HANDBOOK OF

APPLIED FISHERIES REPRODUCTIVE BIOLOGY FOR STOCK ASSESSMENT AND MANAGEMENT

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Preface

Most of the world marine fishery resources are overexploited. Despite the technical measures implemented over the past decade and more, many depleted stocks have failed to recover. During this period there has been a growing awareness that the traditional indicators of stock viability are inadequate because they do not appropriately consider the capacity of a population to annually produce viable eggs and larvae (Stock Reproductive Potential, SRP) which is considered to be extremely important for fish stock recovery. In addition, egg production is also influenced by environmental conditions such as water temperature and food supply; the exact effects of which remain to be accurately quantified.

The NAFO Reproductive Potential Working Group emerged in 2000 based on the apparent need to explore and improve indicators of stock viability linked to reproduction and recruitment, following a recommendation of the Symposium on Variations in Maturation, Growth, Condition and Spawning Stock Biomass Production in Groundfish hosted by the NAFO Scientific Council in 1998 in Lisbon (Portugal). In June 2007 arose FRESH (Fish Reproduction and Fisheries, FA0601), a COST[1] Action of 4-year duration to establish a network of European and North-Atlantic researchers to co-operate on the improvement of i) knowledge on fish reproduction in relation to fisheries and ii) current assessment methodology in order to promote sustainable exploitation of marine fishery resources. The Action formed a focal point for the disparate national, European and North-American research initiatives in this research area. Further, it aimed to assist in the development of future marine policy objectives, such as the recovery of overexploited fish stocks.

Both scientific groups collaborated and met together between 2007 and 2011. During these meetings, it was concluded that reproductive potential of fish stocks is an essential biological characteristic which should be included in quantitative assessments so as to provide fisheries managers with realistic tools for predicting and managing towards fish stock recovery. Fish reproduction is a broad field and a large number of past and present research projects are focus on examining the linkages between fish reproductive success and the subsequent population dynamics. However, full international agreement of preferred methods fails to exist, few protocols are standardized and a great amount of data recorded by different fisheries laboratories are not comparable because they are not homogenous or specific protocols are unknown. Attending all these issues, during the FRESH-NAFO meeting celebrated in Palermo (2007), the need of standardization and cross calibration of the protocols used in different laboratories was detected as a priority task for fisheries researchers. This was the ground to generate a handbook that explicitly described standard protocols to collect and analyze data to study stock reproductive potential. Since then, a concerted effort has been made to call the best

[1] COST (European Cooperation in Science and Technology), see http://www.cost.eu/

international specialists on reproductive potential together in order to write this ***Handbook of Applied Fisheries Reproductive Biology for Stock Assessment and Management***.

This handbook is a compendium of the most substantial aspects of fish reproductive biology applied to stock assessment and management and it contains the procedures to carry out different studies on it. It is an applied book set aside for fisheries researchers, PhD students and lecturers, and is intended not only for advanced fisheries institutions, but also institutes that are embarking on improving their knowledge of the reproductive capacity of their fisheries. The main objective is to provide practical knowledge for studying that subject, although brief theoretical basic notes are included to support technical and methodological contents. Thus, it offers readers the specific knowledge to carry out research in that field in a simple, pedagogical and standard way.

The handbook is structured in five chapters that compile fundamental aspects to be considered on fish reproductive biology studies that can be implemented in stock assessments and management: i) general overview of fish reproductive biology, ii) data collection and statistics for reproductive biology, iii) maturity, iv) egg production, and v) elasmobranch reproductive potential. Additionally, it includes a Glossary with definition of standard terminology commonly used in these types of studies.

The handbook will be published as a free digital book in the institutional repository of the Spanish National Research Council (*Agencia Estatal Consejo Superior de Investigaciones Científicas*), Digital CSIC (http://digital.csic.es). Each chapter will be published as an individual entity (file) and once all of them are edited, they will be compiled in a single e-book. Now, you have in your hands the ***Chapter 3: Maturity***, the second one in coming to light. We hope you enjoy the reading and find the chapter useful for your research studies.

Editors

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Chapter 3

Maturity

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Maturity

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3.1. Introduction

Sexual maturation refers to the process of becoming mature, i.e. capable of sexual reproduction; in humans, it corresponds to puberty. Thus, maturation happens once in a lifetime, when individual goes from the juvenile to the adult stage. Maturation entails complex physiological changes that lead to an important variation in energy distribution and represents a critical transition in the life of an individual (Wootton, 1998). Before maturing, all assimilated energy is allocated for growth and somatic maintenance, nevertheless, after maturation, a considerable part of this energy is diverted to development and maintenance of gonads and gametes, as well as sexual behaviour (Kooijman, 2000). As a result of that energetic balance, growth and maturation are strongly linked and when growth rates are inadequate in the juvenile phase, maturation timing can be modified (Morita et al., 2005) or even inhibited (Boulcott and Wright, 2008). Fish life history is a trade-off of reproductive success with adult growth and mortality risk (Charnov & Berrigan, 1991 and references therein). Maturation involves changes in morphology, behaviour and/or habitat, in some cases even great annual migrations to spawning areas. In summary, maturation requires important energetic, ecological, physiological, anatomical, biochemical and endocrinological adaptations (Rocha, et al., 2008).

Age or size at maturity join to growth rate and natural mortality are the main life-history traits that define population dynamics and are determinants for population responses to environmental forcing. Fish presents multiple reproductive strategies. Species can be gonochoristic, sequential or simultaneous hermaphrodites that can present different gonad morphology and sex functionality (BOX 3.1) or even parthenogenetic (with asexual reproduction). Most species are oviparous, although ovoviviparous and viviparous species also exists. Some species spawn only once in a life (semelparous) while others do it several times (iteroparous). This great variety of reproductive strategies (genetically determined) leads to a wide range of ages/sizes of maturation in wild fish. Long lived species tend to mature at an older age and larger size than short lived species. In fact the onset of sexual maturity may determine the reproductive life duration (Wootton, 1998 and references therein). Generally is assumed that late maturation implies more vulnerability to exploitation (Jennings et al., 1999), especially when fishery targets towards large individuals.

One of the main criteria for judging the status of an exploited fish population is the size of the spawning stock or spawning stock biomass (SSB) and maturity data are the basic information to estimate it, therefore it is essential in fisheries assessment and management. On the other hand, the last aim of fisheries assessment is to predict population dynamics in natural systems, for that purpose, to know how many individuals of the stock are actually contributing to offspring production (only mature and active individuals) is required.

The study of fish reproductive ecology, including maturation, needs a well-designed sampling adapted to species-specific reproductive strategy, in terms of spatial and temporal coverage [Section 3.2.1] as well as in terms of sampling intensity [Section 3.2.2]. Age-length stock-structure has to be considered for maturity analysis, especially when data are going to be used in fisheries assessment because demographic models used in fisheries assessment are based on length and age composition of commercial catches and fishing surveys [section 3.3.1 and 3.3.2].

Besides, maturation, and subsequently growth, usually differs among males and females due to the different physiological implications that this process has in both sexes, hence the necessity of taking into account sex ratio in this type of studies [section 3.3.3].

Gonad morphology is the most common and direct index to determine gonad developmental stage and therefore fish maturity stage, based on both macroscopic [Section 3.4.1] and microscopic [Section 3.4.2] features. Gametogenesis in fish [Section 3.4.2.1] is the process by which diploid germinal cells undergo meitoic division and differentiation to form mature haploid gametes. The gonad structure and components change during this process, and therefore gonad external and internal morphology are the best indicatives of gonad maturation. There are several techniques to analyze gonad structure, from histological methods [Section 3.4.2.2] to whole mounts analysis [Section 3.4.2.3]. In any case, for stock assessment and management purposes, maturity staging keys must be validated and calibrated among laboratories [Section 3.4.3] to homogenize criteria.

Gametogenesis implies gonad growth, because of this the relative gonad weight (gonadosomatic index) can act as a good proxy of maturity stage in some species. In the case of capital breeders, gonad growth is at the expense of depletion of energetic reserves accumulated in other tissues (liver, muscle, mesenteric fat, etc.) (McBride et al., 2015), thus, certain somatic indices like hepatosomatic index, Fulton's condition factor or mesenteric fat index can also be used as maturity indicators in that species show this energy strategy. These two previous methods require the individual slaughter, subsequently cannot be used if individuals must be kept alive. In experimental situations where non-lethal techniques are required, there are alternative methods like biopsies, ultrasonography, blood hormone tests or even analysis of gene expression (Gomez et al., 1999; Martin-Robichaud & Rommens, 2001; Norberg et al., 1989; Viñas & Piferrer, 2008; Witthames et al., 2009, Novelo & Tiersch, 2012), but they are not addressed in the present chapter because they are outside the usual methodological scope of current fisheries assessment.

The proportion of mature individuals increases with age and size from zero to one hundred per cent. The curve of cumulative frequency of maturation is called maturity ogive and is used to determine the proportion of individuals that are mature or immature at certain age or size. There is a variety of methods to estimate maturity ogives that allow obtaining different parameters as the age/size at first maturation, at 50% of maturity or 100% of maturity [Section 3.5.1]. Differences in maturity stage assignation due to methodological differences among laboratories have to be estimated in order to validate maturity ogives [Section 3.5.3] and reduce uncertainty of maturity parameters calculated from it.

Age and size at maturity are key parameters in fisheries stock assessment and can vary not only between cohorts but also between sexes. Growth, maturation and mortality are sexually dimorphic in many species, so estimating sexes-combined or separated maturity ogives can impact the perception of the stock status [Section 3.5.3]. In fact, the mechanisms which activate maturation are not yet fully understood and vary between species. Attending to previous studies, the onset of fish maturation can be determined by several factors: biometric factors such as size, age or weight (Sohn & Crews, 1977; McCormick & Naiman, 1984; Vallin & Nissling, 2000), energy accumulation (Kadri et al., 1996; Svedäng et al., 1996), social interactions (Hofmann et al., 1999; Hobbs et al., 2004) or a

combination of them. Additionally, maturation as well as growth are conditioned by exogenous factors which can be physicochemical such as temperature, salinity, pH or photoperiod (Huber & Bengtson, 1999; Taranger et al., 1999; Dhillon & Fox , 2004; Panfili et al., 2006; Imsland et al., 2007; Alcaraz & Garcia-Berthou, 2007; Tobin & Wright, 2011), biological as food availability or stock density(Bowen et al., 1991; Reimers et al., 1993; Booth, 1995; Helser, & Almeida, 1997) and anthropogenic factors like pollution or fishing that also may have a profound impact on reproductive traits, including maturation (Kime, 1995 & references therein; Grift et al., 2003; Mollet et al., 2007; Lyche et al., 2010; Meier et al., 2011). Special considerations are required to estimate maturity ogive in the case of hermaphrodites that show specific sexual strategies that frequently modulated by social factors [Section 3.5.4].

Fish presents a diversity of tactics, i.e. they can modulate their strategy in response to environmental conditions (Potts & Wootton, 1989) because of phenotypic plasticity of life-history traits. The range of phenotypes produced by a given genotype under different environmental conditions is represented by the reaction norm (Griffiths et al., 2000). It can be used for disentangling genetic and plastic effects when genetic data are not available (Heino & Dieckmann, 2008) because, in theory, changes in the reaction norm reflect evolutionary changes that can be induced both by natural but also anthropogenic factors like fishing. Size-selective fishing is a well-documented evolutionary force that alters population dynamic and affects life-history traits (Jorgensen et al., 2007). Maturation shifts could affect reproductive success and population sustainability, for example early maturation may lead to the decrease of reproductive potential due to reduction of fecundity and/or offspring viability (Vallin & Nissling, 2000 and references therein; Green, 2008; Cooper et al., 2013). Resilience of fish stocks depends on attributes not only of the species but also the stock; so, to understand reproductive ecology of fish populations is essential for an effective stock assessment and fisheries management (King & McFarlane, 2003).

Most of fisheries regulators are aware of the necessity of collecting this information for correct management; consequently most countries involved in fisheries management all around the world collect maturity data. However, universal guidelines for calculating and reporting maturity estimates from the collected data have not been agreed. Specific workshops have been carried out to improve data collection, methodology and quality assurance with the last aim of establishing at least a set of best practices to be used when producing estimates from maturity data. This chapter tries to compile the most relevant techniques to estimate fish maturity in order to be applied in fisheries stock assessment and management. Finally, it provides some recommendations not only to estimate maturity ogives, but also to carry out other reproductive studies [Section 3.5.5].

