
2	12	86.1%	14.4%	-0.14
3	2	83.3%	10.8%	-0.17
4	NA	NA	NA	NA
All	40	92.5%	8.1%	-0.03

In the 2006 otolith workshop it was noticed that difficulties in age determination become more relevant for the otoliths from the second half of the year (Percentage of agreement of 90.7 % and CV of 14.1%), than for the first half. These arise from the need to discriminate true winter rings from summer and autumn checks at this time of the year when the expected total annual growth is not yet fully achieved. For this anchovy, this makes it harder to differentiate ages 1 from older anchovies in the second half of the year.

2.3.6 Annual Growth pattern in length and growth parameters

Arithmetic mean length (and weight) at age in the series of catches of the Spanish spring fishery for ages 1, 2, 3, 4 and 5 are 14.6 cm (20.8 g), 16.7 cm (31.4 g), 17.5 cm (38.0 g), 17.8 cm (40.9 g) and 18.1 cm (42.4 g) respectively, with CV in length of about 4.5% for age-1 and about 3% for older ages (in weight CV are about 17% for age-1 and about 12% for older ages). Figure 2.11a shows no major tendencies in the annual mean length at age in the catches of this fishery throughout the time series. Figure 2.11b shows the fitting of the Von Bertalanffy growth curve ($L_{inf}= 18.28$ cm, *K*=0.79 and *t0*=1,Table 2.5).

When using the information from the direct surveys of the population in May, in particular the DEPM survey, we find that overall (inter-annual) arithmetic mean length (and weight) at age for ages 1, 2, 3+ are 13.2 cm (15.7 g), 15.9 cm (27.8 g) and 16.7 cm (33.9 g), with CV in length of about 5% for all ages (and about 15% in weight). This means that on average the mean sizes at age in the population are smaller than those caught by the spring Spanish fishery by about one cm for ages 1 and 2. Figure 2.11c shows some slightly declining tendency in the population mean lengths at ages throughout the time series estimated by the DEPM, particularly in the most recent years (significant for ages 1 and 2). In 2007 and 2012 abnormally low mean lengths at age 3 were estimated at about 14.5 cm (below the mean length at age-2 of their year classes by 2.3 and 0.8 cm respectively). For the fitting of the Von

Bertalanffy growth curve those two abnormally low values at age-3 were omitted, resulting in L_{inf} = 17.85 cm, *K*=0.85 and *t0*=0.60 (Figure 2.11d, Table 2.5).

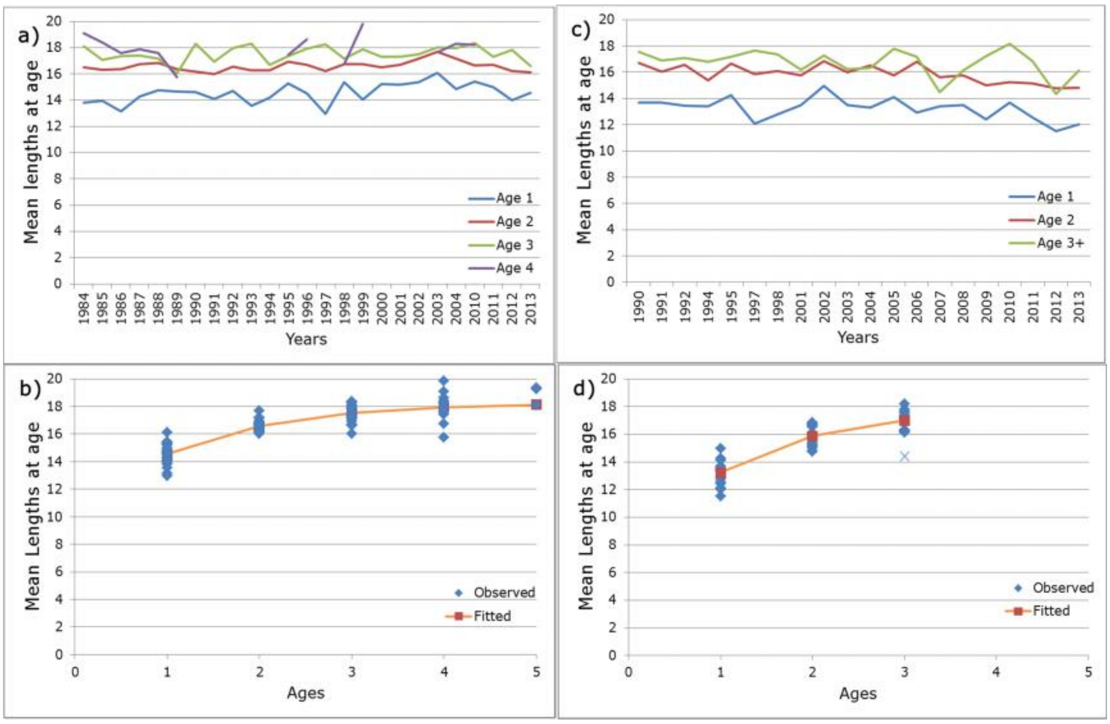


Figure 2.11: Left panels: Anchovy length at age in the catches of the Spanish spring fishery; a) Mean length at age series in the catches (1984-2013); b) Fitted von Bertalanffy growth curve to the mean length at age in the catches. Right panels: Anchovy length at age in the DEPM Spring surveys; c) Mean length at age series in the survey (1990-2013); d) Fitted von Bertalanffy growth curve to the mean length at age in the survey. By symbol x we denote two abnormal mean lengths at age 3 omitted from the VBGF fitting.

Table 2.5: Growth of the Bay of Biscay anchovy in comparison with other anchovies of the same genus (Engraulis): VBG parameters and respective estimation of length at ages 1 to 4. Source refers to the reference where the parameters came from. All works were based on otolith examination, except for those followed by LBA (length-based analysis). SST stands for annual mean sea surface temperature (in Celsius degrees). ϕ' refers to the Pauly and Munro (1984) growth index. Length size is in cm and it can correspond either to total length (TL), fork length (FL) or standard Length (SL). *L1/Linf* is the ratio of the mean length at age 1 over *Linf*.

	Geographic Region		SST		VBG parameters				Mean lengths at age (cm)						
Scientific name	Area	Latitude	(°C)	Length	L inf.	K	t0	ϕ'	Age 1	Age 2	Age 3	Age 4	Mean(1+2)	L1/Linf	Source
E. encrasicolus	North Sea	51°N	12.0	TL	20.59	1.14	0.1	2.68	14.6	18.7	20.0	20.4	16.6	71%	Blaszczyk P. (1999)
E. encrasicolus	Bay of Biscay	44°N	16.0	TL	18.28	0.79	1.00	2.42	14.5	16.6	17.5	17.9	15.6	80%	Uriarte *et al* . (this work, catches)
E. encrasicolus	Bay of Biscay	44°N	16.0	TL	17.85	0.85	0.60	2.43	13.3	15.9	17.0	17.5	14.6	74%	Uriarte *et al.* (this work, surveys)
E. encrasicolus	Bay of Biscay	44°N	16.0	TL	17.25	0.92	1.05	2.44	14.6	16.2	16.8	17.1	15.4	85%	Hernandez *et al* . (2009)
E. encrasicolus	Bay of Biscay	44°N	16.0	TL	24.33	0.33	0.7	2.29	10.4	14.3	17.2	19.2	12.4	43%	Cendrero *et al* . (1981)
E. encrasicolus	Bay of Biscay	44°N	16.0	TL	22.00	0.54	0.1	2.42	9.8	14.9	17.9	19.6	12.3	44%	Guerault and Avrilla (1974)
E. encrasicolus	Cadiz (Spain)	36°N	18.5	TL	18.69	0.89	0.02	2.49	11.1	15.6	17.4	18.2	13.4	60%	Bellido *et al* . (2000) (LBA)
E. encrasicolus	NW Mediterran.	41°N	18.6	TL	18.19	0.45	1.09	2.17	11.1	13.6	15.3	16.3	12.4	61%	Pertierra (1987)
E. encrasicolus	NW Mediterran.	41°N	18.6	TL	20.60	0.38	0.94	2.21	10.7	13.9	16.0	17.4	12.3	52%	Pertierra (1987)
E. encrasicolus	NW Mediterran.	41°N	18.6	TL	19.10	0.35	1.45	2.11	11.0	13.4	15.1	16.3	12.2	57%	Morales-Nin & Pertierra (1990)
E. encrasicolus	Sicilian waters	37°N.	19.2	TL	18.60	0.29	1.81	2.00	10.4	12.4	14.0	15.2	11.4	56%	Basilone *et al* . (2004)
E. encrasicolus	Adriatic Sea	42°N	17.6	TL	19.40	0.57	0.50	2.33	11.1	14.7	16.8	17.9	12.9	57%	Sinovčić G. (2000)
E. encrasicolus	Adriatic Sea	42°N	17.6	TL	16.15	0.40	2.04	2.02	11.4	12.9	14.0	14.7	12.1	70%	Arneri *et al* . (2011)
E. encrasicolus	Aegean & Ionian	38°N	18.0	TL	17.50	0.51	0.89	2.19	10.8	13.5	15.1	16.0	12.1	62%	Machias *et al* . (2000)
E. encrasicolus	Black sea	44°N	20.0	TL	19.10	0.35	1.45	2.11	11.0	13.4	15.1	16.3	12.2	57%	Karacam & Düzgünes (1990)
E. encrasicolus	Black sea	44°N	20.0	TL	16.77	0.32	2.07	1.96	10.5	12.3	13.5	14.4	11.4	63%	Erkoyuncu & Ozdamar (1989)
E. encrasicolus	Tunisian waters	37°N.	19.0	FL	19.16	0.32	1.68	2.07	11.1	13.3	14.9	16.1	12.2	58%	Khemiri *et al.* (2007)
E. encrasicolus	Tunisian waters	34°N.	19.0	FL	17.19	0.36	1.01	2.03	8.9	11.4	13.1	14.4	10.1	51%	Khemiri *et al.* (2007)
E. encrasicolus*	SW African waters	33°S	16.9	FL	12.10	0.97	1.32	2.15	10.8	11.6	11.9	12.0	11.2	89%	Melo (1984)
E. encrasicolus*	SW African waters	33°S	16.9	FL	13.00	1.33	0.17	2.35	10.2	12.3	12.8	12.9	11.3	79%	Melo (1984)
E. encrasicolus*	SW African waters	33°S	16.9	FL	14.00	1.49	0.00	2.47	10.8	13.3	13.8	14.0	12.1	77%	Melo (1984)
E. mordax**	Perú (Center)	12°S	18.0	TL	20.49	0.88	0.00	2.57	12.0	17.0	19.0	19.9	14.5	58%	Palomares *et al.* (1987)
E. mordax**	Perú (South)	17°S	16.0	TL	17.50	1.40	0.18	2.63	14.2	16.7	17.3	17.4	15.4	81%	Morales-Nin (1989)
E. mordax**	Chile	19°S	16.0	TL	19.04	0.73	0.02	2.42	10.0	14.7	16.9	18.0	12.3	52%	Aguayo (1976)
E. mordax**	Chile	25-33°S	15.5	TL	19.50	0.55	1.00	2.32	13.0	15.7	17.3	18.2	14.3	66%	Canales and Leal (2009)
E. mordax**	Chile	37°S	12.5	TL	20.80	0.44	0.39	2.28	9.6	13.6	16.2	17.8	11.6	46%	Cubillos *et al.* (2002) (LBA)
E. mordax	California	37°N.	15.0	SL	16.56	0.30	1.71	1.91	9.2	11.1	12.5	13.6	10.2	56%	Spratt (1975)
E.anchoita	Argent.\Uruguay	35°S	16.4		17.30	0.71	0.9	2.33	12.7	15.1	16.2	16.8	13.9	74%	Brandhorst *et al.* (1974)
E. Japonicus	YellowSea\China	35°N	20.0	TL	15.50	0.60	1.0	2.16	10.8	12.9	14.1	14.7	11.9	70%	Iversen *et al.* (1993)

*Formerly E. capensis ** Formerly E. rigens

2.4 Discussion

2.4.1 The validation methods

Our validation was successful in applying the method of tracking strong year classes, particularly for that of 1982 in the spring Spanish Fishery (Figure 2.3). Certainly, the moderate fishing effort at the beginning of the study and the occurrence of a strong (1982) cohort, followed by several medium or weak year classes, allowed us to track it up to the age of 5. In the past, Uriarte and Astudillo (1987) reported similar progressions of modal lengths in catches in this fishery, also indicative of the passing of spasmodic strong year classes. In 1987 however, the fishing effort increased with the addition of the French pelagic fishery and subsequently age-3 and older became scarcer. Application of this method afterwards would have been more difficult. For instance, the 1989 strong cohort was only followed up to the age of 3. Since then, many cohorts are not seen at the age of 4 (of the 19 cohorts from 1986 to 2004, only 8 cohorts were seen at the age of 4).

The incomplete collection of otolith of the 1982 cohort required additional validations, as the one achieved with the 1989 year class and the support from surveys. The early detection of the 1989 year class in the catches of the second half of 1989 (at age-0) and the monitoring achieved during 1990 and 1991 up to the age of 2 (Figure 2.4) validated that type I corresponded to the age of 1. In addition, the combination of the former method with direct monitoring of several year classes between 1987 and 1992 in surveys culminated the validation procedure, because the strong increases of the population between years ought to be due to sharp increases in the populations at age-1 (with type I otoliths), as it was actually found by the surveys. The use of microstructure analysis by Aldanondo *et al.* (in press) has added a new validation of the first annulus deposition for this anchovy. Finally, our former validation of yearly growth pattern was confirmed and complemented with the monitoring of the occurrence of edge types throughout the year (Figure 2.8).

Using surveys to support the validation of the ageing method is particularly suitable for short lived species as the typical strong fluctuations in recruitments (which constitute the major part of the population) should result in rather parallel biomass fluctuations, provided no major catchability problems affect the surveys. Therefore, age composition should support the interpretation of the biomass fluctuations being caused by recruitment fluctuations. For short lived species with ageing difficulties, but with a routine surveying of the population, this validation method could be used to elucidate between competing ageing procedures. We used this method to validate our initial ageing procedure and subsequently to corroborate the historical performance of the application of such ageing method. The corroboration was expanded to the cross correlation between surveys indexes of juvenile (age-0) and subsequent age-1 adults abundances carried out in autumn and spring respectively. This emphasises the great potential of support from surveys when dealing with age validation problems for short-lived species.

The historical corroboration presented, making use of the whole series of age structures of catches and population estimates, were dependent not only on the precision and accuracy of the age determinations, but also on the variability of the yearly catchability of the fishery and of surveys (plus that of natural mortality). Effectively, for correlation between successive age groups of the same year class in catches to be significant, a rather constant fishing mortality at age (and hence fishing effort and catchability & selectivity at age) between successive years and throughout the series is required so that the proportionality between catches of successive age groups remains rather invariant in the time series. However, the fishing mortality exerted by the fishery is known to have changed quite a lot since 1987 (ICES 2014) and it was probably subject to yearly random variability. In support of the latter it is worth noting that CV of the historical series of catches of the Spanish Purse Seine fishery is far lower than the CV of survey biomass estimates since 1987. For this reason we suspect that a major part of the wide variability found in Figure 2.9, between pairs of catches of successive age groups of the same year classes, is probably due the yearly changes in effective fishing mortality beyond any inherent errors in the age determination. In addition, the drop of significance of such relationship for the Spanish purse seine fishery when the last four years are added must be indicative of an intense change in the level of fishing mortalities in recent years as a result of the strong drop in fishing effort and reduction of the fisheries during the first quarter and the second half of the year as a result of the implementation of a management plan (ICES 2014). This might induce a higher survival at age-2 than before the closure of the fishery, leading to the abnormally higher catch rates at age-2 over age-1 in recent years compared to the ratio in the previous time series.

In parallel, the proportionality (linearity) of abundances indexes of successive age groups of the same year classes relies on the assumption of constant catchabilities at age at the time of the surveys. Although this constancy is presumed, the existence of occasional strong yearly catchability phenomena is also acknowledged in all surveys series, as revealed by the occasional strong divergences in the series of the two spring surveys (ICES 2014). Therefore a relevant part of the wide variability between indexes of juveniles in autumn and of age-1 in the following spring, or in the relationship between relative changes in successive spring biomass estimates and the odds of the proportion at age-1 in the second year (Figure 2.10) must be due to yearly catchability random realisation errors.

Finally, in addition to the former considerations, variability in natural mortality should induce process error in the correlation between catches or abundances indexes of successive age groups of the same year classes and this is also likely to be present in the variability observed in the scatter plots of catches and abundances at age of Figure 2.9 and respectively.

Therefore the corroboration methods used in this work are rough approaches subject to quite a few observation and process errors. The fact that all the relationships turned out to be significant proved both that the age readings were globally accurate and that observation and process errors affecting those relationships were not strong enough to mask the expected relationships.

2.4.2 The growth pattern of otoliths and the age determination criteria

All these approaches demonstrated that annuli consist of a hyaline zone (either single or composite zone) plus a wide opaque growth zone (occasionally at age-1 disrupted by an intermediate check --- the C15 discussed later on).

For this anchovy, the otolith growth is intense until the end of its second year of life (i.e. for the first 18-20 months), being followed by the far reduced increments at ages 2 and older, which become gradually and steadily narrower. Moreover, the decrease in size of the opaque growth at age-1 is noticeable compared to that at age-0 (to about

50%). As such, the majority of otoliths correspond to growth at these two young ages (furthermore the growth at age-0 alone usually accounts for the major part of the otolith).

Certainly this pattern respects the general principle by which year growth increments in otoliths gradually decrease (Morales-Nin and Panfili 2002), but the particular way this anchovy respect this pattern is the first general scheme age readers should be aware of for the examination of these otoliths. The more sustained growth (though still slightly decreasing) increments of the otoliths of older fishes (ages 2 and older) is a rather common pattern for many species which, after the initial sharp decreases of increments, tend to smooth the rate of decreasing sizes or to stabilise gradually (Cappo *et al.* 2000; Fowler 1995), related to the fact that otoliths keep continuously growing with age (Campana and Thorrod 2001).

Besides the general annual growth pattern of the anchovy otolith, there is large individual variability in the size of annual opaque growth increments by ages, with CVs about 33%. Much of this variability is due to the inverse relationship between the growth achieved during the first year of life (at age-0 until the first hyaline annulus) and the second and third years of life (at ages 1 and 2). Such a negative relationship was first shown for this anchovy population by Petitgas and Grelier (2003). It is indicative of the compensatory growth (the smaller fish tend to catch up with larger ones), which is observed in other marine species, also as a result of following the von Bertalanffy model or similar growth patterns (Taylor 1962; Ricker 1975; Xiao 1994). This phenomena explains the high plasticity of the relative growth pattern shown by this anchovy at ages 1 and 2 (Figure 2.6c).

2.4.3 Seasonal otolith growth pattern

General maximum of occurrence of hyaline zone for all ages corresponded with the period of coldest sea waters in late winter time, whilst maximum occurrence of opaque growth edges occurred with increasing temperatures during late spring and summer time, which is the typical pattern for minimum and maximum growth of most fish species at mid latitudes (Beckman and Wilson 1995; Cappo *et al.* 2000), including several small pelagics, such as Tunisian anchovy (Khemiri *et al.* 2007), Californian anchovy (Mallicote & Parrish 1981), tapertail anchovy (He *et al.* 2008). However, the seasonal development of the marginal growth of otoliths, as shown by the edge formation in time, differs remarkably between ages, with the young anchovy (the 1 year-old) resuming opaque growth earlier (since March) than the older (at late spring or early summer). Furthermore, the transition between ON/OW (which here implies surpassing about 33% of the annual expected growth increment) is achieved by the 1 year-old at the beginning of June, while for ages 2 and 3 is achieved at the beginning of July and August respectively. Such a tendency for the younger fishes to resume growth earlier than older fishes seems to be a widespread feature (Williams and Bedford 1974; Holden and Raitt 1975), also affecting clupeids such as Baltic sprat, herring and Atlantic sardine (ICES, 2008a, 2008b; ICES 2011). Given that most of the expected annual opaque increment of the otoliths is achieved by all ages by the end of September, this implies that otoliths of the 1 year-old make most of its growth from March to September in about 7 months, while the 2- and 3- year-olds do it from June and July to September, i.e. in about 4 and 3 months respectively. As such, there is some parallelism between the decreasing gain in length by age and the decreasing time of opaque growth formation in otoliths by ages. In addition, we have observed some variability in the starting and ending time of opaque edge formation (as for the age-2 of 1989 cohort). Such variability in the dates of starting and ending of the otolith opaque growth formation is likely to be due to environmental factors and this has also been observed in other species (Beckman and Wilson 1995; Pearson 1996; Høie *et al.* 2009).

Understanding the seasonal development of the marginal edge by ages was relevant to improve the accuracy of age determination, particularly to discriminate age-1 from older during spring, when most of the 1 year-old fishes will show opaque narrow edge type whilst older fishes are still laying down hyaline edges, or even widening or enhancing them (as noted also for the acoustic surveys in May by Petitgas and Grellier 2003). For this reason, the expected edge type by ages during the year has become the second key criteria for age determination for this anchovy. The differential dynamic of otolith growth and edge formation by ages during the year has already been noted in other fishes, including cupleoids (Williams and Bedford 1974; ICES, 2008a, 2008b; ICES 2011), although we have few analyses of this issue for small short-lived pelagics; this was not examined for Chilean anchovy (Aguayo 1976) nor for E. Capensis (Melo 1984) and was refuted for the Southwestern pilchard (Thomas 1983). Due to its relevance for this anchovy, we are of the opinion that it could be worth considering further this issue for improving age determination procedures in small short-lived pelagic fishes of other areas too. As mentioned by Campana (2001) "age assignment is a function not only of annulus count, but of edge type in relation to date of collection and assigned birth date". Notice that two age groups (age-1 and age-2+) can be enough to sufficiently capture the main demographic structure of these short-lived populations for integrated assessment purposes (Ibaibarriaga *et al.* 2008; 2011) and for such discrimination the edge criteria can be of great help.

2.4.4 Checks

The major difficulties in age determination arise from the correct identification of false checks in the otoliths. We have found that in the case of anchovy in the Bay of Biscay, most false checks are recurrent and hence typically identifiable so that readers can get familiar with them. This is third base knowledge which basically improves the accuracy and precision of the age determinations.

Many fish species lay down checks in their otoliths associated to non-periodic stresses such as environmental (storms, cooling, starvation), life history transitions, endogenous processes (hatching, maturation, spawning) or cyclic environmental issues (Pannella 1971; Campana and Neilson 1985; Casselman 1987). The incidence of checks is particularly common in young ages of many fish species (from age-0 to age-2) (Swan and Gordon 2001; Waldron and Kerstan 2001; Santiago and Arrizabalaga 2005), including pelagics (Thomas 1983; 1984), and hence probably affects the predominant age groups of short-lived species. Therefore our observations of several checks in this anchovy were not surprising.

Since the vast majority of the juveniles (age-0) sampled during the September acoustic survey (Boyra 2013) are completely opaque without any checks, checks C05 and C08 are to be formed in autumn (October) or in late autumn or winter (November-December) respectively. In autumn anchovy juveniles have to recruit from the superficial layers of waters over wide regions of the Bay of Biscay to deeper and more coastal waters where they will be wintering (Uriarte *et al.* 2001;Irigioien *et al.* 2007). This change might induce a first check deposition in some of the juveniles in cases of being abrupt (for instance after storms), associated either to a change in water temperature or to temporal poor feeding conditions. This possibility has been supported by the bioenergetics modelling of otolith biomineralisation applied to this anchovy by Pecquerie *et al.* (2012) who were able to simulate the formation of a false check (like C08) in the age-0 group of anchovies by simulating poor feeding conditions before winter. These types of checks prior the first annulus have also been reported in south west African pilchard and anchovy (Thomas 1983; Melo 1984) and in Chilean anchovy (Aguayo 1976).

Later on during winter, the vigorous growth rate of the 0 group may result in several individuals not forming a single hyaline annulus but a composite structure of hyaline zones alternated by one or two short resuming of opaque growth (split rings) if food and temperature environment allows it. This should explain the rather common observations of these composite hyaline marks of the first annulus in this anchovy. These split rings are commonly associated with the first hyaline annulus of many fish species (Panfili *et al.* 2002; Santiago and Arrizabalaga 2005) and have also been reported for pilchard off South West Africa (Thomas 1983;1984).

The most common check and the only one studied here in detail is the one laid down at the middle of the second opaque growth (C15). We have shown that, if laid down, it becomes evident at age-1 in July, so it should be formed basically in June coinciding with the peak spawning time of the 1 year-old anchovies (Motos 1996) and after a substantial opaque growth of the otolith margin. On the contrary, the older fishes which start maturation and reach peak spawning earlier (April) do not resume the opaque growth of the otolith before spawning, but mainly in June-July. Furthermore, they seem to enhance and widen the hyaline zone (up to HW) during the spring. Hence, C15 could be a spawning check laid down by the fast-growing 1 year-old anchovy after its initial opaque growth during spring, whilst older anchovies might be merging the hyaline winter annulus with the spawning check (resulting in the widening of the hyaline zone during spring). This probably reflects the different relative investment of energy in growth and reproduction of the younger versus the older anchovies; with the former investing relatively more in growth while the latter more in reproduction (suggested also by the dynamic energetic budget modelling of this anchovy -- Pecquerie *et al.* 2009). Spawning checks are reported to occur in many fishes (Pannella 1980; Casselman 1987; Campana and Nielson 1985), including pelagics like South African anchovy (Melo 1984; Waldron 1994) and Chilean anchovy (Aguayo 1976). In addition, a check within the opaque growth of the second year of life (age-1) is reported for Pacific anchovy (Collins and Spratt 1969) and for

Mediterranean anchovy (Pertierra 1987). In some cases, if spawning is close to winter time, it may form a composite hyaline zone of the spawning check plus the winter annulus or it can be merged in older fishes, for instance in cod otoliths (Panella 1971) or northern Pike scales (Casselman 1987); something which may be happening with the older anchovies here as well. A similar case is reported for south west African pilchard for which hyaline rings in the period from winter to spring tend to coalesce into a broad hyaline zone (Thomas 1983; 1984).

Our study corroborated that false checks are not equally laid down in all fishes or in all year classes, as reported for other species too (Thomas 1983;1984; Campana and Nielson 1985; Casselman 1987). For instance, the most common check, C15, was found usually in less than 50% of the otoliths. This irregular incidence impeded the formulation of a simple rule based purely on the counting of every hyaline zone.

The irregular occurrence of checks during the second year of life (i.e. for the age-1 group) makes discrimination of age-1 from older ages difficult in fishes caught in summer and autumn time, when the expected total annual growth is not yet completed although close to finishing. The presence of checks, C15 or C18 could lead to misinterpreting them as additional winter rings and lead the reader to allocate them to an older age class. Or the reverse, it may also happen that some true winter rings might be taken as checks and lead the reader to allocate them younger than true ages (though this is rarer as hyaline annulus are more pronounced). In these cases, the prior knowledge of the most common checks, and the criteria of conformity with the typical annual growth pattern should be helpful: if the doubtful checks are false then their assumption should allow for a better fitting to the typical annual (and seasonal expected marginal) growth of the otolith than to the alternative of assuming them as true hyaline annulus.

2.4.5 Verification:

There are no agreed acceptable levels of precision in age determination studies (Panfili *et al.* 2002), although CV between 0% and 16% are common (median about 7%; Campana 2001). For this anchovy the overall level of precision is high, particularly for ages 0 and 1 (with agreements higher than 93% and CV lower than 10% and negligible bias), while for older anchovies precision levels are poorer (but agreements still higher than 80% and CV around 10-15%, with negligible bias for age-

2 and low for age-3, the latter about -0.14). Nevertheless, results from past workshops show that the overall precision for all ages is better for the first half of the year when much of the fishery and the surveys take place. This, along with the fact that age-1 supposes usually the major fraction of total biomass, minimises the impact of the lower ageing precision level for older ages. Furthermore, by taking into account that splitting the age structure in age-1 and age-2+ can be enough for this type of short-lived population (Ibaibarriaga *et al.* 2008; 2011), the actual levels of precision can be adequate for assessment purposes.

2.4.6 Annual Growth patterns in length and growth parameters

Since the current methodology of age determination was applied in the late eighties, the mean lengths at age have remained almost invariant throughout the time series in the catches of the spring Spanish fishery. As such, a first fitting of the VBG curve parameters in 1988 (Anon -1988) to the mean lengths at age of Spanish fishery in the period 1984-1987 resulted in the same VBG parameters as the ones obtained here, with a K of about 0.79 and L_{inf} of around 18.3 cm. Our results are also similar to those of Hernandez *et al.* (2009) -- working with the Spanish fishery too -- and of Vaz *et al.* (2002) for the acoustic surveys in the Bay of Biscay. However these results contrasted with those published by Cendrero *et al.* (1981) which resulted in length at age-1 of about 10.5 cm and at age-2 of about 14.35 cm in catches (i.e. the latter corresponds to those obtained with the current method for mean length at age-1). In addition, the application of the current method of age determination from otoliths implied a shift in the perception of the major age groups supporting the Spanish spring catches, by which age-1 - formerly almost absent - became very abundant (Anon. 1993). As there was no collection of otoliths available prior to 1984, it has not been possible to identify the actual causes of such change but given the constancy of the lengths at age in this fishery for the last 30 years and the fact that the length distributions in catches in the seventies were similar to those occurring in the following decades (Uriarte and Astudillo 1987), with the first length modes around the mean length at age-1 resulting with the current ageing method, it is presumed that the differences were due to a changes in the reading criteria. Probably many of the age-1 were aged 2, perhaps due to interpreting that the well-developed opaque margin edge of age-1 occurring in spring corresponded with growth of a previous year. The differences in the perceived mean lengths at ages 2 and 3 (major constituent of catches) resulted in a VBGF fitting with smaller *K* and higher L_{inf} than ours () and this necessarily resulted in the smaller mean length at age-1 and bigger length at age-4 (both barely observed in their catch at age composition). Gerault and Avrilla (1974) had worked the anchovies in the Bay of Biscay on the French shelf close to the Gironde, where anchovies are usually smaller as this is a major nursery area (Motos *et al.* 1996 and Vaz *et al.* 2002); It is probably for this reason that they obtained the different VBG parameters for the smaller sizes at ages 1 and 2 than in the current study, but some differences in the ageing criteria cannot be discarded either.

Our study evidenced lower mean lengths at age (and weights) in the population (as estimates from the DEPM surveys) than in the catches of the concurrent spring Spanish fishery, so that they resulted in a slightly higher *K* and lower L_{inf} (Table 2.5). The reason for these differences is related to the spatial pattern of the Spanish fishery as it operates in the regions of deep waters rather close to the Spanish coasts where the bigger anchovies are found (Uriarte *et al.* 1996; Ibaibarriaga *et al.* 2013). In contrast, the surveys systematically cover all the areas occupied by the population and the major part of it is often placed around the Northern coastal shelf close to the Gironde river mouth where age-1 predominates and the mean lengths at ages are smaller than in the remaining areas (Motos *et al.* 1996 and Vaz *et al.* 2002; Ibaibarriaga *et al.* 2013). As this spatial pattern has not changed over this time, the difference from both sources has persisted throughout the time series. From the DEPM survey there is some decreasing tendency in the mean lengths at ages for the period 1990-2013, particularly for ages 1 and 2 in recent years, which seemed also noticeable in the Acoustics, particularly for the age-1 (Duhamel personal communication). The reason of such tendency is uncertain and deserves further analysis which exceeded the scope of this paper.

This anchovy population has an intense growth rate in comparison with other populations of the same species or with other Engraulidae (Table 2.5). The rather high L_{inf} and K leads it to have among the highest ϕ' parameter of this species, only equalled or surpassed by the anchovy in the North Sea and by the anchovy in Cadiz. However, looking at the mean of lengths at ages 1 and 2 only the *E. encrasicolus* in the North Sea seems to be bigger (Blaszczyk, 1999); a more recent comparison with otoliths from the IBTS surveys in the North Sea along the Dutch coasts shows rather comparable mean lengths at age however (Petitgas *et al.* 2012). In spite of several populations having similar or higher L_{inf} in the Mediterranean, the low *K* rates means they have smaller mean lengths at ages 1 and 2 and lower ϕ'. So, average sizes and growth of these populations are smaller. The *E. encrasicolus* of South Africa has a higher *K* rate but smaller L_{inf} so that despite its rather similar ϕ', mean sizes at ages 1 and 2 are also smaller. Peruvían anchovy and Northern Chile are the ones which have rather similar growth rates and mean lengths at ages 1 and 2 (Aguayo 1975; Palomares 1987; Morales Nin 1989a). Most other Engraulidae report smaller sizes at ages 1 and 2 regardless of their actual growth rates or ϕ'values (Hoedt 1990; Tiroba *et al.* 1990), except for the large tropical anchovy *Thryssa hamiltoni* (Hoedt 1992). Anchovies inhabiting estuarine areas tend also to be smaller (Iversen *et al.* 1993; Newberger and Houde 1995; He 2008).

Certainly the intense growth of this anchovy is well reflected in the great proportion of its asymptotic length reached at the end of its first year of life (L1/L_{inf} around 75-80%,Table 2.5), as well as in the intense otolith growth at age-0 (which supposes the major part of the otolith structure). Furthermore, Aldanondo *et al.* (2011), analyzing micro increments in the otoliths of juveniles, has shown that this anchovy can reach a size of 6-8 cm in about 70-90 days. All these reflect the life strategy of this short-lived anchovy, i.e., very intense growth during its first year of life, so that it maximizes its size at the age of first maturity (i.e. at age-1 -- Motos 1996) and hence the reproductive output of its first spawning season. This coupled with a still substantial growth during its second year of life should maximize the overall reproductive output across its expected life, because survivors at older ages become negligible.

2.4.7 Final remarks

This work has focused on the procedure of age determination of anchovy, which relies on the knowledge of three key biological processes: the annual growth pattern of otoliths (matching approximately the expected relative size of opaque zones by ages), the dynamic seasonal formation of the margin and expected occurrence of edge types by ages throughout the year and, third, knowledge of the most common checks and their time of formation. This knowledge was achieved through the validation process we have presented; in addition, we have confirmed by posterior corroboration that the age procedure resulted in a rather accurate ageing. The rules we apply to allocate age are common to many species but when applied with the former knowledge, precision and accuracy seems to be globally satisfactory for this anchovy population. Certainly, age determination from macro examination of otoliths will always have some degree of subjectivity, particularly because it has to take into account the irregular occurrence of false checks. Therefore our approach consisted in becoming familiar with the type of checks usually found and understanding their timing of formation, so that they can be identified as much as possible while occurring, according to their position and intensity of deposition. Age determination becomes therefore a partially subjective decision according to the “best” fitting of the typical growth pattern observed in the past by ages for this anchovy (during our validation study) on the observed growth pattern and margin edge type of the new examined otoliths, given the date of capture. Therefore, no single simple and invariant criterion in terms of absolute number of hyaline zones would be valid for this anchovy. Furthermore, our observations that not all anchovies lay down checks and that their incidence may change also between year classes, discard the idea of getting it in future. Failures to understand this dynamic plasticity of the otolith formation in anchovies may explain part of the difficulties of using them in other species and part of the large variability found in the VBG parameters.

Growth Appendix A: Otoliths of European anchovy in the Bay of Biscay: seasonal characterization by age classes.

This appendix provides a description of the seasonal growth of anchovy otoliths: seasonal characterization by age classes, as seen by incident light on whole mounted otoliths over black slides (including some pictures by seasons at the end).

Age-0

Quarters	Main features of Age-0 otoliths and occasional deviations
Q1	Not present
Q2	Not present
Q3	Opaque – entirely opaque otoliths up to the edge or occasionally faint hyaline edges (OH) of increasing occurrence as the autumn progresses. The small size of anchovies and their otoliths, usually sampled from schools of pure juvenile fishes, make samples very homogeneous, being evident that those fishes are juveniles 0 group. Occasional Deviations: sometimes the outer opaque zone shows up in a white of lesser intensity than inner zones and occasionally there can be some inner checks of easy recognition due to their faint nature.
Q4	Similar to third quarter. At the end of the year narrow hyaline edges (HN) can be observed.

Age-1

Quarters	Main features of Age-1 otoliths and occasional deviations
Q1	In January some anchovies still show entirely opaque otoliths but a majority of them show hyaline edges either OH (in formation) or HN due to the winter growth detention. In February the majority are HN and by March most of them have already laid down a narrow opaque edge (ON) (Fig. A1. Age-1 winter), laying behind the first winter hyaline zone. Occasional deviations: In some cases the winter zone is a composite of successive hyaline zones, typically two or three alternated by one or two narrow opaque zones, resulting in a double or triple hyaline rings conforming the first Winter mark (see Fig. 9 of the paper)
Q2	By April almost all *1-year-old* anchovies will show a narrow opaque edge (ON) after the first winter hyaline zone. During the spring the edge will be growing continuously, becoming a wide opaque edge (OW) by May or during June (varying between years) (Fig. A1 Age-1 May ON). By the end of June most of them will already achieve a OW edge, surpassing 1/3 of their annual expected growth. Occasional deviations: In June some 1 year-olds may show a partially hyaline edge (OH or even HN), corresponding to the formation of a spawning check. Not all fishes lay down this check and its incidence changes across years, ranging between 15% and 60% for the YC 1982-88 (mean incidence of about 40%). The type of opaque edge achieved by the 1 year-old anchovies in spring, or occasionally the faint hyalinity in June (OH) in case of laying the spawning check, make them clearly distinguishable from the 2 year-old which in turn show hyaline (HN or HW edges) throughout spring, without starting a neat opaque edge deposition of the current year (except some in June).

Q3	Intense growth of the opaque edge (OW) happens during summer months July (Fig. A1 Age-1 July OW) and August, so that by September about 80-90% of the total expected annual otolith growth for age-1 is already achieved (Fig. A1 Age-1 August), corresponding with a very wide opaque edge (OW). Occasional deviations: There are always some 1 year-olds with semi-hyaline edges (OH) not entirely visible all around the otolith. If the anchovy laid down a spawning check this will usually be seen from July. The distinction between spawning check and true winter hyaline zone should be made on the following basis a) judging if the distance to the former winter hyaline zone matches with the expected annual opaque growth of 1 year-old anchovies or is less than expected and b) judging the intensity of this hyaline mark (as faint), though if well marked then the reader should base its judgment only on the **a** criteria. Both are a bit subjective criteria and this is probably the time of major difficulties for age determination of the 1 year-old fishes. Besides this around the time of the spawning check or later on the intensity of the white colour of the opaque growth band may occasionally weaken (becoming a bit more grey as if there were two different growth colour bands in the year) (see example for an age-2 fish in Figure 9 of the paper: image A2.1 and for an age-3 fish in Fig. A1 caught in Aug.-Sept)
Q4	The growth band of the *1 year-old* has been completed, with opaque edges (OW) at the beginning of the period and more hyaline (usually OH or HN) at the end of the period (Fig. A1 Age-1 Oct-Nov). Occasional deviations: Same comments as for Q3 and it should be noticed that occasionally there can be some interruptions and resuming of growth resulting in some usually faint checks at the end of the 1-year-old otolith opaque growth (checks 18). (Fig. A1 Age-2 in winter and in Aug - Sep as well as Age-4 Oct-Nov show C18)

Age-2

Quarters	Main features of Age-2 otoliths and occasional deviations
Q1	Otoliths with the two first annuli formed having its second hyaline zone just at the edge either in formation OH (Fig. A1 Age-2 in winter) or as hyaline narrow zones HN. Occasional deviations: In few cases the second hyaline zone is not yet formed having an edge entirely opaque. And in some cases within the opaque growth zone of the previous year checks C12 / 15 or more rarely 18 can be seen (see Fig.9 for several examples and the checks C18 in Fig. A1 Age-2 in winter – very faint)
Q2	Otoliths with the two first annuli formed having its second hyaline zone just at the edge either as narrow hyaline zones (HN), mainly in April, or as wide hyaline zones with increasing occurrence from April to June (Fig. A1 Age-2 in April-May). By the end of May and in June some otoliths will start the deposition of the opaque growth zone showing either HO or ON edge types. Occasional deviations: the starting date of appearance of the opaque edge may change from year to year ranging, the earliest about mid-May and usually not later than end of June.
Q3	In July most of the otolith edges are already opaque, either narrow or wide zones (ON or OW) even though there might still be some hyaline wide edges (HW) (Fig. A1 Age-2 -July). By the end of July and in August edges will show up as wide opaque growth bands (OW) and by September most of the expected annual growth of the otoliths will be achieved (Fig. A1 Age-2 – August-Sept). Occasional deviations: By September a few age-2 otoliths may show partially hyaline edges (OH).
Q4	The expected annual growth of the otoliths for age-2 will be achieved. Edges will show up either as wide opaque (OW) partially hyalines (OH) or a few as narrow hyaline edges (HN). (Fig. A1 Age-2 –Oct-Nov).

Age-3 (and older)

Quarters	Main features of Age-3+ otoliths and occasional deviations
Q1	Otoliths with the three first annuli formed having its third hyaline zone just at the edge either in formation OH or as hyaline narrow zones HN (Fig. A1 Age-3 and Age-4 in winter). Occasional deviations: In a few cases the third hyaline zone may not yet be, formed having an edge entirely opaque. And in some cases within the opaque growth zone of its second year of life (of the age 1) the previous year checks C12 / 15 (or more rarely C18) can be seen (see examples in Fig. 9 of the paper).
Q2	Otoliths with the three first annuli formed having its third hyaline zone just at the edge either as narrow hyaline zones (HN), mainly in April-May, or as wide hyaline zones with increasing occurrence from April to June (Fig. A1 Age-3 and Age-4 in April-May).By June some otoliths will start the deposition of the opaque growth zone showing either HO or ON edge types. Occasional deviations: the starting date of appearance of the opaque edge may change from year to year, but less than at younger ages, usually between June or July. Within the growth band of the otolith during its second year of life previous year checks C12 / 15 o more rarely C18 can be seen (see examples in Fig. 9 A3 and A3.2)
Q3	The three first annuli are formed and the opaque growth zone of the year is being formed: In July some HW edges coexists with ON edges (Fig. A1 Age-3 July On). In August edges will show up as wide opaque growth bands (OW) and by September most of the expected annual growth of the otoliths will be achieved (Fig. A1 Age-3 and Age-4 in Aug-Sept). Occasional deviations: By September a few age-3+ otoliths may show partially hyaline edges (OH).

Q4	The expected annual growth of the otoliths for age-3+ will be achieved. Edges will show up either as wide opaque (OW) partially hyalines (OH) or a few as narrow hyaline edges (HN). (Fig. A1 Age-3 and Age-4 in Oct-Nov).

Figure A.1: Pictures of typical otoliths by age group throughout the year: Ages 1 and 2. Pictures from left to right by ages: Age-1: 07/03/1990; 18/05/1990; 18/07/1990; 26/09/1990;14/11/1984. Age-2: 19/03/1984; 16/05/1991; 12/07/1989; 02/08/1989; 14/11/1984.

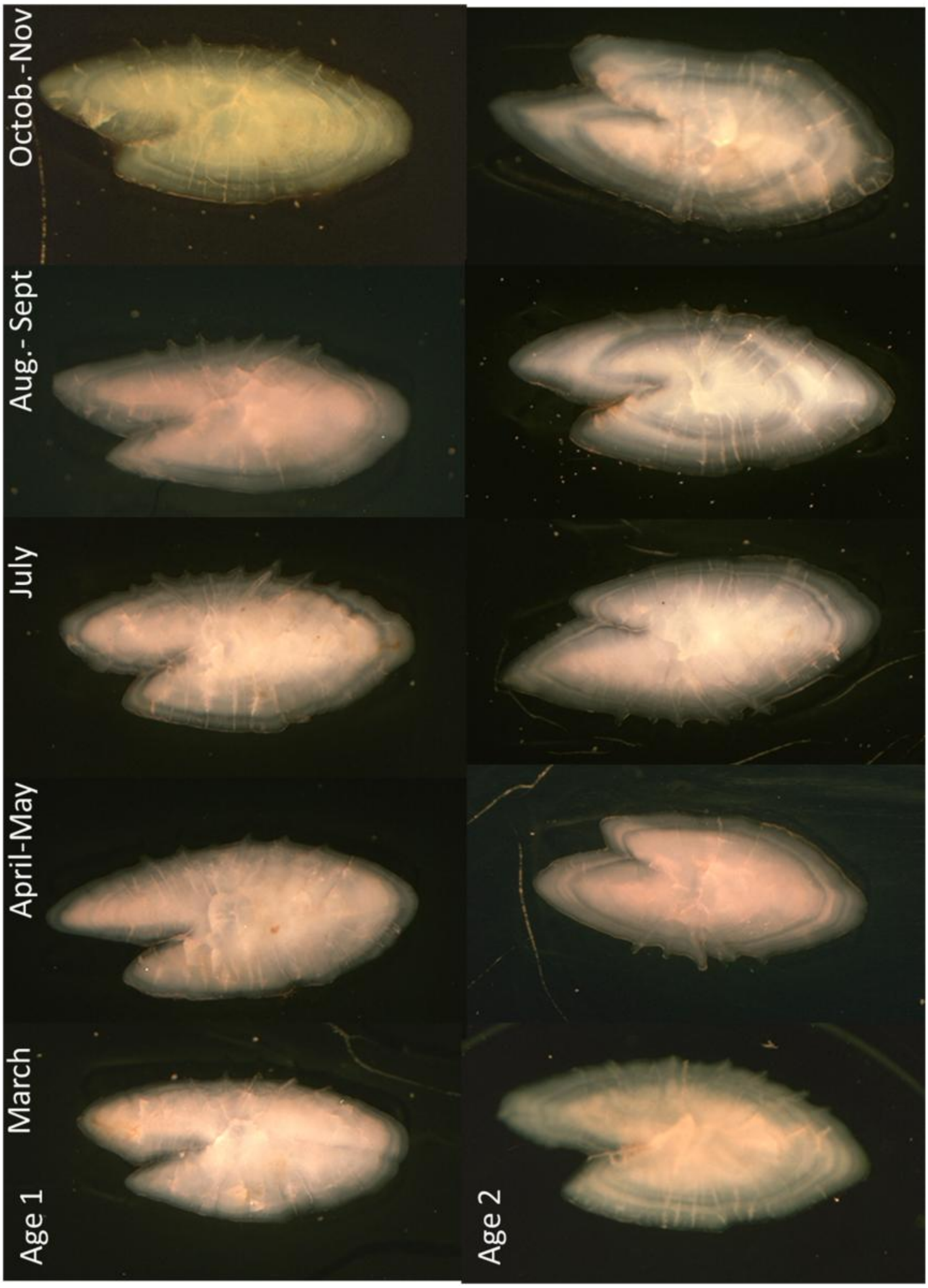
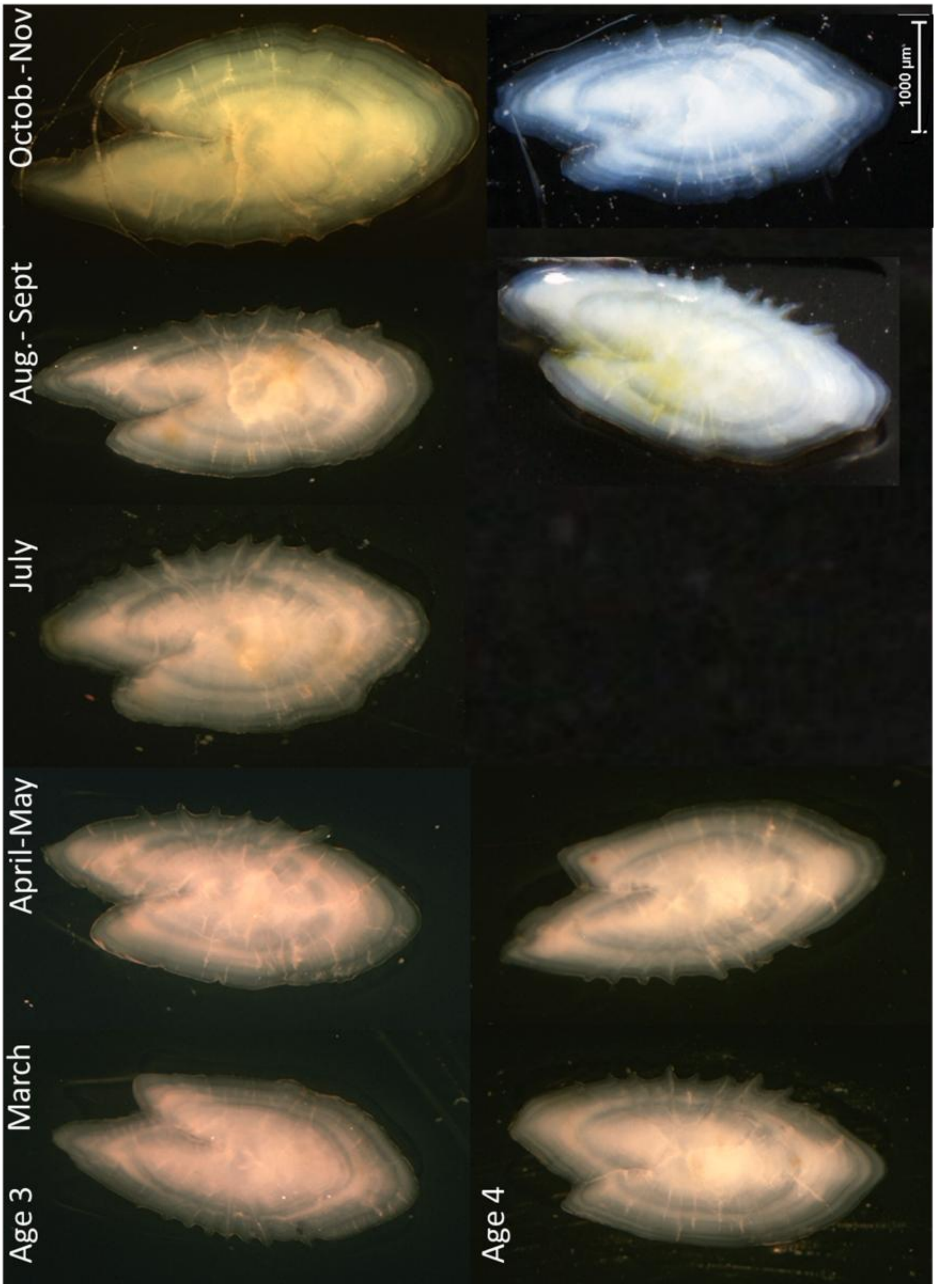


Figure A.1 (Cont…): Pictures of typical otoliths by age group throughout the year: Ages 3 and 4. Pictures from left to right by ages: Age-3: 18/03/1992; 22/05/1992; 26/07/1985; 04/09/1985; 14/11/1984. Age-4: 14/03/1986; 30/04/1986; NA; 27/09/2010; 18/11/2003.



3 Reproduction: A re-evaluation of the spawning fraction estimation procedures for Bay of Biscay anchovy, a species with short inter-spawning intervals

3.1 Introduction

The daily egg production method (DEPM) is currently used for estimating the spawning biomass of indeterminate spawning fish (Stratoudakis *et al.* 2006), according to its original formula (Lasker 1985; Parker 1980), i.e. dividing the daily egg production at sea by the population daily fecundity. Thus, adult reproductive parameters such as batch fecundity and spawning fraction; which are needed to estimate population daily fecundity, are crucial for a successful application of DEPM.

Among the adult parameters, spawning fraction (S), or the fraction of mature females spawning per day, is probably the most relevant and, at the same time, most difficult parameter to be obtained (Stratoudakis *et al.* 2006), requiring a time-consuming and expensive histological analysis. This parameter is usually estimated by the proportion per sample of mature females with post-ovulatory follicles (POFs) pertaining to an identifiable daily spawning class (Hunter and Macewicz 1985). Alternatively, S can also be obtained from the proportion of imminent pre-spawning females, as detected by the presence of ovaries undergoing final oocyte maturation (FOM) (Dickerson *et al.* 1992; Lowerre-Barbieri *et al.* 1996; McBride *et al.* 2002; Priede and Watson 1993; Roumillat and Brouwer 2004; Yamada *et al.* 1998; Yoneda *et al.* 2002; Zeldis and Francis 1998). However, in small pelagic fish S is rarely estimated using females undergoing FOM as those females can be often oversampled due to species spawning behavior (Ganias, 2008). Consequently, there are few examples for small pelagic species where S was estimated using the proportion of imminent pre-spawning females (Lo *et al.* 2005; Luo and Musick 1991; Macewicz *et al.* 1996; Rogers *et al.* 2003; Ward *et al.* 2001).

Application of the post-ovulatory follicle method for the estimation of S requires understanding the degeneration process of POFs through time, as POFs have to be aged, i.e. assigned to a daily spawning cohort (daily class) according to their degeneration state. However, many stocks lack studies on the degeneration process of POFs and, therefore, either assume or adapt former studies. In particular, the pioneer studies in aquaria relating the POF states with past daily spawning classes for northern anchovy *Engraulis mordax*, by Hunter and Goldberg (1980) and Hunter and Macewicz (1985), guided most of the subsequent applications of the post-ovulatory follicle method for estimating spawning fractions of anchovies worldwide (Cubillos *et* *al.* 2007; Dimmlich *et al.* 2009; Pájaro *et al.* 2009; Stratoudakis *et al.* 2006). Adaptation of the original description or new validations of the correspondences between POF degeneration and the daily spawning classes for other species were usually achieved by repeated sampling of schools at sea over a 24-hour cycle (Alheit *et al.* 1984; Goldberg el al. 1984; Macewicz el al. 1996; Roumillat and Brouwer 2004) or for several days, but by sampling at limited periods throughout the day (Funamoto and Aoki 2002; Ganias *et al.* 2003, 2007). In a few cases new experiments in aquaria were used to age POF degeneration (Alday *et al.* 2008; Dickerson *et al.* 1992; Fitzhugh and Hettler 1995; Pérez *et al.* 1992).

The Bay of Biscay anchovy (*Engraulis encrasicolus*) is a batch spawner of indeterminate fecundity (Motos 1996). Santiago and Sanz (1992) noted that day_0 (actively spawning) females around peak spawning time (between 20:00 and 04:00 hours) were sampled in higher proportions than the other daily spawning classes; This is a quite typical phenomena which occurs with small pelagic fishes called oversampling of day_0 (Alheit *et al.* 1984; Picquelle and Stauffer 1985; Ganias 2008). As sampling at the beginning of the series used to take place mostly at night, in order to avoid this oversampling, the day_0 females were discarded from the estimation of S. As such, since the beginning of the applications of the DEPM to the Bay of Biscay anchovy (starting in 1987, Motos *et al.* 2005; Santiago and Sanz 1992; Somarakis *et al.* 2004), S was derived from the average proportion of the day_1 spawners (Motos 1996; Sanz *et al.* 1992), although since 1994, the day_2 spawners have also been included. Examination of the state of POF degeneration and the assignment of POF ages was directly done by an expert judgment in a single step following the descriptions of Hunter and Macewicz (1985). This resulted in a mean S of 0.25 (ranging from 0.17 to 0.33) in May and early June, during the DEPM surveys (Somarakis *et al.* 2004). Recently, a validation of POF degeneration stages with time was made available for this population (Alday *et al.* 2008), indicating a faster degeneration process of POFs than previously thought. This study suggested that the spawning fraction could be higher than formerly estimated. This demanded a revision of the basis for ageing POFs, i.e. allocating them to past spawning daily classes, as well as a revision of the estimators used for spawning fraction, including here an evaluation of whether day_0 spawners could be included in estimates of S.

In addition, the reproductive dynamics of fishes with medium-high spawning fractions have features of particular interest for the estimation of S, such as: a) an increasing negative (complementary) relationship between the incidence of day_0 and day_1 spawners in the samples. This might confer some stable properties to an S estimator based on their combined incidence, given that a negative covariance should reduce the variance of a combined estimator (Ganias *et al.* 2003). b) an increasing number of females displaying the co-occurrence of both pre- and post-spawning stages, allowing the direct estimation of spawning frequency (or interspawning intervals) on an individual basis. This co-occurrence has been reported for many scombroids and other groups (Dickerson *et al.* 1992; Lowerre-Barbieri *et al.* 1996; Macewicz and Hunter 1993; McBride *et al.* 2002; Schaefer 1996; Yamada *et al.* 1998; Yoneda *et al.* 2002) and also for some small pelagic species including Bay of Biscay anchovy (Motos 1996).

The goal of this study is to review the methodology used to estimate the spawning fraction of the Bay of Biscay anchovy as an example of a species with indeterminate fecundity and short interspawning intervals. Towards this aim, our first objective is to incorporate all the available knowledge, for this anchovy, on final oocyte maturation (Motos 1996, updated here) and POF degeneration (Alday *et al.* 2008) over time, in an improved system of allocating mature females to daily spawning classes. To achieve this, a two phase procedure which splits the staging of oocyte maturation and POF degeneration from their ageing, or allocation to a daily spawning class, was adopted. For the second step, a matrix system for allocating females to daily pre/post spawning daily classes based on time of capture and stage of oocyte maturation/POF degeneration is proposed. A second objective is to evaluate alternative S estimators regarding their precision and robustness to the potential bias induced by the oversampling of active spawning females and their aggregation pattern: in total five estimators were evaluated: four estimators based on the proportions of daily spawning classes and a fifth based on the individual spawning frequencies of females. Lastly, based on the main results obtained above, the S estimates of past DEPM surveys since 1990 are revisited; along with an examination of the role that temperature or female size may play in determining the level of S.

3.2 Material and methods

3.2.1 DEPM adult samples and processing

The set of ovaries obtained for 14 DEPM surveys between 1990 and 2007 (ICES 2004, 2006, 2007, Motos 1996; Motos *et al.* 2005; Somarakis *et al.* 2004) were reviewed, accounting for a total of 12,837 females and 503 samples (Table 1). No survey was undertaken in 1993 and for some years adult samples were either not available or have not yet been analyzed. Samples of less than 8 females (10 samples) were not taken into account for the sample-based estimations and, thus, 493 samples were used for such estimates. Samples were collected throughout the day from various sources: opportunistic sampling provided by the commercial purse seine fleet, ad-hoc chartered purse seiners, or by research vessels from pelagic trawlers (Figure 3.1). Most of the purse seine samples (295) were collected at night, whilst most of pelagic trawls (208) were undertaken during the day. However, both types of gears also provided samples collected at other times of the day (Figure 3.1).

Table 3.1: Number of samples per gear and survey analyzed in this study. The original references and the description of the applications of these surveys and their results were summarized in Somarakis *et al.* (2004) and in ICES (2004, 2006 and 2007). NMT: Number of samples from pelagic trawling; NPS: Number of samples from purse seining; N: Total number of samples; N(NF8+): Number of samples having at least 8 females; NFMT: Number of females from pelagic trawling; NFPS: Number of females from purse seining; NF: Total number of females.

Year	Month	NPT	NPS	N	N(NF8+)	NFPT	NFPS	NF
1990	**May**	4	32	36	36	109	1046	1155
1990	**June**	0	34	34	34	0	804	804
1991	**May**	11	31	42	42	291	796	1087
1992	**May**	17	14	31	31	434	403	837
1994	**May**	16	12	28	28	403	314	717
1995	**May**	0	33	33	31	0	670	670
1997	**May**	20	21	41	41	523	600	1123
1998	**May**	20	27	47	47	512	687	1199
2001	**May**	14	19	33	33	341	524	865
2002	**May**	24	11	35	33	595	259	854
2003	**May**	22	14	36	35	546	384	930
2004	**May**	31	10	41	40	760	224	984
2005	**May**	19	10	29	26	502	173	675
2007	**May**	10	27	37	36	291	646	937

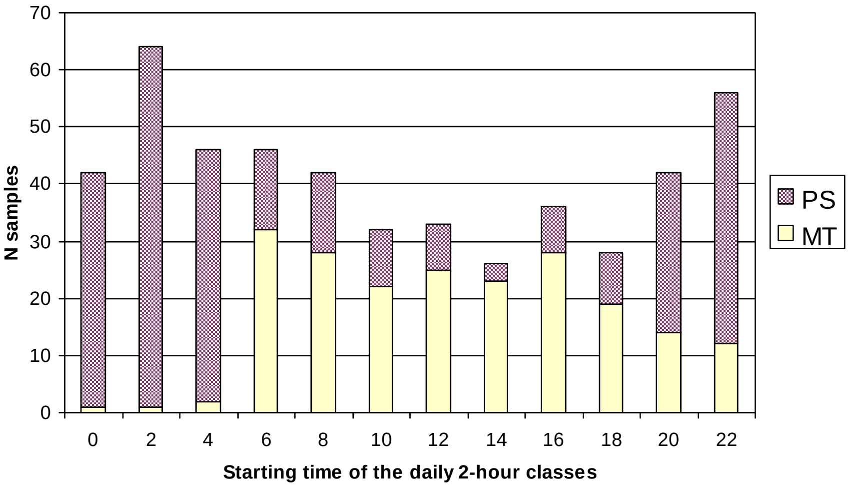


Figure 3.1: Number of samples (N samples) per fishing gear by time of day. PS: purse seines; MT: pelagic trawls.

The samples consisted of a minimum of 1 kg or 60 anchovies taken at random from the catch (including hydrated females when present) with the aim of obtaining 25 non-hydrated mature females, increasing the sample size up to a maximum of 120 anchovies when necessary in order to attain this goal. The females were immediately preserved in formaldehyde for subsequent analysis in the laboratory or processed fresh directly onboard. For each individual, total length, total weight, sex and gonad weight (in the case of females) were recorded. Ovaries were kept in a formaldehyde solution and processing followed standard histological preparation techniques (Hunter and Macewicz, 1985). Lengths and weights for individuals preserved in formaldehyde were corrected for changes due to preservation by ad-hoc calculated correction factors. Sea surface temperature was available for 242 (49%) of the samples, either recorded while fishing, or from in situ measurements recorded +/- 2 days at the position of the fishing haul.

3.2.2 Definition of the daily spawning classes

In the Bay of Biscay, anchovies spawn between 18:00 to 04:00 with a clear daily spawning peak at around 23:00 hours (Alday *et al.* 2008; ICES 2004; Motos 1996). Daily spawning classes are defined for the group of females which will or have spawned the same night of a daily cycle starting and ending at 06:00 hours. A total of five daily spawning classes, relative to the day of capture, can be identified in the samples: day_-1 is the spawning class that will spawn in the night of the following day after capture; day_0 defines the group that will spawn or has spawned on the day of capture, and day_1, day_2 and day_3 are the daily spawning classes of females which have spawned one, two or three nights previously to the day of capture. In reality, day_3 is a plus group, named day_3+, which includes any female having spawned more than two nights before (grouping here mature females without any POF).

3.2.3 Histological indicators of spawning

The first step for assigning females to a daily spawning class is to assess their gonad histological slides in terms of stage of oocyte development (stages 1-8) as well as stage of POF degeneration (stages I-VII) (Table 2). Classification of oocyte development (Table 2), was based on the previous works of Hunter and Macewicz (1985) and Motos (1996). As pointed out by these authors, the stages of final oocyte maturation (FOM) which lead to ovulation (oocyte stages 5 to 8) develop in a day and a half (Figure 3.2) and provide information about the timing of the next batch. The degeneration of POFs was classified into seven stages using the criteria of Alday *et al.* (2008, 2010) (Table 2). For the range of temperatures in the Bay of Biscay during the DEPM surveys (13-20 ºC), complete resorption of the POFs occurs in about 2.5 days; therefore POF stages can identify spawning activity up to 2.5 days before capture (Figure 3.3).

