Don't put all your eggs in one basket!

Reproductive strategies and fecundity regulation in temperate marine teleosts

Cindy J.G. van Damme

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Don’t put all your eggs in one basket!

In fisheries management the spawning stock biomass (SSB) is an important indicator of the status of exploited fish stocks. Knowledge on the reproductive biology is essential to estimate SSB. A large variety of reproductive strategies is found. In marine fish two extreme strategies are known, capital spawners which have a determinate fecundity (no de novo oocyte recruitment during spawning), and income spawners which have an indeterminate fecundity (de novo oocyte recruitment during spawning). In this thesis fecundity regulating mechanisms are studied in commercial fish species with contrasting life history.

In capital spawning plaice *Pleuronectes platessa* and herring *Clupea harengus*, which spawn in autumn and/or winter, oocyte maturation starts around April when daylight length increases. Both species recruit a high number of oocytes which are down-regulated in the course of time in relation to the available energy. After the summer feeding period, when energy levels are highest, plaice shows a second recruitment phase. In herring, no difference was observed in the oocyte development between autumn and winter spawners, although winter spawners continue developing oocytes and spawn fewer but larger eggs. The income breeding horse mackerel *Trachurus trachurus* utilises food resources during spawning although the first batch of spawned eggs is developed on stored energy.

Food availability, through the body condition, is the most important factor regulating fecundity. In situations where food is available during the spawning season traditional determinate spawners may switch to a pseudo-indeterminate fecundity style. In conclusion this thesis shows that fecundity type of marine fish females is not fixed at the species level but represents a plastic response to the environment through food availability and energy allocation.

Ikke legg alle eggene dine i samme kurv!

I fiskeriforvaltning er gytebestanden en viktig indikator på statusen på beskattede fiskebestander og kunnskap om reproduksjonsbiologi er avgjørende for å anslå gytebestandens størrelse. Det finnes et stort utvalg av reproduktive strategier. Hos marin fisk er to ytterpunkter kjent, kapitalgytere som har en forhåndsbestemt fekunditet (ingen nyrekruttering av oocytter under gyting) og inntektsgytere som ikke har en forhåndsbestemt fekunditet (nyrekruttering av oocytter under gyting). Denne avhandlingen omhandler fekunditetsregulerende mekanismer i kommersielle fiskearter med forskjellig livshistorie.

For rødspette *Pleuronectes platessa* og sild *Clupea harengus*, som gyter på høsten og vinteren, starter modningen av oocyttene rundt april når dagslyslengden øker.

Begge arter starter ut med å rekruttere et høyt antall oocytter som siden blir nedregulert i forhold til tilgjengelig energi. Når energinivået er på det høyeste etter fôringsperioden på sommeren, har rødspetten en ny periode med rekruttering av oocytter. I sild ble det ikke observert noen forskjell i oocyttutviklingen mellom høst- og vintergytere, selv om vintergytere fortsatte å utvikle oocytter og gyte færre, men større egg. Hestemakrell *Trachurus trachurus* utnytter matressurser under gyting selv om den første porsjonen av egg er utviklet på lagret energi.

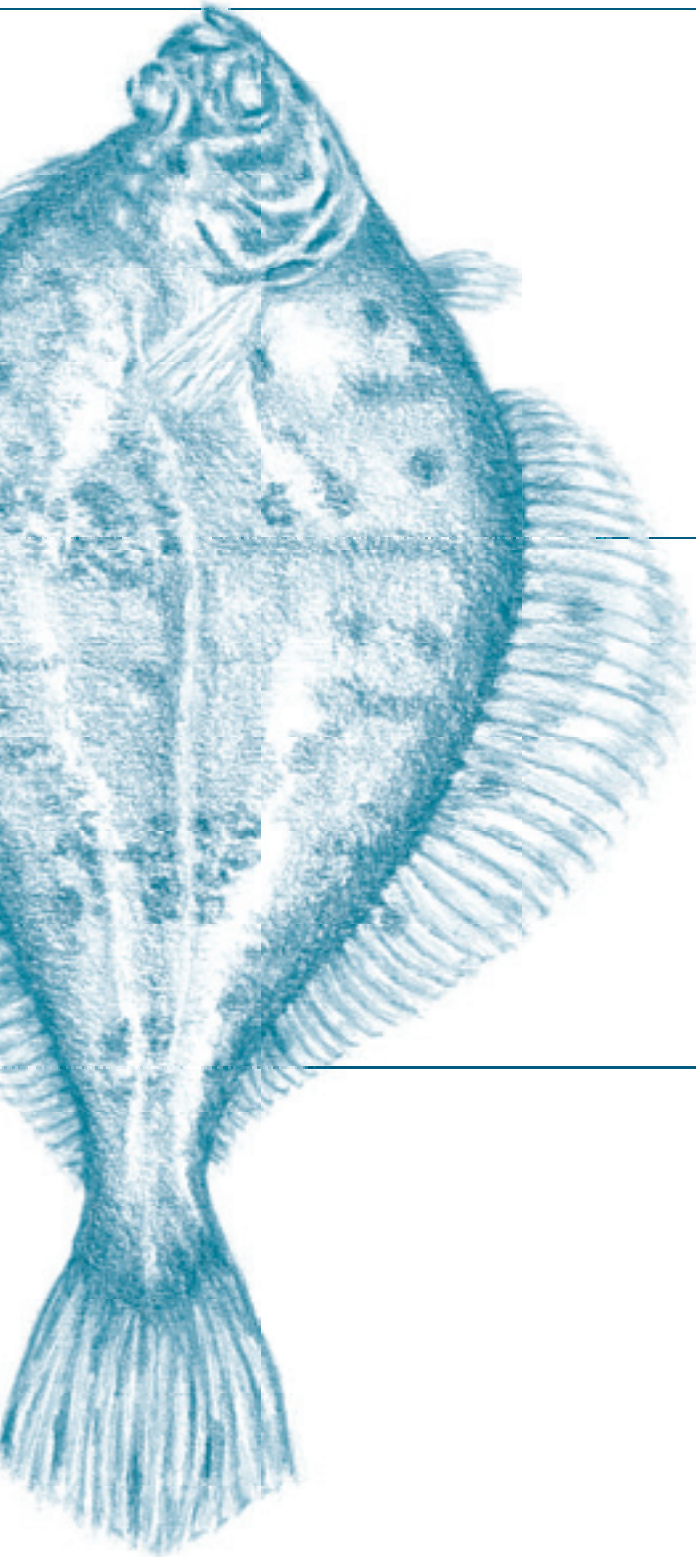
Den viktigste faktoren som regulerer fekunditeten er fiskens kondisjon som i sin tur er et resultat av mattilbudet. I situasjoner der mat er tilgjengelig under gytetidspunktet kan tradisjonelle determinante gytere bytte til en pseudo-indeterminant fekunditetstype. Avhandlingen konkluderer med at fekunditetstypen ikke er fast på artsnivå, men kan endres som et svar på miljøet gjennom mattilgjengelighet og energiallokering.

Stop niet al je eieren in dezelfde mand!

In visserijmanagement is de paaibiomassa (SSB) een belangrijke indicator voor de bepaling van de status van een beviste vispopulatie. Kennis van de reproductie biologie is essentieel voor een goede bepaling van de SSB. Er is een grote variëteit van reproductie strategieën. Er zijn twee extreme strategieën bekent in de voortplanting van mariene vissen, kapitale paaiers welke een bepaalde fecunditeit (tijdens de paaiperiode worden er geen nieuwe eicellen ontwikkeld) hebben en inkomende paaiers welke een onbepaalde fecunditeit (tijdens het paaien worden er nieuwe eicellen gerekruteerd) hebben. In dit proefschrift worden de regulatie mechanismen van de fecunditeit onderzocht in commerciële vissoorten met verschillende leefwijzen.

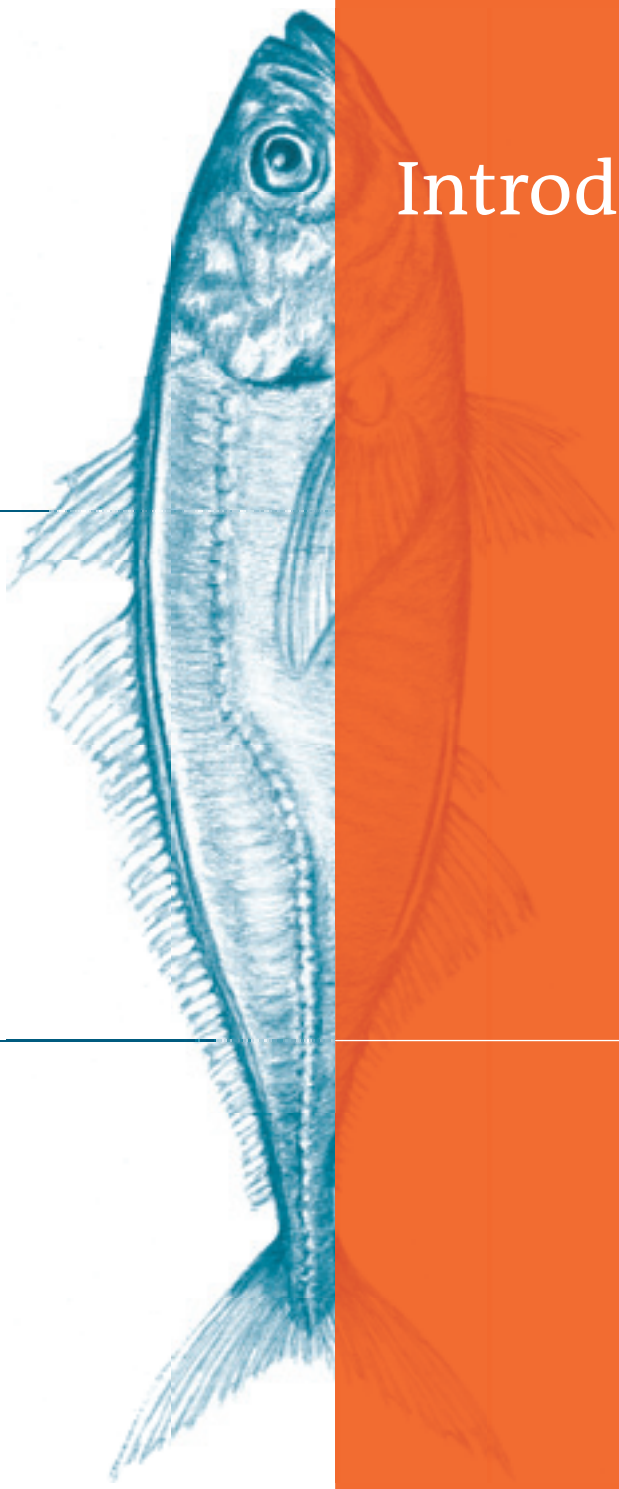
De kapitale paaiers schol *Pleuronectes platessa* en haring *Clupea harengus* paaien in de herfst en/of winter maar de ontwikkeling van de eicellen begint in april, op het moment dat de daglengte toeneemt. Beide soorten starten met de ontwikkeling van een groot aantal eicellen. Het aantal eicellen wordt tijdens de ontwikkelingsperiode naar beneden gereguleerd afhankelijk van de hoeveelheid energie beschikbaar voor de voortplanting. Na de voedingsperiode in de zomer is de hoeveelheid beschikbare energie op het hoogste niveau, en op dat moment is er in schol een tweede rekrutering van nieuwe ontwikkelende eicellen. De ontwikkeling van eicellen in haring is hetzelfde in winter- en herfstpaaiers. Maar winterpaaiers hebben een langere eicel ontwikkelingsperiode en paaien minder maar grotere eieren in vergelijking met herfstpaaiende haring. Horsmakreel ***T****rachurus trachurus* is een inkomende paaier en neemt voedsel op tijdens de paaiperiode, hoewel de eerste groep eicellen ontwikkeld wordt uit opgeslagen energie reserves.

Voedselbeschikbaarheid via lichaamsconditie is de belangrijkste factor in de regulatie van fecunditeit. In situaties waar voedsel beschikbaar is tijdens de paaiperiode kunnen traditionele bepaalde paaiers een pseudo-onbepaald fecunditeitstype laten zien. Concluderend laat dit proefschrift zien dat het fecunditeitstype van marine vissen niet vast ligt op soortniveau maar dat het een plastische respons is op omgevingsfactoren via voedselbeschikbaarheid en energie allocatie.



Chapter 1

Introduction



Cindy J.G. van Damme

Fisheries management and reproduction biology

Fisheries has been important as a food source since ancient times and has always had a high social and economic importance. Since long it has also been understood that fisheries needs to be managed in order to keep sustainable fish stocks and in recent times also sustainable ecosystems. For successful management, the fisheries manager needs to be informed about the status of the stock and the fisheries. In fisheries management stocks of commercial important fish species are monitored continuously.

Fisheries management

The Food and Agricultural Organization of the United Nations (FAO) has proposed a working definition: "The integrated process of information gathering, analysis, planning, consultation, decision-making, allocation of resources and formulation and implementation, with enforcement as necessary, of regulations or rules which govern fisheries activities in order to ensure the continued productivity of the resources and accomplishment of other fisheries objectives." (FAO, 1997). This has been implemented as single species management to ensure the adult stock of a commercial species remains at a sustainable level to produce enough juveniles that will recruit to the adult stock.

In the north Atlantic Ocean and adjacent waters the International Council of the Exploration of the Sea (ICES) is the prime source of information for fisheries management. Within ICES, scientists from countries around the north Atlantic collect data and prepare advice for the managing bodies.

The biomass of the adult population of exploited species – the spawning stock biomass SSB - is an important indicator of the status of the stock. The SSB can be estimated by fisheries dependent and fisheries independent methods (Egg Production Methods). Knowledge on the reproductive biology of the commercial species is of paramount importance to assess the accuracy of the estimates of SSB and the credibility of the assumptions made in the estimation.

Fisheries dependent estimate of SSB

Fisheries scientist routinely estimate the population biomass and the exploitation rates from fishery dependent data such as landing statistics in combination with the age composition of the international landings based on biological samples. Biological samples and fisheries exploitation data is combined in the Stock Assessment (see text next page).

Stock assessment

Stock assessment provides fisheries management with the data needed for the management of fish stocks. In a stock assessment data on the numbers of each age group that have been caught each year are used to estimate the population numbers and fishing mortality rate at age for each year. Combined with information on the weight at age, and the age at maturation, it allows to estimate the population biomass and the spawning stock biomass. The input data are collected from biological samples taken from the fish landed by the fisheries and augmented by data collected during standard surveys. Landing statistics and fishing effort data is also included.

Stock assessment provides biological reference points for fisheries management, such as Maximum Sustainable Yield (MSY, maximum biomass that can be extracted but still leaves a sustainable stock that is able to reproduce) and Fmsy (fishing mortality at MSY).

Maturation is an important process for which knowledge is required to calculate the SSB. Maturation is assessed from the development stage of the gonads, ovary or testis, of the fish. The development stage of gonads is often assessed macroscopically based on a number of morphological criteria, since the development stage of the individual oocytes and sperm cells (10s-100s µm) is not visible by the eye. Hence, there is a need to validate the macroscopic assessed development stage by histological studies. More over macroscopic gonad development stages are assessed throughout the year, thus in various stages of oocyte development. It is important to know the reproductive strategy and the way oocytes develop through the year in order to assess if the sampled female is a juvenile, a first time spawner or an adult spawning for the 2nd time or more, and if she will be able to spawn her eggs in the spawning period (some maturing females will abort maturation and resorb the oocytes).

Fecundity (total number of oocytes produced by a female) is another parameter needed to estimate the total egg production of the spawning stock. Ideally fecundity should be measured just prior to spawning, but this is not always possible. Fecundity may change depending on the development of the oocytes and it is important to know at which stage of the development of the oocytes the fecundity is sampled in order to gain a reliable estimate of the egg production.

Understanding the oocyte maturation cycle and mechanism of regulation of fecundity is vital for the understanding of the stock reproductive potential for an accurate assessment of SSB.

Egg production methods

Fecundity can also be used to obtain a fisheries independent estimate of the spawning population from egg and larval surveys in Egg Production Methods (EPM). Eggs and larvae of many commercial stocks occur in the water column and can be quantitatively sampled using plankton nets. This method is routinely carried out to improve the actual assessment, e.g. directly in the assessment of targeted stocks (e.g. anchovy *Engraulis encrasicolus* (Ibaibarriaga *et al.*, 2008) and mackerel *Scomber scombrus* (Simmonds *et al.*, 2010)), to tune the stock assessment (e.g. horse mackerel *Trachurus trachurus* (ICES, 2012a)), provide useful information for the stock assessment process (e.g. cod *Gadus morhua* (Armstrong *et al.*, 2001) and plaice (Armstrong *et al.*, 2001, Damme *et al.*, 2009a)) or to provide a fisheries independent check on the estimates based on fisheries data (e.g. plaice *Pleuronectes platessa* (Damme *et al.* 2009a)).

Egg production methods (EPM) are an important tool in fisheries management and can be used in recovery situations where fishing effort is reduced to rebuild the SSB and thus no market samples are available (Armstrong and Witthames, 2012, Bernal *et al.*, 2012, Kraus *et al.*, 2012). In addition, the estimate of SSB obtained from the egg production methods is not influenced by movements of fish out of the management area after spawning. EPM can be used in different ways:

1. as an index directly in the assessment,
2. to provide information on trends of a stock without being directly used in the assessment or
3. to provide information on spatial and temporal shifts in the distribution of the stock (Bernal *et al.*, 2012).

The underlying principal of EPM is simple: if you know how many eggs are spawned in a spawning season and if you know how many eggs are produced by a single female in that spawning season you can calculate the size of the spawning stock (Bernal *et al.*, 2012). Although the principle is a simple one, there are some drawbacks: you need to get a good estimate of the total egg production, covering the whole spawning area of the target stock with high sampling intensity, e.g. for the horse mackerel western stock the spawning area runs from south of Portugal up to west of Scotland (ICES, 2011), and you need to have a good estimate of average fecundity of an individual female over the whole spawning area. This requires a large effort in both survey and laboratory time.

Despite the high cost, EPM are used widely because they provide not only a fisheries-independent (egg sampling is only influenced by natural mortality, unlike biological sampling of the fish which also is influenced by fishing mortality) estimate of the spawning stock biomass (SSB) of the targeted commercial species but also information on the spawning biology and habitat of non-commercial species spawning (Fives *et al.*, 2001, Ibaibarriaga *et al.*, 2007, Valavanis, 2008).

There are different EPM of which the Annual Egg Production method (AEPM) and the Daily Egg Production Method (DEPM) are the most commonly used. The AEPM has been used successfully to estimate spawning stock biomass in determinant spawning fish (Parker, 1980, Lasker, 1985, Armstrong *et al.*, 1988, Hunter and Lo, 1993, Armstrong *et al.*, 2001) and is based on dividing the population annual fecundity (total number of eggs produced by the stock in one spawning period) by the average individual fecundity. DEPM has been used to assess SSB of indeterminate pelagic fish (Lasker, 1985) and is based on dividing the daily population fecundity (total numbers of eggs produced by the stock in one day) by the individual batch fecundity (number of oocytes in a single batch) and spawning fraction (the fraction of the stock that spawned on the day the daily egg production was estimated). For the AEPM and DEPM to be used as a reliable tool in fisheries management it is necessary to have a good insight in the reproductive strategy of the targeted species. Understanding the mechanism of regulation of fecundity enhances the understanding of the stock reproductive potential.

Reproductive strategies and fecundity regulation

Reproduction is an important part of the life cycle of organisms. It is also the most costly event in the life cycle (Rijnsdorp, 1990, Smith *et al.*, 1990) and the trade-off between survival and reproduction is an important topic in the life-history theory (Roff, 2000, Stearns, 2000). Capital and income breeding are the extremes of the continuum of reproductive strategies (Bonnet *et al.*, 1998, Drent and Daan, 1980). Capital breeders acquire the energy needed for reproduction prior to the spawning period. As such, they can cope with unpredictable environmental conditions through long-term storage of energy (Calow, 1979) or can spawn during periods when food availability for the adults is low. Income breeders acquire the energy needed for reproduction during the spawning period itself (Jönsson, 1997). It has been suggested that large organisms can cope with large energy storage and are capital breeders while small organisms cannot carry

large energy reserves and are thus income breeders (Klaassen, 2002).

Marine fish show a wide range of reproductive strategies (Murua and Saborido-Rey, 2003), with different reproductive traits. Depending on the environment and geographical location, reproductive traits may differ between fish species and among populations within a species (Stearns, 2000). It remains unclear which underlying mechanisms regulate these variations. Are these variations in traits caused by genetic differences due to local adaptations or are they a result of phenotypic plasticity in response to local differences in environment?

According to life history theory, reproductive strategies evolved to maximizing the production of viable offspring. Hence, fish trade-off their survival probability with their future reproduction success (Stearns, 2000, Roff, 2000). Basically this can be divided in two different trade-offs; 1) reproductive investment against adult growth and mortality (Roff, 2000, Stearns, 2000) and 2) eggs size against egg number (Sargent *et al.*, 1987, Johnson *et al.*, 2010). These trade-offs are related to food availability and mortality risk during different life stages (adults, eggs and larvae).

Within the range of reproductive strategies different fecundity types are found: determinate and indeterminate fecundity type. Female fish with a determinate fecundity type recruit all the oocytes of one maturation cycle to the vitellogenic stock before the onset of spawning (Murua and Saborido-Rey, 2003, Kjesbu, 2009). Indeterminate females keep recruiting new oocytes to the vitellogenic stock after spawning has commenced (Kjesbu, 2009, Murua and Saborido-Rey, 2003). However, irrespective of reproductive strategy or fecundity type, oocytes undergo the same development pattern in marine teleosts (Tyler and Sumpter, 1996). Photoperiod is an important cue for production of chemicals and hormones which trigger reproduction and oocyte maturation in fish (Migaud *et al.*, 2010). Increase or decrease in daylight length and light intensity occurs when the sun crosses the equator, tropic of Capricorn or tropic of Cancer at the spring and autumn equinox. This is suggested as the trigger for the start of oocyte maturation (Kjesbu *et al.*, 2010). The duration of the oocyte maturation is dependent on the final oocyte or egg size, the development rate of the oocytes and is influenced by temperature that affects the development rate of oocytes and metabolic rate of the female. The vitellogenic development stage is the main phase of oocyte growth, which can account for 95% of the final egg size. In capital breeders the vitellogenic growth phase may take up to 9 months, whereas in income breeders this growth phase maybe weeks or even days (Tyler and Sumpter, 1996).

Reproduction requires different chemical compounds, lipids and protein. Lipids storage are believed to be mostly used for maintenance while storage proteins are used for ovary development (Bradford, 1993). However, for the growth of oocytes females need access to both lipids and protein since the yolk of eggs consists of both chemical components (Kamler, 2008, Heming and Buddington, 1988). Hence, fecundity is dependent on the surplus energy, e.g. the total energy in lipids and proteins that is available for reproduction and somatic growth during a reproductive cycle (Rijnsdorp, 1990). In poor environmental conditions and low surplus energy levels iteroparous fish may skip spawning one event in order to increase fitness and survival and probability of spawning in future events (Rijnsdorp, 1990, Rideout *et al.*, 2005).

Most of the oocyte maturation process in determinate spawners takes place before the spawning period and final maturation occurs just before spawning a batch of oocytes. For this the females need to have a large ovary to store a large number of vitellogenic oocytes at the same time. These females also need a large energy store to sustain the oocyte growth during the spawning period. Due to the high energy storage determinate spawners show a seasonally variable and high seasonal amplitude in body condition while this is less in indeterminate spawners (Rijnsdorp and Witthames, 2005). The female must have either enough energy stored or a high nutritional-rich food supply during the spawning period (Hunter and Leong, 1981).

Thesis outline

Knowledge on the reproductive biology, oocyte maturation cycle, fecundity development and reproductive strategy, are important for an accurate assessment of fish stocks. Reproductive data are used directly in the assessments but also assumptions of stock reproduction (such as constant fecundity) are used. Insight in the processes of fecundity regulation are thus of vital importance for assessing SSB.

In this PhD-thesis female reproductive strategies of a variety of fish species with contrasting life histories are investigated focussing on the underlying mechanisms that determine reproductive strategy and fecundity. Fecundity type, egg size and fecundity are related to information on food availability and body condition to explain the differences in reproductive strategies between species and between populations within a species. It also shows and discusses the possibilities for the use of reproductive strategy and fecundity regulation in EPM.

The main questions addressed in this PhD-thesis are:

- What are the underlying mechanisms regulating fecundity and reproductive strategy?
- What are the implications of the different fecundity types and reproductive strategies on the inter-annual variation in population egg production?
- What are the implications of the different fecundity types and reproductive strategies for the use in fisheries management?

These questions are addressed in a numbter of separate chapters. The second chapter focusses on the differences in oocyte maturation and fecundity regulation between recruit, or first-time, and repeat, which have spawned at least once before in their lifetime, spawning females in capital breeding plaice. Recruit spawners are thought to be characterized by lower fecundity (Kjesbu *et al.*, 1998, Wright and Trippel, 2009, Skjaeraasen *et al.*, 2010), smaller egg size (Kennedy *et al.*, 2007a) compared to repeat spawners. The full oocyte maturation cycle from the onset of oocyte maturation, cortical alveoli stage, to the final maturation stages just prior to spawning, and the mechanisms of fecundity regulation are analysed. The hypothesis that fecundity is higher in repeat spawners compared to recruit spawners and that fecundity is already different at the onset of oocyte maturation is tested.