3.2. Data collection: sampling strategy/design

3.2.1. Spatial and temporal coverage

Spatial and temporal aspects of a sampling design of maturity data should be in line with the purpose of the study. When the aim is to collect data for the estimation of maturity ogives, spawning season and spawning location should be known. If that is not the case, samples should be collected throughout the year, to allow the determination of temporal patterns in maturity. The period with higher proportion of actively spawning individuals corresponds to the spawning season. More detailed temporal coverage may ascertain daily cycles in spawning. Studies have indicated that spawning may be synchronised to occur at specific hours in some species, for example at dusk (Bernal *et al*., 2011 and references therein). To determine the spawning locations and avoid biased sampling due to limited overlap of of mature and immature individuals a wide spatial sampling coverage is needed (Murua *et al*., 2003).

Data can be collected both during scientific surveys as well as on board of commercial vessels (market samples). Survey data are limited by low temporal coverage and are appropriate for species with a limited spawning season. Market samples may offer high temporal coverage but in many cases a poor spatial resolution, when the fisheries operate mainly on the spawning grounds. Furthermore, fish length distribution (and consequently, fish age) in market samples are often biased, due to minimum landing sizes (MLS) or high-grading practices. When the lower length classes are scarcely represented in the samples, the maturity ogive will be biased, particularly if the MLS is close to the length at first maturity.

Market samples are more appropriate for species characterized by prolonged reproductive season. However, it is important to assess if these samples are representative i) of the stock spatial distribution (i.e. a good overlap between the stock and the fisheries spatial distribution) and ii) of the length range when the maturation process starts.

Ideally, market samples should be compared with surveys samples, if the later has a good coverage of the spatial stocks distribution and coverage of the pre-spawning season. Whenever the two sources of samples do not show systematic differences, data can be merged from both sources, taking into consideration possible differences in gear selectivity.

In case of species with determinate fecundity the samples should be collected during the pre-spawning season in contrast with indeterminate species, whose best sampling time coincides with the peak of spawning activity (Chapter 2).

3.2.2. Sampling intensity

As for any other statistical analysis, the number of samples should be representative of the population. The sampling design needs to ensure that the two extremes of ogive curve (i.e. immature individual and larger/older individuals) are well represented, but the length classes corresponding to individuals that mature for the first time are the most important to sample. The transitional length classes in which some individuals in the population are still immature and

other are maturing should be also well represented, although other aspects of the sample should be taken in to account, such as the sex, and the location. In Figure 3.1., the low number of samples of the length classes larger than the length of first maturity, prevent a good fit to a standard sigmoid curve.

Moreover, the intervals included in a length class should be in line with the size range of the species and compatible with the length intervals used for the other biological parameters. Usually 0.5 cm length classes for small fish as anchovy and sardine, 1cm for most of the fish stocks assessed in the north Atlantic and larger length range for larger species might be considered.

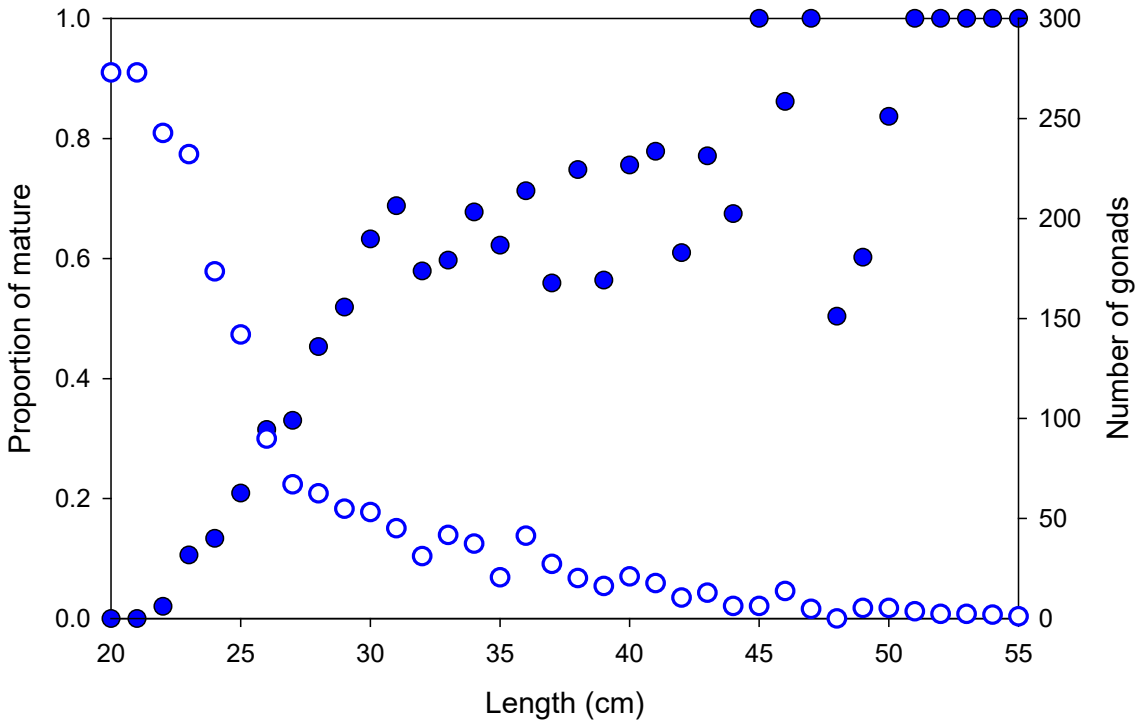


Figure 3.1

Proportion of mature by length class (close circles) and respective numbers of observed gonads (open circles).

3.3. Age-length stock structure

3.3.1. Length/Age stock composition

The demographic models used in fisheries stock assessment are based on length composition of commercial catches and fishing surveys, which are afterwards transformed in age by means of the age-length keys (Section 3.3.2) using the information obtained from calcified structures (e.g. otoliths, scales, vertebra, spines or ilicium). Length/Age (L/A) stock composition is a key characteristic of age-structured populations, by contrast to small pelagic species that present short-life cycles (e.g., anchovy, sardine; see below). A broad L/A stock structure composed by several age classes – which implies a broad reproductive stock – is a 'biological insurance' to buffer the effects of external forces (Hsieh *et al*., 2010 and references therein). For instance, populations with several reproductive age classes (e.g. cod) are able to withstand prolonged periods of adverse conditions for reproduction than species with few classes (e.g. sardine). Moreover, a broad L/A stock structure can act as a filter of the environmental stochasticity generating cycles in the population size (i.e., 'cohort resonance effect', Bjørnstad *et al*., 2004). This mechanism is produced through spreading the stochastic forcing of the recruitment over age classes by means of internal processes such as inter- or intra-cohort interactions (Bjørnstad *et al*., 1999; 2004; Fromentin & Fonteneau, 2001; Hidalgo *et al*., 2011).

A broad L/A stock composition is considered a 'diverse' demographic structure, which enjoys age-related differences in the ecological and biological characteristics of the individuals. For instance, individuals of different age show differences in timing for reproduction and maturity, as well as differences in the spatial locations of spawning grounds (e.g., Hutchings & Myers, 1993; Marteinsdottir & Petursdottir, 1995; Begg & Marteinsdottir, 2002; Rindorf & Lewy, 2006; Wright & Trippel, 2009). These ontogenetic variations are known to affect the reproductive success and the recruitment variability. Additionally, older individuals (larger, more experienced and generally in greater physiological condition) produce higher quantity and/or quality of eggs, which would favour the survival of the offspring (e.g., Solemdal, 1997; Marteinsdottir & Steinarsson, 1998; Marshall *et al*., 1999; Berkeley *et al*., 2004a; 2004b; Venturelli *et al*., 2010). These properties are known as bet-hedging strategies and can dampen the effect of marine environmental stochasticity and help stabilize fish populations. The association between bet-hedging and age structure is often referred as maternal effects in fishes (Trippel, 1998; Hsieh *et al*., 2010).

For fisheries management purposes, L/A stock composition is inherently linked to the stock unit definition (i.e, 'a sub-set of one species having the same growth and mortality parameters and inhabiting a particular geographical area', Sparre & Venema, 1997). In essence, the demographic and the spatial features of the L/A stock composition are closely related. First, the spatial structures of marine fish populations can encompass a wide range of configurations, including patchy populations, networks, and meta-populations (*sensu* Kritzer & Sale, 2004; Ciannelli *et al*., 2013). Asynchronous fluctuations in population sub-units (if they exist) can dampen the overall effect of the environmental forcing when exchanges between the sub-units exist (Begg & Marteinsdóttir, 2002; Hilborn *et al*., 2003; Schindler *et al*., 2010). Additionally,

marine fishes show often ontogenetic and sex specific differences in the spatial distribution mainly due to both density-dependent and environmental-driven habitat selection (Bartolino *et al*., 2011).

One of the hot topics nowadays in fisheries science is to further understand which are the biological, ecological and evolutionary consequences of the size-selective erosion of both the spatial structure of populations and their demography (so called "age truncation effect", Anderson *et al*., 2008; Ottersen, 2008; Hsieh *et al*., 2010, Hidalgo *et al*. 2011; 2012 and review of Jorgensen *et al*., 2009 for evolutionary implications). As a general pattern, size-selective harvesting results in populations being more dependent on the abundance of the younger age classes, which tighten the link between the population dynamics and the recruitment strength (Marteinsdottir & Thorarinsson, 1998; Brander, 2005; Ottersen *et al*., 2006; Planque *et al*., 2010; Hidalgo *et al*., 2011; 2012). Consequently, fish populations are more dependent on the environmental variability (also see Brunel, 2010), with a subsequent effect on population fluctuations (Hsieh *et al*., 2006; Anderson *et al*., 2008). Additionally, this effect could be magnified by the low reproductive success of young age classes through an undermining of bet-hedging strategies and maternal effects. Finally, fishing can affect spatial distribution through both removing patches or population sub-units or reducing the heterogeneity of the spatial distribution (Perry *et al*., 2010; Ciannelli *et al*., 2013). These two effects reduce the spreading of early life stages in space and time, and may make them more vulnerable to variability in the environmental conditions (Begg & Marteinsdottir, 2002; Hsieh *et al*, 2008; Perry *et al*., 2010). Classic management strategies tend to rely on a minimum landing size to guarantee fish reach sexual maturity. However, current strategies aim at protecting the age diversity of the stock structure by reducing the fishing impact on older and more expedited age classes. New gear technologies to improve selectivity, or seasonal and spatial management of critical periods such as reproduction, are among the most common management strategies to diminish the impact of fishing over the more productive age classes.

Several descriptors can be used to summarize the information of the stock structure, and particularly the spawning stock that is the relevant in terms of maturity studies. Among the most common include the mean length, the mean age and the Shannon diversity index of the spawning stock.

The mean length (L_{SSB}. Eq. 3.1) and mean age (A_{SSB}. Eq. 3.2) of the spawners are calculated as the weighted average of length and age, respectively, in the spawners' abundance as following:

$$L_{SSB,t} = \frac{\sum_{l=l_{\min}}^{l=l_{\max}} l M_{l,t} N_{l,t}}{\sum_{l=l_{\min}}^{l=l_{\max}} M_{l,t} N_{l,t}} \quad (3.1)$$

$$A_{SSB,t} = \frac{\sum_{a=a_{\min}}^{a=a_{\max}} a M_{a,t} N_{a,t}}{\sum_{a=a_{\min}}^{a=a_{\max}} M_{a,t} N_{a,t}} \quad (3.2)$$

where l_{min}(a_{min}) and l_{max}(a_{max}) are, respectively, the length (age) of the youngest and the oldest length (age) group contributing to the abundance of spawners in year t; $M_{l,t}$ ($M_{a,t}$) is the proportion of mature at length l (age a) at time t; and $N_{l,t}$ ($N_{a,t}$) is the number of fish at length l (age a) and time t.

Age diversity of a spawning stock can be calculated using the Shannon diversity index (H_{SSB}, Shannon, 1948). The index summarizes the homogeneity of age classes in the spawning stock. H_{SSB} is considered

independent of stock size and describes both the number of age classes in the spawning stock and the diversity of the distribution of fish among cohorts (Marteinsdottir & Thorarinsson, 1998). H_{SSB} is calculated as follows:

$$H_{SSB} = -\sum_{a=1}^{n} p_a \log(p_a) \qquad (3.3)$$

where n is the number of age classes, p_a is the proportion of age class a in the total spawning abundance.