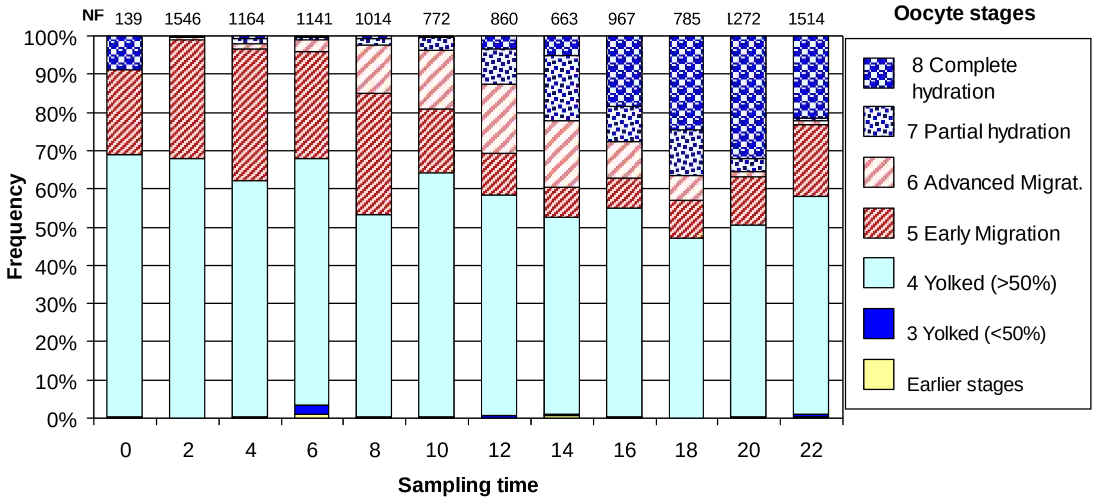
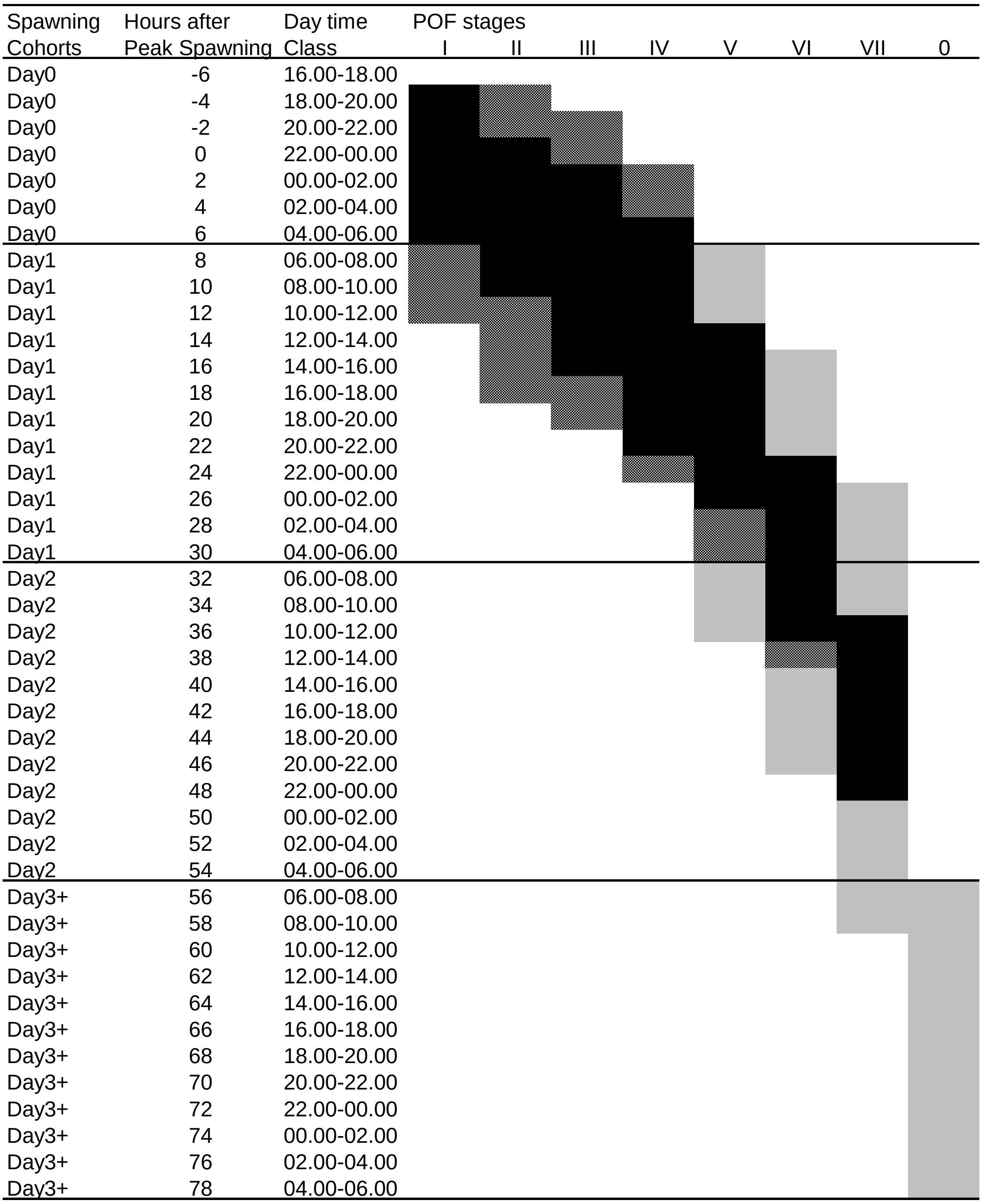


Figure 3.2: Percentages of ovarian stages based on developmental stage of the most advanced oocytes by sampling time, including, above each bar, the number of females (NF) examined by time class. See Table 2 for further descriptions of oocyte stages.



Spawning Cohorts	Hours after Peak Spawning	Day time Class	POF stages							
			I	II	III	IV	V	VI	VII	0
Day0	-6	16.00-18.00								
Day0	-4	18.00-20.00	■	▨						
Day0	-2	20.00-22.00	■	▨	▨					
Day0	0	22.00-00.00	■	■	▨					
Day0	2	00.00-02.00	■	■	■	▨				
Day0	4	02.00-04.00	■	■	■	▨				
Day0	6	04.00-06.00	■	■	■	■				
Day1	8	06.00-08.00	▨	■	■	■	░			
Day1	10	08.00-10.00	▨	■	■	■	░			
Day1	12	10.00-12.00	▨	▨	■	■	░			
Day1	14	12.00-14.00		▨	■	■	■			
Day1	16	14.00-16.00		▨	■	■	■	░		
Day1	18	16.00-18.00		▨	▨	■	■	░		
Day1	20	18.00-20.00			▨	■	■	░		
Day1	22	20.00-22.00				■	■	░		
Day1	24	22.00-00.00				▨	■	■		
Day1	26	00.00-02.00					■	■	░	
Day1	28	02.00-04.00					▨	■	░	
Day1	30	04.00-06.00					▨	■	░	
Day2	32	06.00-08.00					░	■	░	
Day2	34	08.00-10.00					░	■	░	
Day2	36	10.00-12.00					░	■	■	
Day2	38	12.00-14.00						▨	■	
Day2	40	14.00-16.00						░	■	
Day2	42	16.00-18.00						░	■	
Day2	44	18.00-20.00						░	■	
Day2	46	20.00-22.00						░	■	
Day2	48	22.00-00.00							■	
Day2	50	00.00-02.00							░	
Day2	52	02.00-04.00							░	
Day2	54	04.00-06.00							░	
Day3+	56	06.00-08.00							░	░
Day3+	58	08.00-10.00							░	░
Day3+	60	10.00-12.00								░
Day3+	62	12.00-14.00								░
Day3+	64	14.00-16.00								░
Day3+	66	16.00-18.00								░
Day3+	68	18.00-20.00								░
Day3+	70	20.00-22.00								░
Day3+	72	22.00-00.00								░
Day3+	74	00.00-02.00								░
Day3+	76	02.00-04.00								░
Day3+	78	04.00-06.00								░

Figure 3.3: Summary of the occurrence and duration of the different POF stages and general correspondence to spawning classes, adapted from the work of Alday *et al.* (2008), with indication of their periods of maximum occurrence (■), of reliable allocation to a spawning class (▨) and of the overlapping periods between successive daily spawning classes (░). Non filled rectangles for POF stage I between 18:00 hours of day_0 and 18:00 hours of day_1 refer to the period of the day with null or negligible occurrence of the stage.

Table 3.2: Double histological characterization of anchovy ovaries based (A) on oocyte development (Hunter & Macewitz, 1985) and (B) on POFs degeneration (POF stages are described in detail in Alday *et al.* 2008, 2010).

Histological Classifications	**Reproductive Phase**	**Stages Code**	**Histological Features of Oocyte Development / Degeneration of POF stages**
A- Classification based on developmental stage of the most advanced batch of oocytes	**Inmature**	**1**	Only unyolked oocytes. Oogonia and Primary growth
	Early Developping	**2**	The most advanced are partially yolked oocytes.
	Late Developping	**3**	Less than 50% of the slide surface occupied by yolked oocytes.
	Fully Developped	**4**	Yolked: 50% or more of the slide surface occupied by yolked oocytes.
	Final Oocyte Maturation (FOM) (pre-spawning)	**5**	Initial nucleus migration or germinal vesicle migration
		6	Advanced nucleus migration: Arrival to animal pole and nucleus disintegration
		7	Partially hydrated oocytes: Yolk plates visible. Partially hydration.Yolk coalescence
		8	Complete hydration: Completely hydrated oval/star-shaped oocytes.
B- Classification based on postovulatory follicles (POFs)	**Partial Post-spawning (with POFs)**	**I**	New recently collapsed POFs
		II	First signs of POFs degeneration. First vacuoles in granulose cells
		III	Small vacuoles affecting ≤50% of granulose cells
		IV	Massive incidence of medium size vacuoles in granulose cells
		V	Reduction/absence of lumen. Cell walls rupture of granulose cells
		VI	Very reduced POF (to about 50% of original size). Few vacuoles and few pycnotic nuclei.
		VII	Tissue remains. No cells. Some pycnotic nuclei.
	No POFs	**0**	Ovaries with no partial post-spawning signs (No POFs)

3.2.4 Ageing Matrix system to assign females to daily spawning classes

The second step to complete the process of determining spawning classes is to assign the former histological stages to pre-defined daily spawning classes according to the occurrence of those histological stages in time relative to peak spawning time -as summarized in figure 2 from Motos (1996) –updated here- for final oocyte maturation and in figure 3 from Alday et al (2008) for POF stages-. This was done defining an ageing matrix system which provided the probability of a female being in a daily spawning class depending on the ovary histological stage and the sampling time. A first matrix is proposed for the females undergoing final oocyte maturation (stages 5 to 8), which serves to allocate them to day_-1 or day_0 pre-spawning classes (Table 3), whilst a second one is proposed for the classification based on the degeneration of POFs which serves to allocate females to day_0, day_1, day_2 or day_3+ post-spawning classes (Table 4). As such, the double staging coupled with the ageing matrix system allowed, in principle, each female to belong to two daily spawning classes; one pre-spawning and one post-spawning. Further details follow.

Table 3.3: Matrix of percentages used to assign females to pre spawning daily classes (day_-1 and day_0) based on the development stage of their most advanced batch of oocytes and the time of capture. Ovaries with oocytes in the primary and secondary growth phase (stages 1-4) could not be allocated to any pre-spawning class and therefore they are omitted from the table.

Stages of Oocyte maturation	Time interval	Day_-1	Day_0
5 Early Migration	(00-02)	100%	0%
	(02-04)	100%	0%
	(04-06)	100%	0%
	(06-08)	0%	100%
	(08-10)	0%	100%
	(10-12)	0%	100%
	(12-14)	33%	67%
	(14-16)	67%	33%
	(16-18)	100%	0%
	(18-20)	100%	0%
	(20-22)	100%	0%
	(22-00)	100%	0%
6 Advanced Migration	(00-02)	100%	0%
	(02-04)	100%	0%
	(04-06)	100%	0%
	(06-08)	0%	100%
	(08-10)	0%	100%
	(10-12)	0%	100%
	(12-14)	0%	100%
	(14-16)	0%	100%
	(16-18)	0%	100%
	(18-20)	0%	100%
	(20-22)	0%	100%
	(22-00)	0%	100%
7 Partial Hydration	(00-02)	0%	100%
	(02-04)	0%	100%
	(04-06)	0%	100%
	(06-08)	0%	100%
	(08-10)	0%	100%
	(10-12)	0%	100%
	(12-14)	0%	100%
	(14-16)	0%	100%
	(16-18)	0%	100%
	(18-20)	0%	100%
	(20-22)	0%	100%
	(22-00)	0%	100%
8 Complete Hydration	(00-02)	0%	100%
	(02-04)	0%	100%
	(04-06)	0%	100%
	(06-08)	0%	100%
	(08-10)	0%	100%
	(10-12)	0%	100%
	(12-14)	0%	100%
	(14-16)	0%	100%
	(16-18)	0%	100%
	(18-20)	0%	100%
	(20-22)	0%	100%
	(22-00)	0%	100%

Table 3.4: Matrix of percentages used to assign females to post spawning daily classes (from day_0 to day_3+) based on the degeneration state of their POFs and the time of capture. a) POF stages I to IV and b) POF stages V to VII and 0.

a) Stages of POFs	Time interval	Day_0	Day_1	Day_2	Day_3+
I	(00-02)	100%	0%	0%	0%
	(02-04)	100%	0%	0%	0%
	(04-06)	100%	0%	0%	0%
	(06-08)	0%	100%	0%	0%
	(08-10)	0%	100%	0%	0%
	(10-12)	0%	100%	0%	0%
	(12-14)	0%	100%	0%	0%
	(14-16)	0%	100%	0%	0%
	(16-18)	0%	100%	0%	0%
	(18-20)	100%	0%	0%	0%
	(20-22)	100%	0%	0%	0%
	(22-00)	100%	0%	0%	0%
II	(00-02)	100%	0%	0%	0%
	(02-04)	100%	0%	0%	0%
	(04-06)	100%	0%	0%	0%
	(06-08)	0%	100%	0%	0%
	(08-10)	0%	100%	0%	0%
	(10-12)	0%	100%	0%	0%
	(12-14)	0%	100%	0%	0%
	(14-16)	0%	100%	0%	0%
	(16-18)	0%	100%	0%	0%
	(18-20)	100%	0%	0%	0%
	(20-22)	100%	0%	0%	0%
	(22-00)	100%	0%	0%	0%
III	(00-02)	100%	0%	0%	0%
	(02-04)	100%	0%	0%	0%
	(04-06)	100%	0%	0%	0%
	(06-08)	0%	100%	0%	0%
	(08-10)	0%	100%	0%	0%
	(10-12)	0%	100%	0%	0%
	(12-14)	0%	100%	0%	0%
	(14-16)	0%	100%	0%	0%
	(16-18)	0%	100%	0%	0%
	(18-20)	0%	100%	0%	0%
	(20-22)	100%	0%	0%	0%
	(22-00)	100%	0%	0%	0%
IV	(00-02)	100%	0%	0%	0%
	(02-04)	100%	0%	0%	0%
	(04-06)	100%	0%	0%	0%
	(06-08)	0%	100%	0%	0%
	(08-10)	0%	100%	0%	0%
	(10-12)	0%	100%	0%	0%
	(12-14)	0%	100%	0%	0%
	(14-16)	0%	100%	0%	0%
	(16-18)	0%	100%	0%	0%
	(18-20)	0%	100%	0%	0%
	(20-22)	0%	100%	0%	0%
	(22-00)	0%	100%	0%	0%

b) Stages of POFs	Time interval	Day_0	Day_1	Day_2	Day_3+
V	(00-02)	0%	100%	0%	0%
	(02-04)	0%	100%	0%	0%
	(04-06)	0%	100%	0%	0%
	(06-08)	0%	25%	75%	0%
	(08-10)	0%	50%	50%	0%
	(10-12)	0%	75%	25%	0%
	(12-14)	0%	100%	0%	0%
	(14-16)	0%	100%	0%	0%
	(16-18)	0%	100%	0%	0%
	(18-20)	0%	100%	0%	0%
	(20-22)	0%	100%	0%	0%
	(22-00)	0%	100%	0%	0%
VI	(00-02)	0%	100%	0%	0%
	(02-04)	0%	100%	0%	0%
	(04-06)	0%	100%	0%	0%
	(06-08)	0%	0%	100%	0%
	(08-10)	0%	0%	100%	0%
	(10-12)	0%	0%	100%	0%
	(12-14)	0%	0%	100%	0%
	(14-16)	0%	20%	80%	0%
	(16-18)	0%	40%	60%	0%
	(18-20)	0%	60%	40%	0%
	(20-22)	0%	80%	20%	0%
	(22-00)	0%	100%	0%	0%
VII	(00-02)	0%	16.7%	83%	0%
	(02-04)	0%	33.3%	67%	0%
	(04-06)	0%	50.0%	50%	0%
	(06-08)	0%	0%	67%	33.3%
	(08-10)	0%	0%	83%	16.7%
	(10-12)	0%	0%	100%	0%
	(12-14)	0%	0%	100%	0%
	(14-16)	0%	0%	100%	0%
	(16-18)	0%	0%	100%	0%
	(18-20)	0%	0%	100%	0%
	(20-22)	0%	0%	100%	0%
	(22-00)	0%	0%	100%	0%
0	(00-02)	0%	0%	0%	100%
	(02-04)	0%	0%	0%	100%
	(04-06)	0%	0%	0%	100%
	(06-08)	0%	0%	0%	100%
	(08-10)	0%	0%	0%	100%
	(10-12)	0%	0%	0%	100%
	(12-14)	0%	0%	0%	100%
	(14-16)	0%	0%	0%	100%
	(16-18)	0%	0%	0%	100%
	(18-20)	0%	0%	0%	100%
	(20-22)	0%	0%	0%	100%
	(22-00)	0%	0%	0%	100%

3.2.4.1 Ageing matrix based on oocyte development stages

Preparation for spawning begins with the nuclear (germinal vesicle) migration to the animal pole of the oocyte (stage 5) between 1 and 1.5 days before spawning (Motos 1996). From Figure 3.2 it is evident that minimum occurrence of this stage occurs in the three 2-hour time classes from 12:00 to 18:00 hours, coincident with the maximum occurrence of the advanced nuclear migration (stage 6) and the start of its decline at 16 hours. From this we assume that all day-0 females have already entered the advance nuclear migration and hydration phases prior to spawning (stages 6 to 8) at 16:00. Therefore, all females having gonads with oocytes in stage 5 sampled between 16:00 and 06:00 hours, will spawn in the night of the following day and belong to day_-1; whereas females in this stage occurring between 06:00 and 12:00 hours will culminate the final oocyte maturation and will spawn on the day of capture, belonging to day_0 (Table 3). In addition, the non complete disappearance of stage 5 at the maximum occurrence of stage 6, between 12 and 16:00, is probably due to the start of the early nuclear migration in females which will spawn the following day after 12.00 hours, compensating thus the departure from the stage 5 of the day_0 females to stage 6. For this reason a transition (overlapping) period of co-occurrence of day_0 females leaving stage 5 to enter stage 6 with those day_-1 just recruited to the stage 5 is allowed between 12:00 and 16:00 hours; this is reflected in Table 3 by the gradual complementary sharing percentages of stage 5 on the two consecutive daily spawning classes (day_-1 and day_0) over this period.

Ovaries showing oocytes in advanced nuclear migration (stage 6) vanish around 00:00 hours (Figure 3.2), after which an increasing occurrence is seen as result of the recruitment from the stage 5 (initial nuclear migration). This means that ovaries in stage 6 found between 00:00 and 06:00 hours are indicative of a spawning in the following night (day_-1 spawning class), whilst stage 6 seen between 06:00 and 00:00 hours will reach spawning that day (day_0 class). Oocytes in hydration phases (Stages 7 and 8), which last less than a day, are indicative of an imminent spawning in the next few hours; they start recruitment from their earlier stages after 06:00 hours and vanish after spawning, well before the next 06:00 hours of the night of spawning, thus both stages are indicative of a spawning during the day of capture (day_0) (Table 3).

3.2.4.2 Ageing matrix based on POFs degeneration stages

Based on Alday *et al.* (2008), the first four POF stages (I to IV) last less than a day, appearing in the samples before 06:00 hours (as day_0 class after the spawning) and ending during the following day (day_1) (Figure 3.3). As spawning starts at 18:00, just after the sharp minimum occurrence of POF stages I and II at 16:00 hours (in Alday *et al.* 2008), the occurrence of these stages between 18:00 and 06:00 hours is allocated to day_0 and between 06:00 and 18:00 hours to day_1 (Table 4). For stages

III and IV, a marked minimum occurrence in the field samples was detected by Alday *et al.* (2008) at 20:00 hours and at 00:00 hours respectively. For these stages, the period of minimum occurrence was used to separate their allocation into successive daily classes (either to day_0, between the respective minimums and 06:00 hours, or to day_1 at the complementary part of the day) (Table 4**)**.

For the older POF stages, which can last 24 hours or longer (stages V to VII), direct allocation to a single spawning class can be only applied for the periods around their maximum occurrence, when no overlap between successive daily spawning classes can occur (Figure 3.3). However, as these POF stages last for a day or more, some overlap of consecutive daily spawning classes may occur during the entry and exit periods in these stages (corresponding with the shaded areas in Figure 3.3 (Alday *et al.* 2008), given that the entry process finishes later than the exit starts. In these overlapping periods some assumptions need to be made in order to allocate these stages to spawning classes. For simplicity, symmetrical and gradual transition percentages for the allocation to daily spawning classes over the overlapping periods are proposed, as suggested in Alday *et al.* (2008). For example, stage V has a maximum occurrence mainly between 12:00 and 02:00 hours (Figure 3.3); thus, within this period, all females with stage V POFs belong to the day_1 spawning class. However, recruitment to this stage may start at 06:00 hours, as day_1 spawners; and they may not be finished leaving this stage until 12:00 hours of the second day after spawning, as members of the day_2 spawning class (Alday *et al.* 2008). Hence, for the three 2-hour classes between 06:00 and 12:00 hours, day_1 females recruiting to the POF stage V were given increasing percentages of occurrence within the respective classes of 25%, 50% and 75%. Complementary symmetrical decreasing percentages were for day_2 females leaving POF stage V (Table 4).

Finally, a similar approach was followed for the overlapping periods of the POF stages VI and VII, occurring between 14:00 hours and 22:00 hours and between 00:00 and 10:00 hours respectively (Alday *et al.* 2008): Gradually increasing percentages were set for the recruiting spawning classes to the stages (i.e. to day_1 in the case of stage VI and to day_1 and day_2, depending upon the time of capture, in the case of stage VII) (Table 4).

For ageing stages within overlapping periods, a random allocation of the female to one of the two potential daily spawning classes was made in proportion to the percentages set in the ageing matrices (Tables 3 and 4).

3.2.5 Spawning fraction estimators based on the proportions of daily spawning classes

Day_0 and day_1 spawning classes were selected for evaluating the spawning fraction. The other daily spawning classes were discarded: Day_-1 cannot be used as it is detected incompletely in a 24-hour cycle whilst the day_2 spawning class requires increasing assumptions for the long overlapping periods of the POF stages belonging to it (mainly stages VI and VII), in this way making its identification far more uncertain than that of day_1 (Alday *et al.* 2008). In addition, day_2 shows an irregular occurrence throughout the day (see results) which prevents its use for the estimation of S. Finally, four estimators of the spawning fraction (S) based on the proportions of the day_0 and day_1 spawning classes were tested (Table 5): Estimators S(0) and S(1) refer to the proportions of day_0 and day_1 spawning classes in the samples respectively. Estimator S(0+1) is the mean proportion of day_0 and day_1 in the samples. And finally, estimator S(1).Corr is, in principle, similar to the original estimator proposed by Picquelle and Stauffer (1985) for the proportions of day_1 spawning class, corrected for oversampling of day_0. However, the corrected number of mature females in the denominator is not $2 \cdot n'_1 + n'_{2+}$ as originally proposed, but $n - n'_0 + n'_1$, in which the sub-indices refer to spawning classes; n refers to the total number of mature females in the sample and n'_{2+} refer to either day_2 or day_3+ spawning classes. Both expressions are equal only if day_2+ totally excludes the day_0 females. This distinction is relevant for high spawning fractions, as there will be an increasing amount of day_2+ females (even with POFs) also belonging to the day_0 spawning class. Unless they are removed from the day_2+ spawning classes, the denominator will be inflated and thus S(1).Corr will underestimate the true S. For the purposes of clarification, we will call day_2+.Mixed the complete day_2+ group of spawning classes, whilst day_2+.Pure will be used only for the females of the day2+ group which are not spawning during the day of capture (i.e. excluding day_0 females). For %day_2+.Mixed and %day_2+.Pure we will refer to their respective mean percentage per sample (note that these are not S estimators as they do not refer to a single spawning class). In all cases the mature population upon which to base the former S estimates should comprise the spawning capable females (stages 3-8 in table 2). In practice, however, as the immature females were so scarce (just 0.7% of the total population) they were allowed to join the denominator of the S estimators, resulting in S at the population level, not restricted to the mature population.

Estimates of the mean spawning fraction of a group of samples (either grouped by survey, or by sampling time or by mean weight of females) and its variance were produced based upon the proxy of a sampling proportional to abundance (for a judgment sampling scheme) proposed by Picquelle and Stauffer (1985) (its equation 5), in which weighting factors per sample were set equal to 1 when the number of females m_i in the sample was higher than a threshold value (20 in this paper) or equal to $m_i/20$ otherwise. The expected variance of an estimator based on the combined occurrence of several daily spawning classes – such as S(0+1)- in terms of the covariance of the spawning classes, was described by Ganias *et al.* (2003).

Potential relationships between the individual S values and several covariates, such as sampling gear, sampling time, size of the females and the year-survey factor were tested using generalized linear models (McCullagh and Nelder 1989).

Table 3.5: Definition of the estimators of spawning fraction tested in this work, at population and sample level. Population estimates are the weighted mean of sample estimates (see text). The two columns to the right indicate the relative bias (*RBias*) expected from samples obtained from a given time of the day according to the oversampling of day_0 females occurring at that time, for two types of negative effect that the oversampling of day_0 may have on the other daily spawning classes, being either a random effect on all other spawning classes or directly affecting only day_1 (repulsion) (see text). The total number of mature females per sample is n and those observed belonging to different spawning classes are named n'_0, n'_1, for day_0 and day_1 respectively. S refers to the true (unbiased) spawning fraction of the population and f means spawning frequency (in terms of interspawning intervals in days).

Estimator (Names)	Description at population level (across samples)	Sample estimate (at time t)	Relative Bias (Names)	E(RBias(random)) (at time t)	E(RBias(repulsion)) (at time t)
S(0)	Mean percentage of day_0 cohort	$S(0)_t = \frac{n'_0}{n}$	$RBias(\hat{S}(0)_t)$	$\frac{\hat{S}(0)_t}{S} - 1$	$\frac{\hat{S}(0)_t}{S} - 1$
S(1)	Mean percentage of day_1 cohort	$S(1)_t = \frac{n'_1}{n}$	$RBias(\hat{S}(1)_t)$	$-Rbias(\hat{S}(0)_t)\left(\frac{S}{1-S}\right)$	$-Rbias(\hat{S}(0)_t)$
S(1).Corr	Mean percentage of day_1 cohort corrected for random oversampling of day_0	$S(1)_t.\mathrm{Corr} = \frac{n'_1}{n - n'_0 + n'_1}$	$RBias(\hat{S}(1)_t.Corr)$	unbiased	$-Rbias(\hat{S}(0)_t)$
S(0+1)	Mean percentage of day_0 or day_1 cohorts	$S(0+1)_t = \frac{n'_0 + n'_1}{2n}$	$RBias(\hat{S}(0+1)_t)$	$Rbias(\hat{S}(0)_t)\frac{1-2S}{2\cdot(1-S)}$	unbiased
S(*f*)	Mean S of females according to their individual spawning frequency (*f*), assuming a maximum *f* of 3	$S(f)_t = \sum_{f=1}^{3} 1/f * P(f)$	$RBias(\hat{S}(f)_t)$	unbiased	unbiased

3.2.6 Spawning aggregation patterns, oversampling and properties of the estimators

The statistical properties of the four estimators considered above, depend upon the type of aggregation and distribution of the spawning classes between schools: A clumped distribution of the daily spawning classes will tend to increase the variance in comparison to random or uniform distributions of the classes across samples. This was analyzed by correlation matrices between the proportions of the spawning classes and by the normal and standardized Morisita's index of dispersion (Ganias 2008; Krebs 1999). This index can be used to test if the spawning classes are distributed at random across samples by a Chi-square test (Krebs 1999). For its application, all sample sizes were homogenized (Ganias 2008) by re-scaling them to 20 females (which is an arbitrary value chosen at the 10th percentile of all available sample sizes, leading therefore in most cases to a reduction of the actual sample size and hence losing some of the potential power of the test for the amount of data available). For random distributions, the standardized Morisita index gives values of around 0, with 95% confidence limits at +0.5 and -0.5, whereas values greater than 0.5 indicate a clumped pattern and values less than -0.5 a uniform distribution pattern (Krebs 1999).

In addition, these estimators will be unbiased only if no oversampling of day_0 spawners occurs throughout the day. However, if oversampling of day_0 occurs at a certain period of the day then some of the estimators would be biased for the samples collected from that period of the day. Oversampling of day_0 at a given time *t* means that the proportion of the day_0 females in the samples is on average higher than the expected one (i.e. than S). In this paper, oversampling is assessed in relative terms, as the relative oversampling of day_0 throughout the day (i.e. the relative bias in the proportion of day_0 class in the samples taken at any time *t*, $RBias(\hat{S}(0)_t) = \left(\hat{S}(0)_t / S\right) - 1$, with S referring to the true unbiased spawning fraction of the population -- see the evaluation procedure below). As shown in Table 5, the bias of the S estimators, based on samples taken at a certain time of the day, will depend upon the magnitude and type of this oversampling: If random oversampling of day_0 occurs, i.e. the oversampling of day_0 has a negative random effect on all remaining daily classes (proportional to their true abundance), then S(1).Corr will be the only unbiased estimator (see Appendix for demonstrations). In this case, the biases of the other estimators are proportional to the relative oversampling of day_0 at the sampling time *t* ($RBias(\hat{S}(0)_t)$) and depend also upon the actual value of S. For S<0.5 the relative biases of S(1) and S(0+1) are smaller than the relative bias of S(0) whilst for S values above 0.25, S(0+1) is less biased than S(1) (Figure 3.4). Alternatively, if oversampling of day_0 has a parallel negative effect only on day_1 (repulsion type) then S(0+1) will be the only unbiased estimator (Table 5). Notice that for S=0.5 the only type of oversampling should be that of "repulsion" because the only two complementary daily spawning classes are day_0 and day_1, given that day_2 will be at the same time the day_0 spawning class. Examination of correlations between daily spawning classes clarified the type of oversampling encountered.

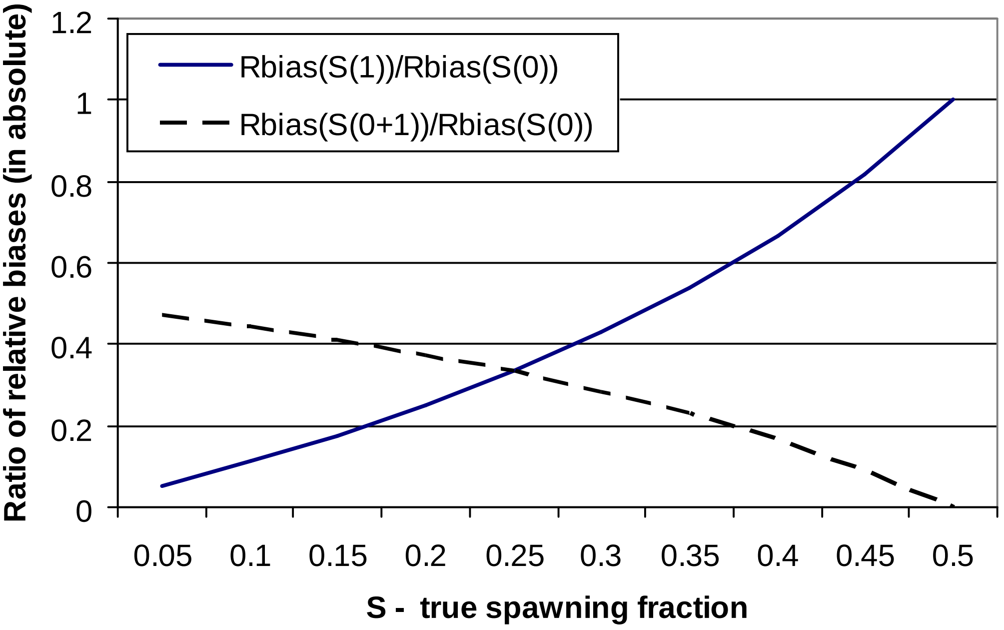


Figure 3.4: Absolute value of the Ratio of the relative biases of S(1) and S(0+1) on the relative oversampling of day_0 (*Rbias(S(0)*) as a function of true S, for the null hypothesis of a random negative effect of the oversampling of day_0 in an undersampling of day_1 and day_2+ spawning classes.

Evaluation of the potential oversampling of day_0, which is suspected to happen mainly around spawning time, was made both visually, throughout a 24-hour cycle, and quantitatively by fitting a logistic model to the absolute oversampling as a function of the time to peak spawning time (From 15 hours prior to 15 hours after spawning time):

$$S_t = S + O \cdot \frac{\exp\left(B*(I-|t|)\right)}{\left(1+\exp\left(B*(I-|t|)\right)\right)} \qquad \text{Eq. 3.1}$$

where S_t is the observed spawning fraction at time *t*, based on day_0 (or day_1 for the observations more than 7 hours after spawning time). *S* is the expected level of spawning fraction outside the oversampling period, *O* is the maximum oversampling effect, by which *S* can be increased on average during the oversampling period, *I* is the inflection time of the logistic equation, offset from the peak spawning time, for which the oversampling effect attains half of its maximum value, $|t|$ is the time in hours (in absolute terms) from the sampling time to the peak spawning time (23:00 hours), and finally *B* is the parameter defining the steepness of the logistic curve. From equation 3.1, the expected relative oversampling of day_0 at any time *t* would be $S_t/S-1$.

As oversampling of day_0 changes with sampling time, the overall final bias of any of these estimators across samples will be the mean of the individual biases of the sample-estimates across all effective sampling time intervals. For example, for the case of S(0+1) estimator the overall relative bias will be:

$$\text{Mean}\left(RBias\ (\hat{S}(0+1)) \right) = \frac{\sum_{t=1}^{24} N_t \cdot RBias\ (\hat{S}(0+1)_t)}{\sum_{t=1}^{24} N_t} \qquad \text{Eq. 3.2}$$

Where N_t is the number of samples in the time interval *t*, and $Rbias\ (\hat{S}(0+1)_t)$ is the expected relative bias at time *t* (Table 5) as a function of S and the relative oversampling of day_0 expected at that time ($S_t/S-1$). From the former equation, it follows that the bias corrected S(0+1) estimator over all samples will be simply:

$$S(0+1).BiasCorr = \frac{S(0+1)}{1+Mean(RBias\ (\hat{S}(0+1)))} \qquad \text{Eq. 3.3}$$

with variance (neglecting the errors in the mean relative bias) equal to the original variance divided by the square of the denominator.

3.2.7 Spawning fraction estimator based upon individual female spawning frequencies

For species expected to have short spawning intervals, as for this anchovy, many of the females can receive double assignments to pre- and post-spawning daily classes which should permit an estimation of their respective spawning frequency (measured in terms of inter-spawning intervals *ISI*, or days passed between successive spawning events; Ganias –this volume-). This double assignment allows an estimation of S for the population based upon the average of the individual spawning fraction of females, deduced from the reciprocal of the spawning frequencies (*1/f*), as follows:

$$S(f)=\sum_{f=1}^{\inf}\left(1/f\right)\cdot P\left(f\right) \qquad \text{Eq. 3.4}$$

where *P(f)* is the probability of a female having a spawning frequency of *f* days.

With variance:

$$Var(S(f))=\sum_{f=1}^{\inf}\left(1/f\right)^{2}\cdot Var(P\left(f\right))+2\cdot\sum_{f=1}^{\inf}\sum_{f'<f}^{\inf}\left(1/f\right)\cdot\left(1/f'\right)\cdot Cov(P\left(f\right),P\left(f'\right)) \ .$$

Eq. 3.5

where f and f' refer to different spawning frequencies.

In our case, we restricted the expression to the first three daily interspawning intervals, i.e. for f ranging from 1 to 3, assuming that all day_3+ spawned during the night of the third day after their previous spawning (Table 5). Under these circumstances, the estimator is simplified to:

$S(f) = 1\cdot P(f = 1) + 0.5\cdot P(f = 2) + 0.333\cdot P(f = 3)$ Eq. 3.6

where $P(f=1)$ is the probability of a female spawning every day and was estimated as the frequency of day_0 within the day_1 spawning females $P(f = 1|$ day_1$)$ = P(day_0 | day_1). $P(f = 2)$ is the probability of a female spawning every other day and was estimated as $[1- P(f=1)]\cdot P(f = 2)$ | day_2), where $P(f = 2 |$ day_2$)$ = P(day_0 | day_2) was the probability of a female of the day_2 class of spawning again in that day (i.e. in the second night after their previous spawning). This was estimated by the frequency of day_2 females also being day_0 females, as estimated during the first part of the day (from 06:00 to 17:59 hours). The selection of the first half of the day_2 for estimating the proportion of day_0 females within it was because in this way the spawning period was excluded totally and enabled day_0 to be identified completely.

Finally, P(f = 3) is the probability of past-spawning females spawning every third day, which was estimated as [1- P(f = 1)] · [1- P(f = 2 | day_2)], where [1- P(f = 2 | day_2)] was the probability of a female of the day_2 spawning class spawning during the following night (i.e. during the third night after their previous spawning). Using the P(f = 2 | day_2) complement avoided having to make direct use of the most likely noisy direct proportion estimates of day_3 females. Notice that in this way a covariance term between P(f = 2) and P(f = 3) was generated.

Given the simplifications assumed to apply this formula and the little variability in the S estimates between years arising from the estimators based on the prevalence of daily spawning classes (see results), we applied this formula to the pooled set of females across the years (not on a survey by survey basis) in an exploratory way, just for the purposes of verification and discussion.

3.3 Results

The spawning dynamics of actively spawning females in the population throughout a five-day period is shown in Figure 3.5a. as the proportion of each daily spawning class in the population, overlaying the relative contribution to each class of the females in a particular ovary maturity stage according to the development of their oocytes. There is an overall consistency of the proportions of day_0 and day_1 spawning classes at around 40%. The incomplete detection of the day_-1 is clearly associated with the early nucleus migration in oocytes during the second half of the day (stage 5). Day_0 females clearly show the progression from early nucleus migration to the hydration stages. Day_0 – after spawning– and day_1 females were composed of females with ovaries showing in majority yolked oocytes (stage 4), although at the end of day_1 more females with oocytes in early nuclear migration (stage 5) were observed. The percentages for day_2 were more irregular than for the former daily classes, being greater than 40% for the first half of the day and markedly lower from 18:00 hours onwards. This endorsed the rejection of this spawning class for the estimation of S.

The initial higher proportion of day_2 might be due to potential ageing confusion with the day_3 spawners across the overlapping period of POF stage VII. The subsequent decrease was related to the advanced maturity stages of the ovary of many of these females (with oocytes in nuclear migration or hydrated), indicating that most of the day_2 females may resume spawning that night. Similarly, when looking at the occurrence of POF stages by daily classes (Figure 3.5b), it can be observed that most day_0 females prior to spawning showed ovaries with POF stages VI and VII, typical of the day_2 spawning class. Most of the identified day_3+ females had no POFs but the oocytes were undergoing FOM. The very few immature or developing females appeared grouped in the day_3+ spawning class.

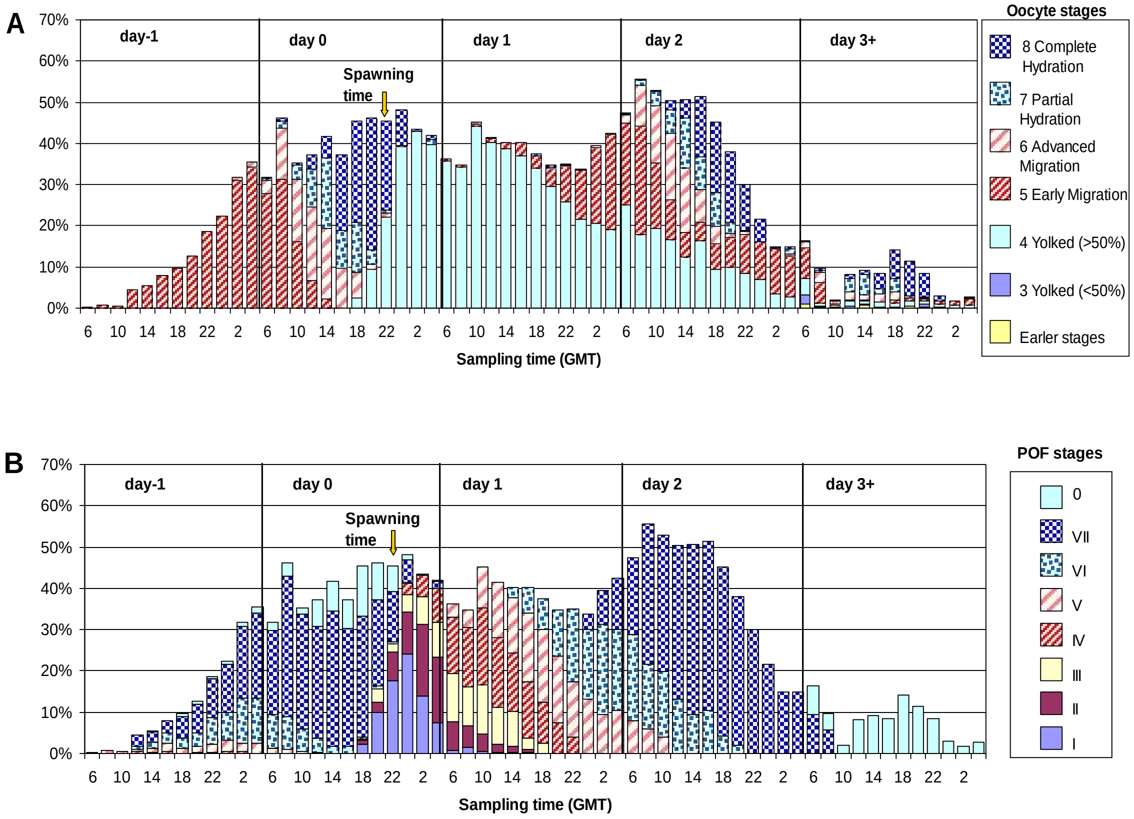


Figure 3.5: Percentages of females assigned to the daily spawning classes displayed consecutively throughout a five day spawning cycle, showing the relative contribution to the daily classes of the ovary maturity stages depending on A) the developmental of the most advanced oocytes and B) the stage of POF degeneration by sampling time classes (2 hour intervals). Stages are defined in Table 2. GMT = Greenwich meridian time.

Figure 3.6 shows some potential oversampling of actively spawning females (day_0) around peak spawning time, as indicated by a rise in the average S(0) to a maximum average value of 0.485, i.e. an increase above the overall mean S of 0.39-0.4. Conversely, some undersampling of the day_1 appears to be apparent around peak spawning time. As the variance of S(0), 5 hours either side of spawning time, was higher than outside these periods (Cochran's C test: 0.6189, $P<0.001$), we performed a Kruskal-Wallis test to compare the median S estimate presented in Figure 3.6, grouping them 5 hours either side of the spawning time (at 23:00 hours), 5 hours either side of the following spawning event (24 hours later) and all remaining estimates outside these time ranges. The medians of the S(0) and S(1) estimates 5 hours either side of the peak spawning times were higher and lower, respectively, than the medians of the S(0) and S(1) estimates outside these periods ($P=0.001$ and 0.062, respectively). The logistic equation fitted to the oversampling of day_0 on the S estimates, 15 hours either side of the peak spawning time, showed a dome-shaped curve (Figure 3.6), defining a rise in spawning frequencies of about 0.067 (parameter O, S.E.=0.021) over the normal S estimate (S=0.391, S.E.=0.012). The oversampling phenomena lasts for about 10 hours (5 hours either side of the peak spawning time, as pointed out by the inflection point I=4.938 hours, with S.E.=0.80, and by the curvature parameter B=1.952, with S.E.=3.37).

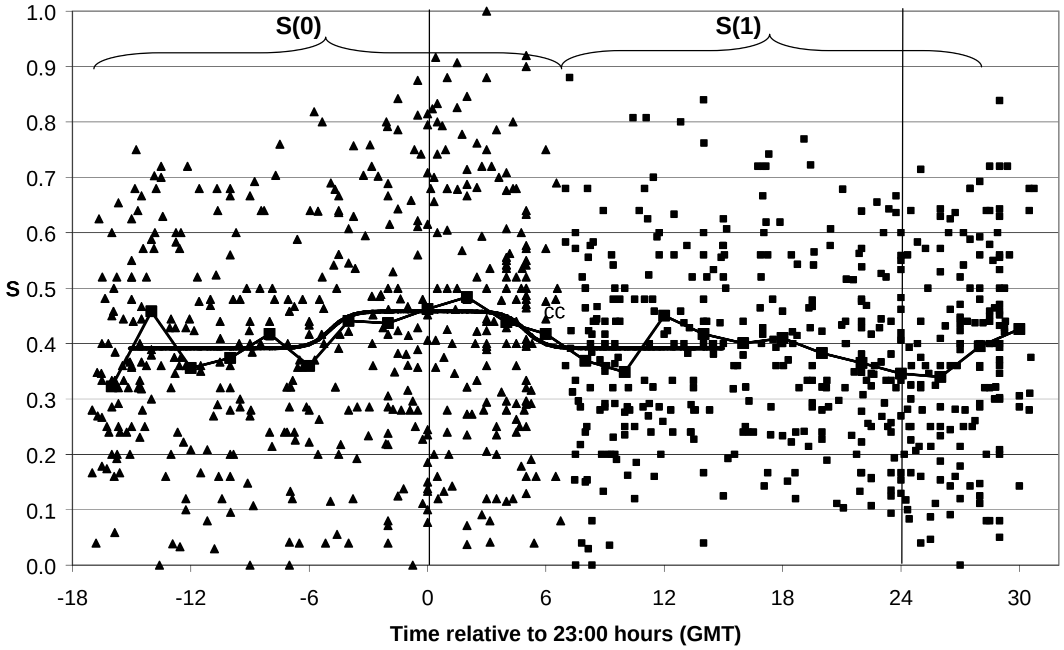


Figure 3.6: Individual spawning fraction estimates per sample by sampling time relative to the peak spawning time at 23:00 hours for the S(0) and S(1)estimators, together with their mean values in 2-hour classes (line with black rectangles) and the fitting of the oversampling effect with a logistic model (continuous black line). Triangles refer to S(0) estimates and squares to S(1) estimates.

The correlations of S(0) with S(1) and with the percentage of occurrence of all remaining classes (%day_2+.Pure) were significantly negative for all times of the day (Table 6), but reaching the maximum around spawning time. Estimator S(1) however, was positively but non significantly correlated with the %day_2+.Pure estimator around spawning time. These observations suggest that oversampling of day_0 negatively affects all of the remaining spawning classes, as corresponds with a random type of oversampling of day_0. The Chi-square values for the Morisita index (Table 7), led to the rejection of the random distribution of spawning classes across samples, except for the joint day(0+1) classes (with P=1). The standardized Morisita index revealed that the individual spawning classes (day_0 and day_1), as the remaining classes (day_2+), tended to show clumped (aggregated) distributions (with values higher than 0.5), whilst day(0+1) tended towards a uniform distribution (with a value lower than -0.5). These aggregation patterns were consistent for all times of the day.

Table 3.6: Correlation matrix for the S(0) and S(1) sample estimates of spawning fraction with all other S estimators and with the joint percentage of the day_2 and day 3+ spawning classes per samples (%day_2+.Mixed or %day_2+.Pure), for two periods of the day: A) during the spawning time (23:00 hours +/-5 hours) (217 samples), and B) for the remaining part of the day (276 samples). Bold values correspond with statistically significant correlation values (at alpha 0.01).

A- Spawning Period	**S(1)**	**S(1).Cor**	**S(0+1)**	**%day_2+.Mixed**	**%day_2+.Pure**
S(0)	**-0.7901**	0.0397	**0.6879**	-0.1123	**-0.6956**
S(1)		**0.4669**	-0.0987	-0.1750	0.1151

B-Non Spawning Period	**S(1)**	**S(1).Cor**	**S(0+1)**	**%day_2+.Mixed**	**%day_2+.Pure**
S(0)	**-0.6676**	0.0792	**0.4794**	**0.2995**	**-0.4773**
S(1)		**0.6473**	**0.3333**	**-0.6635**	**-0.3347**

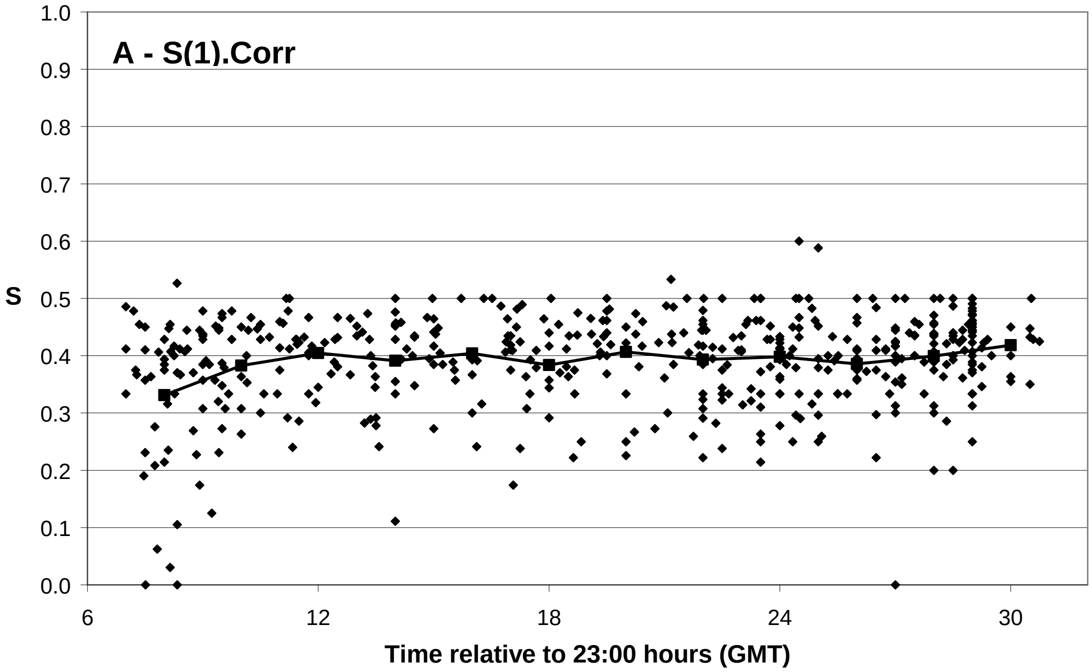
Table 3.7: Morisita index of dispersion (with its Chi-square and probability of that value being due to a random effect) and standardized Morisita index of dispersion for the different spawning classes, for two periods of the day: A) during the spawning time (23:00 hours +/-5 hours) (217 samples), and B) for the remaining part of the day (276 samples).

A- Spawning Period	**day_0**	**day_1**	**days_(0+1)**	**day_2+.Mixed**	**day_2+.Pure**
Morisita Index (MI)	1.1464	1.0795	0.9692	1.2655	1.2803
Chi-square of MI	505.30	340.10	107.09	591.51	443.11
Prob(Chi-square)	3.12E-25	1.42E-07	1	1.12E-36	8.73E-18
Standardized Morisita Index	0.5003	0.5001	-0.5100	0.5005	0.5005

B- Non Spawning Period	**day_0**	**day_1**	**days_(0+1)**	**day_2+.Mixed**	**day_2+.Pure**
Morisita Index (MI)	1.0969	1.0586	0.9710	1.0966	1.2462
Chi-square of MI	483.18	404.99	148.15	556.69	559.57
Prob(Chi-square)	1.24E-13	5.32E-07	1	2.89E-21	1.38E-21
Standardized Morisita Index	0.5001	0.5001	-0.5095	0.5001	0.5004

The variability of the individual spawning fraction estimates between samples obtained from the direct estimators S(0) and S(1) was very high, ranging between 0 and 1, whilst it decreased sharply for S(1).Corr and S(0+1) (Figure 3.6 and Figure 3.7 respectively). The latter estimator provided the least variable estimates as most of the very low S values, which appeared in the S(1).Corr estimates, disappeared. The associated coefficients of variation of the pooled estimates (across all samples of all surveys) were high for S(0) and S(1) (CV of 50% and 45% respectively), lower for the

S(1).Corr estimator (CV=22%) and lowest for the S(0+1) estimator (CV=18%) (Table 8), with the latter being significantly lower than the former (F = 1.33034 P-value = 0.0016).



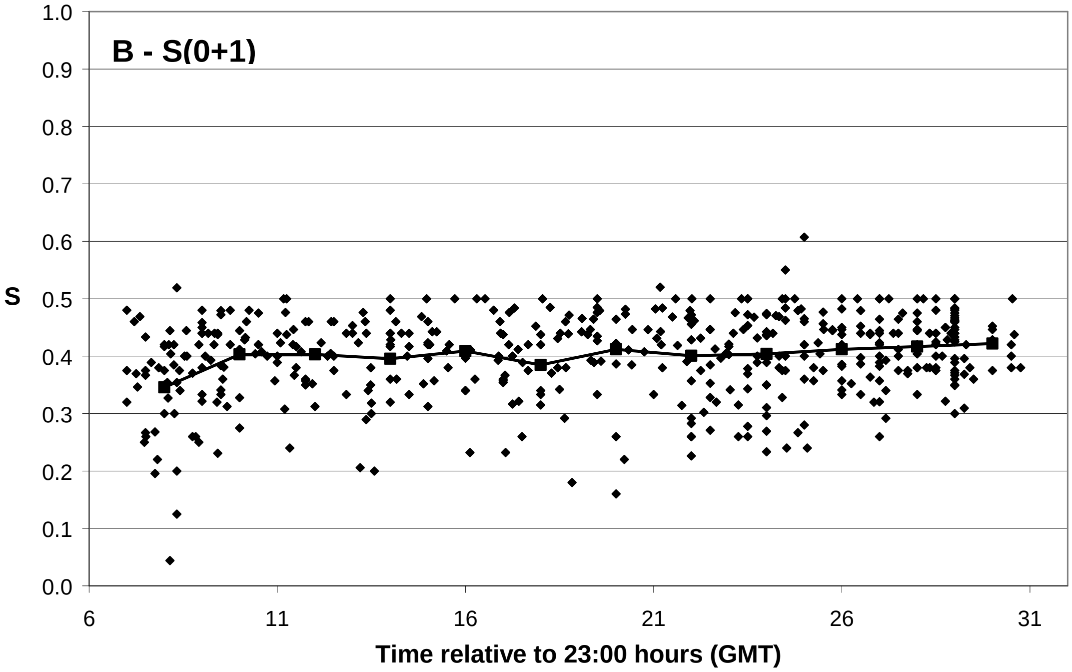


Figure 3.7: Individual spawning fraction estimates per sample by sampling time relative to the peak spawning time at 23:00 hours for the S(1).Corr (A) and S(0+1) (B) estimators, together with their mean values in 2-hour time classes (line with black rectangles).

The survey-based estimates of S (Table 8) revealed low standard errors and low variability in the spawning fraction between surveys and years, around an overall mean of 0.39 (CV=6%) for S(1).Corr and of 0.40 (CV=4.5%) for S(0+1). The estimates did not significantly differ between years. The Mean relative bias of the estimates by surveys, for the random type of negative effect of the oversampling of day_0, were minimal for S(0+1), intermediate for S(1) and maximal for S(0) (Table 8). Application of these mean relative biases to correct the original estimates, produced revisions closer to the S(1).Corr estimates in 9 out of 14 surveys for the S(0) and S(0+1) estimators and in 7 cases for the S(1) estimator.

Table 3.8: Spawning fraction for the four estimators by survey with their standard errors (S.E.), mean relative bias (*RBias*) and bias corrected estimates. Bottom lines refer to the pooled estimates (all samples from all surveys together) and the inter-annual (inter-survey) means, with their standard deviations (S.D.) and coefficients of variation (CV).

Year/Survey	**S(0)**	**S.E.**	**RBias(S(0))**	**S(0).BiasCorr**	**S.E.**	**S(1)**	**S.E.**	**RBias(S(1))**	**S(1).BiasCorr**	**S.E.**	**S(1).Corr**	**S.E.**	**S(0+1)**	**S.E.**	**RBias(S(0+1))**	**S(0+1).BiasCorr**	**S.E.**
1990 May	**0.393**	0.033	0.1327	0.347	0.029	**0.349**	0.021	-0.0853	0.382	0.023	**0.367**	0.014	**0.371**	0.015	0.0237	0.362	0.015
1990 June	**0.405**	0.042	0.1229	0.361	0.038	**0.316**	0.026	-0.0790	0.343	0.028	**0.332**	0.015	**0.360**	0.014	0.0219	0.353	0.013
1991	**0.443**	0.031	0.1161	0.397	0.028	**0.376**	0.023	-0.0747	0.407	0.025	**0.400**	0.009	**0.410**	0.009	0.0207	0.401	0.009
1992	**0.374**	0.038	0.0584	0.353	0.036	**0.455**	0.034	-0.0376	0.473	0.036	**0.413**	0.011	**0.414**	0.010	0.0104	0.410	0.010
1994	**0.420**	0.041	0.0934	0.384	0.038	**0.407**	0.037	-0.0601	0.433	0.040	**0.404**	0.014	**0.413**	0.012	0.0167	0.406	0.012
1995	**0.429**	0.031	0.0847	0.395	0.029	**0.391**	0.025	-0.0545	0.413	0.026	**0.403**	0.011	**0.410**	0.010	0.0151	0.404	0.010
1997	**0.395**	0.019	0.0394	0.380	0.019	**0.389**	0.023	-0.0253	0.399	0.023	**0.384**	0.013	**0.392**	0.011	0.0070	0.390	0.011
1998	**0.418**	0.027	0.0751	0.389	0.025	**0.388**	0.025	-0.0483	0.407	0.026	**0.386**	0.013	**0.403**	0.009	0.0134	0.397	0.009
2001	**0.375**	0.035	0.0663	0.352	0.033	**0.441**	0.034	-0.0426	0.460	0.035	**0.406**	0.018	**0.408**	0.014	0.0118	0.403	0.014
2002	**0.363**	0.034	0.0481	0.346	0.032	**0.443**	0.027	-0.0309	0.457	0.028	**0.409**	0.012	**0.403**	0.012	0.0086	0.400	0.012
2003	**0.501**	0.035	0.0798	0.464	0.033	**0.350**	0.029	-0.0513	0.369	0.031	**0.403**	0.015	**0.426**	0.011	0.0143	0.420	0.010
2004	**0.448**	0.035	0.0823	0.414	0.033	**0.320**	0.025	-0.0529	0.338	0.026	**0.368**	0.016	**0.384**	0.013	0.0147	0.378	0.013
2005	**0.379**	0.046	0.0393	0.365	0.044	**0.449**	0.036	-0.0253	0.461	0.037	**0.421**	0.013	**0.414**	0.015	0.0070	0.411	0.015
2007	**0.471**	0.047	0.1008	0.428	0.043	**0.349**	0.037	-0.0648	0.373	0.039	**0.381**	0.017	**0.410**	0.012	0.0180	0.403	0.011
Pooled estimates	**0.417**	0.009	0.0822	0.385	0.009	**0.384**	0.008	-0.053	0.406	0.008	**0.390**	0.004	**0.401**	0.003	0.0147	0.395	0.003
Pool S.D.	**0.209**			0.187		**0.172**			0.179		**0.085**		**0.072**			0.071	
Pool C.V.	**50.1%**			48.5%		**44.7%**			44.2%		**21.9%**		**18.0%**			17.9%	
Interannual Mean	**0.415**	0.035	0.081	0.384	0.033	**0.387**	0.029	-0.052	0.408	0.030	**0.391**	0.014	**0.401**	0.012	0.015	0.396	0.012
Interannual S.D.	**0.040**			0.034		**0.047**			0.044		**0.024**		**0.018**			0.019	
Interannual C.V.	**9.7%**			8.9%		**12.2%**			10.8%		**6.0%**		**4.5%**			4.8%	

Concerning the estimations based upon spawning frequency of individual females, the probability of a female spawning every day, P(*f* = 1), was almost negligible, about 0.7% (34 out of 4882 females). The value of P(*f* = 2 | day_2) was 60.49% (for 2779 females), and thus P(*f* = 2), the probability of a female spawning every other day, was 60.07%. Consequently the complementary probability of past-spawning females spawning every third day was 39.24%. Hence, the overall estimation of S(*f*), based upon the inter-spawning intervals of individual females, was 0.438 with a very low coefficient of variation (CV) of about 0.4% when using a binomial approach, which rose to about 0.8% when variation among samples in the conditional probabilities were included. The small CVs are due to the large number of females (7661) and samples used in the estimation.

Sea surface temperature has a weak but significant relationship with the S(0) and S(1) estimators with opposite signs (Table 9). But, as no significant correlation was observed with the S(1).Corr or with S(0+1), the role of temperature on S was discarded. Sex ratio was negatively related to the S(0), S(1).Corr and S(0+1), whilst mean weight of females was positively related to S(1).Corr and S(0+1).

Table 3.9: Correlation matrix of the four estimators of spawning fraction with selected covariates of the samples: mean weight of mature females (Wfem), observed sex ratio in mass R(obs), and the sea surface temperature at the station (SST). N refers to the available number of samples and bold values correspond with statistically significant correlation values (at alpha 0.05).

	Wfem	**R(obs)**	**SST**
N	493	493	242
S(0)	0.066	**-0.290**	**0.164**
S(1)	**0.180**	**0.123**	**-0.171**
S(1).Corr	**0.333**	**-0.180**	-0.081
S(0+1)	**0.307**	**-0.271**	0.032

The fitting of the spawning fraction (with logistic regressions) as a function of the survey, sampling gear, sampling time and mean weight of females showed that sampling gear did not affect any of the S estimators (Table 10). Estimates of S(0) changed significantly with survey and sampling time; the latter was consistent with the oversampling phenomena described above. On the other hand, .the estimate of

S(1).Corr. depended on the weight of females and on sampling time, but not on the survey, and finally S(0+1) only depended on the weight of females. Sampling time was not significant in the full model for S(0+1) and the subsequent removal of survey and sampling gear from the analysis of S(0+1) confirmed its non-significant role (final P=0.0726). For the S(1).Corr estimator, the marginal significance of sampling time arose from the 06:00-08:00 hours class (see the means in Figure 3.7a). When this time class was removed from the model, sampling time became non-significant for S(1).Corr. The mean weight of females had a small but significantly positive effect on S(1).Corr and S(0+1) estimates (Figure 3.8). Nevertheless, most of the variability in S remained unexplained, as the logistic models fitted above only explained 7.5% of the original deviance of the data.

Table 3.10: Probability of Chi-square values of several covariates being due to random effects (Likelihood ratio test) while fitting logistic regressions to the S(0), S(1).Corr and S(0+1) estimators. Covariates are survey, sampling gear (Gear), sampling time (in steps of 2 hours), and the mean weight of females (Wfem). The total number of samples was 493. Df means degrees of freedom used in the fitting.

Covariates	**Df**	**S(0)**	**S(1).Corr**	**S(0+1)**
Wfem	1	0.5352	0.0000	0.0004
Gear	1	0.1142	0.6876	0.6364
Survey	13	0.0000	0.1422	0.2446
Sampling time	11	0.0000	0.0284	0.0945

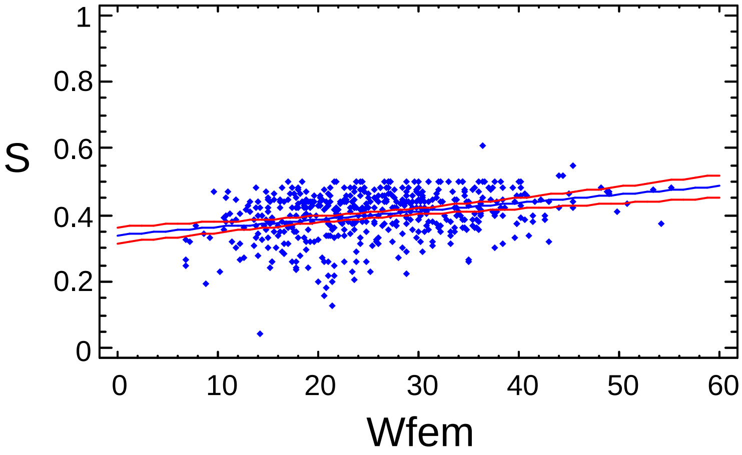


Figure 3.8: Relationship between spawning fraction (from the S(0+1) estimator) and the mean weight of females (Wfem, in grams). The equation of the fitted model is S = exp(eta)/(1+exp(eta)), where eta = - 0.6621+ 0.0101*Wfem.