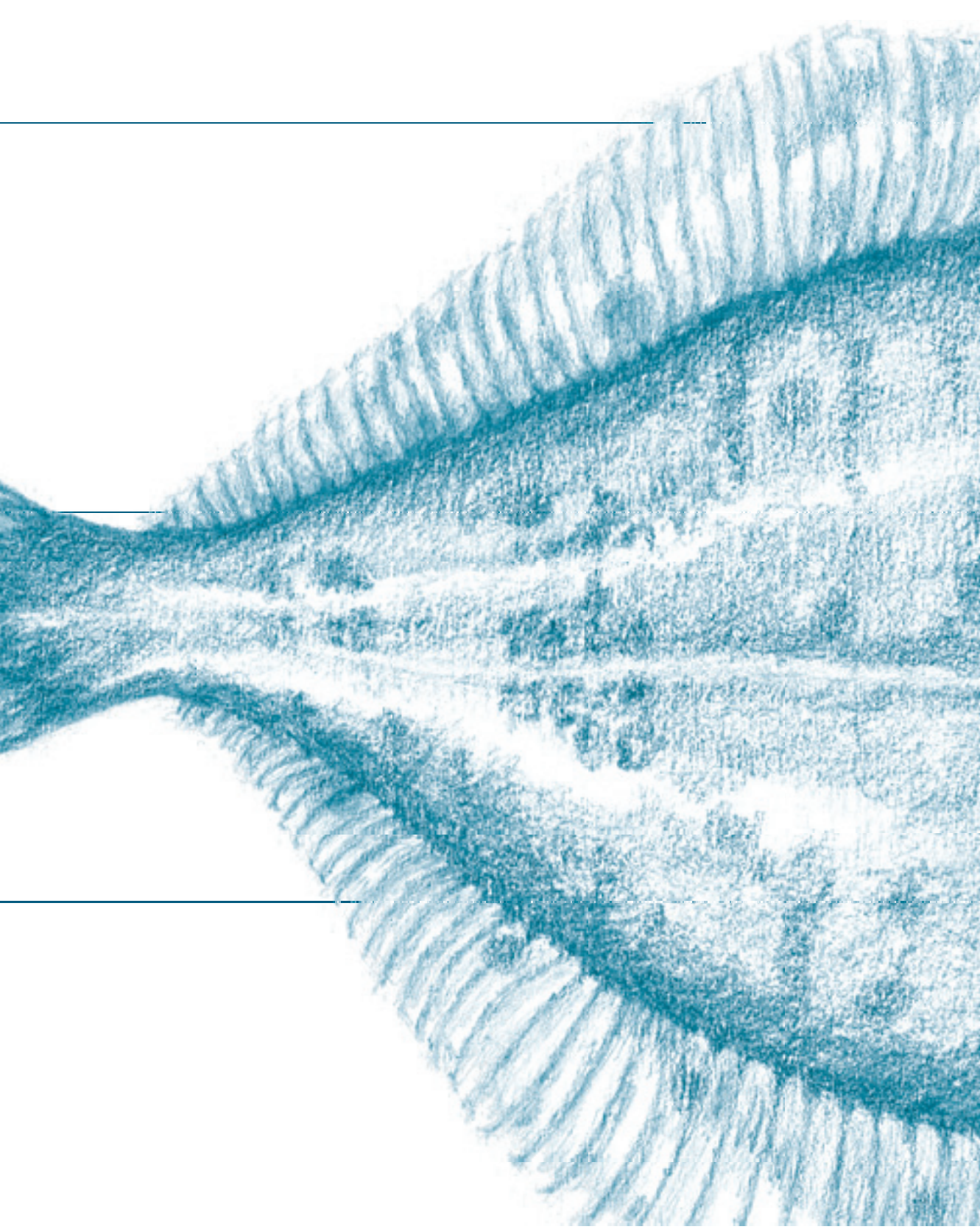
The objective of chapter three is to investigate the underlying mechanisms of the plasticity in reproduction of Atlantic herring *Clupea harengus*. Atlantic herring are capital breeders but populations have contrasting spawning strategies with regard to spawning seasons, egg sizes and spawning areas (Iles, 1964). In the North Sea all spawning types start maturation in April-May (Iles, 1964). Throughout the maturation cycle oocyte size gradually increases and development of oocytes in the different spawning types continues. Iles (1964) suggested that autumn spawners keep developing the oocytes until they are ready to be spawned in September/October, whereas winter and spring spawners stop the development in September and have a resting period through autumn (and winter for spring spawners) followed by further oocyte development just prior to their spawning season. The longer maturation period in winter and spring spawners results in larger oocytes but lower fecundities compared to autumn spawners (Baxter, 1959, Baxter, 1963, Zijlstra, 1973). The mechanisms determining fecundity as a basis to understand causes of interannual and stock variability in fecundity and

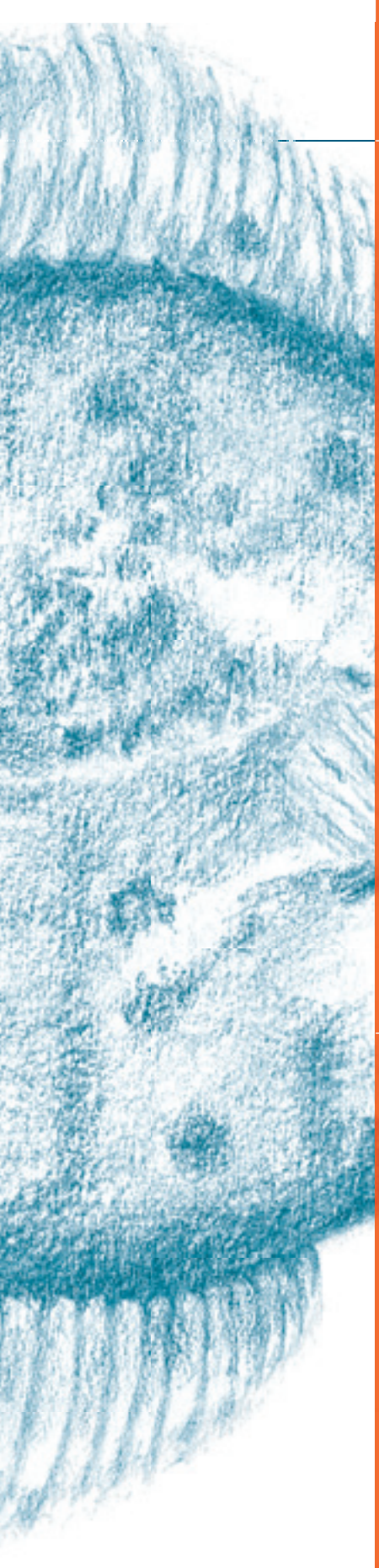
associated oocyte growth are investigated and the hypothesis of a resting period in winter spawners tested.

In the fourth chapter the objective is to investigate the underlying mechanisms regulating oocyte maturation and fecundity in horse mackerel. In the north eastern Atlantic horse mackerel has an eight month long spawning period (Abaunza *et al.*, 2003, Dransfeld *et al.*, 2005). Spawning starts in late winter along the Portuguese coast and moves progressively north ward until it finishes in the summer west of Scotland (ICES, 2011). Currently for horse mackerel the annual egg production is used to aid the management of the stock, although horse mackerel is considered to be an income breeder and thus showing an indeterminate fecundity type (Karlou-Riga and Economidis, 1997, Gordo *et al.*, 2008, Ndjaula *et al.*, 2009). The assumptions that horse mackerel has an indeterminate spawning strategy and lipid content or body condition are reliably indices of fecundity in indeterminate spawners are tested. Also the reliability of the use of horse mackerel fecundity in EPM is discussed.

In chapter five the use of fecundity in the AEPM is tested. A series of egg surveys and fecundity estimates from 1948, 1950, 1987, 1988 and 2004 were analysed and compared to the traditional SSB estimates to investigate relative trends in the North Sea plaice stock and variability in the spatial pattern of spawning.

The objective of the sixth chapter is to review the female reproductive strategy of a variety of fish species and relate the fecundity type, egg size and fecundity with information on the food, survival and environment of the adult and larvae. A conceptual model of fecundity regulation is presented to test the hypothesis that food availability through body condition is the most important factor regulating fecundity and reproductive strategy.





Chapter 2

Fecundity regulation and oocyte maturation in recruit and repeat spawners in a capital spawning teleost

Cindy J.G. van Damme
Olav S. Kjesbu
Adriaan D. Rijnsdorp

Abstract

The maturation cycle and the fecundity regulation mechanism is studied in a capital spawning fish using North Sea plaice *Pleuronectes platessa* as an example. Plaice is an ideal study object as it stops feeding during the spawning period and utilises its body reserves laid down during the feeding period for reproduction. Oocyte maturation already starts in March, i.e. 9-10 months prior to spawning with recruitment of previtellogenic oocytes into the vitellogenic stage. First time (recruit) spawners had larger previtellogenic oocytes when being introduced to the vitellogenic stock. Also, we found that recruit and repeat spawners started off with a higher number of vitellogenic oocytes and subsequently down-regulated the number of developing oocytes. But after the summer feeding period, when surplus energy

Sammendrag

I dette arbeidet har vi studert modningssyklusen og mekanismen for fekunditetsregulering hos rødspette *Pleuronectes platessa* fra Nordsjøen. Rødspette er et ideelt studieobjekt og et eksempel på kapitalgytende fisk som stopper å spise i gyteperioden og bruker sine oppsparte energireserver til reproduksjon. Rekruttering av previtellogene oocytter til det vitellogene stadiet starter allerede i mars, dvs. 9-10 måneder før gyting. Førstegangsgytere hadde større previtellogene oocytter når de ble rekruttert til det vitellogene stadiet. Vi fant også ut at både førstegangs og flergangsgyterne begynte med et høyt antall vitellogene oocytter som siden ble nedregulert i antall før videreutvikling. Etter fòringsperioden på sommeren, da energioverskuddet var på det høyeste, økte antall vitellogene oocytter. Dette tyder

Samenvatting

De eicel ontwikkelingscyclus en het fecunditeit regulatie mechanisme is bestudeerd in kapitaal paaiende vis, met behulp van Noordzee schol *Pleuronectes platessa* als voorbeeld. Schol is een ideaal studieobject omdat het stopt met het opnemen van eten tijdens de paaiperiode en gebruik maakt van haar lichaam reserves, welke zijn opgenomen tijdens de voedingsperiode voor het paaien. Eicel ontwikkeling begint al in maart, dat wil zeggen 9-10 maanden vóór het paaien, met de rekrutering van previtellogene eicellen naar de vitellogenic fase. De previtellogene eicellen die naar de vitellogene fase rekruteren van de vrouwtjes die voor de eerste keer paaien (rekruut paaiers) waren groter dan van vrouwtjes die al eerder gepaaid hadden (herhaal paaiers). Ook, vonden we dat herhaal paaiers begonnen met een hoger aantal vitellogene eicellen. Dit hoge aantal eicellen werd vervolgens naar beneden gereguleerd tijdens

is at the highest level, the number of vitellogenetic oocytes increased suggesting a 2nd recruitment phase of previtellogenic oocytes to the vitellogenic stock. Repeat spawners showed faster oocyte growth rate and less fecundity down-regulation. In consequence this category of spawners spawn eggs early in the season, i.e. December, while recruit spawners release eggs about one month later. In essence this article documents between-seasonal trends in reproductive tactics, which appear important in understanding the dynamics of variation in the stock reproductive potential in iteroparous fish species, and should therefore be relevant in discussions on recovery plans for depleted fish stocks.

på en ny rekrutteringsfase hvor previtellogene oocytter blir rekrutert til den vitellogene poolen. Flergangsgytere viste raskere oocyttvekst og mindre nedregulering av fekunditeten. Konsekvensen av dette er at flergangsgytere gyter eggene tidlig i sesongen, dvs. desember, mens førstegangsgytere gyter eggene omtrent en måned senere. Denne artikkelen dokumenterer i hovedsak sesongbaserte trender i reproduktiv strategi, noe som er viktig for å forstå dynamikken i hvordan reproduksjonspotensialet varier hos fiskearter som i løpet av sitt liv kan gyte flere ganger. Dette er relevant kunnskap når man diskuterer beskatningsplaner for utarmete fiskebestander

de ontwikkeling van eicellen. Maar na de zomer voedingsperiode, wanneer de overtollige energie op het hoogste niveau is, steeg het aantal vitellogene eicellen. Dit suggereert een 2e fase van rekrutering van previtellogene eicellen naar de vitellogene voorraad. Herhaal paaiers lieten een snellere eicel groei zien en minder fecunditeit bijstelling. Gevolg is dat deze categorie van paaiers eieren vroeger in het seizoen paaien, dat wil zeggen in december, terwijl de rekruut paaiers de eieren ongeveer een maand later paaien. In dit artikel worden seizoensgebonden trends in voortplantingsstrategie gepresenteerd, die belangrijk zijn in het inzicht in de dynamiek van variatie in het populatie reproductieve potentieel in iteroparous vissoorten, en derhalve relevant zijn in discussies over herstelplannen voor uitgeputte visbestanden.

Introduction

Reproduction requires high energy investment of the fish, which needs to be carefully balanced with somatic growth (Rijnsdorp 1990, Smith 1990). Capital spawners gain the surplus energy for reproduction well before spawning and use stored energy during the subsequent release of gametes (Stearns 1992, Stephens *et al.* 2009). Most capital spawners like herring *Clupea harengus* (Damme *et al.* 2009b, Kurita *et al.* 2003) and cod *Gadus morhua* (Kjesbu *et al.* 2010) have a long oocyte maturation period. The questions arise when and what triggers the onset of oocyte maturation. Herring starts oocyte maturation at the spring equinox (Damme *et al.* 2009b) to spawn in autumn or winter, while cod starts maturation at the autumn equinox and spawns in spring (Kjesbu *et al.* 2010). Plaice *Pleuronectes platessa* spawn in winter (Damme *et al.* 2009a, Rijnsdorp 1991, Simpson 1959) and have a long oocyte maturation period with the start of oocyte maturation already in spring (Barr 1963, Deniel 1981).

Capital spawners generally have a determinate fecundity (the standing stock of advanced vitellogenic oocytes) type (Calow 1979, Damme *et al.* in prep). In determinate spawners, vitellogenic oocytes are clearly separated from precursor previtellogenic oocytes well before the onset of spawning (Tyler and Sumpter 1996). At the onset of the oocyte maturation fecundity is high. During the maturation cycle fecundity is down-regulated through follicular atresia in dependence of the energy reserves of the female (Damme *et al.* 2009b, Kennedy *et al.* 2007b, Kurita *et al.* 2003).

First-time or recruit spawners are thought to be characterized by lower fecundity (Kjesbu *et al.* 1998, Skjaeraasen *et al.* 2010, Wright and Trippel 2009) and smaller egg size and lower egg dry weight (Kennedy *et al.* 2007a) compared to repeat spawners, which have spawned at least once before in their lifetime. For spawning stock assessments and management of stocks it is thus important to distinguish between recruit and repeat spawners. It is impossible to distinguish recruit from repeat spawners by macroscopic examination of the ovaries, although the probability can be estimated from their length and age (Kjesbu and Holm 1994, Rijnsdorp *et al.* 2010). Other means to separate these two categories might be the ovarian wall thickness, which is greater in repeat spawners (Lowerre-Barbieri *et al.* 2011) or spawning zones in otoliths (Rollefsen 1934). Both of these methods suffer from variability between individuals and years.

In this paper we analysed differences in oocyte maturation and fecundity regulation between recruit and repeat spawning females. Plaice is a capital spawner (Rijnsdorp 1991) with a determinate fecundity type (Armstrong *et al.* 2001, Kennedy *et al.* 2007b,

Urban 1991). During spawning, pelagic eggs are released in batches at intervals of 2 to 5 days (Rijnsdorp 1989, Urban 1991). The duration of the spawning period of an individual female is 4 to 6 weeks (Rijnsdorp 1989). Spawning plaice are widely distributed throughout the English Channel and the southern and central North Sea (Buchanan-Wollaston 1923, Harding and Nichols 1987, Houghton and Harding 1976) although rarely beyond the 50m depth contour (Harding *et al.* 1978). Timing of spawning in plaice is dependent on the latitude. Spawning is progressively later moving northwards through the North Sea. Peak spawning occurs in December in the eastern English Channel, mid-January in the Southern Bight, and in February-March in the more northern regions (Bagenal 1966, Damme *et al.* 2009a, Harding *et al.* 1978). Fecundity varies between the spawning areas, with fecundity decreasing from the eastern English Channel to the German Bight (Damme *et al.* 2009a, Horwood *et al.* 1986, Rijnsdorp 1991). However interannual variation in fecundity can be considerable (Horwood *et al.* 1986, Rijnsdorp 1991).

Predictable patterns are found in the seasonal and spatial distribution of plaice (Rijnsdorp *et al.* 2006) which relate to the migration patterns between spawning and feeding areas and the recruitment (Bolle *et al.* 2005, Hunter *et al.* 2003a). The nursery grounds are inshore on sandy flats (Beek *et al.* 1989, Zijlstra 1972) and plaice gradually move offshore as they grow larger (Wimpeny 1953). In summer the mature plaice are found in the southern and central North Sea for their summer feeding. In late autumn mature plaice migrate to their respective spawning grounds in the English Channel and southern North Sea.

Most fecundity studies so far have focused on the period just prior to spawning (Bagenal 1966, Horwood *et al.* 1986, Rijnsdorp 1991) or sampled once during the oocyte maturation and at spawning (Kennedy *et al.* 2007b). This study will quantitatively analyse the full oocyte maturation cycle from the onset of oocyte maturation, cortical alveoli stage, till the final maturation stages at spawning, and analyse the mechanisms of fecundity regulation. We test the hypothesis that fecundity is higher in repeat spawners compared to recruit spawners and that fecundity is already different at the onset of oocyte maturation. Also we tested that fecundity is regulated to the available energy reserves during the oocyte maturation cycle. We show the importance of atresia and fecundity regulation for the use in SSB estimates based on egg production methods. Understanding the maturation cycle and mechanism of regulation of fecundity enhances the understanding of the stock reproductive potential, and is therefore

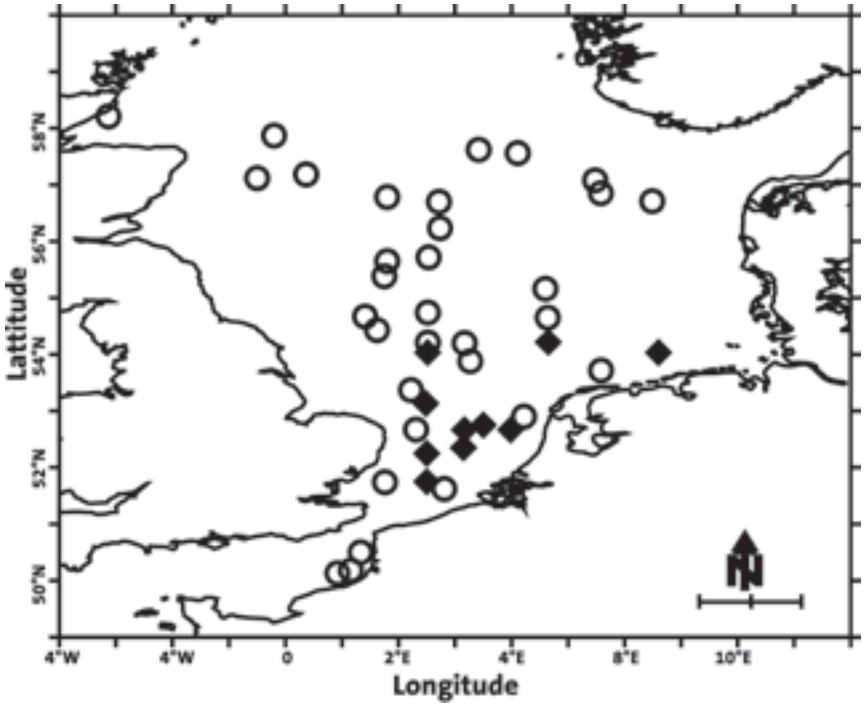
important for the management and recovery of the intensively fished stock.

Methods

Collection of samples

Female plaice were sampled monthly across the North Sea from February 2008 till February 2009 using commercial and research vessels (FIGURE 2.1). This mix of vessel types allowed for the full temporal range of oocyte development to be sampled. Because adult plaice of different spawning grounds mix on the summer feeding grounds (Bolle *et al.* 2005, De Veen 1978, Hunter *et al.* 2003b) no distinction was made between the different spawning populations. Only samples of fresh fish were used, and these were kept on ice prior to examination.

FIGURE 2.1



Sampling locations (♦ commercial and O research vessels).

Probability of recruit spawning

Length at first maturation of female plaice is between 20 and 35 cm (Rijnsdorp 1989, Walraven *et al.* 2010). Samples were taken from three length classes (T 2.1): 1 20 – 26 cm; 2 27 – 34 cm; 3 >35 cm, to ensure recruit and repeat spawning fish were sampled.

TABLE 2.1

Numbers of female plaice by length class sampled per month. (Length classes: 1 recruit; 2 recruit or repeat and 3 repeat spawners.)

length class	2008											2009		total
	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	
1	1	3	6	10	8	8	7	11	14	10	0	3	1	82
2	3	8	20	20	10	10	8	19	18	19	10	16	1	162
3	5	3	14	20	10	10	8	20	20	20	10	10	5	155
All	9	14	40	50	28	28	23	50	52	49	20	29	7	399

Spawning females in length class 1 are likely to be recruit spawners, females in length class 2 could be either recruit or repeat spawners and spawning females in length class 3 are likely to be repeat spawners. The probability of the female being a recruit spawner, *Prec*, is also estimated from the probability of being mature MTal at an age a and length l, conditional on the probability of being immature in the previous year, and taking account of the length increment Δl during the past year (Barot *et al.* 2004, Rijnsdorp *et al.* 2010):

$$P_{rec} = \frac{MT_{al} - MT_{a-1,l-\Delta l}}{1 - MT_{a-1,l-\Delta l}} \qquad [2.1]$$

Body condition

Biological parameters reported were total fish length (TL, to the nearest 0.1 cm), total weight (W, 1 g), macroscopic maturity stage, ovary (OW, 0.1 g), liver (LW, 1 g) and gutted (1 g) weight. Stomach fullness was noted as 0=empty, 1=filled, 2=full and 3=bursting. Age

(years) was determined from the otoliths taking 1 January as birthday. Lipid content of the whole body, excluding the ovary, was measured following the Bligh and Dyer method (Bligh and Dyer 1959). Hepatosomatic index (HSI) was calculated as (Ma *et al.* 1998):

$$HSI = 10000 * LW/TL^3 \quad [2.2]$$

Gonadosomatic index (GSI) was calculated as:

$$GSI = 10000 * LW/TL^3 \quad [2.3]$$

Condition of the fish was expressed Fulton's K (Heincke 1908):

$$K = W/TL^3 \quad [2.4]$$

Fecundity estimation

Different preparations and methods were used to investigate the fecundity of plaice dependent on their stage of oocyte development. Plaice at the beginning of the maturation cycle (February - May) and immature or early developing recruit spawners had their whole ovaries collected and fixed in 3.6% buffered formaldehyde. A sample of the ovaries was dehydrated in ethanol, embedded in Technovit resin and mounted on microscopic slides following standard procedures. The histological sections (4 µm) were coloured with toluidine blue in order to detail the development of the oocytes and check the correctness of the macroscopic maturity stage determination. The early development stages of the oocytes (cortical alveoli) and atretic oocytes were measured using the histological sections (n = 20). As oocytes shrink during histological processing, all diameters were corrected for shrinkage (7%) (Ma *et al.* 1998). Thereafter the size of the leading cohort (10 largest diameters) of previtellogenic oocytes was estimated in both recruit and repeat spawners.

For the plaice which had started true vitellogenesis (ovary development could be seen macroscopically) duplicate samples of 150 µg (samples containing at least 100 vitellogenic oocytes) were taken and fixed in 3.6% buffered formaldehyde. All oocytes were subsequently measured and counted in whole mount analysis using ImageJ (Rasband 1997-2008). Oocyte packing density (OPD) was calculated as:

$$OPD = N/s \quad [2.5]$$

where N is the number of vitellogenic follicles in the pipette subsample and s is the subsample weight (g). Potential fecundity (F_p) was calculated as:

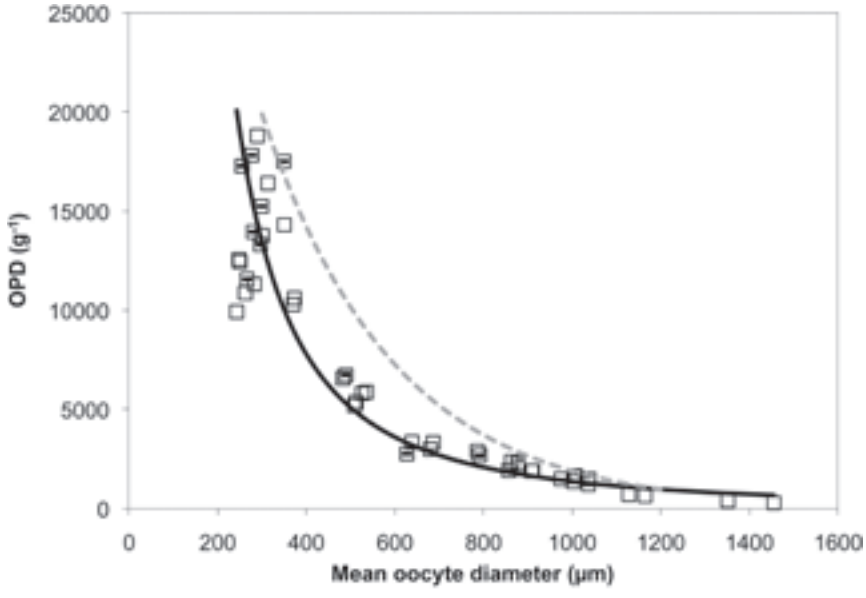
$$F_p = OPD * OW \quad [2.6]$$

Relative fecundity F_r was calculated as:

$$F_r = F_p / TL^3 \quad [2.7]$$

A total of 50 samples of plaice throughout the maturation cycle were used to establish the auto-diametric relationship between oocyte density and mean oocyte diameter (OD). This auto-diametric fecundity method (Thorsen and Kjesbu 2001) was then used for the remainder of the samples (n = 226). Of each sample 100 oocyte diameters were measured. Using the estimated relation between oocyte density and oocyte diameter (FIGURE 2.2) the mean oocyte diameter was converted to oocyte density and then potential and relative fecundity found using equations 6 and 7, respectively.

FIGURE 2.2



Mean vitellogenic oocytes diameter (OD) versus the number of vitellogenic oocytes per gramme of fresh ovary (OPD) for plaice. (Auto-diametric fecundity relationship NG= 7*108 * OD-1.9013; Auto-diametric relationship for Irish Sea plaice (Kennedy *et al.* 2007b))

Regulation of fecundity

Regulation of fecundity was reconstructed for a typical fish in each of the three length classes. Because a fish increase in size during the oocyte maturation cycle, the size of the fish was corrected for the growth increment since the start of the oocyte maturation cycle. The growth increment was estimated using the Von Bertalanffy growth curve:

$$G = T_{\infty}(1 - \varepsilon^{-K}) + TL(\varepsilon^{-K} - 1) \quad [2.8]$$

where G is growth increment, TL∞ is the asymptotic length and K the velocity to reach this asymptotic length. For a typical female plaice K = 0.25 and TL ∞ =55 (Walraven *et al.* 2010). It was assumed seasonal growth follows a cosines pattern (Fonds *et al.* 1992) and length at the start of maturation (TL_0) was calculated as:

$$TL_0 = TL - \left(\frac{cos\beta(M/_{12} * \pi)}{2} * G\right) \quad [2.9]$$

Where M is month. Fecundity standardised to the size at the start of the maturation period (F_{r0}) was calculated as:

$$F_{r0} = F_p / TL_0^3 \quad [2.10]$$

Statistical analyses were performed in SAS and R.

The dependence of fecundity on size and body condition over time was studied using a linear regression model:

$$lnF_p = \beta_0 + \beta_1 lnTL + \beta_2 M + \beta_7 P_{rec} + \beta_{13} K*M + \beta_{14} P_{rec}*M + \beta_{16} P_{rec}*K*M + \varepsilon \quad [2.11]$$

where ε is a normally distributed error term. Month was considered as a fixed factor in the model.

Condition factors such as lipid content is not a standard measurement estimated during standard surveys and market samplings, however age is a standard measurement. A model [12] including age was also developed and tested on the time series of North Sea plaice fecundity data (spawning years 1947-1948, (Simpson 1959); 1982-1985, (Rijnsdorp 1991); 2004, (Damme *et al.* 2009a); and 2009, this study).

$$lnF_p = \beta_0 + \beta_1 lnTL + \beta_2 M + \beta_3 A + \beta_4 K + \beta_5 P_{rec} + \beta_6 lnTL*M + \beta_7 lnTL*A + \beta_8 lnTL*K + \beta_9 K*M + \beta_{10} K*A + \beta_{11} K*P_{rec} + \varepsilon \quad [2.12]$$

where A is age. Model selection was based on the AIC information criterion using the stepwise backward selection approach. Residual patterns were checked for normality

and influential observations (Cook's distance).

Results

Body condition

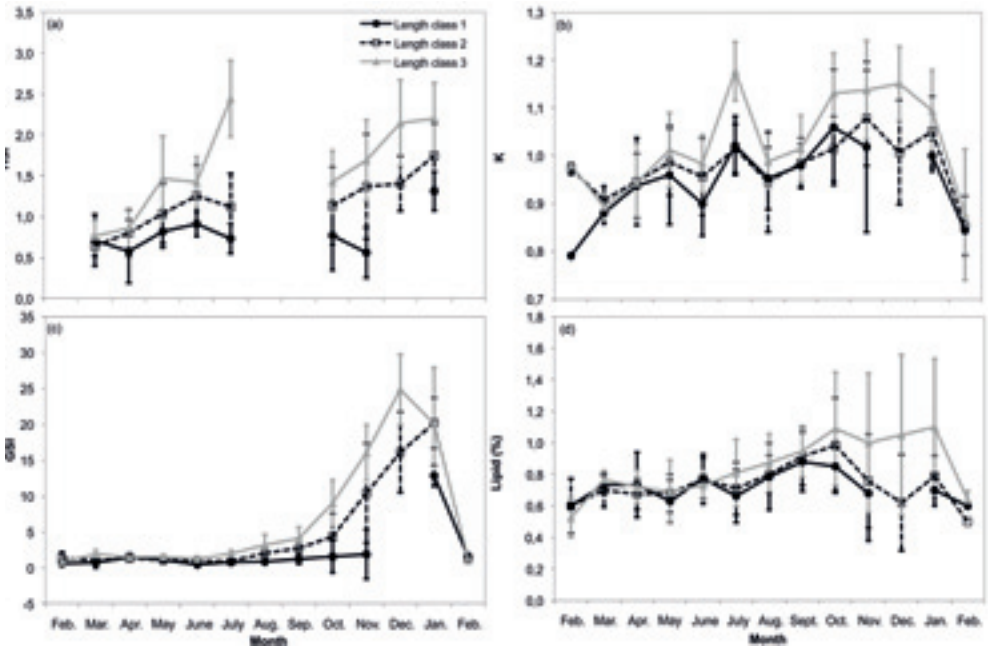
A total of 399 female plaice, of which 21% in length class 1, 41% in length class 2 and 39% in length class 3, were sampled (TABLE 2.1). Length class 1 is below the minimum landing size for plaice and it is difficult to collect samples in this length class, hence the lower numbers. As noted, samples were collected monthly year-round for all length classes, except in December for length class 1. Most samples were collected by commercial vessels, except those in August, September and February which were from demersal research surveys.