3.3.2. Age-length key

The ALK is a matrix showing, for each length class of fish in a particular stock, the distribution of age frequencies (or the relative number of individuals at each age). The preponderant sampling procedure to build an ALK is based in two steps. First, fish are randomly sampled to measure their length and second, sub-samples of the fish measured are processed for age determination. The ALK is generated through ageing (from calcified structures) of n fish of such sub-samples from the entire population. The number of fish in each length group can either be fixed or proportional to the total number of fish in that length group according to the statistical sampling design. Once such a key is available, samples of fish from the same population that were only measured for length can be distributed over age groups and with such a way the age composition can be raised to the overall catch.

ALK matrix is most frequently used in stock assessments and generally in fishery management and is probably the best way of routinely handling length-data when the growth is well known. In principle, a representative length recording for length composition is straightforward and costs are minimal in comparison with age determination. Age determination is more difficult and time consuming due to the complexities of collecting and interpreting the calcified structures. Fish stock assessments are frequently based on ALK approach. For this reason, age estimations must be as accurate and precise as possible, be based on international criteria defined by experts and validated directly or indirectly (e.g. mark- recapture, marginal increment analysis, captive rearing, modal progression analysis, radiochemical dating).

Changes in age diversity can impact reproductive potential (Marteinsdottir & Thorarinsson, 1998; Secor, 2000). Increased diversity in age composition may result in broader spatial and temporal distribution of spawning, due to differences in the spawning time, duration and location of different age classes (e.g., Marteinsdottir & Thorarinsson, 1998; Gerritsen *et al*., 2006; Section 3.3.1). Changes in the proportion of fish at each age in the population depend on: individual variation in growth rates, variation in mortality rates at different ages and sizes- natural or fishing induced- and variability in year-class strength. Therefore, the ALK established for one year should not be applied to the length composition for another year without risk of error. The most important factor of the ALK is to establish, for each length group, the proportion of each age in that group. Some length-groups (mainly younger and oldest ones) are most uncertain about age because usually it is difficult to obtain sufficient samples and the reading of their otoliths presents more difficulties (e.g. definition of the first ring in the younger's; narrowness of last annuli in old specimens) and thus contribute most to the variance in the estimated age distribution. In fact, it may be prudent to concentrate sampling effort in aforementioned length-groups (Gulland and Rosenberg 1992). Additionaly, knowledge of gear

selectivity is very important and should be used to correct length-frequency distributions of samples, due to differences in catchability between different sizes (Jennings *et al.*, 2001).

Spatial differences in age-length structure and distinct demographic parameters must be taken into account when an ALK for a species is established. For example, fish of a certain age might have a larger mean length in one area than another as a result of differential growth rates (Munk *et al*., 2000; McGrath & Scott, 2008), size- specific migration (Tallman *et al*., 2002) and selection –induced by fishing or natural mortality (Hüssy *et al*., 2003; Hutchings *et al*, 2007; Stearns, 1989; Heikinheimo & Mikkola, 2004; Abrams & Rowe, 1996). Also, for certain length classes, differences in abundances in different areas (nursery grounds, spawning grounds) could be observed. These biases can impact the ALK of a population if they are not accounted for. Gerritsen et al. (2006) proposed that if the number of age samples is not proportional to the local abundance of fish, to eliminate bias the aged samples should be weighted by the abundance in each region before they are combined into an ALK. This method could be applied to survey data, as well as data from commercial sources.

However, Gulland & Rosenberg (1992) supported that unless there is something unusual about the spatial patterns of growth, the relation between age and length will be the same for all groups. There is still ongoing debate about the uncertainties, the bias and errors that might be arisen during an assessment from several sources e.g. ageing errors (Gulland & Rosenberg, 1992; Bertignac & De Pontual, 2007; Reeves, 2003), inappropriate sampling methodology (Gulland & Rosenberg, 1992; Vigneu & Mahevas, 2004), statistical fitting or choice of model. These potential bias sources could have impact in all of the age-based parameters, not only maturity, but also yield, growth or mortality and, subsequently, into assessment and management (Morison *et al*., 2005, Vigneau & Mahevas, 2004).

3.3.3. Sex ratio

Stock assessment generally assume a sex ratio of 1:1. However, demographic studies have shown that this is not always true. Studies on a multitude of freshwater and marine species have shown that sex ratios are often skewed within and between cohorts and spawning aggregations (Pitcher & Hart, 1993 and references therein; Hunt, 1996; Morgan & Trippel, 1996). As a general pattern in fish, males have been found to be more numerous among the younger year classes while females dominate the older year classes. The causes for skewed sex ratios have been suggested to stem from differences in size and age linked natural mortality between the sexes (Jakobsen & Ajiad, 1999), differential predation (Britton & Moser, 1982), size selective and unequal removal of the sexes by fishing (Hamilton, *et al*., 2007; Heppell *et al*., 2006; Shepherd *et al.*, 2010) and sex exchange (Jennings *et al*., 2001; Jong *et al*., 2009). Sex ratios have also been shown to change over time. In the NEA cod stock, proportion of males increased in the mature stock during 1950-2000 (Nash *et al*., 2008), which was likely due to the long-lasting size-selective erosion of older age classes (Ottersen, 2008) with higher percentages of females

Changes in sex ratios can affect stock dynamics and reproductive success in many ways. Fertilization success of cod was shown to depend on male abundance where proportion of fertilized eggs declined with reduction in number of spawning males per female (Rowe *et al*., 2004). Unequal sex ratios may also play an important role in a phenomenon called “Allee effects”, a situation where population

growth declines when population size falls below a threshold value (Frank & Brickman, 2000; Hutchings & Reynolds, 2003). In such situations, regeneration may be hindered by low abundance of either sex. Sex ratios have also been linked to changes in aggression and spawning behaviour, that may consequently lead to unequal removal of sexes by fishing (i.e. see below). In this sense, competition and aggressive behaviour is predicted to correlate with abundance of each sex (Jirotkul, 1999; Grant *et al*., 2000). For example, in experimental studies on Japanese medaka (*Oryzias latipes*), male aggression was shown to increase in concordance with increased relative abundance of males (Clark & Grant, 2010) while in Sand gobies (*Pomatoschistus minutus*) and two-spotted gobie (*Gobiusculus flavescens*), intrasexual competition among females decreased with increasing relative abundance of males (Forsgren *et al*., 2004; Kvarnemo *et al*., 1995).

The case of hermaphrodite fish deserves special attention. The Sex Allocation Theory (Charnov, 1982; Munday *et al*., 2006) hypothesized that in hermaphrodite fishes, sex ratios vary depending on the favoured sex at each time. Part of this theory is the Size Advantage Hypothesis that predicts hermaphrodites change sex so as to maximize their reproductive output (Ghiselin, 1969). As a result, sex change may occur when fish of specific sex has attained greater size at age and is more likely to express a higher reproductive potential that the fish of the other sex (review in Provost & Jensen, 2015).

In simultaneous hermaphrodites (BOX 3.1), sex ratio is always 1:1. However, in sequential hermaphrodites, the sex change is an important factor to be considered for sex-ratio estimations, because it could lead to an age/length biased sex-ratio, depending on the specific sexual strategies (BOX 3.1.). Moreover, hermaphrodite fish stocks can be more impacted by exploitation; usually size selective and focused on larger specimens, because it has deep impact on operational sex ratios and timing of sex change that could lead to a reduction of reproductive success and could possibly alter SSB structure (Alonzo & Mangel, 2004; Hamilton *et al*., 2011). The impact of fishing mortality will depend on the sex change mechanism (Alonzo & Mangel, 2005). Simultaneous and bi-directional hermaphrodites may compensate the fishing effect on sex ratio acting as male or female depending on the stock situation. In the same way, social control of sex change may reduce the impact of size selection on sex ratio (Villegas-Ríos, 2013 and references therein). However, the timing, length and age, of sex-change will be altered, putting reproductive potential and success at risk. Sequential hermaphrodites are especially sensitive to size selective harvesting, as was well documented by Hamilton *et al.* (2007) for California sheephead (*Semicossyphus pulcher*). This species suffered a reduction of length and age at sex-change, as well as an increase of proportion of females in those areas more intensely exploited. Anyway, as said above, the plasticity of sex change strategies may buffer the impact of fishing mortality.

The demographic role of sex ratios has not received deserved attention in the literature. One likely reason is the fact that this trait is not easily estimated. Most information on commercially exploited fish stocks stems from catch data. In terms of sex ratios, these may be severely biased due to the time, location and nature of sampling (type of gear). For example, sampling of mature and spawning fish may represent biased sex ratios in populations that consist of sexes that mature at different ages (Jakobsen & Ajiad, 1999). Differences in size or behaviour, where one sex is more active than the other, may also lead to skewed estimates of sex ratios, simply due to different susceptibility to

the fishing gear employed (Rowe & Hutchings, 2003). Males of both graysby (*Epinephelus cruentatus*) and cod (*Gadus morhua*) have been shown to be more susceptible to fishing due to greater aggression and activity (Côté, 2003; Rowe & Hutchings, 2003). As a result, management procedures rarely focus on strategies that aim to maintain or achieve specific sex ratio that guarantees reproductive success and sustainability of the stock in time. In hermaphrodite fishes, management is an even greater challenge than among gonochoristic species. Generally, among species that do not change sex, the size of the spawning stock is based on numbers of mature fish and both sexes are considered to be impacted equally by the fishery. As discussed above, this is likely to be an over simplification. Furthermore, in terms of sex changing species it is difficult to estimate the effects of selectivity by fishing gears or management when sex ratios change with size, age or because of social events. Heppell *et al*., (2006) presented two models to test the effect of highly skewed sex ratios on stock status and reference points estimation for protogynous fish, although these models could be usefull for gonochoristic species too.

Further studies, including behavioural observations and tagging experiments that collect information on the sexes without enforcing selection of different fishing gears, are needed to demonstrate changes in sex ratios between age groups, cohorts, seasons and areas. All stocks assessments involving our main fishing stocks should have access to accurate data on demographic, temporal and spatial variation in sex ratios.

BOX 3.1. How can hermaphodites be recognized?

Functional hermaphrodite refers to those individuals that effectively act as mature male and mature female at some time of its life. Hermaphrodite specimen can present mature male and female gonadal tissue at the same time (simultaneous hermaphrodite) or sequentially (sequential hermaphrodites) An exception of sequential hermaphroditism is bi-directionality, i.e. individuals may change its functional sex in both directions (male to female and viceversa) during its life time, although this strategy has been mainly reported in captivity specimens.

To diagnose functional hermaphroditism in fish, Sadovy & Liu (2008) established a list of criteria divided in:

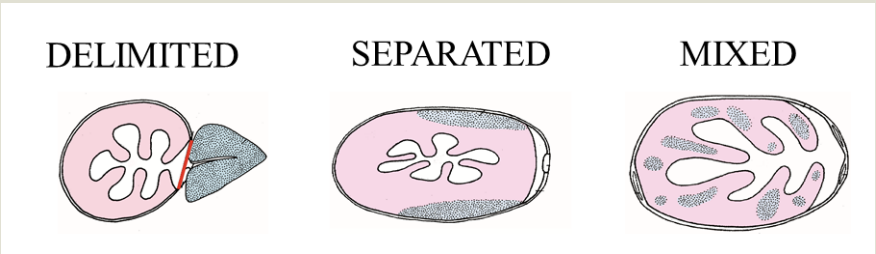
- Strong criteria: Histological identification of sexual transition or simultaneous occurrence of both mature sexes in a gonad and field or captivity observations of functional sex change
- Weak criteria: Observation of bisexual gonadal phase with either sexes or one of them being immature, length/age biased sex ratio and sexual dimorphism. These weak evidences may support the strong ones but are not conclusive by themselves.

Hermaphrodites can present three different types of gonad morphology, depending on the configuration of germinal tissue (Sadovy & Shapiro, 1987):

- Delimited: testicular and ovarian tissues are separated by a membrane of connective tissue.
- Separated undelimited: testicular and ovarian tissues are separated within the gonad, but a connective tissue barrier does not exist.
- Mixed undelimited: testicular and ovarian tissues are mixed all through the gonad

The mechanism of sex change can be genetically constrained (at specific length or age) or socially controlled (Alonzo & Mangel, 2005). In the first case, Allsop & West (2003) found that relative size at sex change is invariant in animals and has been estimated as 0.79 from the total length in sequential hermaphrodite fish.

Exceptional cases of hermaphroditism can be observed in many teleost gonochoristic species, but they are just abnormalities with uncertain causes (hormonal disruptors and pollution, among others).



Different types of gonad morphology in hermaphrodites. Ligth blue dotted areas correspond to testicular tissue, pink areas to ovarian tissue and red line to connective membrane (adapted from Sadovy and Shapiro, 1987).