3.4 Discussion

The anchovy in the Bay of Biscay display a fast spawning dynamic, with about 99% of the population being mature -spawning capable- in May-June (Motos 1996), and with most of the anchovies spawning every other day and the remainder probably every third day. This corresponds with a spawning fraction around 0.4, which is about 60% higher than the previous average estimates reported for this population (ICES 2006, 2007; Motos 1996; Somarakis *et al.* 2004). The current revision of the spawning fraction was made possible due to the biological knowledge behind the definition of the ageing (stage-spawning class) matrix system. Understanding the dynamics of final oocyte maturation and of the degeneration of POFs in time permitted reliable and complete identification of the two retained daily spawning classes for the estimation of spawning fraction, day_0 and day_1. The dynamics of oocyte maturation permits complete identification of the day_0 cohort, although not of day_-1. The degeneration of POFs in time described by Alday *et al.* (2008), permits the allocation of the POF stages to spawning cohorts quite accurately for the main range of daytime intervals. In particular, identification of day_1 was far more certain than day_2 or day_3, as the older the POFs are, the higher the uncertainty in their actual age (Alday *et al.* 2008). The latter derives from the overlapping periods of POF stages V to VII, in which the allocation of females to a spawning cohort is not unique and some assumptions have to be made. As a result of this, identification of day_1 has some little unsolved uncertainties at the end of the day when allocation of POF stages to this spawning class is not unique. Nevertheless, Appendix 2 shows a sensitivity analysis to potential alternative settings of the ageing matrix, accounting for uncertainties in the actual allocation (%) to day_1 in the overlapping periods of POF stages; it is confirmed that the present spawning fraction estimates were robust to these uncertainties, as several plausible alternative settings resulted consistently in values of S(1) between 0.38 and 0.40.

The validity of the present S estimates, and hence of the ageing matrix system, was in addition supported by the consistency of the relative proportions of day_0 and day_1 spawning classes (around 0.4) throughout most of the day (with the exception of the hours around peak spawning time, Figure 3.5). This indicated roughly similar estimates of the spawning fractions S(0) and S(1), as should be expected. Discrepancies with the proportion of the day_2 spawning class for parts of the day (in Figure 3.5), which indicated a higher S for the first half of the day and a lower S during the evening and night, were attributed to the potential confusion with the day_3 spawning class, as reported for other species (Cubillos *et al.* 2007), and to the recruitment to the next spawning, respectively. The former feature would be related to the ageing uncertainties throughout the long overlapping periods of the POF stages defining this class, whilst the latter was evident due to the fact that most of the females showing POFs of the day_2 spawning class had oocytes undergoing final maturation which corresponded with the day_0 pre-spawning class (resuming the spawning in that night). Additional support for the current S estimates comes from the estimation of spawning fraction based on the individual spawning frequency of females which resulted in a value (S*(f)* = 0.438) very close to those produced by the standard methods based upon the proportions of day_0 and day_1 spawning classes. The slightly higher S value produced from the individual spawning frequency of females compared to the standard estimates can be understood by some violation of the assumptions that ageing of day 2 was accurate and that all females will spawn again in at least every three days. If some of the identified day_2 females (in POF stage VII) were in fact day_3, or if some of the day_3 (with no POFs) females in fact belonged to the day_4, this would imply some reduction of the S(*f*) estimate, leading to a closer result.

One of the reasons for the current S estimates of supposing an upward correction over the previous estimates is due to the revision of the degeneration rates of POFs for this anchovy, being somewhat faster than those for the northern anchovy (Alday *et al.* 2008). However, this difference was also originated partly from undue or subjective errors in the direct allocation of a large variety of POFs to daily spawning classes, as practiced in the past. The current matrix system for ageing ovaries stages based on their oocytes and POFs is particularly well designed for cases when the collection of adult samples from several sources provides samples from throughout the day. This sampling strategy increases the variety of ovary stages, based on oocyte development or POF degeneration, that can be allocated to the same daily spawning classes and requires a good deal of expertise if staging and ageing are made simultaneously. Separating ovary staging from the ageing makes the process more objective, as staging only describes the histological features of the ovary whilst ageing only assigns ovary stages to spawning classes by an automated procedure based upon biological knowledge. The process then becomes more repeatable for subsequent cross-checking of ovary (oocyte and POF) stages and for any potential future revision of the stage-spawning class matrices according to new ageing criteria. Hence, we particularly recommend this type of approach for cases when adult samples are obtained throughout the day. In addition, given the important role that the validation of the degeneration of anchovy POFs in temperate waters has played in this review, we recommend: a) that POF degeneration is validated for the range of temperatures each stock inhabits; and b) that the results of Alday *et al.* (2008) for the same anchovy species spawning in a similar range of temperatures are used when validation is not feasible.

In comparison to other species, the present spawning fraction estimates were, in general, higher than those estimated for other Engraulidae stocks in temperate waters (with SST between 12-20 ºC), which rarely exceeded 33% (Alheit *et al.* 1984; Armstrong *et al.* 1988; Cubillos *et al.* 2007; Dimmlich *et al.* 2009; Hampton 1996; Hunter and Macewicz 1985; Pájaro *et al.* 2009; Somarakis *et al.* 2004). Nevertheless, in areas with warmer waters (ranging between 15-25ºC), the spawning fraction may be as high as 50%, or even higher (Clarke 1987; Funamoto and Aoki 2002; Funamoto et al. 2004; Luo and Musick 1991; Rogers *et al.* 2003; Takasuka *et al.* 2005; Wright 1992). Therefore, the high spawning frequencies found for the Bay of Biscay anchovy are compatible with the biology of Engraulidae and Clupeidae. Many of these species can be considered as “income breeders” which obtain the required energy to sustain high spawning frequencies from feeding prior to and during the spawning period (Clarke 1987; Ganias 2009; Luo and Musick 1991; Maack and George 1999; Somarakis 2005; Somarakis *et al.* 2000; Wang and Houde 1994).

This anchovy displays spawning behavior that leads to the oversampling of the most active (day_0) females; but the amount of oversampling is low, adding on average about 0.07 to the normal S, 5 hours either side of the peak spawning time (23:00 hours) (Figure 3.6). The start of the oversampling of day_0, around 18:00, coincides with the period when the great majority of day_0s have completed hydration (ovary stage 8) and finishes around 04:00 hours when the majority of POF degeneration reaches stage II. This oversampling is less intense than previously perceived by Santiago and Sanz (1992) and lower than that reported for most of the Engraulidae (see review in Ganias 2008, which suggest an incidence of day_0 of 3 times day_1 for Engraulidae and Cupleidae). However it is consistent with some sardine stocks for which oversampling is negligible, or does not exist, such as the Iberian Sardine (García *et al.* 1992), California sardine (Lo *et al.* 2005; Macewicz *et al.* 1996) and Australian sardine (Ward *et al.* 2001).

In the case of the Bay of Biscay anchovy, as with many other species, oversampling of day_0 is a result of the ephemeral spawning aggregation of fish, by which spawning takes place in differentiated spawning schools, with the most actively spawning females accompanied by a larger group of males (Ganias 2008). This aggregation is reflected in the skewed sex ratios of these schools, as the higher the fraction of actively spawning females, the lower the sex ratio in the sample ($r = -0.29$, $P<0.001$,Table 3.9), and in the clumped distribution of day_0 females, i.e. day_0 segregates from the rest of the spawning classes. However, contrary to other studies (Alheit *et al.* 1984; Ganias 2008), in the case of the Bay of Biscay anchovy, clumped distributions were also shown by all spawning classes all day round (Table 7). This means that all of the schools sampled were structured according to the spawning condition of the anchovies, so random distributions of the remaining (other than day_0) spawning classes were not seen within these schools. This is probably a feature characteristic of populations with high spawning fraction, as this makes all (pre and post) spawning classes highly complementary; This leads to the strong negative correlations between daily spawning classes detected for all classes in our study (Table 3.6). The exception between day_0 (S(0) and %day_2+.Mixed was certainly due to the partial overlapping of these two classes (as many of the day_2+.Mixed were also day_0).

The clumped distribution of the spawning classes explains the wide variances of the S estimators based on single daily spawning classes (S(0) and S(1)). In addition, the parallel strong negative correlation resulting between day_0 and day_1 explains why the joint distribution of these two classes approached a uniform distribution (according to the standardized Morisita index of dispersion) as each one compensates for the deviations from a random distribution of the other. Similarly this also explains the strong reduction of variance resulting for the joint S(0+1) estimator. In fact, the estimate of variance of S(0+1) (0.052) matched perfectly with the one expected for an estimator of S based on a combination of two daily spawning classes (Ganias *et al.* 2003) in which the negative covariance of the two daily classes is to be subtracted twice from the addition of the individual variances (resulting in a variance of 0.053). The low variance of S(1).Corr is harder to understand as it is based on a single spawning class. Therefore, this low variance must be due to the mathematical correction of the denominator (substitution of day_0 by day_1 females to account for the oversampling of day_0). Nevertheless S(0+1) was a slightly more precise estimator of spawning fraction than S(1).Corr.

The negative correlations between S(0) and S(1) and between S(0) and %day_2+.Pure peaked and became relatively similar around the spawning time. This implies that oversampling of day_0 was made at the expense of all of the remaining daily classes approximately in proportion to their abundances. This corresponds with the random type of oversampling of day_0, based on all of the other spawning classes, for which the unbiased estimator is only S(1).Corr. However, in this case S(0+1) was, in practical terms, almost unbiased, with very low relative bias estimates by surveys. For instance, the relative bias of S(0+1) for the whole set of historical samples was 1.5% of the true S (Table 3.8); this means a very small absolute bias of just 0.006. Thus, from the pooled estimate of S(0+1) of 0.401, the unbiased estimate of S was 0.395, a value very close to that given by S(1).Corr, of 0.390, which implies a negligible difference considering the variability of the estimates. Such a small bias was due, as predicted by Figure 3.4, to the moderate oversampling of day_0 at peak spawning time and to the high value of S. In addition oversampling (and hence bias) was limited to 10 out of the 24 hours of the day. The high S necessarily implied that the oversampling of day_0 spawners was mostly reflected (in absolute terms) in the undersampling of day_1 spawners as they were the only two non-overlapping daily classes in the population; this largely compensated for their relative biases around spawning time and made the estimator S(0+1) almost entirely unbiased. Another way of looking at this is that the closer S is to 0.5 the closer S(0+1) is to becoming the only unbiased estimator in case of oversampling, as for S=0.5 the only potential type of oversampling is that of repulsion.

Therefore, we conclude that for species which have a high spawning fraction (usually above 0.33, as many small pelagic species –including scombroids - in temperate and tropical areas), little oversampling of day_0 , and/or a strong negative correlation between day_0 and day_1, S(0+1) can be considered as a robust and simple estimator of S. This produced the most precise estimator of S with a negligible bias which can ultimately be corrected according to the formulae presented before. Note that in addition the S(0+1) is robust to overlapping of day_0 and day_1 classes, whenever those females are included in both spawning classes. On the other hand, S(1).Corr was unbiased, given the random type of oversampling, but a bit less precise and neglected a great part of the information from the samples (day_0 class) in its estimate, whilst S(0+1) makes direct use of all available information without any correction. Including day_0 (advanced maturity stages and recent POFs) and day_1 in the spawning fraction estimates is a practice not often followed, although several examples are found in the literature (Dickerson *et al.* 1992; Lowerre-Barbieri *et al.* 1996; Macewicz *et al.* 1996; McBride *et al.* 2002; Roumillat and Brouwer 2004; Schaefer 1996; Taylor *et al.* 1998; Yamada *et al.* 1998; Ward *et al.* 2001). Given the benefits of the joint use of these two daily spawning classes for species of high spawning fractions, S(0+1) (corrected for bias if necessary) may be the preferred estimator to be used in this type of circumstance.

The estimates of S produced by S(1).Corr and S(0+1) estimators were independent of sampling gear and of sampling time, except for the 06:00 hour class in the case of S(1).Corr, which showed a lower S. This exception might be related to an incorrect allocation to daily classes in the overlapping period of the POF stage V, in that hour class. Certainly, if recruitment of day 1 to stage V was more abrupt than proposed here in the overlapping 2-hour class range, the difference in S in the 06:00 hour class would be reduced.

In addition, the S estimates showed a great stability over the years with only a slight sensitivity to the mean weight of mature females. This contrasts with the results of most of the applications on other Engraulidae and Clupeidae populations where the spawning fraction was shown to vary remarkably between years and areas (Shelton *et al.* 1993; Somarakis et al. 2004; Stratoudakis *et al.* 2006), or as a function of female mean weight (Claramunt *et al.* 2007; Ganias 2008; Ganias *et al.* 2003). For example, Claramunt *et al.* (2007) showed an expected relative increase in spawning fraction for Engraulidae of about +50% for females increasing from 20 g to 30 g, while our relationship suggested a minor change of about 7.5%. This lack of sensitivity is congruent with the almost invariant S across the years, despite oscillations in the proportion of one-year old fish in the population throughout this period (ICES 2009). From an evolutionary point of view, this result can be expected for a short living species with very little survivorship at the age of 2 because in this case optimum spawning capacity should not be delayed later than one-year-old fish.

All these results suggest that during the season of the DEPM applications in the Bay of Biscay (May/early June), the spawning fraction was rather constant, supporting the idea of the “biorhythm hypothesis” postulated by Hunter and Lo (1997), which states that the spawning fraction could be a rather constant reproductive feature for mature females in similar habitat conditions. In any case, our results suggest that refinements in the ageing of POFs, either by a better comprehension of their degeneration over time or by separating the staging of POFs from their ageing process, reduces the variability of the different spawning fraction estimates between years and surveys. Finally, this study endorses other possibilities for estimating the spawning fraction, such as from a Bayesian perspective under the null hypothesis of a somewhat invariant S throughout time.

Monitoring of the spawning populations of pelagic fish stocks through the DEPM requires precise biological knowledge of the reproductive biology and dynamics of the spawning of stocks for survey design and parameter estimation (Lasker 1985, Stratoudakis *et al.* 2006). This study exemplifies how good knowledge on the timing of final oocyte maturation and degeneration of POFs can result in improved assignation of actively spawning females to pre and post spawning classes and leads ultimately to a revision of the spawning fraction estimates for DEPM applications At the same time, this study demonstrates the fast spawning dynamics of this anchovy (high S, strong aggregative pattern of daily spawning classes and oversampling of active spawners); which has helped in the understanding of statistical properties of the S estimators (variance and bias) and selection of the optimal estimators (S(1).Corr and S(0+1)). Finally the methods presented here have allowed an alternative estimation of S based upon the individual spawning frequencies of females, which can be of particular interest for species with fast spawning dynamics.

Reproduction Appendix A: The bias of the Spawning fraction (S) estimators at a sampling time

It is shown here how the bias of the different S estimators from samples taken at a given time of the day (in Table 5) will depend upon the amount of oversampling of day_0 existing at that time, the type of this oversampling and on the actual value of the spawning fraction of the population S.

Given a sample of size n taken at time t, let n'_0, n'_1 and n'_{2+} denote the observed number of individuals pertaining to day_0, day_1 and day_2+ in the sample respectively, and $\hat{n}'_0$, $\hat{n}'_1$ and $\hat{n}'_{2+}$ the expected numbers of individuals of those spawning classes which should have been taken in the sample according the expected mean oversampling of the day_0 at that time. Furthermore, let n_0, n_1 and n_{2+} denote the expected numbers of individuals of those spawning classes which should have been taken in the sample if there was no oversampling of the day_0. And finally Let ¤_t denote the average oversampling (in absolute numbers) of day_0 individuals at sampling time *t*. From this it follows that the expected number of day_0 individuals in the sample ($\hat{n}'_0$) is $n_0 + \text{¤}_t$ (with $n_0 = S \cdot n$) and the actual observation n'_0 will be a number from the binomial distribution B($(\hat{n}'_0 / n), n$). In all cases spawning classes should exclude from one another and add up to n so that $n = \sum_{i=0}^{2+} n_i = \sum_{i=0}^{2+} n'_i, \sum_{i=0}^{2+} \hat{n}'_i$, and day_0 and day_1 should be completely enumerated at the expense of overlapping with day_2+ (so day_2+ excludes those being at the same time day_0 or day_1).

The expected bias, at time *t*, of the spawning fraction estimators considered in this paper will depend upon the oversampling type:

<u>When the oversampling of day_0 has a negative random effect on all remaining daily spawning classes</u> (n_1 and n_{2+}) the undersampling of the latter classes will be

proportional to their true relative abundance. In this way, the expected sampled day_1 individuals ($\hat{n}'_1$) at time *t* will be

$$\hat{n}'_1 = n_1 - ¤_t \left(\frac{n_1}{n_1 + n_{2+}} \right) = n_1 - ¤_t \left(\frac{n_1}{n - n_0} \right) = n_1 - ¤_t \left(\frac{S}{1-S} \right)$$

That last equality follows from the definition of $S = \frac{n_0}{n} = \frac{n_1}{n}$.

For each sample estimator of S we will have the following expected estimate ($\hat{S}(*)_t$) and relative bias:

$$\hat{S}(0)_t = \frac{\hat{n}'_0}{n} = \frac{n_0 + ¤_t}{n} = S + \frac{¤_t}{n}$$

with relative bias: $$Rbias\ (\hat{S}(0)_t) = \frac{\hat{S}(0)_t}{S} - 1 = \frac{¤_t}{S \cdot n} = \frac{¤_t}{n_0} \qquad \text{Eq. (A.1)}$$

$$\hat{S}(1)_t = \frac{\hat{n}'_1}{n} = \frac{n_1 - ¤_t \left(\frac{n_1}{n_1 + n_{2+}} \right)}{n} = S - \frac{¤_t \left(\frac{S}{1-S} \right)}{n}$$

with relative bias:

$$Rbias(\hat{S}(1)_t) = \frac{\hat{S}(1)_t}{S} - 1 = -\frac{¤_t}{S \cdot n}\left(\frac{S}{1-S} \right) = -Rbias(\hat{S}(0)_t)\left(\frac{S}{1-S} \right) \qquad \text{Eq.(A.2)}$$

$$\hat{S}(1)_t.Corr = \frac{\hat{n}'_1}{n - \hat{n}'_0 + \hat{n}'_1} = \frac{n_1 - ¤_t \left(\frac{n_1}{n_1 + n_{2+}} \right)}{n - n_0 + n_1 - ¤_t - ¤_t \frac{n_1}{n_1 + n_{2+}}}$$

As *n₁* should equal *n₀* they cancel each other out and this formula is reduced to:

$$\hat{S}(1)_t.Corr = \frac{n_1 - ¤_t \left(\frac{S}{1-S} \right)}{n - ¤_t \left(1 + \frac{S}{1-S} \right)} = \frac{n_1 - ¤_t \left(\frac{S}{1-S} \right)}{n - ¤_t \left(\frac{1}{1-S} \right)} = S$$

so $\hat{S}(1).Corr$ is unbiased at any time, as expected.

And:

$$\hat{S}(0+1)_t = \frac{\hat{n}'_0 + \hat{n}'_1}{2n} = \frac{n_0 + ¤_t + n_1 - ¤_t\left(\frac{n_1}{n_1 + n_{2+}}\right)}{2n}$$

because $S = \frac{n_0 + n_1}{2n}$, it follows that:

$$\hat{S}(0+1)_t = S + \frac{¤_t\left(1 - \frac{n_1}{n_1 + n_{2+}}\right)}{2n} = S + \frac{¤_t\left(1 - \frac{S}{1-S}\right)}{2n} = S + \frac{¤_t\left(\frac{1-2S}{1-S}\right)}{2n}$$

with relative bias:

$$Rbias(\hat{S}(0+1)_t) = \frac{\hat{S}(0+1)_t}{S} - 1 = \frac{¤_t}{2S \cdot n}\,\frac{1-2S}{1-S} = Rbias(\hat{S}(0)_t)\,\frac{1-2S}{2\cdot(1-S)} \qquad \text{Eq. (A.3)}$$

<u>When the oversampling of day_0 has a negative effect only on n_1 (repulsion effect)</u> the biases shown in Table 3.5 are derived as above just by taking into account that then oversampling of day_0 individuals and the undersampling of day_1 will be of equal magnitude, but opposite signs ($+/-¤_t$), whilst $\hat{n}'_{2+} = n_{2+}$.

In this circumstance it is easy to show that S(0+1) will be unbiased, as the sampling errors of n_1 and n_0 will compensate for each other. All other estimators will be equally biased by the relative oversampling of day_0.

As the expected observed day_0 and day_1 individuals at time t $\hat{n}'_0, \hat{n}'_1$ will be $n_0 + ¤_t$ and $n_1 - ¤_t$ respectively, in this case we will have:

The relative bias of S(0) is equal to equation A.1.

Next for $S(1)_t$

$$\hat{S}(1)_t = \frac{n_1 - ¤_t}{n} = S - \frac{¤_t}{n}$$

with relative bias $Rbias\ (\hat{S}(1)_t) = -Rbias\ (\hat{S}(0)_t)$

and for

$$\hat{S}(1)_t.Corr = \frac{n_1 - ¤_t}{n - \hat{n}'_0 + \hat{n}'_1} = \frac{n_1 - ¤_t}{n} = S - \frac{¤_t}{n}$$

with relative bias $Rbias\ (\hat{S}(1)_t.Corr) = -Rbias\ (\hat{S}(0)_t)$

and finally

$$\hat{S}(0+1)_t = \frac{(n_0 + ¤_t) + (n_1 - ¤_t)}{2n} = \frac{n_0 + n_1}{2n} = S \text{ is unbiased}$$

Reproduction Appendix B: Sensitivity of the spawning fraction S(1) estimates to alternative allocations of POF stages to past spawning cohorts in their overlapping periods

As there is almost no potential confusion in the identification of the ovary maturity stages from the oocyte maturation (stages 5 to 8) and from the early degeneration of POFs (stages I to IV), day_0 is estimated with high certainty. For that reason the present sensitivity analysis is solely applied to day_1. The approach is a simplification of the estimation of S(1) that avoids the sample-based procedure by pooling all sampled females and estimating the frequency of day_1 fish in each 2-hour class. This is similar to the procedure followed for Figure 5.5 of this paper. This was easily accomplished by the product of the matrix of POF stage frequencies, defined for every 2-hour class for the whole data base and the matrix of allocation of POF stages to the day_1 cohort defined for the same hour classes (Table 3.4). The result was just a vector of the proportions of the day_1 spawning cohort by 2-hour class.

Outside the overlapping periods there was no doubt as to which daily spawning cohort a POF stage should be allocated to. For this reason, the analysis was reduced to identifying the potential alternative allocations of POFs between day_1 and day_2+ in the overlapping periods of the POF stages V, VI and VII by time class. These are presented by columns in Table A.1 (upper panel) followed in the same column (bottom panel) by the resulting vector of the percentage of day_1 by 2-hour classes. The mean value on the bottom line is the arithmetic mean of the column and reflects a value close to the one expected to arise from an even sampling all throughout the day.

The alternative options for the allocation of POFs to day_1 and day_ 2 considered for the overlapping periods were:

a) Current selection: a low percentage of day_1 fish from 06:00 to 10:00 hours and from 00:00 to 04:00 hours; the latter was probably related to undersampling resulting in a global S of about 38.5%.

b) Minimum day_1: 0% allocated to day_1 across all overlapping periods resulted in a global mean of 32.7%.

c) Maximum day_1: 100% allocated to day_1 across all overlapping periods resulted in a global mean of 46.4%.

d) Abrupt recruitment of day_1 to stage V at 06:00 hours: a transition vector in the overlapping period of 50%, 75% and 90% for this POF stage. This possibility, which resulted in a higher S estimate at the beginning of the day between 06:00 and 10:00 hours, was more consistent with the subsequent estimates in time and resulted in a global S mean of 39.1%.

e) Delayed recruitment to stage VI: fish recruited to stage VI in just three time classes from 16:00 to 22:00 hours, with a 25%, 50% and 75% dispatch rule (instead of throughout the four time classes starting at 14:00 hours). This possibility implies a small reduction of S(1) at these times of the day and a global reduction of S(1) to a mean of 38.0%.

f) Abrupt recruitment to stage VII: fish recruited to stage VII at 00:00 hours in the second night after spawning; this was plausible according to the collected field data (Figure 2 of Alday *et al.* 2008). This may have been due to many of the day_2 fish recruiting to the next spawning cohort, so the relative occurrence of day_1 fish in POF VII after the peak spawning time may have been greater than the gradual allocation selected in the paper. We propose starting with 40% at 00:00 hours for day_1 and increasing by steps of 10% per time class (upper panel Table B. 1). This alternative raised the S(1) estimates after spawning time to about 40% and the global mean to 39.6%

Finally adding the options *d* and *f* above (both positively affecting the S(1) estimate) led to a global S(1) mean of 40.2%, and joining the three alternatives led to a global S(1) mean of 39.6%.

The options *d*, *e* and *f* are plausible alternatives that the experiments of Alday *et al.* (2008 and their Figure 1) could not discard. They were originally subjected to debate among the authors, but no conclusive evidence was obtained about their preference over simpler formulations. For simplicity and parsimony, these alternatives were not adopted and, whenever in doubt, the authors selected gradual symmetrical recruitment processes throughout the overlapping periods, a position considered easy to defend and of little implications for the global S estimate (as shown here).

In summary, this sensitivity analysis showed that S(1) had to be between 32.7% and 46.4%, but the most sensible alternatives which were in dispute versus the simple gradual allocation percentages proposed in this paper gave overall estimates S(1) ranging between 38% and 40.2%; these results are very close to the proportion of day_1 cohorts in the paper (38.5%).

Table B. 1: Upper panel: potential alternatives for the allocation of POFs to day_1 for the overlapping periods of POF stages V, VI and VII by hour classes. Bottom panel: resulting percentages of day_1 by 2-hour classes. Final row: simple mean percentages by alternative configurations.

	Option -->	**a**	**b**	**c**	**d**	**e**	**f**	**d+f**	**d+e+f**
Hour Class	**POF Stage**	**Current**	**Min**	**Max**	**AbruptRec(V)**	**ShorterRec(VI)**	**AbruptRec(VII)**	**AbruptRec(V+VII)**	**Mixed**
(06-08)	V	25.0%	0.0%	100.0%	50.0%	25.0%	25.0%	50.0%	50.0%
(08-10)	V	50.0%	0.0%	100.0%	75.0%	50.0%	50.0%	75.0%	75.0%
(10-12)	V	75.0%	0.0%	100.0%	90.0%	75.0%	75.0%	90.0%	90.0%
(12-14)	V	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
(14-16)	VI	20.0%	0.0%	100.0%	20.0%	0.0%	20.0%	20.0%	0.0%
(16-18)	VI	40.0%	0.0%	100.0%	40.0%	25.0%	40.0%	40.0%	25.0%
(18-20)	VI	60.0%	0.0%	100.0%	60.0%	50.0%	60.0%	60.0%	50.0%
(20-22)	VI	80.0%	0.0%	100.0%	80.0%	75.0%	80.0%	80.0%	75.0%
(22-00)	VII	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
(00-02)	VII	16.7%	0.0%	100.0%	16.7%	16.7%	40.0%	40.0%	40.0%
(02-04)	VII	33.3%	0.0%	100.0%	33.3%	33.3%	50.0%	50.0%	50.0%
(04-06)	VII	50.0%	0.0%	100.0%	50.0%	50.0%	60.0%	60.0%	60.0%

Hour Class	**Cohort**	**Incidence**	**Incidence**	**Incidence**	**Incidence**	**Incidence**	**Incidence**	**Incidence**	**Incidence**
(06-08)	Day 1	**35.8%**	**33.0%**	**44.1%**	**38.6%**	**35.8%**	**35.8%**	**38.6%**	**38.6%**
(08-10)	Day 1	**35.6%**	**30.5%**	**40.7%**	**38.2%**	**35.6%**	**35.6%**	**38.2%**	**38.2%**
(10-12)	Day 1	**45.5%**	**35.2%**	**49.0%**	**47.6%**	**45.5%**	**45.5%**	**47.6%**	**47.6%**
(12-14)	Day 1	**41.4%**	**41.4%**	**41.4%**	**41.4%**	**41.4%**	**41.4%**	**41.4%**	**41.4%**
(14-16)	Day 1	**40.1%**	**37.7%**	**49.8%**	**40.1%**	**37.7%**	**40.1%**	**40.1%**	**37.7%**
(16-18)	Day 1	**40.8%**	**34.1%**	**50.9%**	**40.8%**	**38.3%**	**40.8%**	**40.8%**	**38.3%**
(18-20)	Day 1	**37.0%**	**30.1%**	**41.7%**	**37.0%**	**35.9%**	**37.0%**	**37.0%**	**35.9%**
(20-22)	Day 1	**34.1%**	**23.6%**	**36.7%**	**34.1%**	**33.4%**	**34.1%**	**34.1%**	**33.4%**
(22-00)	Day 1	**34.9%**	**34.9%**	**34.9%**	**34.9%**	**34.9%**	**34.9%**	**34.9%**	**34.9%**
(00-02)	Day 1	**34.2%**	**30.0%**	**55.3%**	**34.2%**	**34.2%**	**40.1%**	**40.1%**	**40.1%**
(02-04)	Day 1	**39.0%**	**31.2%**	**54.6%**	**39.0%**	**39.0%**	**42.9%**	**42.9%**	**42.9%**
(04-06)	Day 1	**43.7%**	**30.1%**	**57.4%**	**43.7%**	**43.7%**	**46.5%**	**46.5%**	**46.5%**
Average	**Day 1**	**38.5%**	**32.7%**	**46.4%**	**39.1%**	**38.0%**	**39.6%**	**40.2%**	**39.6%**

4 Demographic studies: Anchovy Population at age estimates and variances from the application of the Daily Egg Production Method

4.1 Introduction

Stock structure information allows for a better analytical assessment of the population in terms of recruitment estimates, profiles of fishing mortality at age and growth estimates among others (Gulland, 1983; Hilborn and Walters 1992). As a result age disaggregated abundance indices are usually preferred over synthetic abundance indices.

Egg surveys require adult sampling to convert egg production into biomass estimates through the estimation of the adult population relative fecundity (eggs/gram) (Gunderson 1993). The Daily Egg Production Method (DEPM; Parker 1980, Stauffer & Picquelle, 1980, Lasker, 1985) was developed and has traditionally been used to estimate the spawning biomass, but not to obtain population at age estimates (Alheit - 1993; Stratoudakis *et al.* 1996). However, the adult sampling can potentially be used to describe the spatial length and age structure of the population and ultimately to derive the spawning population at age. However, this usually is not done. Therefore making use of the adult sampling to provide information about the demography of the spawning population or to obtain directly spawning population at age estimates is still a challenge worth pursuing for egg production methods (Dickey-Collas *et al.* 2012). In addition, if immature fish are present on the spawning grounds a raise of the DEPM spawning population estimates to total population at age over the spawning grounds could be possible if maturity at age or at length estimates were estimated.

The sampling of pelagic fishes, during the DEPM is basically cluster sampling of fishes of rather similar sizes which aggregate in schools (Picquelle y Stauffer, 1985; Fréon and Misund 1999), i.e., clusters correspond to schools. Sampling design can be ambiguous as far as “judgement sampling” is typically carried out, whereby sampling tries to be proportional to the adult abundance along the track of the cruises, but in addition tries to obtain some sampling over the entire spawning area (Jessen 1978). Estimation of adult parameters has been usually based on design-based estimator of single stage cluster sampling (or double sampling for the subsampling within hauls) (Picquelle and Stauffer 1985).

There can be several reasons to not derive population at age estimates from the adult sampling of egg surveys. In some cases limited adult sampling can prevent obtaining the sufficient spatial representation of the adult population (and demographic structure). In others there are doubts about the balance of the spatial sampling and the weighting factors applicable per sample. Although the typical “judgement sampling” tries to be proportional to abundance, this is often very difficult to achieve given the strong aggregation patterns of the small pelagics while, in addition, the survey has tried to assure some sampling over the entire spawning area (Jessen 1978). When the adult sampling is unbalanced, i.e. the amount of samples in space is not proportional to the spatial distribution of biomass, weighting factors per samples proportional to abundance they represent should be employed to get unbiased estimate of the population parameters, or alternatively some strata can be defined to absorb much of the potential spatial demographic heterogeneity of the population (Picquelle & Stauffer 1985). There are other cases where an insufficient otolith sampling (or man power limitations to get it on board) or incorrect age determination procedures simply discourage from obtaining population at age estimates.

The spawning stock biomass (SSB) of the Bay of Biscay anchovy is being monitored through the DEPM since 1987 (Santiago & Sanz 1992, Somarakis et al 2004, Motos *et al.* 2005, Santos *et al. in press*; ICES 2014), with a gap in 1993. It has not always been possible to obtain an adult sampling of the population in parallel to the egg surveys; this was not achieved for instance in 1996, 1999 and 2000 and for these years egg production to biomass regression estimators have been applied (ICES 2001), under the assumption of a constant daily fecundity in the series. For the other years a major goal of the adult surveys in addition to produce biomass estimates was to infer the age composition of the population, as an extension of the DEPM. There are several features of the Bay of Biscay anchovy that makes it particularly suitable for such an essay:

1. Anchovy become mature when reaching its first year of life and those first spawners overlap with the spawning season of adults during May and June (Cort et al, 1976, Motos *et al.* 1991, Lucio & Uriarte 1990, Motos 1996). This implies that a single survey in these months serve to estimate the total spawning population or SSB and this value equals the total population since all age classes are fully mature. Hence there is no need of maturity ogive estimates to infer the total population at age estimates.
2. In May, when the egg survey has been traditionally applied, daily fecundity of the population (eggs/gram) is rather invariant in space and across ages (Motos

> & Uriarte, 1991; Motos 1996; Uriarte *et al.* 2012). This confers a strong robustness to the DEPM estimator of SSB (small sensitivity to the age composition of the population or to the goodness of the spatial judgement sampling) and at the same time makes eggs/area proportional to biomass/area. The latter feature will be used, in this study, to infer weighting factors for the individual samples within strata of the surveys.

This study describes some procedures to extend the DEPM to estimate spawning population at age and corresponding variances. It also presents some generalisation for cases of populations having ages with partial maturity (not fully mature) but for which a raise of the spawning population to total population at age over the spawning grounds is desired. By partial maturity we refer to cases where some fraction of the age classes is immature (or has not reached maturity at survey time) and hence such fraction does not contribute to the spawning. In all cases, the adult sampling, performed in parallel to the egg sampling, should be adapted to extract information on adult sizes (length) and ages (and maturity if required).

The paper is structured in four sections. First the method to expand the DEPM to obtain population at age estimates is described, along with the generalization to total population for the cases where some ages show partial maturity. This will be presented along with the estimation procedures of the new parameters required and with considerations about the estimation of weighting factors per sample. Second, an application to the Bay of Biscay anchovy in 2009 is illustrated as an example. Third, the application of the method to the series of DEPM surveys since 1990 is summarised. And finally, a general discussion on the strengths and weakness of the procedures is made.

4.2 Methods

4.2.1 Estimators of the spawning population at age from the DEPM

According to the Daily Egg Production method - DEPM - (Parker, 1980, Stauffer and Picquelle, 1980, Lasker 1985) the Spawning Stock Biomass (SSB) is estimated as:

$$SSB = \frac{Daily\_Egg\_production}{PopulationDailyFecundity\,(DF'_t\,)} = k\,\frac{A \cdot E0}{R' \cdot S' \cdot (F'/W'_f\,)} = k\,\frac{A \cdot E0 \cdot W'_f}{R' \cdot S' \cdot F'}$$

Eq. 4.1

where ***A*** refers to the spawning area, ***E0*** to the daily egg production per surface unit (in other papers called *P0*), ***R´*** is the sex ratio in mass, ***S´*** is the spawning fraction, or the fraction of mature females spawning per day. ***F´*** is the batch fecundity, or number of eggs released daily per spawning females, and $\boldsymbol{W'_f}$ refers to the mean weight of mature females. All the adult parameters have an upper comma apostrophe symbol ´ to indicate that they are calculated in terms of mass (i.e., per average tonne or kilogramme of the population) and to differentiate from those which do not have it as they will be based in numbers, i.e. per average fish in the population. The meaning of these symbols and all others appearing throughout the paper can be seen inTable 4.1.

From the DEPM formulation the spawning population in numbers (***SSP***) is estimated as the ratio between ***SSB*** and the mean weight of the mature fishes ($\boldsymbol{W_t}$) in the population:

$$SSP = \frac{SSB}{W_t} = k\,\frac{A \cdot E0 \cdot W'_f}{R' \cdot S' \cdot F' \cdot W_t}\,.$$

Eq.

4.2

The estimation of the Spawning Population in numbers at age ($\boldsymbol{SSP_a}$) is derived by multiplying ***SSP*** by the proportion in numbers of each age in the mature population ($\boldsymbol{P_a}$):

$$SSP_a = SSP \cdot P_a\,.$$

Eq. 4.3

Table 4.1: Symbols and conventions used for the formulation of the Spawning Population in numbers at age in the paper.

a	Suffix to refer a certain age of the fishes
l	Suffix to refer a certain length class of the fishes
n	number of adult samples implied in the estimation
i	Suffix to refer to an individual sample.
f , *t*	Suffixes to discriminate between females and all fishes (total of males and females) respectively
DEPM	Daily Egg Production Method.
SSB	Spawning Stock Biomass (in mass)
SSP	Spawning Stock Population (in numbers).
SSPa	Spawning Stock Population at age *a* (in numbers).
DF_t , DF'_t	Daily Fecundity of the population in terms of numbers (eggs/anchovy) and in terms of mass (eggs per gram)
DF_f , DF'_f	Daily fecundity of mature females in terms of numbers and mass respectively.
k	Conversion factor of grams to metric tonnes
A	Sampled area during the egg survey (in surface units)
E0	Daily Egg production per surface unit
R, R'	Sex ratio (fraction of the population that are mature females) in numbers and in mass.
S, S'	Spawning fraction of mature females spawning per day (in numbers and mass of the mature females respectively).
F, F'	Batch Fecundity in terms of numbers (number of eggs spawned per mature female per batch) and (number of eggs spawned per gram of mature female per batch).
W_f, W'_f	Average Weight of mature female in the population (numbers) or per unit of mass of the population (mass) (the latter is the Average Weight of individual mature female in the average tonne or kilogram of the spawning population).
W_t, W'_t	Average Weight of mature fish in the population (numbers) or per unit of mass of the population (mass).
TWt, TW't	Total mean weight of fishes (regardless of its maturity condition) in the population (numbers) or per unit of mass of the population (mass).
P_l , P'_l	Proportion of fishes at length ***l*** in the mature population, in numbers or in mass respectively.
TP_l , TP'_l	Proportion of fishes at length l in the total population (mature and inmature fishes), inhabiting the spawning areas, in numbers or in mass respectively.
TMP, TMP'	Proportion of fishes which are mature either in numbers or per unit of mass in the total population.
TMPa , TP'a	Proportion of fishes which are mature at age a, either in numbers or per unit of mass in the total population.
P_a, P'_a	Proportion of fishes at age ***a*** in the mature population, in numbers or per unit of mass respectively.
TPa, TP'a	Proportion of fishes at age a in the total population (mature and inmature fishes) inhabiting the spawning areas, in numbers and mass respectively.
m_{al} , m_l , $m_\cdot$	number of fishes aged at age ***a*** and length ***l*** , total number of fishes aged length ***l*** and Total number of fishes aged for the construction of an age length key.
q_{al}	fraction of fishes at age ***a*** in length ***l*** , as resulting from the age-length key of the mature population (MALK) or the total population (TALK)
TM_i, TM'_i	School size (or Cluster size) corresponding to the haul producing a sample ***i*** in terms of numbers or mass of fishes of the target species.
M_i, M'_i	Weighting factors for sample ***i*** proportional to abundance in numbers or in biomass of mature fishes of the target species represented by the sample.
m_i	Number of fishes analysed for the parameter estimations from sample ***i*** (size of the subsample processed from sample ***i***).
Y , y_i	Estimate of any parameter above in the population or in a sample respectively.
TPm, ***TPm’***	mature fraction in number of fishes or in mass in the population (either for females or for total males & females) at spawning grounds
TPm_a, ***TPm'_a***	maturity at age ***a***, mature fraction in number of fishes or in mass by ages in the population at spawning grounds
PPS	refers to Probability Proportional to Size sampling typical of cluster sampling

The bias and variance estimators associated to the above Equations (4.2 and 4.3) DEPM Population in numbers (*SSP*) and Population at age estimates ($\boldsymbol{SSP_a}$) are deduced by the delta method (Seber 1982) and are presented inTable 4.2.

Alternative formulations of the spawning population *SSP* are possible. The simples one is to base the DEPM with parameters in terms of numbers instead of biomass: It suffices to omit the mean weight of females from the original formulation of the DEPM (Eq.4.1) and to base all the other adult parameter in terms of numbers (i.e. per average mature female in the population) and not in mass (i.e. sex ratio in numbers, spawning fraction in numbers and batch fecundity for the average mature female in the population).

$$SSP = \frac{A \cdot E0}{R \cdot S \cdot F} \qquad \text{Eq. 4.2.b}$$

We have preferred to expand the DEPM with another parameter ($\mathbf{W_t}$) instead of this alternative because the procedure proposed for **SSP** is decided to rely and pass through the **SSB** estimate produced by the original DEPM formulation, which is the standard output of the method and, shows little sensitivity to the actual weighting factors of the samples. In this way, we estimate populations at age as an expansion of the robust SSB estimate produce by the original DEPM formulation, assuring consistency with the original formulation.

Spawning Biomass at age estimates ($\boldsymbol{SSB_a}$) can be obtained by multiplying the DEPM ***SSB*** estimate by the proportion in mass of each age in the spawning biomass ($\boldsymbol{P'_a}$):

$$SSB_a = SSB \cdot P'_a = k\,\frac{A \cdot E0 \cdot W'_f}{R' \cdot S' \cdot F'} \cdot P'_a \,. \qquad \text{Eq. 4.4.a}$$

With variance estimates parallel to those of $\boldsymbol{SSP_a}$ in Table 4.2 with the omission of the term for W_t and replacing P_a by P'_a . This is a natural extension of the DEPM as good as the one proposed here for $\boldsymbol{SSP_a}$, which requires direct estimations by samples of the $\boldsymbol{P'_{ai}}$, in parallel to the other DEPM parameters.

Alternatively, Spawning Biomass at age can also be estimated as the product of the spawning population at age estimates ($\boldsymbol{SSP_a}$) by their respective mean weight at age estimates ($\boldsymbol{W_a}$):

$$SSB_a = SSP_a \cdot W_a \qquad \text{Eq. 4.4.b}$$

Consistency of both approaches (Eq. 4a and 4b) for estimating Spawning Biomass at age estimates (***SSB$_a$***) will be evaluated.

Table 4.2: DEPM Population and Population at age estimators (in numbers) with associated bias and variance estimators (inferred by the delta method -- Seber, 1982).

Estimate	*SSP* Spawning Population (numbers)	*SSPa* (Population at age estimate)
Estimator	$SSP = \frac{SSB}{W_t} = \frac{A\,E0\,W_f}{R'\,S\,F\,W_t}$	$SSP_a = SSP \cdot P_a$
b (bias)	$SSP \cdot (\,CV(W_t\,)^2 + COVS\,(*,W_t\,)\,)$	$SSP_a \cdot (\,COVS\,(*,P_a)\,)$
Variance	$SSP^2 \cdot (\,CV(SSB\,)^2 + CV(W_t\,)^2$ $+\,2COVS\,(*,W_t)\,)$	$SSP_a{}^2 \cdot (\,CV(SSP\,)^2 + CV(\,P_a\,)^2$ $+\,2COVS\,(*,P_a)\,)$
COVS. Relative Covariance term in the above expressions	$COVS\,(*,W_t\,) =$ $-\frac{COV(E0,W_t)}{Eo\,W_t} - \frac{COV(W_f\,,W_t)}{W_f\,W_t}$ $+\frac{COV(R',W_t)}{R'\,W_t} + \frac{COV(F,W_t)}{F\,W_t} + \frac{COV(S,W_t)}{S\,W_t}$	$COVS\,(*,P_a\,) =$ $\frac{COV(E0,P_a)}{Eo\,P_a} + \frac{COV(W_f\,,P_a)}{W_f\,P_a} - \frac{COV(W_t\,,P_a)}{W_t\,P_a}$ $-\frac{COV(R',P_a)}{R\,P_a} - \frac{COV(F,P_a)}{F\,P_a} - \frac{COV(S,P_a)}{S\,P_a}$

4.2.2 Total Spawning Population at age at the spawning grounds in the presence of partial maturity

Application of the previous approaches to estimate spawning population at age in the presence of partial maturity should be straightforward. In fact, estimation of the reproductive parameters for the application of the DEPM, imply splitting the random fish samples from the fishing hauls in two fractions, one of immature and the other of mature fishes, upon which the parameters of the Daily Fecundity are obtained.

A typical difficulty in these situations with partial maturity is the precise definition of maturity for males, since usually they are not subject to histological gonad analysis. Uncertainty in the definition of the maturity of males can be overcome making the assumption of similar maturity between sexes and a 1:1 sex ratio in numbers (and obtaining the equivalent ratio in weight according to the relative mean weights by sexes across samples of both mature and immature fishes). In this way spawning biomass and population at age estimates can simply be obtained based on the biological features of mature females following the procedures described above, under the assumption of similar maturity and age composition between sexes.

When a sufficiently good adult sampling over the spawning grounds is available, that information can be used to infer the composition at age not only of the mature population but also comprising all immature fishes sampled at the spawning grounds. These estimates would equal total population at age for those ages fully mature and of those maturing age classes present at the spawning grounds and which have a similar chance of being fished during the cruise as the adult fishes have. We will call Total Population at the Spawning ground (***TSP***) to the sum of age classes ($\boldsymbol{TSP_a}$) contributing to the spawning in the surveyed area, being either fully or partly mature. If the partially mature age classes are fully present at the spawning grounds then the TSPa estimates would equal the Total population at age at the entire population level.

In order to get Total Population at age estimates over the spawning grounds, both mature and immature fishes should be sampled at random and their relative proportions per sample estimated, so that the Total Proportion of mature fishes in mass over the spawning grounds (***TPm'***) can be deduced, as well as the total mean weight of fishes (***TW_t***) and their total proportions at age (in numbers) (***TP_a***), regardless of their maturity condition. Accordingly, Total Biomass over the spawning grounds (***TSB***) can be deduced from the SSB as:

$$TSB = \frac{SSB}{TPm'} = \frac{A\,E0 \cdot W'_f}{R'\,S'\,F' \cdot TPm'} \qquad \text{Eq 4.5}$$

With variance parallel to the one detailed in Table 4.2 for SSP, but replacing the terms in ***Wt*** by ***TPm'***.

Estimates for total Population at spawning grounds (***TSP***) will be deduced dividing the former by the total mean weight of fishes (***TW_t***):

$$TSP = \frac{SSB}{TPm' \cdot TW_t} = \frac{A\,E0 \cdot W'_f}{R'\,S'\,F'\,TPm'\,TW_t} \qquad \text{Eq 4.6}$$

With variance parallel to the one detailed in Table 4.2 for SSP but replacing the terms in ***W_t*** by two terms one in ***TPm'*** and the other in ***TW_t***.

And the estimates for the Total population at age in the spawning grounds (***TSP_a***) will be:

$$TSP_a = TSP \cdot TP_a = \frac{SSB}{TPm' \cdot TW_t} \cdot TP_a = \frac{A\,E0 \cdot W'_f}{R'\,S'\,F'\,TPm'\,TW_t} \cdot TP_a \qquad \text{Eq 4.7}$$

With variance parallel to the one given in the last column of Table 4.2 for ***SSPa***, replacing ***SSP*** by ***TSP*** and ***Pa*** by ***TPa*** and replacing as before the terms in ***W_t*** by two terms one in ***TPm'*** and the other in ***TW_t***.

An alternative way of producing Total population at age at the spawning grounds is possible when the mature Population at age in numbers is already achieved via the former section procedures. In those cases estimates of the fraction in numbers of mature fishes by ages (***TPm_a***) can convert the spawning population at age into Total population at age at the spawning grounds (***TSP_a***), for selected ages, as:

$$TSP_a = \frac{SSP_a}{TPm_a} = \frac{SSP \cdot P_a}{TPm_a} \qquad \text{Eq 4.7.b}$$

With variance parallel to the one detailed in Table 4.2 for SSP, but adding a term for $\boldsymbol{TPm_a}$ being dealt as the terms in $\boldsymbol{W_t}$.

4.2.3 Parameter and variance estimates

The extension of the DEPM described in the previous subsection requires some additional parameters like the mean weight, and the proportions by age and, if necessary, the fraction of mature fishes. These will be estimated from mean of the individual sample estimates. In this section, estimation of these parameters is described based on single stage cluster sampling, or two phase cluster sampling (when working first for lengths and next the age-length keys), usually for clusters of unequal sizes (Cochran 1977; Lhor 2010).

4.2.3.1 Mean weight of mature fishes in the population W_t

This is the key parameter to convert biomass into population estimates (numbers; see Equation 4.2). The general procedure to obtain the DEPM adult parameters at the population level from cluster sampling (Picquelle and Stauffer 1985) applies similarly to the estimate of the overall mean weight:

$$W_t = \frac{\sum_{i=1}^{n} M_i w_i}{\sum_{i=1}^{n} M_i} \quad , \qquad \text{Eq. 4.8}$$

where w_i is the overall mean weight per sample i, M_i is the weighting factor for sample i (see section 2.4) and n is the number of stations.

The variance of the overall mean weight is estimated as:

$$\hat{V}ar(W_t) = \frac{\sum_{i=1}^{n} M_i^2 (w_i - W_t)^2}{\overline{M}^2 n(n-1)} = \frac{n \sum_{i=1}^{n} M_i^2 (w_i - W_t)^2}{(n-1)\left(\sum_{i=1}^{n} M_i\right)^2} . \qquad \text{Eq. 4.9}$$

From the latter expression the within samples fraction of the variance, which arises from the sub-sampling of fishes from each fishing hauls to produce each biological sample, is omitted because of the negligible sampling fraction (i.e the finite population correction) (Cochran 1977).

In order to get unbiased estimates of $\boldsymbol{W_t}$ the weighting factors per sample $\boldsymbol{M_i}$ should be proportional to the abundance in the area represented by the sample, in terms of numbers of mature fishes (*see the following section*).

The covariance to any other parameter $\boldsymbol{Y}$ implied in the estimator of $\boldsymbol{SSP}$ and $\boldsymbol{SSP_a}$ will be equal to:

$$COV(W_t Z) = \frac{\sum_{i=1}^{n} M_{wi} M_{yi} (w_i - W_t)(y_i - Y)}{\overline{M}_w \overline{M}_y n(n-1)} \qquad \text{Eq. 4.10}$$

Where $\boldsymbol{w_i}$ and $\boldsymbol{y_i}$ are the mean values in sample $\boldsymbol{i}$ of parameters $\boldsymbol{W_t}$ and $\boldsymbol{Y}$; and $\boldsymbol{M_{wi}}$ and $\boldsymbol{M_{yi}}$ are the weighting factors for sample $\boldsymbol{i}$ corresponding to $\boldsymbol{W_t}$ and $\boldsymbol{Y}$ parameters respectively. These weighting factors will be different for the parameters estimated in terms of mass (as those used in the SSB estimator) in comparison to those estimated per individual fish in the spawning population (as the additional parameters required for the $\boldsymbol{SSP}$ and $\boldsymbol{SSP_a}$). For instance *R'* is the sex ratio of mature females in the population in terms of mass not in numbers, whereas the mean weight of fishes in the population ($\boldsymbol{W_t}$) concerns to the average individual fish in the mature population, hence if for the former parameter the weighting factor for sample $\boldsymbol{i}$ is $\boldsymbol{M'_i}$, for the latter its weighting factor $\boldsymbol{M_i}$ will be $\boldsymbol{M'_i / w_i}$.

From here it follows that the average mean weight of mature fishes in the population is the harmonic weighted average of sample mean weights, when the abundance weighting factors per sample are in terms of mass instead of numbers:

$$W_t = \frac{\sum_{i=1}^{n} M_{wi} w_i}{\sum_{i=1}^{n} M_{wi}} = \frac{\sum_{i=1}^{n} \frac{M'_i}{w_i} w_i}{\sum_{i=1}^{n} \frac{M'_i}{w_i}} = \frac{\sum_{i=1}^{n} M'_i}{\sum_{i=1}^{n} \frac{M'_i}{w_i}} \qquad \text{Eq. 4.8.b}$$

Since the harmonic mean is always smaller than the arithmetic mean, this implies that using abundance indexes in terms of mass as if they were in terms of numbers will lead to overestimates of the actual average weight of fishes in the population (and hence to underestimates of the SSP). Therefore proper weighting of adult samples is capital for the correct estimation of the parameters referred to average individual fishes.

4.2.3.2 Total mean weight of the population at the spawning grounds: TW_t

In the presence of partial maturity, the biological sampling should produce the mean weight of all fishes present in the sample (TW_{ti}) as well as for the mature fraction of fishes in the samples (W_{ti}). Estimation of the total mean weight of fishes at the spawning grounds TW_t is produced parallel to the estimation procedure described above for W_t but applying weighting factors proportional, not to the abundance of the mature population but of the total population in the area the samples represent (see section 2.4).

4.2.3.3 Proportion in numbers at age in the mature population P_a

If sufficient sampling of otoliths is obtained from every adult sample, then direct estimates of the different proportions at age can be estimated per sample P_{ai}. And, by cluster sampling, the population estimate and variance of P_a would be analogous to Equations 4.8 and 4.9 by substitution of W_t and w_i by P_a and P_{ai} respectively. Weighting factors should be the same as those selected for the estimation of the average fish weight, i.e. proportional to the abundance of the population in numbers in the area the samples represent.

For short lived species, like anchovy, with population composed of very few age classes (usually not more than 3), a representative sub-sampling of otoliths per fishing haul can be easily obtained as to assure sufficient probability of detecting any age class in case of being present. For instance with a sub sampling of 50 randomly taken otoliths per sample, an age with an occurrence of 5% will be detected in 93% of the cases. However, this sub sampling is sometimes not feasible due to practical operative reasons during the surveys or for long lived species which have far more age classes. In these cases construction of an Age Length Key (ALK) and application of it to the (overall or per sample) length distributions can be preferred.

Age-Length Keys (ALK) are commonly used for the estimation of the composition of catches at age from the length distribution of catches (Fridriksson, 1934), which correspond with a typical *double* or *two phase sampling* (Cochran 1977, Tanaka 1953). The first phase is usually a simple random sampling aiming at producing the length distribution, a variable strongly related with the age of individual fishes. The second sampling is a stratified random sample of ages within length classes that serves to estimate the proportion of ages by length, the ALK. The desired age composition is the stratified estimate produced from the second sample taking the size of the strata classes from the length composition obtained through the first phase sampling. Many of the published applications of double sampling to infer the age composition of catches are based on the assumption that length is a multinomial distributed variable being obtained through a first simple random sampling (Southward, 1976; Kimura 1977; Gavaris & Gavaris 1983; Lai, 1987; Horppila & Peltonen, 1992). However in many fishery situations, and in particular in the case of small pelagic fishes, landings and/or fishing hauls in a survey arise from schools which are clusters of fishes of rather similar sizes (lengths) and required the application of cluster sampling (Picquelle and Stauffer; 1985; Pennington and Helle 2011). This is applicable also to the cases of bottom trawl surveys (Pennington and Vølstad 1994; Aanes and Pennington 2003). Therefore the adult survey will produce at best a random sampling of clusters of fishes (each of rather similar lengths) from the population. The higher the intra-cluster correlation, the more important will be the cluster effect and hence variance estimates should incorporate the error due to intra-cluster correlation of lengths (Cochran 1977; Sen 1986; Tomlinson *et al.* 1992; Pennington and Vølstad 1994).

Here below we described the formulation we have used for the double sampling of cluster of lengths for posterior either cluster random or stratified (ALK) sampling for ages.

The estimation of the proportion by ages via ALK can be obtained either globally or on sample by sample basis: The first and most common case is that of using the length sampling available for all samples to estimate the mean length distribution of the (mature) population (P_l, relative frequency) through appropriate weighting of samples (as before weighting proportional to the abundance of the population in numbers represented by the samples) (see Tomlinson *et al.* 1992). The frequencies of ages at length from the ALK (q_{al}) multiply then the mean length distribution to infer the proportion by ages in the (mature) population. A second approach is to multiply each sample by the ALK to obtain estimates of proportions at age expected for each sample (P_{ai}), being afterwards averaged overall all samples with weighting factors proportional to population abundance in numbers represented by the sample (as before). The second estimator is the weighted average of the P_{ai} (parallel to the general equation 4.8). The equivalence of both approaches for the estimation of P_a is shown below:

$$P_a = \sum_{l=1}^{L} P_l \cdot q_{al} = \sum_{l=1}^{L} \frac{\sum_{i=1}^{n} M_i P_{li}}{\sum_{i=1}^{n} M_i} \cdot q_{al} = \frac{\sum_{i=1}^{n} M_i \cdot \sum_{l=1}^{L} \left(P_{li} \cdot q_{al} \right)}{\sum_{i=1}^{n} M_i} = \frac{\sum_{i=1}^{n} M_i P_{ai}}{\sum_{i=1}^{n} M_i}, \qquad \text{Eq. 4.11}$$

with **P_{li}** being the relative frequency of length **l** in sample **i**. In this expression, the estimation of the relative frequencies of individuals by lengths **P_l** in the population is implicitly shown to be the weighted average of the sample estimates of **P_l**, with the usual weighting proportional to numbers (M_i).

The variance estimator for the first approach (first expression of Eq 4.11) is given by:

$$\hat{Var}(Pa) = \frac{\sum_{l=1}^{L} q_{al}^{2} \sum_{i=1}^{n} M_i^{2} \left(p_{li} - P_l \right)^{2}}{\overline{M}^{2}\, n(n-1)} +$$

$$+ \frac{2 \sum_{l=1}^{L} \sum_{h=l+1}^{L} q_{al}\, q_{ah} \sum_{i=1}^{n} M_i^{2} \left(p_{li} - P_l \right)\left(p_{hi} - P_h \right)}{\overline{M}^{2}\, n(n-1)} + \sum_{l=1}^{L} \frac{P_l^{2}\, q_{al} (1 - q_{al})(1 - f_{2l})}{(m_l - 1)} \qquad \text{Eq. 4.12}$$

This estimate is slightly upwards biased and its demonstration is given in **Appendix 1**.

The three components of variance of the expression above correspond to among-samples variability in length, cluster effects or covariance of lengths within samples, and the uncertainty in the age length key respectively. The second term of the variance under the assumption of multinomial length distribution would vanish. The third term includes a finite population correction per length ($1\text{-}f_{2l}$) for the case when otoliths are obtained from the individuals producing the length distribution, however in most of the cases this correction is negligible and can be omitted.

An equivalent expression of the above one, which is more suitable for the second approach of Pa estimation (last expression of Eq. 4.11) is:

$$\hat{Var}(Pa) = \frac{\sum_{i=1}^{n} M_i^{2} \left(p_{ai} - P_a \right)^{2}}{\overline{M}^{2}\, n(n-1)} + \sum_{l=1}^{L} \frac{P_l^{2}\, q_{al} (1 - q_{al})(1 - f_{2l})}{(m_l - 1)} \qquad \text{Eq. 4.13}$$

Where P_{ai} is the estimate of P_a in sample i, not from its own sub-sample of otoliths but from the application of the ALK to its length distribution. The first component of the variance is the familiar among cluster estimation of variance of Pa for unequal cluster sizes (parallel to equation 4.9). This first component of variance equals the first two components of equation 4.12 (see **Appendix 1**). The second term equals the third term of Eq. 4.12 and accounts for the additional uncertainty due to the ALK while estimating the P_{ai}. This additional component of variance due to the ALK is estimated under the assumption of multinomial distribution of ages within lengths.

All the above parameters implied in the estimation of the population in numbers at age, $\mathbf{W_t}$, $\mathbf{P_a}$, $\mathbf{P_l}$ and their respective sample estimates, as well as the $\mathbf{q_{al}}$ estimates of the ALK, refer to the mature population.

We will compare the amount of age readings with the effective sampling in order to assess the efficiency of the actual sampling design. The effective sampling size refers to the equivalent amount of age determinations which would be required by random sampling to obtain similar variances as the ones obtained by the actual followed sampling design (Aanes and Penington, 2003; Pennington and Helle 2011).

4.2.3.4 Mean proportions at age in the total population at the spawning grounds (TP_a)

Estimation of TP_a can be made in the presence of partial maturity in complete parallelism to the estimation procedures described for P_a, whenever the TP_{ai} are obtained per samples (either from a direct collection of otoliths or by applying to the sample length distribution -- TP_{li} -- a common age length key for all type of fishes regardless of their maturity condition -- TALK). Certainly, the estimation requires weighting factors proportional not to the abundance of the mature population but to the total population in the area the samples represent (see section 4.2.4).

4.2.3.5 Mean weight and length at age

These two biological features of the mature age classes are estimated by the application of either of the two equivalent formulations below (using as example the mean weight at age):

$$W_a = \frac{\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l}{\sum_{l=1}^{L} P_l \cdot q_{al}} = \frac{\sum_{i=1}^{n} M_i \cdot P_{ai} \cdot W_{ai}}{\sum_{i=1}^{n} M_i \cdot P_{ai}} \qquad \text{Eq. 4.14}$$

Being the left side expression applicable when the $\boldsymbol{W_a}$ is to be deduced with ALK procedures while the latter is a sample based (cluster) estimate of these biological features. The second expression can be applied when biological sampling and age readings are available as to produce $\boldsymbol{W_{ai}}$ for each sample, in such cases the Variance of $\boldsymbol{W_{ai}}$ can be derived using equation 4.9, taking $\boldsymbol{M_i \cdot P_{ai}}$ as the estimate of cluster size. When the estimates are based on the use of age length key with estimates of weight at length then the variance can be estimated by applying the delta method to the first expression of equation 4.16, which is a ratio of two related estimates (see **Appendix 2** for details):

$$\hat{V}ar(Wa) = \frac{\sum_{i=1}^{n} M_i^2 \left(P_{ai} \cdot W_{ai} - P_a \cdot W_a\right)^2}{P_a^2 \cdot \overline{M}^2 n \cdot (n-1)} + \sum_{l=1}^{L} \frac{\left(P_l \cdot W_l\right)^2 q_{al}(1-q_{al})(1-f_{2l})}{P_a^2 \cdot (m_l - 1)} \qquad \text{Eq. 4.15}$$

$$+ \sum_{l=1}^{L} \frac{\left(P_l \cdot q_{al}\right)^2 \cdot Var(W_l)(1-f_{wl})}{P_a^2 \cdot m_{wl}} + W_a^{\,2} \cdot \frac{Var(P_a)}{P_a^2} - 2 \cdot W_a \cdot {}^{\circ} \cdot S_{Pa \cdot Wa} \cdot S_{Pa} / P_a^{\,2}$$

Where, the first component is the among samples estimation of the variance of the numerator for unequal cluster sizes. The second component accounts for the additional uncertainty due to the ALK and the third component accounts for the variance of the mean weights within length category. The forth term is the estimated variance of the denominator of equation 4.14, which equals to $\boldsymbol{P_a}$ (with variance estimated by equation 4.12), and the last term is the covariance between numerator and denominator respectively. Notice that $\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l$ is replaced everywhere by the product of $P_a * W_a$.

We will compare the former estimate with the following intuitive one:

$$\hat{V}ar(Wa) = \frac{\sum_{i=1}^{n} M_i^2 \cdot P_{ai}^{\,2} \left(W_{ai} - W_a\right)^2}{P_a^2 \cdot \overline{M}^2 n \cdot (n-1)} + \sum_{l=1}^{L} \frac{\left(P_l \cdot W_l\right)^2 q_{al}(1-q_{al})(1-f_{2l})}{P_a^2 \cdot (m_l - 1)} + \sum_{l=1}^{L} \frac{P_{la}^2 \cdot Var(W_l)(1-f_{wl})}{m_{wl}}$$

Eq. 4.16

4.2.3.6 Proportion in Mass at age in the mature population P'_a

Parallel procedures to the estimation of the proportions at age in numbers in the population can be applied to estimate the proportion at age in mass of the spawning biomass: If sufficient sampling of otoliths and individual weighting of fishes is obtained from every adult sample, then direct estimates of the different proportions at age in mass can be estimated per sample ($P'_{a,i}$), with population estimates from the cluster means and variance according to equations 4.8 and 4.9 by substitution of $\boldsymbol{W_t}$ and $\boldsymbol{w_i}$ by P'_a .and $P'_{a,i}$. Weighting factors should be proportional to the biomass in the area the samples represent (*M'i*) (see section 2.4).

If ages and weight are only available on a reduced selected (sub) sampling (second phase sampling --not on all individuals or samples), then the estimation is to be supported by the ALK and the length weight relationship (or simply by estimates of mean weight at length). Again the estimates can be obtained either globally or on sample by sample basis, by using any of the following equivalent expressions (for verification see Appendix 1 – A.1.c):

$$P'_a = \sum_{l=1}^{L} P'_l \cdot q_{al} = \frac{\sum_{l=1}^{L} P_l \cdot W_l \cdot q_{al}}{\sum_{l=1}^{L} P_l \cdot W_l} = \frac{\sum_{i=1}^{n} M'_i \, P'_{ai}}{\sum_{i=1}^{n} M'_i} \qquad \text{Eq. 4.17}$$

The first and second expression of Eq. 4.17 use the length sampling available for all samples to estimate the mean relative frequency distribution of the biomass by length for the (mature) population ($\boldsymbol{P'_l}$,) with the support of the ALK and the sub-sampling for weight. For the $\boldsymbol{P_l}$ the weighting of samples is proportional to the abundance of the population in numbers represented by the sample ($M_i = M'_i / W_{t,i}$). The third approach is just making use of the ALK and weight sampling to obtain proportions of biomass by ages for each sample ($\boldsymbol{P'_{ai}}$), being afterwards averaged overall all samples with weighting factors proportional to population abundance in mass represented by the sample (M'_i). The latter estimator is the weighted average of the $\boldsymbol{P'_{ai}}$ (parallel to the general equation 4.8). Notice that the denominator of the second expression is just an stratified estimate of the overall mean weight of fishes in the population (W_t).