HSI, condition, GSI and lipid content changed over the year (FIGURE 2.3). HSI, condition factor K and GSI increased from February till December and decreased after that till February. HSI and K showed a peak in July in length class 3. No other studies showed a peak in body condition in July and drop in August, but Rijnsdorp (1990) showed that surplus production in plaice peaks in July. HSI increased significantly over time (FIGURE 2.3A; ANOVA, length class 1 $P=0.047$; length class 2 $P<0.001$; length class 3 $P<0.001$) and differed between the length classes (ANOVA, $P<0.001$). K significantly changed over time for all length classes (FIGURE 2.3B; ANOVA, length class 1 $P=0.018$; length class 2 $P<0.001$; length class 3 $P<0.001$) and was different between the length classes (ANOVA, $P<0.001$). GSI remained at the same, low level until July but then increased until spawning (FIGURE 2.3C). GSI increased significantly over time in all length classes (ANOVA, length class 1 $P<0.001$; length class 2 $P<0.001$; length class 3 $P<0.001$). GSI was highest in January in length class 1 and 2, but peaked in December in length class 3. There was no evidence of differences between the length classes from February till July but GSI was significantly different from August till January (ANOVA, $P<0.001$), with the lowest GSI in length class 1 and the highest in length class 3 (FIGURE 2.3C). Lipid content showed a slight but insignificant increase from February till October in length class 1 (FIGURE 2.3D; ANOVA, $P=0.112$). Lipid content increased significantly in the larger length classes (ANOVA, $P<0.001$, length class 2 and 3). After October lipid content dropped in length classes 1 and 2, but remained at the same high level in length class 3 until January but then dropped markedly. So, over the year lipid content was the same regardless of female size, except prior and during spawning when the larger females had a

significant higher lipid content (ANOVA, P<0.001).

FIGURE 2.3

Body condition of plaice; (a) HSI; (b) Fulton's K; (c) GSI; and (d) lipid content. Error bars are standard deviations.



In all length classes the highest frequency of empty stomachs appeared from October till February. In December all stomachs were empty but in January some stomachs contained a few remains. From April till September almost all stomachs contained remains of feeding. No differences were found between the length classes.

Fecundity regulation

The largest previtellogenic oocytes were found in the smallest length class (FIGURE 2.4; ANOVA, P=0.027). Hence, previtellogenic oocyte size decreases with length of the female. Oocyte maturation had started in all females in all length classes in March (FIGURE 2.5) but cortical alveoli were detected as early as February for some gonads. Oocyte size increased during maturation for all length classes (FIGURE 2.5). Oocyte size was bigger in the largest length class in all months until December. Oocyte size dropped in

the largest fish in January while length class 1 and 2 reached the biggest oocyte size in January.

FIGURE 2.4

Previtellogenic oocyte size for the different length classes; 1) recruit; 2) recruit or repeat; and 3) repeat spawning females.

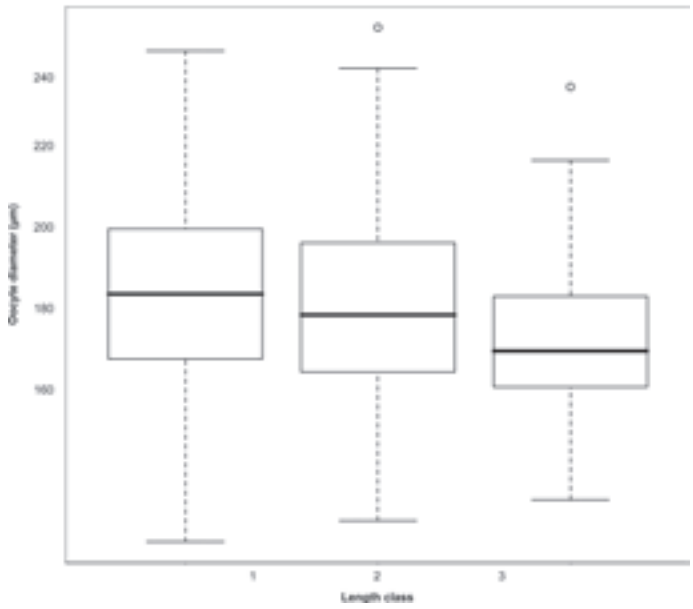
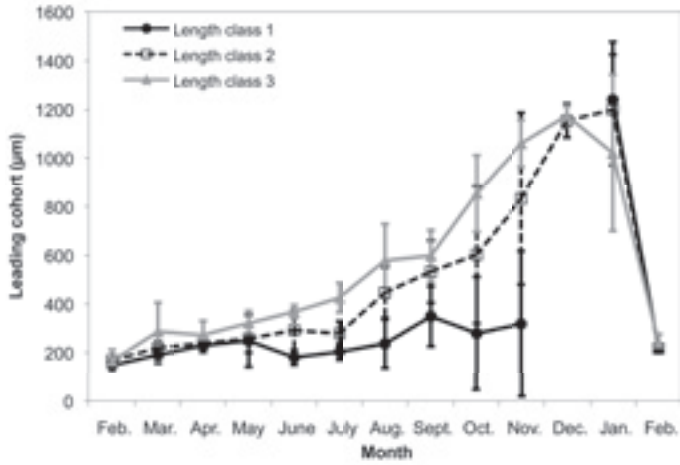


FIGURE 2.5

Oocyte development through maturation in plaice.



During maturation oocyte size increased and fecundity decreased due to down-regulation through atresia (FIGURE 2.6). Atretic oocytes were found at different maturity stages. The number of atretic oocytes in the samples was low, < 5% of all oocytes in the sample. However, from oocyte leading cohort 600 µm fecundity seems to increase again (FIGURE 2.6). Fecundity corrected for growth decreased from February till July, but from August-September fecundity increases again (FIGURE 2.7). This increase is seen in all length classes though it is less clear in length class 1. In August-September oocyte leading cohort is around 600 µm in length classes 2 and 3 (FIGURE 2.5). Cortical alveoli were also found from July till December in the three length classes.

FIGURE 2.6

Regulation of fecundity in different length classes of plaice

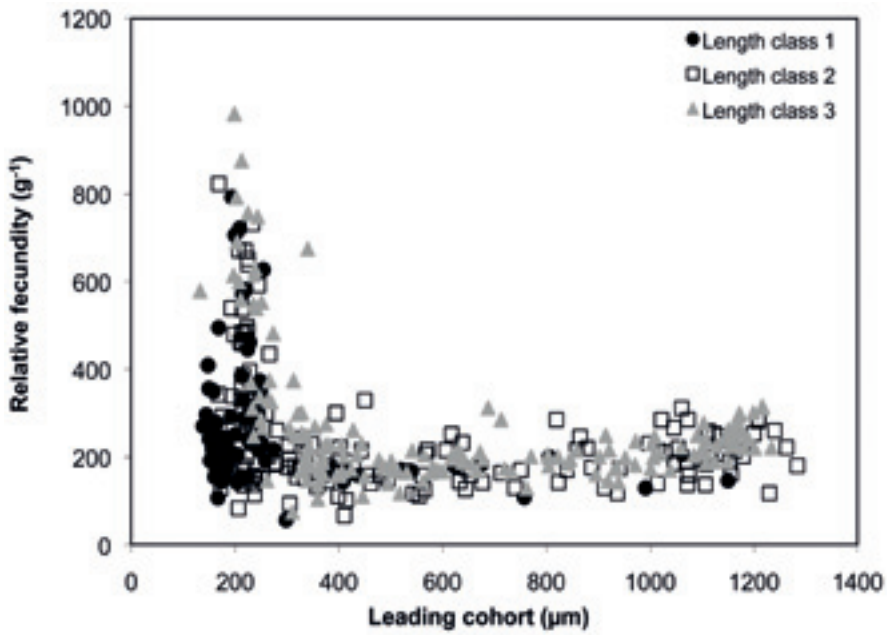
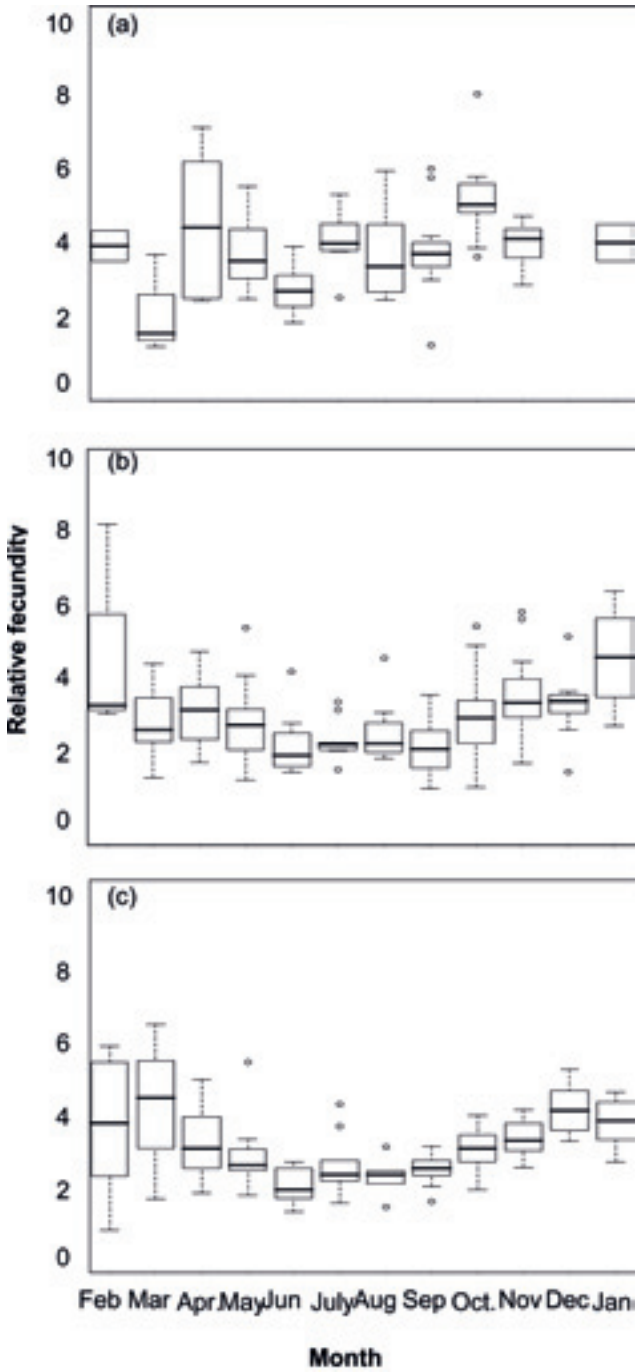


FIGURE 2.7

Relative fecundity standardised to the size of the fish at the start of the maturation period in (a) recruit, (b) recruit or repeat and (c) repeat spawners.



Fecundity

Potential fecundity, measured just prior to the spawning time, was different between the length classes (TABLE 2.2 AND FIGURE 2.8). In length class 3 potential fecundity was 81% higher compared to length class 1. Based on the average potential fecundity expected body condition was calculated using the relationships in figure 2.8. Both expected lipid content and K were higher in the bigger length class (68 and 12% above the lowest values seen in length class 1, respectively) (TABLE 2.2 AND FIGURE 2.8). However, the actually measured body condition was higher compared to the expected condition in length class 1 and 2, while the measured condition was lower compared to the expected in length class 3 (TABLE 2.2).

The selected fecundity model with the lowest AIC was:

$$lnF_p = lnTL + M + P_{rec} + K*M \qquad [2.13]$$

The significant explanatory variables explained about 78% of the variance in Fp (TABLE 2.3). Fp increased with body size and condition as well as probability of being a recruit spawner. As fecundity is down regulated during oocyte maturation each month has an increasing negative effect on Fp. Age had a negative effect on Fp but the impact is small and, except for age 8, not significant.

TABLE 2.2

Length class	Potential fecundity (N oocytes)	Lipid (%)	K	Gear
1	42100 (13900)	0.65 (0.36)	1.00 (0.91)	Gulf III, 20 cm nose cone diameter, 270 µm mesh
2	72200 (25600)	0.73 (0.60)	1.03 (0.94)	Gulf VII, 53 cm length, 28 cm nose cone diameter, 270 µm mesh
3	218100 (116400)	1.09 (1.75)	1.12 (1.31)	Gulf III, 53 cm length, 20 cm nose cone diameter, 270 µm mesh

Potential fecundity and body condition in different length classes; 1) recruit; 2) recruit or repeat; and 3) repeat spawning females. (In brackets for potential fecundity is standard deviation; for body condition expected condition parameters calculated from relationships in Fig. 8.)

FIGURE 2.8

Body condition, (a) lipid content and (b) relative condition factor, regulating potential fecundity prior to spawning.

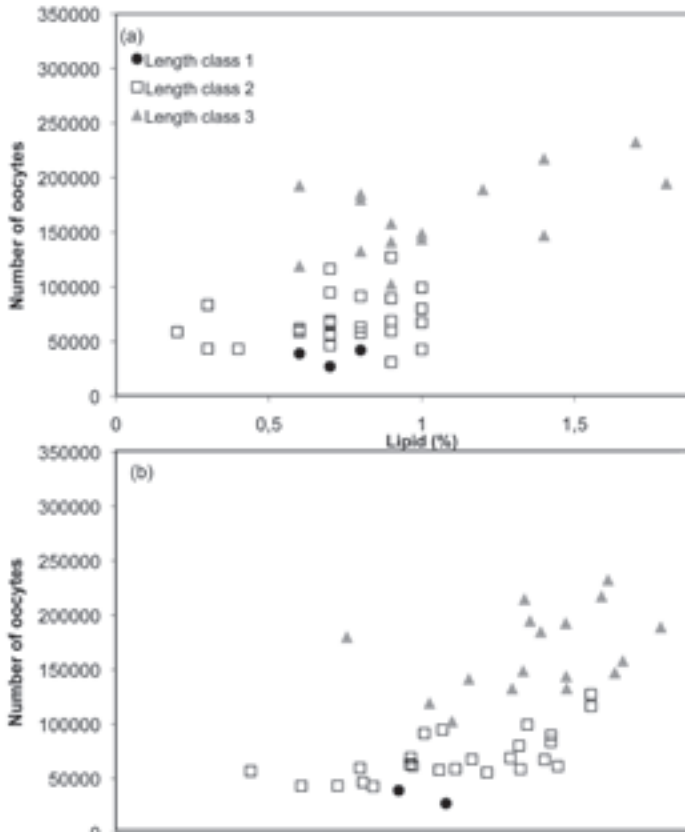


TABLE 2.3

Results of the model selection of the fecundity size relationships of equation

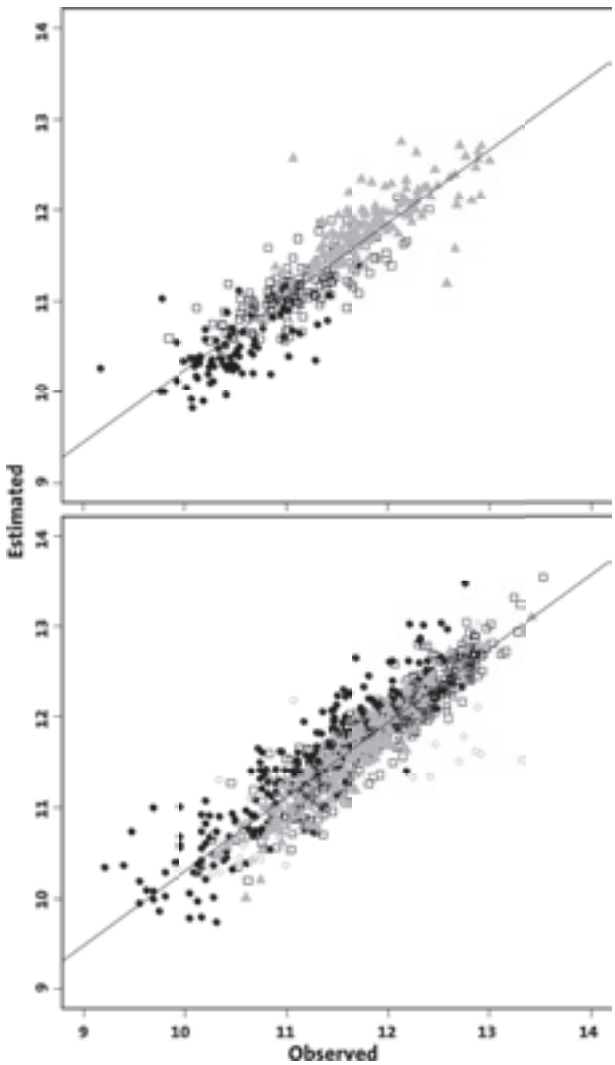
Model	DF	AIC	R^2
Equation [13]	29	248.81	0.787
Equation [13] + K	29	248.81	0.787
Equation [13] + P_{rec}*M	41	252.44	0.791
Equation [13] + P_{rec}*K*M	42	253.51	0.791

The simplified model applicable to statutory field data was:

$$lnF_p = lnTL + M + A + K + lnTL*M + lnTL*K + K*M + K*A \quad [2.14]$$

This combination of effectors explained 80% of the variance in Fp. In the absence of lipid and leading cohort data, Fp increased with body size and K. The previous negative effect of age on potential fecundity was strengthened. The predictive strength of the two models appeared comparable (FIGURE 2.9).

FIGURE 2.9



Observed and estimated fecundity for plaice using (a) all variables (● length class 1; □ length class 2; and ▲ length class 3); and (b) without lipid content and leading cohort (● spawning years 1947-1948; □ spawning years 1982-1985; ▲ spawning year 2004; and □ spawning year 2009).

Discussion

This study shows that oocyte maturation in plaice already starts in March and some of the bigger females have cortical alveoli stage as early as February. Oocyte size increases from March onwards until spawning. Repeat spawning larger females show a faster oocyte growth and reach maximum oocyte size already in December and spawn earlier compared to smaller recruit spawners, which spawn in January. Previtellogenic oocyte size is largest in the smaller recruit spawners, suggesting that the mean size of the recruiting previtellogenic oocytes decrease with size, e.g. with the number of times that the female has been spawning. Since the leading cohort reach the same maximum size, this suggest that large fish invest more resources per oocyte that smaller females (this will however be a relatively small difference). Body condition indices (K, lipid, HIS) increased throughout the summer feeding period until spawning in winter with the largest size class reaching the highest body condition indices.

Regardless of size, all females start with a high number of vitellogenic oocytes at the start of the maturation cycle and during the maturation the number of vitellogenic oocytes is down-regulated. Down-regulation occurs at the start of the oocyte maturation cycle, while after the summer feeding period fecundity increases again.

Plaice is a capital spawner which only feeds when on the summer feeding grounds (Rijnsdorp 1991). Liver weight and lipid content increased during the summer months and dropped during the spawning period in winter (Dawson and Grimm 1980, Rijnsdorp and Ibelings 1989). Lipid content is higher in the larger repeat spawning females. Walraven *et al.* 2010 showed that larger females have a higher lipid content compared to smaller females during the spawning season. Storage lipids are mostly used for maintenance of the fish (Bradford 1993, Dawson and Grimm 1980) but lipids are also important at the start of oocyte maturation (Patino and Sullivan 2002). Rijnsdorp (1990) showed that 50% of the energy used for gonad growth in plaice originates from reserves that are built up during the somatic growth period. The onset of oocyte maturation is already in March. This is before the surplus energy storage and relative body condition have reached the maximum. Body condition increased from April and dropped to the lowest level in winter after spawning. Previous studies show an increase in body condition from April to October and a decrease during spawning in winter (Bromley 2000, Costopoulos and Fonds 1989, Rijnsdorp 1990). No other studies showed a peak in body condition in July and drop in August, but Rijnsdorp (1990) showed that surplus production in plaice peaks in July. The body condition changed from 0.8

to 1.2, this is within the range found for well-fed plaice (Costopoulos and Fonds 1989, Rijnsdorp 1990). Repeat spawners has a significant higher body condition compared to recruit spawners. Bromley (2000) showed the highest and lowest body condition showed extremer peaks at older ages, with the extreme peaks at ages 10 and 11. Most samples in this study were collected between the ages 2 and 8.

Females start at the onset of the oocyte maturation cycle with a high number of oocytes. During the maturation cycle the number of oocytes is regulated according to the surplus energy available. Fecundity increases with size and females divide the surplus energy reserves between current fecundity and somatic growth to increase future fecundity (Gadgil and Bossert 1970, Rijnsdorp 1990, Schaffer 1974), thus at the beginning of the oocyte maturation females start with a high number of oocytes to be able to spawn the highest fecundity possible in the current spawning season. The mechanism of fecundity down regulation during the oocyte maturation has been found in other plaice populations, such as the Irish Sea plaice (Kennedy *et al.* 2007b, Kennedy *et al.* 2008) as well as other capital spawners e.g. herring (Damme *et al.* 2009b, Kurita *et al.* 2003) and cod (Thorsen *et al.* 2006). However, none of these studies have shown an increase of fecundity after the summer feeding period as found in the present study. After the initial down-regulation female plaice are able to recruit previtellogenic oocytes to the vitellogenic stock when high surplus energy levels are available after the summer feeding period, showing plasticity in the fecundity regulation.

The surplus energy available for oocyte development in recruit spawners is less compared to the larger repeat spawners, as shown by a higher body condition, lipid content and liver weight in the larger females. Surplus production increases with size of the fish (Rijnsdorp 1990) thus for smaller recruit spawning investing in somatic growth increases the chance of producing a higher size-specific fecundity in later years.

The positive effects of length and body condition during the oocyte maturation period are also shown in the fecundity models. However in the models age has a negative effect on potential fecundity which seems to contradict the positive effect of size. Over time plaice started spawning at younger ages (Rijnsdorp 1993, Rijnsdorp *et al.* 2005, Walraven *et al.* 2010). The negative effect of age on fecundity is probably due to the higher probability of being a repeat spawner at younger ages. Over time fecundity at length has increased (Damme *et al.* 2009a, Rijnsdorp 1991). But maturing at younger ages and thus smaller sizes has a negative effect on fecundity.

The higher number of vitellogenic oocytes at the onset of the maturation, the lower

down-regulation and higher surplus energy reserves of the repeat spawning females results in a higher fecundity at the end of the oocyte maturation cycle. Potential fecundity is 81% higher in repeat spawners compared to recruit spawners. Lipid content and relative condition factor are, repsectively 63% and 8% higher in repeat spawners. The migration to the spawning ground (Jones *et al.* 1979) and final maturation of the oocytes just prior to spawning is an energy demanding process (Kjesbu and Kryvi 1993), and during the spawning season the body condition of the females rapidly decreases. The higher surplus energy reserves in the larger repeat spawners ensures these females have enough energy for the final maturation and spawning of a higher total number of oocytes. The higher surplus energy reserve in the repeat spawning females also results in a bigger (3%) spawned egg size (Fox *et al.* 2003, Kennedy *et al.* 2007a, Rijnsdorp 1989).

The measured lipid content and condition factor of recruit spawners are higher compared to the expected. Only the females with high body condition will be able to spawn for the first time at a smaller length and younger age. In larger repeat spawning females the measured body condition was lower compared to the expected. Larger females are able to recover from a bigger depletion of the energy reserves (Bromley 2000).

The start of oocyte maturation is similar in recruit and repeat spawners. But repeat spawners exhibit advanced oocyte growth during the maturation cycle. Oocyte size prior to final maturation and spawning is the same in recruit and repeat spawners. Through the earlier oocyte growth, repeat spawners reach maximum oocyte size sooner compared to recruit spawners. Smaller recruit spawners invest a relative larger proportion of the annual surplus energy in somatic growth (Rijnsdorp 1990). Hence, interannual variations in feeding conditions will have a larger effect on the increase in body size during the oocyte maturation period in small fish as compared to large fish. Smaller fish will therefore keep a relatively large stock of small vitellogenic oocytes till later in the feeding season to reduce the cost of atresia of the access number of oocytes relative to their body size if feeding conditions turn out to be poor. Larger repeat spawners show less somatic growth and have less uncertainty about the total number of oocytes to develop. Repeat spawners exhibit advanced oocyte growth and are therefore ready to start spawning in December, while recruit spawners reach maximum oocyte size later and start spawning in January. Previous studies have also shown larger females start spawning before smaller (Rijnsdorp 1989, Simpson 1959). Larger females

have higher fecundity and need to spawn a larger number of eggs. Larger plaice have a higher batch fecundity (Kennedy *et al.* 2007a, Urban 1991), but larger females also produce more batches (Kennedy *et al.* 2007a). For the spawning of a higher number of batches larger females probably have a longer individual spawning period. Hence larger females need to start spawning earlier in order for the larvae of the latest batches to still hatch at a time with favourable feeding conditions.

The leading cohort of the standing stock of previtellogenic oocytes is depended on fish size. The recruit spawning females have the largest leading cohort of previtellogenic oocytes. When females mature, the largest previtellogenic oocytes recruit to the vitellogenic stock first. In larger repeat spawners, only smaller sized previtellogenic oocytes are available, the previtellogenic oocytes are 4% smaller in repeat spawners. The repeat spawning fish will need to invest more energy at the onset of oocyte maturation compared to the recruit spawners. However, the oocyte size difference (4%) at the onset of maturation is small and compared to the total oocyte size increase to spawning (±600% increase in size), the extra energy needed for the smaller oocyte size at the start of vitellogenesis will be relatively small. This is the first time that a length-dependent difference in leading cohort of the previtellogenic oocyte stock has been shown in fish.

The onset of the oocyte maturation in plaice is already in March. Fish in temperate areas have a seasonal entrainment in their reproductive biology but the complex network regulating this process is not yet fully understood. Migaud *et al.* (2010) suggest that photoperiod is an important factor for production of chemicals and hormones that trigger reproduction in fish. In March the sun crosses the equator at the spring equinox and at the northern hemisphere daylight length increases considerably. The onset of oocyte maturation in plaice is probably triggered by the increasing daylight length in March.

This study shows there are differences in reproductive biology between recruit and repeat spawners. Recruit spawners recruit larger previtellogenic oocyte to the vitellogenic stock. However repeat spawners have higher surplus energy levels and lower down-regulation and oocyte growth is faster. Repeat spawners have therefor the ability to spawn more oocytes earlier in the spawning season. However, the onset of oocyte maturation is the same in recruit and repeat spawners and this is probably triggered by the increase in daylight length in spring. After the onset of oocyte maturation fecundity is down-regulated to surplus energy reserves. However after the summer feeding

period when surplus energy levels are highest female plaice are able to recruit previtellogenic oocytes again to the vitellogenic stock. Plaice is highly flexible in the regulation of fecundity to available surplus energy.