3.4 Maturity staging

The ovary maturity stage is an important biological parameter to study in respect to the reproductive biology and ecology of fish, as well as for fisheries research. Data from maturity staging is used to develop maturity ogives which are essential for further estimation of length/age at maturity (L_{50}, A_{50}), spawning stock biomass and total egg production. Information from maturity studies can also be used to make temporal analyses of life-history traits of fish stocks. Thus, the adequate estimation of the maturity stage is a critical step for the reliability of these estimations on which biological criteria for assessing and managing fisheries are largely founded.

Data from maturity staging is also used to describe the reproductive cycle of a species as well as to monitor long-term changes in the reproductive cycle. Therefore, the maturity stage should be estimated according to the strategy of reproduction of the species. The reproduction strategy in marine fishes is defined as the combination of several components of the breeding systems, i.e., semelparous-iteroparous, total spawner-batch spawner, external-internal fertilization, etc. (Murua & Saborido-Rey, 2003). Noteworthy, the sampling strategy for maturity staging should be in turn design based on the reproductive strategy of the species. The timing of sampling in this case is crucial, because the probability of identifying an omitted spawning individual during the pre-spawning season is higher than once the spawning season has begun. Skip spawning implies that skipping individual will not take part in the spawning of that year and should not be included in the spawning biomass. It is also important to take into account species behaviour when sampling for maturity staging, i.e., many species form spawning aggregations where immature individuals may be underrepresented, this has to be accounted for in order to avoid a biased maturity ogive.

Another main issue is the precision and bias in the maturity staging. The maturity staging is mostly based on maturity scales that have been used without a histological validation. A great number of published works have used specific gonad maturity scales for particular species few of them have associated their scales with those defined in other papers. However, as Núñez & Duponchelle (2009) have pointed out, comparing them can be confusing, since they range from simple three-stage scales to more detailed nine-stage ones. To address this issue, these authors have provided a universal scale to unify the classification of the maturation assignment in fishes. In order to contribute to the already assumed need for standardization, it seems opportune to propose a scale that follows the universal terminology for reproductive classification in fishes proposed by Brown-Peterson *et al*. (2011).

Maturity stages should be, if possible, assessed in a common manner across closely related species. The maturity staging should be consistent i.e. maturity scales should be based on objective and universal characteristic of the gonad, ideally, characteristics which can be assessed macroscopically as this is less costly and labour intensive than microscopic maturity staging.

3.4.1 Macroscopic Maturity Staging

Typically, in oviparous bony fishes (oviparous species that release ova from the female reproductive tract to the external environment, where they are fertilized), there are 5 maturity stages both in females and males:

Table 3.1.

Description of macroscopic characteristics of the gonadal phases in the reproductive cycle of females and males (based on Brown Peterson et al., 2011 and adapted by WKMATCH, 2014)

Development stage	Description females	Description males
Immature	Ovaries are small and more or less translucent, sometimes lightly pink. Oocytes are not distinguishable to the naked eye. Transverse sections show little gonad volume.	Testes are small and more or less translucent, sometimes lightly pink. Sperm are not distinguishable to the naked eye. Transverse sections show little gonad volume.
Developing	Ovaries are increasing in size, becoming larger and more consistent. The ovary usually turns to a yellow, orange or pink color. Individual oocytes are still not macroscopically distinguishable. External blood vessels start to develop around the ovaries.	Testes are increasing in size, becoming larger and more consistent. The testes remain whitish. Individual sperm are still not macroscopically distinguishable.
Spawning capable	Ovaries are much bigger and voluminous; vascularization is very apparent. The granular consistency of the ovary can be appreciated externally, since yellow vitellogenic oocytes are individually distinguished.	Testes are much bigger and voluminous; vascularization is very apparent. In the testes, accumulation of sperm in the spermatic ducts is also macroscopically visible.
Actively spawning	The transparent hydrated eggs of the ovaries are visible through the ovarian wall.	The large amount of sperm in the testis is easily released towards the external medium when the fish abdomen is pressed, even very lightly.
Regressing	Ovaries are still large but almost empty. They are flaccid, show a wrinkled gonad wall, and are usually grayish in color.	Testes are still large but almost empty. They are flaccid, show a wrinkled gonad wall, and are usually grayish in color.
Regenerating	The macroscopic aspects of the ovaries in the regenerating phase are very similar to those in the immature phase, but the transverse section tends to be larger and the gonad wall thicker. They tend to be more opaque than immature gonads.	The macroscopic aspects of the testes in the regenerating phase are very similar to those in the immature phase, but the transverse section tends to be larger and the gonad wall thicker. They tend to be more opaque than immature gonads.
Abnormal	The ovaries may possess abnormal traits that causes at least partly reduced fecundity.	The reproductive tissue of testes may partly turn into adipose or only one lobe developed.

immature, developing, spawning, regressing and regenerating (Table 3.1) These stages are based on the most common visible macroscopic characteristics of the gonad in the different phases of the reproductive cycle for female and male fishes. Noteworthy, some development stages are not easily distinguished macroscopically in some species, mainly because of the lack of differentiating visible structures, i.e., immature and regenerating are most frequently misleading stages in some species while in others immature and omitted spawning may be difficult to distinguish from one another. Again, these misleading stages are related with the reproductive strategy of the species.

When applying this general classification to other species, minor alterations may be necessary depending on the specific reproductive biology of the species in question. For example, zygoparous (an intermediate stages between oviparity and viviparity where fertilized ova are retained within the female reproductive tract for short periods of time) and embryoparous (when the embryo may develop to an advanced state before its release from the female reproductive tract to the external environment) (Wourms *et al.*, 1988) may require modified descriptions and (or) additional maturity stages.

However, and as Falk-Petersen (2005) pointed out, basic developmental mechanisms of teleosts are similar; the differences rely on the timing of oocyte and embryo developmental events and their control by genetic, physiological and environmental factors. In Falk-Petersen (2005), studies of the early development of marine fishes are summarized and compared, but there are many published studies that classify the embryonic stages of a specific species or genus.

3.4.2 Microscopic Maturity Staging

Although the sex and reproductive status of specimens can usually be macroscopically determined, a microscopic analysis, i.e., histology and whole-mounts, is often performed in order to provide a more accurate analysis of the reproductive characteristics and the annual reproductive cycle of the species. Macroscopic inspection is based on alterations in ovary size and appearance, whereas histological methods evaluate changes in oocyte stages at cellular level. Microscopic maturity staging is a useful tool to validate the macroscopic maturity staging and to describe gonad developmental stages (Table 3.2). This is highly advantageous in cases where the assignment of macroscopically determined maturity stages are uncertain as can be frequently the case during specific periods in the reproductive cycle (Section 3.4.1 for examples). Thus microscopic maturity staging is considered to give a more reliable estimate of the maturity ogive as it avoids misinterpretation, which can be frequently the case, during macroscopic staging (Section 3.5). Microscopic maturity staging usually involves either histological processing or whole mount based methods.

3.4.2.1. Gametogenesis

Oogenesis

The first phase in egg formation or the meiotic transformation of oogonia into a primary oocyte is considered oogenesis in a *sensu strictu*; being the following phases of oocyte development part of folliculogenesis process (Wallace & Selman, 1981; Mayer *et al.*, 1988) (Figure 3.2.). The term oogenesis is, however, often used in a broader sense to encompass all stages from division of oogonia to final maturation of oocytes (Kjesbu, 2009). Here the term oogenesis refers to the process of oocyte development and maturation from primary growth oocyte to hydrated oocyte.

The stages of development in the oocytes are usually staged following the criteria established by Wallace & Selman (1981) and West (1990). Primary growth oocyte stage includes the Chromatin nucleolar and the Perinucleolar stages. Chromatine nucleolar oocytes have a big nucleus that may contain several small nucleoli, although often there is an outstanding one. The scant cytoplasm is basophilic and homogeneus (Figure 3.3.A). In the perinucleolar oocytes the nucleus contains several peripheral nucleoli, located around the nuclear membrane. The cytoplasm gradually loses its basophilia (Figure 3.3.B). Secondary growth

Table 3.2.

Description of microscopic characteristics of the gonadal phases in the reproductive cycle of females and males (based on Brown-Peterson et al., 2011).

Development stage	Description females	Description males
Immature	Only oogonia and Primary Growth oocytes present. Ovarian wall thin and distribution of the oocytes very compact.	Only primary spermatogonia present. Testicular lobules without or with a very little lumen.
Early Developing	Only primary Growth and Cortical Alveoli oocytes.	Primary and secondary spermatogonia. Some primary spermatocytes.
Developing	Also primary and secondary Vitellogenic oocytes. No tertiary vitellogenic oocytes neither postovulatory follicles.	Also secondary spermatocytes, spermatids and spermatozoa within the cysts. No spermatozoa in the lobular lumen neither in sperm ducts. Germinal epithelium continuous through the whole test.
Spawning capable	Tertiary vitellogenic and/or germinal vesicle migration oocytes are present. In batch spawners postovulatory follicles can also be detected.	All stages of spermatogenesis can be present. Spermatozoa can appear in the lumen of the lobules and in the sperm ducts. Germinal epithelium can be discontinuous in lobules near ducts.
Actively spawning	Germinal vesicle breakdown and/or hydrated oocytes are present.	Large amount of sperm in the sperm ducts and lobular lumens. Germinal epithelium discontinuous throughout the testes.
Regressing	Atretic oocytes and postovulatory follicles. Some primary growth, cortical alveoli or vitellogenic oocytes can be present.	Residual spermatozoa and few scattered spermatocysts can be present in the testes. Regeneration of the germinal epithelium with spermatogonial proliferation in the periphery of the testes.
Regenerating	Only oogonia and Primary Growth oocytes present. Ovarian wall thick and distribution of the oocytes with some spaces. Degenerating postovulatory follicles can be present.	Only primary spermatogonia present. Testicular lobules with lumen. Germinal epithelium continuous.Very few residual sperm can remain in the testes.

oocyte stages start with the Cortical alveoli stage and continues with the Vitellogenic stages. The oocytes in the cortical alveoli stage show a granular and more acidophilic cytoplasm (Figure 3.3.C). Before the formation of the Cortical alveoli in some species small lipid droplets can already be detected on the perinulear ooplasma. The highly acidophilic zona radiate become apparent. The first vitellogenic stage is characterised by the appearance of small yolk granules on the periphery of the cytoplasm. The cortical alveoli has a greater distribution than in the previous stage and the lipid droplets, when present, increase greatly in size. During the second Vitellogenic stage there is an increase in number, size, and distribution of the yolk granules, which occupy virtually all the cytoplasm. The third Vitellogenic stage show much thicker yolk granules and lipid droplets (Figure 3.3.D). The oocyte maturation process is included in the oocyte secondary growth phase although some authors also called this process the third growth stage (Grier, 2000). Maturation ends with the migration of the nucleus towards the animal pole (Figure 3.3.E), its

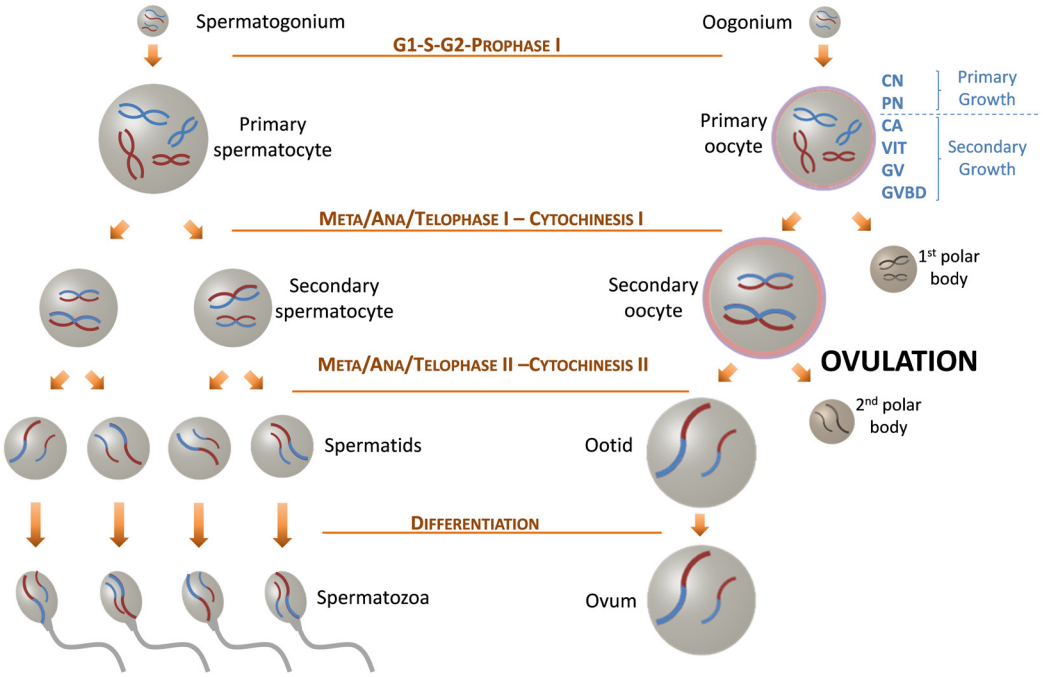


Figure 3.2.