The variance of this estimator using the ALK and the mean weights by lengths is deduced by analogy of the first expression with that of $\boldsymbol{P_a}$ estimate and of the second expression with that of $\boldsymbol{W_a}$ estimate (as a ratio of two related variables, see also **Appendix 2**), therefore we will have:

$$\hat{V}ar(P'_a) = \frac{\sum_{i=1}^{n} M'^{2}_{i} (p'_{ai} - P'_a)^2}{\overline{M}'^{2} n(n-1)} + \sum_{l=1}^{L} \frac{P'^{2}_{l} \; q_{al}(1-q_{al})(1-f_{2l})}{(m_l - 1)}$$

$$+ \sum_{l=1}^{L} \frac{(P_l \cdot q_{al})^2 \cdot Var(W_l)(1-f_{wl})}{W_t^2 \cdot m_{wl}} + P'^{\,2}_{a} \cdot \frac{Var(W_t)}{W_t^2} - 2 \cdot P'_a \cdot \frac{COV\left(\sum_{l=1}^{L} (P_{l,i} \cdot q_{al} \cdot W_l), W_{t,i} \right)}{W_t^2}$$

Eq. 4.18

Where ***p'***$\boldsymbol{p'_{ai}}$ is the estimate of $\boldsymbol{P_a}$ in sample ***i***, not from its own biological sampling but from the application of the ALK to its length distribution. The first component is the among samples estimation of the variance of the $\boldsymbol{p'_{ai}}$ estimates for unequal cluster sizes. The second component accounts for the additional uncertainty due to the ALK and the third component accounts for the variance of the mean weights within length category. The last two terms are, first, the estimated variance of the denominator of the second expression of equation 4.17, which equals to $\boldsymbol{W_t}$ with variance estimated by equation 4.9 above, and the last terms is the subtraction due to the correlation of the numerator and denominator.

4.2.3.7 Proportion of Mature fishes over the spawning grounds TPm' and Maturity at age TPm'_a

Maturity of individual fishes is usually established by the presence of early yolked oocytes or more advanced states of maturing oocytes so that the fish is capable of spawning sometime after the sampling (Brown-Peterson *et al.* 2011; Lowerre-Barbieri *et al.* 2011). These individuals may have already spawned earlier in the spawning season being occasionally evidenced from the presence of postovulatory follicles within the gonads. Since for the fecundity parameters a random subsample of mature fishes is to be obtained from each sample of the target fish species collected during the cruise, then individual estimates of the proportion of mature fishes per unit of mass for each sample will be available (TPm'_i). Consequently, an estimate of the average maturity per unit of mass of the stock at the spawning grounds is to be calculated as the weighted average of the sample estimates of maturity, making use of equations 4.8 and 9 for the mean and variances respectively. Weighting factors per sample will be proportional to the total biomass represented by the fishing hauls (of both mature and immature fishes- TM'_i)[1].

Selection of the subsample of the mature fishes of the target species is on board established macroscopically either *a visu* using a mature key or making use of a quantitative indicator such as the GSI (duly calibrated) (Somarakis *et al.* 2004). Subsequently in the laboratory the histological examination of individuals should serve to verify the maturity of each individual.

The proportion of mature fishes by age classes (in numbers, TPm_a) can be obtained by the quotient between the mature fishes at age ***a*** and the total number of fishes sampled of that age, under the assumption of a binomial distribution. That information will be available if for the fishes selected for age determination, their maturity is also obtained.

In addition to the simple former approach, when the degree of maturity of fishes is closely related to the size of fishes, even within an age class, then stratified to length

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[1] Notice that if no independent estimation of the total abundance represented by the each sample TM'_i is available (as for instance from an acoustic surveys) then those weighting factors should be deduced from the indicators of the abundance of the mature population represented by each sample (as used for the adult reproductive parameters M'_i) by dividing such weighting factor by the proportion of mature fishes in the sample (TPm'_i). In these circumstances:

$$TPm' = \frac{\sum_{i=1}^{n} TM'_i \, TPm'_i}{\sum_{i=1}^{n} TM'_i} = \frac{\sum_{i=1}^{n} \frac{M'_i}{TPm'_i} TPm'_i}{\sum_{i=1}^{n} \frac{M'_i}{TPm'_i}} = \frac{\sum_{i=1}^{n} M'_i}{\sum_{i=1}^{n} \frac{M'_i}{TPm'_i}}$$

Hence the proportion of mature fishes in the population becomes the harmonic weighted average of sample proportions of maturity weighted to the biomass of mature fishes represented by each sample. If in any sample the maturity would be 0 then the average maturity would become 0 as the denominator goes to infinity; this exemplifies that an egg survey cannot estimate the total population of both mature and immature fishes unless either immature fishes are well mixed with the adult spawning fishes or an independent estimate of the total biomass abundance represented by each haul is estimated by indicators not dependent on egg abundances (as for instance acoustics).

estimates can be more precise for TPm_a estimation. Those estimates will be often produced via mature length keys (MLK) and age length keys (ALK), not only due the former biological reason but also because sampling estimates of that parameter would be of too high cost obtaining or simply because maturity at length was generated on a different set of fishes than those for age determination, etc. These estimates are:

$$TPm_a = \frac{\sum_{l=1}^{L} TP_l \cdot TPm_{a,l}}{\sum_{l=1}^{L} TP_l \cdot Tq_{a,l}} \qquad \text{Eq 4.19}$$

With $TPm_{a,l}$ being the proportion of mature fishes of age *a* in legth ***l*** (over the total number of fishes at length ***l*** -all ages- for which maturity and age were assessed), and $Tq_{a,l}$ is the proportion of age *a* at length ***l*** which correspond with the ALK constructed over the total, mature and immature, fishes. This expression is a division of two related variables for which the variance can be obtained by the delta method (see the complete expression in Appendix 3).

In case of independency of maturity at length from the age of fishes then the former expression can be estimated by

$$TPm_a = \frac{\sum_{l=1}^{L} TP_l \cdot Tq_{a,l} \cdot TPm_l}{\sum_{l=1}^{L} TP_l \cdot Tq_{a,l}} \qquad \text{Eq 4.20}$$

For which TPm_l is the proportion of mature fishes by length. This expression is parallel to the one used for the estimation of the mean weight at age and therefore the variance will be similar to equation 4.15 by substituting the terms in P_l by TP_l, q_{al} by Tq_{al} and W_l by TPm_l (and applying a binomial variance to the TPm_l parameter or the predicted variance from a fitting of a logistic model for the maturity by length). In Appendix 3 a full justification of the variances of population estimates total maturity in mass and of maturity at age (in numbers) is provided.

4.2.4 Sample Weighting factors and sampling design of EPM surveys

In EPM surveys, the variables of interest (such as reproductive or demographic parameters) are typically spatially structured and whenever possible they should be addressed by spatial stratification. However, in some cases stratification may not be possible or some spatial heterogeneity of the variables across samples is still noticeable within a given stratum, in those cases proper weighting of samples should be considered in order to obtain unbiased estimates (Cochran 1977; Lhor 2010; Aanes and Voltad 2015). For a given strata, two approaches are typically followed for the allocation of weighting factors per samples: in the first case, fishing is assumed to produce random sampling of fish schools (the primary cluster units) of different sizes upon which to make inferences applying equations 4.8 and 4.9 for the variables of interest. Cluster size (*M'*) can be taken either from the catch obtained of the target species in a standard fishing hauls or from an acoustic estimate of the schools size. However in many pelagic surveys it is rather difficult to design a "standard" pelagic fishing haul as to produce catches proportional to school sizes and for EPM surveys acoustic devices are not always available as to provide information about the size of the fished schools. In those circumstances, or alternatively, many authors assume that differences in school sizes can be disregarded (as not informative enough) and set cluster sizes equal to 1 for all samples, which lead to produce population estimates from the simple arithmetic mean of the measured variables by samples. In the second case, survey fishing should aim at producing sampling proportional to fish abundance (sampling probability proportional to size –PPS-, to *M'i*) for which simple mean of sample estimates are unbiased (Cochran 1977[2]). However such unweighted mean estimates can be biased as far as actual cluster sizes (the sample weighting factors) may actually change (and matter) and the effective sampling does not match the presumed sampling (either random or pps) (Cochran 1977; Aanes and Pennington 2003; Cotter 2009a; Lhor 2010).

In most of EPM surveys, the selection of fishing locations is usually made under judgement sampling (Picquelle and Stauffer, 1985) and hence the statistical properties of such sampling are unclear (Jessen 1978). Under judgement sampling, location of

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[2] Cochran 1977 equations 11.39 and 11.40 in page 308.

hauls and the intensity of fishing is made according to a variety of indications on the presence of fishes, such as historical background on the spatial distribution of fishes, echoes of fish schools from acoustic devices, egg abundances in the plankton hauls, information from commercial fleet etc, while at the same time trying to provide some sampling over most of the surveyed spawning areas. In this way, judgement sampling scheme may result in mixture between PPS (with proportionality to population size or abundance) and systematic sampling over the surveyed area. Therefore it is most likely that sampling probabilities will change across the surveyed area as a function of available fishing time by areas (related as well with the speed of surveying) and the pattern of biomass spatial distribution. We propose to evaluate *a posteriori* the actual sampling intensity (probability) throughout the surveyed area, by discrete sub-regions, in order to estimate sample weighting factors proportional to size, leading to unbiased estimates, as follows:

In an EPM survey, egg production (E0) by sub-regions is an indicator proportional to the spawning biomass in those sub-regions (from Eq.1) provided the Daily Fecundity (DF) can be assumed constant across all sub-regions. Furthermore egg abundance is directly proportional to egg production whenever temperature and egg mortality are constant across the sub-regions (McGarvey and Kinloch 2001). Hence egg abundance divided by the DF by sub-regions (in case of suspecting it is different) will also be proportional to biomass. We use the ratio between the amount of samples (n) and any of the two latter indicators of biomass F(SSB), as best suited by sub-regions (k) (n_k/f(SSB_k)), to check the proportionality of sampling to biomass in space. If the ratios by sub-regions are approximately constant then the sampling was proportional to abundance in space and the sampling is shelf weighting (and equal cluster size *M'i* can be allocated to them for mean and variances estimates). If the ratios differ then we infer that the actual probability of selection of samples from the different sub-regions was different and we adopt those ratios as estimators of the relative probability of inclusion of samples (Ψk) from the different sub-regions. Probablity Ψk represents the capacity of obtaining a sample per unit of mass in sub-region *k*. For the case of cluster sampling with unequal probabilities, the Hansen–Hurwitz (1943) estimator produces unbiased estimate for sampling with replacement (Cochran 1977; Lohr 2010). Though repeated sampling of the same cluster is impossible in our surveys such estimator would still be applicable here because for very large populations, as fish resources, the likelihood of repeating any sample would always be negligible. For clusters of equal sizes such estimator implies weighting every sample by reciprocal of the inclusion probability $1/\Psi i$. This means that individual weighting factors of samples by sub-regions should be proportional to $f(SSB_k)/n_k$, i.e, to the fraction of the total spawning biomass represented by a sample unit of that sub-region. By adopting $f(SSB_k)/n_k$ as the value of cluster size in mass ($M'i$) by sample, equations 4.8 and 4.9 are equal to the Hansen–Hurwitz estimators for the mean and variance per subunit element (either per kilogram or per fish) (see **Appendix 4** for the equivalence with equations 6.10 and 6.12 in Lohr 2010). This leads to the very intuitive result of population parameters resulting from the mean of sample estimates weighted to the proportion of biomass each sample represents. The estimator is almost unbiased provided the proportionality of the indicator to biomass is precise (Cochran 1977).

In summary, in the absence of a reliable cluster size indicator and for a given surveyed area for which stratification is not possible, but where some structuring of the variables of interest is still suspected or noticeable, we will check if sampling in space can be assumed pps. For *k* sub-regions of rather apparent homogenous values of the concerned variables for which the survey produced at least 1 or more samples, we will check if the amount of fishing hauls is proportional to the proxy of spawning biomass by sub-regions, so that all the following ratios are approximately equal:

$$\frac{f(SSB_1)}{n_1};\frac{f(SSB_h)}{n_h};\frac{f(SSB_k)}{n_k} \qquad \text{Eq. 4.21}$$

If they were similar then pps could be assumed. If they were not similar we will adopt those ratios as estimators of relative cluster size of every sample by sub-regions ($M'_{i,h} = M'_h = f(SSB_h)/n_h$) i.e. a value proportional to the biomass each sample represents). Thus the sum of sampled cluster sizes by sub-regions will be proportional to their respective Biomasses:

$$\frac{f(SSB_1)}{\sum_{i=1}^{n1} M'_{i,1}} = ... = \frac{f(SSB_h)}{\sum_{i=1}^{nh} M'_{i,h}} = ... = \frac{f(SSB_k)}{\sum_{i=1}^{nk} M'_{i,k}} \qquad \text{Eq. 4.22}$$

i.e, the biomass of second stage units represented by the first stage sampled units per sub-regions would be proportional to the biomass per sub-regions. This is equivalent to say that, for a PPS sampling for units of un-equal sample size, the proportion in mass of second stage units represented by the first stage sampled units per sub-regions over the total effective sampling should be similar to the proportion of biomass by regions.

$$\frac{f(SSB_h)}{\sum_{h=1}^{K} f(SSB_h)} = \frac{\sum_{i=1}^{nh} M'_{i,h}}{\sum_{h=1}^{K}\sum_{i=1}^{nh} M'_{i,h}} \qquad \text{Eq. 4.23}$$

Equation 4.23 evidences why having the relative distribution of biomass in space suffices to check for pps sampling, not relying therefore on the actual biomass levels (in absolute terms) by sub-regions. For the same reason our estimates of proportions by age do not depend on the actual absolute levels of biomass, but only on the relative distribution of biomass in space.

Certainly this is an approximation for the cases when no direct estimate of cluster size is available within a stratum but where we have an indication of biomass distribution across sub-regions in the strata. Notice in addition that we are assuming that the samples available by sub-regions represent equally (randomly) the biological characteristics of the fishes within sub-regions, so that allocation to every sample of the former cluster size ($M'_{i,h} = M'_h$) will approach the condition for unbiased estimates. Furthermore, if acoustic estimates of individual school sizes were available for the samples within sub-regions, they could be taken into account (if desired) for a relative weighting of samples within the region, so that the equations 4.22 and 4.23 will still be respected.

For the parameters directly affecting the population at age estimates the above biomass weighting factors are simply divided by the mean weight of fishes in each sample so that they become proportional to the abundance of the population in numbers in the area they represent.

$$M_{i,h} = \frac{M'_h}{w_i} \qquad \text{Eq. 4.25}$$

Weighting factors proportional, not to the biomass of the mature population but, to the total biomass (mature and immature) in the area the samples represent TM'_i, can be obtained either directly from acoustic information or, if not available, from eggs parallel to the way described above, by dividing M'_i by the proportion of mature fishes in the samples (TPm'_i). And finally the required weighting for the total population in numbers represented by each sample would be simply the TM'_i divided by the respective Tw_i .

4.2.5 Bias of the biological parameters

All previous estimates of the biological parameters are ratio estimates of the form ***R=Y/X***, which are slightly biased of the order of 1/n (being n the number of samples available for the estimation of the parameter. According to Cochran (1977) (ec. 6.39, pag. 162) the amount of bias included in those estimates equals to:

$$bias(R) = -Cov(R, X) / \overline{X} \qquad \text{Eq. 4.26}$$

For the mean of cluster estimates of parameters: we have that for $\boldsymbol{W_t}$, P_a , $\boldsymbol{TP_a}$ and P'_a the denominators are $\boldsymbol{M_i}$, $\boldsymbol{M_i}$, $\boldsymbol{TM_i}$ and $\boldsymbol{M'_i}$ respectively; For Wa and Tla the denominators are $P_a \cdot \overline{M}$. And for ***Tpm'*** is $\boldsymbol{TM'_i}$ etc. So in general the larger the correlation between the parameter and the cluster sizes the larger will be the bias. Nevertheless, the bias can be neglected if it is smaller than 0.1 times the standard error of the estimates, and its influence is still modest if it is smaller than 0.2 times the standard error (Cochran 1977). We will check the amount of bias included in our estimates.

4.3 Applications

The methods outlined above are first applied to 2009 DEPM survey on the Bay of Biscay anchovy, as an example to illustrate the estimation of weighting factors per samples and the estimation of the population and biomass by ages with their variances, along with the key biological parameters (mean weight and length). Sensitivity to the weighting factors per samples is also presented. And a comparative between estimates based on single stage cluster sampling of the proportions at age by samples and estimates based on double sampling for stratification of ages by lengths (via ALK) is also presented.

Next the application of the method to the whole time series of the DEPM since 1990 is summarised, with inclusion of a general sensitivity analysis to weighting factors per samples.

4.3.1 Application to the DEPM survey in 2009

4.3.1.1 The survey, spatial structuring and biomass estimates

Santos *et al.* (2009) implemented in May 2009 the DEPM to the Bay of Biscay anchovy (BIOMAN survey) by combining an egg sampling on board R/V Investigador, with a simultaneous collection of adult samples by pelagic trawling on board the R/V Enma Bardan (Table 4.3). Spawning occurred throughout the southeast surveyed area. Relative high concentrations of eggs appeared to the North of 45º52’N mostly between coast and the isoline of 80m depth, in region under the influence of the Garonne river (here called the Garonne region), whilst the rest of the spawning spread out to the South over the French continental shelf regions and expanded towards the west along the Spanish continental shelf break and adjacent offshore areas (Figure 4.1). No clear discontinuity between the different spawning grounds was found and a single estimate of the Total Daily Egg Production (DEP) was derived for the entire spawning area, following standard procedures (DEP = 1.5346*E12, CV = 13%) (Santos *et al.* 2009; reviewed in Santos *et al.* 2013).

From the 38 pelagic trawl hauls performed, anchovy was found in 33 but only 31 were of sufficient sampling size as to be used for the DEPM analysis (Santos *et al.* 2009). The set of adult samples covered most of the spawning areas (Figure 4.1b). For every fishing haul, parallel to the sampling for fecundity, a random sampling for age determination of about 60 otoliths was produced (Table 4.4), from which proportions at age (in numbers and in mass) and mean weight and length by age per sample were obtained.

Table 4.3: Egg and adult anchovy sampling in May 2009 for the application of the Daily Egg Production method (DEPM – BIOMAN survey) (Santos *et al.* 2009). Egg samples are made with Pairovet hauls, while adult sampling with pelagic trawling.

Parameter to estimate	Vessel	Date	Samples	Selected samples
Total egg production & Spawning area	R/V Investigador	5-25 May	409	409
Daily fecundity & Numbers at age	R/V E. Bardán	6-25 May	38	31

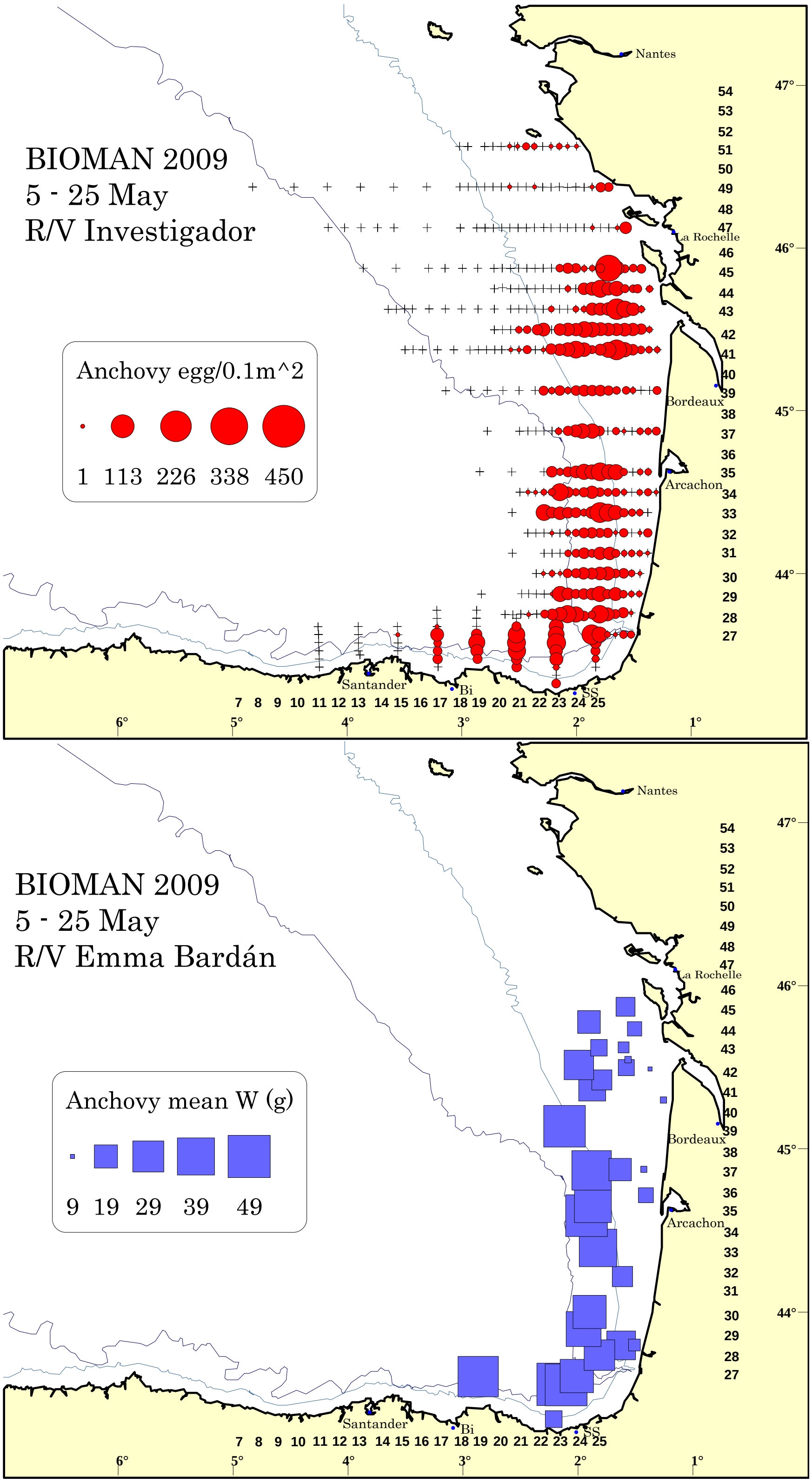


Figure 4.1: BIOMAN 1994: Left: Map of Plankton stations and anchovy egg abundances (eggs/ 0.1m2) of the DEPM survey BIOMAN09 obtained with PairoVET hauls (2 Calvet nets). Right: Anchovy adult samples used for population at age estimates with indication of their mean weight.

Table 4.4: Samples and the parameter estimates of mean weight and proportions at age, along with their estimates of proxies for sample cluster size proportional to biomass (M´i) and proportional to numbers (Mi =M´i / Wt). Lat and Long in degesimals, Samp.Size is the sample size of otoliths per sample, Pa_a is the proportion at age ***a***, Wa is the mean weight at age ***a***, Regions of random sampling with equal weighting factors. ThreshF is a threshold down weighting factor for sample size if less than 30 (=Samp.Size/30).

Code	localDate	Lat	Long	W (g)	Samp.Size	Pa_1	Pa_2	Pa_3	Pa_4	W1	W2	W3	W4	Regions	ThreshF	M'i	Mi
5001	06/05/2009	43.3443	-2.2060	13.5	53	0.943	0.038	0.019	0.000	13.1	18.0	27.2		SE	1.00	1.00	73.69
5005	08/05/2009	43.6050	-2.8629	44.5	60	0.050	0.283	0.617	0.050	23.8	43.1	46.5	48.7	SW	1.00	2.83	63.66
5007	08/05/2009	43.5575	-2.1678	48.0	63	0.032	0.333	0.571	0.063	36.4	47.0	49.3	48.0	SW	1.00	2.83	58.22
5008	08/05/2009	43.5543	-2.0980	47.4	60	0.050	0.450	0.483	0.017	26.2	48.1	49.1	44.3	SW	1.00	2.83	59.70
5009	09/05/2009	43.6087	-2.0050	32.1	60	0.500	0.117	0.367	0.017	20.2	41.9	44.5	51.3	SW	1.00	2.83	88.07
5010	09/05/2009	43.7963	-1.6190	25.2	60	0.800	0.117	0.067	0.017	22.3	33.3	41.8	44.4	SW	1.00	2.83	110.99
5011	10/05/2009	43.7367	-1.8082	27.7	60	0.750	0.117	0.117	0.017	22.1	43.7	46.4	40.9	SW	1.00	2.83	101.05
5013	10/05/2009	43.8962	-1.9460	34.4	68	0.368	0.235	0.368	0.029	22.4	38.6	43.5	35.9	SW	1.00	2.83	81.64
5014	11/05/2009	44.0013	-1.8937	31.9	70	0.300	0.329	0.271	0.100	24.9	33.9	36.1	35.0	SW	1.00	2.83	88.79
5016	11/05/2009	44.2182	-1.6080	16.2	60	0.950	0.050	0.000	0.000	15.4	30.8			SE	1.00	1.00	61.54
5017	12/05/2009	44.3937	-1.8208	39.2	57	0.053	0.316	0.544	0.088	23.1	38.1	41.2	39.8	SW	1.00	2.83	72.29
5019	13/05/2009	43.7987	-1.5035	10.8	60	0.983	0.017	0.000	0.000	10.7	16.3			SE	1.00	1.00	92.42
5020	18/05/2009	44.5913	-1.9205	45.8	46	0.000	0.239	0.674	0.087		45.3	45.8	46.8	SW	1.00	2.83	59.99
5021	19/05/2009	44.6620	-1.8649	38.2	80	0.050	0.338	0.550	0.063	25.2	37.8	39.9	36.6	SW	1.00	2.83	74.32
5022	19/05/2009	44.7165	-1.4038	12.4	60	0.867	0.117	0.017	0.000	11.5	18.6	16.2		SE	1.00	1.00	80.52
5023	19/05/2009	44.8687	-1.8762	41.8	80	0.038	0.300	0.563	0.100	25.1	41.7	42.5	43.4	SW	1.00	2.83	66.31
5024	19/05/2009	44.8737	-1.6277	18.2	60	0.883	0.100	0.017	0.000	17.6	21.1	34.9		SE	1.00	1.00	54.95
5025	20/05/2009	44.8754	-1.4220	9.4	60	0.967	0.033	0.000	0.000	9.2	14.6			SE	1.00	1.00	106.72
5026	20/05/2009	45.1392	-2.1120	46.8	60	0.000	0.250	0.683	0.067		45.5	47.2	47.2	SW	1.00	2.83	60.28
5027	21/05/2009	45.3762	-1.8714	23.2	19	0.737	0.211	0.000	0.053	19.6	30.1		46.1	NE	0.63	2.41	104.01
5028	21/05/2009	45.4230	-1.7870	15.7	86	0.767	0.233	0.000	0.000	15.0	18.1			NE	1.00	2.41	149.93
5029	22/05/2009	45.5147	-1.9867	26.5	60	0.683	0.217	0.083	0.017	21.5	33.7	45.8	41.7	NW	1.00	2.84	107.31
5030	22/05/2009	45.3000	-1.2510	9.3	65	0.862	0.123	0.015	0.000	8.4	14.7	15.5		NE	1.00	2.41	257.02
5031	22/05/2009	45.4903	-1.3680	9.3	57	0.737	0.053	0.193	0.018	7.2	12.0	15.6	18.8	NE	1.00	2.41	260.35
5032	23/05/2009	45.4985	-1.5743	13.1	60	0.833	0.133	0.033	0.000	12.6	15.9	15.2		NE	1.00	2.41	184.04
5033	23/05/2009	45.5475	-1.5592	9.3	80	0.938	0.063	0.000	0.000	9.0	13.3			NE	1.00	2.41	256.20
5034	23/05/2009	45.6223	-1.5978	10.5	60	0.800	0.183	0.017	0.000	9.7	13.6	12.4		NE	1.00	2.41	229.61
5035	24/05/2009	45.6202	-1.8125	13.4	60	0.867	0.083	0.050	0.000	12.8	15.3	19.3		NE	1.00	2.41	180.46
5036	24/05/2009	45.7780	-1.8992	18.2	70	0.643	0.286	0.043	0.029	16.0	20.4	26.1	31.2	NE	1.00	2.41	130.32
5037	24/05/2009	45.8712	-1.5810	15.1	10	0.300	0.600	0.100	0.000	13.4	15.3	19.2		NE	0.33	2.41	159.34
5038	25/05/2009	45.7362	-1.5025	12.0	63	0.302	0.603	0.079	0.016	9.3	13.0	14.4	12.4	NE	1.00	2.41	200.41

The anchovy population was clearly structured in space according to size, with small anchovies close to coast and big anchovies at deeper waters (Figure 4.1). In addition, the reproductive capacity of the coastal small anchovies was lower than that of the bigger ones at the western regions, with daily fecundity values of about 89 and 137 eggs per gram (CVs of 10% and 7%) respectively. Regarding the age composition, at the outer shelf and offshore spawning grounds 2- and 3-year-old anchovies usually predominated over young 1-year-old anchovies. Along the coastal regions the reverse happened except for the Garonne region where the 2-year-old anchovies still dominated in some samples (Figure 4.2a). In fact, age length keys were different between the Garonne region and the remainder regions (Table 4.5). The age length keys were not required for the estimation of the age composition because every sample had its own collection of otoliths, but they will be used later on in a comparative exercise basing the estimations on ALK procedures (see below).

Table 4.5: Age Length Key by areas: Garonne and Rest.

Area	NE - Garonne				
Counts by ages	Final Age				
Tot Length (mm)	1	2	3	4	Total
95-99	5				5
100-104	28				28
105-109	62				62
110-114	73	1			74
115-119	64	7			71
120-124	67	15		1	83
125-129	59	37	5		101
130-134	53	25	8		86
135-139	25	21	6	1	53
140-144	19	8	5		32
145-149	11	1			12
150-154	3	3	1		7
155-159	1	5	2	1	9
160-164		3			3
165-169		1			1
170-174					0
175-179		1		1	2
180-184					0
185-189					0
190-194				1	1
195-200					0
Total general	470	128	27	5	630

Area	REST (SW+SE+NW)				
Counts by ages	Final Age				
Tot Length (mm)	1	2	3	4	Total
95-99	1				1
100-104	3				3
105-109	16				16
110-114	39				39
115-119	47				47
120-124	65				65
125-129	61	2			63
130-134	47	2	1		50
135-139	45	3			48
140-144	65	4			69
145-149	65	5			70
150-154	51	3	2		56
155-159	34	10			44
160-164	14	17	4	1	36
165-169	7	18	14	4	43
170-174	1	41	48	10	100
175-179	1	51	77	7	136
180-184		52	116	12	180
185-189		34	72	7	113
190-194		11	32	3	46
195-200		1	14	3	18
Total general	562	254	380	47	1243

All this reflected some reproductive and age spatial structuring of the population which might require careful weighting of samples in case of the sampling not being balanced along with the spatial distribution of the population.

Quantification of key biological processes determining the dynamics and the assessment of the anchovy population in the Bay of Biscay: growth, reproduction, demography and natural mortality.

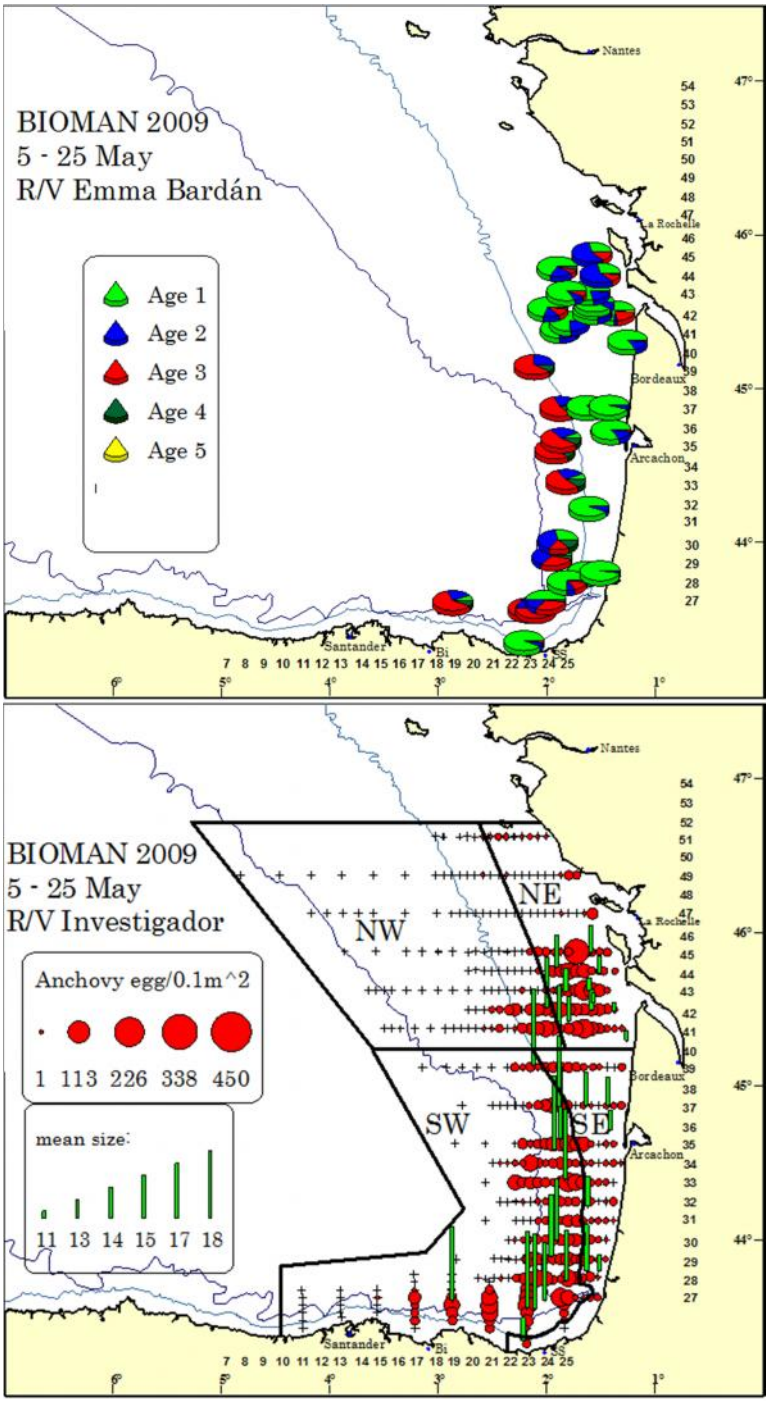


Figure 4.2: Age composition per sample (right panel) and regionalization of the Egg abundance distribution according to the mean size and age of anchovies per fishing hauls in space, with indication of mean length of anchovies per fishing haul.

4.3.1.2 Checking PPS sampling and setting individual weighting factors

In order to check if the sampling was balanced to the spatial abundance of the population we split the area in four regions aiming at accounting for the differences in reproductive and size-age composition of anchovies in space (Figure 4.2b): East or Coastal areas where small anchovies dominated, were split South and North of 45º22’N, extending in the North-eastern region (NE) up to the isoline of 80m and in the South-eastern region (SE) up to the 100 m depth. The western areas, over the shelf and offshore, where big anchovies dominated were also split at the same latitude (NW and SW) covering the areas until the western limits of the spawning areas.

Table 4.6: Checking PPS sampling and derivation of individual weighting factors per samples for the regional approach by areas and regions in BIOMAN2009, making use of the egg abundance and Daily Fecundity by regions.

	Areas	East (coastal areas)		West (shelf and offshore areas)	
	Region	NE (Garonne)	SE	SW	NW
1	Total egg abundance	1.14.E+12	2.59.E+11	2.44.E+12	1.88E+11
2	Relative egg abundance (%)	28%	6%	61%	5%
3	Daily Fecundity (DF) by areas	89.26	89.26	137.08	137.08
4	Proxy SSB by region	1.28.E+10	2.90.E+09	1.78.E+10	1.37.E+09
5	Relative Proxy SSB per region (%)	37%	8%	51%	4%
6	Nº of adult samples	11	6	13	1
7	Proxy of SSB per sample (M’i)	0.033	0.014	0.039	0.039
8	Rescaled M’i (relative to region SE)	**2.41**	**1.00**	**2.83**	**2.84**
9	Weighting factor proportional to the population in numbers (Mi) by sample	2.41/wi	1/wi	2.83/wi	2.84/wi

Table 4.6 shows that the egg abundance was highest in the SW region (line 1), followed by the Garonne region (NE), whilst the NW and SE regions had minor egg abundances. By taking these abundances as proxy of Egg Production, and dividing them by the estimates of Daily Fecundity by areas (line 3) we would obtain an indicator (proxy) of spawning biomass by region (lines 4 and 5). Here we have assumed that DF was equal between regions within areas, something we could only check and corroborated for the Eastern (coastal) area – not shown--. The proportionality of adult sampling to this biomass indicator by regions is checked in line 7 by the quotient of the SSB proxy (line 5) over the amount of available samples by regions (line 6). In case of balance sampling this quotient has to be constant across regions (Equation 4.21), which is not the case. Therefore this implies that adult sampling is considered unbalanced (not PPS), although for the current example it seems not far from balanced except for region SE which results in a lower ratio of

biomass per sample than the other regions i.e. in the region occurred oversampling compared to the others. In practice, a larger amount of samples would have been required to sample in the other regions to produce a balanced sampling, in particular by about 2.5 or 2.8 times larger than in the SE area (line 8). Such quotient (line 7) account for the biomass each sample by region represents of the total SSB and is a proxy of the inverse of the inclusion probability of a sample by the scheme of sampling carried out by the survey. This value, directly or rescaled (relative to a region, line 8), are the weighting factors proportional to cluster size in mass (**M'i**) used as abundance weighting factor per sample. This value divided by the mean weight of each sample will be proportional to the population in numbers each sample represent, i.e. the proxy of cluster size in numbers (**Mi**) required for the estimates of the population in numbers at age (line 9). The resulting values for each sample are given in Table 4.4).

4.3.1.3 Population at age estimates in 2009 and variances

Estimates of the anchovy population at age are shown in Table 4.7: The adopted procedure **(Case A)** was the pooled estimation procedure over the whole set of samples**.** The Total daily Egg Production (E0 of equation 4.1), the adult parameters and SSB are the ones reported by Santos *et al.* (2013) to ICES. All reproductive parameters and variances were obtained by weighted means of sample's parameters (with equations 4.8 and 4.9 and the **M'i** for every sample by regions as deduced above). The key parameters for the population at age estimates (mean weight and proportions at age) were similarly obtained but with weighting factors proportional to abundance in numbers of anchovies represented by the samples (**Mi**) (Table 4.4). In order to allow inspection of results by areas we show as well an alternative processing based on stratification in two strata Garonne and all other regions (rest area) (**Cases B** in Table 4.7) based on the same weighting factors by samples as used for Case A. Selection of these two strata for the exercise was made on the basis of their different age structure by length (Table 4.5). To that purpose we produced E0 by strata equal to the Total Egg Production times the fraction of egg abundances by strata, both with equal CVs (such that the addition across strata of variances would equal to the original one over the total area). It clearly shows the remarkable smaller sizes of anchovies in the Garonne area as well as its younger age composition in comparison with the remainder regions (Rest area). The differences between the estimates of the two procedures are minima (always less than 7% of change).

The Coefficients of variation (CV) gradually increases from SSB to the Total Population (in numbers, SSP) and to the population in numbers at age (SSPa), as the different parameters required for the estimations (Wt and Pa) are included. For the Pooled processing it was found that the major contribution to the variance of the spawning population in numbers (SSP) comes from the SSB which accounts for about 50% of this variance; the second contribution (29%) arises from the term due to the mean weight of fishes and finally about 21% comes from the covariation of the latter parameter Wt with those included in SSB (2COVS terms in Equation of Table 4.2). Obviously, the highest co-variations with the mean weight are shown by Wf and F, but they contribute with opposite signs to the 2COVS term and therefore they almost vanish; hence the role of this term in determining the variance of the total population in numbers (SSP) is not too large.

As expected from statistical inference theory, Table 4.7 shows that the higher the proportions at age the more precise are their estimates (see estimates in both strata and for the pool estimates). As we had otoliths per sample CV of Pa derived entirely from the cluster variance estimates (Eq.4.8 & 4.9). Pa CVs were in the range 8-22% except for age 3 in Garonne region (43%). CVs for the population in numbers at age and biomasses by age ranged between 16% and 27% for the pooled processing or the addition of strata processing. For the population at age estimates (SSPa), the largest contribution to the variance comes from the variance of SSP for all ages. The variances of SSPa can also be higher for the very poor abundant age classes, as for age 3 in the Garonne region (CV about 54%) due to the larger variance of the Pa.

Similar considerations apply to the percentages at age in mass (P’a) and estimates of the biomass by ages. In fact as P’a tend to be more balanced (i.e., differing less between ages than Pa), the CVs are often slightly smaller than for Pa.

Table 4.7: Biomass and Population at age estimates of anchovy in 2009 according to the two different procedures: Case **A** weighted pooled estimate of all samples and Case **B** Area stratified based estimates for the Garonne and Rest of the area (with weighted procedures within the strata). The estimation of reference corresponds with Option A and the changes of option B relative to option A are shown in the last column. Age production by strata was not estimated but roughly approached by allocating the Total egg Production over the whole are to the strata in proportion to the egg abundance by strata (with variances inflated to result in the total strata estimates with similar CV as the pooled Ptot estimate) (see acronyms in Table 4.1).

CASE	A		B		B		Stratified		
Weighting Procedure	**Weighted**		**Weighted**		**Weighted**		**Weighted**		
W.factors for SSB params?	Yes		Yes		Yes		Yes		
W.factors for params by age?	Yes		Yes		Yes		Yes		**Relative to A**
SUMMARY	**POOLED**		**Garonne**		**Rest**		**Addition**		**Changes**
Parameter	**estimate**	**CV**	**estimate**	**CV**	**estimate**	**CV**	**estimate**	**CV**	**in Params**
Total Egg Production (E0)	**1.53E+12**		**4.36E+11**		**1.10E+12**		**1.53E+12**		**0.0%**
S.e. & C.V. (%)	1.99E+11	13.0%	7.35E+10	16.9%	1.85E+11	16.9%	1.99E+11	13.0%	
Sex ratio (R')	**0.57**		**0.55**		**0.57**		**0.56**		**-0.3%**
S.e. & C.V. (%)	0.0203	3.6%	0.0062	1.1%	0.0298	5.2%	0.0187	3.3%	
Spawninf fraction (S')	**0.43**		**0.38**		**0.45**		**0.43**		**-0.6%**
S.e. & C.V. (%)	0.0146	3.4%	0.0332	8.8%	0.0089	2.0%	0.0138	3.2%	
Batch Fecundity (F')	**14,097**		**5,868**		**18,443**		**13,670**		**-3.0%**
S.e. & C.V. (%)	1,575	11.2%	789	13.5%	1,377	7.5%	906	6.6%	
Female mean weight (W'f)	**28.45**		**13.98**		**36.08**		**27.70**		**-2.6%**
S.e. & C.V. (%)	2.71	9.5%	1.30	9.3%	2.38	6.6%	1.55	5.6%	
Daily Fecundity (DF)	**119.99**		**87.28**		**133.10**		**115.71**		**-3.6%**
S.e. & C.V. (%)	7.85	6.5%	10.51	12.0%	8.44	6.3%	6.58	5.7%	
BIOMASS (Tons) (SSB)	**12,853**		**5,071**		**8,291**		**13,362**		**4.0%**
S.e. & C.V. (%)	1,869	14.5%	1,051	20.7%	1,493	18.0%	1,826	13.7%	
Mean Weight (Wt)	**19.42**		**12.04**		**28.96**		**18.90**		**-2.7%**
S.e. & C.V. (%)	2.18	11.2%	0.96	8.0%	2.87	9.9%	1.30	6.9%	
Population (millions) (SSP)	**666**		**423**		**289**		**712**		**6.9%**
S.e. & C.V. (%)	138	20.7%	106	25.1%	62	21.5%	123	17.3%	
Proportion at age 1 (Pa1)	**0.646**		**0.750**		**0.513**		**0.654**		**1.2%**
S.e. & C.V. (%)	0.056	8.6%	0.061	8.1%	0.087	16.9%	0.050	7.7%	
Proportion at age 2 (Pa2)	**0.191**		**0.191**		**0.188**		**0.190**		**-0.4%**
S.e. & C.V. (%)	0.032	16.8%	0.054	28.4%	0.027	14.6%	0.034	18.0%	
Proportion at age 3+ (Pa3+)	**0.164**		**0.059**		**0.300**		**0.156**		**-4.4%**
S.e. & C.V. (%)	0.037	22.5%	0.025	42.5%	0.062	20.7%	0.029	18.7%	
Numbers at age 1 (SSPa1)	**432.2**		**318**		**150**		**468**		**8.2%**
S.e. & C.V. (%)	114.4	26.5%	88.2	27.8%	50.4	33.6%	101.6	21.7%	
Numbers at age 2 (SSPa2)	**126**		**80**		**54**		**134**		**5.9%**
S.e. & C.V. (%)	28.9	22.8%	25.3	31.4%	10.2	19.0%	27.2	20.3%	
Numbers at age 3 (SSPa3+)	**108**		**25**		**85**		**111**		**2.7%**
S.e. & C.V. (%)	21.3	19.8%	13.8	54.3%	18.1	21.2%	22.8	20.6%	
Prop. at age 1 (in mass) (P'a1)	**0.419**		**0.642**		**0.289**		**0.423**		**0.9%**
S.e. & C.V. (%)	0.062	14.7%	0.067	10.4%	0.069	23.9%	0.050	11.8%	
Prop. at age 2 (in mass) (P'a2)	**0.258**		**0.270**		**0.251**		**0.258**		**0.2%**
S.e. & C.V. (%)	0.025	9.8%	0.059	22.0%	0.022	8.7%	0.026	10.2%	
Prop. at age 3 (in mass) (P'a3+)	**0.323**		**0.088**		**0.459**		**0.318**		**-1.3%**
S.e. & C.V. (%)	0.053	16.4%	0.031	34.9%	0.057	12.5%	0.037	11.7%	
SSB at age 1 (Tons)	**5,434**		**3,256**		**2,438**		**5,693**		**4.8%**
S.e. & C.V. (%)	1,242	22.9%	767	23.6%	769	31.5%	1,086	19.1%	
SSB at age 2 (Tons)	**3,305**		**1,359**		**2,070**		**3,430**		**3.8%**
S.e. & C.V. (%)	535	16.2%	372	27.4%	384	18.6%	535	15.6%	
SSB at age 3 (Tons)	**4,113**		**456**		**3,782**		**4,238**		**3.0%**
S.e. & C.V. (%)	814	19.8%	205	44.9%	800	21.2%	826	19.5%	
Biological Features	**estimate**	CV	**estimate**	CV	**estimate**	CV	**estimate**	CV	
Weight at age 1 (g) (W1)	**12.78**		**10.73**		**16.60**		**12.62**		**-1.3%**
S.e. & C.V. (%)	1.07	8.4%	0.94	8.8%	1.69	10.2%	0.84	6.7%	
Weight at age 2 (g) (W2)	**25.17**		**15.27**		**38.72**		**24.64**		**-2.1%**
S.e. & C.V. (%)	3.09	12.3%	1.15	7.5%	1.64	4.2%	0.95	3.9%	
Weight at age 3 (g) (W3+)	**38.81**		**17.26**		**43.92**		**37.81**		**-2.6%**
S.e. & C.V. (%)	2.81	7.2%	1.67	9.7%	1.06	2.4%	0.91	2.4%	
Lenght at age 1 (mm)	**123.79**		**118.15**		**134.31**		**123.34**		**-0.4%**
S.e. & C.V. (%)	3.00	2.4%	2.89	2.4%	4.20	3.1%	2.38	1.9%	
Lenght at age 2 (mm)	**149.03**		**131.22**		**173.33**		**148.06**		**-0.7%**
S.e. & C.V. (%)	5.70	3.8%	2.59	2.0%	1.97	1.1%	1.74	1.2%	
Length at age 3 (mm)	**172.28**		**136.67**		**180.70**		**170.61**		**-1.0%**
S.e. & C.V. (%)	4.50	2.6%	3.24	2.4%	0.92	0.5%	1.03	0.6%	

Comparison of the CVs between the adopted pooled and the potential stratified processing reveals that the later processing could have resulted in a reduction of the CVs for all parameters (provided the precision of the regional Daily Egg Production wouldn't rise the overall CV of E0 from the addition across strata, as assumed here). However such regionalization of the Egg production estimates was not implemented and as such the pooled estimate was adopted, obtaining thus a fair average of the parameter values and variances over the whole area

For comparative purposes we estimated the proportions at age in the population via ALK by regions (using those in Table 4.5), as if we wouldn`t have had any individual age readings by samples (Table 4.8). For the pooled processing appearing in Table 4.8, we used a single ALK, addition of the two regional ALK. We obtained consistent estimates of the Variance of Pa via ALK (Equation 4.12) compared to the one based on the direct observations per samples (Equation 4.9), either over the total area or by regions (Table 4.8), with some reduction for the variances estimates from the ALK procedures (in particular for age 2 in the Garonne area). The variance components of Pa by areas according to equation 4.12 (Figure 4.3) reveals that the two first terms are the key components whilst the contribution of the ALK becomes only relevant for the poorly represented ages, like ages 3 and 4 in the Garonne area. The addition of the first two terms of the variance accounts for the cluster component of the variance (equal to the first term of Eq.4.13) and they suppose generally the largest part of the variance of Pa over the two areas Garonne and Rest (accounting for 88%, 82% and 67% and for 99%, 88% and 97% for ages 1, 2 and 3+ in the Garonne and Rest areas respectively). The negative role of the covariation of lengths within samples resulting for age 1 in the Garonne area (i.e. aggregation effect of length within samples for age 1) means that in this area length at age 1 is very homogenously present between samples and reduces the cluster variance, whilst for the other cases the covariation of length at age within samples is larger and increases the cluster variances. In summary we obtained, as expected, consistent Pa and Var (Pa) estimates from either direct observations from samples or from ALKs with some moderate reduction for certain ages in the latter procedure. This comes from a partial reduction of the cluster variance (loss of variability between samples) when ALKs are applied, not being fully counterbalanced by the addition of an ALK component of variance.

The biases associated to the all previous estimates resulted always below +/-3% of the parameter estimates (mean +/- 1%), and the typical bias relative to the standard errors of the estimates were mostly below +/- 10% (mean of +/- 6%), with highest bias associated to the estimates of the population in numbers at age 3 (14% of its standard error). Therefore in practice they can be neglected (Cochran 1977).

Table 4.8: Comparison of Proportion at age estimates (in number and mass upper tables) and mean weight and length at age estimates (bottom tables) via ad hoc cluster sampling of these parameters (Case A) or via Age Lenth Key applied to the sampling of lengths (Case B), either for single pooled area (left Columns), for the Garonne area (middle columns) or for the Remainder areas (Rest) (right columns).

CASE	A		B		A		B		A		B	
Weighting Procedure	**Weighted**		**Weighted (via ALK)**		**Weighted**		**Weighted (via ALK)**		**Weighted**		**Weighted (via ALK)**	
W.factors for SSB params?	Yes		Yes		Yes		Yes		Yes		Yes	
W.factors for params by age?	Yes		Yes		Yes		Yes		Yes		Yes	
Strata?	**Pooled**		**Pooled**		**Garonne**		**Garonne**		**Rest**		**Rest**	
Biological Features	**estimate**	CV	**estimate**	CV	**estimate**	CV	**estimate**	CV	**estimate**	CV	**estimate**	CV
Proportion at age 1 (Pa1)	**0.646**		**0.661**		**0.750**		**0.781**		**0.513**		**0.510**	
S.e. & C.V. (%)	0.056	8.6%	0.054	8.1%	0.061	8.1%	0.042	5.3%	0.087	16.9%	0.087	17.1%
Proportion at age 2 (Pa2)	**0.191**		**0.182**		**0.191**		**0.176**		**0.188**		**0.188**	
S.e. & C.V. (%)	0.032	16.8%	0.023	12.5%	0.054	28.4%	0.032	18.0%	0.027	14.6%	0.030	16.0%
Proportion at age 3+ (Pa3+)	**0.164**		**0.157**		**0.059**		**0.043**		**0.300**		**0.302**	
S.e. & C.V. (%)	0.037	22.5%	0.037	23.4%	0.025	42.5%	0.012	28.0%	0.062	20.7%	0.060	19.7%
Prop. at age 1 (in mass) (P'a1)	**0.419**		**0.433**		**0.642**		**0.688**		**0.289**		**0.283**	
S.e. & C.V. (%)	0.062	14.7%	0.062	14.2%	0.067	10.4%	0.045	6.6%	0.069	23.9%	0.067	23.5%
Prop. at age 2 (in mass) (P'a2)	**0.258**		**0.245**		**0.270**		**0.233**		**0.251**		**0.252**	
S.e. & C.V. (%)	0.025	9.8%	0.019	7.9%	0.059	22.0%	0.030	12.9%	0.022	8.7%	0.023	9.2%
Prop. at age 3 (in mass) (P'a3+)	**0.323**		**0.322**		**0.088**		**0.079**		**0.459**		**0.464**	
S.e. & C.V. (%)	0.053	16.4%	0.052	16.0%	0.031	34.9%	0.022	27.9%	0.057	12.5%	0.053	11.4%

Strata?	**Pooled**		**Pooled**		**Garonne**		**Garonne**		**Rest**		**Rest**	
Biological Features	**estimate**	CV	**estimate**	CV	**estimate**	CV	**estimate**	CV	**estimate**	CV	**estimate**	CV
Weight at age 1 (g) (W1)	**12.78**		**12.91**		**10.73**		**10.90**		**16.60**		**16.76**	
S.e. & C.V. (%)	1.07	8.4%	1.01	7.8%	0.94	8.8%	0.77	7.1%	1.69	10.2%	1.63	9.7%
Weight at age 2 (g) (W2)	**25.17**		**25.97**		**15.27**		**15.13**		**38.72**		**39.06**	
S.e. & C.V. (%)	3.09	12.3%	3.28	12.6%	1.15	7.5%	1.32	8.7%	1.64	4.2%	2.40	6.2%
Weight at age 3 (g) (W3+)	**38.81**		**40.55**		**17.26**		**18.86**		**43.92**		**44.27**	
S.e. & C.V. (%)	2.81	7.2%	2.23	5.5%	1.67	9.7%	3.26	17.3%	1.06	2.4%	1.58	3.6%
Lenght at age 1 (mm)	**123.79**		**124.43**		**118.15**		**119.09**		**134.31**		**134.64**	
S.e. & C.V. (%)	3.00	2.4%	2.73	2.2%	2.89	2.4%	2.33	2.0%	4.20	3.1%	4.04	3.0%
Lenght at age 2 (mm)	**149.03**		**150.78**		**131.22**		**131.78**		**173.33**		**173.72**	
S.e. & C.V. (%)	5.70	3.8%	4.74	3.1%	2.59	2.0%	1.87	1.4%	1.97	1.1%	1.97	1.1%
Length at age 3 (mm)	**172.28**		**175.26**		**136.67**		**139.88**		**180.70**		**181.33**	
S.e. & C.V. (%)	4.50	2.6%	2.57	1.5%	3.24	2.4%	3.82	2.7%	0.92	0.5%	1.04	0.6%

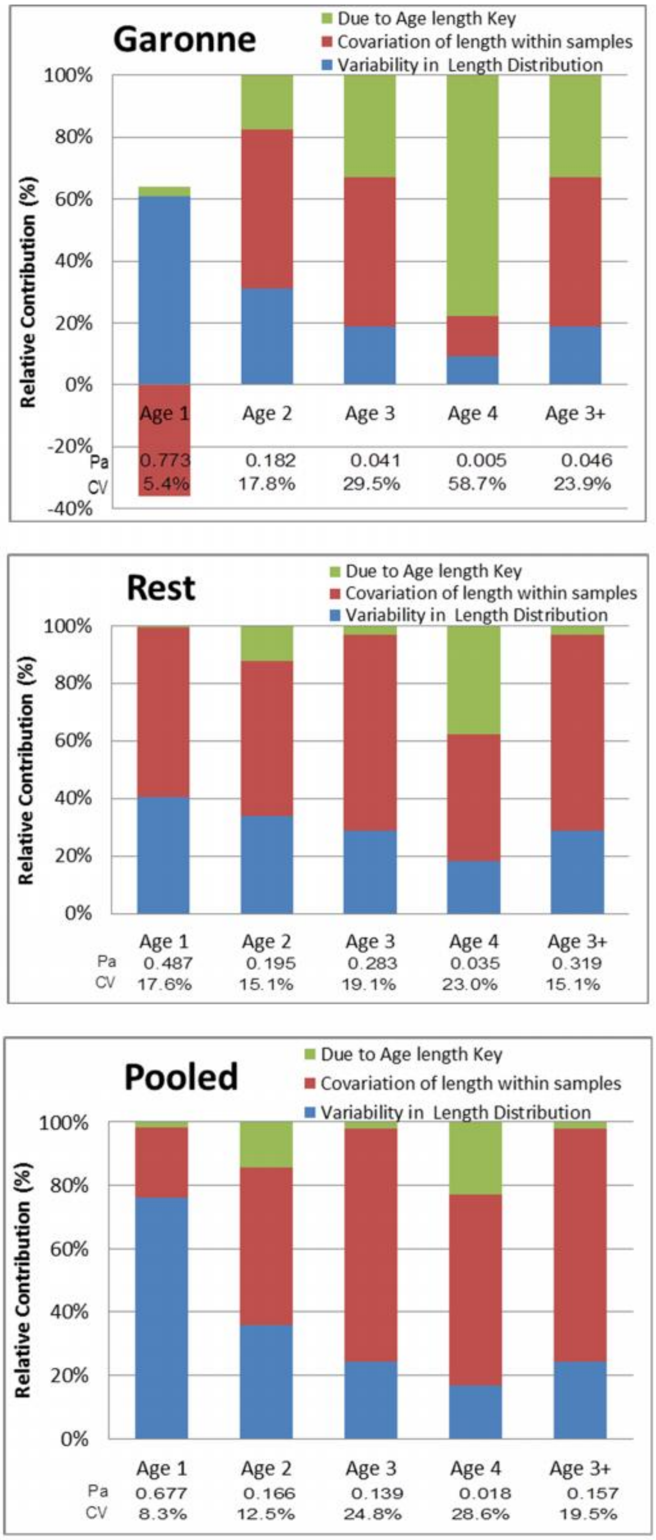


Figure 4.3: Components of variance for the Proportion at age estimates by areas and overall area (Pooled estimates). Cluster variance arises from the addition of the variability in length distribution between samples plus the covariation in length within samples and it amounts for the largest component of the Pa Variance (except for age 4 in Garonne area)

4.3.1.4 Sensitivity analysis to weighting factors

We explored, in Table 4.9, the sensitivity to omitting the weighting factors per sample in two steps: First, for omitting only the weighting factors for the estimation of the reproductive parameters only (i.e., affecting the SSB estimates) (semi weighted procedure - Case B) and second omitting weighting factors for the estimation of all the parameters, i.e. those affecting the SSB and the population at age estimates (SSPa) (Unweighted procedure - Case C). Omitting weighting factors means setting them all equal to 1. For the semi weighted procedure, SSB estimates were affected by about a 3% increase by the omission of weighting factors in the estimation of the reproductive parameters. Even though F' and W'f were similarly affected by about 9% their covariation in the numerator and denominator of Equation 4.1 counterbalance for their effects leading to the minimal changes in DF and SSB. Naturally in this case B, population at age estimates are just parallel affected by about 3% due to the disturbance induced in the SSB estimate. A complete unweighted procedure (Case C) results in additional changes in the remaining parameters, amplifying the former effect in SSB: first it reduces mean weight estimates (by about 9%) and leads to an increase of the total population (SSP) by about 13%. Next it increases the percentages at age 1 in the population by about 9% at the expense of reducing the Pa of ages 2 and 3+, and this results in a final increase of the SSPa1 of about 23% and a reduction of the SSPa2 and SSPa3+ by about 2% and 12% respectively. Similarly, the proportions in mass of age 1 increased by about 18% and led the SSBa1 to increase by about 21%. Certainly the rise in the estimates of the population at age 1 reflects the over sampling occurring at the SE region, where small and younger anchovies dominated, compared to the western areas. The increasing levels of relative errors of the estimates shows the gradual propagation of errors from the SSB estimates to the SSPa and SSBa estimates as the different parameters (Wt and Pa, or P'a) are included for the estimates.

This example shows the sensitivity of our population at age estimates to the weighted procedure of samples and hence the need for a suitable selection of weighting factors to produce unbiased estimates. It also shows the successive accumulation of errors from the SSB to the population at age estimates (SSPa and SSBa).

Table 4.9: Pooled Biomass and Population at age estimates of anchovy in 2009 according to the three different weighting procedures of the individual samples. Case A: pooled weighted procedure for SSB and Population at age estimate. Case B: Pooled semi-weighted procedure, i.e. unweighted for the estimation of SSB (for the reproductive parameters) but weighted for the parameters affecting the population at age estimates (i.e, weighted for Wt, Pa, P'a). Case C: Pooled unweighted procedure for both SSB and Population at age parameters (i.e. letting weighting factors equal to 1 for all samples). The estimation of reference is Case A and the changes relative to option A for cases B and C.