Acknowledgements

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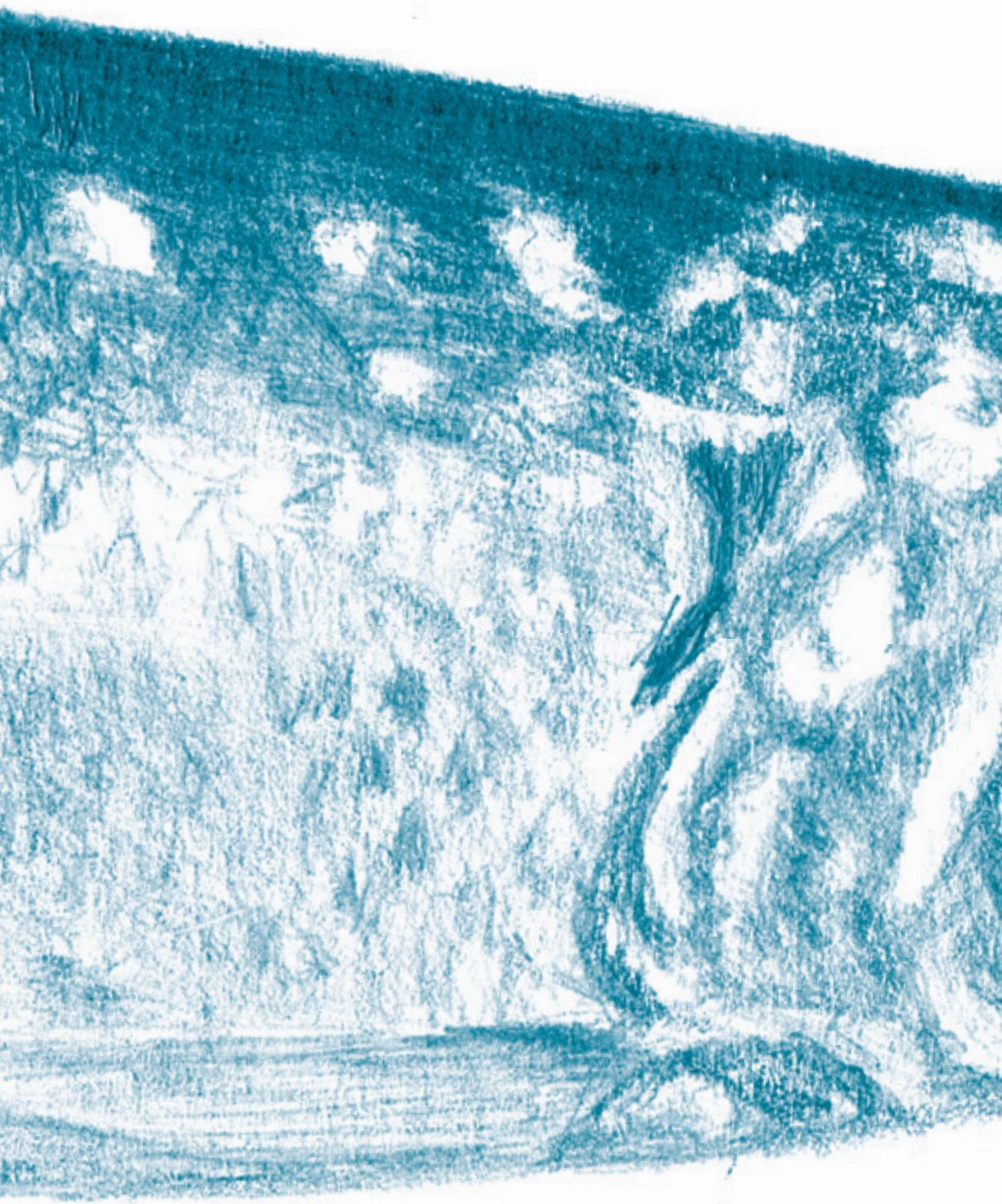

Chapter 3

Fecundity, atresia, and spawning strategies of Atlantic herring *Clupea harengus*

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Abstract

Atlantic herring *Clupea harengus* have contrasting spawning strategies with apparently genetically similar fish "choosing" different spawning seasons, different egg size and different spawning areas. In the North Sea both autumn and winter spawning herring share the same summer feeding area, but have different spawning areas. Females of both spawning types start their oocyte development in April-May. Oocyte development is influenced by the body energy content; during the maturation cycle fecundity is down-regulated through atresia in relation to the actual body condition. Hence, fecundity estimates

Sammendrag

Atlantisk sild *Clupea harengus* har ulike gytestrategier. Tilsynelatende genetisk like fisk "velger" ulike gytetider, eggstørrelse og gyteområder. I Nordsjøen har høst og vinter-gytende sild samme beiteområdet om sommeren, men de har likevel forskjellige gyteområder. Hunnfisk fra begge gyteområder starter oocyttutviklingen i april–mai. Modningen av oocyttene er påvirket av kroppens energiinnhold; under modningssyklusen blir fekunditeten nedregulert gjennom atresi i forhold til den faktiske energitilstand. Fekunditetsestimater må derfor sees i forhold til tidspunktet for prøvetaking. Nedregulering over hele

Samenvatting

Atlantische haringen *Clupea harengus* hebben contrasterende paaistrategieën, waarbij genetisch vergelijkbare vissen "kiezen" voor verschillende paaiseizoenen, verschillende eigroottes en verschillende paaigebieden. In de Noordzee, delen herfst en winter paaiende haring hetzelfde voedselgebied in de zomer maar hebben ze verschillende paaigebieden. Vrouwtjes van beide paaitypen begin hun eicel ontwikkeling in april-mei. De eicel ontwikkeling wordt beïnvloed door de lichaamsenergiereserves; tijdens de eicel ontwikkelingscyclus wordt fecunditeit door atresia naar beneden gereguleerd in verhouding tot de werkelijke lichaamsconditie. Fecunditeit schattingen moeten dus rekening houden

must account for the relative time of sampling. The down-regulation over the whole maturation period is approximately 20% in autumn and 50% in winter spawning herring. The development of the oocytes is the same for both spawning strategies until autumn when autumn spawners spawn a larger number of small eggs. In winter spawners, oocyte development and down-regulation of fecundity continues, resulting in larger eggs and lower number spawned. In theory autumn and winter spawners could therefore switch spawning strategies indicating a high level of reproductive plasticity.

modningsperioden er ca 20% for høst- og 50% for vintergytende sild. Utviklingen av oocyttene er den samme for begge gytestrategier frem til høsten når høstgytende sild gyter et større antall små egg. Hos vintergytende sild fortsetter oocyttutviklingen og nedreguleringen av fekunditeten, noe som resulterer i at eggene blir større, men også færre. I teorien kunne derfor høst og vintergytende sild bytte gytestrategier, noe som indikerer en høy grad av reproduktiv plastisitet.

met het tijdstip van bemonstering. De regulering van de fecunditeit over de gehele ontwikkelingscyclus is ongeveer 20% afname in de herfst en 50% afname in de winter paaiende haring. Tot in de herfst is de ontwikkeling van de eicellen hetzelfde voor beide paaistrategieën, wanneer de herfst paaiers een groter aantal kleine eieren paaien. In de winter paaiers gaan de eicel ontwikkeling en naar beneden regulatie van de fecunditeit door, wat resulteert in grotere eieren, maar een kleiner aantal dat in de winter wordt gepaaid. In theorie kunnen herfst en winter paaiers schakelen tussen paaistrategieën, wat een hoog niveau van reproductieve plasticiteit aangeeft.

Introduction

Atlantic herring *Clupea harengus* have contrasting spawning strategies with regard to spawning seasons, egg sizes and spawning areas (Iles 1964). The underlying reason for this plasticity in reproduction is yet unknown. Within the North Sea, spring, autumn and winter spawning stocks are found that aggregate on the summer feeding ground in the northern and central North Sea (Boeke 1906; Cushing and Burd 1957; Zijlstra 1969) and then segregate during their autumn migration to their respective spawning grounds. In the northern and central North Sea, herring spawn in autumn, with major spawning grounds around Shetland and Orkney and in the Buchan area (Cushing 1958; Baxter 1959; Baxter 1963). In the southern North Sea and eastern English Channel the so-called 'Downs'-herring spawn during the winter. In addition some spring spawning herring can be found in the North Sea, these do not spawn in the North Sea proper but tend to spawn in the coastal fringe (Roel *et al.* 2004).

All spawning types start maturation in April-May (Iles 1964). Throughout the maturation cycle oocyte size gradually increases and development of oocytes in the different spawning types continues. Iles (1964) suggested that autumn spawners keep developing the oocytes until they are ready to be spawned in September/October, whereas winter and spring spawners stop the development in September and have a resting period through autumn (and winter for spring spawners) followed by further oocyte development just prior to their spawning season. The longer maturation period in winter and spring spawners results in larger oocytes but lower fecundities (Baxter 1959; Baxter 1963, Zijlstra 1973). Within a spawning type, the variation of oocyte size in repeat spawners is small, but in recruit spawners the oocyte size increases with length (Óskarsson *et al.* 2002).

In capital spawners like Atlantic herring, fecundity, the standing stock of vitellogenic follicles, is regulated in relation to body energy reserves. This surplus energy, which is gained during the feeding period prior to spawning, is allocated to somatic growth and reproduction (Rijnsdorp 1990; Kurita *et al.* 2003). In herring, the energy uptake occurs during spring and summer (Iles 1964). Herring fecundity has been shown to increase with increasing length (Hickling 1940; Polder and Zijlstra 1959; Burd and Howlett 1974). A feeding experiment with Norwegian spring spawning herring showed no effect of feeding intensity on oocyte size and relative fecundity (oocytes per gramme fish) but lower feeding intensity and weight resulted in lower potential fecundity (total number

of oocytes per fish) (Ma *et al.* 1998). The most recent study of North Sea herring fecundity was performed by Almatar and Bailey (1989) and they did not question the idea that development pauses in later spawners. Since then techniques and concepts to investigate fecundity and oocyte growth in marine fish have further developed, e.g. using image analysis for estimating oocyte size and number (Thorsen and Kjesbu 2001) and the concept of down-regulation of fecundity through atresia (Kurita *et al.* 2003; Óskarsson and Taggart 2006; Kennedy *et al.* 2007b) influenced by earlier findings by Vladykov (1956) and others (see Kjesbu 2009). At the start of the maturation cycle oocyte size is small and fecundity is high. During maturation oocytes increase in size and fecundity decreases. In Norwegian spring spawning herring it has been found that this down-regulation of fecundity is about 56% during the period of vitellogenesis (Kurita *et al.* 2003). Following these studies, it is likely that for North Sea herring, the intensity of the down-regulation changes over time and thus maturity stage (oocyte size) needs to be taken into account when estimating fecundity.

In this study we compare the autumn and winter spawning strategies in North Sea herring using these techniques and concepts. We analyse the mechanisms determining fecundity as a basis to understand causes of interannual and stock variability in fecundity and associated oocyte growth. We test the hypothesis of a resting period in winter spawners and the absence of atresia in North Sea herring, which seems to conflict with findings in other herring stocks such as Norwegian spring spawning herring where oocyte growth is continuous throughout the maturation cycle and fecundity is down-regulated by atresia (Kurita *et al.* 2003). If such an arrest in oocyte growth is non-existent other factors, such as time and level of down-regulation, could explain the difference in reproductive output between the two North Sea herring spawning components in question.

Methods

North Sea herring were sampled monthly from May till December in 2006 and 2007 from commercial and research vessels. This mix of vessel types allowed for the full temporal range of oocyte development to be sampled. The herring were sampled from across the North Sea (FIGURE 3.1). Only samples of fresh fish were used, and these were kept on ice prior to examination. Biological parameters measured were total fish length (TL, to the nearest 0.5 cm), total weight (W, 1 g), maturity, ovary (OW,

0.1 g) and gutted (1 g) weight. Stomach fullness was noted as 1=empty, 2=filled, 3=full and 4=bursting. Age was determined from the otoliths, and spawner type (autumn, winter or spring) by using the microstructure of the core of the otoliths (Clausen *et al.* 2007). Lipid content was measured using a Distell fish fat meter (Kent 1990). Lipid was measured on both sides of the fish and the average of both readings was taken as the lipid content. The fat meter was calibrated using lipid samples of 69 herring following the Bligh and Dyer method (Bligh and Dyer 1959). The fat meter gave higher readings at high lipid content (>15%), as was found by Kent (1990), but the relationship was consistent over the full data range (Lipid Bligh and Dyer = 0.3 * Lipid fat meter + 9.6, R2=0.54). Gonad somatic index (GSI, %) was calculated as:

$$GSI = 100 * OW * (W - OW)^{-1} \quad [3.1]$$

where OW is individual ovary weight and W is individual total weight. Condition of the fish was expressed as the relative condition factor (Le Cren 1951; Hansen and Nate 2005):

$$Kn = W / Wr \quad [3.2]$$

where Kn is the relative condition factor and Wr is the estimated weight of a herring based on population weight - length relationship in July using data from the acoustic herring surveys (ICES 2006a; 2007a) as general reference point.

Different preparations and methods were used to investigate the fecundity of herring dependent on their stage of oocyte development. Herring at the beginning of the maturation cycle (spring) had their whole ovaries collected and fixed in 3.6% buffered formaldehyde. A sample of the ovaries was dehydrated in ethanol, embedded in Technovit resin and mounted on microscopic slides following standard procedures. The histological sections (4 µm) were coloured with toluidine blue in order to detail the development of the oocytes and check the correctness of the macroscopic maturity stage determination. The early development stages of the oocytes (in May) were measured using the histological sections. Oocytes shrink during the histological processing and therefore the oocyte diameters taken from the histological slides were corrected for shrinkage (7%) according to Ma *et al.* (1998).

For the herring, that had started true vitellogenesis, duplicate pipette (Drummond Scientific Company, Wiretrol II) samples of 100 µg were taken and fixed in 3.6% buffered formaldehyde (Witthames *et al.* 2009). In these pipette samples, all oocytes were

measured and counted in whole mount analysis using ImageJ (Rasband 1997-2008). Oocyte density and potential fecundity was calculated as:

$$NG = N/s \quad [3.3]$$

where NG is number of oocytes per gramme ovary tissue (oocyte density), N is the number of vitellogenic follicles in the pipette subsample and s is the subsample weight.

$$F_p = NG * OW \quad [3.4]$$

where Fp is potential fecundity. From this relative fecundity F_r was calculated:

$$F_r = F_P / W \quad [3.5]$$

Total 66 samples of autumn spawners and 51 of winter spawning herring were used to estimate the auto-diametric relationship between oocyte density and oocyte diameter (OD). The auto-diametric fecundity method (Thorsen and Kjesbu 2001) was then used for the remainder of the samples. Of each sample 100 oocyte diameters were measured. Using the estimated relation between oocyte density and oocyte diameter, the mean oocyte diameter was converted to oocyte density and then potential fecundity using equations 4 and 5.

Oocyte development between the different spawning types was not only compared using oocyte diameter measurements but it was also checked if oocytes of exactly the same size (diameter) were in the same developmental phase. A number of 7 autumn and 10 winter spawners were examined for size-specific oocyte development within a wide oocyte size range but with comparable development. From each fish, three single oocytes were selected in histological sections and analyzed. The criteria used for the development stage of the oocytes were the size of the leading cohort (10 biggest) of yolk granules, the volume fraction of yolk granules and the thickness of the chorion, as described in Óskarsson *et al.* (2002).

Statistical analyses were performed in SAS and R. The dependence of fecundity on size and body condition over time was studied using a linear regression model:

$$lnFp = \beta_0 + \beta_1 lnTL + \beta_2 Y + \beta_3 A + \beta_4 S + \beta_5 LC + \beta_6 K_n + \beta_7 L + \beta\ lnTL*Y + \beta_9 lnTL*A + \beta_{10} lnTL*S + \beta_{11} lnTL*LC + \beta_{12} K_n*Y + \beta_{13} L*Y + \varepsilon \quad [3.6]$$

where Y is year, A is age, S is spawning type, LC is leading cohort (mean diameter of the 10% biggest oocytes), L is lipid content and ε is a normally distributed error term. Year, age and spawning type were considered as factors in the model. Since not all of these

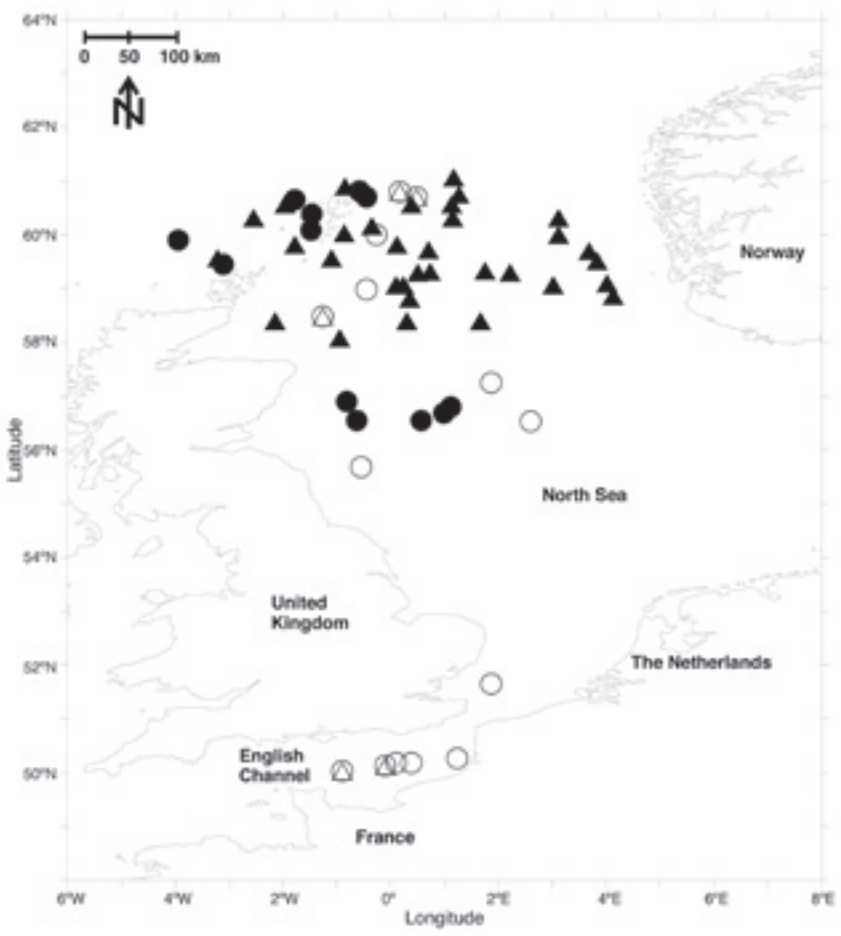
parameters are measured during the standard herring surveys and market sampling programmes a reduced model was tested. In order to be able to use the model for predictions the factor year was also removed.

$$lnFp=\beta_0+\beta_1 lnTL+M+A+S+\beta_2 K+\beta_3 lnTL*M+\beta_4 lnTL*A+\beta_5 lnTL*S+\beta_6 K*M+\varepsilon \quad [3.7]$$

where M is month. Model selection was based on the AIC information criterion using the stepwise backward selection approach.

FIGURE 3.1.

Sampling locations in 2006 (○ commercial and ● research vessels) and 2007 (Δ commercial and ▲ research vessels).



Results

A total of 655 herring, of which 45% were autumn and 55% were winter spawners, were sampled (TABLE 3.1). From October till December only winter spawners were sampled, hence the slightly higher percentage of winter spawners in the samples. No statistical differences were found in the weight - length relationship between autumn and winter spawners and between years (ANOVA; year P = 0.379, DF = 286; spawning type P = 0.825, DF = 286). Results were comparable to the results found during the yearly North Sea herring acoustic survey (ICES 2008a). Kn was higher in 2006 compared to 2007 both for autumn (ANOVA; P = 0.002, DF = 291) and winter spawners (ANOVA; P < 0.001, DF = 358). The lipid content was also higher in 2006 for autumn (ANOVA; P < 0.001, DF = 291) and winter spawners (ANOVA; P = 0.001, DF = 334). Thus, the condition of herring was evidently higher in 2006 compared to 2007. Within each year no significant difference in condition was found between the spawning types (FIGURE 3.2A AND FIGURE 3.2B). Both condition factor and lipid content showed a high increase from May to June, when herring start feeding in the central North Sea (FIGURE 3.2A-D). During June to August, body condition remained the same and dropped in September for autumn spawners and in November for winter spawners (FIGURE 3.2A). Of all the herring examined only 4% had food remains in the stomach, either remains of plankton or fish scales. GSI was significantly different both between years (ANOVA; P < 0.001, DF = 291 autumn spawners; DF = 358 winter spawners) and spawning types (FIGURE 3.2E and FIGURE 3.2F; ANOVA; P< 0.001, DF = 347, and P = 0.001, DF = 302 for 2006 and 2007, respectively). As expected, GSI increased during maturation and was higher prior to spawning in winter spawners (FIGURE 3.2E and FIGURE 3.2F). No significant difference in the relationship between oocyte diameter and oocyte density was found between the two spawning types (FIGURE 3.3; ANOVA, P=0.614, DF = 113).

TABLE 3.1

Numbers of herring sampled per month in 2006 and 2007.

Spawning type	Year	5	6	7	8	9	11	12	Total
Autumn	2006		38	35	28	48			149
Winter	2006		43	7	14		90	48	202
Autumn	2007	17	26	81		21			145
Winter	2007	14	21	74		26		24	202

FIGURE 3.2

Body condition of autumn (—♦—) and winter (--□--) spawning North Sea herring; relative condition factor and 95% confidence intervals in (a) 2006 and (b) 2007; lipid content in (c) 2006 and (d) 2007; and GSI in (e) 2006 and (f) 2007.

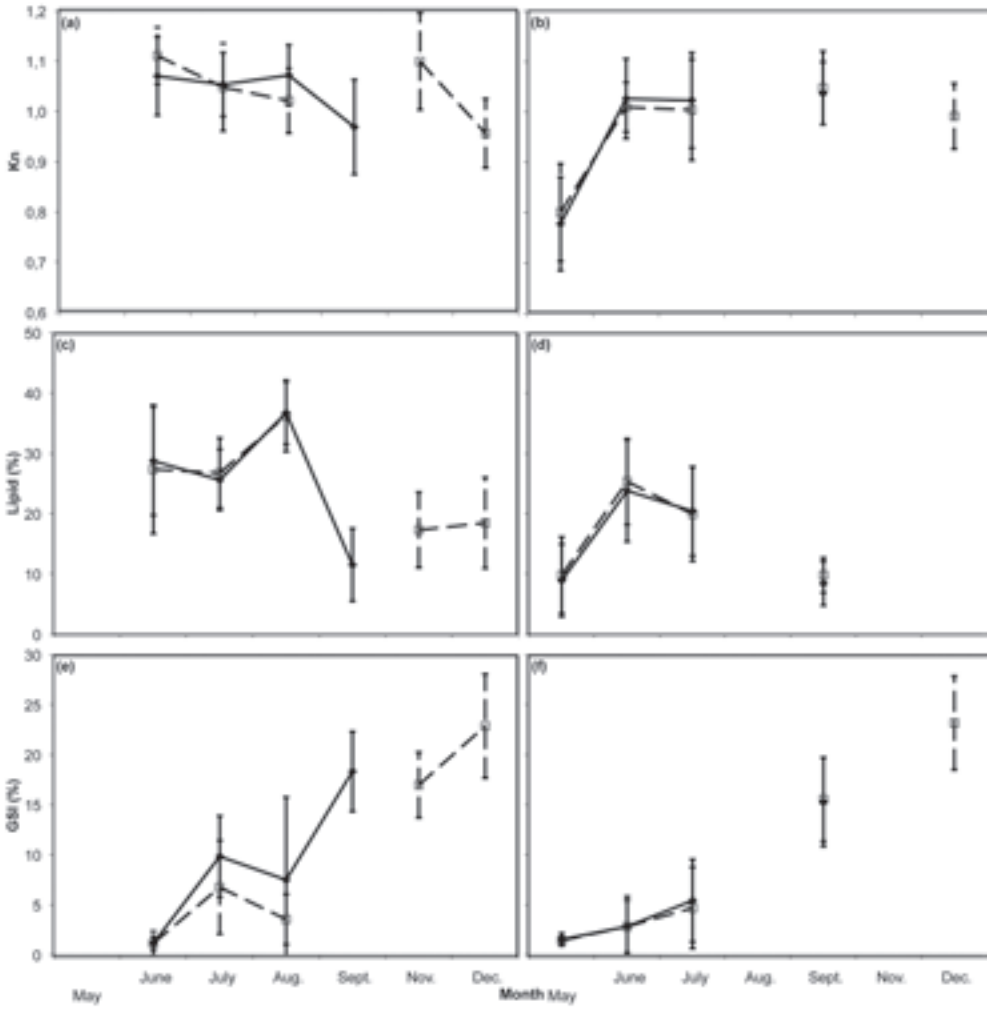
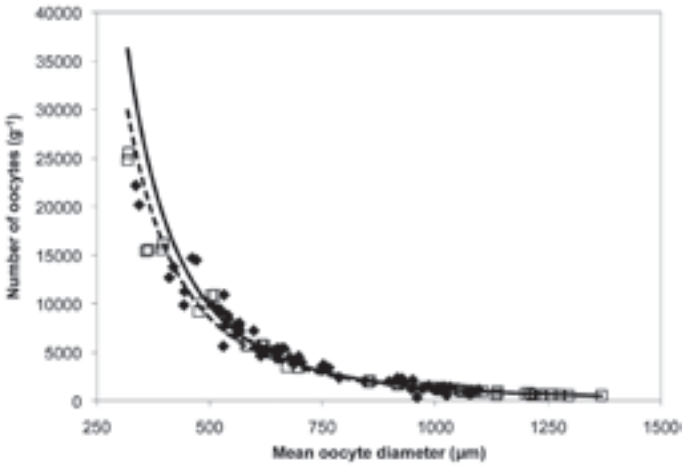


TABLE 3.2

Auto-diametric fecundity relationship between oocyte density (NG) and oocyte diameter (OD) for autumn and winter spawning North Sea herring (NG= α * ODß).

Spawning type	α (*10-11)	ß	R^2
Autumn	6.464	-2.892	0.910
Winter	2.536	-2.766	0.987

FIGURE 3.3



Mean vitellogenic oocytes diameter (OD) versus the number of vitellogenic oocytes per gramme of fresh ovary (NG) for autumn (—♦—, n = 66) and winter (--□--, n = 50) spawning herring.

TABLE 3.3

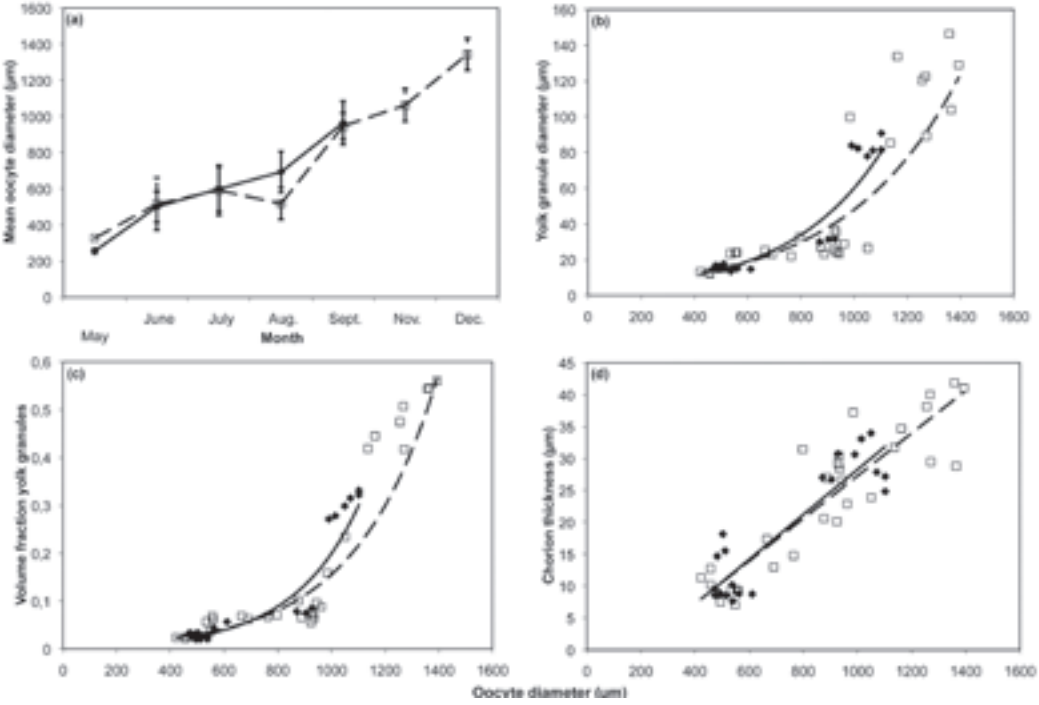
Spawning type	Month	Oocyte diameter (µm)	Relative fecundity (g-1)	Weight (g)	Potential fecundity (N oocytes)	Change (%)
Autumn	5	400	434	196	84920	
	6	500	415	203	84324	-0.2
	7	599	400	207	82724	-2
	8	694	385	225	86678	+5
	9	965	343	196	67341	-22
	Total					-21
Winter	5	400	432	152	65641	
	6	516	396	214	84549	+30
	7	591	376	196	73936	-13
	8	519	395	178	70262	-5
	9	946	283	174	49153	-30
	11	1064	252	159	40106	-18
	12	1345	178	174	30894	-23
	Total					-53

Down-regulation of fecundity during the maturation cycle prior to spawning. Oocyte diameters and body weights used are the mean for each month for both autumn and winter spawners and both years combined. Relative fecundity (per ovary-free weight) calculated using the down-regulation relationships; autumn spawners Fr = -0.16*OD + 494 and winter spawners Fr = -0.26*OD + 532

The auto-diametric relationship between oocyte diameter and oocyte density was determined (TABLE 3.2) and used to determine fecundity as previously described. Potential fecundity, measured just prior to the spawning time, was the same in both years, but autumn spawners had a higher potential fecundity compared to the winter spawners (TABLE 3.3). Autumn spawners spawned oocytes at a smaller size (TABLE 3.3).