Gametogenesis process in teleost fish

breakdown and a further rapid size increase of the oocyte caused by the uptake of fluid (Figure 3.3.F) (Wallace & Selman, 1981). Ovulation occurs when the follicle breaks after hydration. The remaining follicles are then called postovulatory follicles (POFs). The postovulatory follicles appear as structures folded over onto themselves and degenerate through several stages (Hunter & Macewicz, 1985). This mechanism is considered to differ from the atretic process (Wood & Van Der Kraak, 2003).

Spermatogenesis

The process of spermatogenesis is quite similar in most of the teleosts (Figure 3.2). The male germinal epithelium is normally composed of spermatocytes that are formed when a single clone of primary spermatogonia is enclosed by Sertoli cells. The germ cells develop synchronously inside these cysts. At the end of the process, the cysts open and the spermatozoa are released into the lobular lumen. This well-known type of spermatogenesis is called cystic because the whole process happens inside these cysts. Spermatogenesis, however, does not follow this pattern in all species. Spermatocysts can open and release developing germ cells into the lobular lumen before they become spermatozoa. This semi-cystic spermatogenesis has been described for the first time in the genera Ophidion (Mattei *et al*., 1993) and afterwards in many other phylogenetically widely separated species, as in Scorpaena (Muñoz *et al*., 2002c; Sàbat *et al*., 2009).

The development stage of the male gametes can be determined following Grier (1981) and Grier & Uribe-Aranzábal (2009). Transmission electron microscopy (TEM) can complete the information obtained histologically. In this sense and given the small size of the gametes, this kind of analysis becomes indispensable to study the sperm structure.

The primary spermatogonia appear individually or in small groups (Figure 3.4.A), having a major round nucleus which can contain one or few nucleoli. The secondary spermatogia are greater in number and

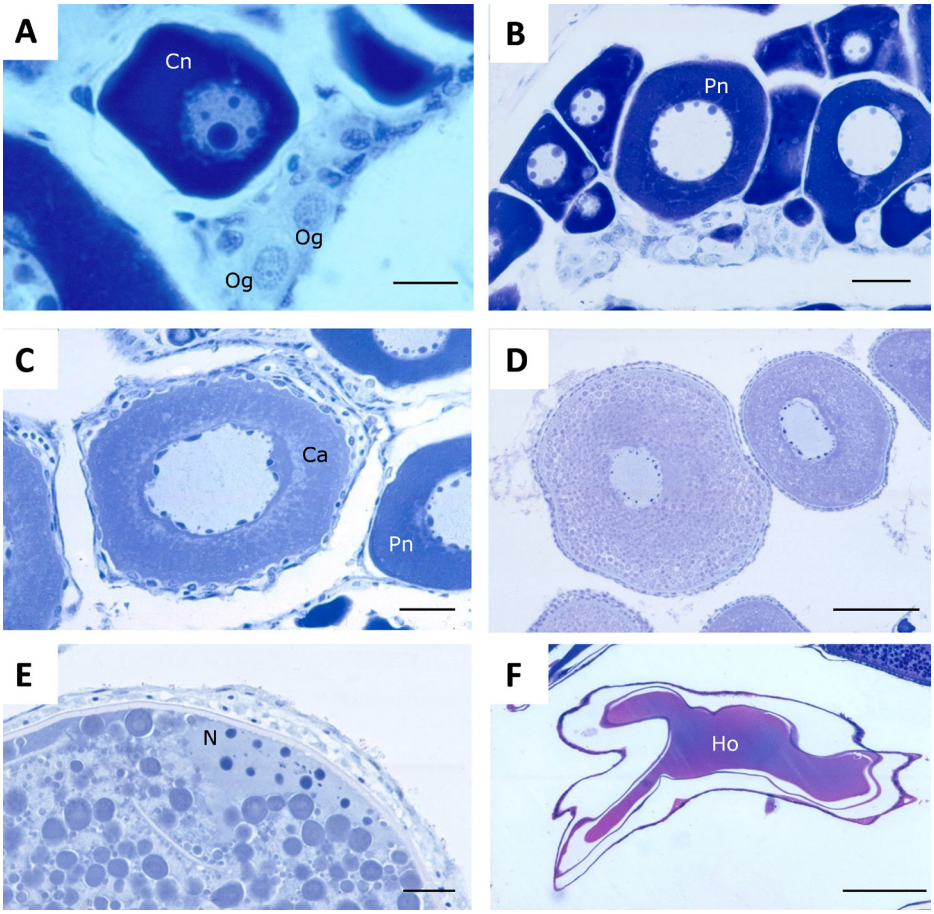


Figure 3.3.

Oogenesis in Scorpaena sp. (photo courtesy of Maria Sàbat). A: Oogonia and chromatin-nucleolar oocyte. Toluidine blue. Bar = 10µm. B: Perinucleolar oocyte. Toluidine blue. Bar = 30µm. C: Cortical alveoli oocyte. Toluidine blue. Bar = 30µm. D: Vitellogenic oocytes. Basic fuchsin-methylene blue. Bar = 200µm. E: Migratory nucleus oocyte. Toluidine blue. Bar = 30µm. F: Hydrated oocyte. Basic fuchsin-methylene blue. Bar = 200µm. Ca = cortical alveoli oocyte; Cn = chromatin-nucleolar oocyte; Ho = hydrated oocyte; N = nucleus; Og = oogonia; Pn = perinucleolar oocyte

always enclosed into a cyst surrounded by Sertoli cells. The nucleus may still contain nucleolus but it is smaller than in the previous stage. The morphology of the nucleus of the primary spermatocytes varies as the cell proceeds through the prophase of the meiosis, being the most easily identifiable phase the pachytene stage, marked by the synaptonemal complexes (Figure 3.4.A, B). The secondary spermatocytes, with a smaller nuclear size, are very difficult to observe because the time between the first and second division is short. In the spermatids there is the condensation of the chromatin, which implies a smaller size of the nucleus. The cytoplasm also reduces considerably. The flagellum starts to growth but it is difficult to detect by optical microscopy (Figure 3.4.A, B, C). Spermatozoa have a more or less rounded and small head and the flagellum (Figure 3.4.C).

3.4.2.2. Histological processing and staining

In the histological processing a section of ovary is embedded in wax or paraffin and thin sections are cut and mounted on slides for viewing under a microscope. The sections are stained during processing in order to highlight specific structures within the ovary.

Fixation, is the most important stage for an histology processing and should be carried out as soon as possible after removal of the tissues or soon after death. Formaldehyde is the most used chemical for all routine fixations, but it is carcinogenic, i.e. adequate security measures are required to work with it. Nowadays non-carcinogenic fixatives are commercialized, as those based on glyoxal, acrolein or carbodiimides among others; however, they can alter slightly the structure of the gonad and their impact on the studied tissue should be carefully tested before replacing formaldehyde with any of them.

When embedding in resin, the cross section of ovary is dehydrated in alcohol solutions of increasing strength (70%, 90%, 96%) for several hours (32, 16,8) followed by infiltration of resin using ascending concentration (50%, 100%) for several days (2, 2). Finally, the cross section of ovary is placed in a mould and embedded in resin mixed with hardener to obtain resin blocks. Sections are then cut (width usually about 4µm) from the resin block using a microtome these are then stained and mounted on glass slides.

Processing using as media paraffin also involes dehydration through ascending grades of alcohols, "clearing" in a wax miscible agent (chloroform, xylol) and finally impregnation with wax. After this, blocks are sectioned with a microtome in a specific thickness depending on their stage of maturity, since with more mature gonads it is more difficult to get a very thin section. Before staining, sections must be hydrated with a decreasing alcoholic series.

Most histological analyses on reproductive biology of fishes are routinely stained using the hematoxylin- eosin method due to its simplicity and standardization, but sometimes it is necessary to complete the information with other staining

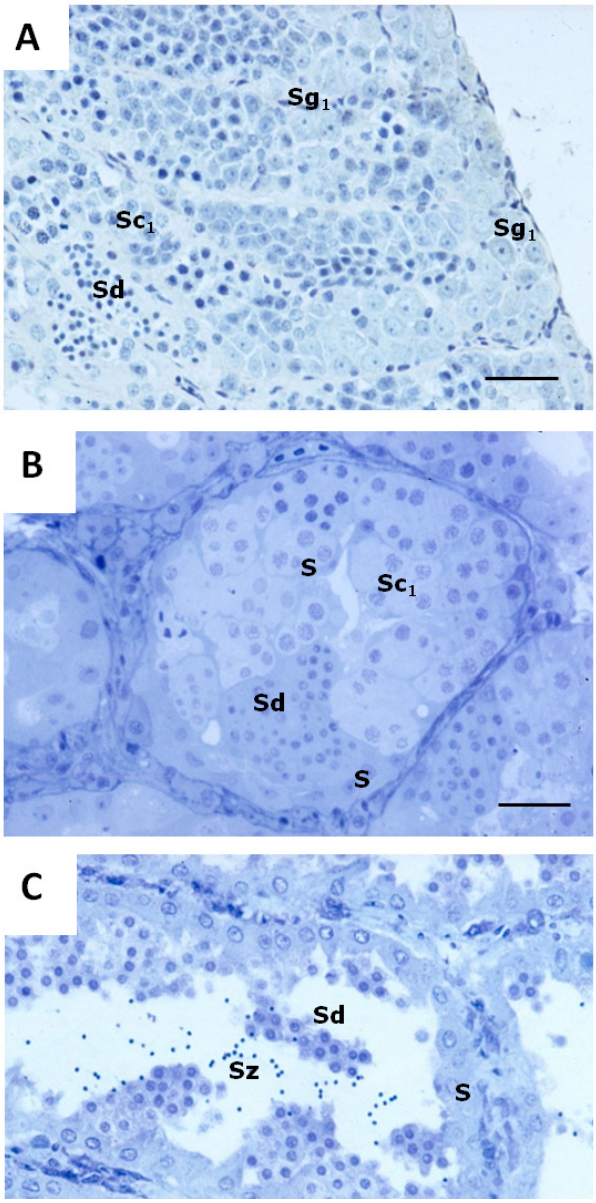


Figure 3.4.

Spermatogenesis in Scorpaena sp. (photo courtesy of Maria Sàbat). A: Toluidine blue. Bar = 30µm. B: Methylene blue. Bar = 30µm. C: PAS. Bar = 30µm. S = Sertoli cell; Sc1 = primary spermatocytes; Sd = spermatids; Sg1 = primary spermatogonia; Sz = spermatozoa.

methods. For instance, the Mallory's trichrome staining highlights the zona radiata and facilitates the analysis of its continuity, and so, the detection of early atretic oocytes (Muñoz *et al.*, 2010). Another example is related with the evolutionary trends towards viviparity, which are usually accompanied by small size and scarcity of cortical alveoli (Takemura *et al.*, 1987; Muñoz *et al.*, 2002a; b) which makes their

detection difficult. However, these structures become highlighted with the periodic acid-Schiff (PAS) stain, making this technique very useful to detect oocytes in the cortical alveoli stage in zygoparous, embryoparous, or viviparous species. In the case of the males, or in females with intraovarian sperm storage, spermatozoa can be highlighted with the Mallory's trichromic staining (Muñoz *et al*., 1999).

3.4.2.3. Maturity staging from whole mounts

Whole mount techniques (Kjesbu, 1991) are usually simple and based on ovarian tissue spread gently on the bottom of a petri dish and thereafter observed under the microscope at a moderate level of magnification. Fresh tissue is kept in isotonic sea water (around 1.07%) which can easily be made by mixing one part sea water with two parts of fresh water. If fixed tissue is used, it is important to allow enough time for fixation to complete before size measurements are performed since fixation usually causes swelling or shrinkage.