CASE	A		B			C		
Weighting Procedure	**Weighted**		**SemiWeighted**			**Unweighted**		
W.factors for SSB params?	Yes		No			No		
W.factors for params by age?	Yes		Yes		**Relative to A**	No		**Relative to A**
SUMMARY	**Pooled**		**Pooled**		**Changes**	**Pooled**		**Changes**
Parameter	**estimate**	**CV**	**estimate**	**CV**	**in Params**	**estimate**	**CV**	**in Params**
Total Egg Production (E0)	**1.53E+12**		**1.53E+12**		0.0%	**1.53E+12**		0.0%
S.e. & C.V. (%)	1.99E+11	13.0%	1.99E+11	13.0%		1.99E+11	13.0%	
Sex ratio (R')	**0.57**		**0.57**		0.2%	**0.57**		0.2%
S.e. & C.V. (%)	0.0203	3.6%	0.0170	3.0%		0.0170	3.0%	
Spawninf fraction (S')	**0.43**		**0.42**		-1.9%	**0.42**		-1.9%
S.e. & C.V. (%)	0.0146	3.4%	0.0142	3.4%		0.0142	3.4%	
Batch Fecundity (F')	**14,097**		**12,682**		-10.0%	**12,682**		-10.0%
S.e. & C.V. (%)	1,575	11.2%	1,500	11.8%		1,500	11.8%	
Female mean weight (W'f)	**28.45**		**25.92**		-8.9%	**25.92**		-8.9%
S.e. & C.V. (%)	2.71	9.5%	2.59	10.0%		2.59	10.0%	
Daily Fecundity (DF)	**119.99**		**116.44**		-3.0%	**116.44**		-3.0%
S.e. & C.V. (%)	7.85	6.5%	7.32	6.3%		7.32	6.3%	
BIOMASS (Tons) (SSB)	**12,853**		**13,245**		3.0%	**13,245**		3.0%
S.e. & C.V. (%)	1,869	14.5%	1,911	14.4%		1,911	14.4%	
Mean Weight (Wt)	**19.42**		**19.42**		0.0%	**17.66**		-9.1%
S.e. & C.V. (%)	2.18	11.2%	2.18	11.2%		1.78	10.1%	
Population (millions) (SSP)	**666**		**687**		3.1%	**753**		13.0%
S.e. & C.V. (%)	138	20.7%	142	20.6%		149	19.8%	
Proportion at age 1 (Pa1)	**0.646**		**0.646**		0.0%	**0.707**		9.5%
S.e. & C.V. (%)	0.056	8.6%	0.056	8.6%		0.051	7.2%	
Proportion at age 2 (Pa2)	**0.191**		**0.191**		0.0%	**0.166**		-13.1%
S.e. & C.V. (%)	0.032	16.8%	0.032	16.8%		0.030	18.3%	
Proportion at age 3+ (Pa3+)	**0.164**		**0.164**		0.0%	**0.127**		-22.3%
S.e. & C.V. (%)	0.037	22.5%	0.037	22.5%		0.031	24.1%	
Numbers at age 1 (SSPa1)	**432**		**445**		3.1%	**533**		23.4%
S.e. & C.V. (%)	114.4	26.5%	117.8	26.5%		129.2	24.2%	
Numbers at age 2 (SSPa2)	**126**		**130**		3.0%	**124**		-1.5%
S.e. & C.V. (%)	28.9	22.8%	29.6	22.7%		28.5	22.9%	
Numbers at age 3 (SSPa3+)	**108**		**111**		3.0%	**95**		-11.7%
S.e. & C.V. (%)	21.3	19.8%	21.7	19.6%		20.2	21.2%	
Prop. at age 1 (in mass) (P'a1)	**0.419**		**0.419**		0.0%	**0.496**		18.3%
S.e. & C.V. (%)	0.062	14.7%	0.062	14.7%		0.065	13.2%	
Prop. at age 2 (in mass) (P'a2)	**0.258**		**0.258**		0.0%	**0.228**		-11.6%
S.e. & C.V. (%)	0.025	9.8%	0.025	9.8%		0.025	10.9%	
Prop. at age 3 (in mass) (P'a3+)	**0.323**		**0.323**		0.0%	**0.276**		-14.4%
S.e. & C.V. (%)	0.053	16.4%	0.053	16.4%		0.052	18.7%	
SSB at age 1 (Tons)	**5,434**		**5,600**		3.1%	**6,594**		21.3%
S.e. & C.V. (%)	1,242	22.9%	1,278	22.8%		1,420	21.5%	
SSB at age 2 (Tons)	**3,305**		**3,406**		3.1%	**3,013**		-8.8%
S.e. & C.V. (%)	535	16.2%	548	16.1%		499	16.5%	
SSB at age 3 (Tons)	**4,113**		**4,238**		3.0%	**3,637**		-11.6%
S.e. & C.V. (%)	814	19.8%	833	19.7%		774	21.3%	
Biological Features	**estimate**	CV	**estimate**	CV		**estimate**	CV	
Weight at age 1 (g) (W1)	**12.78**		**12.78**		0.0%	**12.49**		-2.3%
S.e. & C.V. (%)	1.07	8.4%	1.07	8.4%		0.88	7.0%	
Weight at age 2 (g) (W2)	**25.17**		**25.17**		0.0%	**24.00**		-4.7%
S.e. & C.V. (%)	3.09	12.3%	3.09	12.3%		2.82	11.7%	
Weight at age 3 (g) (W3+)	**38.81**		**38.81**		0.0%	**37.98**		-2.2%
S.e. & C.V. (%)	2.81	7.2%	2.81	7.2%		3.01	7.9%	
Lenght at age 1 (mm)	**123.79**		**123.79**		0.0%	**123.42**		-0.3%
S.e. & C.V. (%)	3.00	2.4%	3.00	2.4%		2.51	2.0%	
Lenght at age 2 (mm)	**149.03**		**149.03**		0.0%	**147.15**		-1.3%
S.e. & C.V. (%)	5.70	3.8%	5.70	3.8%		5.24	3.6%	
Length at age 3 (mm)	**172.28**		**172.28**		0.0%	**170.91**		-0.8%
S.e. & C.V. (%)	4.50	2.6%	4.50	2.6%		4.87	2.8%	

4.3.1.5 Mean weight and length at age

Mean length and weight at age pooled over all area and by strata are shown in Table 4.7**,** from Eq.4.14 (second expression). As for Pa, mean weight and length at age differed between strata, being significantly smaller the fishes at the Garonne strata for every age class. The pooled mean estimates (for the entire surveyed area) were rather robust and not too sensitive to the weighting procedures of individual sample's estimates (Table 4.9). Variances for Weight at age appearing in Table 4.7 are those based on Eq. 4.9, taking Mi*Pai as the sample weighting factor (i.e. equal to the first term of Eq. 4.16**)**, since we use the actual observations of mean length and weight at age per samples from the individual biological sampling. The CVs of these means are rather small in particular for the lengths, the latter reflecting the narrow range of individual lengths compared to weights.

For comparative purposes we estimated mean weight and length at age via ALK by regions, as if we wouldn`t have had any individual age readings by samples. Table 4.8 shows that ALK estimates of means and variances of weights and lengths by age were very consistent with the original estimates, except for age 3 in the Garonne area where the CV via ALK sharply increases. The values in Table 4.8 correspond to applying Eq.4.16 for the Variance estimation of the mean weight at age. The major contributions to the total variance of weights at age via ALK (from Eq.4.16) comes from the first two terms (i.e., from the cluster variance among samples and the ALK component), whilst the contribution from its third term (i.e., from the variability of individuals weights within each length class is for all ages smaller than 3% and therefore negligible.

The estimates of variance arising from Eq. 4.15 are compared with those of Eq. 4.16 in Table 4.10 by variance components. Eq. 4.15 is the most complete estimator deduced from the delta method. The result concerning Eq. 4.15 can be obtained in two ways regarding the correction for the correlation between numerator and denominator (from the fifth term of Eq. 4.15). If the coefficient is applied to the estimates of $S_{Pa \cdot Wa}$ comprising the contribution from the ALK and length weight relationships (as believed it should be done) then the variance estimates result in slightly smaller values than those from Equation 4.16 and in some cases smaller than the original estimates from the actual observations by samples (see results of a.2, b.2 and c.2 in Table 4.10).

If the correlation is applied to the $S_{Pa \cdot Wa}$ estimates not comprising the contribution from the ALK and length weight relationships, then variances result to be slightly bigger than those from Equation 4.16 and usually above the original estimates from the actual observations by samples at least by strata (see results of a.3 and b.3 in Table 4.10), though not over the whole area. And finally, the results from Eq. 4.16 are placed in the middle of both approaches, being by strata still above the original variance estimates (and hence conservative), but not for the whole area. This means that the simple and intuitive estimate from equation 4.16 based on the cluster Variance of the mean weight by samples (after application of the ALK by sample) is a mixture of the two approaches for the application of correlation in Equation 4.15 and what supposes an approach sufficiently good to that variance estimation (being at the same time the simplest one to be calculated). The analysis by strata showed that for the respective not too big ALKs the variance estimates from Eq. 4.16 or 4.15 (option alternative) can be conservative (higher than that from the actual cluster observations of Wa). However for the total strata the big pooled ALK size makes the variances smaller than ones from cluster observations (see also some discussion on this afterwards).

The biases associated to the mean length and weight at age resulted always below +/- 3% of the parameter estimates (mean +/- 1%), and the typical bias relative to the standard errors of the estimates were mostly well below +/- 20% (mean of +/- 7% pooled, but mean of +/-12% in the Garonne region), with the only biases exceeding the threshold of 20% corresponding to the mean length and weight at age 3 (26% and 28% of their standard errors respectively). Therefore in practice biases can be generally neglected (Cochran 1977).

Table 4.10: Comparison of the Weight at age and CV estimates by strata (Garonne – a - and Rest –b-) and overall area (-c-) from the original processing of observations by samples and from the use of ALKs. Weight estimates are deduced according to equations 4.14 (for both estimation procedures) and for the variance of the ALK pure estimates according to either equation 4.16 or 4.15 (the latter with two alternatives for the fifth term of the variance—see text).

	a) GARONNE Region					**b) REST or remainder regions**				
	Age 1	Age 2	Age 3	Age 4	**Age 3+**	Age 1	Age 2	Age 3	Age 4	**Age 3+**
Strata Cluster Mean of Weight at age by hauls	**10.83**	**15.48**	NA	NA	**17.73**	**16.69**	**38.65**	NA	NA	**43.91**
CV	**8.8%**	**7.5%**	NA	NA	**9.7%**	**10.2%**	**4.2%**	NA	NA	**2.4%**
ALK ESTIMATES GARONNE	Age 1	Age 2	Age 3	Age 4	**Age 3+**	Age 1	Age 2	Age 3	Age 4	**Age 3+**
ALK estimates of Wmean at age	**11.11**	**15.27**	**19.05**	**19.17**	**19.17**	**17.14**	**39.22**	**44.43**	**44.32**	**44.32**
a.1 Variance estimate Equation 16										
Term 1 - Cluster Variance(of Wai))	0.5612	0.5731	3.7480	5.7350	3.0998	2.5832	1.4550	0.3126	0.6065	0.2547
Term 2- Var(Wa from ALK) / Pa^2	0.0703	1.2129	8.2740	89.4743	7.5933	0.0990	4.2555	2.0483	38.3201	2.0891
Term 3 - Sum(Pal^2*Var(Wl)) / Pa^2	0.0109	0.0236	0.0298	0.0453	0.0247	0.0143	0.0482	0.0731	0.0656	0.0586
Total Variance	0.6423	1.8096	12.0518	95.2546	10.7178	2.6965	5.7587	2.4340	38.9923	2.4025
Se	0.80	1.35	3.47	9.76	3.27	1.64	2.40	1.56	6.24	1.55
CV	**7.2%**	**8.8%**	**18.2%**	**50.9%**	**17.1%**	**9.6%**	**6.1%**	**3.5%**	**14.1%**	**3.5%**
a.2 Variance estimate Equation 15										
Term 1- ClusterVariance of Pai*Wail/ Pa^2	0.04	8.38	34.36	54.66	28.44	7.66	38.65	73.26	64.00	58.73
Term 2- Var(Wa from ALK) / Pa^2	0.070	1.213	8.274	89.474	7.593	0.099	4.255	2.048	38.320	2.089
Term 3- Sum(Pal^2*Var(Wl)) / Pa^2	0.011	0.024	0.030	0.045	0.025	0.014	0.048	0.073	0.066	0.059
Term 4- Wa^2*Var(Pa)/Pa^2	0.362	7.383	31.544	141.567	27.000	9.103	34.852	72.016	100.153	58.190
Term 5- - 2· Ratio * Ro * Sy·Sx /Pa^2	0.335	-16.377	-70.422	-276.934	-59.916	-14.259	-76.225	-147.010	-201.517	-118.808
Total Variance	0.82	0.63	3.79	8.81	3.14	2.61	1.58	0.39	1.02	0.26
Se	0.91	0.79	1.95	2.97	1.77	1.62	1.26	0.62	1.01	0.51
CV	**8.2%**	**5.2%**	**10.2%**	**15.5%**	**9.2%**	**9.4%**	**3.2%**	**1.4%**	**2.3%**	**1.2%**
a.3 Alternative for Equation 15										
Term 5- - 2· Ratio * Ro * Sy·Sx /Pa^2	0.196	-13.891	-51.742	-80.341	-42.806	-14.135	-67.838	-142.814	-125.979	-114.507
Total Variance	0.683	3.112	22.466	205.402	20.251	2.739	9.967	4.586	76.560	4.565
Se	0.83	1.76	4.74	14.33	4.50	1.65	3.16	2.14	8.75	2.14
CV	**7.4%**	**11.6%**	**24.9%**	**74.8%**	**23.5%**	**9.7%**	**8.1%**	**4.8%**	**19.7%**	**4.8%**

	c) ALL AREA (all regions)				
	Age 1	Age 2	Age 3	Age 4	**Age 3+**
Cluster Mean of Weight at age by hauls	**12.78**	**25.17**	NA	NA	**38.81**
CV	**8.4%**	**12.3%**	NA	NA	**7.2%**
	Age 1	Age 2	Age 3	Age 4	**Age 3+**
ALK estimates of Wmean at age	**13.14**	**27.53**	**41.20**	**41.19**	**41.19**
b.1 Variance estimate Equation 16					
Term 1 - Cluster Variance(of Wai))	0.8922	5.7472	1.4833	1.1393	1.1830
Term 2- Var(Wa from ALK) / Pa^2	0.0330	1.4528	1.7488	31.2059	1.7720
Term 3 - Sum(Pal^2*Var(Wl)) / Pa^2	0.0100	0.0196	0.0574	0.0534	0.0459
Total Variance	0.9352	7.2196	3.2896	32.3986	3.0010
Se	0.97	2.69	1.81	5.69	1.73
CV	**7.4%**	**9.8%**	**4.4%**	**13.8%**	**4.2%**
b.2 Variance estimate Equation 15					
Term 1- ClusterVariance of Pai*Wail/ Pa^2	0.74	25.65	124.13	122.78	99.35
Term 2- Var(Wa from ALK) / Pa^2	0.033	1.453	1.749	31.206	1.772
Term 3- Sum(Pal^2*Var(Wl)) / Pa^2	0.010	0.020	0.057	0.053	0.046
Term 4- Wa^2*Var(Pa)/Pa^2	1.186	11.757	104.305	137.850	83.915
Term 5- - 2· Ratio * Ro * Sy·Sx /Pa^2	-1.048	-33.282	-228.767	-290.696	-183.942
Total Variance	0.92	5.60	1.48	1.20	1.14
Se	0.96	2.37	1.22	1.09	1.07
CV	**7.3%**	**8.6%**	**3.0%**	**2.7%**	**2.6%**
b.3 Alternative for Equation 15					
Term 5- - 2· Ratio * Ro * Sy·Sx /Pa^2	-1.017	-29.983	-224.679	-227.944	-179.887
Total Variance	0.955	8.894	5.567	63.948	5.193
Se	0.98	2.98	2.36	8.00	2.28
CV	**7.4%**	**10.8%**	**5.7%**	**19.4%**	**5.5%**

4.3.2 Application of the method to the historical series of DEPM surveys on anchovy

4.3.2.1 Synopsis of the DEPM surveying

The current method for population at age estimates has been consistently applied since 1990, although the DEPM for SSB estimation and provisional estimates of population in numbers at age started in 1987 (Somarakis *et al.* 2004; Motos *et al.* 1991; 2005; Santos *et al. in press*) (Table 4.11). In the surveys, adult samples are collected from various sources in parallel to the egg sampling either from an ad hoc rented fishing boat (initially purse seiner and in the last decade a pelagic trawler), from a parallel acoustic survey (usually a French survey, -- Massé *et al., in press* — as in 1994 and years 1997-2006) or from opportunistic samples supplied by the commercial fleet (mostly Spanish purse seiners) (Table 4.11). From the complete set of available samples a posterior selection is made for processing purposes according to their spatial overlapping and synchronicity with the egg sampling. The amount of anchovy adult samples per survey retained and actually used for the estimation of the parameters varied between 21 (2013) to 60 (1997). For these various sources the scheme of sampling was usually a mixing between judgement and opportunistic sampling. In some cases the spawning frequency (S) was not available for all samples as in 1991, 1994-95.

Number of strata (for SSB estimations) and regions (for generation of weighting factors per samples) are given in Table 4.11. Further details can be found in the original survey reports (see references in the table as well). Between 1990 and 1998 the estimates were stratified in 2 (or a maximum of 3) strata according to the egg distribution and mean weight of anchovy in space, but since 2001 single estimates over the whole surveyed area are produced (no stratification) (Santos *et al. in press*). In all cases regionalization of the egg abundance over a minimum of 2 to a maximum of 5 regions was examined in order to check for the proportionality of sampling to the spatial distribution of biomass (PPS), taking the egg abundance or this value divided by the daily fecundity, as the proxies of biomass by regions (in case of stratified, estimates the biomass by strata were used too). In case of PPS the ratio of the proxy of biomass over the amount of samples by regions should be equal across regions and so will be the individual weighting factors per samples (from those ratios). The table shows the actual mean and coefficient of variation (CV) of the individual weighting factors proportional to mass (*M'*). The actual CV(*M'*) is an indicator of how much balanced (PPS) the actual sampling was (for PPS CV should be 0). Another indication of the balance of the actual sampling is deduced from the ratio of the mean *M'* of the samples in the region(s) with the smallest anchovies over the mean *M'* of all samples over the entire surveyed area (column headed as Small/Mean in Table 4.11). In some years the actual sampling resulted close to PPS with CVs close to 0 and Small/Mean ratios close 1 (as for instance 2002, 2004, 2005 or 2012) but in many other years the sampling was rather unbalanced (particularly in the nineties and 2003 and 2006).

Direct sampling of anchovies from the haul collected during BIOMAN survey consisted of a minimum of 1 kg or 60 anchovies taken at random from the catch with the aim of obtaining 25 non-hydrated mature females (for histological examination), increasing the sample size up to a maximum of 120 anchovies if necessary in order to attain this goal. For each individual, total length, total weight, sex and gonad weight (in the case of females) were recorded and usually otoliths were extracted from the first 50 anchovies sampled (and in recent years from every individual) for posterior age determination. For the samples with otoliths the actual mean number of otoliths collected per sample is reported in Table 4.11. The rest of samples coming from other sources consisted also of random sample from haul catches but were directly preserved in formaldehyde and did not have any collection of otoliths. To the latter group of samples either a global or a regional ALK was applied to their length distribution to produce their age composition. Corrections for the gain in weight and the reduction in length arising from the preservation of the anchovies in formaldehyde solution were applied.

Table 4.11: Adult sampling for the implementation of the DEPM survey series (BIOMAN series) since 1990 in the Bay of Biscay, with information of: Amount of anchovy samples available (Anc.Samples) and Retained for the estimations, with indication of their origin, (from the survey itself, DEPM, for a parallel acoustic survey, Acoustic, or from opportunistic sampling from the fleet, Fleet); the adopted stratification procedures (SSB processing either single strata, all area pooled, or several strata) and whether the estimation was weighted or unweighted at the strata level; total number of regions for definition of weighting factors (including the adopted strata); adopted indicator proxy of biomass by regions (Weighting to); Means ample weighting factor in mass (Mean *M’*) and its CV; and the Ratio between *M’i* for the samples in the areas with small fishes over the mean *M’i* of all samples (Small/Mean). Average number of fishes per sample (Sample size (#)); Procedure for age determination (either by hauls or by ALK – with indication of the number of regional ALKs built up-- or a mixture of both procedures); Total amount of age readings for the adopted ALKs; mean number of otoliths available by hauls for those samples having their own collection of otoliths. Effective sample size (Samp.size), equivalent to the size of a random sampling producing similar CV for Pa under multinomial distribution assumption. Last column provide indication of the original references, though the series was partly published in Somarakis et al (2004); Motos *et al.* (2005) and Santos *et al.* (2015), with a review of the SSB estimates in Santos *et al.* (2013).

BIOMAN SURVEY SERIES		Adult Sampling		Sources of retained			SSB Processing		SSPa Processing (Checking PPS by sample Weighting Factors M')						Sample	Age Determination	Tot.ALK	By haul	Effective	Original Source
Year	**Date**	Anc.Samples	Retained	DEPM	Acoustic	Fleet	Strata	weighted?	Regions	Weighting to	Mean M'	CV(M`)	Small.M'	Small/Mean	Size (#)	By Hauls / # ALKs	Otoliths	Otoliths	Samp.Size	References
1987	2 - 7 June	35			na	na														Santiago & Sanz 1992
1988	21 - 28 May	82			na	na														Santiago & Sanz 1992
1989	10 - 21 May	35			na	na														Motos et al. 1991
1989	14-24 June	13			na	na														Motos et al. 1991
1990	4 - 15 May	49	51	19	4	28	2 Strata	No	4	SSB + EggAb	1.96	0.93	3.63	1.85	83.0	2ALK(51)	978	na	500	Motos & Uriarte 1991
1990	29 May- 15 June	51	46	18		28	2 Strata	No	2	SSB										Motos & Uriarte 1991
1991	16May-07Jun	57	54 (28.S)	14	na	40	2 Strata	No	3	SSB + EggAb	8.22	0.73	12.81	1.56	78.0	2ALK(54)	1042	na	100	Motos & Uriarte 1992
1992	16May-13Jun	31	31	15	2	14	2 Strata	No	3	SSB + EggAb	2.16	0.99	6.50	3.00	91.0	2ALK(31)	745	na	250	Motos & Uriarte 1993
1993	No Survey	none			na	na														
1994	17 May-3Jun	57	35(28.S)	1	17	17	2 Strata	No	4	SSB+Egg+Acoust.	10.25	1.41	24.92	2.43	70.0	Hauls(23)+1ALK(12)	438	33.3	50	Motos et al. 1995
1995	11 - 25 May	48	36(33.S)	8	na	28	Pooled	Yes	2	EggAb	1.46	0.94	5.10	3.49	70.0	1 ALK	552		100	Motos et al. 1996
1996	18 - 30 May	none	na	na	na	na														
1997	9 - 21 May	66	60		24	36	3 Strata	No	3	SSB	1.21	0.18	1.31	1.08	69.0	3 ALK	1556		200	Uriarte et al. 1999
1998	18 May - 8 Jun	69	47		18	29	3 Strata	No	5	SSB + EggAb	3.62	0.80	6.89	1.90	67.0	Hauls(18)+4ALK(29)	1969	40.0	300	Uriarte et al. 1999
1999	22 May - 5 Jun	none	na	na	na	na														
2000	2- 20 May	none	na	na	na	na														
2001	14-May - 8 Jun	61	47	0	25	22	Pooled	Yes	4	EggAb/DF	3.49	0.90	1.92	0.55	56.9	Hauls(24)+1ALK(23)	1021	20.0	80	Uriarte et al. 2002
2002	6 - 21 May	35	35	0	24	11	Pooled	Yes	2	EggAb	1.07	0.28	1.25	1.17	57.6	2ALK(35)	1126		80	Santos et al. 2003
2003	22 May-9Jun	36	36	14	22	0	Pooled	Yes	3	EggAb/DF	2.08	0.73	4.70	2.26	79.0	Hauls(13)+1ALK(23)	639	48.8	125	Santos et al. 2004
2004	2 - 17 May	63	29	14	5	10	Pooled	Yes	3	EggAb/DF	2.74	0.35	3.28	1.20	65.4	Hauls(14)&2ALK(15)	1228	68.5	110	Santos et al. 2005
2005	8 - 28 May	30	23	8	11	4	Pooled	Yes	2	EggAb/DF	1.00	0.02	1.00	1.00	62.1	Hauls(9)&2ALK(14)	775	56.2	100	Santos et al. 2005
2006	4 - 24 May	47	37	3	11	23	Pooled	Yes	4	EggAb	2.62	0.49	4.84	1.84	65.8	Hauls(5)+1ALK(32)	1466	91.0		Santos et al. 2006
2007	3-23 May	44	30	10	0	20	Pooled	Yes	5	EggAb	1.17	0.37	1.00	0.86	68.1	by Hauls (30)	1977	57.5	100	Santos et al. 2007
2008	6-26 May	29	22	22	na	na	Pooled	Yes	2	EggAb	1.27	0.34	1.00	0.79	77.0	by Hauls (22)	1583	72.0	100	Santos et al. 2008
2009	5 - 25 May	38	31	31	na	na	Pooled	Yes	4	EggAb/DF	2.25	0.33	1.91	0.85	60.4	by Hauls (31)	1876	60.2	150	Santos et al. 2009
2010	5 - 20 May	39	36	30	na	6	Pooled	Yes	2	EggAb/DF	1.36	0.41	2.19	1.61	71.8	Hauls(30)&1ALK(6)	1870	74.1	400	Santos et al. 2010
2011	6 - 27 May	49	49	43	na	6	Pooled	Yes	4	EggAb/DF	1.51	0.67	1.99	1.32	68.0	Hauls(41)&1ALK(8)	2837	63.3	200	Santos et al. 2011
2012	10 - 30 May	28	24	24	na	na	Pooled	Yes	6	EggAb/DF	1.90	0.60	1.49	0.78	67.8	by Hauls	1679	67.3	40	Santos et al. 2012
2013	9-28 May	22	21	21	na	na	Pooled	Yes	3	EggAb/DF	1.66	0.45	2.12	1.28	79.4	by Hauls	1661	79.1	40	Santos et al. 2013
2014	5-24 May	48	47	41	na	6	Pooled	No	2	EggAb	1.28	0.34	1.84	1.44	58.4	Hauls&1ALK(6)	2405	58.4	160	Santos et al. 2014

Table 4.12 Series of Anchovy population at age estimates and mean weight and length at age from the DEPM series in the Bay of Biscay. Variance for CVs for the proportions in mass and the biomasses by ages are not available for the 1990-92 and 1995, and the variance for the mean weight and length at age of those years were deduced from the Sd of the length at age distribution (not consistently with current procedures).

	1990	1991	1992	1994	1995	1997	1998	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
W.factors for SSB params?	0	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
W.factors for params by age?	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SSPa Processing (Pool/Stratified)	Stratified	Stratified	Stratified	Stratified	POOLED	POOLED	Stratified	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED
Parameter	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate
BIOMASS (Tons) (SSB)	**74,371**	**13,295**	**60,332**	**37,080**	**36,432**	**28,341**	**77,658**	**75,826**	**22,462**	**16,117**	**11,496**	**4,832**	**22,590**	**13,064**	**12,898**	**12,853**	**31,206**	**135,556**	**26,473**	**53,856**	**89,150**
S.e. & C.V. (%)	0.21	0.27	0.13	0.20	0.16	0.10	0.11	0.13	0.15	0.17	0.12	0.20	0.24	0.18	0.20	0.15	0.14	0.15	0.20	0.18	0.12
Mean Weight (Wt)	**16.12**	**19.91**	**15.36**	**19.88**	**20.74**	**13.97**	**15.69**	**20.21**	**30.24**	**19.19**	**18.25**	**25.18**	**18.25**	**19.21**	**23.20**	**19.42**	**17.77**	**14.22**	**15.53**	**15.89**	**16.97**
C.V. (%)	0.02	0.04	0.07	0.04	0.06	0.07	0.06	0.08	0.05	0.07	0.10	0.06	0.12	0.08	0.09	0.11	0.08	0.10	0.14	0.15	0.07
Population (millions) (SSP)	**4,618**	**671**	**3,986**	**1,864**	**1,764**	**2,030**	**4,965**	**3,761**	**742**	**836**	**632**	**192**	**1,237**	**682**	**559**	**666**	**1,758**	**9,548**	**1,720**	**3,403**	**5,253**
C.V. (%)	0.23	0.26	0.19	0.22	0.17	0.13	0.18	0.16	0.16	0.19	0.18	0.22	0.27	0.20	0.22	0.21	0.19	0.19	0.26	0.25	0.15
Proportion at age 1 (Pa1)	**0.976**	**0.704**	**0.965**	**0.719**	**0.853**	**0.855**	**0.879**	**0.705**	**0.263**	**0.806**	**0.881**	**0.374**	**0.829**	**0.703**	**0.429**	**0.646**	**0.868**	**0.895**	**0.503**	**0.620**	**0.734**
C.V. (%)	0.00	0.04	0.01	0.06	0.04	0.03	0.02	0.07	0.19	0.05	0.03	0.13	0.05	0.06	0.12	0.09	0.02	0.02	0.16	0.14	0.05
Proportion at age 2 (Pa2)	**0.021**	**0.292**	**0.033**	**0.267**	**0.125**	**0.142**	**0.113**	**0.271**	**0.605**	**0.144**	**0.093**	**0.602**	**0.130**	**0.258**	**0.505**	**0.191**	**0.125**	**0.103**	**0.480**	**0.288**	**0.210**
C.V. (%)	0.18	0.10	0.23	0.15	0.25	0.18	0.15	0.17	0.07	0.18	0.25	0.07	0.25	0.14	0.09	0.17	0.12	0.18	0.16	0.19	0.13
Proportion at age 3+ (Pa3+)	**0.003**	**0.005**	**0.003**	**0.014**	**0.022**	**0.004**	**0.008**	**0.024**	**0.131**	**0.050**	**0.027**	**0.024**	**0.041**	**0.039**	**0.065**	**0.164**	**0.007**	**0.002**	**0.017**	**0.092**	**0.056**
C.V. (%)	0.29	0.37	0.54	0.65	0.32	0.54	0.29	0.28	0.13	0.26	0.26	0.37	0.27	0.55	0.17	0.23	0.30	0.38	0.39	0.33	0.18
Numbers at age 1 (SSPa1)	**4,507**	**474**	**3,849**	**1,334**	**1,507**	**1,735**	**4,370**	**2,658**	**195**	**672**	**557**	**72**	**1,024**	**480**	**242**	**432**	**1,527**	**8,541**	**874**	**2,119**	**3,856**
C.V. (%)	0.23	0.28	0.20	0.27	0.19	0.15	0.20	0.21	0.28	0.21	0.21	0.29	0.30	0.22	0.29	0.26	0.20	0.20	0.38	0.37	0.17
Numbers at age 2 (SSPa2)	**95**	**194**	**127**	**505**	**218**	**287**	**556**	**1,014**	**450**	**121**	**58**	**115**	**161**	**175**	**281**	**126**	**219**	**985**	**817**	**976**	**1,105**
C.V. (%)	0.25	0.31	0.20	0.20	0.25	0.15	0.16	0.15	0.15	0.21	0.17	0.21	0.27	0.21	0.21	0.23	0.15	0.21	0.20	0.18	0.15
Numbers at age 3 (SSPa3+)	**12**	**3**	**10**	**26**	**38**	**8**	**40**	**89**	**97**	**43**	**17**	**5**	**51**	**27**	**36**	**108**	**12**	**22**	**30**	**308**	**293**
C.V. (%)	0.44	0.52	0.53	0.24	0.32	0.52	0.32	0.27	0.19	0.26	0.24	0.38	0.29	0.61	0.26	0.20	0.28	0.36	0.47	0.25	0.23
Prop. at age 1 (in mass) (P'a1)	**0.957**	**0.588**	**0.932**	**0.625**	**0.780**	**0.694**	**0.776**	**0.594**	**0.229**	**0.672**	**0.785**	**0.283**	**0.669**	**0.605**	**0.307**	**0.419**	**0.817**	**0.828**	**0.323**	**0.423**	**0.650**
C.V. (%)				0.07		0.05	0.03	0.07	0.19	0.07	0.05	0.15	0.14	0.07	0.17	0.15	0.02	0.03	0.14	0.19	0.06
Prop. at age 2 (in mass) (P'a2)	**0.038**	**0.404**	**0.063**	**0.352**	**0.184**	**0.294**	**0.204**	**0.370**	**0.668**	**0.215**	**0.168**	**0.675**	**0.247**	**0.360**	**0.612**	**0.258**	**0.168**	**0.168**	**0.655**	**0.407**	**0.277**
C.V. (%)				0.12		0.12	0.10	0.10	0.06	0.13	0.21	0.05	0.19	0.12	0.08	0.10	0.10	0.14	0.07	0.12	0.11
Prop. at age 3 (in mass) (P'a3+)	**0.006**	**0.008**	**0.005**	**0.023**	**0.036**	**0.013**	**0.020**	**0.035**	**0.103**	**0.113**	**0.046**	**0.042**	**0.084**	**0.035**	**0.083**	**0.323**	**0.015**	**0.005**	**0.023**	**0.170**	**0.074**
C.V. (%)				0.40		0.41	0.29	0.24	0.19	0.21	0.24	0.38	0.23	0.42	0.18	0.16	0.24	0.36	0.27	0.23	0.14
SSB at age 1 (Tons)	**71,142**	**7,821**	**56,202**	**23,008**	**28,416**	**19,677**	**60,321**	**45,172**	**5,129**	**10,771**	**9,042**	**1,376**	**15,111**	**7,906**	**3,998**	**5,434**	**25,487**	**112,182**	**8,607**	**22,977**	**57,881**
C.V. (%)				0.26		0.12	0.13	0.16	0.24	0.19	0.15	0.26	0.28	0.19	0.27	0.23	0.15	0.15	0.27	0.28	0.14
SSB at age 2 (Tons)	**2,807**	**5,369**	**3,803**	**13,221**	**6,721**	**8,308**	**15,789**	**27,989**	**15,023**	**3,489**	**1,922**	**3,254**	**5,582**	**4,695**	**7,861**	**3,305**	**5,241**	**22,807**	**17,258**	**21,837**	**24,717**
C.V. (%)				0.19		0.15	0.14	0.15	0.16	0.21	0.19	0.20	0.30	0.21	0.21	0.16	0.15	0.21	0.20	0.20	0.15
SSB at age 3 (Tons)	**422**	**106**	**327**	**851**	**1,295**	**356**	**1,547**	**2,665**	**2,309**	**1,857**	**532**	**203**	**1,897**	**463**	**1,064**	**4,113**	**482**	**694**	**609**	**9,045**	**6,555**
C.V. (%)				0.23		0.42	0.31	0.27	0.24	0.27	0.24	0.43	0.32	0.46	0.27	0.20	0.26	0.37	0.34	0.28	0.18

Biological Features				estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate	estimate
Weight at age 1 (g) (W1)	**15.82**	**16.61**	**15.15**	**17.08**	**18.93**	**11.81**	**13.93**	**16.88**	**22.40**	**16.03**	**16.45**	**18.40**	**14.92**	**16.52**	**16.18**	**12.78**	**16.72**	**13.12**	**9.86**	**10.93**	**15.08**
C.V. (%)	0.02	0.04	0.07	0.03	0.02	0.06	0.07	0.09	0.08	0.07	0.10	0.07	0.11	0.09	0.09	0.08	0.08	0.11	0.20	0.15	0.07
Weight at age 2 (g) (W2)	**29.59**	**27.87**	**31.05**	**25.75**	**31.86**	**26.73**	**28.61**	**27.38**	**33.19**	**28.51**	**33.18**	**27.01**	**34.33**	**26.29**	**27.99**	**25.17**	**23.87**	**23.38**	**21.13**	**22.39**	**22.15**
C.V. (%)	0.02	0.02	0.03	0.03	0.11	0.08	0.08	0.03	0.04	0.08	0.08	0.05	0.05	0.06	0.07	0.12	0.04	0.06	0.07	0.09	0.06
Weight at age 3 (g) (W3+)	**34.71**	**33.71**	**33.99**	**33**	**35.65**	**37.86**	**37.19**	**29.62**	**35.73**	**42.96**	**32.14**	**42.35**	**36.85**	**17.90**	**30.24**	**38.81**	**39.54**	**31.47**	**19.25**	**28.90**	**22.18**
C.V. (%)	0.02	0.02	0.04	0.03	0.21	0.70	0.29	0.05	0.14	0.07	0.20	0.17	0.09	0.14	0.09	0.07	0.07	0.09	0.22	0.06	0.12
Lenght at age 1 (mm)	**13.69**	**13.67**	**13.44**	**13.36**	**14.24**	**11.40**	**12.67**	**13.30**	**14.92**	**13.48**	**13.26**	**14.08**	**12.85**	**13.35**	**13.46**	**12.38**	**13.62**	**12.52**	**11.34**	**11.89**	**13.14**
C.V. (%)	0.01	0.01	0.02	0.01	0.05	0.06	0.02	0.03	0.06	0.03	0.03	0.05	0.04	0.03	0.03	0.02	0.03	0.04	0.07	0.05	0.02
Lenght at age 2 (mm)	**16.70**	**16.03**	**16.58**	**15.40**	**16.76**	**15.82**	**15.92**	**15.63**	**16.84**	**15.96**	**16.55**	**15.74**	**16.82**	**15.63**	**15.78**	**14.90**	**15.24**	**15.15**	**14.76**	**14.81**	**14.73**
C.V. (%)	0.01	0.01	0.01	0.01	0.06	0.06	0.08	0.01	0.03	0.08	0.08	0.03	0.04	0.02	0.02	0.04	0.01	0.02	0.02	0.03	0.02
Length at age 3 (mm)	**17.53**	**16.87**	**17.07**	**16.82**	**17.35**	**17.71**	**17.42**	**15.97**	**17.23**	**16.24**	**16.31**	**17.88**	**17.17**	**14.19**	**16.15**	**17.23**	**18.15**	**16.87**	**14.03**	**16.17**	**14.59**
C.V. (%)	0.01	0.01	0.01	0.01	0.06	0.54	0.29	0.01	0.12	0.10	0.16	0.16	0.09	0.04	0.03	0.03	0.02	0.03	0.07	0.02	0.04

4.3.2.2 Series of DEPM Population and Pa estimates

The series of biomass and population at age reveal the dominant role of the 1-year-old recruits in sustaining the anchovy population in the Bay of Biscay (Figure 4.4, Figure 4.5 and Table 4.12). Old age classes (ages 2 and 3+) are usually a minor component, although its relevance is a bit larger when examined in terms of mass instead of numbers (Figure 4.5). Nevertheless, old age classes are rarely dominant; this occurs only after the succession of very big and bad recruitments as in 2001/2002 or 2011/2012.

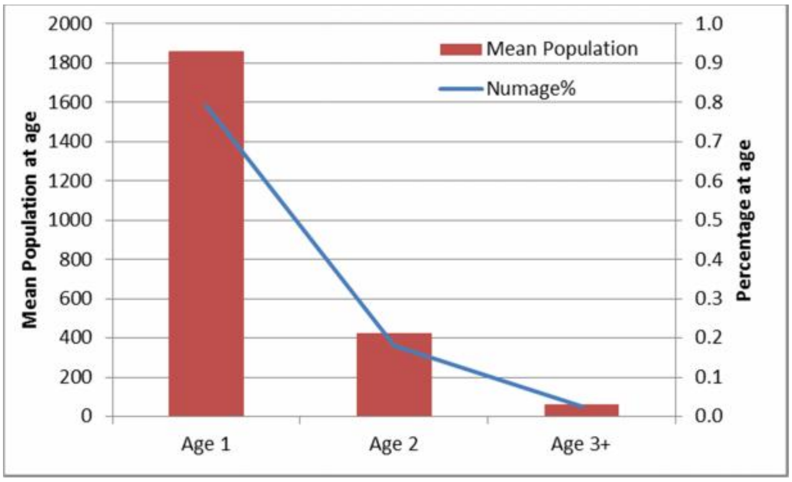


Figure 4.4: Mean population at age estimates in the period 1990-2013 and respective proportions at age.

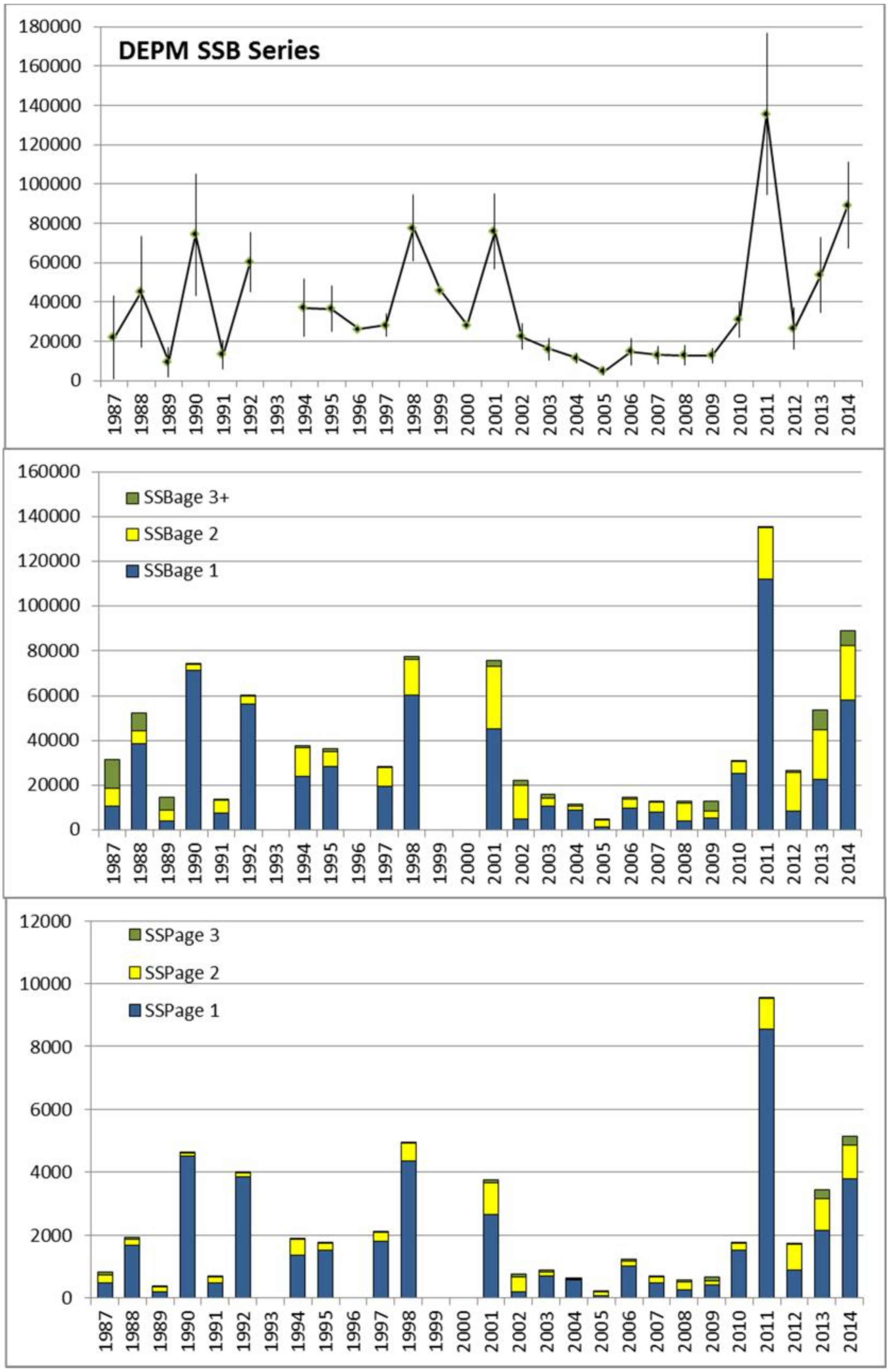


Figure 4.5: Series of estimates of anchovy spawning biomass (SSB) by the application of the DEPM in the bay of Biscay (upper panel, with 95% confidence intervals), series of spawning biomass by age (middle panels) and series of population in numbers at age (bottom panel).

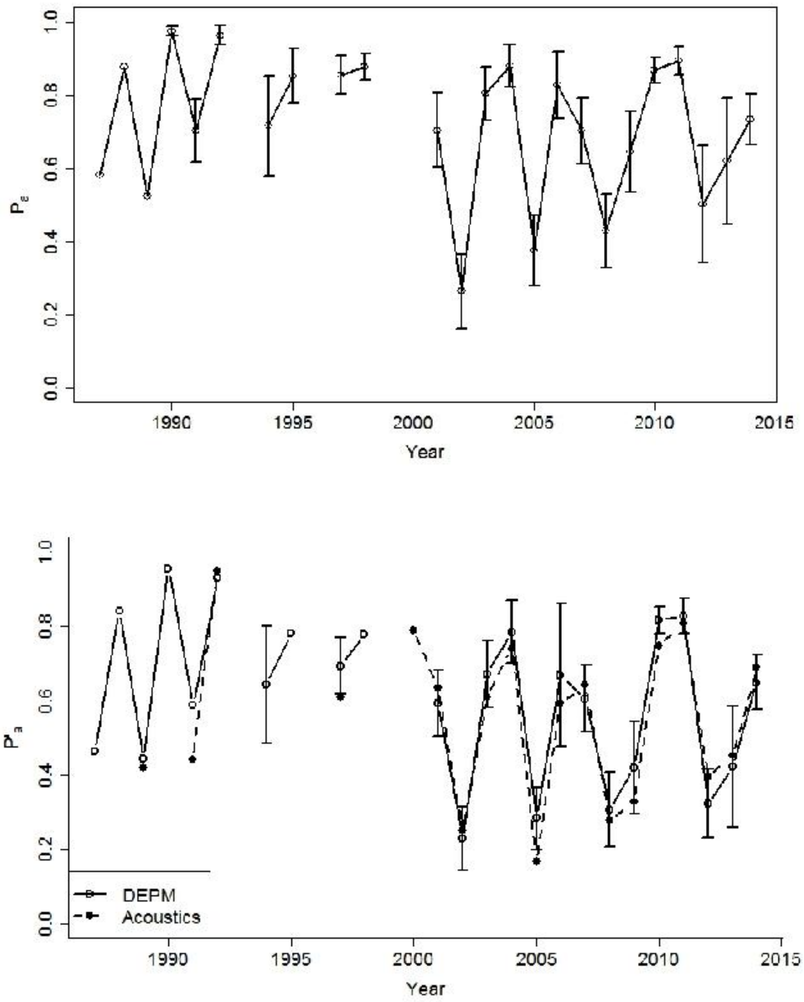


Figure 4.6: Series of proportions at age in the anchovy population estimates by the application of the DEPM in the Bay of Biscay with 95% confidence intervals, in numbers (***Pa*** -- upper panel,) and in mass (***P'a*** bottom panel) (here included the results of the parallel French Acoustic survey Massé et al in press; ICES 2014).

The series (Figure 4.5) also shows the large inter-annual variability of the 1-year-old recruits, typical of the small pelagic species. Such variability is also reflected in the yearly changes of the proportions at age 1 in numbers and in mass in the population (Figure 4.6). Standard errors of the proportions at ages 1 and 2 are almost identical in absolute terms due to the very little proportion of age 3, this leads in general to low CVs for the ages with highest proportions in the population (generally age 1) (Table 4.12). Therefore the lower the Pa the higher the CV estimates **(**Figure 4.7).

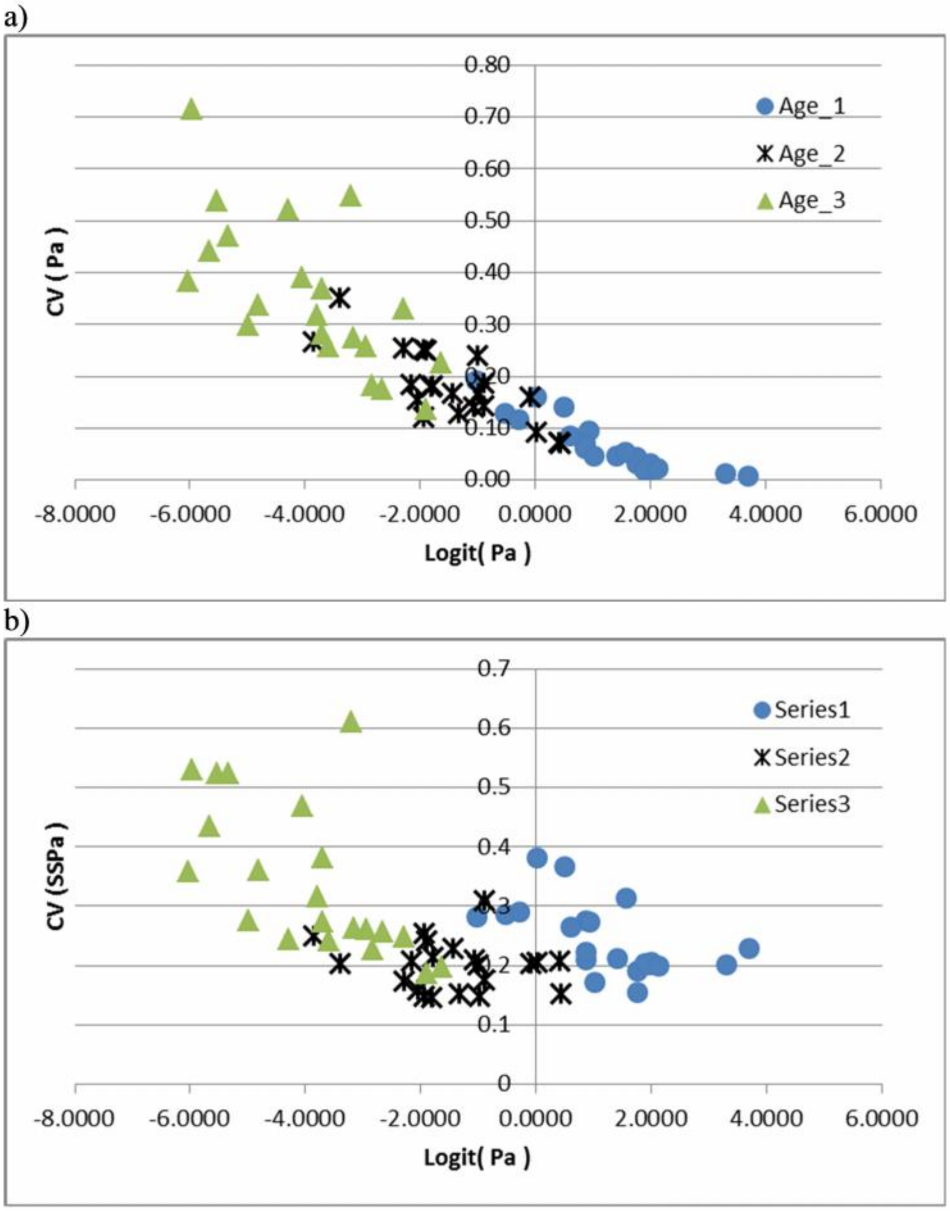


Figure 4.7: Estimates of Coefficients of Variation (CV) of the proportions at ages (Pa) (a) and of the population at age (SSPa) (b) over the entire area (pooled procedure) as function of the logit of Pa.

When ALKs are used, the major component of the variance of the proportions at age is still the cluster variance which usually accounts for about 80 to 100% of the total variance for all ages with values of Pa above 0.2. Below this value the contribution from the ALK tend to increase as Pa tends to 0, though rarely exceeding 50%. Nevertheless the ALK contribution will depend as well on the actual size of the ALK.

The two terms of the cluster variance are positive for Pa values below 0.7, but the second term tend to be negative for higher values of Pa (as was the case in 2009 in the Garonne region). This means that at regions with homogeneous high Pa between samples the overall level of cluster variance can sharply decrease.

The CVs of Pa are generally below 0.2 for age 1, below 0.3 for age 2 and usually below 0.5 for age 3, though in cases of Pa below 0.05 the CVs can exceed 0.5 (Figure 4.7).

The confidence intervals (CI) of the *Pa* estimates included most of the times the independent estimates of *Pa* provided by the parallel French acoustic survey (see Figure 4.6 for *P'a1*, where in 12 out of 15 cases the CI contained the acoustic *P'a1* estimates).

The CVs of the Population at age estimates remain generally below 0.3, particularly for the two main age groups (which have a CV mean of 0.22). The CVs of SSP at age 3 can increase above 0.3 as a result of the poor precision of the Pa estimate for Pa<0.05 (Figure 4.7b). Large CVs (above 0.3) associated to the estimates of the population at age 1 in 2012 and in 2013, correspond to relatively low Pa1 of 0.50 and 0.62 and high spatial variability in the mean weight at age and in Pa1, which lead to some of the highest CV in the series affecting these parameters.

Very similar results were obtained for the CVs of P'a and SSBa.

The actual amount of otoliths read is about 10 times the equivalent effective sampling size (Table 4.11). This reveals that our surveys are not efficient compared to a random sampling of ages (under the assumption of multinomial distribution). However, such inefficiency derives from the cluster aggregations of fishes in schools (they tend to be of similar sizes and ages compared to the population).

4.3.2.3 Sensitivity of the Population at age estimates to the weighted procedure

Weighting is required to obtain unbiased estimates in the presence of unbalanced sampling. If an unweighted procedure would have been applied to the estimates over the entire area (pooled processing) then Population at age would have been changed in amounts ranging between 26% and -44% (Table 4.13). It is evident that relative changes induced on the final population at age estimates arise from the addition of the changes appearing in the total population and those in the proportions at age. This derives from the estimation procedure in Eq. 4.3. The direction (increase/decrease) and intensity of the changes induced by the unweighted procedures depends upon the degree of departure of the actual sampling from the PPS sampling. The larger the departure, the larger the impact on the final estimate of Population at age will be. Figure 4.8 shows such effect for SSPa1. In that figure, the ratio of mean sample weighting factors of the regions with the smallest fish sizes (M.small) over the overall mean *M'* of all samples is used as indicator of the departure from PPS. When the ratio is greater than 1, it means that the regions with the smallest fishes were under-sampled in comparison with the remainder areas for the biomass occupying these regions. In those cases the unweighted procedures would lead to increase *Wt*, decrease *SSP*, decrease *Pa1* and ultimately decrease *SSPa1* (negative effect) (Figure 4.8), with gradually increasing impacts for the respective parameters. When oversampling of these areas occurs the effect is reversed.

Table 4.13: Sensitivity of the Anchovy population at age estimates to adopt an unweighted procedure in terms of relative change over the standard weighted procedure.

	Indices of sampling balance		Sensitivity to UN-Weighting (% Change of)			
Year	CV(M`)	M.Small/M.Mean	Δ% SSB	Δ% SSP	Δ% Pa1	Δ% SSPa1
1990 (May)	0.93	1.85	-1.7%	-18.7%	-3.7%	-21.7%
1991	0.73	1.56	4.6%	-5.4%	-10.1%	-15.1%
1992	0.99	3.00	-8.8%	-26.5%	-4.8%	-29.9%
1993						
1994	1.41	2.43	-7.2%	-19.4%	-31.4%	-44.6%
1995	0.94	3.49	-1.9%	-15.6%	-15.9%	-29.0%
1996						
1997	0.18	1.08	-1.1%	-5.8%	-1.9%	-7.6%
1998	0.80	1.90	-10.2%	-34.7%	-6.8%	-39.2%
1999						
2000						
2001	0.90	0.55	1.7%	5.3%	3.7%	9.0%
2002	0.28	1.17	0.3%	-2.1%	-7.5%	-9.2%
2003	0.73	2.26	-3.5%	-19.4%	-14.3%	-30.7%
2004	0.35	1.20	-2.4%	-6.8%	-2.2%	-8.9%
2005	0.02	1.00	-1.1%	3.2%	9.8%	13.0%
2006	0.49	1.84	-5.1%	-19.6%	-7.8%	-25.7%
2007	0.37	0.86	0.3%	10.8%	5.0%	16.2%
2008	0.34	0.79	1.4%	12.6%	12.5%	26.0%
2009	0.33	0.85	3.0%	13.0%	9.5%	23.4%
2010	0.41	1.61	-6.4%	-15.5%	-3.2%	-18.2%
2011	0.67	1.32	-2.1%	-5.2%	-0.9%	-6.0%
2012	0.60	0.78	-1.6%	4.2%	5.2%	9.1%
2013	0.45	1.28	-1.7%	-6.3%	-3.2%	-9.4%
2014	0.34	1.44	-2.4%	-8.3%	-5.5%	-13.2%

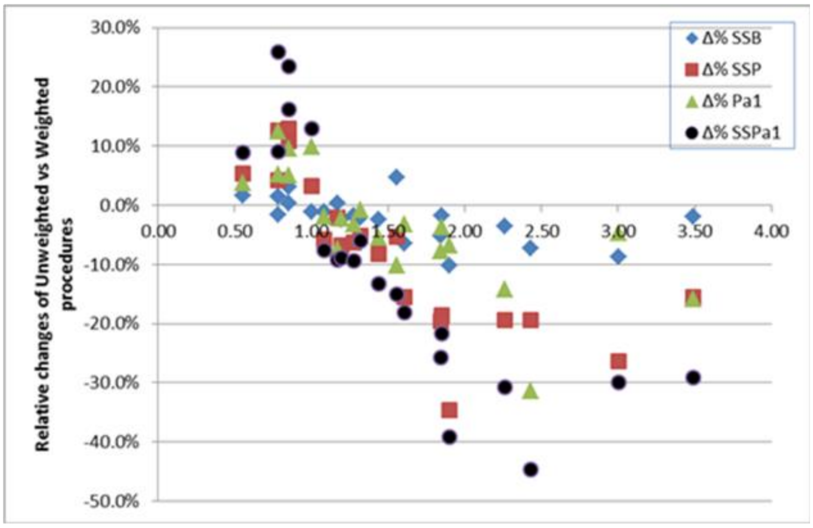


Figure 4.8: Relative changes of the estimates of SSB, SSP, Pa1 and SSPa1 induced by adopting an unweighted procedure instead of a weighted procedure for the estimation of the DEPM parameters over the whole surveyed area (pooled single strata processing). The X axis reflects the divergence of the actual sampling from a PPS sampling as reflected by the Ratio of mean individual weighting factors of samples in the regions with the smallest fish sizes ((M.small) over the overall mean M. A ratio of 1 corresponds to the PPS sampling.

The bias of the estimates throughout the whole series was similar to those mentioned in the 2009 example, i.e. generally below 0.2 times the standard error, and hence they could have been neglected.

4.3.2.4 Mean weight and length at age estimates

Figure 4.9 and Figure 4.10 show the series of anchovy weight and length at age estimates throughout the whole series of the DEPM application. No major tendencies appear for any age group. Mean weight CVs are on average around 10% and usually below 20% for all ages, though for age 3 in particular years (with very low Pa3) CV can exceed that value (Figure 4.11a). Based on equation 4.16, cluster contribution to the variance of mean weight at age is proportional to Pa, whilst the remaining contribution is basically due to the ALK (the lower Pa the higher the ALK contribution to the variance). In all cases the contribution from the variance of weight within length class is negligible (on average 0.6%).

Mean length CVs are on average around 6% and usually below 10% for all ages, though for Pa lower than 0.2 CVs can reach higher values (Figure 4.11b). The confidence intervals, in most of the years, include the estimates produced by the independent parallel French acoustic survey (Duhamel *et al.* 2014).

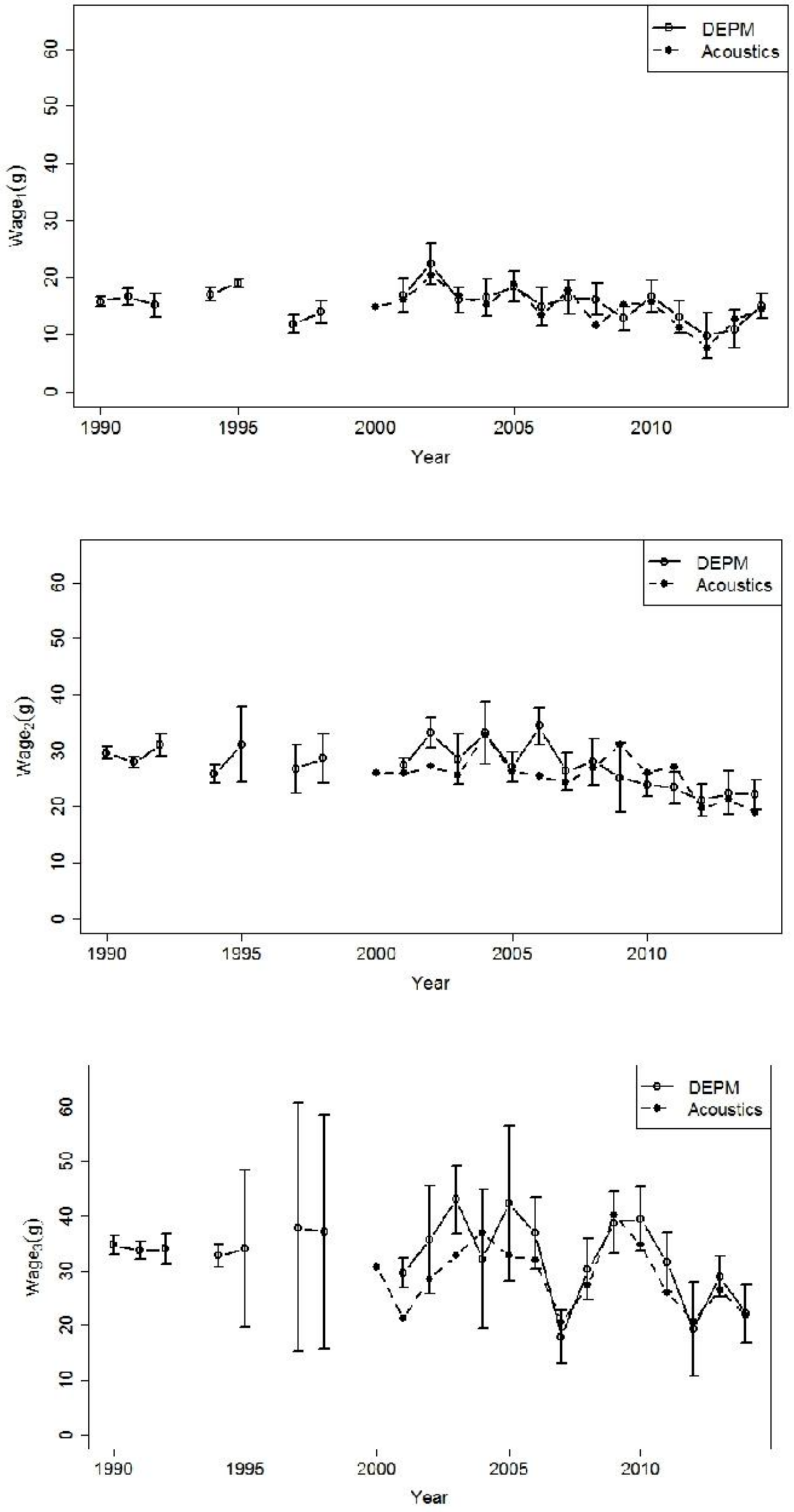


Figure 4.9: Anchovy mean weight at age series with confidence intervals from the application of the DEPM in the Bay of Biscay, overlaying the results of the parallel French Acoustic survey (Duhamel *et al.* 2014; ICES 2014) (before 1997 CVs were not estimated consistently with current procedures).

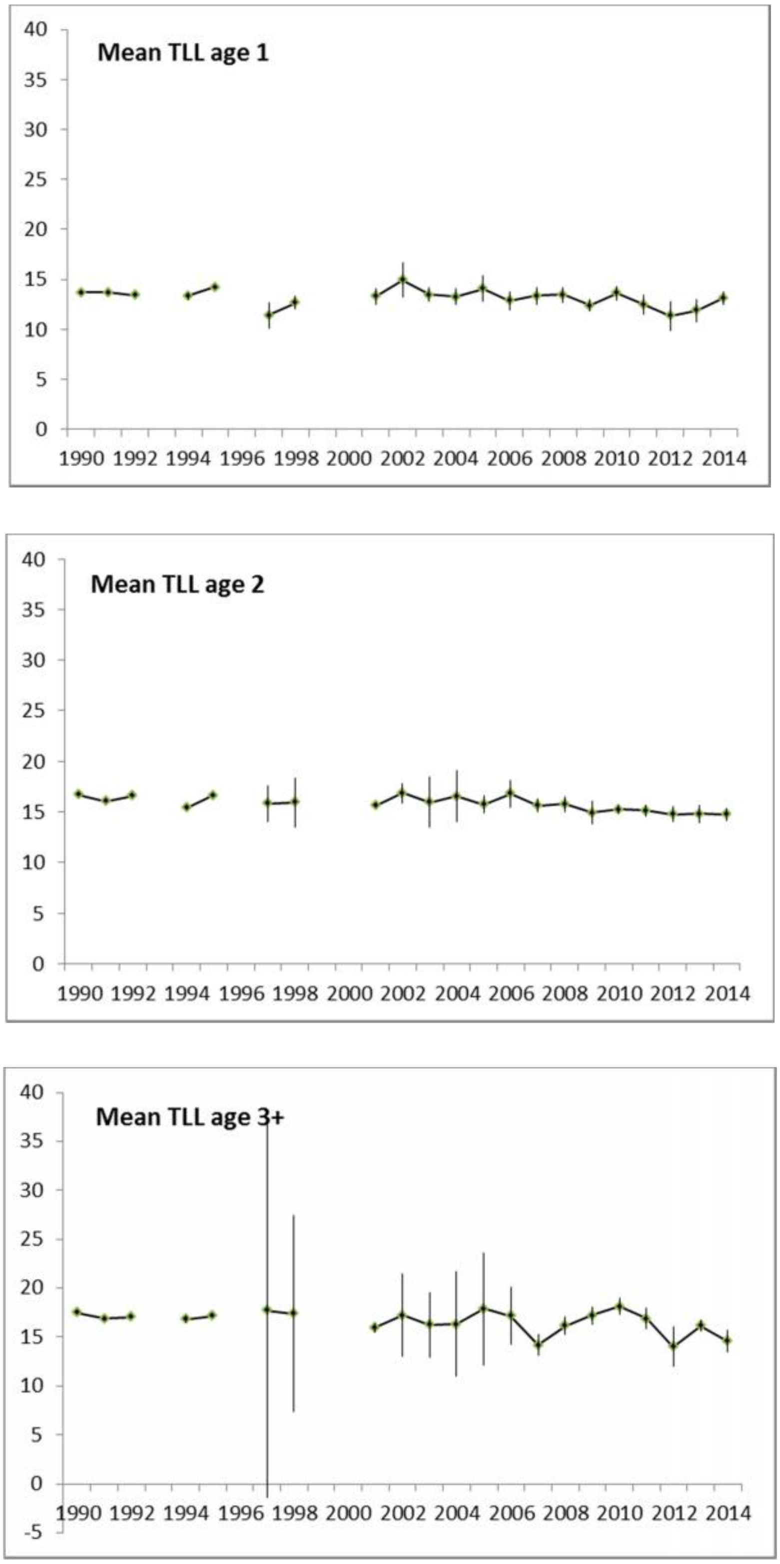
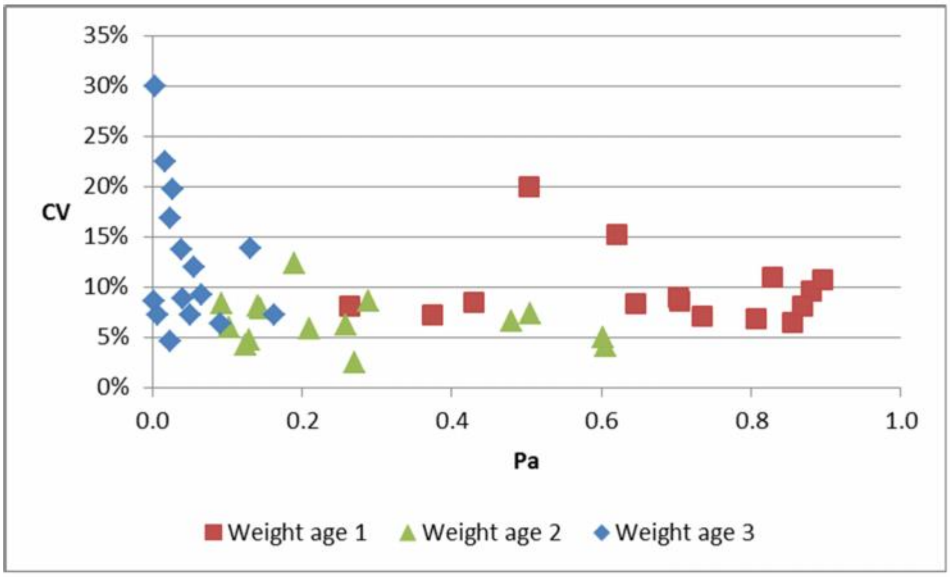


Figure 4.10:Anchovy mean length at age series with confidence intervals from the application of the DEPM in the Bay of Biscay (before 1997 CVs were not estimated).

a) CV of mean weight at age



b) CV of mean length at age

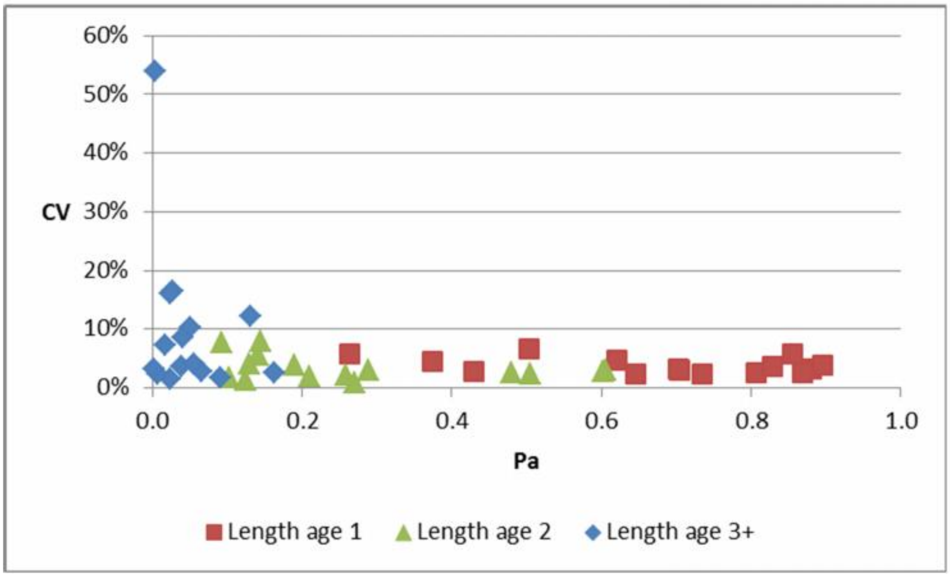


Figure 4.11: CVs series of the mean weight at age (a – Upper panel) and mean length at age (b- bottom panel) derived from the application of the DEPM in the Bay of Biscay in years 1997 and 2001-2014.