FIGURE 3.4

Oocyte development during maturation in autumn (—♦—) and winter (--□--) spawning North Sea herring; (a) mean oocyte diameter over time, (b) mean diameter of the leading cohort of yolk granules, (c) volume fraction of yolk granules in developing oocytes and (d) mean chorion thickness of maturing oocytes.

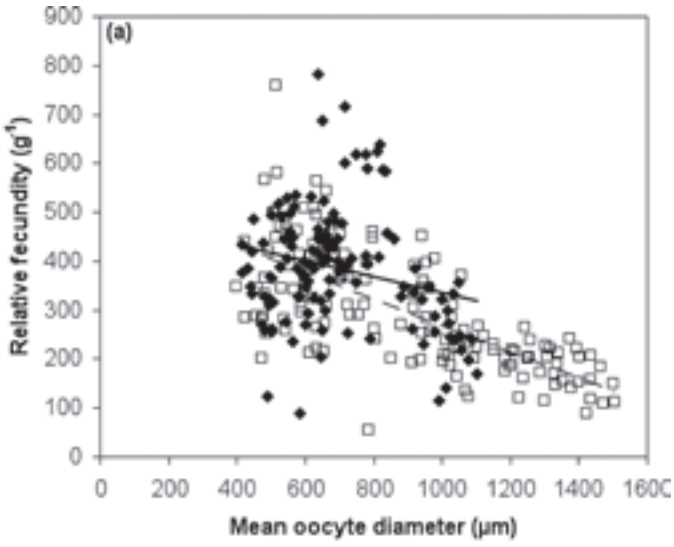


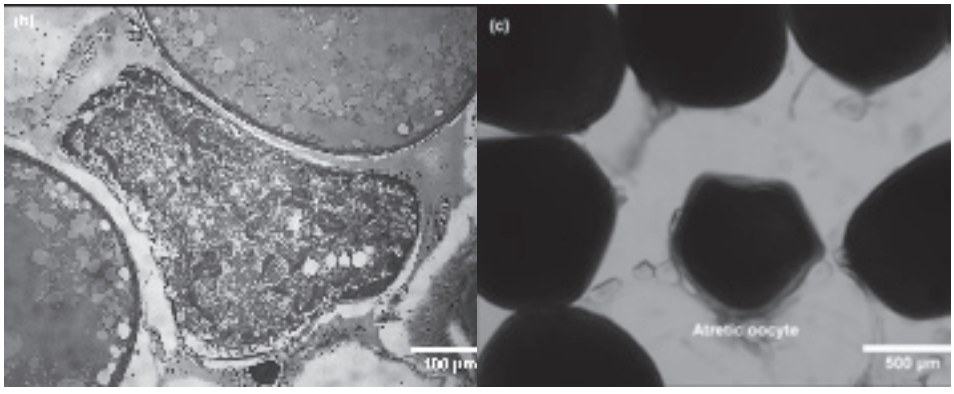
Oocyte size increased during maturation for both autumn and winter spawners and no differences existed in their microscopic development (FIGURE 3.4A-D). Both spawning types had the same oocyte size in May and subsequent oocyte growth was the same over time (ANOVA, P = 0.436, DF = 10; FIGURE 3.4A). In the May samples, 65% had cortical alveoli, which is a sign of early maturation of oocytes (endogenous vitellogenesis;

Kjesbu and Kryvi 1989). Still in September, prior to the spawning of the autumn spawning herring, oocyte size was the same for both spawning types. The size of the yolk granules and the volume of yolk granules inside the oocytes increased with increasing oocyte size (FIGURE 3.4B and FIGURE 3.4C). No significant differences where found between autumn and winter spawners (ANOVA, P = 0.229, DF = 49 and 0.209, DF = 49 for yolk granules size and volume, respectively). Furthermore, the thickness of the chorion increased during maturation (FIGURE 3.4D) but no significant differences were found between the autumn and winter spawners in this respect either (ANOVA, P = 0.156, DF = 49).

FIGURE 3.5

Down-regulation of fecundity in autumn (—♦—) and winter (--□--) spawning North Sea herring through atresia; (a) down-regulation (relative fecundity per ovary-free weight). Micrographs of atretic herring oocytes; (b) in a histological preparation and (c) in a whole mount preparation





During the maturation, oocytes increased in size while fecundity decreased due to down-regulation through atresia (FIGURE 3.5A). Atretic oocytes were found in different maturity stages both in the histological sections (FIGURE 3.5B) as well as the whole mounts (FIGURE 3.5C). The samples contained less than 10 atretic oocytes per sample. The level of down-regulation differed between autumn and winter spawners. As indicated earlier, both spawning types started off with the same oocyte size and fecundity at early vitellogenesis (ANOVA, P=0.048, DF = 285; FIGURE 3.5A). Down-regulation was calculated for autumn and winter spawners using the average oocyte size and body weight per month for both spawning types, setting the initial oocyte size at 400 µm. Overall, from this start of the maturation cycle to just prior to spawning, down-regulation in autumn spawners was 21% while 53% in winter spawners (TABLE 3.3). Furthermore, down-regulation seemed to be lowest at the start of maturation and increased towards spawning.

At the start of the maturation cycle, in May-June, fecundity is high and body condition still low (FIGURE 3.6 and FIGURE 3.7). In summer, body condition rises due to food uptake, while fecundity decreases due to down-regulation. In autumn and winter, body condition decreases due to low food uptake and fecundity is still down-regulated. The selected model with the lowest AIC is:t

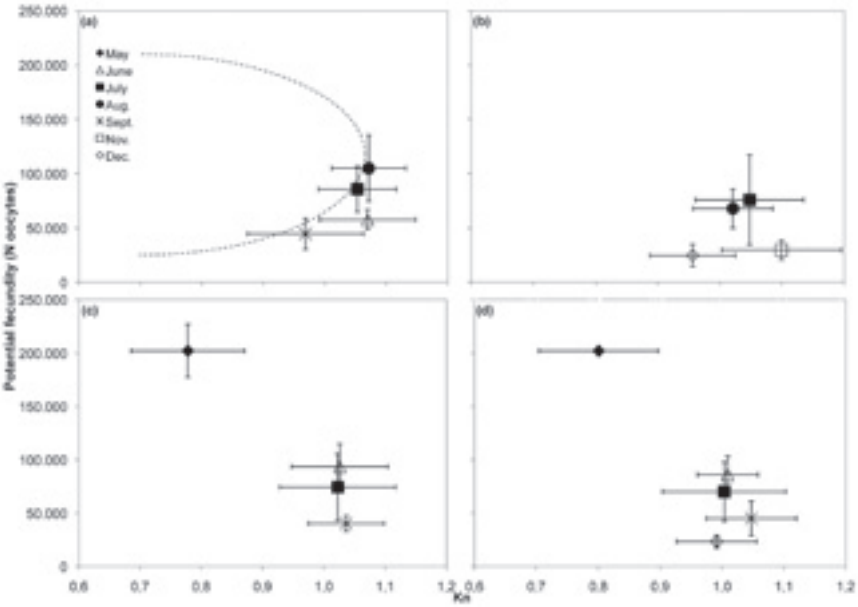
$$lnF_p = lnTL + Y + LC + K_n + L + LC * K_n + L * Y \quad [3.8]$$

and the significant explanatory variables explained about 79% of the variance in Fp (TABLE 3.4). Fp increased with body size and was a function of year (TABLE 3.5). Increasing leading cohort diameter has a negative effect on Fp. Fp increased with relative body condition and lipid content though the estimate is small. Fecundity–at-length did not differ between autumn and winter spawners. Age, either as nominal variable or continuous variable did not affect the fecundity size relationship. Since lipid measurements are generally not available, a simplified model was estimated ignoring lipid and year effects. The selected model is:

$$lnF_p = lnTL + M + K_n + lnTL * M \quad [3.9]$$

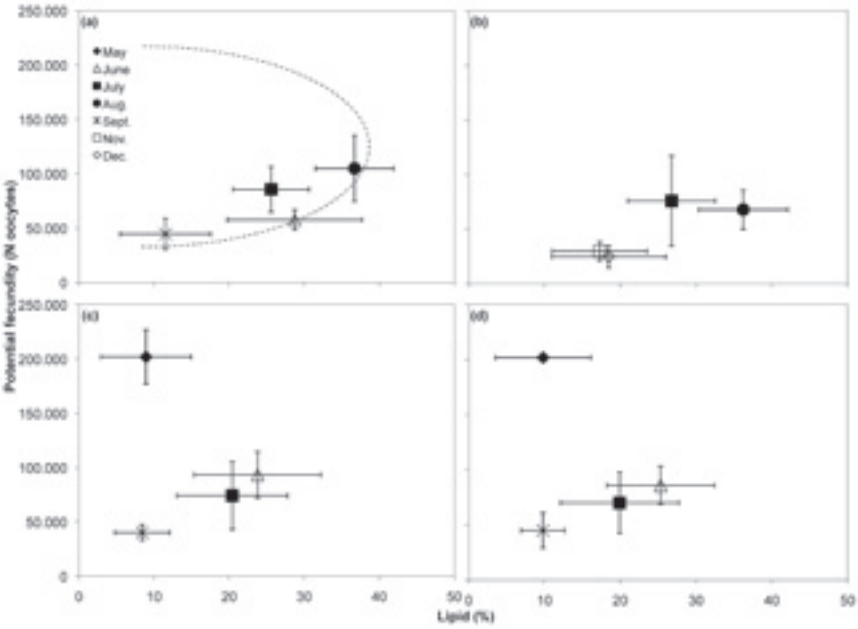
and explained about 78% of the variance in Fp. In absence of lipid, Fp increased with body size and was a function of Kn (TABLE 3.5). The predictive strength of both models is the same (FIGURE 3.8). However both models overestimate at low fecundities and underestimate at high fecundities.

FIGURE 3.6



Relationship between relative body condition and potential fecundity in (a) autumn and (b) winter spawners in 2006 and (c) autumn and (d) winter spawners in 2007. The dashed line drawn in panel a shows the expected curve (see text).

FIGURE 3.7



Relationship between lipid content and potential fecundity in (a) autumn and (b) winter spawners in 2006 and (c) autumn and (d) winter spawning herring in 2007. The dashed line drawn in panel a shows the expected curve (see text).

TABLE 3.4

Results of the model selection of the fecundity size relationships of equation [6].

Model	DF	AIC	R^2
Autumn	6.464	-2.892	0.910
Equation [8]	7	31.33	0.791
Equation [8] + A	15	35.31	0.794
Equation [8] + S	8	32.62	0.791
Equation [8] + Ln(TL)*Y	8	32.93	0.791
Equation [8] + Kn*Y	8	32.57	0.791

FIGURE 3.8

Observed and estimated fecundity for autumn (x) and winter (□) spawners using (a) all variables and (b) without year and lipid. (See Table 3.5 for the model description.)

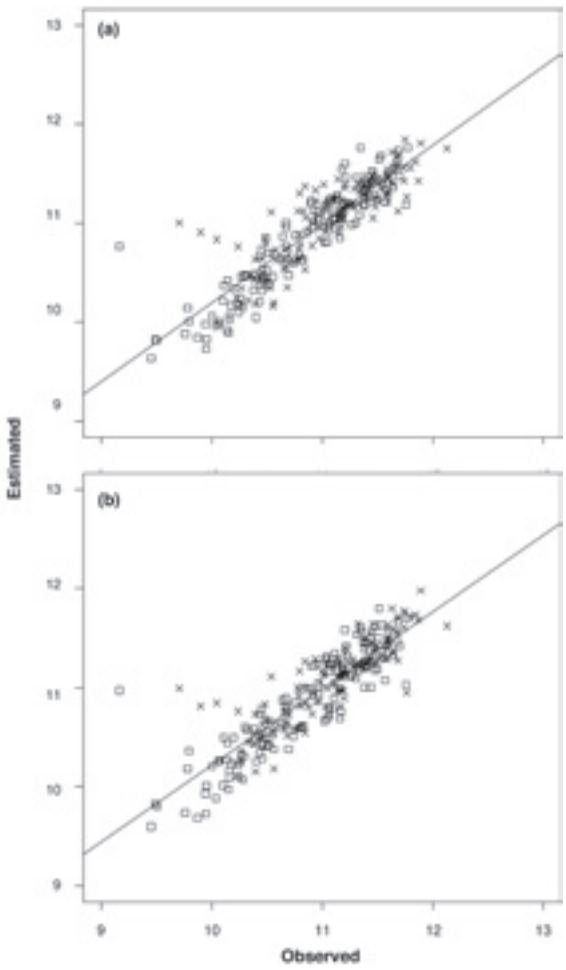


TABLE 3.5

Parameter estimates and P-value of the selected fecundity model (equation [8]) and the model ignoring the differences between years (equation [9]).

Equation [8]			
	Estimate	SE	Significance[a]
Intercept	-8.156	0.893	***
Ln(TL)	5.972	0.235	***
Y	-0.210	0.094	*
LC	-0.002	0.001	***
Kn	0.008	0.532	
L	0.006	0.003	.
LC*Kn	0.001	0.001	*
L*Y	-0.012	0.003	***

Equation [9]			
	Estimate	SE	Significance[a]
Intercept	-20.546	7.620	**
Ln(TL)	9.434	2.247	***
M (June)	16.025	8.389	.
M (July)	14.285	7.672	.
M (August)	12.761	8.221	
M (September)	22.670	7.828	**
M (November)	13.575	8.287	
M (December)	6.766	9.796	
Kn	0.736	0.202	***
Ln(TL)*M (June)	-4.9094	2.4870	*
Ln(TL)*M (July)	-4.4359	2.2700	.
Ln(TL)*M (August)	-3.9222	2.4373	
Ln(TL)*M (September)	-7.0893	2.3175	**
Ln(TL)*M (November)	-4.3803	2.4671	.
Ln(TL)*M (December)	-2.4156	2.9286	

[a] Significance levels: . P<0.1, * P<0.05, ** P<0.01; *** P<0.001.

Discussion

This study shows that both autumn and winter spawning North Sea herring start their maturation cycle at a similar time, i.e., in April or May. Also oocyte development during the maturation cycle is the same for both spawning types, but winter spawners continue to develop their oocytes after autumn spawners have spawned, resulting in bigger eggs. During maturation, fecundity in North Sea herring is down-regulated through atresia which is controlled through body condition. The down-regulating effect of atresia on fecundity has also been shown in other herring spawning stocks, such as Norwegian spring spawning herring (Kurtita *et al.* 2003) and Icelandic summer spawning herring (Óskarsson and Taggart 2006).

In May, 65% of the herring showed signs of early vitellogenesis. The early vitellogenesis is only visible in microscopic analysis of the oocytes. However, the first signs of vitellogenesis by macroscopic analysis appear in June (Iles 1964). The macroscopic determination of maturity ogives is therefore not possible in April and May.

The growth of the yolk granules within the oocytes follows the same pattern for autumn and winter spawners. Yolk granules start with slow development and growth at the beginning of maturation. At the end of the development, yolk granules take up water and growth increases rapidly (Kjesbu and Kryvi 1989; Kurita *et al.* 2003). From the FIGURE 3.4B and FIGURE 3.4C it remains unclear whether the growth of yolk granules follows exponential growth or it is a segmented process that could be described in two separate equations.

This study suggests that the difference between an autumn and winter spawned fish is less obvious than previously thought, therefore the option for individual plasticity cannot be ruled out. The genetic difference is low between the different spawning components within the North Sea (Mariani *et al.* 2005). As oocyte development during the maturation is the same in autumn and winter spawning herring until autumn spawners spawn, both in oocyte size and oocyte structure, it is theoretically possible that autumn and winter spawners could switch between the two spawning strategies. There is no proof or reports of autumn and winter spawners switching between strategies. Brophy *et al.* (2006) suggested that season and location of spawning in herring in the Irish and Celtic Sea are predetermined, possibly through larval imprinting. McQuinn (1997) indicated that herring return to the same spawning grounds, but not necessarily to the natal spawning ground. In the North Sea, populations that recolonise the spawning sites after population collapse generally maintain the previous temporal spawning

pattern, and in recent years all herring sampled whilst spawning in winter originate from winter spawning events (Dickey-Collas *et al.* 2005). Long term observations suggest that environmental variability and latitude may play role in determining spawning strategy (Melvin *et al.* 2009) but the mechanisms are unclear and it is assumed that once an individual has spawned for the first time, it remains fixed as that spawner type for the rest of its life (McQuinn 1997; Melvin *et al.* 2009). Thus current thinking assumes that plasticity occurs in the population, but only in an individual's choice for its first spawning and not in regular annual decisions of each individual herring (Secor *et al.* 2009). This study however suggests that the potential and capability are there for an individual to switch.

Timing of spawning is tuned to the survival of larvae, hence there is a specific time and spatial window for fish to spawn and reproduce successfully. Thus difference in timing of the spawning is as an adaptation to these time windows and not as a result of a physiological process. During the evolution, physiology puts constraints on how an animal can respond to the environment and fill certain niches. Egg size shows a clear seasonal pattern with large eggs in winter and small eggs in summer, both within and across species, and may be related to the yolk reserves needed by the larvae, or the benefit of hatching at a certain size at certain seasons. From this ecological perspective, and the similarity in the physiology shown here, the question is whether spawning types may switch. Although physiologically possible, it is less likely due to the proposed "learned" nature of herring spawning migrations (McQuinn 1997). From this perspective conservatism during the life of an individual is expected.

Although body condition and lipid content decreased in November and December, the winter spawning herring, in contrast to the autumn spawners, still continued developing their oocytes which resulted in bigger oocytes, with more and bigger yolk granules prior to their winter spawning. Iles (1964) suggested, based on macroscopic study of ovaries, that autumn and winter spawning herring start development of their oocytes at the same time, but that winter spawners have a resting phase in autumn and start development again prior to spawning. Whilst our samples show a levelling off in oocyte size from July to August in winter spawners (FIGURE 3.4A), there is a gradual increase from August onwards and no signs of a resting phase in autumn.

This is the first study that has found down-regulation through follicular atresia in North Sea herring, although it has been reported in Norwegian spring spawning (Kurita *et al.* 2003) and Icelandic summer spawning herring (Óskarsson *et al.* 2002; Óskarsson

and Taggart 2006). Previous automated analyses did not pick up atresia but the use of new improved image analysis systems made it possible to detect the atretic oocytes (FIGURE 3.5C, Witthames *et al.* 2009). In whole mounts, atretic oocytes appear irregular in shape, relatively smaller than vitellogenic oocytes and with an uneven transparency (Óskarsson *et al.* 2002). In the fecundity study of Simpson (1951) air dried oocytes were counted. Thus shape, size and transparency could not be used as indicators of atretic oocytes.

The down-regulation over the whole maturation period is relatively high, generally 21% in autumn and 53% in winter spawning herring. Autumn spawners spawn more numerous smaller eggs compared to the winter spawners (Zijlstra 1973), hence the total down-regulation in autumn spawners is less. In some periods of this study (from July to August in autumn spawners and from May to June in winter spawners, TABLE 3.3) fecundity increased. This increase might be due to sampling error resulting in variation in herring size in the samples and thus variation in fecundity. However overall there is down-regulation in fecundity (TABLE 3.3). Kurita *et al.* (2003) has also found this pattern of increasing reduction in Norwegian spring spawning herring and a down-regulation of 56% over the total maturation cycle.

To estimate potential fecundity for the use in spawning stock biomass estimation through annual egg production methods, it is important that fecundity is estimated just prior to spawning or the stage of maturity and thereby the subsequent level of down-regulation is accounted for (Kjesbu 2009). Earlier estimation of fecundity will result in overestimation of fecundity and thus lower estimates of spawning stock biomass (Óskarsson and Taggart 2006; Damme *et al.* 2009a).

In addition to the previously mentioned articles on herring (see above), down-regulation through atresia has been found in other species, such as cod *Gadus morhua* (Thorsen *et al.* 2006), plaice *Pleuronectes platessa* (Kennedy *et al.* 2007b) and sole *Solea solea* (Witthames and Greer Walker 1995). This process is regulated by the mechanism of energy allocation where the fish regulates its body reserves according to a seasonally varying threshold aimed to build up the optimal body reserves needed for reproduction and survival (Rijnsdorp 1990). If energy reserves exceed the threshold, surplus energy will be allocated into somatic growth and reproduction, while fish with body reserves below the threshold will stop growing and invest all surplus energy in improving body reserves (Rijnsdorp 1990; Kjesbu 2009). At the start of the maturation, the future summer food conditions are unknown to an individual and all females

start with the same high number of oocytes. Although herring do not grow in length after maturation as much as many other teleosts (Kjesbu and Witthames 2007; Geffen 2009), they nevertheless take on body weight and thus have to make the same trade-off between somatic growth and reproduction as other capital spawning fish like plaice. Totally 50% of the energy used for gonad growth in plaice originates from reserves that are built up in the somatic growth period (Rijnsdorp 1990). In herring the energy uptake occurs during the rather short spring and summer feeding period (Maravelias and Reid 1997; Slotte 1999). This is also seen in the fecundity model were the importance of the interaction between Kn and month fluctuates but generally decreases towards winter (FIGURE 3.6). At the end of the maturation cycle when the level of energy uptake is determined, fecundity will be fine tuned to the energy content (Kurita *et al.* 2003), hence the higher down-regulation just prior to spawning (TABLE 3.3). However, studies on other Atlantic herring stocks, measuring atresia during maturation, show the intensity of atresia declines just before spawning (Kurita *et al.* 2003; Óskarsson and Taggart 2006). In this study intensity of atresia was not measured directly.

Results of this study suggest that reproductive investment in both spawning types is similar despite temporarily different GSIs. Body condition of winter spawners prior to spawning (December) was lower compared to body condition of autumn spawners prior to their spawning time (September). However, mean ovary weight prior to spawning of winter spawners was comparable to the mean ovary weight of autumn spawners prior to their spawning time, therefore GSI of winter spawners just before spawning was higher compared to the autumn spawners (FIGURE 3.2E and FIGURE 3.2F). Thus, studies of reproductive investment through GSI should also take into account the temporal changes on body condition that occur through seasonal changes in feeding and, not at least, standardise measurements of ovary weight in relation to the developmental stage (Óskarsson *et al.* 2002; Kainge *et al.* 2007).

Potential fecundity prior to spawning in this study was at the same level as in previous studies (Hickling 1940; Baxter 1963; Burd and Howlett 1974) with autumn spawners having a higher fecundity compared to winter spawners. Polder and Zijlstra (1959) stated that variation in fecundity between years is negligible in North Sea herring.

Levels of atresia were low and therefore the finding of atresia does not suggest that North Sea herring skip spawning. This is in contrast with the study of Engelhard and Heino (2005) which suggests based on scale analysis that herring frequently skipped the second spawning season. However, an experiment conducted with Norwegian

spring spawning herring showed that only herring with an extremely low body condition (Kn < 0.6) showed massive atresia and skipped spawning (James Kennedy pers. comm.), supported by related data from the field (Óskarsson *et al.* 2002). In the current study relative condition factor did not fall below 0.78., thus the atresia found is a sign of the down-regulation of fecundity and not the skipping of spawning.

The use of lipid content and body condition as an indicator of fecundity, especially early in the maturation cycle, should be done with caution. In 2006 the start of the Dutch 'maatjes' herring fishing season was postponed to the beginning of June because the lipid content of the herring was too low in May. If lipid content is below 16% herring are not fat enough for the market (Produktschap Vis, Verordening Hollandse Nieuwe 2007, BWBR0023778). Generally herring reach the 16% lipid content threshold the second half of May. The data collected in this study show that over the season in 2006 body condition, both condition factor and lipid content (FIGURE 3.7A and FIGURE 3.7B), were higher compared to 2007. However, no lipid samples were collected in May 2006 but if the start of the 'maatjes' herring season was postponed it can be assumed that lipid content will have been very low (< 16%) in May 2006. Thus, from May to June 2006 the lipid apparently rose sharply (FIGURE 3.7A and FIGURE 3.7B).

Fecundity is regulated by energy uptake and body condition. At the start of the maturation cycle, in spring, fecundity is high and body condition still low. In summer, body condition will rise due to food uptake, while fecundity decreases due to down-regulation. In autumn and winter, body condition will decrease due to low food uptake and fecundity will still fall due to down-regulation (FIGURE 3.6 and FIGURE 3.7). The change in relative body condition was not supported by the stomach content analysis, since almost all the fish had an empty stomach. In 2006, when body condition appeared to be better compared to 2007 (FIGURE 3.2), the relationship between body condition and potential fecundity was not as expected, contrasting to 2007 when the relationship between body condition and potential fecundity was as expected.

In conclusion we have demonstrated that both spawning types start the oocyte development at the same time in spring. During the maturation cycle, fecundity is down-regulated through atresia in relation to the body condition. The development of the oocytes is the same for both spawning strategies until autumn when autumn spawners shed a relatively large number of small eggs. In winter spawners, oocyte development and down-regulation of fecundity continues, resulting in larger eggs and lower fecundity. Thus, in theory autumn and winter spawners could switch spawning

strategies indicating a high level of reproductive plasticity.

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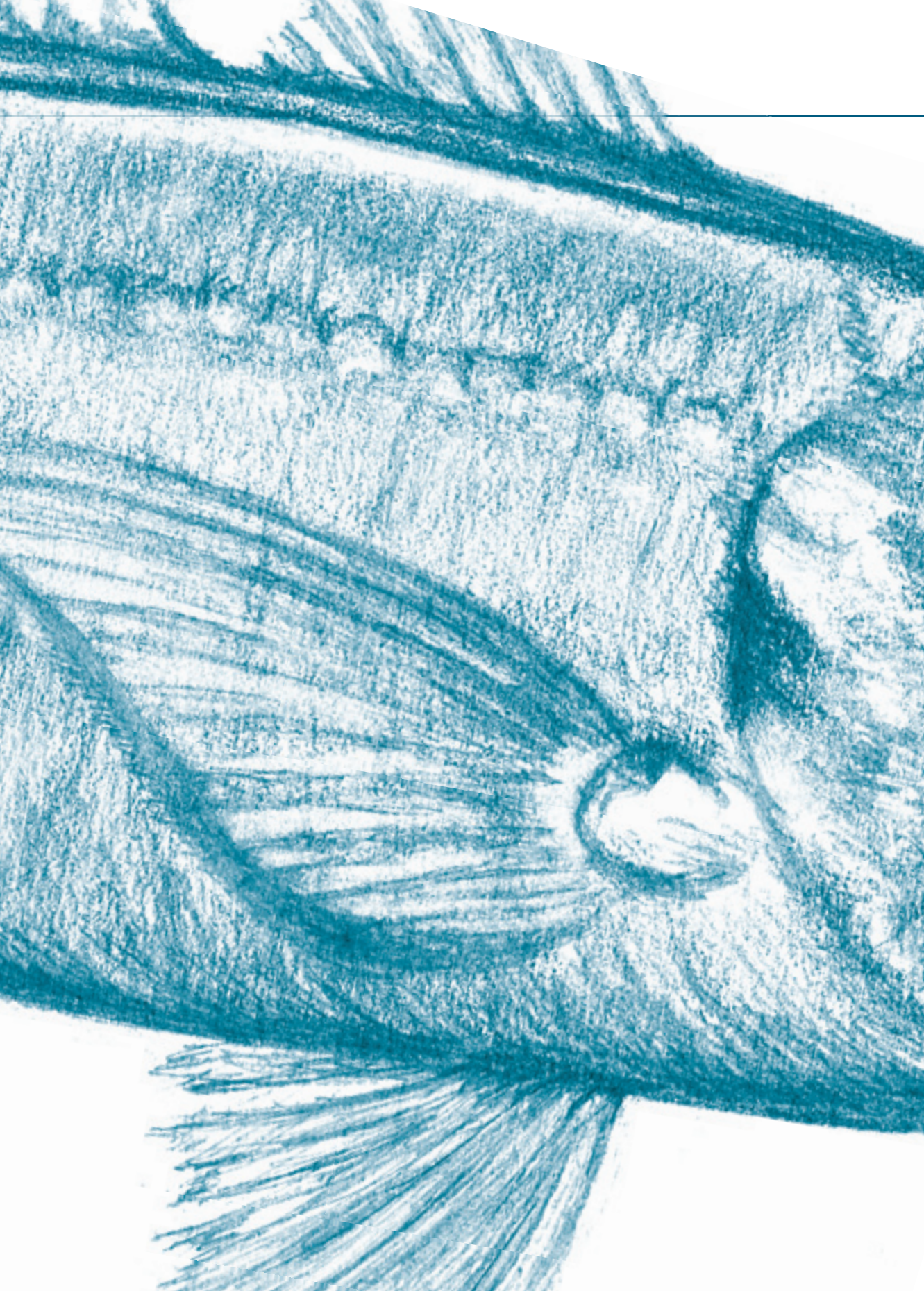
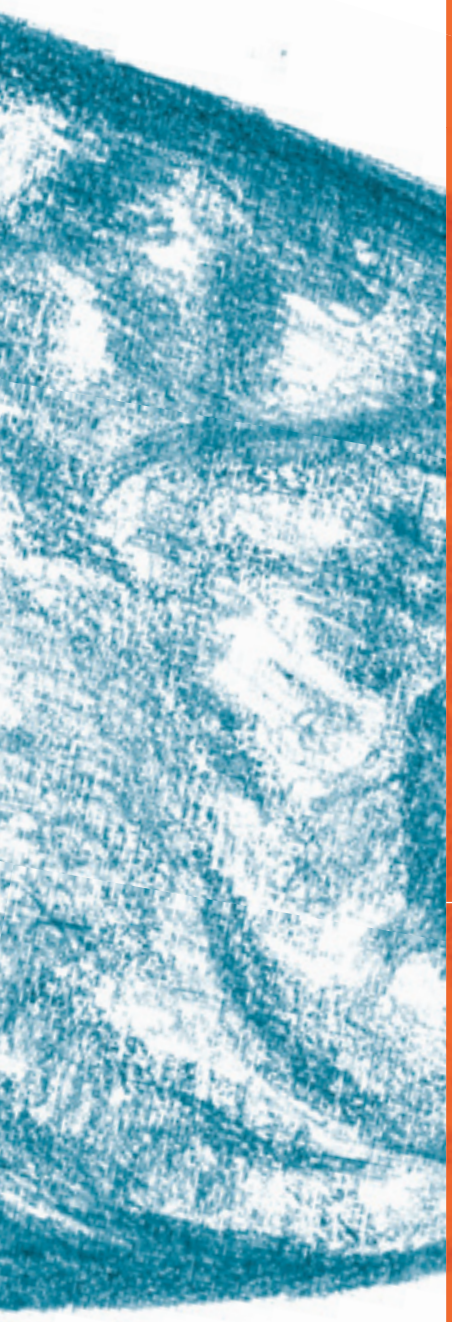


Chapter 4



Fecundity regulation in income breeding horse mackerel

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Abstract

Egg Production Methods have been used successfully in the provision of advice for fisheries management. These methods need accurate and unbiased estimates of fecundity. We explore the reproductive strategy of horse mackerel and evaluate the suitability of EPM to estimate population biomass. Fecundity and fecundity regulation in relation to condition was investigated over a number of years. Fulton's K, lipid content and hepatosomatic index increased after the start of spawning, though decreased again at the end of spawning. The increase in GSI, fecundity and body condition after the onset of spawning support the idea that horse mackerel is an income breeder, utilising food resources during the spawning season.