Compared to histology examinations, whole-mount analysis may for certain purposes be much more efficient both with respect to time and cost (Thorsen and Kjesbu, 2001; Alonso-Fernández *et al*., 2009). Using automated image based particle analysis, oocyte sizes can easily be measured (Chapter 4, page 18). Whole-mount methods are therefore often used to replace or validate macroscopic staging of ovaries. Especially in regards to the size of the largest oocytes in the ovary, the leading cohort (LC), which is a good proxy for the maturity stage of the ovary and the time to start of spawning (Kjesbu, 1991). The LC diameter of the previtellogenic oocytes also permits the identification of specimens that have just completed a spawning season from those that have not yet entered sexual maturity as these oocytes are typically smaller than the previous ones (Witthames *et al*., 2010).

In addition to oocyte size measurements, whole-mounts techniques may also utilize differences in oocyte transparency for oocyte staging. Previtellogenic oocytes and advanced hydrated oocytes are highly transparent, while cortical alveoli oocytes and early vitellogenic oocytes are semi-transparent, and mid and late vitellogenic oocytes are nontransparent (Figure 3.5). Transparency is particularly useful then to separate between early developing and developing specimens (Figure 3.5.A), i.e. oocytes in the cortical alveoli stage or oocytes in early vitellogenesis that are not detectable by the naked eye. It is also useful in separating between immature and regressing/regenerating specimens, when the latter ones contains hydrated oocytes (Figure 3.5.B) or atretic oocytes remaining from the spawning period (Figure 3.5.C). Besides transparency, the shape of oocytes in whole mounts can be used to distinguish between normal and atretic oocytes (Figure 3.5.C). Atretic oocytes are more irregular in shape and displays a peripheral band of high transparency (Óskarsson *et al*., 2002). Also post-ovulatory follicles can be recognized in whole mounts based on their shape and contrast (Figure 3.5.A), allowing identification of spawning individuals.

Oocyte size frequency histograms contain important information about the maturity status of the fish and also about the type of ovary development and type of spawning (Figure 3.6). Fish oocyte development differs profoundly between species. We may consider asynchronous ovary development on one extreme and synchronous development on the other. In addition there are many other type of divisions used, like for example determinate (e.g. herring and cod) and indeterminate (hake) and total spawner (herring) and batch spawner (cod and hake) (Figure 3.6). However, for all species oocyte size frequency histograms will

contain key information both on the type and stage of ovary development.

Oocyte development and spawning of cod (*Gadus morhua*) is well studied and we will therefore use cod as an example to illustrate how information about oocyte size frequency can be interpreted to give information about the maturity status of the fish. In cod, vitellogenesis is initiated several months before spawning starts. In the period between start of vitellogenesis and spawning, the size of the vitellogenic oocytes indicates time to start of spawning. During this period the size of the largest oocytes, the leading cohort, seems to grow with a steady pace and is thus particularly useful for prediction of time to start of spawning (Kjesbu, 1994). In several reports the leading cohort has been defined as the mean of the 10 % largest oocytes (Thorsen & Kjesbu, 2001; Kjesbu *et al*., 2010), or also as the 95 % percentile.

Cod is a determinate batch spawner that typically release 10-20 batches within a spawning season with 2-4 days in-between (Kjesbu, 1989). Maturation of cod typically starts in the autumn and is associated with oocyte growth. The cortical alveolus stage can be regarded as the first major stage that is associated with maturation (Kjesbu & Kryvi, 1989). The cortical alveolus stage typically starts when the oocytes are around 200 µm in diameter. When the oocytes reach a diameter of 200-250 µm further growth is associated with incorporation of large amount of vitellin, and hence from now on the oocytes are said to be in the stage of vitellogenesis. Furthermore, when cod oocytes reach 700-900 µm they are ready for final maturation and subsequent ovulation and spawning. Since all these stages can be associated with oocyte size, oocyte particle analysis can give a quick maturity status of the fish (Figure 3.7).

Since cod is a batch spawner, a new batch coming up can be seen on a size frequency histogram as an isolated group of oocytes that are larger than the remaining vitellogenic oocytes (Figure 3.7). The size frequency data can in such cases also be used to estimate batch fecundity. During the spawning period a size frequency histogram can also indicate the proportion of eggs spawned; when the spawning period starts (Figure 3.7.A) the oocyte size distribution is wide, but as spawning progress (Figure 3.7.B, C, D) the size distribution narrows and the mean oocyte diameter increases (Kjesbu *et al*., 1990).

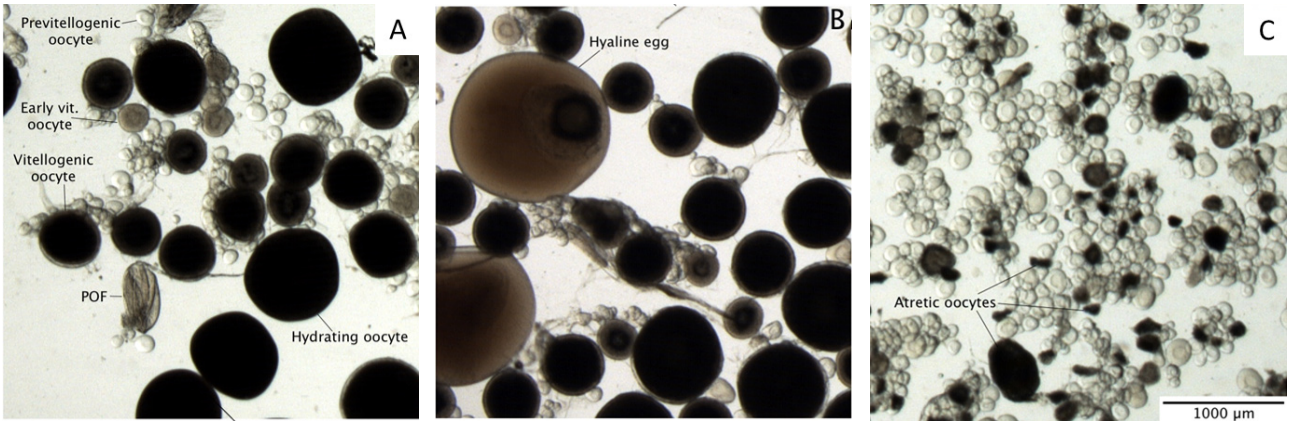


Figure 3.5.

Pictures of whole mount formalin fixed ovary samples from Atlantic mackerel. A) and B) Samples from spawning fish. C) Sample from a spent fish. All pictures have similar magnification.

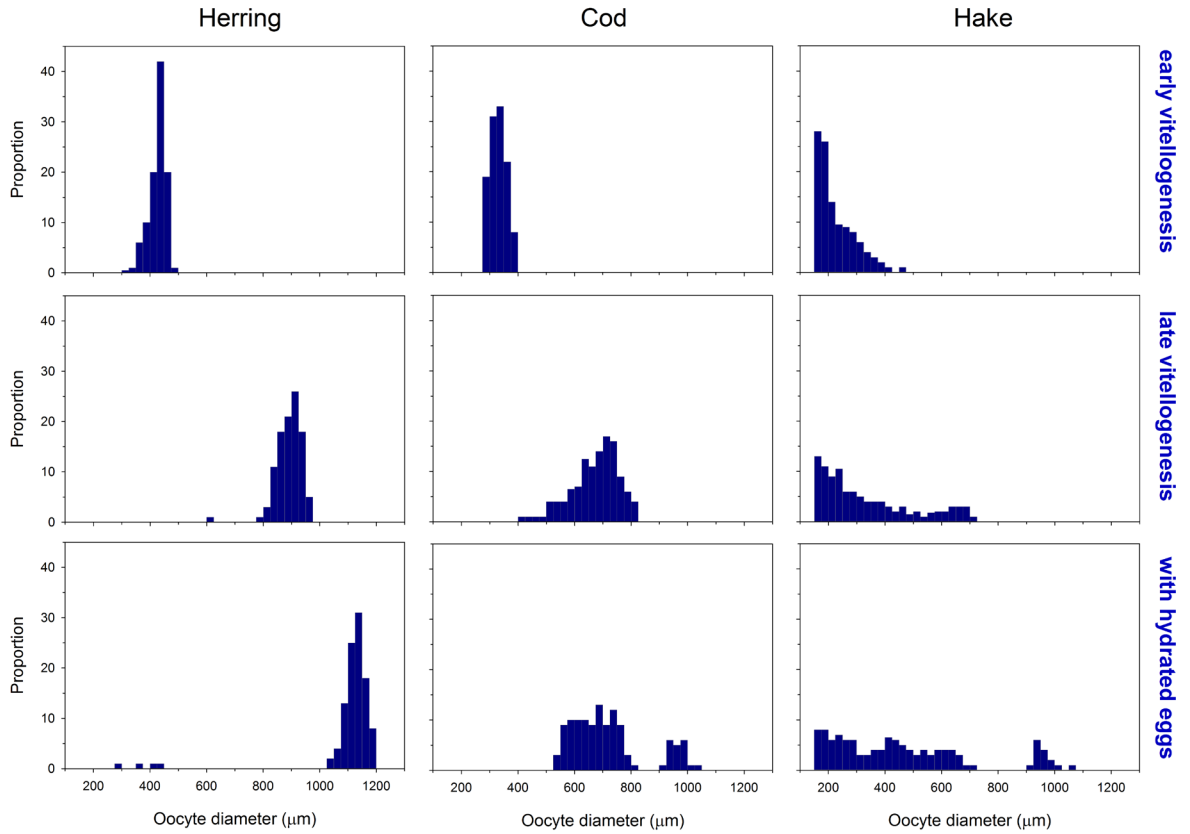


Figure 3.6.

Follicle size frequency histogram for Atlantic herring (Clupea harengus), Atlantic cod (Gadus morhua), and European hake (Merluccius merluccius) in different stages of ovary devlopment (note that lower sizes are limited by measuring limitations).

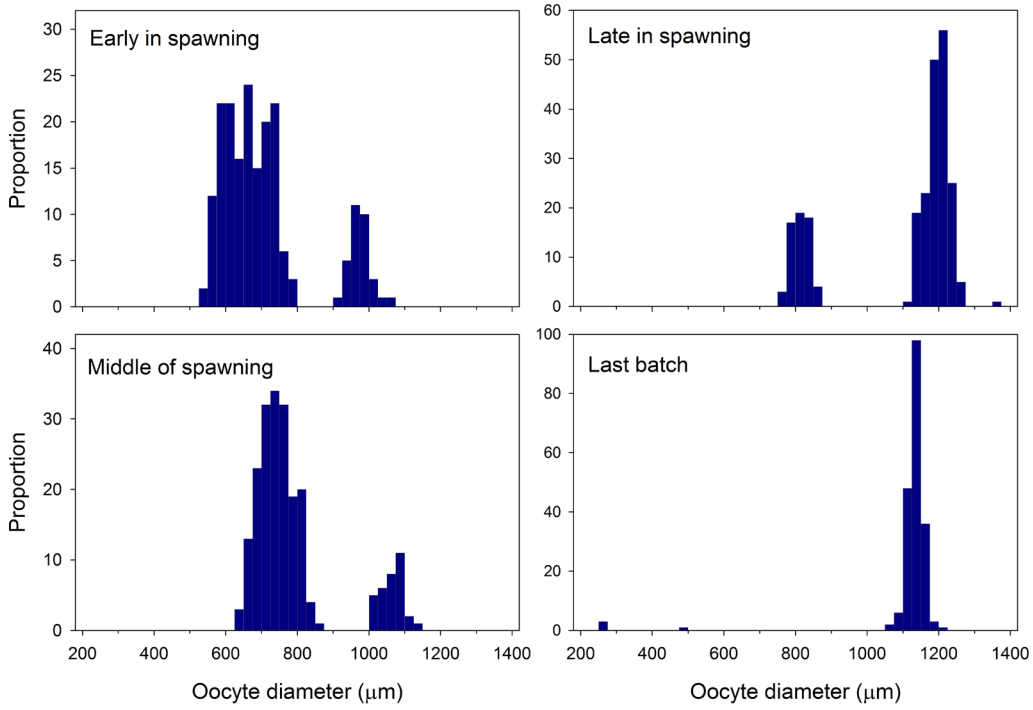


Figure 3.7

Follicle size frequency histograms from cod in the spawning period: A) early spawning, B) middle of spawning, C) late in spawning and D) last batch. All fishes displayed have a new batch (final maturation) coming up on the right hand side. The size distribution of the vitellogenic pool on the left hand side is broad early in the season but progressively narrows as the spawning progress.

3.4.3 Validation and methodological calibration

It is fundamental to calibrate and validate the maturity staging that is carried out in different research institutes dealing with the same fish species. Generally maturity staging on a particular species is carried out by different laboratories in many countries. This data is then combined in one database; the data is then used for stock assessment purposes. It is important that the maturity scale used for each species is consistent across the laboratories/countries involved in the sampling. Thus the maturity staging of individuals should be regularly revisited for calibration and validation.