4.4 Discussion

4.4.1 Sampling design and weighting procedures

The applicability of the current method to extend DEPM to population at age estimates relies on achieving a good spatial coverage of the population to capture the potential spatial heterogeneity of the concerned parameters. Furthermore sampling proportional to fish abundance (PPS sampling) is desirable to allow for unbiased estimates by unweighted means of observed parameters by samples. However during DEPM surveys judgement sampling is typically applied according to the indications of fish abundance along cruise track (from eggs, acoustics etc); the statistical properties of such approach are unclear (Jessen 1978). In the DEPM surveys in the Bay of Biscay, we have usually carried out direct fishing either from the egg sampler boat or from a different chartered boat. But in order to achieve a good coverage of the stock in space we have traditionally included samples from several other sources concomitant with our egg sampling. This has generally assured good spatial coverage of the stock but has complicated at the same time the scheme of sampling, becoming usually not PPS.

In statistical theory the probability of inclusion of any sample in the sampling should be preferably known in advance. However for surveys of pelagic resources the limitations of ship time and spatial aggregation pattern of these resources make hard to know such probability in advance. This affects the efficiency and design of the adult sampling. For a non-balanced sampling (not PPS) of spatially heterogeneous reproductive and/or size /age parameters of fishes, inclusion of proper weighting factors of adult samples is compulsory to get unbiased estimates (Lhor 2010). Usually, if the variability is clearly spatially structured, then stratification of the DEPM survey estimates is the usual practice chosen to maximize homogeneity of the parameters and in those cases the implications of not choosing proper weighting factors within strata is probably minimized and may become irrelevant. But, in some cases stratification may not be possible or some heterogeneity of the parameters is still noticeable within strata and therefore proper weighting of samples will still be desirable to produce unbiased estimates.

The PPS sampling is one of the most common (and usually not checked) assumptions adopted in the application of the DEPM, as for instance with the northern anchovy

(Picquelle & Stauffer, 1985, Bidman, 1986), Peruvian anchovy (Santander *et al.* 1984), anchovy in the Western Mediterranean sea (Palomera & Pertierra, 1993), and the sardine around the Iberian Peninsula Spain (Perez *et al.* 1989; Angelico *et al.* 2015), etc. However the actual judgement sampling achieve by the surveys are often not PPS and in those cases proper estimates of cluster sizes (*Mi*) should enter the parameter estimators in order to get unbiased population reproductive parameters (Hampton 1990). For instance Armstrong et al (1988) found that adult sampling in the DEPM surveys for the southern Benguela anchovy was not sufficiently balanced (not PPS) and acoustic anchovy abundance indexes along transect lines were used to give relative estimators of cluster size. In the 1994 the anchovy DEPM survey made use of acoustic indexes (provided by the parallel French acoustic survey) to allocate cluster sizes to the samples within a given strata.

In the DEPM survey series we have made use of regional egg abundances, directly or scaled by the regional daily fecundity estimates, to produce regional indicators (proxies) of biomass which allow assessing the actual probabilities of inclusion of samples by regions. This allows testing whether the actual sampling was proportional to size (PPS), or not, and inferring weighting factors (cluster size) for the individual samples by regions for the estimation of parameters. This weighted procedure of cluster samples is statistically sound and lead to unbiased estimates as it corresponds with the Hansen–Hurwitz estimator (1943, in Cochran 1977; Lohr 2010). Furthermore an indirect proof of this comes from the fact the DEPM *Pa* and *P’a* estimates were very consistent with the estimates provided by the parallel French acoustic survey on anchovy in the Bay of Biscay (Massé *et al. in press*), as expected from two unbiased estimators methods.

The sensitivity of the DEPM estimates to weighting is minima for SSB and gradually increases through SSP, Pa to SSPa. In the anchovy DEPM series the sensitivity of SSB ranged between 5% and -10%, whilst for SSPa it ranged between 26% and -40%. The little sensitivity of SSB estimates to the actual weighting of samples is due to the fact that DF does not change much in space or across anchovy sizes. This relates with: a) On the one hand, spawning frequency shows little dependency on fish ages or sizes (Uriarte *et al.* 2012) and b) On the other hand, the spatial variability in Wf is counterbalanced by the parallel variability in F and as such F/Wf is rather constant in space even for a spatially size structured resource. Even in cases where DF differs statistically between regions, as in the case study of 2009, the differences are not major and hence the final differences between weighted and unweighted means are moderate (around 3% in the 2009 example). This makes the DEPM a robust estimator of SSB, as a result of little variation of DF across size or age classes. This is certainly valid for the case of the Bay of Biscay anchovy but furthermore this feature was one of the basic assumptions for the development of the Daily Egg Production Method (Parker 1980; Picker and Stauffer 1985; Stratoudakis *et al.* 2006).

Alternatively, the population at age estimates SSPa are highly sensitive to the use of correct weighting factors, because they depend directly on the sample means of fish weight (*Wti*) and proportions at age (*Pai*), both typically structured in space. Hence weighting factors proportional to the abundance the samples represent become essential to obtain unbiased *Wt*, *Pa* and *SSPa*. Furthermore we have showed how relative errors in SSP (*SSB/Wt*) and Pa propagate in a cumulative manner into the errors of *SSPa*. Certainly from statistical theory the unweighted means of clusters of unequal size are biased roughly in proportion to the covariation of cluster size with the variables (Cochran 1977, Pennington and Helle 2011). In our case such covariation beween *Mi* and *Wti* and *Pai* is present, because regionalization and allocation of weighting factors by samples was designed to cope with spatial variability of these parameters for the unbalanced sampled of the survey. In our surveys quite often the regions with smaller sizes (and higher *Pa1*) have lower density of sampling per mass than the remainder regions and it led to assign bigger *M'i* to those samples.

4.4.2 Population and Proportions at age estimates and variances

The population at age estimates produced by the DEPM survey showed since the beginning of the series that 1-year-old fishes are usually the major component of the spawning population (Motos *et al.* 1991; Uriarte *et al.* 1996). Furthermore, the yearly variability of the recruitment at age 1 is well reflected in the *Pa* series.

The estimates of *Pa* were rather precise with CVs usually well below 0.3, particularly for the two dominant ages classes (ages 1 and 2), whilst for age 3 they can become higher particularly when very poorly represented (for *Pa*<0.05). The latter is due to the fact that the lower the *Pa* the harder is its estimation, resulting in imprecise estimates. The variance of *Pa* is mainly due to the cluster component (or aggregation effect of ages within clusters, i.e. similarity of ages within schools), which usually account for about 80-100% of the variability, as reflected in the analysis of years using ALK estimates. Usually both components of the cluster variance (two first terms of Eq. 4.12) are positive, this being an indication of the need (and appropriate election) of the cluster approach to estimate such variance. For very dominant age classes (of Pa>0.8) there is usually a great homogeneity of the age abundance (and lengths at ages) across samples and this leads the second term Eq. 4.12 (the covariation of lengths by samples) to become negative, reducing thus sharply the overall cluster variance (and hence the overall CV) (as shown for the Garonne area in the case example of 2009). For the high amount of otoliths supporting our ALKs, the contribution of the ALK component to the final variance was very small (usually below 20%). Its contribution becomes relevant only for Pa below 0.2, but even in that case its contribution remains usually below 50% of the final variance.

Certainly, in order to estimate the contribution of the ALK to the variances of Pa we have made the assumption that the distribution of ages within length classes follows a binomial distribution. However in pelagic fishes the clumped distribution of fish by sizes in schools (clusters) could affect the age distribution by lengths as well, diverging in this way from the original assumption of binomial distribution within lengths. Furthermore, sampling the otoliths is rarely done independently in each length class, but available samples do provide otoliths for several contiguous length classes. As such otolith sampling is probably not sufficiently independent. In these circumstances, if ages are clustered, the ALK cannot avoid cluster effects present in the available samples for otoliths, enlarging thus the actual variance associated to the ALK compared to that derived from the assumption of binomial distribution. This is exacerbated if the ALK is constructed from few samples providing a lot of otoliths. All these considerations generate what is called “overdispersion” (Kvist *et al.* 2001) and gradually deviate from the standard assumption of multinomial distribution of ages by lengths in the ALK, and require more complex approximations than the ones considered here. These considerations have pushed other authors to proceed in a simulation context abandoning the analytical approaches followed in our work (Morton and Bravington 2008; Aanes and Vølstad 2015) or simply to assure collection of otoliths in all sample hauls as we tend to do in recent years. For the 2009 example we showed some reduction of variances for the ALK based estimation compared to that based on actual observation of ages by samples, which is probably indicative of some overdispersion occurring between samples, not sufficiently compensated with the additional component of variance from the ALK, based on the multinomial approach.

The comparison of the actual amount of otoliths we read with the equivalent effective sampling size revealed that our survey was not efficient compared to random sampling of ages, as a result of the cluster aggregations of fishes of similar sizes and ages by schools (the so-called positive intra-cluster correlation -- Cochran, 1977). This implies, at the same time, that there would be no practical (and cheap) procedure of managing such random sample of ages due to the aggregative behaviour of fishes (Pennington and Helle 2011).

Population at age estimates: The precision of the estimates of the Population at age was satisfactory as well, as the CVs remained generally below 0.3. They do not decrease as Pa increases but stay on average around 0.22 for the two main age groups. This happens because the variance of SSPa depends, in addition to the variance of Pa, on the variances of SSB and Wt. For the age classes poorly represented however the CV of SSPa can increase above 0.3 as a result of the increased variance of Pa (for Pa<0.05).

All former comments on the estimates and CVs of Pa and SSPa applied similarly to the P’a and SSBa estimates.

4.4.3 Mean weight and length at age estimates and variances

The biological sampling during the DEPM has allowed to directly estimate the mean weight and length at age of anchovies. Currently this is the longest available series of these population biological features for this population. The CVs of the weight and length at age estimates are rather precise, generally below 0.2, with few exceptions for the very poor abundant year classes (mainly at age 3, with Pa<0.05). For the estimation of variances using ALK we consider sufficient the estimates produced by equation 4.16. When using ALK for the estimation of these parameters with variances, Eq. 4.15 resulted as well in consistent low CVs for the most abundant age classes. However those CVs sharply rose up for the most rare age classes (with *Pa* usually below 10%), due to the increase in the ALK variance contribution (inversely to Pa Figure 4.12). At very low Pa, the uncertainties surrounding the ALK and the length at age distributions might be too high, so that little advantages can be obtained by pursuing such approach for the estimation of the mean and variances by ages. Usually a few individual fishes have been collected of those age classes and in such cases a simple mean and standard error of their weights and lengths (as if they had been taken randomly) might be a sufficient approach.

Part of the yearly variability observed in the mean weight and length at age of the population results from the spatial heterogeneity of these parameters (perhaps coupled to some differential spatial dynamics of the stock). Typically the age structure of the population in the main nursery area (the Gironde region- Motos *et al.* 1996) will very much affect the final estimates of the biological features by age because in this area anchovies usually have smaller sizes than in the remainder regions. For instance in 2009 there was a significant proportion of age 2 anchovies in the Gironde region (Pa2=0.19) with mean weight about half the estimates in the rest of the area. As a result of this mean weight at age 2 in 2009 was well below average and its CV was the highest in the series. In relation to this the CV of the mean weights at age obtained from the single pooled ALK over the whole surveyed area (despite the observed differences in the ALK by strata) (in Table 4.10) leads to underestimate the actual variance of the Wa either from equation 4.15 or 4.16 compared to that obtained from the clusters observations (Eq. 4.9). This means that the spatial heterogeneity is being reduced by the single ALK for the pooled area processing. Therefore the actual data show overdispersion of Wa among clusters beyond their differences in length distributions.

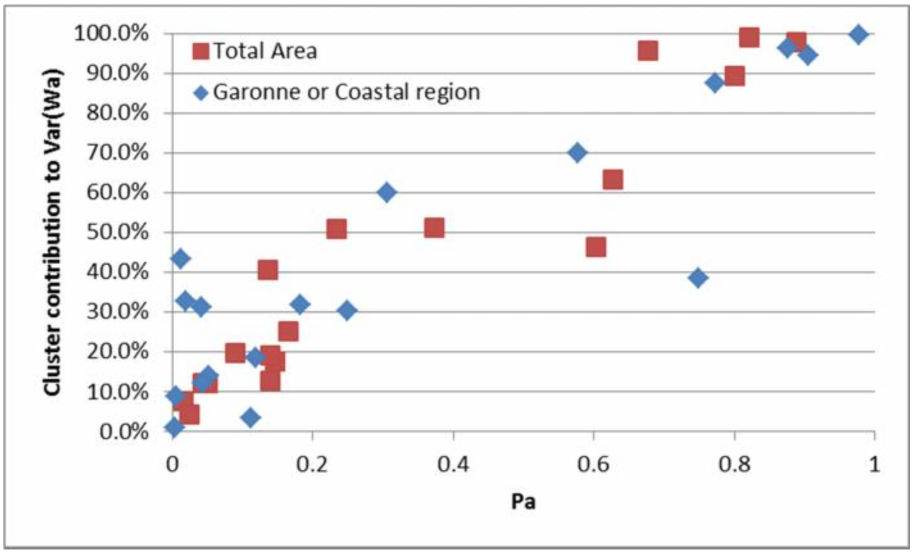


Figure 4.12: Cluster contribution to the Variance of the Mean weight at age as a function of Pa for a procedure based on ALK for estimates over the entire area or restricted to the Coastal or Garonne region. The complementary contribution is basically the ALK contribution. Values correspond to exercises with the surveys made in 1994, 2002, 2003, 2004, 2006 and 2009.

These results evidenced that behind the synoptic population parameters and biological features of the stock reported in this paper there is quite big spatial heterogeneity worth of detailed examination. Some research on the spatial dynamics of this stock and the heterogeneity of biological parameters in the space has already been carried out (Vaz *et al.* 2002; Lehuta *et al.*, 2010; Ibaibarriaga *et al.*, 2013). However such kind of research was out of the scope of this work.

4.4.4 Applicability of the method to other resources and alternative approaches

In spite that, based on the adult sampling, DEPM surveys could have been naturally extended, as shown above, to produce Population in numbers at age or simply biomass at age, this has been rarely pursued or implemented. The reason for not extending the DEPM up to the estimates of population or biomass at age is probably the limited adult sampling often achieved during EPM surveys, or the complicated design of such sampling.

The only application we know producing estimates of the population in numbers (not at age) is the one on Baltic sprat (Krauss and Koster 2004; Haslob *et al.* 2012) which have developed a length structured daily fecundity estimation per fish in the presence of length dependent mature population. This alternative formulation of the DEPM produce estimates of the total stock (mature and immature) in the surveyed spawning areas. The approach is close to the one proposed here when maturity is stratified by length (using Eq. 4.5 but with the adult parameters per fish, not by mass, resulting from a weighted mean across lengths, see also Appendix 3). The method worked well and gave results globally consistent with an acoustic survey. We have preferred to expand the DEPM SSB with another parameter (*Wt*) (and with the proportion of mature fishes *TPm'*, in the presence of partial maturity) to infer *SSP*, instead of that alternative, because we decided to pass through the SSB estimate produced by the original DEPM formulation which is shown to be robust to the actual weighting factors of the samples. In this way, we estimate populations (total or by age) as an expansion of the robust SSB estimate of the original DEPM formulation.

Certainly situations of incomplete maturity are often encountered for many engraulidae and cupleoids (Ganias *et al.* 2003; Kraus and Koster 2004; Silva *et al.* 2006) and scombroids as mackerel or carangidae as horse mackerel (Abaunza *et al.* 2003), where some of the youngest ages occurring in the spawning grounds are immature. The process of defining the fraction of mature individuals has to be well established and documented since the beginning in order to avoid potential bias (Brown-Peterson *et al.* 2011; Lowerre-Barbieri *et al.* 2011). Usually macroscopic maturity scales or gonad somatic index criteria are applied to define the set of potential mature fishes appearing in a random sample from a fishing haul. However, a careful calibration of these indicators of maturity is required for a proper use of them (Dickerson *et al.* 1992; Somarakis *et al.* 2004b; Costa 2009; Ferrery *et al.* 2009). In our opinion, application of these criteria to define the fraction of mature individuals should assure that almost no mature fish will be allocated to the immature fraction. Subsequently histological examination of gonads would establish if any of the retained females should be joined with the immature fraction of the sample. Following this practice population estimates of all mature and immature fishes can be straight forward deduced by the methods presented in this paper.

Application of partial maturity at age to SSPa over ages for which maturity is relatively high (for instance higher than 30%) has the advantage of obtaining reliable age structure of the total population older than a threshold age from the survey which is a direct age structured index of abundance for a subset of ages directly useful as input in most of the age structured assessment modelling frameworks, i.e. not an aggregate SSB index dependent on the general maturity ogives in the assessment. For age classes with very low percentages of mature fishes, the reliability of the survey to estimate the total population at those ages would be poor, as the reliability of low percentages is usually poor. In the extreme nothing can be said of an age class fully immature except if it were entirely distributed at the spawning grounds randomly mixed with the adults, something seldom happening (Lowerre-Barbieri *et al.* 2011).

4.4.5 Final considerations

We have shown that DEPM can naturally be extended to produce population at age (in numbers or biomass) provided adult sampling sufficiently covers the spatial distribution of the spawning stock. Since the beginning of the implementation of the DEPM on anchovy in the Bay of Biscay, the survey included among its objectives that of obtaining population at age estimates, in addition to biomass, as a way to enhance its contribution to the assessment of the stock. The survey has featured the typical biological characteristics of this short-lived species, as for instance its fluctuating nature because of being sustained basically by the 1-year-old fishes (Motos *et al.* 1991; Uriarte *et al.* 1996; Motos *et al.* 2005: Santos *et al. in press*). Since then the survey biomass and population estimates are two pillar inputs of the integrated assessment carried out by ICES; and the CVs of the *Pa* were used to guide the formulation of priors of the Integrated Bayesian assessment (Ibaibarriaga *et al.* 2008; 2011; ICES 2014).

The good agreement between DEPM and Acoustics estimates of the percentages at age (*Pa*) in the population gives confidence on the unbiased application of the two methods. Such comparison only depends on the relative spatial distribution of the population and consistent age determinations. Therefore this comparison was the one selected for this study. However the agreement in terms of Population at age estimates was poorer (not shown) as they depend on the final Biomass produced by the methods, and for this anchovy the absolute levels of the acoustics tend to be higher than that of the DEPM, as evidenced in the integrated assessment of the stock (Ibaibarriaga *et al.* 2013; ICES 2014). Nevertheless the relative tendencies shown by the two series are generally parallel, showing a statistically significant correlation (For total SSP r=0.64, n=18 and p<0.01). Conceptually, it worth noting the parallelism and independency between the DEPM and acoustics surveys when applied at spawning time on a full mature population (as this anchovy): The DEPM obtain regional eggs abundances as indicator of biomass in space whilst the acoustic uses the regional acoustic energy of the target species to that purpose; They both use their biomass indicators to weight the adult biological samples of the survey in space. And finally the two methods will produce the final biomass (and population estimates) from those two indicators using their own scaling factors, i.e. the daily fecundity and the target strength respectively.

The method presented follows the standards of single stage cluster sampling, or two phase cluster sampling (when working first for lengths and next the age-length keys), usually with clusters of unequal sizes (Cochran 1977; Lhor 2010). The result presented confirm the adequacy of cluster sampling for the estimates of length distribution and age composition and their variances for this small pelagic species, like anchovy, as advocated since the beginning for the DEPM (Picquelle and Stauffer 1985), as well as for many types of surveys (Pennington and Vølstad 1994; Aanes and Pennington 2003; Cotter 2009). In the current application we have made some simplifications: a) we omitted the finite population corrections affecting the first sampling level for lengths, both at the population level and at the haul level because, compared to the population, our sampling is negligible and because, at the haul level, there was no need to raise our samples to the total catch of the haul (as we disregarded that information as not indicative of fish abundance and we considered our random sample of sufficient size as duly represent the haul information). B) we have omitted the variance associated to the weighting factors which are actually estimates (not observations) resulting from the posterior regionalization of the survey. Similar practices of posterior poststratification are also applied in other pelagic surveys (Petitgas *et al.* 2003), but this simplification omits part of the variance associated to our estimates. In order to deal properly with such variance, some objective formulation of the regionalization of the egg abundance coupled with bootstrapping of the observations will have been required. We are of the opinion that this simplification will have just induced a minor reduction of the variance estimates, though certainly regionalization for the estimation of weighting factors remains some subjective step in the procedure described here.

Alternative ways to address the spatial variability, other than the cluster sampling theory (and beyond stratification or regionalization), could come from modelling approaches of the spatial variability of the concerned Egg and Adult parameters (Stratoudakis *et al.* 2006). This has been pursued for eggs with GAMS or geostatistics (Borchers *et al.* 1997; Fletcher and Sumner 1998; Bernal *et al.* 2011;) and for adults with GLMs and GAMs as well (Kvist 2001; ICES 2004; Ibaibarriaga *et al.* 2013) and it is a developing area for Egg Surveys (Dickey-Collas 2012). We have not followed these other approaches just to provide a simple extension of the originally designed Daily Egg Production Method, but there can be gains in precision by pursuing those alternative modelling approaches (along with some risk of bias as well). Certainly the more complicate the modelling approach, the more the need of good statistical skills for the user will be.

DEPM surveys, like acoustics or bottom trawl surveys, can produce several population indicators beyond biomass estimates, as population age structure, mean weight and length at age, reproductive parameters (F and S), or accurate estimates of the fractions of mature fishes (from the histological examination of gonads required by the DEPM) or even maturity at age or by length (Lmat50% for instance) (Doray *et al. in press*; Cotter 2009b). All these indicators are of increasing relevance in the context of Qualitative descriptors for determining good environmental status requested by the EU Marine Strategy Framework Directive (Directive 2008/56/EC). They might be regarded as long term indicators of population status. Such information can support ecological analysis on the role that abundance, demography and ecosystem productivity may play on determining the reproductive potential of stocks (Dickey Collas *et al.* 2012).

Demography Appendix 1: Variance of proportions at age (Pa).

A.1.a. Variance of the Population proportion at age (Pa).

Double sampling to estimate a population parameter is a well known and unbiased procedure to get a stratified estimate of a parameter, where the strata are estimated by the first sampling and the parameter estimates by strata classes are produced by the second sampling (Neyman 1938, Cochran 1969). This demonstration follows that of Cochran (1969) (Theorem 12.2) and for the scope of simplicity we assume here that the size of the clusters are all equal.

First sampling is a random sampling of clusters of fishes, aggregated according to their size (i.e. length) which is strongly related to the age of the fishes. Usually a small sample of the whole cluster is made to get a length frequency distributions per cluster. A second sampling is a random sub sampling of otoliths within length classes which might be at the same time a subsample of the former or just an independent parallel sample. Therefore, the lengths follow a clumped distribution by clusters. Ages within length are assumed to follow a binomial distribution. Finally usually the length sampling per cluster is far more extensive than the age sampling and can be considered independent one from the other. In addition ALK are built up so that ages are grouped from several distinct samples and are this way independent of any concrete length sample.

Circumflex sign represents Population estimates, whereas the symbols not bearing that sign are the true population parameters.

The expected value of Pa for all the possible second samples is

$$\hat{P}_a = \sum_{l=1}^{L} \hat{P}_l Q_{al} = \sum_{l=1}^{L} P_l Q_{al} + \sum_{l=1}^{L} (\hat{P}_l - P_l) Q_{al}$$

Where the second term reflects the bias or error arising from the first sampling. The expected variance of Pa by double sampling conditioned to the first sampling it will be equal to the variance produced by the second sampling plus the bias of the first one:

$$E\,[(\,\hat{p}_a - P_a\,)^2 \,|\, \hat{p}_l\,] = \sum_{l=1}^{L} \frac{\hat{p}_l^{\,2}\, q_{al}\,(1-q_{al})(1-f_{2l})}{(\,m_l - 1)} + \left[\,\sum_{l=1}^{L}(\,\hat{p}_l - P_l\,)Q_{al}\,\right]^2$$

Now taking expectations of the above expression for the possible first sampling realizations (and leaving fixed the size of all subsamples of the second sampling of ages) we will produce expected variance of this double sampling procedure:

To make that we first recall that

$$E\,(\,\hat{p}_l\,)^2 = p_l^{\,2} + Var(\,\hat{p}_l\,)$$

And also that the variance by subsampling lengths of cluster units of unequal size is

$$Var(\,\hat{p}_l\,) = (\,1 - f_1\,)\frac{\sum_{i=1}^{n} M_i^{\,2}(\,p_{li} - \hat{p}_l\,)^2}{\overline{M}^2\, n\,(n-1)} + f_1\frac{\sum_{i=1}^{n} M_i^{\,2}\, p_{li}(\,1 - p_{li}\,)(1-f_{2i}\,)}{\overline{M}^2\, n\,(\,m_i - 1)}$$

$$Var(\,\hat{p}_l\,) \approx \frac{\sum_{i=1}^{n} M_i^{\,2}(\,p_{li} - \hat{p}_l\,)^2}{\overline{M}^2\, n\,(n-1)}$$

(Cochran 1977, from equation 11.30 divided by M0^2 -- example of page 305--), where the second expression is a sufficient one when the first sampling of clusters is negligible in comparison with the total population size.

In this way we will have that

$$E_1\left[\,\sum_{l=1}^{L}\frac{\hat{p}_l^{\,2}\, q_{al}\,(1-q_{al})(1-f_{2l})}{(\,m_l - 1)}\,\right] =$$

$$= \sum_{l=1}^{L}\frac{P_l^{\,2}\, q_{al}\,(1-q_{al})(1-f_{2l})}{(\,m_l - 1)} + \sum_{l=1}^{L}\frac{Var\,(\,\hat{p}_l\,)\,q_{al}\,(1-q_{al})(1-f_{2l})}{(\,m_l - 1)}$$

And also

$$E_1\left[\sum_{l=1}^{L}(\hat{p}_l - P_l)Q_{al}\right]^2 =$$

$$= E_1\left[\sum_{l=1}^{L} Q_{al}^{2}(\hat{p}_l - P_l)^2 + 2\sum_{l=1}^{L}\sum_{h=l+1}^{L} Q_{al}\,Q_{ah}(\hat{p}_l - P_l)(\hat{p}_h - P_h)\right] =$$

$$= \sum_{l=1}^{L} Q_{al}^{2}\,Var(\hat{p}_l) + 2\sum_{l=1}^{L}\sum_{h=l+1}^{L} Q_{al}\,Q_{ah}\,Covar(\hat{p}_l,\hat{p}_h) =$$

$$= \frac{\sum_{l=1}^{L} Q_{al}^{2}\sum_{i=1}^{n} M_i^{2}(p_{li} - \hat{p}_l)^2}{\overline{M}^{2}\,n(n-1)} + \frac{2\sum_{l=1}^{L}\sum_{h=l+1}^{L} Q_{al}\,Q_{ah}\sum_{i=1}^{n} M_i^{2}(p_{li} - \hat{p}_l)(p_{hi} - \hat{p}_h)}{\overline{M}^{2}\,n(n-1)}$$

Therefore the addition of both terms gives the expected variance for a double sampling of lengths (first phase) and ages (second phase) for clusters of length distribution (with clusters of unequal sizes):

$$Var(\hat{p}_a) = \sum_{l=1}^{L}\frac{P_l^{2}\,q_{al}(1 - qSUBal)(1 - f_{2l})}{(m_l - 1)} + \sum_{l=1}^{L}\frac{Var(\hat{p}_l)\,q_{al}(1 - q_{al})(1 - f_{2l})}{(m_l - 1)} +$$

$$+ \frac{\sum_{l=1}^{L} Q_{al}^{2}\sum_{i=1}^{n} M_i^{2}(p_{li} - \hat{p}_l)^2}{\overline{M}^{2}\,n(n-1)} + \frac{2\sum_{l=1}^{L}\sum_{h=l+1}^{L} Q_{al}\,Q_{ah}\sum_{i=1}^{n} M_i^{2}(p_{li} - \hat{p}_l)(p_{hi} - \hat{p}_h)}{\overline{M}^{2}\,n(n-1)}$$

and as estimator of this variance we have:

$$\hat{V}ar(\hat{p}_a) = \sum_{l=1}^{L}\frac{\hat{p}_l^{2}\,q_{al}(1 - q_{al})(1 - f_{2l})}{(m_l - 1)}$$

$$+ \frac{\sum_{l=1}^{L} q_{al}^{2}\sum_{i=1}^{n} M_i^{2}(p_{li} - \hat{p}_l)^2}{\overline{M}^{2}\,n(n-1)} + \frac{2\sum_{l=1}^{L}\sum_{h=l+1}^{L} q_{al}\,q_{ah}\sum_{i=1}^{n} M_i^{2}(p_{li} - \hat{p}_l)(p_{hi} - \hat{p}_h)}{\overline{M}^{2}\,n(n-1)}$$

$$- \sum_{l=1}^{L} Var(\hat{p}_l)\,Var(q_{al})$$

where the first term estimates the first line and the remaining part all the rest. The last negative term derives from the substitution of the value of Q_{al}^2 by q_{al}^2 , as in addition to Q_{al}^2 it will contain its variance and it will imply some over estimate of the variance estimate, which might be corrected by such subtraction. However as the product of both variances will be far less than any other terms in the expression, it could be omitted. Hence this proves that **equation 4.12** of the main text is approximately a sufficient estimator of the variance of the proportions at age estimates when the age-lenth key is used for such purpose in a two phase sampling.

This demonstration has followed the method of Cochran 1969) but it can also be demonstrate following Kimura (1977) and Cochran (1977).

A.1.b. Equivalence of equations 4.12 and 4.13:

As the las term of both equations are identical then it is only necessary to show that the first term of **equation 4.13** equals the first two terms of **equation 4.12**. Furthermore as denominators are equal should just justify the equality of the numerators. By developing the first term of **equation 4.13** and omitting (by simplicity) the weighting factors or cluster sizes (Mi), we have:

$$\sum_{i=1}^{n}(p_{ai}-P_a)^2=\sum_{i=1}^{n}\left[\sum_{l=1}^{L}(P_{li}\,q_{al})-\sum_{l=1}^{L}(P_l\,q_{al})\right]^2=$$

$$=\sum_{i=1}^{n}\left[\sum_{l=1}^{L}(P_{li}\,q_{al}-P_l\,q_{al})\right]^2=\sum_{i=1}^{n}\left[\sum_{l=1}^{L}q_{al}(P_{li}-P_l)\right]^2=$$

$$=\sum_{i=1}^{n}\left[\sum_{l=1}^{L}q_{al}^{\,2}(P_{li}-P_l)^2+2\left(\sum_{l=1}^{L}q_{al}(P_{li}-P_l)\right)\left(\sum_{h=l+1}^{L}q_{ah}(P_{hi}-P_h)\right)\right]=$$

$$=\sum_{i=1}^{n}\sum_{l=1}^{L}q_{al}^{\,2}(P_{li}-P_l)^2+2\sum_{i=1}^{n}\sum_{l=1}^{L}\sum_{h=l+1}^{L}q_{al}\,q_{ah}(P_{li}-P_l)(P_{hi}-P_h)=$$

$$=\sum_{l=1}^{L}q_{al}^{\,2}\sum_{i=1}^{n}(P_{li}-P_l)^2+2\sum_{l=1}^{L}\sum_{h=l+1}^{L}q_{al}\,q_{ah}\sum_{i=1}^{n}(P_{li}-P_l)(P_{hi}-P_h)$$

So numerator of the first term of **equation 4.13** equals to the numerators of the first two terms of **equation 4.12** and this completes the proof.

A.1.c Equivalence of the different estimators of Proportion in Mass at age in the mature population P'_a

Equation 4.17 states that the estimates can be obtained either globally or on sample by sample basis, by using any of the following equivalent expressions:

Eq. 17
$$P'_a = \sum_{l=1}^{L} P'_l \cdot q_{al} = \frac{\sum_{l=1}^{L} P_l \cdot W_l \cdot q_{al}}{\sum_{l=1}^{L} P_l \cdot W_l} = \frac{\sum_{i=1}^{n} M'_i \, P'_{ai}}{\sum_{i=1}^{n} M'_i}$$

The first and second expression use the length sampling available for all samples (P_l) to estimate the mean relative frequency distribution of the biomass by length for the (mature) population (P'_l,) with the support of the ALK and the sampling for weight. The third approach is just applying the ALK and weight sampling to the $P_{l,i}$ to obtain proportions of biomass by ages for each sample ($P'_{a,i}$), being afterwards averaged overall all samples with weighting factors proportional to population abundance in mass represented by the sample (M'_i).

Notice that for P_l the weighting of samples is proportional to the abundance of the population in numbers represented by the sample (M_i) and these are just the result of dividing those in mass by the mean weights of the fishes in the samples (M'_i / $W_{t,i}$), so that $M'_i = M_i \cdot W_{t,i}$.

Notice in addition that the denominator of the second expression is just an stratified estimate of the overall mean weight of fishes in the population (W_t).

From there we have that expression A and B below are equal

Expression A: $$P'_a = \frac{\sum_{l=1}^{L} P_l \cdot W_l \cdot q_{al}}{\sum_{l=1}^{L} P_l \cdot W_l} = \frac{\sum_{l=1}^{L} P_l \cdot W_l \cdot q_{al}}{W_t}$$

Expression B:

$$P'_a = \frac{\sum_{i=1}^{n} M'_i \, P'_{ai}}{\sum_{i=1}^{n} M'_i} = \frac{\sum_{i=1}^{n} M_i \cdot W_{t,i} \cdot \left[\sum_{l=1}^{L} P_{l,i} \cdot W_l \cdot q_{al}\right] \Big/ W_{t,i}}{\left(\sum_{i=1}^{n} M_i \cdot W_{t,i}\right) \cdot \sum_{i=1}^{n} M_i \Big/ \sum_{i=1}^{n} M_i} = \frac{\sum_{i=1}^{n} M_i \cdot \sum_{l=1}^{L} P_{l,i} \cdot W_l \cdot q_{al}}{W_t \cdot \sum_{i=1}^{n} M_i}$$

$$= \frac{\sum_{l=1}^{L} \left(\sum_{i=1}^{n} M_i \cdot P_{l,i}\right) \cdot W_l \cdot q_{a,l}}{W_t \cdot \sum_{i=1}^{n} M_i} = \frac{\sum_{l=1}^{L} P_l \cdot W_l \cdot q_{al}}{W_t}$$

and this proves equation 4.17 of the main text:

Demography Appendix 2: Variance of mean Weight at age using ALK

When mean weight at age $\boldsymbol{W_a}$ is deduced from the application of age-length keys (q_{al}) and mean weight at length (W_l) to the weighted pool length distribution (P_l) from the available fishing sets of the target fish species, the estimate (**equation 4.14**) is given by:

$$W_a = \frac{\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l}{\sum_{l=1}^{L} P_l \cdot q_{al}} = \frac{\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l}{P_a}$$

Eq. A2.1

As for the rest of the paper we assume that fishing hauls are clusters of length distributions, which are averaged across the sampled area according to an index of abundance each sample represents. As the W_a estimator is a ratio of two related estimates, its variance can be obtained by applying the delta method, as:

Eq A2.2:

$$\boldsymbol{Var(Num/Deno) = (Num/Deno)^2 \cdot \left[CV(Num)^2 + CV(Deno)^2 - 2\frac{COV(Num, Deno)}{Num \cdot Deno}\right]}$$

this is:

Eq.A2.3:

$$Var(W_a) = \left(\frac{\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l}{P_a} \right)^2 \cdot \left[\frac{Var(\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l)}{(\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l)^2} + \frac{Var(P_a)}{P_a^2} - 2\frac{COV\left(\sum_{l=1}^{L} P_{l,i} \cdot q_{a,l} \cdot W_l, P_{a,i}\right)}{\left(\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l\right) \cdot P_a} \right]$$

which results in

Eq. A2.4:

$$Var(W_a)=\frac{Var(\sum_{l=1}^{L} P_l\cdot q_{al}\cdot W_l)}{P_a^2}+W_a^{\,2}\cdot\frac{Var(P_a)}{P_a^2}-2\cdot W_a^{\,2}\cdot\frac{COV\left(\sum_{l=1}^{L}P_{l,i}\cdot q_{a,l}\cdot W_l\,,P_{a,i}\right)}{\left(\sum_{l=1}^{L}P_l\cdot q_{al}\cdot W_l\right)\cdot P_a}$$

By calling $FW_{l,a}=q_{al}\cdot W_l$ to the portion of the total mean weight at length ***l*** corresponding to the age ***a***, we see that $Var(\sum_{l=1}^{L}P_l\cdot q_{al}\cdot W_l)=Var(\sum_{l=1}^{L}P_l\cdot FW_{a,l})$ which is a estimator from a two phase sampling first for length and next independent subsampling for ages within length and for weights within lengths. This estimators is analogous to $Var(P_a)=Var(\sum_{l=1}^{L}P_l\cdot q_{al})$ shown in equation 4.13 of the main text, and therefore we will have by analogy that

Eq. A2.5

$$Var(\sum_{l=1}^{L}P_l\cdot FW_{a,l})=\frac{\sum_{i=1}^{n}M_i^{\,2}\left(\sum_{l=1}^{L}P_{l,i}\cdot FW_{a,l}-\sum_{l=1}^{L}P_l\cdot FW_{a,l}\right)^2}{\overline{M}^2\,n\,(n-1)}+\sum_{l=1}^{L}P_l^{\,2}\cdot Var(FW_{a,l})$$

Now assuming independency of the sampling for weights within lengths from that of ages within lengths, being usually the former far larger than that latter, we will have that

Eq. A2.6

$$Var(FW_{a,l})=FW_{a,l}^{\;2}\cdot\left(\frac{Var(q_{a,l})}{q_{a,l}^{\;2}}+\frac{Var(W_l)}{W_l^2}\right)=W_l^2\cdot Var(q_{a,l})+q_{a,l}^{\;2}\cdot Var(W_l)$$

Notice that replacing $\sum_{l=1}^{L}P_l\cdot q_{al}\cdot W_l$ by $P_a\cdot W_a$ in the denominator of the relative covariance term of Eq. A2.4 and the covariance $COV\left(\sum_{l=1}^{L}P_{l,i}\cdot q_{a,l}\cdot W_l\,,P_{a,i}\right)$ by the product of the coefficient of correlation between the numerators and denominator of expression A2.1 and the respective variances, $° \cdot S_{Pa \cdot Wa} \cdot S_{Pa}$, the covariance term of equation a A2.4can be written as well as:

Eq A2.7:

$$2 \cdot W_a^{\,2} \cdot \frac{COV\left(\sum_{l=1}^{L} P_{l,i} \cdot q_{a,l} \cdot W_l , P_{a,i} \right)}{P_a \cdot W_a \cdot P_a} = 2 \cdot W_a \cdot \frac{COV\left(\sum_{l=1}^{L} P_{l,i} \cdot q_{al} \cdot W_l , P_{ai} \right)}{P_a^2}$$

$$= 2 \cdot W_a \cdot \frac{COV(P_{ai} \cdot W_{ai}, P_{ai})}{P_a^2} = 2 \cdot W_a \cdot ° \cdot S_{Pa \cdot Wa} \cdot S_{Pa} / P_a^{\,2}$$

Now by inserting equation A2.6 into A2.5 and taking the sample estimates of the variances for qal and Wl according to the size of the subsamples for ages and weights and inserting such a result into A2.4 we have the following expression

Eq. A2.8

$$Var(Wa) = \frac{\sum_{i=1}^{n} M_i^2 \left(\sum_{l=1}^{L} P_{l,i} \cdot q_{al} \cdot W_l - \sum_{l=1}^{L} P_{l,} \cdot q_{al} \cdot W_l \right)^2}{P_a^2 \cdot \overline{M}^2 n \cdot (n-1)} + \sum_{l=1}^{L} \frac{(P_l \cdot W_l)^2 \, q_{al} (1-q_{al})(1-f_{2l})}{P_a^2 \cdot (m_l - 1)}$$

$$+ \sum_{l=1}^{L} \frac{(P_l \cdot q_{al})^2 \cdot Var(W_l)(1-f_{wl})}{P_a^2 \cdot m_{wl}} + W_a^{\,2} \cdot \frac{Var(P_a)}{P_a^2} - 2 \cdot W_a \cdot \frac{COV\left(\sum_{l=1}^{L} P_{l,i} \cdot q_{al} \cdot W_l , P_{ai} \right)}{P_a^2}$$

Notice that this expression is entirely parallel to the one given for the variance of a ratio in Cochran (1977) – equation 6.13 -, when neglecting the population correction sampling factor.

And, replacing $\sum_{l=1}^{L} P_l \cdot q_{al} \cdot W_l$ by $P_a \cdot W_a$ and the covariance term by the final expression from the equation A2.7 and rearranging we will obtain equation 4.15 of the main text of the paper:

Eq. A2.9 (equivalent to **equation 4.15** of the main text):

$$Var(Wa) = \frac{\sum_{i=1}^{n} M_i^2 \left(P_{ai} \cdot W_{ai} - P_a \cdot W_a\right)^2}{P_a^2 \cdot \overline{M}^2 n \cdot (n-1)} + \sum_{l=1}^{L} \frac{\left(P_l \cdot W_l\right)^2 q_{al} (1-q_{al})(1-f_{2l})}{P_a^2 \cdot (m_l - 1)}$$

$$+ \sum_{l=1}^{L} \frac{\left(P_l \cdot q_{al}\right)^2 \cdot Var(W_l)(1-f_{wl})}{P_a^2 \cdot m_{wl}} + W_a^{\ 2} \cdot \frac{Var(P_a)}{P_a^2} - 2 \cdot W_a \cdot \circ \cdot S_{Pa \cdot Wa} \cdot S_{Pa} / P_a^{\ 2}$$

And finally, notice that

- the weighting factors per sample for the numerator as for the denominator are the Mi (proportional to numbers)
- we suggest to apply the coefficient of correlation in the covariance term to all the numerator and denominator variance estimates including the contributions arising from the ALK (terms with Var(qal) in Eq A2.9 and for the Var(Pa) including the component due to ALK as estimated in **equation 4.15** of the main text.

Demography Appendix 3: Proportion of mature fishes in the population in mass and in numbers.

If estimates of TPa and TPm_a can not be obtained per sample with sufficient precision (or it is too costly), then estimates of this parameters can be obtained by length stratified estimates of maturity and age composition. It would then be convenient to assure that ALK for all fishes is obtained at random (TALK) (named as $Tq_{a,l}$) and next that a mature age length key is also produced (MALK) (named as $q_{a,l}$ along the main text and former Appendixes) so that this is a fraction of the TALK. In those cases with both keys a single IMALK (immature-mature Age-length key) (named as $TPm_{a,l}$) can be produced for the whole population or by strata as convenient.

The fraction of mature fishes over the total population in total (by mass, TPm') or by ages (in numbers, TPm_a) will be often calculated via mature length keys and age length keys, particularly for TPm_a because sampling estimates of that parameter would be of too high cost obtaining. These estimates are:

Eq. A.3.1

$$TPm' = \sum_{l=1}^{L} TP'_l \cdot TPm_l = \frac{\sum_{l=1}^{L} TP_l \cdot W_l \cdot TPm_l}{\sum_{l=1}^{L} TP_l \cdot W_l} = \frac{\sum_{l=1}^{L} TP_l \cdot W_l \cdot TPm_l}{TW_t}$$

And

$$TPm_a = \frac{\sum_{l=1}^{L} TP_l \cdot Tq_{a,l} \cdot TPm_l}{\sum_{l=1}^{L} TP_l \cdot Tq_{a,l}}$$

Eq A.3.2

in case of maturity only depends on length (not on age), Or

$$TPm_a = \frac{\sum_{l=1}^{L} TP_l \cdot TPm_{a,l}}{\sum_{l=1}^{L} TP_l \cdot Tq_{a,l}}$$

Eq A.3.3

in case of dependency of maturity on length and age. With $TPm_{a,l}$ being the proportion of mature fishes of age *a* in legth ***l*** (over the total number of fishes at length ***l*** -all ages- for which maturity and age were assessed), and $Tq_{a,l}$ is the proportion of age *a* at length ***l*** which correspond with the ALK constructed over the total (mature and immature) fishes). Notice that the denominator of the last two expressions is just TP_a

The former three expressions are division of two related variables for which the variance can be obtained by the delta method. IN practice as equations A.3.1 and A.3.2 are analogous to the estimator of mean weight at age (in terms of the numerator being a fraction of the denominator), we can derive the variance of those estimators as shown in Appendix2 for the mean weight at age. By such derivation we would obtain the following parallel expressions of variance:

For ***TPm'*** Eq. A.3.4[3]:

$$Var(TPm') = \frac{\sum_{i=1}^{n} TM_i^2 \left(\sum_{l=1}^{L} TP_{l,i} \cdot TPm_l \cdot W_l - \sum_{l=1}^{L} TP_{l,} \cdot TPm_l \cdot W_l \right)^2}{TW_t^2 \cdot \overline{TM}^2 n \cdot (n-1)}$$

$$+ \sum_{l=1}^{L} \frac{(P_l \cdot W_l)^2 \cdot TPm_l (1 - TPm_l)(1 - f_{ml})}{TW_t^2 \cdot (m_l - 1)} + \sum_{l=1}^{L} \frac{(P_l \cdot TPm_l)^2 \cdot Var(W_l)(1 - f_{wl})}{TW_t^2 \cdot (m_{wl} - 1)}$$

$$+ TPm'^2 \cdot \frac{Var(W_t)}{TW_t^2} - 2 \cdot TPm' \cdot \frac{COV\left(\left(\sum_{l=1}^{L} P_{l,i} \cdot TPm_l \cdot W_l \right), TW_{t,i} \right)}{TW_t^2}$$

---

[3] Notice that $\sum_{l=1}^{L} TP_{l,i} \cdot TPm_l \cdot W_l$ can be replaced by $TPm'_i \cdot TW_{t,i}$ and hence, the first component of the *Var(TPm')* can also be written and estimated as:

$$\frac{\sum_{i=1}^{n} TM_i^2 \left(\sum_{l=1}^{L} TP_{l,i} \cdot TPm_l \cdot W_l - \sum_{l=1}^{L} TP_{l,} \cdot TPm_l \cdot W_l \right)^2}{TW_t^2 \cdot \overline{TM}^2 n \cdot (n-1)} = \frac{\sum_{i=1}^{n} TM_i^2 \left(\frac{TW_{t,i}^2}{TW_t^2} \cdot TPm'_i - TPm' \right)^2}{\overline{TM}^2 n \cdot (n-1)}$$

Where TPm_l is the proportion mature at length $\boldsymbol{l}$ assuming this value is identical in terms of mass or in numbers (if that were not the case then replace above TPm_l by TPm'_l).

And for ***TPma*** when there is independency between maturity at length and age,

Eq. A.3.5[4]:

$$Var(TPm_a) = \frac{\sum_{i=1}^{n} TM_i^2 \left(\sum_{l=1}^{L} TP_{l,i} \cdot TPm_l \cdot Tq_{a,l} - \sum_{l=1}^{L} TP_{l,} \cdot TPm_l \cdot Tq_{a,l} \right)^2}{TP_a^2 \cdot \overline{TM}^2 n \cdot (n-1)}$$
$$+ \sum_{l=1}^{L} \frac{\left(P_l \cdot Tq_{a,l}\right)^2 \cdot TPm_l\,(1 - TPm_l)(1 - f_{ml})}{TP_a^2 \cdot (m_l - 1)} + \sum_{l=1}^{L} \frac{\left(P_l \cdot TPm_l\right)^2 \cdot Tq_{a,l}\,(1 - Tq_{a,l})(1 - f_{al})}{TP_a^2 \cdot (m_{al} - 1)}$$
$$+ TPm_a{}^2 \cdot \frac{Var(TP_a)}{TP_a^2} - 2 \cdot TPm_a \cdot \frac{COV\left(\left(\sum_{l=1}^{L} P_{l,i} \cdot TPm_l \cdot Tq_{a,l} \right), TP_{a,i} \right)}{TP_a^2}$$

Here Var(***TP**$_a$*) will be calculated as for Var(***P**$_a$*) in **equation 4.13** of the main text.

Finally *Var(TPm$_a$)* when there is dependency between maturity and age within length would be:

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[4] Notice that $\sum_{l=1}^{L} TP_{l,i} \cdot TPm_l \cdot Tq_{a,l}$ can be replaced by $TPm'_{a,i} \cdot TP_a$ and hence, the first component of the *Var(TPm$_a$)* can also be written and estimated as:

$$\frac{\sum_{i=1}^{n} TM_i^2 \left(\sum_{l=1}^{L} TP_{l,i} \cdot TPm_l \cdot Tq_{a,l} - \sum_{l=1}^{L} TP_{l,} \cdot TPm_l \cdot Tq_{a,l} \right)^2}{TP_a^2 \cdot \overline{TM}^2 n \cdot (n-1)} = \frac{\sum_{i=1}^{n} TM_i^2 \left(\frac{TP_{a,i}^2}{TP_a^2} \cdot TPm'_{a,i} - TPm'_a \right)^2}{\overline{TM}^2 n \cdot (n-1)}$$

Eq. A.3.6:

$$Var(TPm_a) = \frac{\sum_{i=1}^{n} TM_i^2 \left(\sum_{l=1}^{L} TP_{l,i} \cdot TPm_{a,l} - \sum_{l=1}^{L} TP_{l,} \cdot TPm_{a,l} \right)^2}{TP_a^2 \cdot \overline{TM}^2 n \cdot (n-1)} + \sum_{l=1}^{L} \frac{P_l^2 \cdot TPm_{a,l} (1 - TPm_{a,l})(1 - f_{mal})}{TP_a^2 \cdot (m_{al} - 1)}$$

$$+ TPm_a^2 \cdot \frac{Var(TP_a)}{TP_a^2} - 2 \cdot TPm_a \cdot \frac{COV\left(\left(\sum_{l=1}^{L} P_{l,i} \cdot TPm_{a,l} \right), TP_{a,i} \right)}{TP_a^2}$$

With $TPm_{a,l}$ being the proportion of mature fishes of age *a* in legth ***l*** (over the total number of fishes at length ***l*** -all ages- for which maturity and age were assessed.

Demography Appendix 4: Correspondence of the variance of the Hansen and Hurwitz estimator with the formulae of the Variance for clusters of unequal sizes

In one stage sampling with unequal probability of the primary sampling units (psu), calling Ψ_i the probability of inclusion of any psu (i form n samples), of size *Mi*, the Hansen and Hurwitz (1943) unbiased estimators of the total mean by psu (t_Ψ) of a variable ***y*** measured at every individual within the sample (i.e. for every second sampling unit ssu) is

Eq A.4.1 $$\hat{t}_\Psi = \frac{1}{n}\sum_{i=1}^{n}\frac{t_i}{\Psi_i}$$ (equation 6.7 in Lhor 2010)

Where $t_i = M_i \cdot \bar{y}_i$

But we are interested in the mean of *y* per element or individuals (per ssu), this is

Eq A.4.2 $$\hat{y}_\Psi = \frac{\hat{t}_\Psi}{\hat{M}_{0\Psi}}$$ (equation 6.10 in Lhor 2010)

Where $\hat{M}_{0\Psi} = \frac{1}{n}\sum_{i=1}^{n}\frac{M_i}{\Psi_i}$, which is an estimate of the total number of elements in the population.

As equation A.4.2 is a ratio, the variance of this estimator is given by

Eq. A.4.3 $$\hat{V}(\hat{y}_\Psi) = \frac{\sum_{i=1}^{n}\left(\frac{t_i}{\Psi_i} - \frac{\hat{y}_\Psi \cdot M_i}{\Psi_i}\right)^2}{\left(\hat{M}_{0\Psi}\right)^2 \cdot n \cdot (n-1)}$$ (equation 6.12 in Lhor 2010)

Replacing t_{ii} by $M_i \cdot \bar{y}_i$ we obtain

Eq. A.4.4 $$\hat{V}(\hat{y}_\Psi) = \frac{\sum_{i=1}^{n}\left(\frac{M_i}{\Psi_i}\right)^2 \cdot \left(\bar{y}_i - \hat{y}_\Psi\right)^2}{\left(\hat{M}_{0\Psi}\right)^2 \cdot n \cdot (n-1)}$$

Notice that as $\hat{M}_{0\Psi}$ is the mean of the weighting factors in the numerator ($\frac{M_i}{\Psi_i}$), Eq A.4.4 equals the Equation 4.9 of the main text for the cluster variance per ssu of any variable in the population. In fact in our approach we assume that actual size of any school in mass is unknown and assumed equal for all samples (lets say to C) and for it the corresponding size in numbers would be Mi=C/W$_i$. When estimating means over samples obtained over the whole surveyed area (where several *k* regions with different sampling levels were produced) we estimate the probability of inclusion of samples by regions as Ψ_{ki} ($\Psi_{ki} \approx n_k / f(SSB_k)$). And our final weighing factor by sample proportional to numbers was $\frac{M_i}{\Psi_{ki}} = \frac{C/W_i}{n_k / f(SSB_k)}$. In fact, in the paper we omit the constant C and we call M_i to $\frac{C/W_i}{n_k / f(SSB_k)} \approx \frac{f(SSB_k)}{n_k \cdot W_i}$.

As such, the correspondence of the Equation 4.9 with the Hansen and Hurwitz unbiased estimator of the variance is evidenced.

5 Natural Mortality: Assessing natural mortality of Bay of Biscay anchovy from survey population and biomass estimates

5.1 Introduction

Natural mortality (M) is a key population parameter scaling the population abundance and fishing mortality estimates in standard (age-structured) assessment methods (Hilborn and Walters 1992; Quinn and Deriso 1999, Gislason *et al.* 2010). However, it is difficult to estimate because of the risk of confusion between natural (M) and fishing (F) mortality, or between M and survey catchability at age (Vetter 1988, Quinn and Deriso 1999, Cotter *et al.* 2004; Wang *et al.* 2009). For this reason, the common approach is to apply a constant M value (Hilborn and Walters 1992; Gislason *et al.* 2010; Jorgersen and Holt 2013). Such value is often guessed according to the life span of the species. On other occasions, M is based on published empirical relationships between M and life history parameters valid for comparable groups of species and environments (Beverton 1959; 1992; Pauly 1980; Hoening 1983; Hewit & Hoening 2004). And sometimes it broadens to include size or age-dependent mortality as well (Caddy 1991; Lorenzen 1996; Charnov *et al.* 2001; Charnov and Gillooly 2004; Gislason *et al.* 2008; 2010).

In evolutionary theory, it is often suggested that the extrinsic mortality rate, attributable to external factors like disease or predation, is the major factor that shapes the evolution of life history (and indirectly, of senescence) through fitness optimization (Williams' hypothesis –1957 in Williams *et al.* 2006; Woodhead 1998; Reznick *et al.* 2002; Charnov *et al.* 2001; Jorgensen and Holt 2013). Natural mortality of fish will change throughout the successive life stages, from very high values in the egg, larval and juvenile stages to medium or low values across their mature life span, before increasing again during senescence (Chen and Watanabe 1988, Charnov *et al.* 2001). Several biochemical mechanisms have been proposed to explain the progressive deterioration of the physiological condition of organisms over time associated with senescence, such as the mutation accumulation theory or the antagonistic pleiotropy theory (see reviews and discussions in Woodhead 1998; Williams *et al.* 2006 and Golubev 2009).

Increasing mortality associated with senescence occurs at older ages (Vetter 1988, Woodhead 1998, Reznick *et al.* 2002, Gislason *et al.* 2010), and this is presumed to be particularly noticeable in short-lived clupeoids (Beverton 1963). Measurements of senescent mortality, as part of natural mortality, are difficult to obtain in the wild due to the many errors affecting the observation of fish populations (Quinn and Deriso 1999, Gislason *et al.* 2010). Nevertheless, various senescence patterns of fish, have been reported, ranging from the abrupt senescence of salmon and eels (Woodhead 1998) or of some gobies (Caputo *et al.* 2002) to the gradual senescence of guppies (Reznick *et al.* 2006), *Nothobranchius furzeri* (Terzibasi 2007), gadoids (Sparholt et al 2002; Nielsen 2012) or herring (Tanasichuk 2000; Beverton *et al.* 2004), and to the very slow or even negligible senescence of rockfish and carps (Reznick *et al.* 2002).

The Bay of Biscay anchovy (*Engraulis encrasicolus* L.) is a fast growing and short-lived species, fully mature at age 1, and rarely exceeding its third year of life (Uriarte *et al.* 1996; Petitgas *et al.* 2010). In this region, anchovy is prey, along with other small pelagic fish, for piscivorous species such as hake, megrim, sea bass, tunnidae or cetaceans, among others (Preciado *et al.* 2008; Goñi *et al.* 2011; Lassalle *et al.* 2011; López-López *et al.* 2012). The adult anchovy population is monitored yearly by two independent research surveys: an acoustic survey (Massé *et al.* 1996; *in press*) and an egg survey applying the Daily Egg Production Method - DEPM (Somarakis *et al.* 1994; Motos *et al.* 2005; Santos *et al.* 2011; *in press*). Both surveys provide estimates of biomass and population numbers at age, which are included in the assessment of this stock carried out by ICES (International Council for the Exploration of the Sea). A Bayesian two-stage biomass model (Ibaibarriaga *et al.* 2008) is used for this assessment. Until 2004, ICA (Integrated Catch at age Analysis, Patterson and Melvin, 1996) was used (ICES 2005). Until 2013, M has been presumed to be constant at 1.2 per year for all ages. This value was inferred in the nineties from direct DEPM estimates of the population at age, assuming that they were absolute and unbiased (Uriarte *et al.* 1996; Prouzet *et al.* 1999). While the Bayesian model presumes constant catchability across ages and surveys, ICA calculated the catchability at age which was 50% higher for age 2 than for ages 1 or 3 (ICES 2005) in both surveys. This was not regarded as a realistic outcome, taking into account the standard designs of both the DEPM and acoustic surveys, which included non-selective fishing gears for adults (capable of catching sizes well below minimum anchovy sizes in spring), and the sufficient spatial coverage of anchovy distribution (ICES 2013)[5]. Certainly, an

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[5] According to cruise leaders, there are no reasons to suspect catchability should be different by ages either in the acoustic or in the DEPM surveys. Both surveys cover the entire distribution area and use pelagic fishing trawls non selective at fish sizes above 9 cm (so well below the usual minimum sizes of

alternative explanation of that result could be that natural mortality is not constant over ages.

Due to recruitment failures since 2001 and subsequent low biomass estimates (ICES 2013), the anchovy fishery in the Bay of Biscay was closed between 2005 and 2010. However, since scientific surveys were still ongoing during its closure, they provided a unique opportunity to estimate the actual natural mortality rate and possible patterns in natural mortality at age. Likewise, this closure allowed comparisons to be made between total mortality rates during the closed period and those in the former period of exploitation, in order to get natural and fishing mortality estimates under the assumption that no major changes in M occurred between both periods (Gulland 1983, Vetter 1988; Cook 1994; Sinclair 2001).

In this paper, several methods for estimating natural mortality of the anchovy in the Bay of Biscay were applied. First, a direct analysis of the variance (ANOVA) of total mortality rate (Z) derived from consecutive survey estimates of the population in numbers at age, was performed, where Z in the closure period was equivalent to the natural mortality rate (M). Next, M was estimated by regression of Z on an indicator of fishing mortality (F), which will be derived from the ratio of catches to the survey estimates of abundance. In this procedure, M is calculated from the intercept of Z at zero fishing mortality, which includes information from surveys at other F rates. Finally, an integrated assessment with a seasonal (half-year) separable fishing mortality model was applied to catch and survey data in order to find natural mortality rates and patterns that would optimize data fit, under the assumption of equal survey catchability at age. This approach provides M estimates that best fit with all historical survey information (including the two spring surveys and an acoustic recruitment index started in 2003 – Boyra *et al.* 2013) and the historical catches at age. Therefore the successive methods gradually use a larger amount of information. Furthermore,

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anchovies in May not smaller than 9 cm, with modes between 13 and 15 cm). Coastal areas, preferred by the one year old fishes, are well covered by the surveys and only the very shallow waters (less than 20 m depth) cannot be accessed by the fishing boats, but this area will probably be less than 3% of the remaining offshore well covered area. There is no increasing trend of acoustic or egg abundance towards the coast as to suspect that the proportion of biomass missed in this coastal areas is more than the proportion of the area unsampled by the boats, but probably less. And the DEPM egg sampling reaches rather coastal shallow areas, which allows presuming that no significant under-sampling of egg production in coastal areas occurs.

while the first two approaches assume log-normal errors of the population at age estimates from surveys, the integrated assessment will in addition allow for multinomial errors.

5.2 Material and methods

5.2.1 Surveys

Estimates of population numbers at age are available from the acoustic (PELGAS – Ifremer- Massé *et al.* 1996; in press) and DEPM (BIOMAN–AZTI- Somarakis *et al.* 1994; Motos *et al.* 2005; Santos *et al.* in press) surveys carried out yearly in May since 1987 and 1989, respectively (ICES 2013). DEPM surveys since 1987 and acoustic surveys since 2000 reported population at ages 1, 2 and 3+ (with 3+ referring to fish of age 3 and older), while earlier acoustic estimates reported total biomass and, only occasionally, population numbers at age 1 and 2+ (in 1989, 1991, 1992 and 1997).

In addition, since 2003, an autumn survey was carried out to provide an acoustic index of juvenile anchovy abundance (Boyra *et al.* 2013). This was used as auxiliary information on the level of recruitment at age 0 in the integrated assessments.