Sammendrag

Eggproduksjonsmetoder (EPM) har blitt brukt med hell i rådgivingen av fiskeriforvaltningen. Disse metodene er avhengige av nøyaktige og objektive fekunditets anslag. Vi utforsker reproduktiv strategi hos hestemakrell og kommenterer nytten av EPM som redskap til å anslå populasjonsbiomassen. Fekunditet og fekunditetsregulering i forhold til kondisjon ble undersøkt over flere år. Fulton's K (K), fettinnhold og hepatosomatisk indeks økte etter starten av gytingen, selv om den ble redusert igjen mot slutten av gytingen. Økningen i GSI, fekunditet og kondisjon etter gytestart, støtter ideen om at hestemakrell er en inntektsgyter som utnytter matressurser i løpet av gytesesongen. Men nedgangen i

Samenvatting

Eiproductiemethoden (EPM) zijn met succes gebruikt in het verstrekken van advies voor het visserijbeheer. Deze methoden hebben nauwkeurige en objectieve ramingen van fecunditeit nodig. We onderzoeken de reproductieve strategie van horsmakreel en verkennen de mogelijkheid van EPM voor het schatten van de paaibiomassa. Fecunditeit en fecunditeit regulatie in relatie tot de lichaamsconditie is gedurende een aantal jaren onderzocht. Fulton's K, lipiden niveau en hepatosomatische index gingen omhoog na het start van het paaiseizoen, maar daalden alweer aan het einde van het paaiseizoen. De toename van GSI (gonadosomatische index), fecunditeit en lichaamsconditie na de start van het paaiseizoen ondersteunt het idee dat horsmakreel een inkomst paaier is, die gebruik maakt van energie opgenomen

However, the decline in K and lipid prior to the spawning season suggest the first batch of oocytes is developed on stored energy. Fecundity varied between years and within a spawning season. Over latitude variations in fecundity were small. K and lipid content are not reliable indices as proxy for fecundity. Batch fecundity appears to be heterogeneous across the spawning season but homogeneous across latitude. The homogeneity of batch fecundity over latitude could indicate that the Daily Egg Production Method is an appropriate approach for estimating abundance of a wide ranging species, as horse mackerel.

K og fett før gytestart tyder på at den første gruppen av oocytter utvikles på lagret energi. Fekunditeten varierte mellom år, innenfor en gytesesong og over breddegrad. K og fettinnhold er ikke pålitelige indekser for fekunditet. Porsjonsfekunditeten ser ut til å være heterogent fordelt i gytebestanden og mellom år, og gytebestandens fekunditet er vanskelig å anslå med gjeldende prøvetakingsmetoder. Den daglige eggproduksjonsmetoden er sannsynligvis ikke egnet til å estimere biomasse av en vidtvandrende art som hestemakrell.

uit voedsel tijdens de paaiperiode. De daling in K en lipiden voorafgaand aan de paaiperiode wijzen er echter op dat de eerste groep van eicellen ontwikkeld is uit opgeslagen energie voor de paaiperiode. Fecunditeit varieerde tussen jaren, binnen een paaiseizoen en over breedtegraad. Het is niet mogelijk om K en lipiden niveau als een indexen te gebruiken voor fecunditeit. Groep fecunditeit in heterogeen over de paaipopulatie en over de jaren en de populatie fecunditeit is moeilijk te schatten met de huidige bemonsteringsmethoden. De dagelijkse eiproductiemethode is waarschijnlijk geen adequate aanpak voor het schatten van de paaibiomassa van een soort met een zeer groot paaigebied, zoals horsmakreel.

Introduction

Egg production methods (EPM) are an important tool to estimate fish biomass in many fish stocks (Armstrong and Witthames, 2012, Bernal *et al.*, 2012, Kraus *et al.*, 2012). Despite its large cost, EPM provide fisheries-independent estimates of the spawning stock biomass (SSB) of the targeted commercial species as well as information on the spawning biology and habitat of non-commercial species spawning (Fives *et al.*, 2001, Ibaibarriaga *et al.*, 2007, Valavanis, 2008).

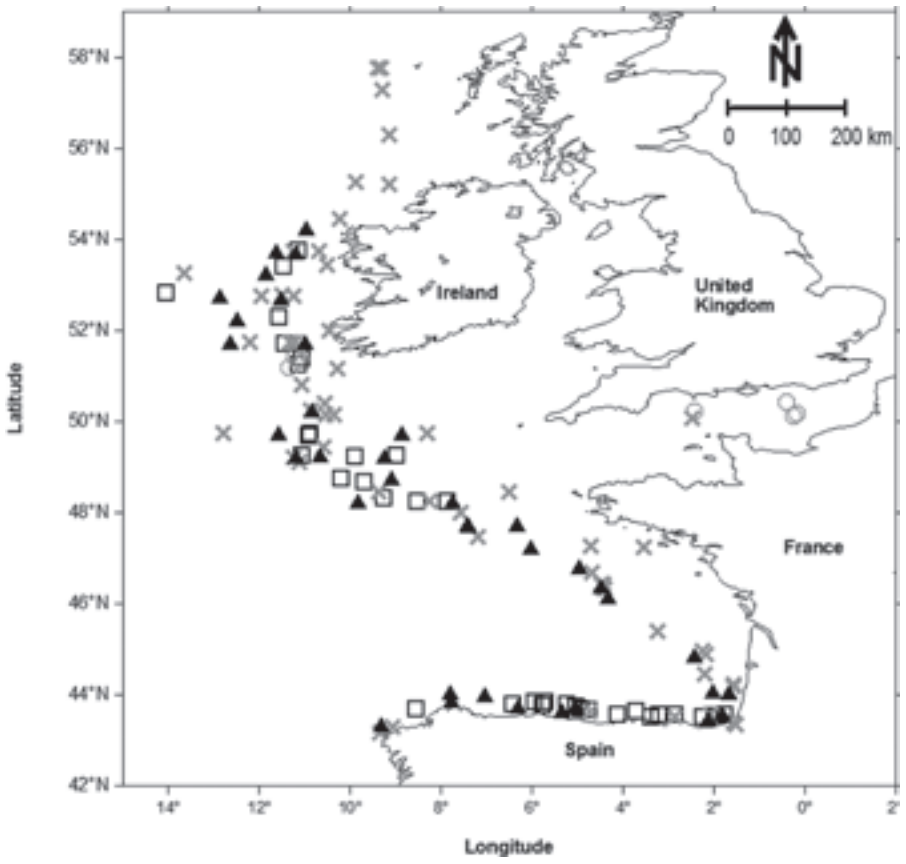
Different EPM have been developed to cope with the range of reproductive strategies in fish, of which the Annual Egg Production Method (AEPM) (e.g Armstrong *et al.*, 2001, Damme *et al.*, 2009) and Daily Egg Production Method (DEPM) (e.g. Macewicz and Hunter, 1993, Stratoudakis *et al.*, 2006, Ward *et al.*, 2011) are the most commonly used. The AEPM requires reliable estimates of the total egg production over the whole spawning season and the total fecundity (the total number of oocytes spawned from the ovary over the whole spawning season, Lockwood *et al.*, 1981). In capital breeders potential fecundity (standing stock of vitellogenic oocytes prior to spawning) is a good estimate of total fecundity. The AEPM can be used to estimate SSB in capital breeders (Armstrong *et al.*, 2001, Damme *et al.*, 2009). The DEPM has been used to estimate SSB of small pelagic fish (Lasker, 1985). The DEPM requires a reliable estimate of daily egg production and batch fecundity (the number of oocytes spawned in a single batch). Batch fecundity can be estimated in both capital and income breeders. For EPM to be used as a reliable tool in fisheries management it is necessary to have a good understanding of the reproductive strategy of the targeted species.

Reproduction is a core process in life and it requires a high investment of energy (Rijnsdorp, 1990, Smith *et al.*, 1990). Fish need to balance their surplus energy (energy available above the maintenance level) between somatic growth and reproduction (Rijnsdorp, 1990). Income breeders use their current uptake of energy for their current reproductive investment (Stephens *et al.*, 2009). But insects and marine zooplankton have been shown to use stored energy to produce a first batch of eggs and are able to utilise current food supplies for the production of subsequent batches to increase their reproductive output (Wessels *et al.*, 2010, Varpe *et al.*, 2009). It has been suggested that large organisms can cope with large energy storage whilst small organisms cannot store and carry large energy reserves and are thus income breeders (Klaassen, 2002).

Horse mackerel *Trachurus trachurus*, is a medium sized pelagic fish, which matures around 20 cm at between 2 and 4 years of age (Abaunza *et al.*, 2003, ICES, 2010). In the

northeastern Atlantic (FIGURE 4.1), the horse mackerel population has an eight month long spawning season (Abaunza *et al.*, 2003, Dransfeld *et al.*, 2005), although duration of an invidual's spawning period is unknown. Spawning in the population starts in late winter along the Portuguese coast and moves progressively northwards until it finishes in the summer west of Scotland (ICES, 2011). It is considered to be an income breeder and has an indeterminate fecundity type (Karlou-Riga and Economidis, 1997, Gordo *et al.*, 2008, Ndjaula *et al.*, 2009). Fish adopting such a spawning strategy augment their fecundity (the standing stock of vitellogenic oocytes in the ovary) from the previtellogenic oocyte population (de novo vitellogenesis) during the spawning season (Greer Walker *et al.*, 1994, Murua and Saborido-Rey, 2003). In this situation potential fecundity is an underestimate of total fecundity.

FIGURE 4.1



Sampling stations of horse mackerel in the western area in □ 2004, ○ before the spawning season 2007, X 2007 and ▲ 2010.

Due to uncertainties in the assessment, the management of horse mackerel is difficult (ICES, 2010). Every three years, an ICES coordinated survey is conducted that covers the whole horse mackerel spawning area and spawning season (FIGURE 4.1; ICES, 2011). This survey is directed at an AEPM for mackerel *Scomber scombrus* (ICES, 2011, Lockwood *et al.*, 1981). Since the potential fecundity of the indeterminate-spawning horse mackerel is an underestimate of the total fecundity, the AEPM cannot be used to reliably assess horse mackerel SSB. The triennial ICES survey does not target the sampling of spawning adult fish, thus the traditional DEPM assessment, which estimates batch fecundity and spawning females from hyaline oocytes and post-ovulatory follicles (POF's) cannot be carried out (Lasker, 1985).

The current stock assessment method uses only the total annual egg production as a relative and imprecise index of SSB, because of the absence of reliable fecundity estimates (ICES, 2010). This approach implies that fecundity is constant over time. This assumption of unvarying fecundity is likely to be incorrect, especially for income breeding species (ICES, 2002). So to improve the information used to provide fisheries advice, greater understanding of the reproductive strategy and the variability in fecundity through space and time, at various resolutions is required.

Body condition and surplus energy content are important factors that regulate fecundity and it is likely that jointly they determine the total annual fecundity of a female (Rijnsdorp, 1990, Damme *et al.*, 2009, Damme *et al.*, in prep). It has been suggested that these factors may be used as an index for interannual variation in horse mackerel fecundity (ICES, 2003a). The relationship between the proxies and fecundity needs to be strong, well described and based on a time-series before the use of the proxies will improve the management advice (De Oliveira *et al.*, 2006).

Body condition of horse mackerel in the Bay of Biscay varies over time but does not appear to change during the spawning season (Lucio and Martin, 1989). This contrasts with other pelagic fish (e.g, herring *Clupea harengus* and Sardinella, Davidson and Marshall, 2010; Hofstede *et al.*, 2007). This is possibly due to the replacement of fat by water, whereby total weight of the fish will not change. Total amount of lipid content might, therefore, give a better indication of fecundity. It has been shown in horse mackerel in captivity that lipid content increases with oocyte development from the cortical alveoli to the migratory nucleus stage (Ndjaula *et al.*, 2009).

This study investigates the oocyte and fecundity development, in relation to several metrics associated with body condition, prior to and during the spawning season

in wild north-eastern Atlantic horse mackerel. It then considers the findings within the context of fecundity regulation and the question of the possible indeterminate spawning strategy of horse mackerel. Observations on total body mass, lipid content and feeding intensity were assessed in relation to the spawning season to assess the value of these factors as indices of total individual egg production. Finally based on the results of the fecundity regulation and spawning strategy we evaluate the suitability of the different EPM's as a reliable estimate of horse mackerel SSB.

Methods

Sample collection

During the 2004, 2007 and 2010 international triennial mackerel and horse mackerel egg survey, samples were collected from freshly caught horse mackerel in pelagic trawls (Damme *et al.*, 2005, ICES, 2007b, 2009). For each female horse mackerel sampled total fish length (LT, 0.1 cm), total body weight (WT, 1 g), gutted weight (WG, 1 g), liver weight (WL, 0.1 g), and ovary weight (WO, 0.1 g) were measured. Ovaries were extracted and samples of 26 µg were taken with a solid displacement pipette (Drummond Scientific Company Wiretrol II) inserted into the ovary, through a cut in the tunica wall (Hunter *et al.*, 1989, Damme *et al.*, 2005, Witthames *et al.*, 2009). Duplicate pipette samples were taken from each fish and preserved separately in 2 ml of 3.6% buffered formaldehyde. Stomachs were removed from the fish and the stomach fullness was estimated. Fullness was divided into four categories: empty (1), partially full (2), full (3) and stuffed (4). After this procedure the whole fish, including intestines and the remains of the ovaries, were frozen separately for lipid content analysis in the laboratory.

From October 2006 until February 2007 horse mackerel females were collected from pelagic freezer trawlers. All fish were frozen hence no fecundity samples could be collected but body condition measurements were carried out.

Body condition measurements

Body condition was calculated as Fulton's condition factor K (Heincke, 1908):

$$K = \frac{W_T}{L_T^3} \qquad [4.1]$$

where WT is total weight and LT is total length. The lipid content analysis was carried

out during the 2004 and 2007 surveys. In the laboratory the whole fish (the carcass including head, skin, bones, intestines and remains of the ovaries) was shredded. The remains were homogenised and duplicate samples were analysed for lipid and water content according to Bligh and Dyer (1959) or its adaptation by Smedes (1999). Gonadosomatic index (GSI, %) was calculated as:

$$GSI = 100 * W_0 * (W_T - W_0)^{-1} \quad [4.2]$$

Hepatosomatic index (HSI, %) was calculated as:

$$HSI = 100 * W_L * W_T^{-1} \quad [4.3]$$

Fecundity analysis

The duplicate pipette samples were randomly distributed and analysed by different institutes participating in the ICES triennial survey, AZTI (Spain), IEO (Spain), IMARES (The Netherlands), IMR (Norway) and MI (Ireland). In addition some images of oocytes and samples were sent around for direct comparison of oocyte diameter measurements and fecundity estimates.

Fecundity is determined by counting the number of vitellogenic oocytes in the ovary sample. Distinguishing between previtellogenic and vitellogenic oocytes is possible by measuring oocyte diameter. For this study the oocyte diameter threshold for vitellogenic oocytes was set at 185 µm (ICES, 2005, Witthames and Greenwood, 2002, Ndjaula *et al.*, 2009). In the pipette samples, all vitellogenic oocytes were measured and counted in whole mount analysis using ImageJ (Rasband, 1997-2008). The larger share of the oocytes was detected by automatic particle analysis while the remaining oocytes were counted manually. Oocytes in early vitellogenesis are transparent and could be difficult to analyse by automatic particle analysis. To enhance the contrast, oocytes in the 2004 survey samples were therefore coloured using PAS-staining (Periodic acid followed by Schiff's reagent) or toluidine blue. The PAS staining is more intense when the oocytes are more advanced in maturation, whereas in toluidine blue all oocyte stages are coloured equally. The oocyte counts and measurements of the stained oocytes were compared to results of unstained oocytes. The results were comparable and it was decided not to stain the 2007 and 2010 oocyte samples (ICES, 2006b).

Data from the whole mount analysis was used to estimate leading cohort (LC), defined as the diameter of the 10% biggest oocytes and fecundity (total number of

vitellogenic oocytes) was calculated using the formula:

$$F = \frac{N}{s} * W_0 \quad [4.4]$$

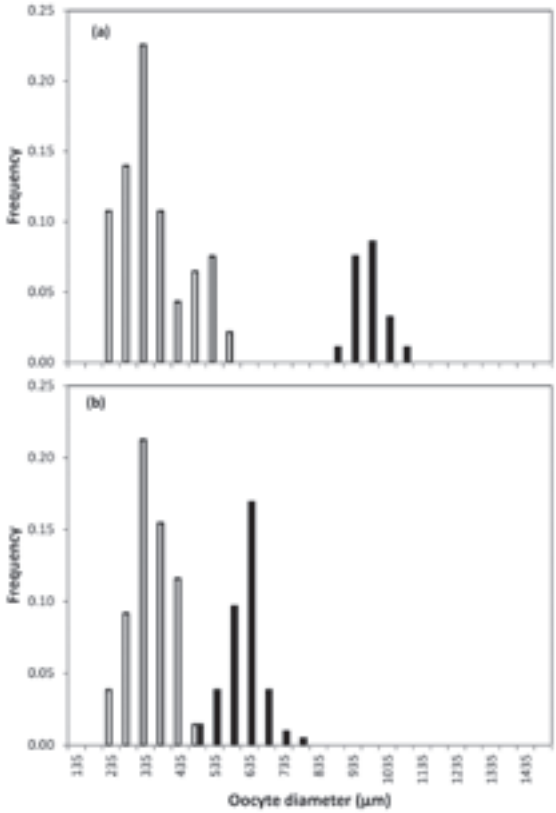
where F is fecundity, N is the total number of vitellogenic oocytes in the pipette subsample, s is the subsample weight (26 µg).

Relative fecundity FR was calculated as:

$$F_R = \frac{F}{W_T - W_0} \quad [4.5]$$

Only samples which showed no sign of spawning were used for the analysis. Macroscopically it can be difficult to assign the maturation stage. The whole mount pipette samples were checked under the microscope for spawning markers, hyaline oocytes or POF's. Whenever spawning markers were encountered, the samples were rejected for fecundity estimation.

FIGURE 4.2



Examples of batch fecundity estimation based on vitellogenic oocytes in horse mackerel in a) a female with a clearly separated batch and b) a female where the batch is visible but not clearly separated from the newly developed vitellogenic oocytes. White bars are the stock of newly developed vitellogenic oocytes, black bars are the maturing vitellogenic oocytes which will be spawned in one batch.

Usually batch fecundity is estimated from counts of hyaline oocytes. However, in our material very few samples contained hyaline oocytes and batch fecundity was estimated from the number of oocytes with diameters >450 µm, which were clearly separated from the main vitellogenic oocyte stock (FIGURE 4.2). Batch fecundity FB was calculated as:

$$F_B = \frac{N_B}{s} * W_0 \qquad [4.6]$$

Where NB is the number of vitellogenic oocytes >450 µm in a batch.

Statistical analyses were performed in SAS (ANOVA; SAS, 2011) and R (GLM; Team, 2011).

Results

Body condition

In total 1,252 females were sampled prior to (250 females) and during (1002 females) the spawning season in the different survey years (TABLE 4.1) covering the whole spawning area (FIGURE 4.1). Mean total fish length differed between the years (ANOVA, $p < 0.001$), fish caught in 2010 were larger compared to 2004 and 2007. K, lipid content, HSI and GSI differed significantly between the years and months (GLM, K: all years $p < 0.008$, all months except November $p < 0.039$; Lipid: all years and months $p < 0.002$; HSI: 2007 $p = 0.05$, all months $p < 0.017$; GSI: all years $p < 0.031$, March – July $p < 0.001$; FIGURE 4.3). K varied between 0.65 and 1.12, and was high from October till January but was lowest in February just prior to the spawning season (FIGURE 4.3A). During the spawning season K increased till June but dropped again at the end on the spawning season in July (FIGURE 4.3A). Lipid content was also high from October till December but decreased in January and February with the lowest lipid content just prior to the spawning season (FIGURE 4.3B). In March at the start of the spawning season the lipid content had increased, but decreased again during the spawning season. At the end of the spawning season, in June and July, lipid content increased again (FIGURE 4.3B). It was not possible to collect the livers from the frozen females that were collected outside the spawning season. During the spawning season HSI increased (FIGURE 4.3C). GSI was low and showed only a slight increase before the start of the spawning season from October till February (FIGURE 4.3D). During the spawning season GSI showed a big increase but dropped again at the end of the spawning season (FIGURE 4.3D).

TABLE 4.1

Numbers of horse mackerel females and average total fish lengths (standard deviation in brackets) sampled during the surveys.

Month	Numbers			Fish length (cm)		
	2004	2007	2010	2004	2007	2010
October	-	50	-	-	26.9 (2.4)	-
November	-	50	-	-	25.4 (2.2)	-
December	-	50	-	-	27.8 (3.1)	-
January	-	50	-	-	27.1 (1.4)	-
February	-	50	-	-	29.1 (1.3)	-
March	122	45	5	28.5 (3.1)	27.2 (3.3)	30.9 (1.8)
April	163	182	55	28.0 (2.6)	28.3 (4.2)	30.8 (3.7)
May	30	139	80	28.3 (2.0)	28.9 (3.6)	31.4 (2.8)
June	60	60	8	30.5 (3.1)	27.5 (2.9)	28.6 (2.3)
July	-	39	16	-	27.7 (1.8)	33.5 (2.1)

Body condition was also variable over the latitudinal range of the spawning area (FIGURE 4.4). K was significantly different over the latitudinal range and between the years (GLM, 48 – 54, 56, 57°N $p < 0.018$). K decreased from south to north (all latitudes have a negative effect on K), though the most northern transect has the highest but also the most variable K values (FIGURE 4.4A). Lipid content increased from south to north (FIGURE 4.4B) and was significantly different over the transects and years (GLM, 44, 48 – 53, 55, 57°N $p < 0.001$). HSI was highly variable and seemed to be highest in the middle of the spawning latitudinal range (GLM, 44, 47 - 57°N $p < 0.025$; FIGURE 4.4C). GSI decreased significantly from south to north (GLM, 47, 48, 50 – 57 $p < 0.001$; FIGURE 4.4D).

Prior to the spawning season, most stomachs were partially full or full, while empty stomachs were only found in October (TABLE 4.2). During the spawning season stomachs were either empty or partially full (TABLE 4.2). Empty stomachs were found in all months but highest numbers were found at the beginning (March) and end (July) of the spawning season (TABLE 4.2). Full stomachs were rare during the spawning season. Fully stuffed stomachs were rare both prior to and during the spawning season (TABLE 4.2). During sampling no signs of regurgitation were found.

75% of females with spawning markers, POF's or hyaline oocytes, had an empty while 22% had a partially full stomach

FIGURE 4.3

Body condition of horse mackerel; a) Fulton's condition factor K, b) lipid content, c) hepatosomatic index and d) gonadosomatic index in □ 2004, X 2007 and ▲ 2010.

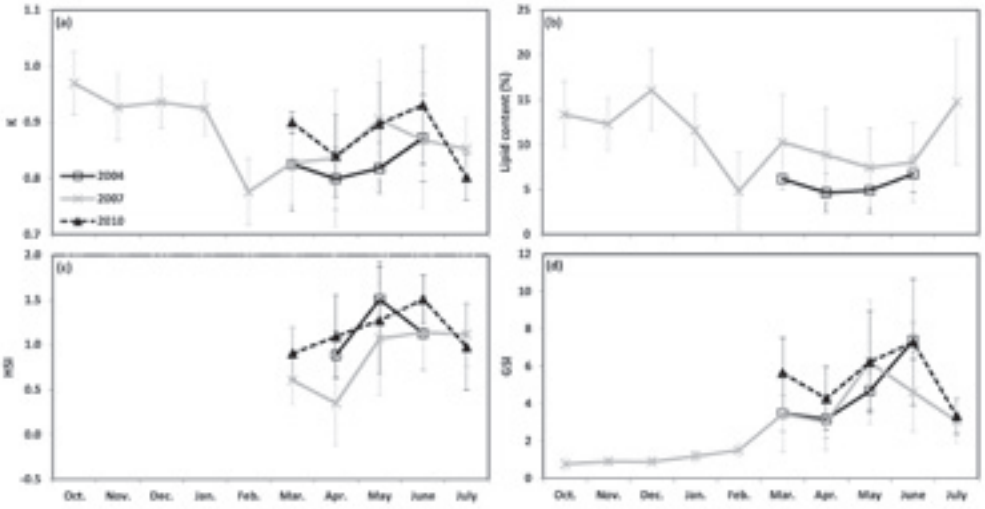


FIGURE 4.4

Body condition of horse mackerel over the latitudinal range; a) Fulton's condition factor K, b) lipid content, c) hepatosomatic index and d) gonadosomatic index in □ 2004, X 2007 and ▲ 2010.

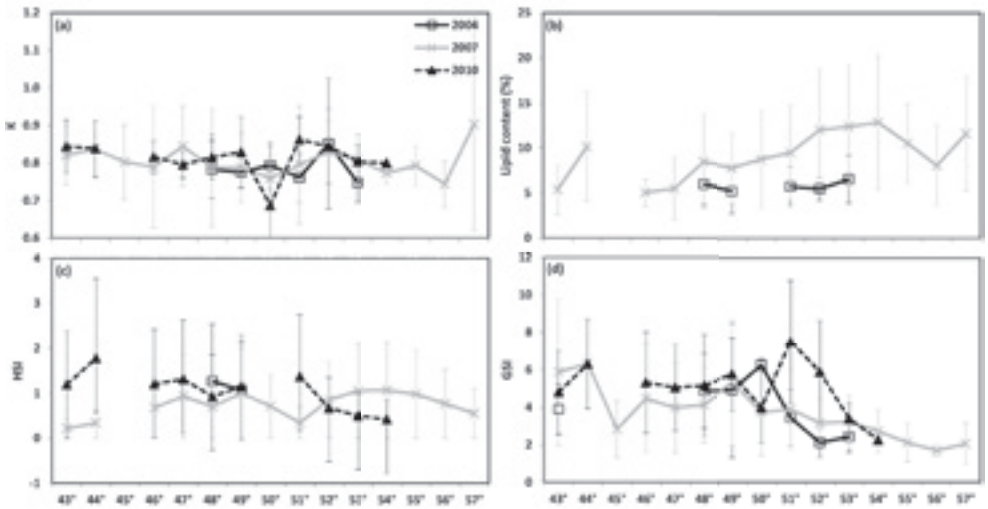


TABLE 4.2

Stomach fullness of spawning horse mackerel.