One way to calibrate maturity staging among different laboratories is to hold workshops where fresh samples are provided for maturity staging exercise. Frozen or fixed samples should be avoided as, due to preservation process, these samples lose some of the objective macroscopic characteristic used to assign the maturity. In calibration exercise the percentage of agreement/disagreement among different laboratories is statistically calculated as well as precision and bias of obtained maturity observation. Generally, microscopic maturity staging is considered as ground truth for the determination of staging errors.

More recently, calibration and validation can be done by using on-line tools, such as WebGR (http://webgr.azti.es/ce/search/myce) where the organiser of a calibration workshop can upload images of gonads. Before the workshop, a plan for collecting samples to be used during the workshop should be setup. The samples used during the workshop should closely reflect the samples typically encountered during the actual maturity sampling. Thus, the samples used during the workshop should cover all the maturity stages and length range of sampled individuals which are found during sampling. The collection of biometrics and the treatment and processing of the gonads should also be similar. All participants individually score maturity stage of each picture on-line using a standard maturity scale. This way discrepancies on maturity staging are detected as well as most problematical stages are identified. Besides, this is an easy and cheap tool which requires only the collection of high quality pictures of the gonads. However, it is clearly shown that maturity staging from pictures is more difficult compared to staging from fresh gonads. Also some of the characteristics of the development stages are difficult to see from pictures whereas they are easy distinguished in fresh material. Thus workshops with staging from fresh material will give a better understanding of the agreement between maturity stagers. A good setup for such a workshop is to use trial-discussion-retrial where participants stage the gonads, then discuss results and stage gonads again.

3.5. Maturity ogives and spawning proportion

3.5.1. Methods: estimating maturity ogives

An ogive is a cumulative frequency line graph (i.e. y-axis values are among 0 and 1) that is used mainly for statistical analysis of quantitative data and allows estimating the percentiles of data distribution.

Maturity ogive is defined as a curve that describes the proportion of fish that is mature at a certain length or age. Ogives are often represented using a logistic (s-shaped) curve, which is defined as:

$$P_i = \frac{1}{1 + e^{-K(X_i - X_{50})}} \qquad (3.4)$$

where P_i is the proportion mature at length or age X_i and K and X_{50} are the parameters to be estimated, k represents the instantaneous rate of maturation, or the slope of the curve, x_{50} represents the age or length at which 50% of the fish are mature (Chen and Paloheimo 1994) and it is often interpreted as the age or length at first maturity (A_{50} and L_{50} respectively). However, it is noteworthy to specify here that some authors consider the age or length of the smallest mature individual observed in the sample to be the age or length at first maturity.

There are various methods to fit a logistic curve but the most common approach is to use a generalised linear model (GLM) with a binomial error distribution and a logit link function. In Box 3.2 there is some R code that illustrates how to fit a GLM to maturity data (Box. 3.2).

Fitting a simple GLM will produce a slope (a) and an intercept (b); the slope corresponds directly to the parameter K in the Eq. 3.4 and the parameter X_{50} (L_{50} or A_{50}) can simply be calculated as follows: $X_{50} = -b/a$. Because L_{50} or A_{50} has a direct biological relevance, it is more common to report this parameter rather than the intercept.

One advantage of using a GLM to fit maturity curves is the availability of a large range of diagnostics tools to identify influential observations, check for overdispersion, goodness of fit, etc (McCullagh and Nelder, 1989; Collett, 2003). Another important advantage of GLMs is the possibility to include additional terms (like sex, year or region) in the model and test if these terms improve the model fit (Gerritsen *et al*., 2003; Armstrong *et al*., 2004). For example in a stock where males mature at a smaller size than females, a logistic curve might fit the combined-sex data poorly (Figure 3.8.A). Introducing the term sex into the model improves the fit (Figure 3.8.B) and adding a length * sex interaction term improves the fit even more (Figure 3.8.C). The interaction term allows the model to fit two curves with different slopes and different intercepts. The Akaike Information Criterion (AIC) is a standard output of GLMs and can be used to select the most appropriate terms to include in the model (Sakamoto *et al*., 1986). The AIC is a measure of the relative quality of a statistical model for a given set of data. It analyses the trade-off between the goodness of fit and the complexity of the model, but it doesn't offer an absolute estimate of the goodness of fit as other models testers do, e.g., the coefficient of determination (R^2). The lower the AIC, the better the model is.

The logistic model is by far the most common approach to fitting maturity ogives; however it

BOX 3.2. R code of GLM for maturity data.

```
# generate some data
mat1 <- rbind(data.frame(lenclass=floor(rnorm(100,20,5)),maturity=0)
 ,data.frame(lenclass=floor(rnorm(100,30,5)),maturity=1))
# fit the model
glm1 <- glm(factor(maturity)~lenclass,family=binomial,data=mat1)
# Calculate L50 and K from the model coefficients
L50 <- - glm1$coef[1]/ glm1$coef[2] # L50 = -Intercept/slope
K <- glm1$coef[2] # K = slope
# plot the observed and modelled proportions mature
fun <- function(x) sum(x)/length(x)
mat2 <- aggregate(list(p=mat1$maturity),list(lenclass=mat1$lenclass),fun)
plot(mat2)
lines(mat2$lenclass,predict(glm1,newdata=mat2, "response"))
```

constrained to be symmetric around X_{50} and have asymptotes at proportions of zero and one. Schnute & Richards (1990) proposed a number of families of curves that can take a wide range of shapes. However, these models have not been widely applied to estimate maturity ogives, perhaps because logistic curves are typically adequate.

When a GLM is used to estimate maturity-at-length, data that have been collected on a length-stratified basis can be used directly without causing bias because within each length class the individuals were randomly sampled. However, using length-stratified data to estimate maturity-at-age can lead to bias because individuals within age classes were not randomly sampled. In that case it may be necessary to weight the observations by the abundance of each length class (Morgan and Hoenig 1997).

GLMs are based on the assumption that the individual observations are independent which will result in an under-estimate of the standard error if fish from the same haul or fishing trip are more similar to each other than to fish of other hauls or trips. Hierarchical or mixed-effects models could be used to account for the variability within and between hauls or trips (Venables and Dichmont, 2004), but in practice these models are not (yet) widely used for maturity estimation.

In some cases a knife-edge or fixed maturity ogives are used (Box 3.3.). The first ones assume that all fish mature at the same length/ age, i.e. the proportion of mature specimens at age (or length) changes abruptly

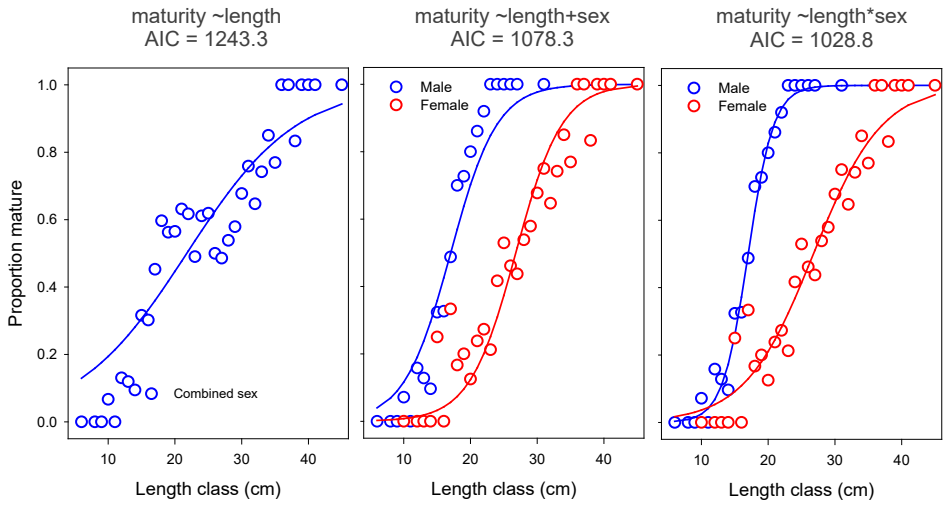


Figure 3.8.

Example of three GLMs fitted to the same data. A) The simple model (with length as the only explanatory variable) fits the data poorly. B) Including the term 'sex' in the model improves the fit (the AIC is lower); the model produces two curves with the same slope but different L_{50}. C) Including 'sex' as an interaction term provides the best fit (lowest AIC); the model consists of two curves with different slopes and different L_{50}. This approach is identical to fitting a separate model for each sex.

from zero to one; for example, all fish younger than age 2 are assumed to be immature and all older fish are considered as mature. The second ones are common maturity ogives but time-invariant that means they do not change over time, so, the same proportion of mature individuals at age (or length) is applied every year.

The use of knife-edge and/or fixed maturity ogives overlooks the actual structure of spawning stock and potential temporal changes in age or size at maturity and could eventually cause serious bias when estimating the number of spawning fish in the stock and stock reproductive potential. Applying knife-edge maturity ogives that overestimates the mature component of the stock and does not accommodate the poorer reproductive fitness of young fish, could seriously overestimate the reproductive capacity of the stock (which is lower in young/ smaller individual of the stock), leading to an underestimation of the impact of fishing and to a biased interpretation of the stock status. However, the use of these alternative ogives should be considered when the sampling level is not ideal, i.e., when the variation of maturity proportion is sampling noise instead of true variation of the stock.

3.5.2 Validated maturity ogives

A correct assignment of maturity stages and thus an unbiased individual classification as immature or mature is of critical importance to investigate maturity.

The judgement of the reproductive status based on the gross anatomy (macroscopic observation) of the ovary or testes is a low cost and quick method for assessing maturity, allowing the analysis of a large amount of samples and therefore ideal for routine monitoring the fish spawning stock biomass. However, the macroscopical judgement does not always mirror the actual gonadal status as some features may not be discernable with the naked eye and consequently misinterpreted. Therefore macroscopical

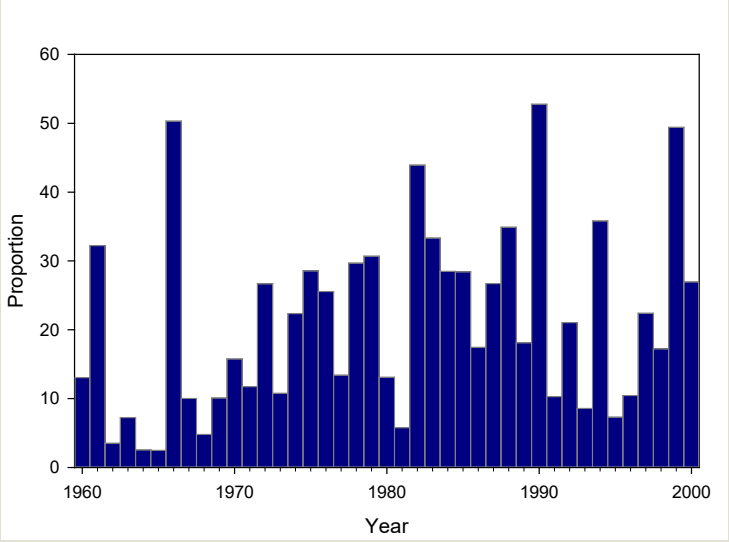
BOX 3.3. The implications of using fised knife EDGE maturity ogives: The case of north sea sole.

(from Bromely, 2003 and refrences therein)

The current analytical assessment of North Sea sole (Solea solea) used a fixed knife-edge maturity ogive to estimate spawning stock biomass (SSB), assuming that all specimens (males and females) are fully mature at 3 years old. However, by 2003, Bromley already alerted about the risk of this practice.

As in most of flatfish, the North Sea sole presents sex-differentiated maturation and males mature younger and smaller than females. Besides, there are many factors affecting maturation that may change year to year depending on the environmental and/or the cohort characteristics. The use of fixed knife-edge maturity ogives in this specie has led, according to Bromley (2003), to an overestimation of the SSB from 2% to 53% between years, depending on the year class attributes (see figure below).

This bias of the estimates of SSB alters the stock-recruitment relationship, calling the reference points based on SSB, commonly used in fisheries management, into question.



The percentage overestimation of the total number of spawning North Sea sole for the period 1960–2000 if it is assumed that 100% rather than 51% of 3-year-olds spawn, i.e., after the market data had been adjustment for sampling bias (based on Bromley, 2003).

maturity data needs to be validated by the means of a more accurate method. In most of the cases, this method is histology, which allows the identification of specific characteristics undetected / misidentified macroscopically. However other methods like whole mount preparations or hormone analyses are sometimes used.