5.2.2 Direct total (Z) and natural mortality (M) estimates.

In a cohort of *N* fish at age a in year y (N_{ay}) subject to a yearly mortality rate Z, the survivors to the next year will be $N_{a+1,y+1} = N_{a,y}e^{-Z_{a,y}}$.

Let $U_{a,y,s}$ denote the number of individuals at age a in year y estimated from survey s. Provided this index is proportional to the true population abundance (***N***) by a catchability coefficient (***Q***), and subject to a log-normal observation error common to all ages ($U_{a,y,s} = Q_{a,s}N_{a,y}e^{\varepsilon'_{s,y}}$), then the log of the ratio of successive age classes estimates in consecutive years is an estimate of the total mortality at age a in year y from survey s, $Z_{a,y,s}$ modified by the change in catchability:

$$\hat{Z}_{a,y,s} = \ln\left[\frac{U_{a,y,s}}{U_{a+1,y+1,s}}\right] = \ln\left[\frac{N_{a,y}\cdot Q_{a,s}\cdot\exp(\pounds'_{s,y})}{N_{a+1,y+1}\cdot Q_{a+1,s}\cdot\exp(\pounds'_{s,y+1})}\right] = Z_{a,y} + \ln\left[\frac{Q_{a,s}}{Q_{a+1,s}}\right] + \pounds_{s,y}$$

We refer to this as a Z estimate. Z estimates are further split into the natural and fishing mortality components:

$$\hat{Z}_{a,y,s} = \ln\left[\frac{U_{a,y,s}}{U_{a+1,y+1,s}}\right] = F_{a,y} + M_{a,y} + \ln\left[\frac{Q_{a,s}}{Q_{a+1,s}}\right] + \pounds_{y,s} \qquad \text{Eq. 5.1}$$

Three Z estimates are derived: Z1+, from ages 1+ to 2+,; Z1, from age 1 to 2; and Z2+, from ages 2+ to 3+. Notice that Z1+ will generally be closer to the Z of the most abundant age classes (in this case, age 1). Since surveys are carried out at spawning time in May, Z estimates refer to mortality rates from May to May. These estimates rely on two assumptions: 1) catchability is similar over age, and 2) survey observation errors follow log-normal distribution and are of similar average magnitude in both surveys.

Consistency of the Z estimates by survey depending on fishing or closure periods was tested by analysis of variance. Year, survey type (DEPM or acoustic), age (1 or 2+) and fishing period (closed or open fishery) were taken as factor variables, under the terms ***Year*** and ***Survey*** and ***Old*** and ***Fishing,*** respectively**.**

We first tested, using ANOVAs, the consistency of the *Z* estimates by surveys across years for all ages

$$\hat{Z}_{a,y,s} = Year_y + Survey_s + [Old] + \pounds \qquad \text{(Model A1, Eq. 5.2)}$$

Old is in brackets because it only applies in the joint analysis of *Z1* and *Z2+*. As the year factor will cover the inter-annual variability in Z due to either natural (ecological) or fishing causes, the former analysis should serve to assess whether or not the Z estimates provided by the two surveys are consistent. This was checked by testing the statistical significance of the ***Survey*** factor and, for the analysis by age, of the ***Survey * Old*** first order interaction**.**

Next, we tested the effect of closure on the overall and age-dependent *Z* values, which during the closure period, will be our direct estimate of the natural mortality rate (M):

$$\hat{Z}_{a,y,s} = Fishing + Survey_s + [Old] + Interactions + \pounds \qquad \text{(Model A2, Eq. 5.3)}$$

As before, ***Old*** only applies when analyzing Z_1 and Z_{2+} together, but not when dealing with Z_{1+}. Interactions are the potential first and second order interactions of the former variables, which were initially checked. Finally, £ is assumed to be a normal random variable with constant variance common to all ages, years and surveys.

5.2.3 Natural mortality estimates from linear models

In order to make use of the whole dataset for the estimation of *M* through a linear model, an indicator of the fishing intensity for each year was obtained from the ratio of the catches between surveys and the mean abundance of the cohort between surveys. This follows from the catch equation:

$$F_{a,y} = \frac{C_{a,y}}{\bar{N}_{a,y}} == \frac{C_{a,y}}{N_{a,y,s} \cdot \left(1 - e^{-Z_{a,y,s}}\right) / Z_{a,y,s}} = \frac{C_{a,y}}{U_{a,y,s} \cdot \left(1 - e^{-Z_{a,y,s}}\right) / Z_{a,y,s}} \cdot Q_s = RC \cdot Q_s \qquad \text{Eq. 5.4}$$

where the coefficient of proportionality of the Relative Catches over survey estimates (*RC*) to *F* equals the catchability coefficient of the surveys (assumed constant across ages) (Q_s). If RC can be estimated then it can be used to calculate *M* from Equation 5.1, as the intercept of the linear model. However, the problem with this approach is that the fitted *Z* will appear in the independent covariate (*RC*). In order to avoid this, we considered two alternative formulations of *RC* and checked the sensitivity to them:

$$RCSurvey2_{a,y} = \frac{C_{a,y}}{\left(U_{a,y,s} + U_{a+1,y+1,s}\right) / 2} \qquad \text{Eq. 5.5}$$

$$RCJoint\,2_{a,y} = \frac{C_{a,y}}{\sum_{s} \left(U_{a,y,s} + U_{a+1,y+1,s}\right) / 4} \qquad \text{Eq. 5.6}$$

The first RC estimator provides an estimate by survey (*RCSurvey2*) using as denominator the arithmetic mean of the abundances provided by the survey at the beginning and end of the Z estimate period. The second estimator *RCJoint2* provides a single joint indicator of fishing intensity for each year from both surveys together, by taking as denominator the average population provided by both surveys during the same period. In the latter case the analysis will be restricted to the years when both surveys were carried out in parallel, so that the two Z_s estimates of the year will be linked by the same *RCJoint* estimate.

In all cases, the catches considered are those between May 15 of year *y* and May 15 of year *y+1*, for the ages *a* and *a+1* in each respective year. Catches at age (in numbers) with their mean weights are reported by season in ICES until the closure of the fishery in 2005 (ICES 2005) and, more recently, in WGHANSA reports (ICES2013).

The following linear model was statistically tested for the different potential significant coefficients:

$$\hat{Z}_{a,y,s} = M_{a,y,s} + F_{a,y,s} + \pounds_{a,y} = M + \left[Old_a\right] + Q_s \cdot RC_{a,y} + Survey + Interact + \pounds_{a,y,s}$$

(Model B, Eq. 5.7)

With ***M*** (natural mortality) being the intercept either at age 1 (M1) or for all ages together (M1+) - depending on the subset of data being analyzed -. ***Old*** is now a dummy variable, being 0 for age 1 and 1 for age 2+. This term will indicate the increase of natural mortality in fish of age 2+ relative to M at age 1 (M1), for the joint analysis of Z_1 and Z_{2+}. ***RC*** accounts for the Relative Catches between surveys of the respective age *a* in year *y*, and coefficient $\boldsymbol{Q_s}$ accounts for proportionality of ***RC*** to fishing mortality *F*. ***Survey*** is a dummy variable, being 0 for DEPM and 1 for acoustics, and this term will reflect any potential effect of the acoustic survey relative to the DEPM on Z estimates. ***Interact*** are the potential first and second order interactions of the former variables, which were initially checked.

For the joint analysis of Z by age (Z1 and Z2+), our assumption about catchability, constant across age and necessary to estimate Z, implies that the first or second order interactions referring to the slope changing by age are not significant as the slope coefficient is the catchability of the survey. Secondly, the intercepts reflecting M are population parameters which should be similarly estimated by the surveys. As such, first order interactions of intercepts with surveys should not be significant. Therefore, the only interaction which could be significant is that of the slopes by survey, which would correspond with the common assumptions in most assessments of different catchabilities by survey. We will refer to the two-slope model as the one allowing different slopes on RCSurvey2 by survey (but with constant slopes across age), while we will refer to the single slope model as the one forcing a common slope for both surveys.

For the ANOVA analysis associated to all analysis above Type III errors were used.

5.2.4 Natural mortality estimates from integrated assessments

Finally, an integrated catch at age analysis with a seasonal (half yearly) separable fishing mortality model was applied to the catch and survey data currently used by ICES for the assessment of this fishery (ICES 2013): Catches in tons and at age on a half-yearly basis, spring surveys; Acoustic and DEPM estimates of total biomass (tons) and populations at age (numbers); and finally, an acoustic survey on juveniles (JUVENA Survey index started in 2003 which it is used to tune the recruitment at age 1 with a power catchability function). The purpose, as before, was to check what levels and patterns of natural mortality at age optimize an integrated assessment, under the assumption of equal catchability at all ages in the surveys. Natural mortality by ages was applied either to a calendar year, going from January to December or from July to June (the latter was checked because the main surveys are carried out in May, so closer to July than to January). The model was fitted using two different approaches: the first one assumes, as before, that observations (catches or surveys estimates –in biomass and by ages-) are subject to log normal errors and the objective function is a direct minimization of a weighted sum of squared residuals (WSSQ fitting - like in ICA analysis -- Patterson and Melvin 1996); the second approach is similar to the former one except that it assumes that all age disaggregated data (catches and population at age estimates) are subject to multinomial errors and hence are input as percentages at ages 1, 2 and 3+. In the latter case, model fitting is achieved by maximization of the log-likelihood (using log-likelihood ratios -- LLHR fitting). Given the general agreement of the two surveys in terms of percentages at age, and the poorer agreement in terms of tendencies of biomasses (ICES 2013), it seems that the multinomial approach for indexes at age is probably a suitable way to deal with the type of errors associated with the survey observations. Details of the model’s tuned and objective functions are given in Appendix A. The two modeling approaches were run in Microsoft Excel, using Solver for optimization of the objective function. Convergence was verified from different starting parameter values. The likelihood of fitted models was calculated (see appendix). Confidence intervals for the natural mortality estimates were obtained using the profile likelihood method. Based on the likelihood ratio test, the 95% confidence interval around the joint optimums of $\hat{M}1$ and $\hat{M}2+$ is defined as the pairs of M1 and M2+ which satisfy the following inequality:

$$\ln(L(\hat{\theta}, \hat{M}1, \hat{M}2+) - \ln(L(\hat{\theta}, M1, M2+) \leq \frac{1}{2}\chi_2^2(.95) \qquad \text{Eq. 5.8}$$

Where $L(\hat{\theta}, \hat{M}1, \hat{M}2+)$ represents the likelihood at the optimum of M1 and M2+ and every other parameter ($\hat{\theta}$), and $\ln(L(\hat{\theta}, M1, M2+)$ is the likelihood for any selected alternative of fixed M1 and M2+ parameters (for the optimum of the remaining parameters - $\hat{\theta}$). And $\chi_2^2(.95)$ is the 0.95 percentile of the Chi-square with 2 degrees of freedom (i.e. 5.9915). Confidence intervals for M1+ were also deduced from equation 5.8 applied to a single M parameter and Chi-square with 1 degree of freedom.

In order to compare the different fitted models, we used the corrected Akaike Information Criterion (AICc), estimated as:

AICc = -2*ln (likelihood) + 2*K + (2*K*(K+1))/(n-K-1), Eq. 5.9

with K being the number of parameters and n being the number of observations.

5.2.5 Sensitivity analysis

Sensitivity to some observations, that looked noisy at first sight and might have become too influential for the particular methods applied, was tested by including and excluding such data (when excluded we will refer to the subset of data). For direct analysis of the raw data during the closure period (2005-2010), the 2005 and 2006 survey data resulted in negative Z estimates between them, indicating that either 2005 estimates were too low or 2006 estimates too high. For the linear models, years with high values of RC (above 0.8) were considered unlikely to be actually happening (due to the difficulty of producing such a fishing impact) which led us to suspect some noisy, excessively low biomass estimates from the surveys during those years. In addition, years 2011 and 2012 were checked for sensitivity as they resulted in extremely different Z estimates by surveys due to a large discrepancy in the 2012 biomass estimates (ICES 2013). For the integrated assessment, analyzing sensitivity to the inclusion/exclusion of the 2012 survey biomass estimates was considered sufficient (the year of maximum biomass divergence –ICES 2013).

Furthermore, we tested the sensitivity of our results to a potential maximum level of errors in the age determination from otoliths. Even though the current ageing method is perceived to be unbiased (Uriarte *et al.* in press), the actual level of error is unknown. From expert advice, a maximum of a 5% level of ageing errors among contiguous age groups (from ages 1 to 3+) was evaluated. Larger errors were considered unlikely and were not compatible with data. Determination of age 0 was considered fully accurate. In addition, given the low percentages of ages 3 in the survey and in the catches, the errors in age 2 were considered to occur more often with age 1 (80% of the cases) than with age 3 (only 20%). The essayed matrix for a 5% of age determination error (named **E**) is shown in Table 5.1. Given a vector with the observed (assigned) age composition **A** (1*4) of a survey or catches, the corrected estimates of the age composition **C** (1*4) is deduced in matrix notation as:

$$\mathbf{C} = \mathbf{A} \cdot \mathbf{E}^{-1} \qquad \text{Eq. 5.10}$$

Corrections were not allowed to reduce the size of any age class below 20% of its original value (before correction) and ad hoc changes were applied to assure those minimums (by restoring enough numbers from the contiguous age class in proportion to the removals produced on the original amount as to still allow 20% of the original quantity). For the catches, corrections were applied to the international catches separately over three periods: before surveys (January – mid-May), after surveys (mid-May - June) and in the second half of the year.

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Table 5.1: Matrix of age determination errors.

	Assigned age				
True Age	**0**	**1**	**2**	**3+**	**Total**
0	1.00	0.00	0.00	0.00	1.00
1	0.00	0.95	0.05	0.00	1.00
2	0.00	0.04	0.95	0.01	1.00
3+	0.00	0.00	0.05	0.95	1.00

5.3 Results

5.3.1 Direct Z and M estimates

The series of Z estimates by surveys show a large inter-annual variability (Figure 5.1). Z estimates did not differ statistically between surveys ($p>0.95$ for Z1+ and $p>0.12$ for the Z by ages, Model A1). During the 2005-2009 closure, Z estimates (proxy of M1+) were markedly lower on average (0.81, CV=13%) than during the fishing periods 1.66 (CV=9%) (Figure 5.2 and Table 5.2). Parallel differences between fishing periods were seen for the Z by ages (Table 5.2) (with p[*Fishing*]<0.002, from Model A.2), and were shown consistently by both surveys (with non-significant interactions of *Fishing* by *Surveys*, $p>0.6$).

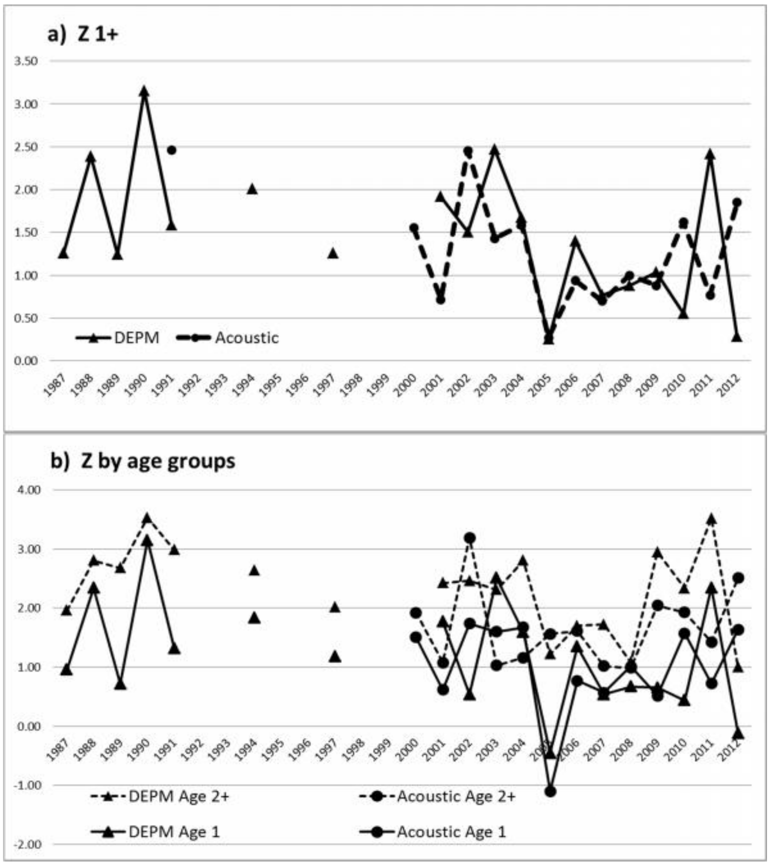


Figure 5.1: Series of Z estimates by surveys for a) overall ages Z1+ and b) by age groups (Z1 and Z2+).

The natural mortalities deduced from the Z estimates by ages during the fishing closure period (proxies of M1 and M2+) were very little affected by the omission of the 2005 and 2006 noisy estimates and turned out to be M1=0.66 (CV=11%) and M2+=1.63 (CV=19%) (Table 5.2). Such big differences in Z by ages were shown consistently by the two surveys (Figure 5.1**,** Figure 5.2 and Table 5.2) in the time series (model A.1) and for the two fishing periods (model A.2) (with P(*Old*)<0.0001 and p(*Old*Survey*)> 0.2 in the two models).

Table 5.2: Mean Z estimates for Z1+, Z1 and Z2+ by fishing periods and surveys or jointly for the two surveys (pooled surveys), for the raw data (a- upper tables) and assuming a 5% ageing error (b- bottom tables). The fishery was closed between July 2005 (with only very small catches in 2006) and December 2009.

a) No Ageing Errors

Data Sources	**DEPM Surveys**			**ACOUSTIC Surveys**			**Pooled Surveys**		
Z Means by periods	**Z1+**	**Z1**	**Z2+**	**Z1+**	**Z1**	**Z2+**	**Z1+**	**Z1**	**Z2+**
N (complete data)	19	19	19	14	13	13	33	32	32
Complete Series	**1.48**	**1.22**	**2.32**	**1.30**	**0.99**	**1.65**	**1.40**	**1.13**	**2.05**
(1987-2012) CV	13%	19%	8%	14%	22%	11%	9%	14%	7%
Fishing Period (1987-2004 & 2010-12)	**1.69**	**1.47**	**2.53**	**1.61**	**1.38**	**1.78**	**1.66**	**1.44**	**2.26**
CV	12%	17%	7%	13%	11%	15%	9%	11%	7%
Closure Period	**0.87**	**0.55**	**1.73**	**0.76**	**0.35**	**1.44**	**0.81**	**0.45**	**1.59**
(2005-2009) CV	21%	53%	19%	17%	106%	14%	13%	50%	12%
Subset Closure Period	**0.89**	**0.62**	**1.92**	**0.86**	**0.70**	**1.35**	**0.88**	**0.66**	**1.63**
(2007-2009) CV	9%	7%	29%	10%	23%	26%	6%	11%	19%

b) 5% Ageing Errors

Data Sources	**DEPM Surveys**			**ACOUSTIC Surveys**			**Pooled Surveys**		
Z Means by periods	**Z1+**	**Z1**	**Z2+**	**Z1+**	**Z1**	**Z2+**	**Z1+**	**Z1**	**Z2+**
N (complete data)	19	19	19	14	13	13	33	32	32
Complete Series	**1.79**	**1.64**	**2.17**	**1.56**	**1.24**	**1.47**	**1.69**	**1.48**	**1.89**
(1987-2012) CV	12%	15%	8%	17%	21%	14%	9%	12%	7%
Fishing Period (1987-2004 & 2010-12)	**2.06**	**1.95**	**2.36**	**1.95**	**1.68**	**1.53**	**2.02**	**1.85**	**2.05**
CV	10%	13%	7%	17%	14%	20%	9%	10%	8%
Closure Period	**1.03**	**0.78**	**1.66**	**0.87**	**0.53**	**1.38**	**0.95**	**0.65**	**1.52**
(2005-2009) CV	18%	35%	20%	16%	76%	15%	12%	35%	13%
Subset Closure Period	**1.05**	**0.85**	**1.88**	**0.98**	**0.92**	**1.27**	**1.01**	**0.88**	**1.58**
(2007-2009) CV	18%	18%	30%	14%	20%	28%	10%	12%	21%

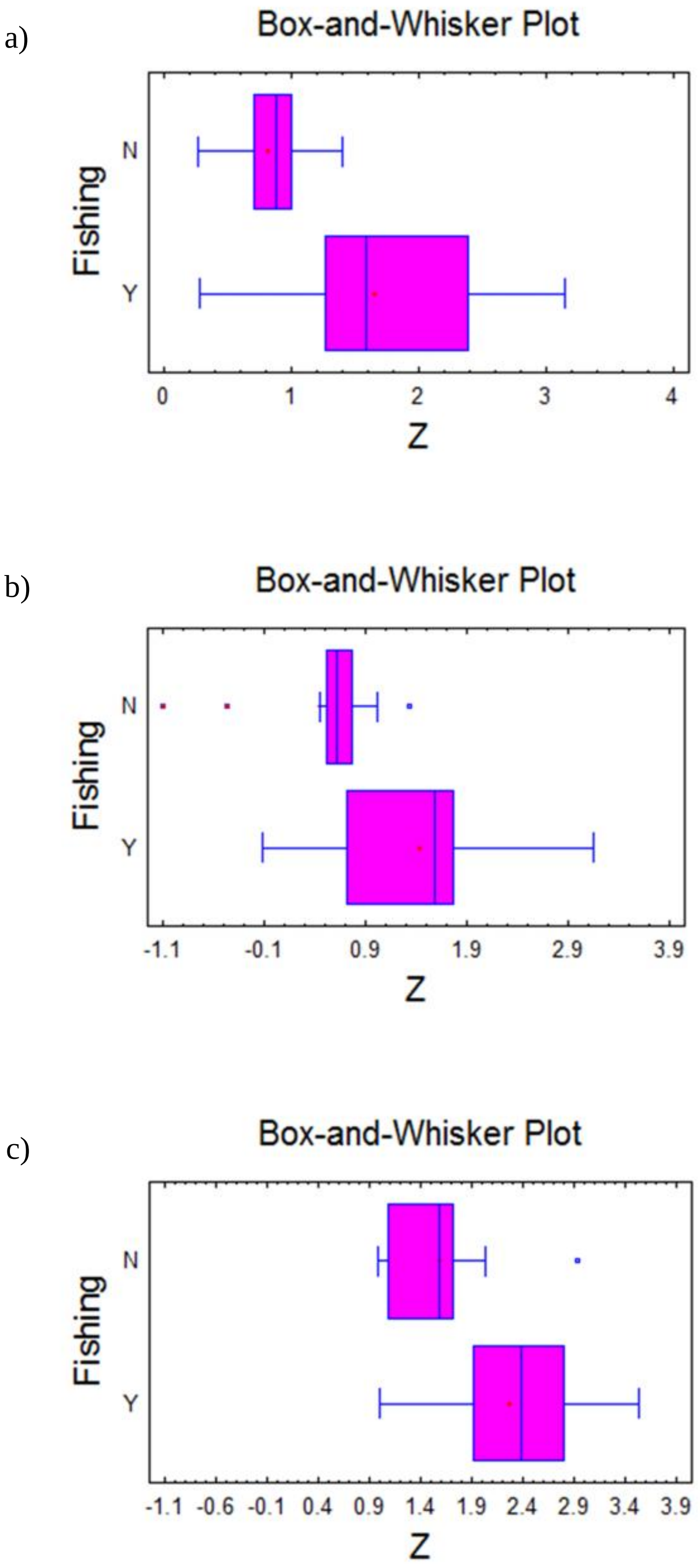


Figure 5.2: Box-and-whisker lot for Z by age (pooling survey estimates). N=No Fishing (Closure Period). Y= Fishing Period.

5.3.2 Natural mortality estimates from linear models

The M estimates (intercepts) did not differ statistically between surveys, neither in the case of Z1+ (p[*Survey*]>0.6) nor by ages, Z1 and Z2+ (p[*Survey*]> 0.3 and p[*OLD*Survey*]>0.25) for any RC index or subset of data. Therefore the requisite for the joint analysis of the Z from the two surveys with this model was verified.

For the total mortality (Z1+), the two slope model on RCSurvey2 (Figure 5.3) resulted in M1+ of 1.05 (CV=19%) (table 5.3), though the slopes were not significantly different between them (p=0.283). This estimate was very close to the mean of the individual M1+ estimates from the surveys, which were 1.14 (CV=26%) for the DEPM and 0.953 (CV=26%) for the acoustic (Table 5.3). Forcing a common slope for the two surveys on RCSurvey2 made the single slope model significant and resulted in a M1+ of about 1.15 (with a CV of 15%). The RCJoint2 estimator results in a M1+ of 0.885 (CV=20%). Removal of the suspicious Z1+ values corresponding to RCSurvey2>0.8 and Year>2011 globally improved the fitting while leading to a reduction of M1+ estimates (Table 5.3 and Figure 5.3). In summary, if the complete set of data points towards an M1+ range of between 0.88 and 1.15, analysis of the subset of data reduced those values to within the range of 0.78-0.92, with RCSurvey2 pointing towards higher M1+ than RCJoint2.

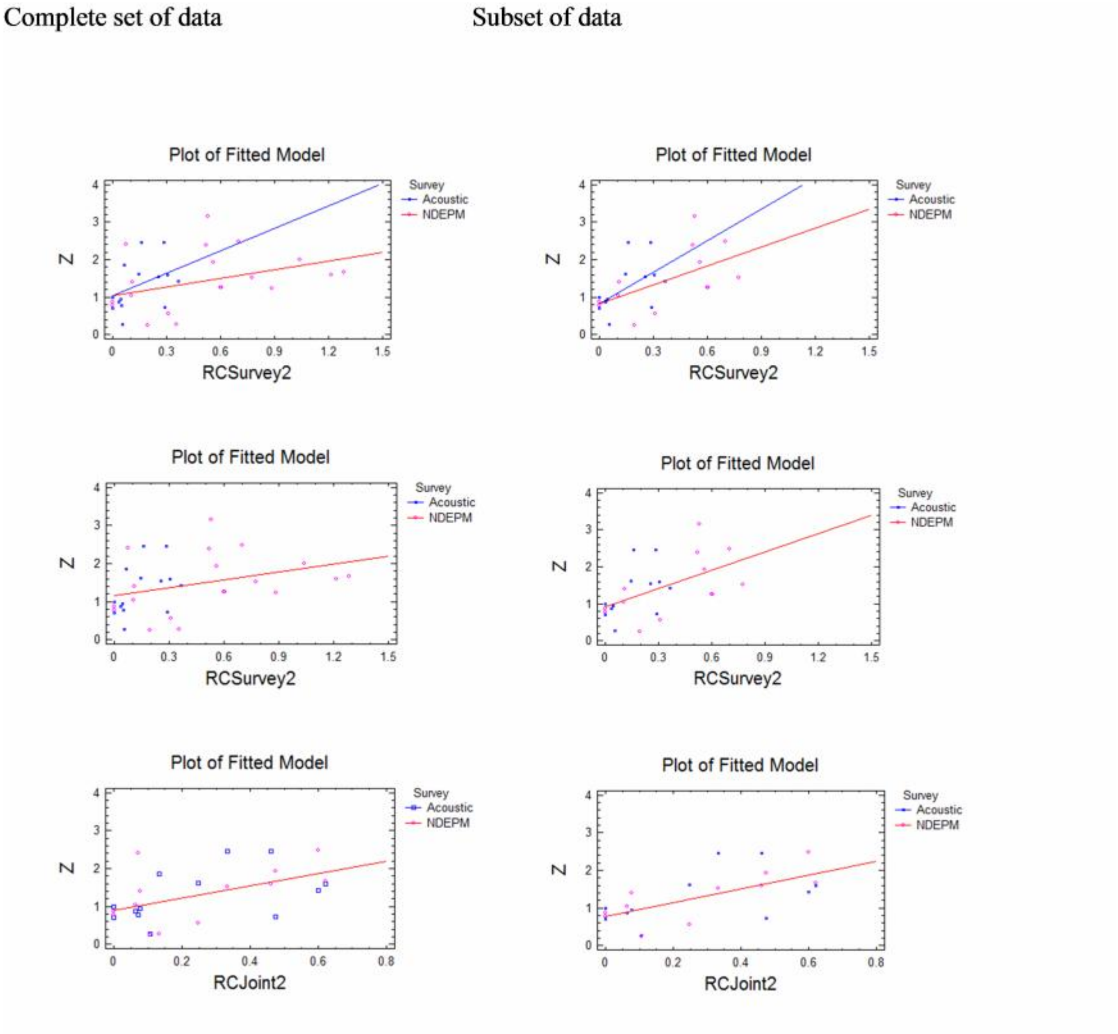


Figure 5.3: Fitted models (Model B1) of total Z estimates (Z1+) as a function of the relative catches between surveys, RCsurvey2 (two and single slope fitting – upper and medium panels) and RCJoint2 (bottom panels), for the complete set of data (left graphs) and for the subset of data (which remove Z values with RC>0.8 and Years>2010) (right graphs).

Table 5.3: Summary results from linear models on overall mortality (Z1+) and estimates of overall Mortality (M1+) from the intercept of the linear models. a) Upper panel analysis for all data, b) bottom panel analysis for data with RC<0.8 and year<2011. Left columns for data assuming no ageing errors, final three columns for cases assuming 5% ageing errors. Values in red and italics are statistically not significant (with p> 0.05).

CASE Z1+	**Estimates by Surveys**		**Pooled Estimates**			**Pooled Estimates & 5% Ageing Errors**		
RC estimator	**RCsurvey2**	**RCsurvey2**	**RCsurvey2**	**RCsurvey2**	**RCJoint2**	**RCsurvey2**	**RCSurvey2**	**RCJoint2**
SURVEY Series	**DEPM**	**Acoustic**	**Pooled**	**Pooled**	**Pooled**	**Pooled**	**Pooled**	**Pooled**
Linear model with	Single-Slope	Single-Slope	Two-Slopes	Single-Slope	Single-Slope	Two-Slopes	Single-Slope	Single-Slope
a) Complete set of Data \ N:	19	14	33	33	26	33	33	26
Intercept (= M1+)	**1.136**	**0.954**	**1.052**	**1.152**	**0.885**	**1.137**	**1.301**	**1.001**
CV	26%	26%	19%	15%	20%	20%	15%	23%
RC slope coefficient	*0.656*	*2.380*	*1.991*	0.694	1.633	3.107	1.048	2.188
CV	69%	55%	62%	49%	33%	44%	36%	32%
Additive Slope component			*-0.915*			*-1.951*		
CV			81%			65%		
Model P-Value	0.1636	0.095	0.0837	0.0496	0.0064	0.0119	0.0099	0.0043
R-Squared	11%	21%	15%	12%	27%	26%	20%	29%
b) Subset of Data \ N:	13	12	25	25	22	24	24	22
Intercept (= M1+)	**0.762**	**0.884**	**0.826**	**0.920**	**0.781**	**0.936**	**1.078**	**0.941**
CV	45%	32%	26%	22%	22%	29%	23%	27%
RC slope coefficient	1.787	*2.583*	2.804	1.651	1.831	3.829	2.103	2.309
CV	41%	54%	44%	33%	27%	38%	34%	31%
Additive Slope component			*-1.130*			*-1.731*		
CV			96%			75%		
Model P-Value	0.0333	0.0927	0.015	0.006	0.0013	0.014	0.0079	0.0039
R-Squared	35%	26%	32%	25%	41%	34%	28%	35%

Analysis of Z by ages (Z1 and Z2+) revealed significant differences in the intercepts (M) by ages (P[*Old*]<0.001) for any RC index: For the case of RCSurvey2, the two slope model (Figure 5.4) pointed to M1=0.94 and M2=1.79, with CVs around 17% and 14% respectively (Table 5.4). These estimates were about the mean of individual surveys estimates (Table 5.4) but the slopes by surveys were not significantly different (p=0.437). The single slope model became fully significant and resulted in very similar values. Using RCJoint2 (Table 5.4 and Figure 5.4 bottom panels) results in slightly lower M1 (at 0.82, CV=23%) and rather similar M2 (at 1.73, CV=17%).

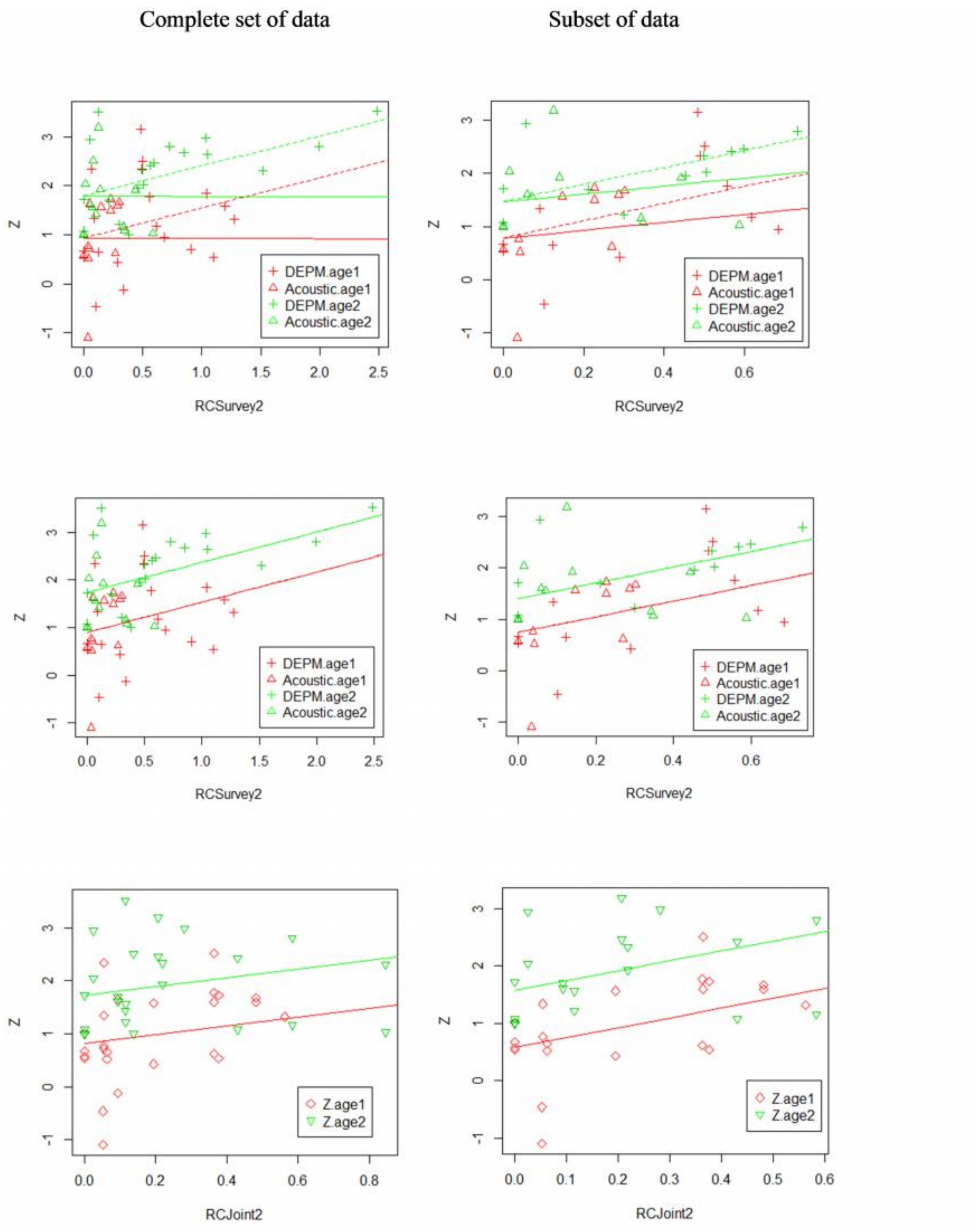


Figure 5.4: Fitted models for the Z by age (Z1 and Z2+) as a function of the relative catches between surveys, RCsurvey2 and RCJoint2 for the complete set of data (left graphs) and for the subset of data (which removes Z values with RC>0.8 and Years>2010) (right graphs).

Table 5.4: Summary results from linear models on estimates of Z by age (from Age 1 to 2 and from Age 2+ to 3+), with estimates of M1 and M2+ from the constant intercepts of the fitted models. a) Upper panel analysis for all data, b) bottom panel analysis for data with RC<0.8 and year<2011. Left columns for data assuming no ageing errors, final three columns for cases assuming 5% ageing errors. Values in red and italics are statistically not significant (with p> 0.05).

CASE Z by ages	**Estimates by Surveys**		**Pooled Estimates**			**Pooled Estimates & 5% Ageing Errors**		
RC estimator	**RCsurvey2**	**RCsurvey2**	**RCsurvey2**	**RCsurvey2**	**RCJoint2**	**RCsurvey2**	**RCsurvey2**	**RCJoint2**
SURVEY Series	DEPM	Acoustic	POOLED	POOLED	POOLED	POOLED	POOLED	POOLED
Linear model with	Single-Slope	Single-Slope	Two-Slopes	Single-Slope	Single-Slope	Two-Slopes	Single-Slope	Single-Slope
a) Complete set of Data \ N:	38	26	64	64	50	64	64	50
Intercept1 (= M1)	**0.951**	**0.933**	**0.940**	**0.900**	**0.822**	**1.373**	**1.319**	**1.113**
CV	23%	26%	17%	17%	23%	12%	12%	19%
OLD (addition for M2+)	0.999	0.644	0.854	0.839	0.908	*0.339*	*0.313*	*0.448*
CV	26%	46%	23%	23%	25%	62%	67%	56%
Intercept2 (= M2+)	**1.951**	**1.577**	**1.794**	**1.738**	**1.730**	**1.713**	**1.631**	**1.561**
CV	18%	24%	14%	14%	17%	16%	16%	21%
RC slope coefficient	0.527	*0.412*	*-0.009*	0.634	*0.818*	*-0.474*	0.418	*0.878*
CV	45%	234%	9593%	31%	64%	172%	33%	62%
Additive Slope component			*0.621*			*0.873*		
CV			128%			90%		
Model P-Value	0.0002	0.0875	0.1942	0.0000	0.0002	0.0045	0.0026	0.048
R-Squared	39%	19%	15%	35%	30%	15%	18%	12%
b) Subset of Data \ N:	23	22	45	45	40	45	45	40
Intercept1 (= M1)	**0.682**	**0.861**	**0.780**	**0.742**	**0.582**	**1.091**	**1.058**	**0.883**
CV	44%	32%	25%	26%	35%	20%	20%	26%
OLD (addition for M2+)	0.754	*0.610*	0.684	0.662	0.997	*0.261*	*0.221*	0.546
CV	41%	54%	32%	33%	23%	93%	110%	48%
Intercept2 (= M2+)	**1.436**	**1.471**	**1.464**	**1.403**	**1.580**	**1.352**	**1.279**	**1.429**
CV	30%	29%	20%	21%	19%	24%	25%	24%
RC slope coefficient	1.748	*0.629*	*0.748*	1.535	1.710	*0.507*	1.413	1.871
CV	36%	163%	116%	32%	35%	171%	38%	36%
Additive Slope component			*0.878*			*1.079*		
CV			91%			76%		
Model P-Value	0.0044	0.1474	0.1474	0.0002	0.0001	0.0306	0.0115	0.0069
R-Squared	42%	18%	18%	33%	41%	14%	16%	24%

Working with the subset of data (Figure 5.4b and Table 5.4b) improved the fittings overall and again led to a reduction of M estimates: For the two slopes model on RCSurvey2, the values reduced to 0.78 for M1 (CV= 25%) and to 1.46 for M2+ (CV=20%), and to very similar values for the single slope model. For the RCJoint2, using the subset data reduces M1 to 0.58 (CV=35%) and M2 to 1.57 (CV=19%). In summary, the complete set of data results in M1 in the range 0.82- 0.94 and M2+ about 1.73, whilst analysis restricted to the subset data reduced those estimates to M1 within the range 0.58-0.78, and M2+ in the range 1.40-1.57, with RCSurvey2 pointing towards higher M1 than RCJoint2.

5.3.3 Natural mortality estimates from integrated assessments

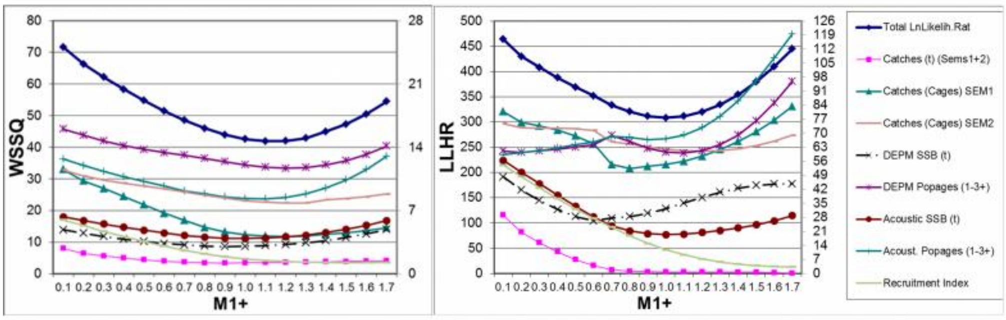
Optimization for a single overall natural mortality (M1+, common for all ages) including all input data resulted in M values of around 1.15 and 1 for the WSSQ and LLHR optimizations respectively (Table 5.5a). In all cases the response surface was rather flat around the optimum (+/- 0.1) being basically guided by the age-structured survey indexes, followed by the catches at age information and finally, to a lesser extent, by the biomass survey indexes -- which in fact favored slightly lower M1+ optimums (around 0.7-1.1) (Figure 5.5a). In all cases the small contribution of JUVENA favored M1+ values at or above the synthetic optimum. The sensitivity of these results to the omission of the 2012 survey estimates was negligible, with optimums differing by less than 1% (not shown for simplicity).

Table 5.5: Summary results of the integrated assessments.

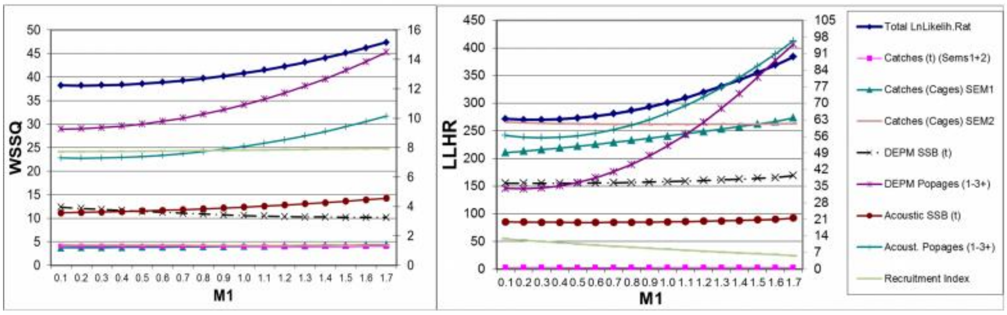
	a) No ageing errors		**b) 5% ageing errors**	
Single M				
	WSSQ	**LLHR**	**WSSQ**	**LLHR**
Mean M1+	**1.148**	**1.000**	**1.188**	**1.044**
CV (aprox)	5.4%	4.3%	6%	4%
Objective Function	42.3093	308.5502	50.1018	317.7327
Total LogLikelihood	-88.1095	-490.0735	-126.5522	-491.5557
AIC	362.2191	1166.1470	439.1043	1169.1115
AICc	419.3563	1223.2842	496.2416	1226.2487
M. by ages	**M. (January to December)**		**M. (January to December)**	
	WSSQ	**LLHR**	**WSSQ**	**LLHR**
Mean M1	**0.172**	**0.262**	**0.733**	**0.582**
CV (aprox)	88%	44%	36%	21%
Mean M2+	**1.467**	**1.376**	**1.326**	**1.263**
CV (aprox)	7.5%	6.1%	9%	6%
Objective Function	38.1907	270.0725	49.1966	64.0470
Total LogLikelihood	-73.1320	-451.5218	-123.5479	-478.6685
AIC	334.2641	1091.0436	435.0958	1145.3369
AICc	392.8215	1149.6009	493.6532	1203.8943
LogLikelihood ratio vs single M	14.9775	38.5517	3.0043	12.8873
Probability of the Ratio	0.0000	0.0000	0.0496	0.0000
M. by ages	**M. (July to June)**		**M. (July to June)**	
	WSSQ	**LLHR**	**WSSQ**	**LLHR**
Mean M1	**0.700**	**0.712**	**0.979**	**0.853**
CV (aprox)	19%	9%	15%	8%
Mean M2+	**1.518**	**1.429**	**1.351**	**1.299**
CV (aprox)	8.0%	6.4%	9%	7%
Objective Function	38.5194	271.5586	49.2783	305.014
Total LogLikelihood	-74.6555	-452.8735	-123.7316	-478.888
AIC	337.3109	1093.7471	435.4632	1145.7757
AICc	395.8683	1152.3045	494.0206	1204.3331
LogLikelihood ratio vs single M	13.4541	37.1999	2.8205	12.6679
Probability of the Ratio	0.0000	0.0000	0.0596	0.0000

WSSQ minimization **LLHR minimization**

a) M1+



b) M1 & M2+ (Jan-Dec)



c) M1 & M2+ (July-June)

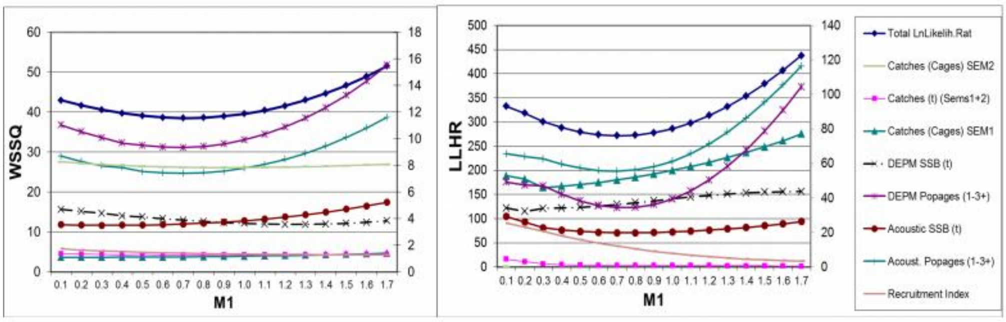
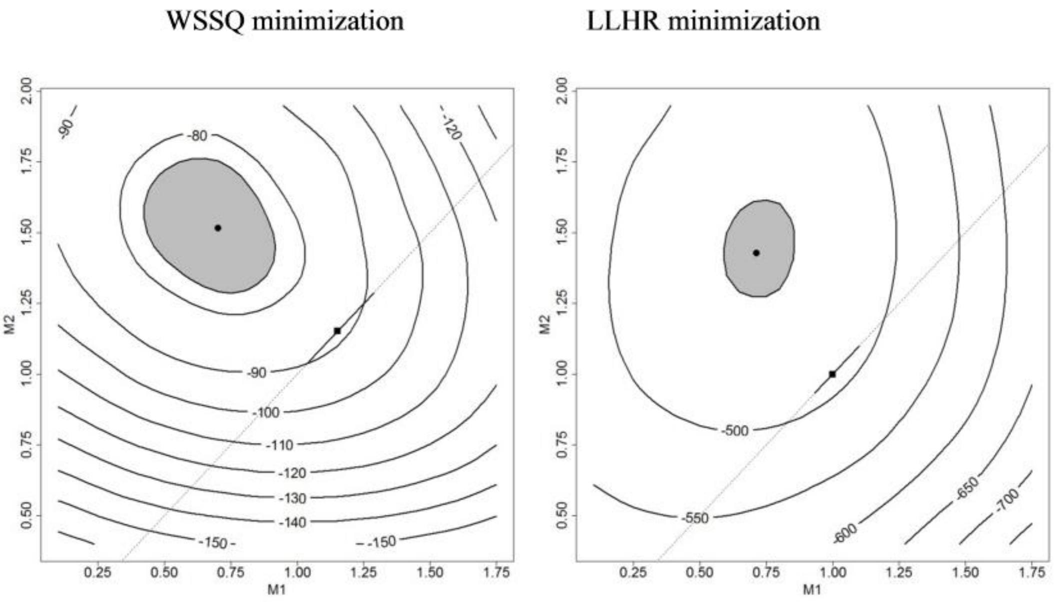


Figure 5.5: Response surfaces (total and partial contribution of the different auxiliary information) for the two objective functions, weighted sum of squares (WSSQ, right panels) and Log-Likelihood Ratios (LLHR, left panels) for a) a range of single Natural Mortality values (M1+) or b) , for a range of natural mortality at age 1 (M1 optimizing M2+) with a natural calendar year (January to December) and c) by age as before but with a calendar year from July to June.

Optimization of natural mortality by ages (M1 and M2+) for a Jan-Dec calendar year suggested for both objective functions that the lower the M1 the better the fitting achieved, though improvements gradually reduced, being minimal below 0.7 (Figure 5.5b) with optimum M1 around 0.15-0.3 and M2+ around 1.35-1.45 (Table 5.5b). Applying a July-June calendar year pointed out optimums for both objective functions of around 0.7 for M1 (Figure 5.5c and Table 5.5c) and 1.4-1.5 for M2+ (see joint confidence intervals by models in Figure 5.6a,b). Results are basically guided by the age-structured survey indexes which provide a rather parallel response, whilst all other inputs are non-informative (almost flat) (Figure 5.5b, c). Compared to the assessments assuming a single natural mortality (M1+), the assessments allowing M at age to be estimated (M1/M2+) get a better fitting (higher likelihood - Figure 5.6) and lower Akaike’s information criterion (Table 5.5). These results were insensitive to the omission of the 2012 survey estimates (not shown).

a) No Errors



b) 5% Ageing Errors

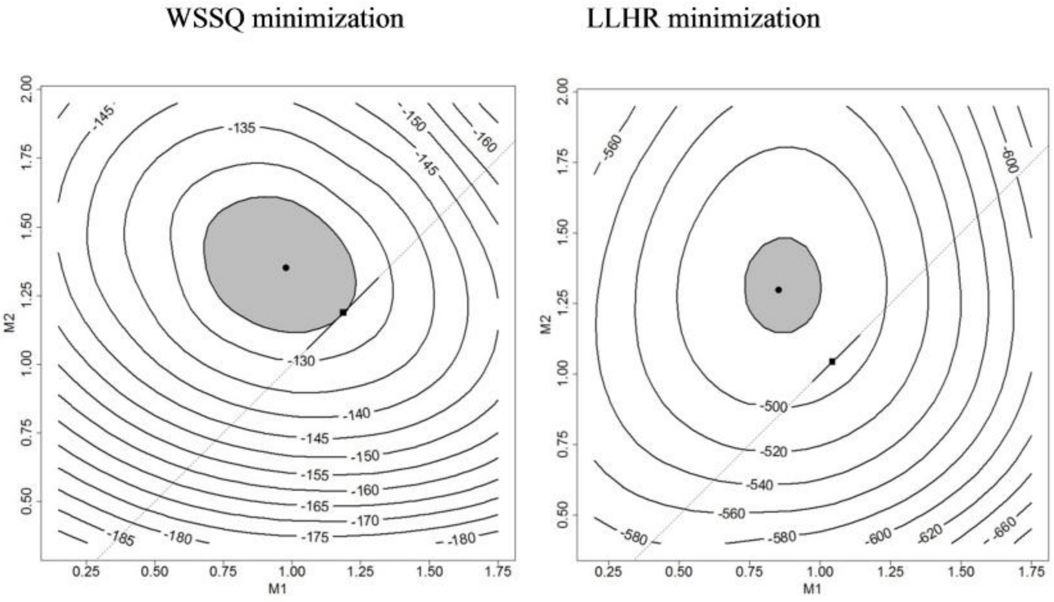
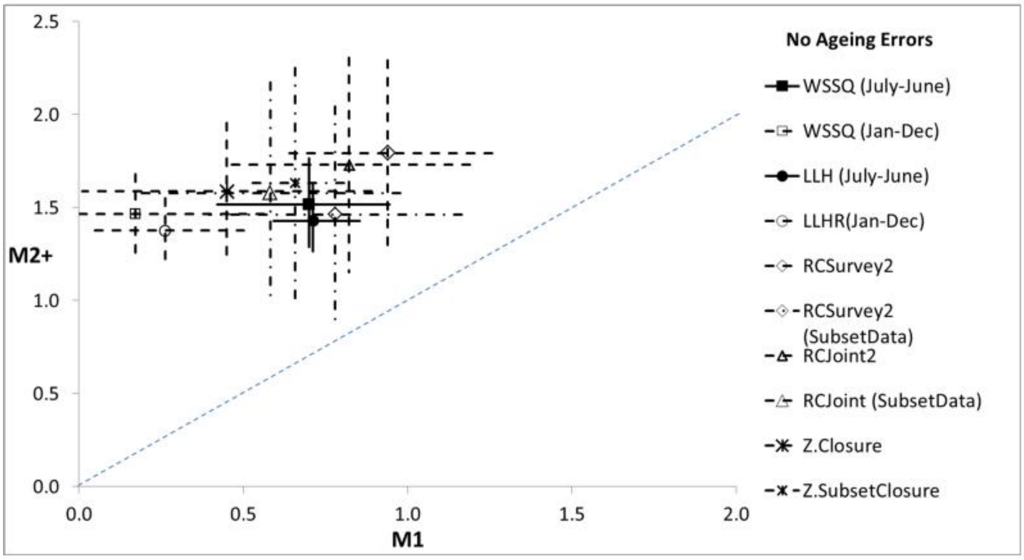


Figure 5.6: Joint likelihood profile contour plots for natural mortality estimates by age (M1 and M2+), deduced from the two integrated assessments based either on weighted sum of squares (WSSQ, left graphs) or on log-likelihood ratios (LLHR right graphs), both based on a calendar year going from July to June, with information about the MLE estimates for M1 and M2+ (black dot) with their joint 95% confidence region (gray area) and MLE for a single natural mortality common to all ages (M1+) and its 95% confidence limits (black square point and lines along the 1:1 dotted line) for a) no ageing error default case (upper graphs) and b) for an assumption of 5% ageing errors (see text for details).

The M at age estimates obtained by the integrated models for the July-June calendar year are consistent with those produced by the direct and linear models previously (Figure 5.7).

a) No ageing errors



b) 5% Ageing Errors

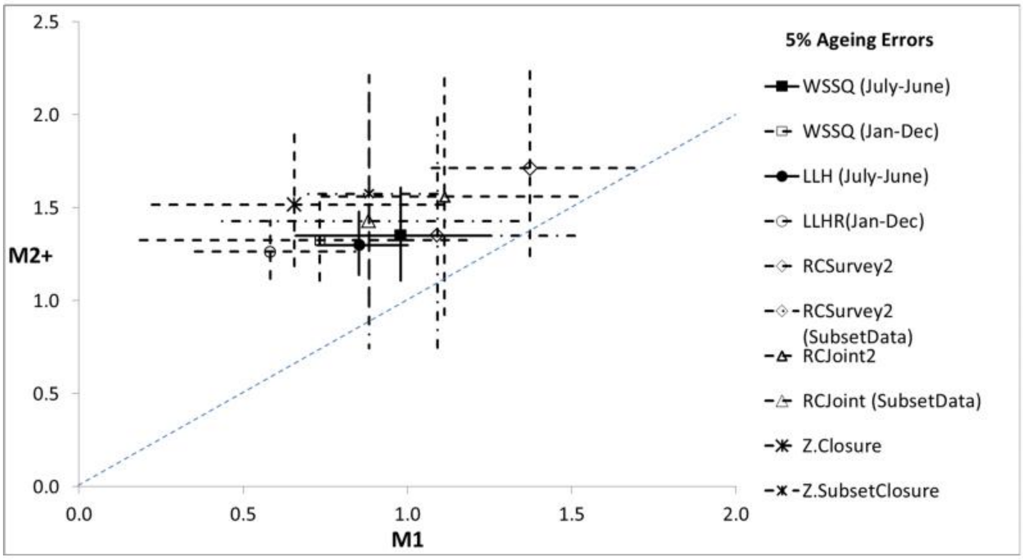


Figure 5.7: Comparison of natural mortality estimates by age (M1 and M2+) for the different estimation methods: a) for cases with no ageing errors b) for cases with 5% ageing errors. A 1:1 dotted line is included to check compatibility with the common assumption of single mortality.

5.3.4 Sensitivity to Ageing Errors

Inclusion of 5% ageing errors did not affect the compatibility of Z1+ estimates by surveys, neither in the raw data analysis (P=0.97) nor in the log-linear models (P=0.71). This compatibility also applied to the analysis of Z by ages where all terms with *Survey* were not significant either. Therefore the joint analysis of the two surveys was again statistically supported.

Consideration of a 5% ageing errors, raised up the M1+ estimates by about 0.14 in direct and lineal model analysis, letting for the subset data all M1+ in the range to 0.94-1.08 (Table 5.2 and Table 5.3). Incorporation of this ageing error into the integrated models leads to minor increases of the original M1+ estimates (by about 0.04), resulting in poorer fits and larger AICc than for the uncorrected data (Table 5.5).

By ages, consideration of a 5% ageing error meant that paired raw estimations of Z1 were still on average below those of Z2+ for both surveys over the whole period (Table 5.2b) (paired t-test p=0.0295), and more intensively during the closure period (p=0.0146). The statistical significance over the historical series is due basically to the DEPM (p<0.01), not to the acoustic (p=0.4572), though for this survey in majority (8 over 13 years), and particularly during the closure (4 over 5 years) Z2+ was higher than Z1.

The correction for a 5% ageing error reduces the differences of M between ages by increasing M1 estimates by about 0.2 and 0.3-0.4 in the direct and lineal model analysis respectively, and by reducing M2+ by a lesser extent (by about 0.08- 0.16). Nevertheless, M1 remains invariantly below M2+, even though statistical significance is sometimes lost (Table 5.3)

Both integrated models, when considering a 5% ageing error, also obtained a reduction of the differences of M by ages by increasing M1 and reducing M2+, but to a larger extent for the January to December calendar year. Nevertheless, M1 remains invariantly below M2+. Certainly, the improvement in fitting achieved by allowing M to change by age is reduced when compared to no aging errors, but the differences are still significant (even though only at alpha 0.06 for the WSSQ) (Figure 5.6) and the

Akaike's information criterion improves (is reduced), therefore still endorsing the M by ages models over the single M1+ models.

Figure 5.7b shows the general consistency and overlapping of the M at age estimates from the different methods essayed before (Figure 5.7).

5.4 Discussion

Estimating natural mortality is one of the main challenges in stock assessment (Vetter 1988; Hilborn and Walters 1992; Gislason *et al.* 2010), and in order to do it, availability of research surveys are essential (Sinclair 2001; Sparholt *et al.* 2002; Zhao *et al.* 2003; Francis 2011). The basic assumption of this paper was that the direct monitoring program since 1987, through two research surveys (23 DEPM and 18 Acoustic surveys) and the closure of the fishery for 5 years should enable the overall level of natural mortality and its pattern by ages to be inferred for the Bay of Biscay anchovy. This was supported by the compatibility of the Z estimates from the two surveys and because, in spite of the large variability inherent to the data, Z differed significantly between the open and closed fishing periods, being lower during the latter period and therefore indicative of the natural mortality levels. Moreover, mortalities at age two and older (Z2+) were significantly higher than at age group one (Z1) throughout the time series, suggesting an increasing pattern of natural mortality by age.

The series of Z estimates have a large inter-annual variability which must be linked either to observation errors in the surveys or to variability in natural and fishing mortality, or both. Observation errors in surveys are evidenced by the fluctuations in Z between consecutive surveys (sometimes passing to negative values) and in the occasional strong divergences of survey estimates (as in 2012). Variability of fishing mortality naturally happens for the fisheries, which tend to stabilize catches when exploiting a highly fluctuating population (like anchovy). Some variability of natural mortality has always been presumed and could be linked to changes in the ecological environment (Vetter 1988; Zwolinski and Demer 2013). For these reasons, we gradually chose improved estimators of natural mortality, allowing for increasing data input and, in principle, greater noise filtering.

The direct and linear models indicate to a natural mortality for all age groups (M1+) of between 0.81 and 1.15, which reduces to 0.78 - 0.92 after filtering the suspected noise. The integrated models indicate out M1+ at about 1.15 for WSSQ and 1.00 for LLHR, i.e., at the upper range of the estimates from the former models. Even though the estimates are not statistically different, there can be several reasons explaining this preference for upper M1+ by the integrated models: Firstly, their results include, in addition to the spring surveys, other auxiliary information which partly favors this upper range of M1+ values, mainly by the recruitment index and to a lesser extent the catches by age. Secondly, it should be pointed out that linear models make inferences of M1+ from the decay between age groups 1+ to 2+, whilst the integrated models fit simultaneously the three age classes (1/2/3+) with the same M1+, and as such the former estimates may be closer to the weighted mean of M by ages (according to their abundance), while the latter to an arithmetic mean (as residuals by ages have equal weights). Hence, weighted M1+ to age class abundances should favor the M1+ at the lower range pointed out beforehand, because M1 is lower, and will serve to better describe the average change of the whole population. In any case, this results in a most likely range going from 0.78 (RCJoint2) to 1 (log-likelihood assessment), which corresponds with an annual survival of between 46% and 37%. If a single number is needed, a rough compromise could be in the middle of the ranges, around 0.9 for M1+ (annual survival rate of 41%).

These results point towards a lower M1+ than the currently assumed value of 1.2 which was calculated in the 1990s on the basis of the DEPM survey alone (Uriarte *et al.* 1996; Prouzet *et al.* 1999). The DEPM survey still suggests, when including all data, a value for M1+ of around 1.14. Moving to an M1+ of 0.9 would imply a reduction of the average historical estimates of SSB by about 30-35%, and an inverse parallel increase of the fishing mortality estimates.

This average natural mortality for adult anchovy in the Bay of Biscay is just slightly below the values of 1.08 and 1.34 which results from applying Hoening’s equation (1983) (for a Tmax of 4) and that of Pauly (1980) (for von Bertalanffy growth parameters Linf, K and t0 about 18.05 cm, 0.77 and 0.72, respectively, as fitted to DEPM survey observations and a mean temperature of 16 ºC). Nevertheless, these

M1+ estimates are rather similar to, albeit slightly higher than, the ones reported for short-lived species of similar growth, such as sprats, some Sardinops or Engraulidae (Beverton 1963, 1992, MacCall 1973; Methot 1989; Iversen *et al.* 1993; Sinovčić 2000), but at a lower level than the ones reported for Peruvian anchovy (Pauly 1987) and *Anchoa mitchilli* (Neweberger & Houde 1995) and other Engraulidae of smaller maximum sizes (Bayliff 1967).

All the analysis improved by allowing M to change with age: The ***Old*** factor was retained in the linear models and the likelihood of the integrated models improved significantly when M was estimated by ages compared to the single common M1+ modeling (Figure 5.6). This led to the conclusion that M1 is significantly lower than M2+ (Figure 5.7): On the one hand, the direct Z estimate and the linear models result in a range of M1 from 0.45 to 0.94 when using the full data set, and a narrower range of about 0.6-0.8 with the most reliable subset of data. This corresponds with annual survival of about 55%-45% for age group. On the other hand, M2+ was consistently about twice the value of M1: 1.59 to 1.79 for M2+ with the full data set and 1.4-1.65 for the most reliable subset of data. This corresponds with an annual survival of about 25%-19% for ages two and older.

Furthermore, integrated assessment indicate M at ages consistent with the former results, but only for the July-June calendar, whilst the Jan-Dec calendar year suggested a better fit at a lower M1 (Figure 5.7). For the latter modeling, such discrepancy arises from the pronounced mismatch between the calendar of application of natural mortality by ages (January to December) and the calendar between survey observations (from May to May) which have to be fitted. Both integrated assessments from July to June (WSSQ and LLHR) produced almost identical estimates as those from the linear models in RCSurvey2 on the most reliable subset of data. This means that the population modeling context of the integrated assessment, including other auxiliary information, has enabled a natural filtering of much of the noise affecting the individual spring survey estimates. In summary, both July-June integrated assessments have captured the pattern of natural mortality at age between survey observations (from May to May) well and are consistent with the former simpler linear models (particularly with those using the reliable subset of data) (Figure 5.7). To clarify, they all pointed towards an M1 of around 0.70 and M2+ of around 1.40 (i.e. survivals about 50% and 25% respectively).