Year	Month	Empty	Partially full	Full	Stuffed
2004	March	0.95	0.05	-	-
	April	0.97	-	0.03	-
	May	0.48	0.33	0.15	0.04
	June	0.82	0.15	0.03	-
2007	October	0.22	0.68	0.10	-
	November	-	0.38	0.62	-
	December	-	0.14	0.86	-
	January	-	0.06	0.94	-
	February	-	0.26	0.74	-
	March	0.81	0.19	-	-
	April	0.28	0.67	0.04	-
	May	0.20	0.47	0.29	0.04
	June	0.51	0.44	0.05	-
	July	0.92	0.08	-	-
2010	May	0.43	0.36	0.14	0.07
	June	0.25	0.25	0.50	-

Fecundity

In 2004 images of ovary samples of 8 females and ovary subsamples of 29 females were sent round the analysing institutes for comparison of the oocyte image analysis method (TABLE 4.3). Based on the images no difference was found in oocyte counts between the institutes (ANOVA, $p = 0.780$). No significant difference was found in the estimation of fecundity of the 29 females between the institutes (ANOVA, $p = 0.051$), but there was however a significant difference in the oocyte diameter measurements (ANOVA, $p = 0.001$). All other duplicate ovary subsamples were analysed randomly by the different institutes. Oocyte diameter measurements and fecundity estimates differed between the analysing institutes (TABLE 4.4), but the institutes analysed different samples. Mean oocyte diameter, LC and FR differed significantly between the institutes in all the years

(ANOVA, Mean diameter: $p < 0.000$; LC: $p < 0.000$; FR: $p < 0.001$). In 2007 the fecundity estimates of institute 2 were much higher compared to the other institutes, while in 2010 the fecundity estimates of institute 5 were much lower compared to the other institutes (TABLE 4.4). Since these differed considerably it was decided not to use these estimates in the fecundity, oocyte diameter and LC analysis.

TABLE 4.3

Institute	Mean oocyte diameter (µm)	FR (oocytes per gram)
3	364.7 (53.1)	648.0 (226.3)
4	439.4 (62.9)	779.8 (399.7)
5	393.4 (55.9)	568.6 (225.0)

Comparison between institutes of horse mackerel oocyte diameter measurements and fecundity estimates (standard deviation in brackets) based on ovary subsamples of 29 females.

TABLE 4.4

Comparison between institutes of horse mackerel oocyte diameter measurements and fecundity estimates based on all analysed ovary subsample analyses.

Year	Institute	Number subsamples analysed	Mean oocyte diameter (µm)	Leading cohort (µm)	F_g (oocytes per gram)
2004	1	-	-	-	-
	2	83	307.0 (45.4)	464.7 (125.6)	607.2 (305.0)
	3	158	361.5 (68.2)	564.8 (94.9)	685.3 (358.2)
	4	48	430.8 (61.7)	-	666.1 (420.3)
	5	191	391.4 (71.1)	594.9 (98.5)	648.5 (376.0)
2007	1	-	-	-	-
	2	121	331.1 (69.2)	525.4 (137.3)	1131.0 (402.7)
	3	236	387.5 (75.1)	387.5 (75.1)	676.6 (354.5)
	4	137	370.3 (50.9)	573.1 (72.6)	635.0 (290.9)
	5	223	449.1 (90.3)	650.5 (149.1)	537.6 (273.5)
2010	1	32	335.1 (48.4)	599.1 (36.6)	1036.6 (462.3)
	2	39	270.0 (47.5)	561.1 (35.2)	1080.0 (467.2)
	3	68	316.4 (41.6)	592.3 (31.9)	1112.1 (505.2)
	4	55	341.1 (44.5)	619.8 (33.5)	1106.4 (450.0)
	5	105	373.9 (45.0)	599.6 (30.4)	743.2 (375.6)

Of the 1002 females sampled during the spawning season 33 contained spawning markers and were not used for the fecundity analysis. All females which had spawning markers were caught during the night.

F and FR were significantly higher in 2010 (GLM, $p < 0.001$; FIGURE 4.5 and TABLE 4.5). F and FR increased significantly from the start of the spawning season, but decreased again towards the end of spawning (GLM, May and June $p < 0.001$). FB was not significantly different between the years (FIGURE 4.5 and TABLE 4.5). However, relative FB was significantly higher in 2007 (GLM, $p < 0.001$; FIGURE 4.5D). Similarly like F, FB increased significantly from the start of the spawning season and decreased again to the end of the spawning (GLM, May and June $p < 0.001$; FIGURES 4.5C & 4.5D).
Both F and FB only showed a significant difference on transects 49 and 51°N (GLM, $p < 0.015$; FIGURE 4.6).

FIGURE 4.5

Fecundity development over the spawning season a) total number of vitellogenic oocytes, b) relative fecundity, c) batch fecundity and d) relative batch fecundity in □ 2004, X 2007 and ▲ 2010.

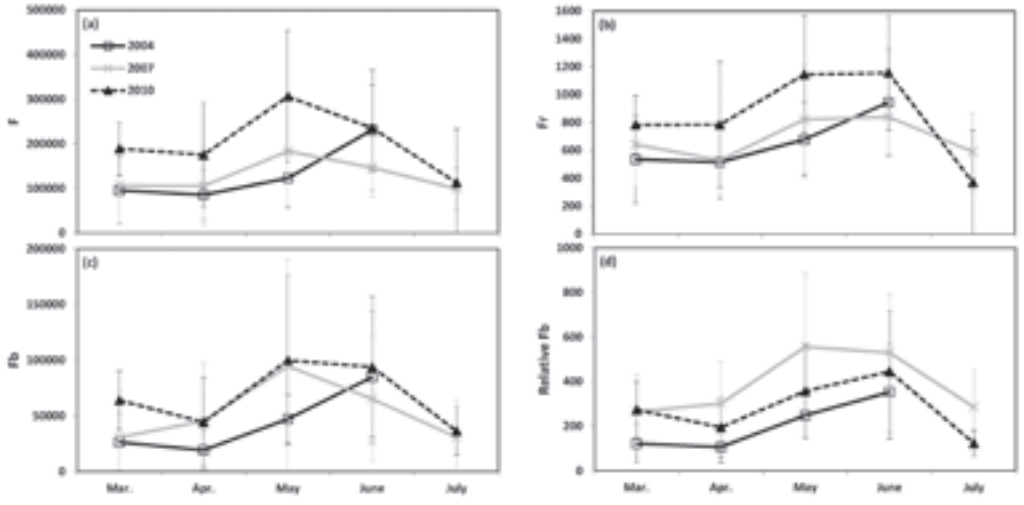
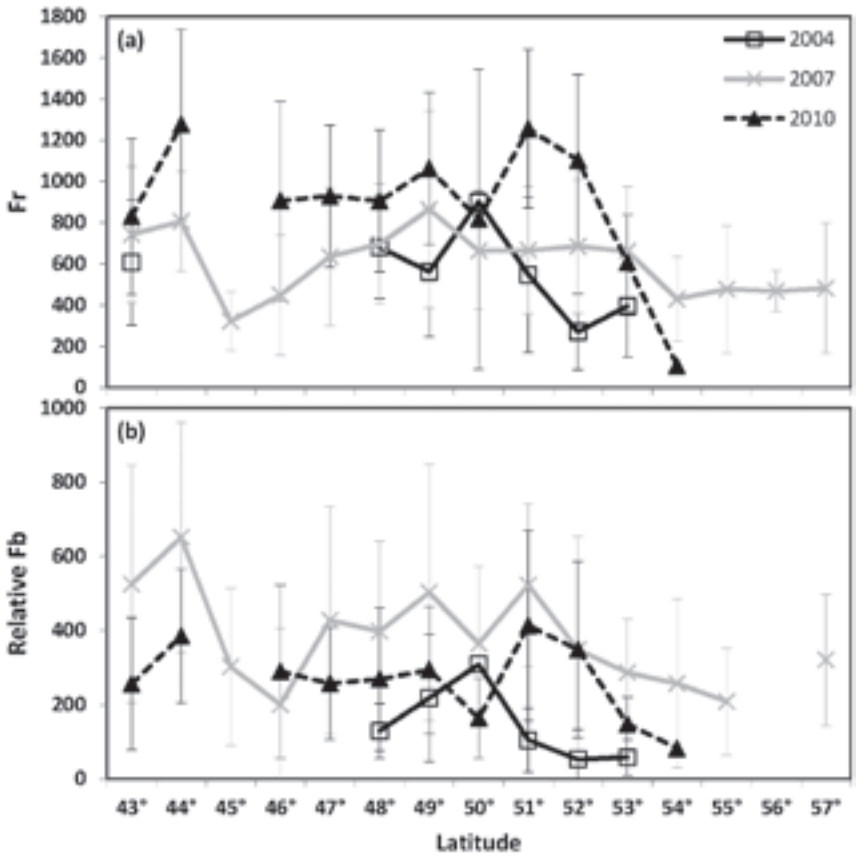


TABLE 4.5

Horse mackerel fecundity and oocyte diameters in the different years (standard deviation in brackets)..

Year	Mean oocyte diameter (µm)	LC	Total fecundity (N of oocytes)	Relative fecundity (N oocytes per gram)	Relative batch fecundity (N oocytes per gram)
2004	352.1 (69.4)	543.3 (115.3)	11.5*104 (9.6*104)	603.5 (342.1)	193.4 (172.7)
2007	385.9 (90.6)	509.7 (159.0)	13.3*104 (10.4*104)	671.1 (337.3)	406.2 (275.7)
2010	316.9 (54.2)	590.2 (37.4)	23.8*104 (14.4*104)	956.8 (457.4)	284.6 (199.3)

FIGURE 4.6

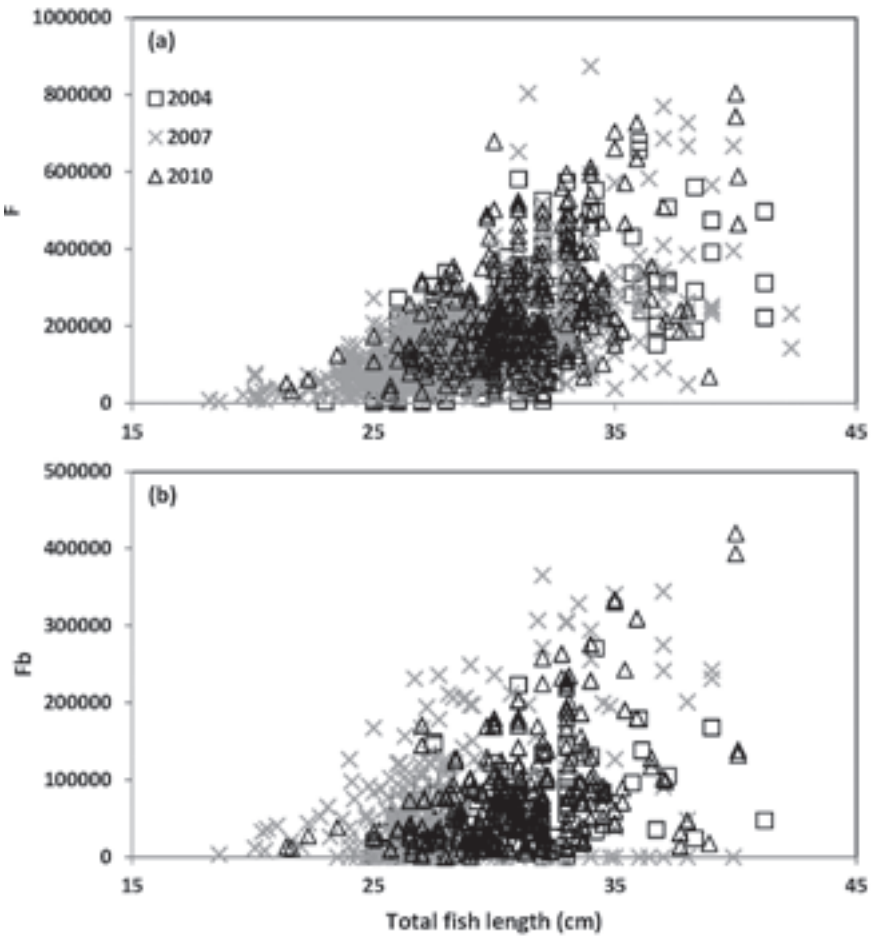


Fecundity development over the latitudinal range a) relative fecundity and b) relative batch fecundity in □ 2004, X 2007 and ▲ 2010.

Mean oocyte diameter and LC were significantly different between the years (GLM, diameter all years $p < 0.001$; LC 2007 $p < 0.001$). Mean oocyte diameter was highest in 2007 and lowest in 2010, while LC was lowest in 2007 (TABLE 4.5).

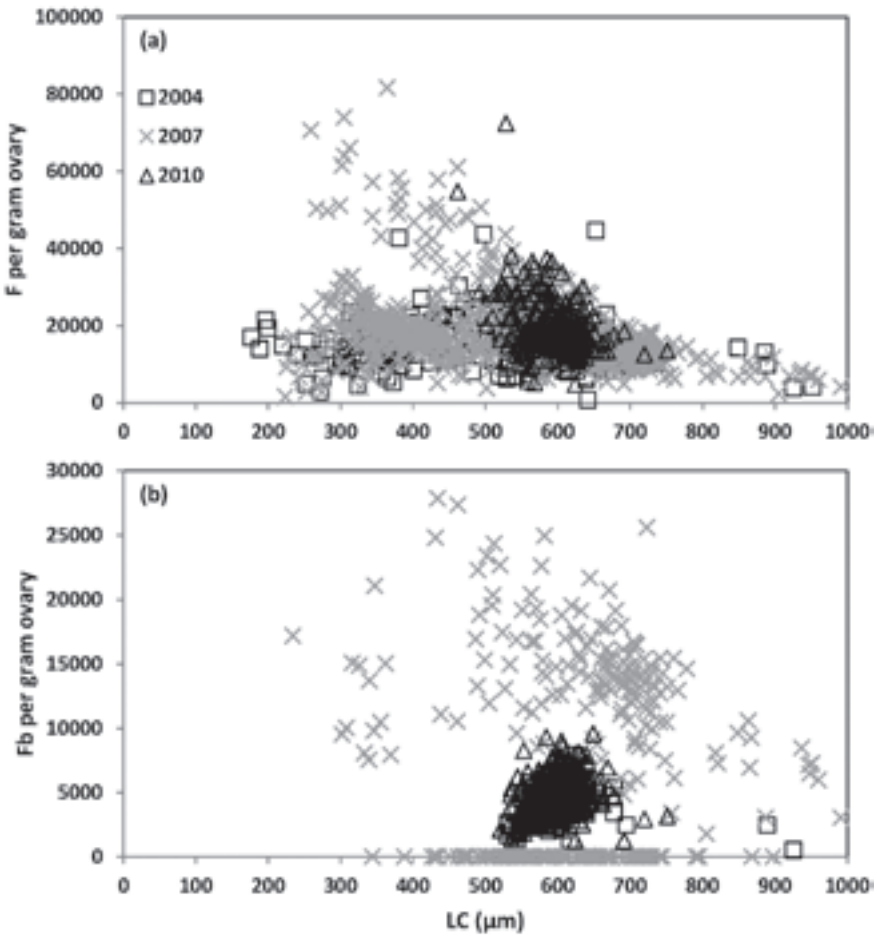
Both F and FB increased with size of the females (FIGURE 4.7). However, the fit of the curve for each year is very poor and no significant differences were found between the years. Relative F seems to decrease with increasing oocyte diameter size (FIGURE 4.8) though the fit is poor, 2010 was significantly different from 2004 and 2007 (GLM, $p < 0.001$). Similarly FB seems to decrease with increasing oocyte diameter though here the fit is also poor (FIGURE 4.8).

FIGURE 4.7



Horse mackerel total number of vitellogenic oocytes and batch fecundity in different length classes during the spawning season in □ 2004, X 2007 and ▲ 2010.

FIGURE 4.8



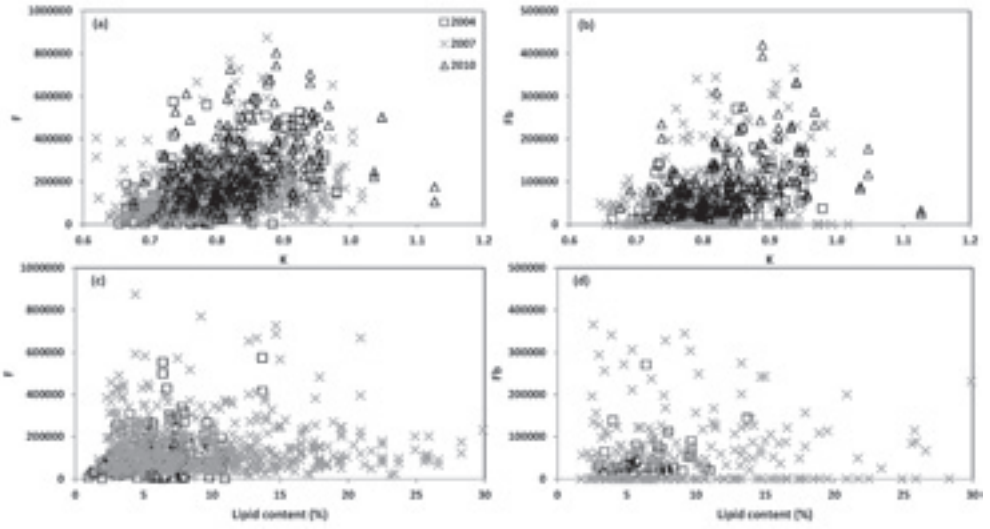
Fecundity down-regulation in horse mackerel in a) relative fecundity and b) relative batch fecundity in □ 2004, X 2007 and ▲ 2010.

Fecundity and body condition

Though the relationship between Fulton's K and fecundity is highly variable, both F and FB seem to increase with increasing K (FIGURE 4.9a & 4.9B). No relationship can be found between lipid content and fecundity (FIGURE 4.9c & 4.9D).

FIGURE 4.9

Body condition, a, b) K and c, d) lipid content, regulating total number of vitellogenic oocytes and batch fecundity during the spawning season in □ 2004, X 2007 and ▲ 2010



Discussion

Our study has shown that horse mackerel exhibits variable reproductive characteristics and body condition changes over space and time. Body condition and lipid content decreased just prior to the spawning season but both increased again after the start of spawning, though body condition decreased again at the end of spawning. HSI increased during spawning but dropped at the end of the spawning season. GSI was very low before the onset of spawning but increased during the spawning season and decreased at the end of spawning. F and FB increased after the onset of spawning and decreased at the end of the spawning season. Relative F and FB seem to decrease with increasing oocyte diameter size in 2007 suggesting down-regulation (reduction of the numbers of vitellogenic oocytes (Óskarsson *et al.*, 2002, Kurita *et al.*, 2003)) of fecundity. F and FB increased with in increasing K, but no relationship was found with lipid content. Bigger females had a higher number of vitellogenic oocytes and produced bigger oocyte batches.

Although the trials with exchanged images and subsamples showed no significant difference in oocyte counting between the analysing institutes, significant differences in oocytes diameter measurement and fecundity estimates were found between the

analysing institutes when comparing the final analysis. Part of this can be explained by methodological approaches. The final fecundity analysis was carried out on different subsamples of the fish. Pipette sampling is carried out on board the survey vessels in varying weather conditions and by different people. Pipette sampling needs to be done carefully with no fluid or air trapped in the pipette. Small differences in the subsamples taken from one fish can lead to big differences when the subsample estimate is raised to the total ovary size to estimate total number of vitellogenic oocytes.

All females with spawning markers were caught during the night, suggesting that horse mackerel spawn at night time. Other studies of horse mackerel do not report spawning time of horse mackerel, but jack mackerel, Trachurus symmetricus, are also night time spawners (Macewicz and Hunter, 1993).

Before the spawning season most females showed signs of feeding, only few empty stomachs were found. At the start and end of the spawning season high numbers of empty stomachs were found. Oocyte final maturation, spawning and mating require high energy and oxygen demands restricting other activities (Kjesbu *et al.*, 1998, Rijnsdorp and Ibelings, 1989). Hence, fish cease feeding during actual spawning. The stomachs containing food items during the spawning season suggest that in between the spawning of oocyte batches horse mackerel does feed. Earlier studies on horse mackerel diet in the Atlantic, North Sea and Adriatic show that many stomachs are empty, especially during the spawning season (Cabral and Murta, 2002, Dahl and Kirkegaard, 1986, Dahl and Kirkegaard, 1987, Jardas *et al.*, 2004, Sahrhage, 1970). These studies also show a clear diurnal feeding pattern. No such pattern was found in this study, probably as most sampling of females occurred during the daytime.

Condition factor K and lipid content dropped significantly just prior to the onset of the spawning season. During the spawning season K, lipid content and HSI increased. This is in contrast to Lucio & Martin (1989), who did not find a change in body condition during the spawning season of horse mackerel in the Bay of Biscay. Horse mackerel in captivity did show an increase in K, lipid content and HSI during oocyte development (Ndjaula *et al.*, 2009). GSI only showed a slight increase before the start of the spawning season and showed a big increase at the start of spawning. The study of captive horse mackerel also showed an increase of GSI with growth of the oocytes (Ndjaula *et al.*, 2009). This seems to suggest that oocyte development and growth before the spawning season is minimal and major oocyte development occurs during the spawning season, suggesting an indeterminate fecundity type. Horse mackerel are also

able to increase their body condition during the spawning season suggesting that they utilise the food resources during the spawning season directly for oocyte development. This supports the idea of horse mackerel having an income breeding strategy and indeterminate fecundity. Other field studies in the Bay of Biscay (Gordo *et al.*, 2008) and the Mediterranean (KarlouRiga and Economidis, 1996) and a study of captive western horse mackerel (Ndjaula *et al.*, 2009) indicated an indeterminate fecundity. Although the fecundity development during the spawning season suggests an indeterminate fecundity, the drop in K and lipid just prior to the start of spawning could indicate that the first batch of oocytes is develop from stored energy. This has not been shown before in indeterminate fish although it has been observed in insects and marine zooplankton (Wessels *et al.*, 2010, Varpe *et al.*, 2009).

Relative FB varied from 193 oocytes per gram female in 2004 to 406 oocytes per gram female in 2007. Batch fecundity found in the southern horse mackerel off Portugal was on average 200 oocytes per gram female (Goncalves *et al.*, 2009, Abaunza *et al.*, 2008a) and 205 oocytes per gram female in the Mediterranean (Karlou-Riga and Economidis, 1997). These studies used the histological method to identify hydrated oocytes and POF's for estimating batch fecundity. Average batch fecundity in 2004 was comparable to the batch fecundities found in the Portuguese and Mediterranean studies, but in 2007 and 2010 the mean batch fecundity was much higher. FB of the 33 females that contained POF's or hydrated oocytes did not differ from the FB of the females without spawning markers. However, POF's and hyaline oocytes are difficult to identify using the image analysis method and can only be reliably identified using histology (P. Goncalves, pers. comm.). Although batch fecundity estimates in this study are higher, the (in this study not significant) decrease in batch fecundity with increasing latitude is also found by (Abaunza *et al.*, 2008a).

Bigger females have a higher batch fecundity. This increase with increasing size has been shown in other studies (Abaunza *et al.*, 2008a, Karlou-Riga and Economidis, 1997).

Lipid content peaked in December prior to spawning. Lucio and Martin (1989) showed a sharp increase in lipid content from October to November, with the peak occurring in November, for horse mackerel in the Bay of Biscay. Lipid content decreased before the onset of spawning and increased during the spawning period. No clear relation is found between lipid content and fecundity. Lipid content will be difficult to use and should not be considered as a reliable proxy for fecundity in a stock assessment. Fecundity seems to increase with increasing K, but the relationship is weak. K is

probably not a reliable proxy to use for fecundity either. Thus it is unlikely that either lipid content or Fulton's K can act as a proxy for fecundity, especially when considering the assertion of De Oliveira *et al.*, (2006) that the correlations must be strong. Recent studies have shown that Fulton's K is not a good proxy for muscle fat (Davidson and Marshall, 2010) or mesenteric fat (McPherson *et al.*, 2011) content. There is also no standard unit for Fulton's K and thus as a metric it is not transferable between fish (Coilin Minto, GMIT, Ireland, pers com). Body condition should be validated against a direct biochemical measurement before they can be used as a proxy (Davidson and Marshall, 2010, McPherson *et al.*, 2011).

Fecundity development prior and during the spawning season and feeding during the spawning season suggests horse mackerel is an income breeder with indeterminate fecundity type. Since potential fecundity will be an underestimate of total fecundity and condition factors are unreliable proxies for total fecundity, the AEPM will not produce a reliable estimate of horse mackerel SSB. The DEPM has been used to estimate SSB of small pelagic income breeders (Somarakis *et al.*, 2004, Lasker, 1985, Ward *et al.*, 2009). However, it has been shown that the DEPM is vulnerable to changes in batch fecundity (Somarakis *et al.*, 2004, Stratoudakis *et al.*, 2006). Peak spawning in horse mackerel occurred in June in all years (ICES, 2012b). FB of horse mackerel varies within a spawning season and on only two of the 15 sampled transects was FB significantly different. Relative FB was significantly different in 2007 compared to the other years. The pelagic species for which DEPM has been used to estimate SSB all have a considerable smaller spawning area, especially when compared to western horse mackerel. But batch fecundity has been found to also vary over time and between stocks (Ganias *et al.*, 2004, Somarakis *et al.*, 2002, Stratoudakis *et al.*, 2006). This suggests that an ad hoc approach to sampling spawning adults is not appropriate. Under the current sampling regime DEPM could be a reliable method for estimating SSB of stocks, such as horse mackerel. However, we advise that before using this method in management strategies, an extensive analysis of an SSB time-series should be carried out to assess the reliability of DEPM for Atlantic horse mackerel.

The results of this study support the idea that horse mackerel is an income breeder and has an indeterminate fecundity. Thus, the annual egg production method is certainly not appropriate to determine trends in spawning adult abundance. Horse mackerel spawning occurs at night. The first batch of oocytes probably develops using stored energy as suggested by the sharp decline in K and lipid content prior to the

spawning season. During the spawning season GSI, K and lipid content increase and drop again at the end of spawning. K and lipid content are not reliable indices to use as a proxy for fecundity in assessments. The homogeneous distribution of batch fecundity over space and time suggests that the Daily Egg Production Method is probably an appropriate approach for estimating the abundance of a wide ranging species, such as horse mackerel. However, to apply the DEPM much greater and more carefully targeted sampling of spawning adults would be required as well as an extensive analysis of SSB estimations based on the available survey data.