Discrepancies between macroscopical and microscopical maturity data have been observed and quantified for different species, such as cod (Vitale *et al*., 2005), horse mackerel (Costa, 2009), sardines and anchovies (Ferreri *et al*., 2009). Misclassification of macroscopic data implies an underestimation or overestimation of the X_{50} and subsequently of the spawning stocks biomass.

While advanced stages (late vitellogenesis and spawning) are easily recognizable and therefore properly judged, incongruences are encountered for individuals at the beginning of the developmental process. The maturing stage in a macroscopical scale should include all the maturing individuals that will eventually spawn during the upcoming spawning season. However, a consistent part of immature specimens showing initial signs of structural modification are often erroneously interpreted as maturing and included in this stage (Vitale *et al*., 2005). Such a mistake obviously overrates the proportion of mature individuals compared with the true population, and consequently leads to an overestimation of the spawning stock biomass. On the other hand, the underestimation of the spawning stock biomass is usually encountered while dealing with post-spawning, regenerating or skip-spawning individuals. These are often macroscopically hardly discernible from immature specimens. When comparing the microscopical and macroscopical staging during the pre-spawning season (when all the reproductively active individuals are supposed to be mature but no spawning has occurred yet), the misjudgement is minimized due to the unmistakable advanced stage of the maturity process and to the unlikelihood to find fish in post-spawning condition. Thus collecting maturity data outside the spawning season may be meaningless as the visual classification of fish maturity state is unreliable and the prevalence of non-maturing individuals hampers the quantification of the reproductive stock (Trippel *et al*., 1997). Being the reliability of visual judgement dependent on the time of sampling, accurate estimations of maturing fish outside the spawning season can only be assured by using microscopical investigations (Saborido-Rey & Junquera, 1998; Kjesbu *et al*., 2003; Vitale *et al*., 2006).

Beside all possible actions to minimise macroscopic misclassification, such as correct sampling season and routine training of observers, validating macroscopic data used for maturity ogive estimation is always recommended.

A correct estimation of the maturity ogives requires that the entity of the disagreement between the two classification methods is ascertained and incorporated in the calculation. When using the macroscopic maturity identification, a subsample of fix number of gonads by length class should be collected for histological analysis.

Once the disagreement, if present, between the maturity ogives calculated using the two methods has been quantified within age or length classes, the difference can be applied as a conversion factor/matrix to correct the macroscopical maturity ogives accordingly.

The differences in the proportion of mature individuals per age/lenght class i between the macroscopic observations and the histological analysis can be used

as an estimate of the bias or as a correction factor of the macroscopic maturity ogive (Vitale *et al*., 2006). The relative bias per age/ length class i, (RB_i) can be defined using the following equation:

$$RB_i = \frac{MV_i - MH_i}{MH_i} \quad (3.5)$$

where MV_i is the percentage of mature fish according to visual inspection (macroscopic observation) per each age/length class i; MH_i is the percentage of mature fish according to histological analysis per each age/length class i.

The absolute bias (AB_i) per age length class can also be calculated:

$$AB_i = MV_i - MH_i \quad (3.6)$$

A corrected maturity ogives (CMO_i) for each age/ lengh class i can be obtained applying the estimated RB_i per age/ length class on the maturity ogives based on visual inspection (VMO_i) as follows:

$$CMO_i = \frac{VMO_i}{1 + RB_i} \quad (3.7)$$

The bias and the corrected maturity ogives should be calculated separately for males and female as the extent of misclassification of maturity stages, using the two methods, may differ between sexes.

3.5.3. Combined vs separated maturity ogives

Despite sex-separated maturity ogives are available for most of the stocks the use of combined ogives (females and males) is currently still used in stock assessment. In some cases, this might represent a potential source of error of indicator of reproductive capacity.

Growth, maturation and mortality are known to be sexually dimorphic in many species and this can severely impact the perceptions of the stock status. The estimation of maturity ogives for each sex separately shows, in most of the species, that males generally mature at younger ages than females (Jorgensen, 1990).

In most of flatfishes, such as plaice (*Pleuronectes platessa*), females grow faster and reach sexual maturation at an older age than males (Kell & Bromley, 2004). Therefore, the use of a sex-combined maturity ogive might not be the best tool to use as an indicator of the reproductive capacity.

Also in gadoids like cod (*Gadus morhua*), growth, maturation and mortality are known to be sexually dimorphic, i.e. earlier maturity and shorter lifespan in males (Tomkiewicz *et al*., 2003 and references therein). Furthermore sex ratio is generally moving towards lower proportion of females in stocks where the fishery selection pattern changed the size/age structure towards smaller/younger individuals, which are mostly males with slower growth rate (Jakobsen & Ajiad, 1999).

In many cases, however, an estimate of SSB based only on females (FSSB) might be might be a more accurate measure of the stock reproductive capacity (Marshall & Saborido-Rey, 2003; Marshall et al., 2006), given the assumption of the relationship between SSB and population's egg production (Box 3.4.).

3.5.4. Ogives in hermaphrodites

To estimate maturity ogive in hermaphrodites, the sexual change strategy (Box 3.2) has to be taken into account.

For simultaneous hermaphrodites, where both sexes

are always present and mature at the same time, maturity ogive is equivalent to that of gonochoristic species, i.e., proportion of mature specimens at age or length. However, in sequential hermaphrodite species it is necessary to estimate the age or length of sex change too. The method to estimate sex change ogive is equivalent to that used to calculate maturity ogive, but replacing the proportion of mature individuals by the proportion of the terminal sex (male or female depending on the sexual pattern). Length (or age) of sexual transition (L_T and A_T respectively) corresponds to the size (or age) at which 50% of the individuals had undergone the transition from the initial sex to the terminal sex (Alonso-Fernández *et al*., 2011).

In this case, the estimation of maturity ogive for SSB calculation is constructed considering exclusively the initial sex, when maturation takes place at first time, thereafter, individual will be considered as mature for the rest of its life. Nevertheless, for estimating FSSB, that is a basic parameter to estimate total egg production (TEP), the sex change ogive has to be considered in different ways depending on the sexual strategy: protandry or protogyny (Figure 3.9). For example, in protandrous species, FSSB is estimated directly from sex change ogive (Figure 3.9.A), while in protogynous species, FSSB is calculated substracting the sex change ogive from the maturity ogive (Figure 3.9.B)

When sex change is socially controlled, the local effect is high (it depends on each group, family or harem) and must be considered in the interpretation of the maturity ogive estimations.

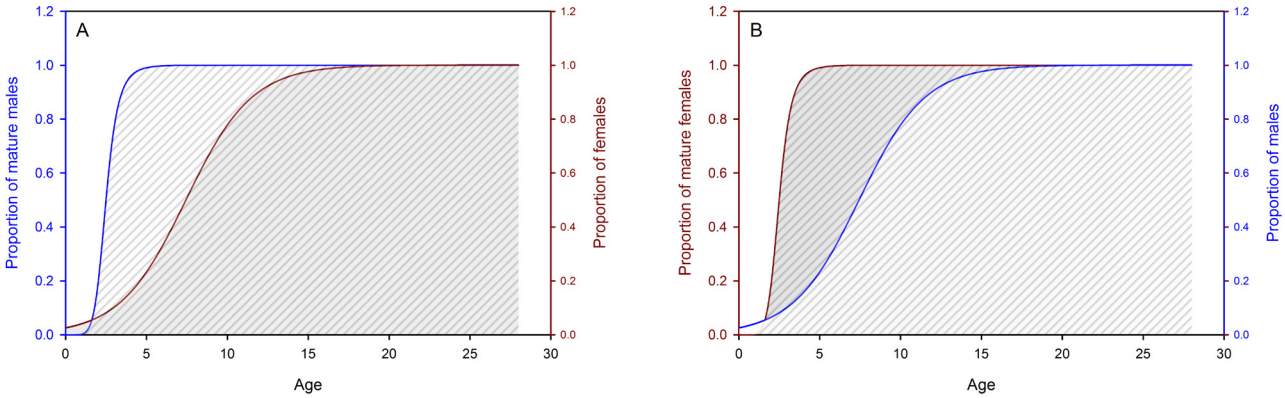


Figure 3.9.

Maturity and sex change ogive for A) protandrous and B) protogynous hermaphrodite species. Blue line and red line denotes males and females respectively. A50: age at maturity, AT: age of sex change. Lined area: proportion of specimens to be considered for SSB estimations. Grey area: proportion of specimens to be considered for FSSB estimates.

BOX 3.4. The cod stock in Kattegat.

The potential differences in the trends of sex-separated and combined spawning biomass is here shown taking as an example the cod stock in the Kattegat (Eastern North Sea) for the period 1991-2007. The FSSB is calculated as following:

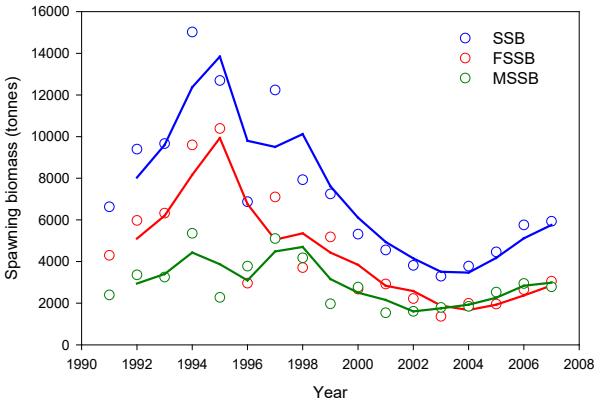
$$FSSB = \sum_{a=i}^{plus\ group} FMO_a \cdot Fw_a \cdot SR_a \cdot N$$

where FMO_a is the proportion of mature females relative to the total number of females (female maturity ogives) at age, FW_a is the female mean weight-at-age, SRa is the sex ratio at age and Na is the total number of individuals in the stocks at a given age. All age groups in the population from i to the plus group should be considered. The male spawning biomass (MSSB) is instead calculated as:

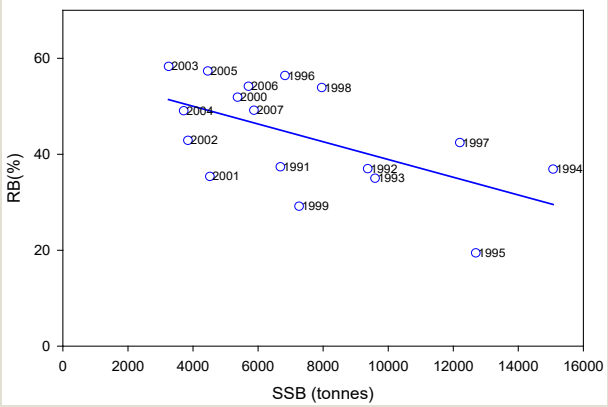
$$MSSB = 1 - FSSB$$

When comparing the time series of both sex-separated and combined SSB (see figure below) it is evident that the difference between the FSSB and MSSB is larger at higher values of SSB, evidencing a prevalence of spawning females. Moving towards the period of lower values of SSB the MSSB may exceed the FSSB, reflecting that the population is skewed towards early maturing males. In this way using a sex combined SSB, introduces different bias depending on the status of the spawning stock. The relative bias (RB) introduced when using SSB based on combined maturity ogives can be calculated as following:

$$RB = \frac{SSB - FSSB}{SSB} \cdot 100$$



Time series of combined (SSB), female (FSSB) and male (MSSB) spawning biomass of Kattegat cod. Lines represent the running average for each plot (data from ICES DATRAS database).



Relationship between the relative bias of using a sex combined ogive and the respective SSB (data from ICES DATRAS database).

3.5.5. Recommendations

To estimate maturity ogives, and also for other studies related with reproduction and maturity, several steps should be considered:

- Appropriate sampling design that covers the temporal and spatial features of the species;
- Adequate sampling level that covers the entire length range of the species, with a good coverage of the length classes that are not fully mature;
- Use a simple and easy to understand macroscopic maturity scale that allows an unequivocal identification of the different stages;
- Validate a macroscopic observation (preferably with histology);
- Correct the macroscopical maturity data with the histological information;
- Estimate the separate sex maturity ogive and evaluate the impact on the SSB estimation between the use of a sex combined and a sex separated ogive;
- Take into account specific methodological aspects for sequential hermaphrodite fish.
- When preparing an age based maturity ogive, take into account the quality of the age readings (e.g., experience of readers, accuracy of the method, precisions between readers, etc.);
- Conduct routinely maturity staging workshops or exchanges (with photos) between laboratories working with the same species. Evaluate the impact of staff discrepancy in the SSB estimation.

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