Our data do not allow staying when this additional mortality at age 2 and older happens during the year, however the sharp decrease of the 2 and 3 year-old is already noticeable in the fishery after spawning, during the second half of the year (ICES 2005; 2013, Uriarte *et al.* 1996): This could have been the result of some permanent emigration of the old (2+) fishes outside of the major fishing grounds, off the Bay of Biscay, but such a possibility has never been shown and it is well known that every spring old fishes concentrate again to spawn in the southeast of the Bay of Biscay (Motos *et al.* 1996) where the surveys take place. Therefore this increasing mortality at age two and older is most likely due to either increased vulnerability to predation at older ages or to natural biological mortality, probably reflecting senescence of anchovies at age 2 and older. No major concentration of predators on adults in early summer has been reported. The Bay of Biscay is an area of bottom up controlling of the upper-trophic-levels (Lassalle *et al.* 2011), with anchovy being one (and not the main) among several small pelagic and other fishes (such as sardine, sprat, horse mackerel, blue whiting, etc) connecting the plankton communities to piscivorous species (Sanchez and Olaso 2004; Preciado *et al.* 2008). It contributes to the diets of demersal species (such as hake, monkfish, megrim and tunnidae but mainly as juveniles (Guichet 1995; Preciado *et al.* 2008; Lezama-Ochoa *et al.* 2010; Goñi *et al.* 2011; 2012; López-López *et al.* 2012) whilst in adult anchovy it seems that some demersal fishes (John dory and hake, etc) and cetaceans prey routinely all year around (Preciado *et al.* 2008; Mahe *et al.* 2007; Lassalle *et al.* 2012; Meynier *et al.* 2008). Therefore, discarding any ecological higher predation on adults, we tend to think that senescence might be occurring at the age of two and older, in accordance with the expectation of observable senescent mortality affecting short-lived cupleoids (Beverton 1963).

We hypothesize that this anchovy population may suffer from “reproductive stress” inducing increased mortality, particularly after its second spawning event. It is known that the large energetic cost of reproduction can induce some varying rates of mortality due to “reproductive stress”, as shown for Cod (Hutchings 2005), or in extreme cases of semelparous species like Pacific salmon *Oncorhynchus*, which reproduce and die, whereby dramatic hormonal changes induce starvation that causes intestine deterioration by necrosis and inflammatory processes during spawning migration (McBride *et al.* 1986). Examples of mortality associated to reproductive stress in short-lived species are common, as in invertebrates (squids and shrimps, Caddy et al 1996) and fishes such as *Aphia minuta* (an extremely short-lived goby where apoptosis of enterocytes is related to post spawning mortality– Caputo *et al.* 2002), capelin *Mallotus villosus* (Vilhjalmsson 1983; Gjosaeter 1998) and in the short-lived gadoid Norway Pout (Nielsen *et al.* 2012). Anchovy in the Bay of Biscay invest much energy in reproduction, having one of the highest spawning frequencies of the Engraulidae, which still increases slightly with size and age (Uriarte *et al.* 2012) and results in a very high daily fecundity (Santos *et al.* 2015). Many of the one year old fishes lay down a spawning check at the end of the spawning season (in early summer) (Uriarte *et al.* 2002; *in press*; Petitgas & Grelier 2003) being probably indicative of the limiting condition of many anchovies after spawning (Pecquerie *et al.* 2012). While they have reached about 88% of their maximum attainable growth (18.3 cm) by the age of 2, spawning starts earlier and probably lasts longer than at age 1, with a likely increase in the number of batch-spawning events during the spawning season (Motos *et al.* 1996; Motos 1996, Pecquerie *et al.* 2009), in accordance with the indications for other Engraulidae (Parrish *et al.* 1986; Claramunt *et al.*2007; Cubillos & Claramunt 2009). On average, the 2 year old anchovies do not resume the opaque edge formation in otoliths until mid-June, beyond the mid-point of the spawning season (Uriarte *et al.* 2002; in press), this being also indicative that much of the energy of this age group is invested in reproduction during the first half of the year. This reproductive strategy of anchovy may result in some reproductive stress at the end of the spawning season for the age 2+ group, which may explain the increase in natural mortality observed in our analysis. As such, this population of anchovies may have evolved in its fluctuating environment by allowing some non-negligible chances of reproducing over two spawning seasons before senescence. This would enhance the opportunities to overcome single (not repeated) environmentally-induced failures of recruitment. This strategy would place anchovy among capelin, which mostly die after their first spawning (Vilhjalmsson 1983; Gjosaeter 1998), and sprats, sardines or sandeels which have progressively longer adult life expectation with several annual reproductive cycles (Cook 1994; Nunes *et al.* 2011, Zwolinski and Demer 2013), up to herrings which seem to have up to eight reproductive seasons during their life history before increased mortality by senesce shows up (as for Norwegian Spring-spawning herring, Beverton *et al.* 2004). To our knowledge, senescence in Engraulidae had only been suggested previously for northern anchovy (from age 2 and older -- MacCall

1973 – based on analysis of catch ratio from catches and surveys) and for the anchovy in the Yellow Sea (from age 3 and older -- Zhao *et al.* 2003 – based on analysis of surveys). We suspect that this pattern of increasing mortality by ages may be applicable to other Engraulidae, whereby assessments following the usual constant mortality assumption often results in non-understandable big changes in catchability by ages in surveys (Giannoulaki *et al.* 2014).

In terms of assessment, the new M estimates would impact the average level of spawning biomass approximately in proportion to the reduction in average M: For instance, for the LLHR modeling, moving from the single M1+ hypothesis at 1.2 to its optimum at 1.00 would reduce mean biomass by about 27%, while moving further moving from the optimum M1+ to its optimal mortality by ages would result in a minor reduction of about 5%. So once M1+ is set at its optimum, a further change to the optimum M by ages does not imply additional major changes to mean biomass estimates. In spite of this, however, this latter change to M by ages will probably have implications in any projections of the population and the fishery which might be required for the provision of advice to managers. For instance, for this anchovy, old populations (composed mainly of ages 2+) will decay faster (with M around 1.4) than young populations (composed mainly of age 1) (with an M around 0.7-0.8). Therefore in terms of management, moving to unbiased M estimates should also improve the quality of advice as noted for other species (Lee *et al.* 2011; Zwolinski *et al.* 2013). Certainly, the perception of fishing mortality will change inversely to the estimation of biomass levels. Furthermore, as the new M estimates change the perception of the dynamics in mass of cohorts, this will affect the estimation of management-related reference points (F0.1, Fmsy etc.).

These results are partly sensitive to the maximum level of ageing errors put for consideration. Correction of such a 5% ageing error moved the direct and linear models estimates of M1+ and of M1 upwards, while slightly reducing M2+. This was associated with a partial loss of the statistical significance of the difference between M1 and M2+ (Figure 5.7b). Integrated models were less affected in the M1+ estimates (remaining just slightly above the original optimums), and also showed a reduction of the differences between M1 and M2+ (Table 5.5). Nevertheless, integrated assessments show that models allowing M by age estimates achieve better fittings than the single M1+ models (with $p<0.056$ for the WSSQ and $p<0.000$ for the LLHR)

(Figure 5.6). Therefore, even after consideration of this maximum level of potential ageing errors, the significant increase pattern of natural mortality at age is still supported by the integrated assessments. In any case, the M estimates will be conditioned by the actual level of ageing errors affecting the observations.

Certainly, the results depend upon the assumption of the constant catchability of surveys across ages, on which the estimations of Z rely (an assumption we have proved to be compatible with the data). Sustaining this is not true would be difficult to understand, given the good spatial coverages of the surveys, and would imply that the prevailing null hypothesis should be on the assumption that M is equal across ages. Biologically, this is known not to be true (Vetter 1988), even though for simplification this has been the null hypothesis for the assessment of the exploited range of ages of most of the long and short-lived fish resources. Although this has already been questioned and revised for the younger ages (Caddy 1991; Abella *et al.* 1997: Gislason *et al.* 2010), it has not been sufficiently revised yet for the inclusion of senescence in the older age classes. And, certainly for short-lived species, senescence should have been presumed to be noticeable not too long after reaching maturity (Beverton 1963). By accepting that the null hypothesis should be that senescence is present, then our analysis shows consistency of the observations with the hypothesis, and we would rely more on the observations of the age structures reported by surveys rather than on doubtful assumptions of constant natural mortality across ages, and finally the assessment can become parsimonious (as in our case with two surveys) in terms of demanding a lesser amount of parameters to infer population sizes (i.e. lesser amount of catchability at age parameters in comparison to the increased number of natural mortality by ages).

A correct selection of the natural mortality serves to properly scale the assessments around true population abundance values and this becomes particularly relevant in the context of relative indexes of population abundances, and even more so for short-lived species. In this paper we have shown that that the overall natural mortality M1+ was somewhat below what had been assumed so far, and furthermore, that natural mortality at ages 2+ is higher than at age 1, in line with expectation of senescence. In achieving this conclusion, the continuous monitoring of the population by two parallel and independent methods for many years, including a period of fishing closure, has been essential, as it has provided sufficient contrast for the analysis. The issue of natural mortality should be revisited periodically when additional years of survey observations and improvements in our base knowledge become available. Relevant improvements may come from better understanding of survey catchability, or of the accuracy of age determinations for this anchovy, besides, for instance, from monitoring biological and biochemical markers of aging and reproductive stress or from further studies on eco-trophic interactions.

NatMort. Appendix 1: Integrated assessment of the Bay of Biscay

We applied a Seasonal Integrated Catch at Age analysis (SICA) on a six-monthly basis, fitting different selectivity patterns to the international fleet operating during each half of the year and tuning the population to the abundance indexes available (as used by ICES 2013): the two spring surveys (Acoustic and DEPM index) and a recruitment acoustic survey on juveniles in autumn. Age structure of both catches and spring surveys go up to age group 3+ (grouping ages 3 and older). The survey on juveniles produces a single index at age 0 in the autumn of any year Y which is entered as an indicator of the strength of the recruits at age 1 in January of year Y+1.

Biomass indexes are dealt with as relative indexes proportional to abundance for which no additional catchability is estimated. In addition, in SICA, a Qflat catchability model is implemented for the purposes of this analysis (i.e. forcing catchability at age of the surveys to be equal for all ages).

We have fitted SICA with the Qflat catchability model for the two surveys allowing to optimize for M1+ (a single natural mortality for all ages) or for M1 and M2+ (i.e. one M for age 1 and another for the plus group of ages 2 and older); the latter is essayed in order to find out what natural mortality pattern optimizes the fitting. In practice, as the model is implemented in Excel, a systematic optimization procedure across a range of M1+ or M1 (including the optimization of M2+) was made. Initially a range of M between 0.1 and 1.7, in steps of 0.1, was covered. Later, a systematic 2D mapping around the optimum was carried out in stopes of 0.05. The results show the values of the objective function optimized for the selected range of M1+ or M1 values (the latter also optimized for M2+) in 1D or 2D as required.

Operating Model

Population and fishing mortality at age: We applied the typical population survival exponential model (Ricker 1975) with catches following the Baranov (1918) equation, subject to a separable model of fishing mortality by age, year and seasonal (half-year) fishery of the form:

$F_{a,y,p} = F_{ref,y,p}.S_{a,p}$ Eq A.B.1

Where $F_{ref,y,p}$ is the fishing mortality in year ***y*** and half-year fishery ***p*** for the age of reference (*ref*), which in this study is age 2 ($F_{ref,y,p} = F_{2,y,p}$).

$S_{a,p}$ is the selectivity for each age typical of every half-year fishery and relative to the age of reference (age 2), which is fixed at 1 (*i.e.* selectivity value of $S_{2,p}$=1).

Catches are modeled up to age 3+ (older ages are negligible) on a half-yearly basis.

Natural Mortality model: Natural mortality can be set common for all years and ages (by setting a constant M1+) or varying between ages 1 and 2+ by a common factor, as follows:

$$M_{2+} = M_1 \cdot Mfactor_{2+} \qquad \text{Eq A.B.2}$$

$Mfactor_{2+}$, if included, is estimated and kept constant across years.

We checked the sensitivity of applying this factor to age 2 from the beginning of the year or from July only.

The modeled average population during the spring period is tuned to the Acoustic and DEPM spawning biomass and population at age estimates. The tuning indices were used as relative (i.e. proportional to abundance by a catchability parameter Q).

Parameters: For an assessment covering the period 1987-2012 (including the first half of 2013), we would have the following parameters for the 26 years (Y) of data: 4 for the numbers at ages in the first year 1987 (ages 1-4, because the 3+ plus groups were modeled as the sum of two age groups --3 and 4+), 26 recruits at age 0 (N0,y) , 2*26+1 for the fishing mortalities at the age of reference (age 2) (one per half year: $F_{2,y,1}$ and $F_{2,y,2}$ and $F_{2,2013,1}$), 5 selectivities at age (the ages on a half-yearly basis: $S_{1,1}$ / $S_{3,1}$ / $S_{0,2}$/ $S_{1,2}$ / $S_{3,2}$), 4 Catchability parameters ($Q._{Ac}$, $Q._{DEPM}$, and Q & *Power* for the Juvenile index), and a minimum of 1 natural mortality (M1+) = 4+26+53+5+4+1= 93 parameters (or 94 if M1 and M2+ were estimated instead of single M1+).

Objective function: two objective functions were defined, one responding to the case of assuming log-normal errors in all tuning data, which was based on a sum of squared log residuals (similar to the Integrated Catch at age Analysis Patterson and Melvin 1996), and the other for the case where age disaggregated observations (of catches or from the spring survey abundance indexes) are split in a biomass component (assuming log-normal error) and in percentages at age (assuming multinomial errors). In the latter, the objective function was based on the log-likelihood ratios (similar to the objective function in Stock Synthesis Model SS3 -Methot and Wetzel 2013). Parallel to the minimization of the objective function, the log-likelihoods and the Akaike information criterion (AIC) were estimated for comparison purposes (see below).

For the analysis assuming log-normal errors of all data catchability of surveys was estimated common (equal) for all ages (flat catchability at age) but independently for each survey, so that a single common catchability by survey is estimated. Both the population in numbers at age and biomass (SSB) indices are used for the fitting. However, the fitting to SSB indices do not require a catchability parameter, because only the population at age estimates derived from the surveys are used to fit the catchability by survey. Modeled SSB as estimated for a survey is just the product of the modeled numbers at age estimates for the survey times the weights at age in the population. In this way, consistency is assured between the catchability at age estimates and SSB estimates for the surveys. In addition, the residual sum of squares between modeled and observed biomass by the surveys contributes to the total fitting even in the years when no age estimates from the surveys were available (as in 1994 and 1998 for the Acoustics or in 1996, 1999 & 2000 for the DEPM). In turn, this implies that the years when only a biomass index is provided by a survey do not contribute to the fitting of the catchabilities at age. As such, 14 out of 16 acoustic estimates are used for tuning the catchabilities at age (because the other 2 cruises have no age index). For this same reason, only 19 out of 22 cruises tune the catchability at age for the DEPM.

The objective function is a sum of squared log residuals (SSQ) defined for the tuning survey indices of biomass and population at age estimates and for the catches at age and catches in tonnes of the different seasonal fisheries defined above.

$$WSSQTotal =$$
$$SSQCapt_{age} + SSQCapt_{weight} + SSQSurveys_{age} + SSQSurveys_{weight} \qquad \text{Eq A.B.3}$$

The SSQ of the catches in tonnes ($SSQCapt_{weight}$) are estimated as:

$$\sum_{1987}^{2013}\sum_{p=1}^{2} \mathrm{a}_{y,p} \cdot \left(Ln(C_{y,p} / \hat{C}_{y,p})\right)^2 \qquad \text{Eq A.B.4}$$

Where the summation reaches the first half of year 2013 and suffix ***p*** refers to the seasonal fishery (i.e. the first or second half of the year). The expected catches in weight are just based on the SOPs (sum of products) of the modeled catches at age and their observed mean weights.

In parallel the residuals to the catches at age (in numbers) ($SSQCapt_{age}$) are estimated as:

$$\sum_{ages}^{3+}\sum_{1987}^{2012}\sum_{p=1}^{2} \mathrm{a}_{a,y,p} \cdot \left(Ln(C_{a,y,p} / \hat{C}_{a,y,p})\right)^2 \qquad \text{Eq A.B.5}$$

Where modeled catches ($\hat{C}_{a,y,p}$) are simply obtained from the Baranov equation over the half-year period.

In addition, for DEPM and Acoustics population at age the estimates, the $SSQSurveys_{age}$ are

$$SSQSurveys_{age} = \sum_{ages}^{3+}\sum_{year}^{2013}\sum_{s}^{surveys} \mathrm{a}_{a,y,s} \cdot \left(Ln(U_{a,y,s} / \hat{U}_{a,y,s})\right)^2 \qquad \text{Eq A.B.6}$$

Where the modeled index is estimated as:

$$\hat{U}_{a,y,s} = Q_s \cdot \hat{N}_{a,y,s} = Q_s \cdot \frac{\hat{N}_{a,y,p=1} \cdot e^{-\sim_v \cdot Z_{a,y,p=1}}}{(\sim_s - \mu_s) \cdot Z_{a,y,p=1}} \cdot \left(1 - e^{-(\sim - \mu) \cdot Z_{a,y,p=1}}\right) \qquad \text{Eq A.B.7}$$

Where, suffix ***s*** refers to the Acoustic or DEPM surveys, suffix ***p=1*** refers to the first half of the year period; and ***a*** and ***y*** for age and year. ***Z*** is total mortality and $\hat{N}_{a,y,s}$ the modeled population in numbers at survey time. And $\sim_s - \mu_s$ are the starting and ending time of the surveys (as fractions of the year). Notice that the survey catchability Q_s is common for all ages. Suffix ***a*** reaches for acoustics age 2+ until 1999 and subsequently to age 3+ as for the whole DEPM series.

And for the aggregate indices of acoustic or DEPM the $SSQSurveys_{weight}$ equals:

$$\sum_{year}^{2013}\sum_{v}^{surveys} \mathrm{a}_{y,s}\cdot\left(Ln(U_{y,s}/\hat{U}_{y,s})\right)^{2} \qquad \text{Eq A.B.8}$$

With the modeled index estimated simply as the sum of products of the estimated index by age by its observed mean weight in the population, as: 8

$$\hat{U}_{y,s}=\sum_{ages}\hat{U}_{a,y,s}\cdot W'_{a,y,s} \qquad \text{Eq A.B.9}$$

where no additional catchability parameters appear.

Weighting factors: tuning data and fishery catches at age can be weighted (with the lambda factors, a): Fitting the catches (in tonnes) of the two halves of the year received equal weighting factors (lambda = 1). Weighting factors for the catches at ages 1 to 3+ were all set equal to 1, but for age 0 it was set equal to 0.01 because these catches are not considered to be actually separable (since they are taken independently of the other ages and are very noisy). Weighting factors for the DEPM and acoustics were set equal to 0.667 as used in ICA; this is made to discount for correlation across ages in the survey estimates as in the standard ICA implementation. The survey biomass estimates by the model were fitted directly without any differential weighting (a =1), therefore acting as a penalty when the total sum of products of the modeled age structured values diverges from the biomass observations.

We carried out a sensitivity analysis to the weighting factors applied to the indexes at age from surveys for which all $\mathrm{a}_{a,y,s}$ were set at 1 (not shown in the paper). Such alternative weighting result in negligible changes of the natural mortality estimates, with changes smaller than 3% both in the estimates as in the range of the 95% confidence intervals.

The Log likelihood estimates (*LLH*) of the fitting to the (o) auxiliary series of (*n*) observations, each subject to log-normal errors, was simply the sum over the observation series of their respective $LLH\_{Observations}$:

$$LLH\_{Observations} = -\sum_{years\_ages}\sum \mathrm{a}_{y,a}\cdot\left(Ln(O_{y,a}/\hat{O}_{y,a})\right)^2 \Big/ (2\pm_o{}^2) - n\cdot\ln(\pm_o\sqrt{2\circledR})$$

Eq A.B.10

Whereby the log standard error $\pm_o$ by series was taken from the square root of the weighted mean squared residuals for each auxiliary observation series.

For the analysis assuming multinomial errors of the age

All assumptions for the modeling were the same as for the sum of squared log errors, except that for the age structure information from surveys and catches, which are entered as percentages at age (in numbers), subject to multinomial errors. Model fitting is achieved by maximization of the log-likelihood (using log-likelihood ratios).

The objective function is a sum of log-likelihood ratios defined for the tuning survey indices of biomass and population at age estimates and for the catches at age and catches in tons of the different seasonal fisheries defined above.

$$L\_Total = L\_Capt_{age} + L\_Capt_{weight} + L\_Surveys_{age} + L\_Surveys_{weight} \qquad \text{Eq A.B.11}$$

Where log-likelihood ratios for the catches in tonnes ($LCapt_{weight}$):

$$L\_Capt_{weight} = \sum_{1987}^{2013}\sum_{p=1}^{2} \mathrm{a}_{a,y,p}\cdot\left(Ln(C_{y,p}/\hat{C}_{y,p})\right)^2 \Big/ (2*CVc\text{^}2) \qquad \text{Eq A.B.12}$$

Which extends the summation up to the first half of year 2013, with modeled catches in weight based only on the SOPs (sum of products) of modeled catches. Suffix *p* refers to the first or the second half of the fisheries year. The assumed *CVc* (equal to log-normal error σ) was 0.1 (i.e. consistent with our assumption of rather precise catch reporting).

The log-likelihood ratios for the observed proportions at age in the catches in numbers (*LCapt*$_{age}$) are:

$$L\_Capt_{age} = \sum_{ages}^{3+} \sum_{year}^{2013} \sum_{p=1}^{2} x_{y,p} \cdot p_{a,y,p} \cdot Ln(p_{a,y,p} / \hat{p}_{a,y,p}) \qquad \text{Eq A.B.13}$$

which extends the summation until the first half of 2013. Suffix ***p*** refers to the first or second half of the fisheries year. The modeled proportion of catches by ages ($\hat{p}_{a,y,p}$) is directly deduced from the ratio of catches at age over the total catch in numbers. The equivalent random sampling size ($x_{y,p}$) of available ages was assumed to be from 100 individuals of all the series of half-year catches (similar to sampling levels adopted by default for the surveys).

For the catches at age 0, as they were considered not separable, a likelihood ratio similar to equation A.B.12 above was applied to the observed over expected catches at age 0, assuming a CV_{age0} of 0.25.

Similarly, the log-likelihood ratios for the Biomass indexes in tons ($L\_Surveys_{weight}$)

$$L\_Surveys_{weight} = \sum_{1987}^{2013} \sum_{s}^{Surveys} \mathrm{a}_{y,s} \cdot \left(Ln(U_{y,s} / \hat{U}_{y,s})\right)^2 \Big/ (2 * CV_s \wedge 2) \qquad \text{Eq A.B.14}$$

with the modeled biomass index ($\hat{U}_{y,s}$) is estimated as the sum of products of modeled populations at age times the global (common) catchability across ages of the surveys (given our Qflat model) and their mean weights at age ($\sum_{ages} Q_s \cdot \hat{N}_{a,y,s} \cdot W'_{a,y,s}$).

Suffix ***s*** is the survey index (referring to either the Acoustic or the DEPM). The assumed CV_s (equal to log-normal error) was 0.25 for both surveys.

In addition, the likelihood ratios for the observed proportions at age in the survey estimates ($\boldsymbol{p_{a,y,s}}$) is estimated as

$$L\_Surveys_{age} = \sum_{ages}^{3+} \sum_{year}^{2013} \sum_{s}^{Surveys} x_{y,s} \cdot p_{a,y,s} \cdot Ln(p_{a,y,s} / \hat{p}_{a,y,s}) \qquad \text{Eq A.B.15}$$

And the modeled proportion at age estimate of the survey ($\hat{p}_{a,y,s}$) derives from the population at age indexes ($\hat{U}_{a,y,s}$ of equation A.B.7) and is estimated as the ratio of the modeled population at age ($\hat{N}_{a,y,s}$) and the total modeled population (in numbers during survey time).

$$\hat{p}_{a,y,s} = \frac{\hat{U}_{a,y,s}}{\sum_{ages} \hat{U}_{a,y,s}} = \frac{Q_s \cdot \hat{N}_{a,y,s}}{\sum_{ages} Q_s \cdot \hat{N}_{a,y,s}} = \frac{\hat{N}_{a,y,s}}{\sum_{ages} \hat{N}_{a,y,s}} \qquad \text{Eq A.B.16}$$

Where suffix ***s*** is the survey index (to refer either the Acoustic or the DEPM), ***a*** and ***y*** stand for age and year respectively.

The equivalent random sampling size ($x_{y,s}$) of available ages was assumed to be 100 individuals for the acoustics series whilst ad hoc yearly sizes were estimated for the DEPM which matched as closely as possible with the yearly reported CVs of the proportions at ages from this survey series, as currently used for the ICES assessment (ICES 2014). The ad hoc equivalent sizes for the DEPM ranged from 40 to 500, with a mean of about 140 individuals. A sensitivity analysis was carried out (not shown) to check the effect of reducing the equivalent sampling size to a maximum of 66 individuals for all input data series of percentages at age, finding out only relatively minor effects on the estimates of the natural mortality (by about 2%), though certainly the range of the confidence intervals was increased at sampling size 66 (by about 20-25%).

No differential weighting factors for the components of the objective function were applied for this second modeling of the observations, except for the weighting factors of the catches at age 0 which were set equal to 0.01 since these catches are not considered to be separable (this is because they are taken independently of the other ages and are very noisy).

The log-likelihood estimates (*LLH*) of the fitting to the (o) auxiliary series of (*n*) observations each was simply the sum over the observation series (o) of every

$LLH\_{Observations}$, which corresponded to either equation A.B.10 for those subject to log-normal errors (catches in tons, surveyed estimates of total population in biomass and the recruitment index) or to the following expression for the age disaggregated observations subject to multinomial errors (catches and populations at age):

$$LLH\_{Observations} = \sum_{years} \ln\left(\frac{x_{y,t}}{x_{y,1}! \cdot x_{y,2}! \cdot x_{y,3+}!} \cdot \hat{p}_{y,1}{}^{x_{y,1}} \cdot \hat{p}_{y,2}{}^{x_{y,2}} \cdot \hat{p}_{y,3+}{}^{x_{y,3+}} \right) \qquad \text{Eq A.B.17}$$

Whereby the $x_{y,t}$ corresponds to the assumed equivalent random sampling size in year y for the auxiliary age disaggregated observation, as described above, and the $x_{y,a}$ to the respective numbers at age as deduced from the observed percentages at age (as $x_{y,a} = x_{y,t} \cdot p_{y,a}$, rounded) and finally the $\hat{p}_{y,a}$ correspond to the expected percentages at age according to the modeling, as described above.

6 General discussion

6.1 How many anchovies are there at sea? (And how much is this related to the biological knowledge on anchovy?)

Considerations from catches

The age reading procedures and the validation reported in this Thesis was basically adopted by the institutes working with this anchovy in the late eighties and nineties through several exchanges and workshops. This changed the perception of the age structure of catches from the previous one, where age 1 was almost absent, to a new one where age 1 was either predominant or similar to that of older ages in the catches. The formerly perceived age structure would have implied that there was some hidden (cryptic) biomass at sea, untouched by the fishery, which would always act as buffer biomass weakening the impacts of the fishery. Whilst the new perceived age structure implies that all age classes were directly exploited by the fishery. Estimates of the virtual population (VPA) from the mean catches at age (by VPA with a natural mortality M of 1.2) of the respective old and validated age composition of catches (Figure 6.1) evidenced that the older age reading procedures would have implied a 154% larger population of anchovies in numbers (and about 70% in biomass) than the currently adopted mean age composition of catches (resulting from the validated age determination procedures). Very similar results are obtained for an M at 0.9, which leads to VPA estimates 135% higher in numbers and 68% higher in biomass.

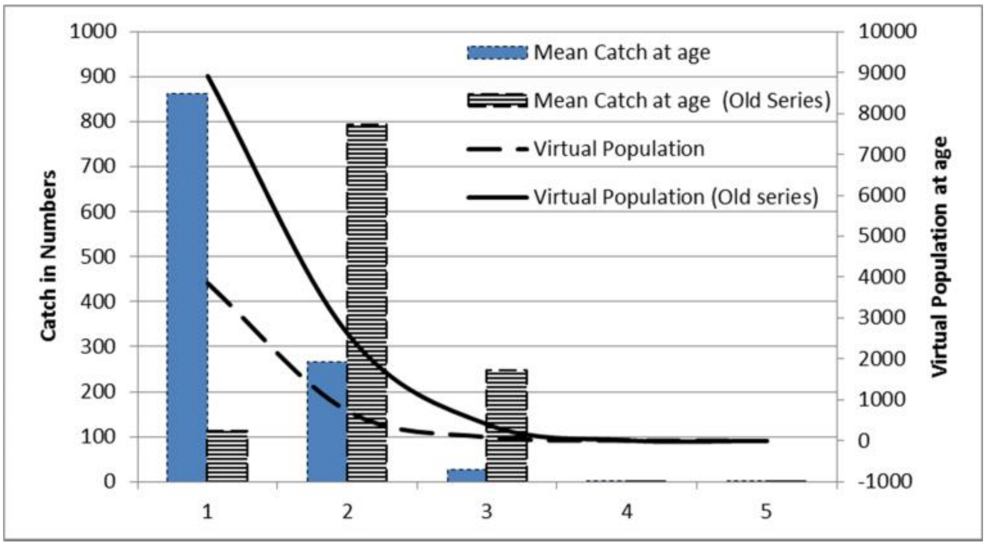


Figure 6.1 Mean catches age for the old series (from 1974 to 1980) and for the new age determination criteria (in the period 1987-1998) (equaling the total amount fish caught in numbers), along with Virtual Population at age estimates for a Natural Mortality equal to 1.2 (assuming survivors at the end of the oldest year is equal to the catch).

Considerations from surveys

During the eighties and nineties the series of the parallel direct surveys of the anchovy population in spring (the DEPM and Acoustic surveys) estimated about the same levels of biomass in absolute terms. However during the first decade of this century it was increasingly evident that DEPM tended to report consistently less biomass than the acoustic surveys. This was visually evident, and estimated by the Bayesian Biomass Modelling of the population (Ibaibarriaga *et al.* 2008;2011) quantified this in about 0.80 and 1.02 for the DEPM and the acoustic catchability respectively (see Figure 1.3 in the introduction)

In some cases we have enter in useless disputes about which of the two methods is more reliable (more precise and unbiased), when in fact both methods are in principle unbiased provided their estimation parameters are all estimated during survey time. And both methods should provide at least relative indicators of abundance when some parameters are not estimated but assumed be constant throughout the series (i.e. the TS with the acoustics or the DF for some years of the DEPM if not available).

In any case there are two natural and complementary ways to overcome these disputes:

The most common and standard way is through Integrated Assessment of all available information on the stock and the fishery (if considered reliable). This allows searching for a balance (best statistical) fitting of the different sources of information, providing in this way the best objective representation of the stock status (Maunder and Punt 2013).

The second approach is trying to improve the reliability of the inputs to the assessment through a careful analysis of the biological knowledge required to provide these inputs, as well as through improvements of the technological tools required to generate the inputs to the assessment (statistical tools or direct sampling technologies, etc.). They can at the end improve the inputs, usually reducing some apparent discrepancies between different data sources. This can also change some of the modelling approaches if requiring more refined process modelling etc. (Maunder and Piner 2015). Though in some cases discrepancies may not disappear and should trigger more research for a better understanding of those differences to achieve better basis for an integrative synthesis of the conflicting inputs.

Some results deriving from the biological basis for age determination and spawning frequency estimates in chapters 2 and 3 of this Thesis suggest that there might be less anchovies at sea than the levels that DEPM and Acoustic estimates pointed out during the nineties and first decade of this century:

First the larger amount of age 2 recorded in the catches at age, and in the population estimates, since the reopening of the fishery in 2010 suggests that the impact of the fishery was higher than perceived formerly. As such the reduction of the fishery subject to the LTMP of anchovy has restored catches at ages 2 and older in the Spring Spanish fishery at higher levels than expected. For the catches actually known to have taken place in the past (and assuming negligible errors in the age reading procedures), this can only happen if the biomass was lower than assessed formerly.

Second, the rising of Spawning frequency from about 0.25 to about 0.4, along with the complete revision of the DEPM parameters, supposed a reduction of the series of

DEPM biomass by about 33% (Santos *et al.* 2013). This instead of reconciling both methods of direct surveying increased their differences in absolute levels (Figure 6.2).

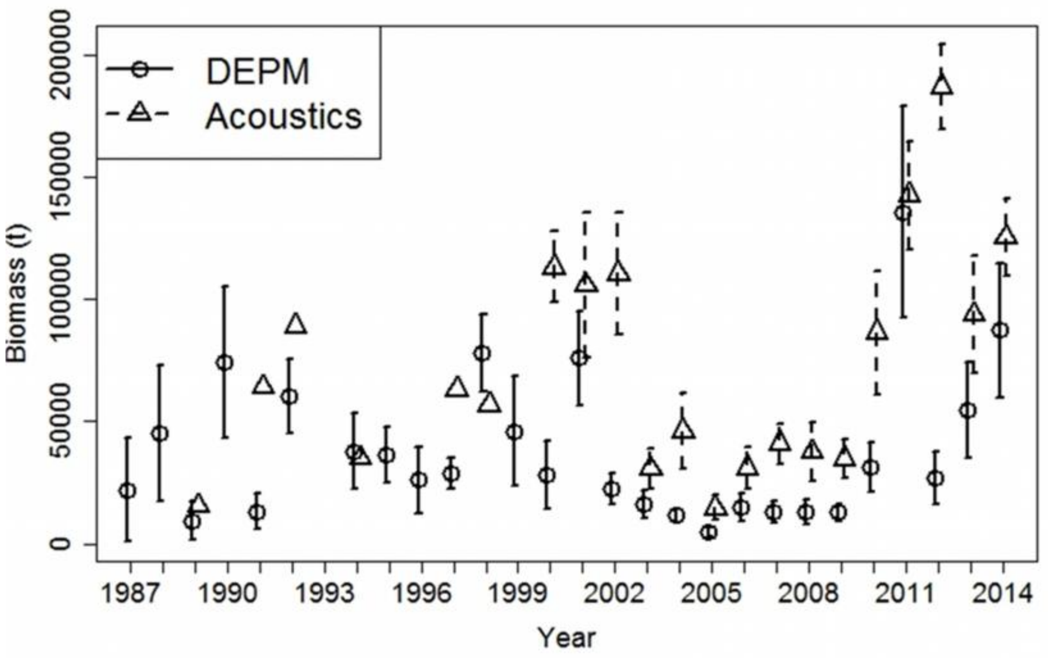


Figure 6.2: Historical series of spawning stock biomass estimates and the corresponding confidence intervals from DEPM (as revised in Santos *et al.* 2013-- solid line and circles) and acoustics (dashed line and triangles), as used by ICES since 2014.

Third, during the closure of the fishery survival at age 2 was higher than expected for the assumed natural mortality at 1.2. This was also shown by the complete analysis of the whole series of surveys and catches (Chapter 4) either by lineal and integrated models (with single M about 0.8-1), lower than formerly assumed. Reducing natural mortality implies a reduction of the average stock biomass at sea (Figure 6.3). A parallel (slightly more pronounced effect) results from reducing M1 (while optimizing for M2) (Figure 6.3).

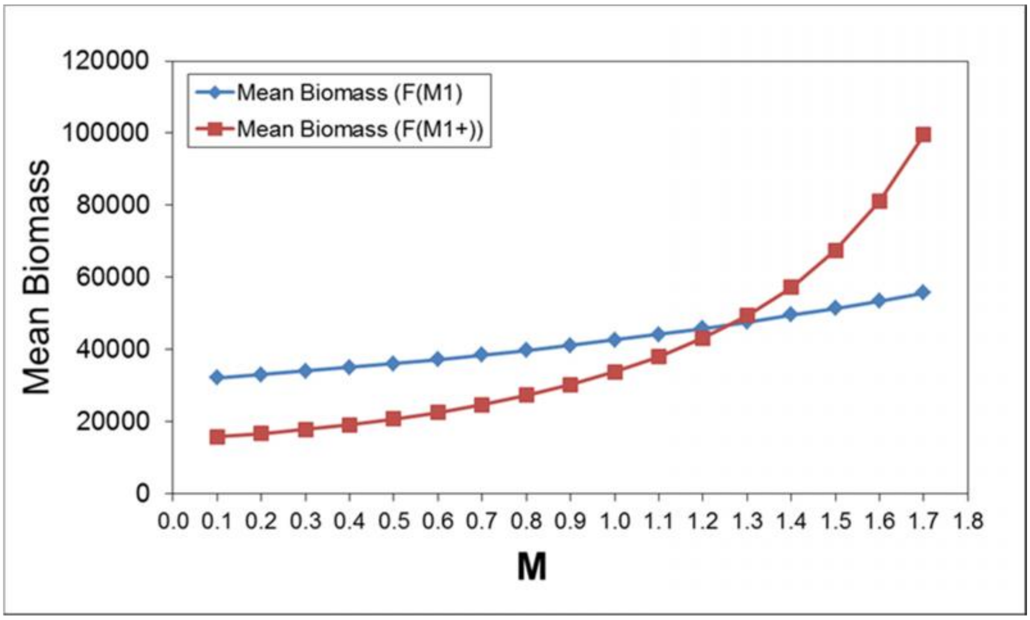


Figure 6.3. Effect on historical mean biomass (SSB) of different values of M (X axis), either single M (M1+, constant for all ages) or of M at age 1 (M1)(conditioned to optimized M2+) for a two-stage M.

Certainly the estimation of M was associated to a change on the assumptions about the catchability of surveys by age. The default setting of catchability models of surveys is to allow such parameter to change with age (Shepherd 1999; Methot *et al.* 2013). However such typical approach was shown (by ICA) to lead to catchabilities at age which were 50% higher for age 2 than for ages 1 or 3 (ICES 2005) in both surveys. Such a result suggested that there was some hidden or “cryptic” biomass to the surveys (parallel to the hidden biomass to the fishery as a result of wrong age determination – see above). However this was not expected from the efficient adult sampling (in terms of fishing gears) and good spatial coverage of the anchovy population by both surveys. Such a result was conditioned to the assumption of a constant M across ages at 1.2. It has been shown that by reverting the assumption to constant catchabilities across ages of surveys we can infer new values of constant M across ages and furthermore a pattern of changing M by ages. Thus the model becomes parsimonious (requiring less parameters) for a similar and/or even a better fitting to the observations of population at age respectively (both during and outside the closure period). Furthermore we will rely more on survey observations than on assumptions. On the other hand selectivity at age becomes flatter at ages 2 and older at spawning time in comparison with the original modelling with changing catchabilities (something closer to the intuitive perception of the fishery and the market preferences for big fishes) (Figure 6.4).

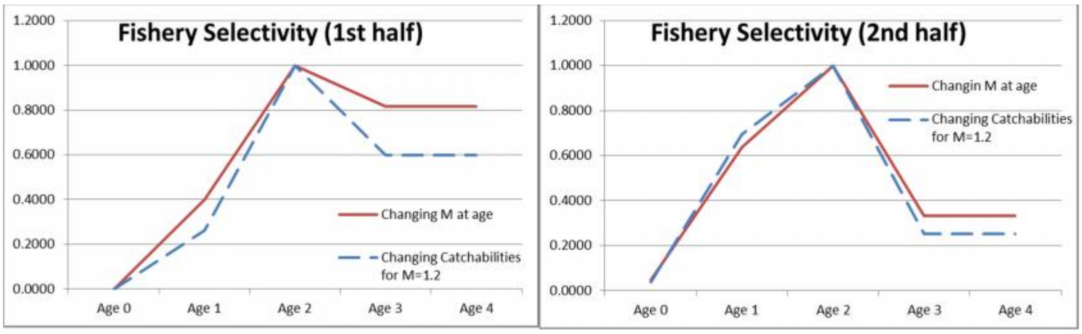


Figure 6.4.Fitted fishery selectivity for the first and second half of the year depending upon a modelling with changing catchabilities at age (for a single M=1.2) or with changing M1 by ages (at optimum WSSQ assessment) for a single catchability by survey)

Certainly in the analysis of M it has been capital the long series of population at age provided by the DEPM (Chapter 4) and the acoustics. Furthermore the changing pattern by ages was shown to be statistically significant for the integrated assessment and robust to moderate levels of ageing errors. In such statistical analysis the use of the CVs inferred form the DEPM for the proportions at age was capital to make statistical inference for the Integrated assessment based on logLikelihood ratio (with multinomial errors). As such correct age determination (Chapter 2) and determination of levels of errors of the proportions at age (Chapter 4) were fundamental to assess the significance of our results on M.

Another example of this critical examination of the data was the revision of the spawning frequency, where thanks to the detailed histological examination of the tank experiments of POFs degeneration by Alday *et al.* (2008), it was demonstrated that the high incidence of day-0 females was not a result of oversampling (as most of the literature defends) but it was an indicator of the actual high level of spawning fraction. If such incidence resulted contradictory to the incidence of post spawning females, it was just because of partial wrong allocation of POFs to past spawning daily cohorts. The revision of the criteria to allocate POF to spawning females made both incidences compatible, changing our understanding of the spawning frequency of females (S) and led ultimately to revise the entire series of the DEPM SSB estimates and the assessment (ICES 2013).

These are just examples of how much scientists should critically examine the inputs to the assessment and models they use, because it is necessary to judge a priori where to put emphasis for the modelling (sensu high/down weighting or sensu narrowing or widening priors) according to the reliability of the data. So, as much attention has to be put at the data inputs as to the modelling. This is the general approach defended in this Thesis.

This does not imply that the discussion about how many anchovies are at sea is solved beyond what the two surveys say. At the contrary, but it is useless discussing which one is best, it is better to look at the basis why the different results are obtained in order to acquire sufficient knowledge of the key parameters as to understand the origin of differences and to make them compatible.

For the DEPM there are several pieces which require further understanding: In recent years S tend to be slightly lower that found in the 2012 revision (Chapter 4). This might be linked to recent high very levels of recruitment and potential density dependence effects on S, but might there be still some remaining inconsistencies in the correct allocation of POFs?. IN addition there are assumptions in the DEPM not sufficiently checked as for instance that all eggs are fertilized and remain in the plankton, which might not be true (Hunter and Lo 1997) and might lead to some sub-estimation of Egg production and hence of SSB.

For the acoustics, the TS of anchovy has not been estimated properly in the Bay of Biscay at spawning time and hence the absolute levels of biomass are unknown. Furthermore in acoustic surveys the TS is not corrected for the changes of the swimblader of fishes with depth. Years with major changes in bathymetrical distribution of anchovy schools can affect in the indication of relative change in SSB in the series (as acknowledge by the experts) (ICES 2014b).

Certainly, going more in detail to the parameters behind the estimates can help much in understanding the differences between both models and can move the assessment to new modelling basis and new results.

In this Thesis the major role played by good knowledge of some biological parameters as growth (and age determination procedures) and reproductive biology has been defended in order to improve the assessment and the understanding of population dynamics. An approach close to the views of Maunder and Piner 2015, where they claim for detailed analysis and studies on the data inputs and on the assumptions of the modelling supporting assessment: “Interpretation of data used in fisheries assessment and management requires the knowledge of population (e.g. growth, natural mortality, and recruitment), fishing (e.g. selectivity), and sampling processes. Without this knowledge, assumptions must be made, either implicitly or explicitly, based on the methods used. Incorrect assumptions can have a substantial impact on stock assessment results and management advice.” This Thesis shows several examples where improvements of biological knowledge and estimation procedures, as well as critical analysis of some assumptions (as catchability) can lead to changes in the assessment and perception of the stock.

6.2 Interactions between Biology and Management: Perspectives.

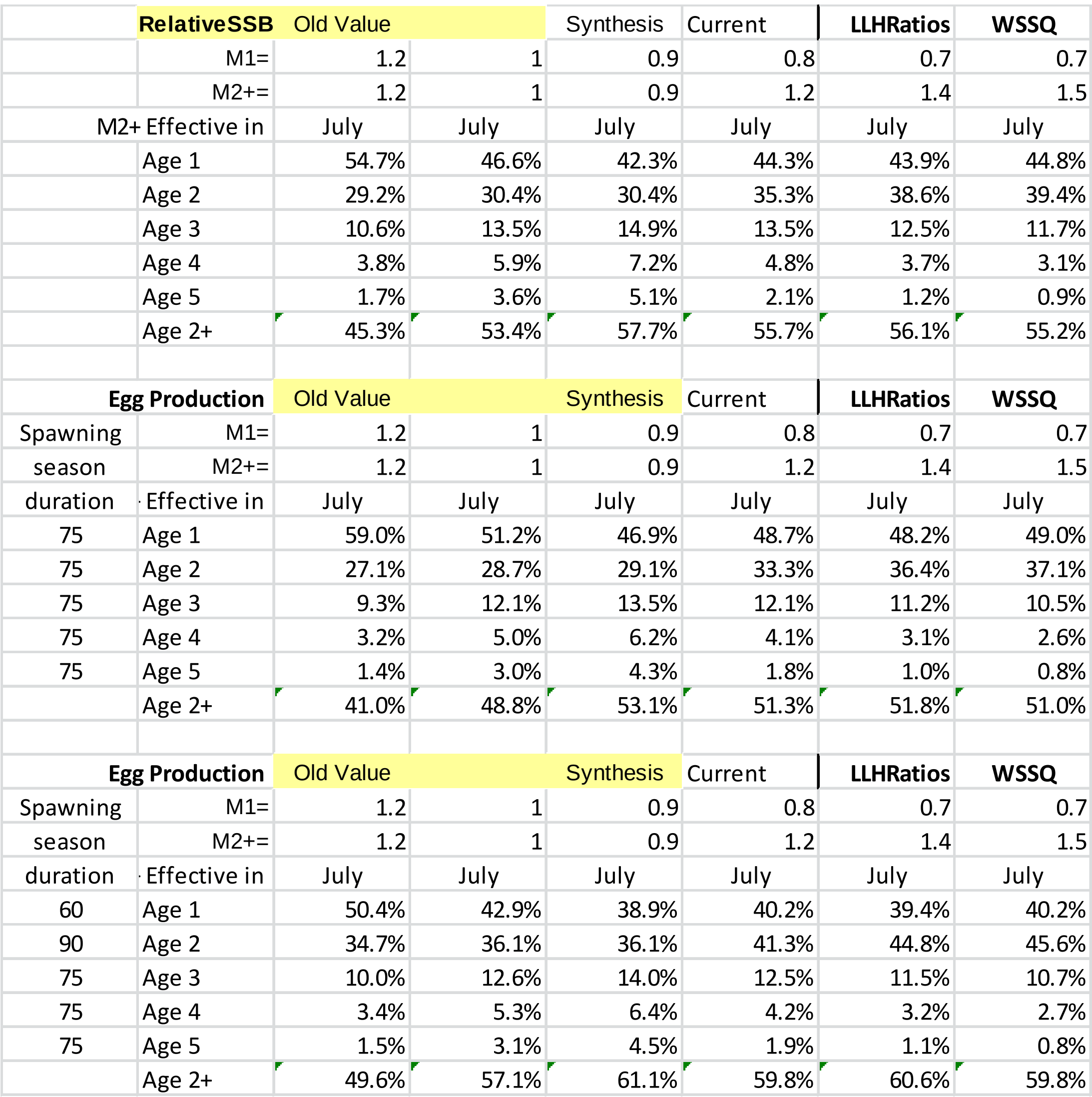
Two major findings of this thesis may result partly contradictory:

a) Spawning frequency does not increase sharply with age but just smoothly. This contrasts with the results on other Engraulidae and Clupeidae populations where the spawning fraction was shown to vary remarkably as a function of female mean weight (Ganias *et al.* 2003; Claramunt *et al.* 2007; Ganias 2008). For example, Claramunt *et al.* (2007) showed an expected relative increase in spawning fraction for Engraulidae of about +50% for females increasing from 20 g to 30 g, while our relationship suggested a minor change of about 7.5%. From an evolutionary point of view, this result can be interpreted as an adaptation of short lived species, so that optimum spawning capacity should not be delayed later than one-year-old fish.

b) The lowest natural mortality occurs between age 1 and age 2, when still growth is intense. From an evolutionary point of view this might be understood as if this population of anchovies would have evolved in its fluctuating environment by allowing some non-negligible chances of reproducing over two spawning seasons before senescence. This would enhance the opportunities to overcome single (not repeated) environmental- induced-failures of recruitment. This second finding claims for a more important role of the age 2 in the population than previously believed.

Table 6.1shows the relative Spawning biomass by ages and the relative contribution to spawning (in terms of egg production) by ages, according to a simple model of Wt, F and S conditioned to the assumption of the duration of the spawning season by ages. In that model Wf is taken a Wt * 1.0736 (where Wt comes from the mean weights at age deduced from the DEPM in Chapter 4 and 1.0736 is the mean raising factor of Wt to Wf, r2=0.9406); F is taken from a linear regression from the historical data of the DEPM, whereby F= A+B*Wf roughly (with A=1759.1 and B=560.82, r2=0.8714) and S comes from the fitting in figure 3.8 applied to the former Wf by ages. In order to assess the relative contribution by ages to spawning we have to assume how long the spawning season by ages is, something not sufficiently studied yet. Motos (1996) considered equal duration of spawning season by ages, with some shifts in the timing of older/bigger fishes over younger/smaller fishes. Pecquerie *et al.* (2009) simulated the growth and reproduction of this anchovy with the Dynamic energy budget theory and obtained that older anchovies probably spawn for a longer time period (about a month more) than age 1, although age 3+ may reduce partly the length of its season (having a duration between that of age 1 and 2). Table 6.1 shows, for different natural mortality patterns by ages, the SSB by ages at virgin state of the anchovy population (top panel) along with the relative egg contribution by ages for their respectively assumed duration of the spawning season, either as assumed by Motos (1996) (medium panel) or as simulated by Pecquerie *et al.* (2009) (bottom panel).

Table 6.1. Relative age contribution (rows) to SSB (upper panel) and to Egg Production either based on equal duration of the spawning season by ages (as suggested by Motos 1996) medium panel) or based on unequal spawning season by ages (as suggested by Pecquerie 2009 – bottom panel), for different levels of natural mortality by ages 1 and 2+ (columns).



	RelativeSSB	Old Value		Synthesis	Current	**LLHRatios**	**WSSQ**
	M1=	1.2	1	0.9	0.8	0.7	0.7
	M2+=	1.2	1	0.9	1.2	1.4	1.5
M2+ Effective in		July	July	July	July	July	July
	Age 1	54.7%	46.6%	42.3%	44.3%	43.9%	44.8%
	Age 2	29.2%	30.4%	30.4%	35.3%	38.6%	39.4%
	Age 3	10.6%	13.5%	14.9%	13.5%	12.5%	11.7%
	Age 4	3.8%	5.9%	7.2%	4.8%	3.7%	3.1%
	Age 5	1.7%	3.6%	5.1%	2.1%	1.2%	0.9%
	Age 2+	45.3%	53.4%	57.7%	55.7%	56.1%	55.2%
	Egg Production	Old Value		Synthesis	Current	**LLHRatios**	**WSSQ**
Spawning	M1=	1.2	1	0.9	0.8	0.7	0.7
season	M2+=	1.2	1	0.9	1.2	1.4	1.5
duration	Effective in	July	July	July	July	July	July
75	Age 1	59.0%	51.2%	46.9%	48.7%	48.2%	49.0%
75	Age 2	27.1%	28.7%	29.1%	33.3%	36.4%	37.1%
75	Age 3	9.3%	12.1%	13.5%	12.1%	11.2%	10.5%
75	Age 4	3.2%	5.0%	6.2%	4.1%	3.1%	2.6%
75	Age 5	1.4%	3.0%	4.3%	1.8%	1.0%	0.8%
	Age 2+	41.0%	48.8%	53.1%	51.3%	51.8%	51.0%
	Egg Production	Old Value		Synthesis	Current	**LLHRatios**	**WSSQ**
Spawning	M1=	1.2	1	0.9	0.8	0.7	0.7
season	M2+=	1.2	1	0.9	1.2	1.4	1.5
duration	Effective in	July	July	July	July	July	July
60	Age 1	50.4%	42.9%	38.9%	40.2%	39.4%	40.2%
90	Age 2	34.7%	36.1%	36.1%	41.3%	44.8%	45.6%
75	Age 3	10.0%	12.6%	14.0%	12.5%	11.5%	10.7%
75	Age 4	3.4%	5.3%	6.4%	4.2%	3.2%	2.7%
75	Age 5	1.5%	3.1%	4.5%	1.9%	1.1%	0.8%
	Age 2+	49.6%	57.1%	61.1%	59.8%	60.6%	59.8%

The old assumption of M=1.2 for all ages results in an SSB at age 1 of about 55% and an egg contribution coming from age 1 of about 59% (for equal duration of the spawning season) or of about 50% (for the unequal duration of the spawning season). When M is taken at 0.9 (equal for all ages) the contribution of age 1 diminishes by about 12% in all cases, and this is roughly maintained by any pattern of natural mortality by ages, whereby the contribution to spawning of ages 2+ become predominant. When moving to a changing pattern of natural mortality by ages, the relative contribution of age 2 to the spawning is maximized compared to the constant natural mortality by ages, although the relative contribution from older ages reduces. So in total the relative contribution of ages 2+ is relative similar for M constant at 0.9 as for varying M at ages.

All these results suggest that, without exploitation, anchovies at ages 2+ may play a role at spawning time rather similar or even slightly greater than that played by age 1 (depending on the actual duration of spawning time by ages). It seems that from evolutionary perspective spawning of anchovies at age 2 matters. This might be related to the presumed better quality of eggs produced by older females (as shown for other species Kennedy *et al.* 2007; Marshall *et al.* 1998; Marteinsdottir and Steinarsson 1998), and to the different (complementary) seasonal and spatial pattern of spawning of older (age 2+) versus younger (age 1) anchovies (Motos *et al.* 1996; Allain *et al.* 2007; Ibaibarriaga *et al.* 2014), which will tend to maximize the chances of matching the good environmental spatio-temporal windows in the Bay (Lasker 1978; Cury and Roy 1989; Borja *et al.* 1996; 1998; 2008). If age structure at spawning time is severely affected by exploitation it is likely that the recruitment will become even more unstable and dependent on environmental fluctuations.

These considerations are partly neglected from the management perspective when basing entirely its goals on SSB (regardless of their age composition). Perhaps a LTM objective could also be stablished in terms of minimizing the affection or distortion of the contribution of age 2 to spawning, either as a target spawning at age 2 per recruit (relative to the virgin state, i.e., as SSB2+_35% or SSB2+_50%, etc) or as target relative contribution of age 2 to spawning over the total Egg production (SSBage2+%). Another way of taking this into account might be setting targets in terms of an escapement policy with a given target escapement to spawning of SSB2+ (though preliminary estimates showed this to be a very restrictive management policy).

Table 6.2 shows that a strategy based on F0.1 (which basically is borrowed from long living species) results in typically accepted level of reduction of spawning Biomass per recruit to about 35-40%, compared to the unexploited status. However it corresponds with very high fishing pressures and a major reduction of the contribution of age 2 to spawning, (of course linked to the particular fishing pattern of this fishery as estimated for each natural mortality pattern --- rows in the table). For small pelagics, moderate levels of exploitation have been often advocated due to their vulnerability to fishing and the need of minimum buffers of spawning biomass (Ulltang 1980, Csirke 1988, Pitcher 1995). In this sense Macer and Sissenwine (1993) stated that the higher the natural mortality the bigger should be kept the percentage of spawning biomass per recruit in relation to the virgin state (the criteria of %SBR). They also indicated that the small pelagic species could be poorly resistant to exploitation and for these species the %SBR corresponding with the Fmed can be as high as 40 % or even in some cases 60 %. Patterson (1992) suggested that a moderate and sustainable rate of exploitation could be reached at 0.67 M. These findings have been partly supported by Zhou *et al.* (2012), which suggest sustainable FMSY at about 0.87·M. Barange *et al.* (2009) reported that F targeting SBR between 40 to 60% are applied all over the world to the management of these pelagic populations. Table 6.2 shows that F_SSB50% or F=0.67·M (Patterson criteria), suppose a major drop in target Fs and substantial increases in the relative contribution of the age 2 to Spawning (in terms of Spawning biomass). In terms of %SBR criteria of Patterson lead to about 66% of SBR% (a rather conservative approach).

Table 6.2. Implication of different exploitation strategies (F_0.1 / F_50%SBR / F=0.6667·M by columns) in terms of Fishing mortality (F), relative contribution Age2 to Spawning and Spawning Biomass per recruit relative to the unexploited state, for different levels of natural mortality and patterns by age (rows) and from assessments assuming either changing catchability at age or constant by ages in surveys.

Changing	Scenario		Survey	Unexploited		Exploitation Strategy_1 (F_0.1)				Exploitation Strategy_2 F_50%SBR			Exploitation Strategy_3 (Patterson Criteria)		
M2+ by ...	M1	M2+	Catchability	Age2+.Contrib.SSB		F.01	Age2+.Contrib.SSB	SBR%		F_SSB50%	Age2+.Contrib.SSB		F=0.6667*M	Age2+.Contrib.SSB	SBR%
Annual	1.2	1.2	by ages	45%		2.98	5%	37%		1.63	14%		0.80	26%	66%
Annual	1.2	1.2	QFLAT	45%		2.95	5%	37%		1.61	15%		0.80	26%	66%
Annual	0.9	0.9	QFLAT	57%		1.85	16%	36%		1.03	29%		0.60	39%	62%
July	0.7	1.52	QFLAT	54%		1.79	19%	37%		1.09	30%		0.60	40%	65%

Recently the examination of the role that small pelagic fishes and other low–trophic level species play on sustaining marine ecosystems has led to recommend low levels of exploitation of these species (well below Fmsy) to minimize the impacts on marine Mammals, seabirds and on other commercially important species (Smith *et al.* 2011).

The original management plan of anchovy (which included a fitted Stock recruitment relationships) showed that a harvest rate of about 30% of SSB would be sustainable, which is even more conservative than the options discussed above (COM 2009; STECF 2008a,b). This harvest was recently shown to be sustainable again looking at the most recent assessment (STECF 2013). The currently adopted HCR allows getting a maximum of 39% at healthy stock state (but drops it to about 30% when it reaches the lowest allowable biomass for fishing). Such harvest rate is in practice below the criteria of Patterson (as the latter results in a HR about 45%). All the testing on sustainability carried out to set up the management and harvest control rules in recent years are rather supportive of the advocated policy of moderate exploitation levels for small pelagic and such management practice would lead to exploitation levels still allowing a relevant contribution of age 2+ to spawning, something which can make sense from an evolutionary point of view even for short lived species (when minimum natural mortalities at age 1 occurs as here).

Final comment: Senescence might be present as soon as age 2 not only for this anchovy, but perhaps in many other anchovies (and short lived species). We have assumed that the typical decreasing pattern of natural mortality at age (of many long living species) could be applicable to short living species. But as claimed by Beverton 1963, senescence could be expected to be noticeable quite soon after spawning for many short living species. In fact some assessment reflect surprising dome shape catchabilities by ages in surveys, which would be indicative of increasing natural mortality by ages (Giannoulaki *et al.* 2014). Therefore, a parabola like model of the pattern of natural mortality by ages for short living species could be more applicable than believed previously. This would have effects in terms of discarding the hidden or “cryptic” biomasses of older age groups, and certainly would affect any quantitative short term forecast of the population in case this is required for the provision of management advice to managers.

7 Conclusions

On the age determination from otoliths and growth

1. Anchovy otoliths show a regular growth pattern which allow identification of different ages classes: Yearly annuli consist of a hyaline zone (either single or composite) and a wide opaque zone, disrupted occasionally by some typical checks (mainly at age-0 and at age-1 at peak spawning time).Age readers need to become familiar with these checks, knowing that not all anchovies lay down the same amount of checks and many of them may not show any.
2. Maximum otolith growth occurs at age 0, and typically, otolith growth at age-1 and age-2 diminishes roughly to about a half and a third of that at previous age, whilst at older ages growth is more sustained. The intense growth during ages 0 and 1 leads to attain about 88% of the asymptotic maximum length at the age of 2.
3. Seasonal formation of the otolith edge follows that of most temperate fishes in the northern hemisphere: Most opaque growth occurs in summer and is minimal (translucent) in winter. However the older the fish the later the opaque otolith growth resumes during the first half of the year. This feature may be of interest to other short-lived species too, as it is useful to distinguish age-1 from older fish during the first half of the year.
4. Age determination, given a date of capture, requires therefore knowledge of the typical annual growth pattern of otoliths, their seasonal edge formation by ages and the most typical checks.
5. The age determination procedure was validated by monitoring very strong year classes in successive catches and surveys, and also by monitoring the seasonal occurrence of edge types. Historical corroboration of the good performance of the ageing method has been shown in the catches and surveys in the period 1987-2013.

On the revision of the Spawning Fraction

6. The good knowledge achieved on the final oocyte maturation and of the degeneration of post-ovulatory follicles allowed improving the accuracy of the allocation of spawning females to daily spawning classes.

7. The incidence of day_0 and day_1 spawners is highly consistent around 0.395 (CV=18%) over a 24-hour cycle, while the incidence of day_2 females is irregular. The joint incidence of day_0 and day_1 spawning females, S(0+1), varied little throughout the study period and was independent of the fishing gear or sampling time.
8. Oversampling of day_0 females occurs +/- 5 hours around peak spawning time (at 23:00 hours), but it is of weak intensity. Much of the oversampling of day_0 affects negatively to the incidence of day_1 past spawning females.
9. For these reasons, the mean proportion of day_0 and day_1, S(0+1), becomes practically an unbiased estimator of S and slightly more precise than the traditional S(1) corrected estimator (based upon the proportion of day_1 females, corrected for oversampling of day_0). If desired such estimator can be corrected for its small bias.
10. An S estimator based upon the reciprocal of the individual spawning frequencies of females confirmed the high estimates of the spawning fraction, around 0.4. The new S estimates revised upward the former estimates by about 60% and implied lower DEPM biomass estimates.
11. Female size had a small but significant influence on S (females increasing from 20 g to 30 g, would increase the S by about 7.5%).

On the population at age estimates

12. DEPM can be extended to provide spawning population at age estimates with variances, by extending the sampling for the additional parameters (Wt, Pa and Wa). The theory of cluster sampling is appropriate to address this task.
13. The series of biomass and population at age estimates revealed the dominant role of the 1-year-old recruits in sustaining the anchovy population. Old age classes (ages 2 and 3+) are usually a minor component, although its relevance is a bit larger when examined in terms of mass instead of numbers. Dominance of old age classes occurs only after the succession of very big and bad recruitments, as in 2001/2002 or 2011/2012.
14. Accuracy of spawning population at age estimates depend basically on obtaining a good spatial adult sampling, reflecting the actual heterogeneity of

the population parameters, and on a correct weighting of samples, proportional to the abundance they represent. For the latter, weighting factors proportional to the egg abundance per area, if necessary corrected by daily fecundity estimates in space, should be considered in DEPM surveys.

15. Mean weight, total (Wt) and by ages (Wa), as well as the Proportion at age estimates (Pa) estimates are independent from the absolute regional SSB estimates, they only depend on the relative distribution of biomass in space. The potential bias in Wt and Pa estimates propagate cumulatively into the spawning population in numbers (SSP) and by ages (SSPa) estimates.
16. The method can also be applied to the estimation of Total population at age over spawning grounds in cases of partial maturity.

On the natural mortality

17. Estimates of total mortality (Z), were consistent between the acoustic and DEPM surveys, under the assumption of constant catchability across age by surveys. And these Z estimates were responsive to fishing, being smaller during the closure period than during the fishing period.
18. The three estimation procedures, of increasing modelling complexity used in the analysis, indicated a mean value of natural mortality for all ages (M1+) in the range 0.8-1.15 (CV between 6 to 20%). This value is smaller than the M1+ formerly adopted (at 1.2) and therefore implies higher F, so bigger fishing impact on the stock, and some smaller stock sizes, than previously assessed.
19. All approaches show that the level of natural mortality at age 2+ roughly doubles that at age 1 (being about 0.7-0.8 vs. 1.4-1.5 respectively).
20. Such increase of the natural mortality at age 2 could be associated to early senescence of short living species as suggested previously by Beverton (1963) and pointed out only for a couple of other anchovy populations previously (as in Pacific coast and in the Yellow Sea). The increased mortality might be due to spawning stress. Anchovy would have therefore an intermediate life history between capelin (which die after it first spawning) and sardines or sprats.
21. Assumption of 5% ageing errors do not change the sign of the difference (M1<M2+) though reduces it and leads to partial loss of statistical

significance. Results are in any case noisy, partly due to observation errors from surveys and certainly to potential inter-annual variability in natural mortality too.

22. The pattern of increasing natural mortality at age might be applicable to other Engraulidae where assessments following the constant mortality assumption results in non-understandable big changes in survey catchabilities by age.

Concluding remarks:

23. The high growth of anchovy up to the age of 2, its higher F and spawning fraction S, and the lower mortality during age 1 until reaching its second spawning (at age 2), suggest that the contribution of age 2 to spawning may be more relevant than previously perceived for a non-harvested anchovy population. This suggests that minimizing the distortion of the contribution of ages 2+ to the spawning might be a precautionary goal for the management of this short lived species.
24. The biological methods and parameters covered in this study have all implied changes in the perception of the population dynamics and on the assessment of this anchovy population. This confirms the relevance of accurate estimation of biological parameters for a correct assessment of populations and their management, and corroborates the Thesis defended at the beginning.

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