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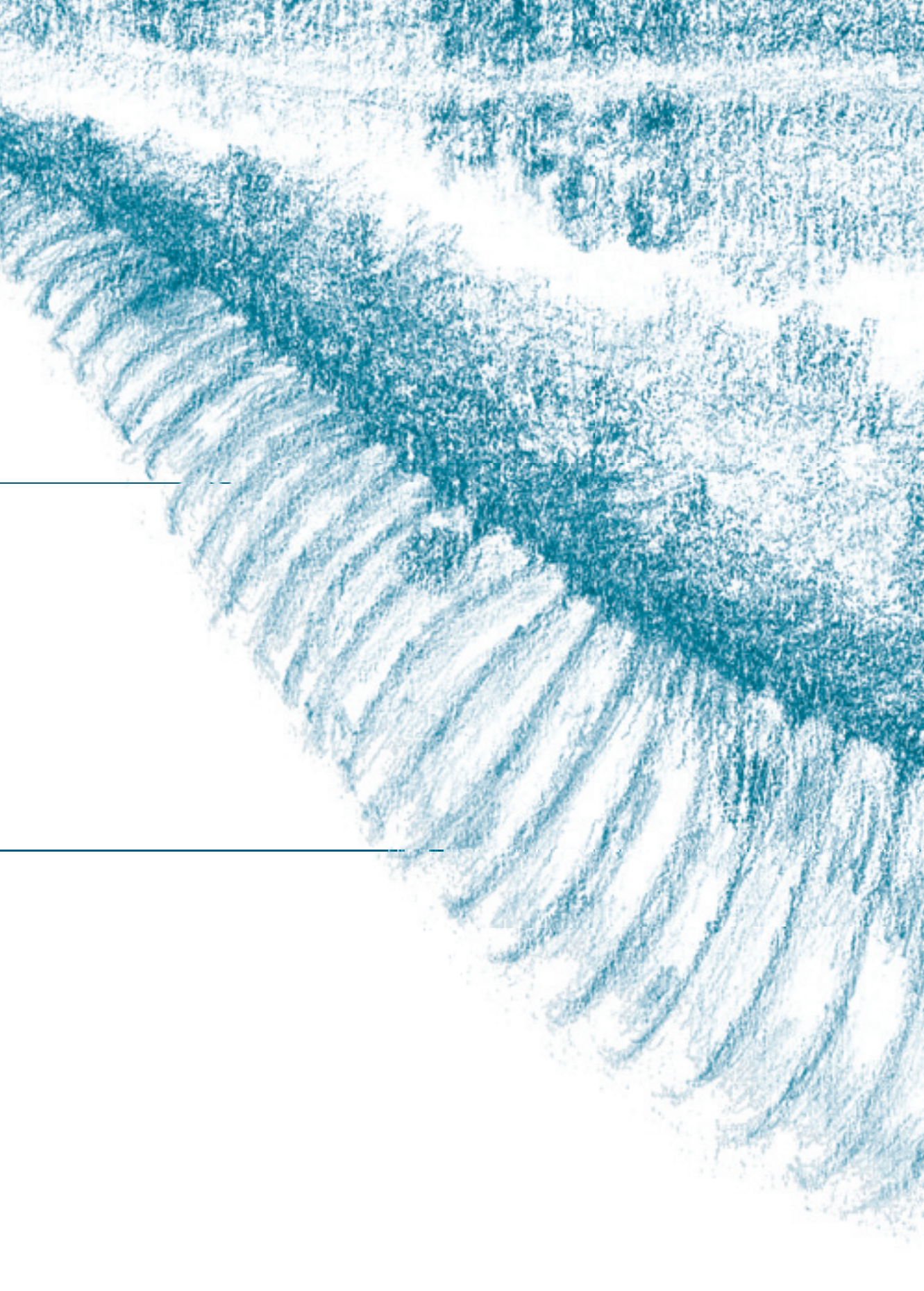

Chapter 5

A reanalysis of North Sea plaice spawning-stock biomass using the annual egg production method

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Abstract

There is uncertainty about the quality of current VPA-based stock assessment for North Sea plaice *Pleuronectes platessa*. Biomass estimates from the Annual Egg Production (AEP) method are compared to current catch-at-age based stock assessments to validate the current and historic perception of exploitation. The AEP method also allows us to investigate the dynamics of the spatial components of plaice in the North Sea. Importantly, we correct for fecundity down-regulation and changes in sex ratio. Estimates from both methods are similar in trend and absolute biomass. On the Dogger Bank there has

Sammendrag

Usikkerhet om kvaliteten på gjeldende metodikk for virtuelle populasjons baserte bestandsberegninger for rødspette i Nordsjøen *Pleuronectes platessa* har ført til ulike bestandsindekser. For å validere gjeldende og historisk oppfatning av beskatningen sammenlignet vi biomasseanslag fra årlig eggproduksjonsmetode (AEP) med gjeldende bestandsvurderinger basert på alder ved fangst. AEP-metoden ble også brukt til å undersøke dynamikken i de romlige komponentene for rødspette i Nordsjøen. Vi korrigerte for nedregulering i fekunditet, og endringer i forhold til kjønn. Estimater fra begge metodene var lik i trenden

Samenvatting

Onzekerheid over de kwaliteit van de huidige virtuele paaibiomassa schatting van de visstand voor Noordzee schol *Pleuronectes platessa* heeft geleid tot gebruik van diverse abundantie indices. Wij vergeleken paaibiomassa schattingen van de jaarlijkse eiproductiemethode (AEP) met de huidige paaibiomassa schatting op basis van de vangst-op-leeftijd voor het valideren van de huidige en historische perceptie van exploitatie van de biomassa. De AEP methode werd ook gebruikt om de dynamiek van de ruimtelijke paaicomponenten van schol in de Noordzee te onderzoeken. Fecunditeit is gecorrigeerd voor de naar beneden regulering en wijzigingen in de sekse ratio over tijd zijn ook

been a dramatic decline in biomass from the 1940s to 2004, and in the Southern Bight the stock appears to increase from the 1980s to 2004, but not yet up to the biomass of the 1940s. There appears to be no major change in timing of spawning of North Sea plaice, throughout the period of high exploitation. We conclude that the AEP method is a useful way to hindcast spatial dynamics of heavily exploited flatfish stocks.

og på absolutt biomasse. På Doggerbank var det en dramatisk nedgang i biomasse fra 1948 og 1950 til 2004, og i det sørlige Bight, syntes bestanden å øke fra 1987 og 1988-2004, selv om den ikke nådde de historisk høye nivåene fra 1948 eller 1950. Tidspunktet for gyting hos rødspette i Nordsjøen synes ikke å ha endret seg i hele perioden med høy beskatning. Vi konkluderer med at AEP-metoden er et nyttig verktøy for tilbakeberegning av romlig dynamikk av tungt beskattede bestander av flyndrefisker.

meegenomen. Schattingen van beide methodes waren vergelijkbaar in trend en absolute paaibiomassa. Op de Doggersbank, was er een dramatische daling in de paaibiomassa van 1948 en 1950 tot 2004, en in de Zuidelijke Bocht bleek de paaibiomassa te stijgen van 1987 en 1988 tot 2004, hoewel deze niet de historisch hoge niveaus van 1948 en 1950 bereikte. Het moment van het paaien van Noordzee schol lijkt niet te zijn veranderd gedurende de periode van hoge exploitatie. We concluderen dat de AEP een bruikbare methode is om de ruimtelijke dynamiek van zwaar uitgebuite platvis paaibiomassa terug te rekenen.

Introduction

The Annual Egg Production (AEP) method has been used successfully to estimate spawning stock biomass in determinant spawning fish (Parker, 1980; Lasker, 1985; Armstrong *et al.*, 1988; Hunter and Lo, 1993; Armstrong *et al.*, 2001) and is based on dividing the population annual egg production by the average individual production. It has proved useful to investigate the trends in SSB in certain stocks (Simpson, 1959; Lockwood *et al.*, 1981; Priede and Walsh, 1991), one off SSB estimates (Bulman *et al.*, 1989; Zeldis, 1993), to compare biomass estimates derived from catch-at-age based stock assessments with fishery independent assessments (Daan, 1981; Heessen and Rijnsdorp, 1989; Horwood, 1993a; b; Armstrong *et al.*, 2001 Zenitani, *et al.*, 2001), or to examine the spatial distribution of spawning components (Fox *et al.*, 2000; Heffernan *et al.*, 2004). A similar technique (the larvae production estimate LPE), has also been used to investigate the trends in stocks with attached or benthic eggs (Nichols *et al.*, 1987; Heath, 1993; Fossum, 1996; Briggs *et al.*, 2002). It can be argued that the SSB estimates from AEP are more useful for fish ecology or management than those derived from aged based models as they are a fisheries-independent method, measure reproductive production directly, have a higher spatial resolution and are not inferred from virtual population analysis derived matrices of numbers and weights at age. Also the assumptions made when calculating an AEP estimates are often different from those made in a virtual population analysis (Armstrong *et al.*, 2001). The AEP method is fisheries-independent assessment and can be used in recovery situations where fishing effort must be reduced to rebuild SSB and thus no market samples are available. In addition, the estimate of SSB obtained from the AEP method is not influenced by movements of fish out of the management area after spawning. There are also some disadvantages; all spawning areas need to be sampled throughout the spawning season and forecast possibilities are limited.

North Sea plaice *Pleuronectes platessa* is a commercially important species and it is mainly exploited by a beam trawl fleet that targets both sole (*Solea solea*) and plaice (Daan, 1997). There is substantial discarding of plaice by the beam trawl fishery and this has complicated both the stock assessment and stock management (Casey, 1996; Rijnsdorp and Millner, 1996; Dickey-Collas *et al.*, 2007).

Spawning plaice are widely distributed throughout the English Channel and the southern and central North Sea (Buchanan-Wollaston, 1923; Bannister *et al.*, 1974; Houghton and Harding, 1976; Harding and Nichols, 1987) although rarely beyond the

50m depth contour (Harding *et al.*, 1978). The nursery grounds are inshore on sandy flats (Zijlstra 1972, Beek *et al.* 1989) and plaice gradually move offshore as they grow larger (Wimpeny, 1953). Spawning commences in December in the eastern English Channel and is progressively later moving northwards through the North Sea (Bagenal, 1966). Peak spawning occurs in mid-January in the Southern Bight, and in February-March in the more northern regions (Simpson, 1959; Harding *et al.*, 1978).

Plaice are determinate batch spawners (Urban, 1991; Armstrong *et al.*, 2001; Murua and Saborido-Rey 2003). Fecundity (the standing stock of advanced vitellogenic follicles) is clearly separated from precursor previtellogenic follicles well before the onset of spawning. We use the term follicle meaning the developing oocyte and the maternal follicle granulosa and theca layers (Tyler and Sumpter, 1996). At the start of maturation fecundity is high. During the maturation cycle fecundity is down-regulated through follicular atresia according to the energy reserves of the female (Kennedy *et al.*, 2007b; 2008) but is not thought to cause further loss after spawning commences (Nash *et al.* 2000). During spawning, pelagic eggs are released in batches (Rijnsdorp, 1989; Urban, 1991). Spawning of an individual female occurs over a period of 4 to 6 weeks (Rijnsdorp, 1989). Fecundity varies between the spawning areas, with fecundity decreasing from the eastern English Channel to the German Bight (Horwood *et al.*, 1986; Rijnsdorp, 1991). However interannual variation in fecundity can be considerable (Horwood *et al.*, 1986; Rijnsdorp, 1991).

The spatial distribution of plaice has reportedly changed in recent years (Keeken *et al,* 2007), and there is anecdotal evidence coming from the fishery that the relative distribution of the adults may also have changed. Plaice in the southern North Sea is partially managed through spatial closures, designed to protect juvenile plaice (Rijnsdorp and Beek, 1991; Pastoors *et al.*, 2000). There are also predictable 5.patterns in the seasonal and spatial distribution of the population, which relate to the migration patterns between spawning and feeding areas and the recruitment (Rijnsdorp *et al.*, 2005). Plaice in the North Sea and English Channel are managed as different stocks, and it is assumed that no mixing occurs (ICES, 2003b) between these stocks. Recent studies show that there is considerable mixing (Hunter *et al.*, 2003b; Bolle *et al.*, 2005) and this has considerable influence on the stock assessments (Kell *et al.*, 2004). The spatial components of spawning can be investigated by the AEP method (Bannister *et al.*, 1974). Thus within this spatial context of both fish and fleet behaviour, the AEP method can provide evidence for changes in relative importance of plaice spawning grounds.

A new estimation of plaice SSB by AEP was also considered necessary because of the uncertainty of the VPA estimates. Large retrospective changes in the estimated absolute levels of plaice SSB in the North Sea have occurred (Pastoors, 2005). Furthermore, stock assessment methodology of North Sea plaice has changed greatly in recent years. Now the stock assessment incorporates discarding of plaice, which is done by the use of raised discard estimates from sampling the fleet in recent years and the use of an inter-annually varying growth model that simulates potential discarding behaviour of the fleets back in time (Keeken *et al.*, 2003; ICES, 2006c). This new method has never been sensitivity tested (STECF, 2005) and further information is required to support the use of this new technique (Dickey-Collas *et al.*, 2007).

In 2004 a series of ichthyoplankton surveys covered the whole North Sea (Taylor *et al.*, 2007; Fox *et al.*, 2008). These surveys targeted cod and plaice egg production with the primary aim of mapping the spawning grounds. In the southern North Sea there were sufficient repeated surveys conducted during the spawning season to allow estimation of plaice SSB by AEP. With this in mind, the fecundity of plaice in the Southern North Sea was also investigated in 2004. Also data from previous ichthyoplankton surveys of the southern North Sea (Simpson, 1959; Heessen and Rijnsdorp, 1989; Land, 1991) were reanalyzed using the same method as for the 2004 data and thus the relative trends in the North Sea plaice stock and variability in the spatial pattern of spawning could be investigated.

Methods

Ichthyoplankton surveys in 2004

From December 2003 to April 2004, 11 ichthyoplankton cruises were carried out (TABLE 5.1). Data from two cruises were not used in this investigation as they were outside the area considered important for plaice spawning (<55.50N) and are therefore not included in the table. A detailed description of the ichthyoplankton surveys is given in previous reports (Taylor *et al.*, 2007). At each station plankton was collected by deploying the sampler in a double-oblique manner to 2 m from the seabed. Hydrographic data were collected at each station. Due to failure of the CTD no temperature data were collected during cruise 1. Based on the data of previous years in the ICES oceanographic database, 9°C was assumed to be the temperature for all samples from that first cruise.

Plankton samples were fixed and stored in 4% formaldehyde solution buffered with

sodium acetate trihydrate (Tucker and Chester, 1984). Eggs were sorted from the samples and counted. Samples containing large quantities of eggs were subsampled using a Folsom splitter following agreed protocols. Eggs were identified to species level using the criteria described by Russell (1976). Plaice eggs were identified based on their size (>1.75 mm diameter) and thick chorion. The developmental stage of the plaice eggs was assigned according to Simpson (1959).

TABLE 5.1

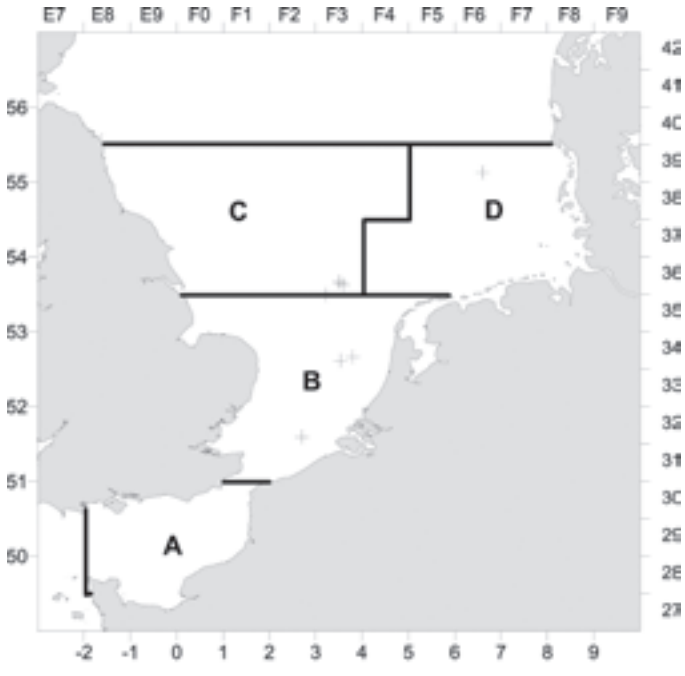
Ichthyoplankton survey dates and gears deployed. Areas surveyed shown in Figure 1. Note two other cruises within this programme took place but were dropped from the analysis and this table because they were outside the area of plaice spawning (<55.50N)

Cruise number	Country / Ship	Start date	End date	Gear	Numbers of hauls	Area surveyed
1	Netherlands Tridens II	15-12-2003	18-12-2003	Gulf III, 20 cm nose cone diameter, 270 µm mesh	77	A,B
2	Netherlands Tridens II	12-01-2004	16-01-2004	Gulf VII, 53 cm length, 28 cm nose cone diameter, 270 µm mesh	66	C,D
3	Germany Alkor	08-01-2004	19-01-2004	Gulf III, 53 cm length, 20 cm nose cone diameter, 270 µm mesh	108	A,B
4	Netherlands Tridens II	19-01-2004	23-01-2004	Gulf III, 20 cm nose cone diameter, 270 µm mesh	92	A,B
5	Netherlands Tridens I	11-02-2004	16-02-2004	Gulf VII, 53 cm length, 28 cm nose cone diameter, 270 µm mesh	69	B, C,D
6	Germany Heincke	16-02-2004	23-02-2004	Bongo, 60 cm diameter, 500 µm mesh	52	B,C,D
7	England Corystes	18-02-2004	08-03-2004	Gulf VII, 76 cm length, 40 cm nose cone diameter, 270 µm mesh	136	C
8	Netherlands Tridens II	01-03-2004	04-03-2004	Gulf VII, 53 cm length, 28 cm nose cone diameter, 270 µm mesh	66	B, C,D
9	Germany Alkor	06-04-2004	13-04-2004	Bongo, 60 cm diameter, 500 µm mesh	54	B,C,D

Estimation of fecundity of North Sea plaice in 2004

In December 2003 and January 2004 female plaice were sampled from three different areas in the southern North Sea (FIGURE 5.1). The samples were collected from a Dutch beam trawler, "ARM 44", and RV "Tridens" (TABLE 5.2). The fish were kept on ice prior to the collection of fecundity samples. Only fresh, mature and ripening (Walsh *et al.*, 1990) females were used for fecundity estimations. The fish were measured and sampled within 24 hours of being caught. Biological parameters collected were total length, total weight, maturity and ovary weight. From each fish duplicate (one of each ovary) fecundity samples with a known volume of 100 µl (Kennedy *et al.*, 2007b) were taken with a solid displacement pipette. The fecundity samples were preserved separately in 2 ml of 3.6% buffered formaldehyde.

FIGURE 5.1



Southern North Sea. Location of plaice sampling for fecundity estimation in December 2003 to January 2004 and area division for the 2004 egg survey. A- Eastern Channel, B- Southern Bight, C- Dogger Bank, D- German Bight. Degrees shown on bottom and left axis, ICES rectangle descriptors on the top and right axis

TABLE 5.2

Sampling of plaice females for estimation of fecundity from December 2003 to January 2004. The sample areas referred to in this table are shown in Figure 1

Sample Number	Sample Area	Sample Date	Number of females
1	Southern Bight (B)	3-12-2003	17
2	Dogger Bank (C)	2-12-2003	19
3	German Bight (D)	11-12-2003	21
4	Dogger Bank (C)	11-12-2003	34
5	Southern Bight (B)	18-12-2003	23
6	Southern Bight (B)	07-01-2004	19
7	Dogger Bank (C)	06-01-2004	26

The opaque oocytes were coloured using PAS staining (Periodic Acid Schiff's reagent) to aid counting and measurement of follicle diameter with an auto image analysis system (GFA, PIAS; Thorsen and Kjesbu, 2001, Damme *et al.*, 2005). The threshold to include vitellogenic or exclude smaller previtellogenic follicles in the fecundity count was set at 450 µm. Prior to image analysis the samples were examined manually, under a dissecting microscope, to identify and reject samples containing spawning markers (hyaline or post-ovulatory follicles (POFS)) (Witthames *et al.*, 2009). The means of follicle count and diameter, in the pair of samples taken from each fish, were used to determine the fecundity and to correct for down-regulation during maturation respectively.

Fecundity of individual fish was calculated using the formula:

$$F=(N*(O/s))/W \qquad [5.1]$$

where F is relative fecundity, N is the number of vitellogenic follicles in the pipette subsample, s is the subsample weight, O is the ovary weight and W the total weight of the fish.

Fecundity was estimated for each of the three areas separately as well as for the whole of the Southern North Sea (see FIGURE 5.1 and methods above). For the whole North Sea fecundity estimates from the separate areas were pooled. For each area female size specific relative fecundity was estimated from a linear regression of relative fecundity against total body weight predicted from the mean area specific female weight (Armstrong *et al.*, 2001).

Down-regulation of fecundity was estimated for the Southern Bight and Dogger Bank

areas. Differences in fecundity and down-regulation between areas were estimated using generalized linear models in R. Linear regression was determined between relative fecundity against the mean follicle diameter. Using the relationship between oocyte diameter and fecundity, down-regulation was estimated using the mean oocyte diameter in December and January. Fecundity was corrected for down-regulation, but to be able to compare the estimates with earlier studies uncorrected fecundity for 2004 was used as well.

Temperature dependent egg development rates

To test the estimate of AEP to assumptions about egg development rates, two models of temperature development rates were compared. Development rates were calculated using the equations:

$$D=(100/(aT+b))+D_0 \quad [5.2]$$

from Ryland and Nichols (1975), and

$$D=a+b*ln(T) \quad [5.3]$$

from Fox *et al.* (2003), where D = Development time till the end of the development stage, T is temperature (°C) and D0, a and b are constants (see TABLE 5.3).

TABLE 5.3

Constants used for the calculation of development time till the end of each stage, using models 2 and 3

Stage	Ryland and Nichols (1975)			Fox *et al.* (2003)	
	a	b	D0	a	b
1A	0.6203	8.9372	-5.5639	5.186	-1.612
1B	2.3629	4.6528	-1.2662	8.002	-2.540
2	2.1274	0.9166	-0.2867	12.819	-4.098
3	1.0642	1.5260	-1.7543	25.398	-8.078
4	0.7299	1.3619	-2.7171	29.880	-9.313
5	0.3150	1.3153	-10.4479	43.853	-14.427

Egg mortality

A mortality rate of eggs must be assumed or calculated to estimate the numbers of eggs at the immediate time of spawning (i.e. time=0). To investigate the sensitivity of assumptions about egg mortality we assumed egg mortality rates were of similar orders to those described in previous studies (e.g. Harding *et al.*, 1978; Heessen and Rijnsdorp, 1989; Land, 1991, Dickey-Collas *et al.*, 2003). Assuming instantaneous daily mortality Z = 0 results in the minimum AEP and assuming Z = 0.29 (approximately the maximum estimated for plaice eggs; Dickey-Collas *et al.*, 2003) results in the likely maximum estimate of AEP. In addition to fixed mortalities, Z was also calculated based on temperature using the equation

$$Ln(Z)=0.40*T-4.79 \quad [5.4]$$

from Dickey-Collas *et al.*, 2003, where Z is instantaneous daily mortality and T temperature (°C) for each haul.

It is also possible to use the decline by stage in an empirically estimated egg production. This leads to high variability in the estimates of Z (Dickey-Collas *et al.*, 2003) but not a great change in the likely maximum or minimum estimates of Z and was therefore not used to calculate daily mortality in this study.

Estimation of annual egg production

The method used in the present study was developed from those described by Heessen and Rijnsdorp (1989) and Armstrong *et al.* (2001). For assumptions used for the estimation of AEP and SSB see TABLE 5.4. For each haul the abundance (numbers per m2) of eggs was calculated and converted to the daily production of plaice eggs by stage (numbers per m2 per day) using the integrated water column temperature (°C) and egg development time. The daily production was back-calculated to the daily production at time of spawning (development stage 1A).

$$P_{t0}=P_{t1A}*exp(-Z_t) \quad [5.5]$$

where P is egg production, t is the time between, t0 (time of spawning) and t1A (median time of egg stage 1A) and Z is the coefficient of daily mortality. Since sampling was continuous throughout each day and night, the mean development duration of stage 1A eggs indicated the half way point (median) of the 1A stage duration (t in EQUATION 5.5).

TABLE 5.4

Assumptions used in the annual egg production and spawning stock biomass estimates.

Step	Assumptions
Egg sampling	• The plankton samplers provide an unbiased estimate of the abundance at a station. • Egg identification and staging were not biased. • Flow meter and CTD measurements were calibrated and unbiased. • The cline in abundance between stations is linear. • The seasonal trend between surveys can be linearly interpolated. • The first and last cruise (unless otherwise stated) occur at almost zero egg production.
Fecundity estimation	• For comparison to previous studies no loss of fecundity though atresia occurred in the North Sea population during spawning in assessment years. • Realised fecundity is fertilized and produces eggs on a 1 for 1 basis. • Fecundity samples collected in each area refer to the spawning population producing eggs.
Egg mortality	• Mortalities were assumed to have similar orders and distributions to those described in previous studies (e.g. Harding *et al.*, 1978; Heessen and Rijnsdorp, 1989; Land, 1991, Dickey-Collas *et al.*, 2003).
Estimation of annual egg production	• The age of the eggs was the median of an egg stage (1A, 1B, 2, 3, 4, and 5). • Mean development duration of stage 1A eggs indicated the half way point of the 1A stage duration. • Egg development rates determined for Irish Sea plaice were applicable to plaice in the North Sea.
Estimating spawning stock biomass	• The ratio of female weight to total SSB weight was estimated to be 0.37 in 1987 and 1988 and 0.60 in 2004. The time series does not go back as far at 1948, so 0.54 (the mean of the early 1960s) was applied.

For each survey area (FIGURE 5.1), the egg production per cruise was calculated by taking the mean production of all ICES rectangles productions weighted by area. Variance in the egg production was calculated by ICES rectangle and these were raised to the areas. The raised variance was used to estimate the CV for all egg productions. Annual production curves by area were estimated following Armstrong *et al.* (2001). The production curves by area were integrated using the equation:

$$AEP = \Sigma(t_{cruise} * P_{cruise}) \quad [5.6]$$

where P is the egg production per cruise and t is the duration of this production

calculated as half the number of days between the midpoint of this cruise and the previous one and added half the number of days between this cruise and the following one. For the first and the last cruises the duration was assumed to be twice the half number of days between the midpoint of the first or last cruise and the following or previous one respectively. The variance of the egg productions was summed as described for the AEP in EQUATION 5.6, this variance was used to estimate the CV for the annual egg production.

Estimating spawning stock biomass in 2004

Female SSB was calculated for the different areas using the area specific population fecundity estimates and the estimated annual egg production by using the equation:

$$SSB_F = (AEP/F) \quad [5.7]$$

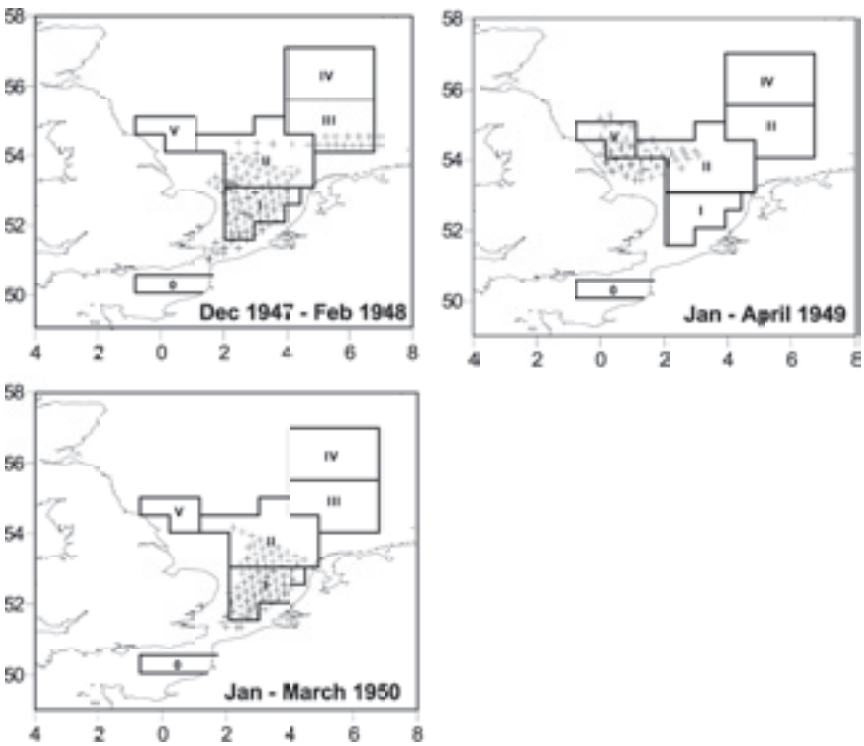
where SSBF is female spawning stock biomass and F is relative population fecundity. When comparing to the standard stock assessment the total SSB of males and females is required. Thus a sex ratio R must be estimated. For this the female only stock assessment (Rijnsdorp IMARES unpublished) was used to estimate the sex ratio by weight between mature males and females. The maturity ogive for males was taken from Rijnsdorp (1989). The ratio of female weight to total SSB weight was estimated for 1980s and 2004. The time series does not go back as far at 1948, so 0.54 (the mean of the early 1960s) was applied. Since no down-regulation estimates are available for the earlier periods it was assumed to be zero. However, for 2004 a SSB estimate was also calculated with a corrected fecundity, to show the impact of down-regulation. Summing all the areas gave a total SSB. To investigate the sensitivity of applying area-specific fecundity estimates, the total SSB was also estimated by summing up the annual productions by area and dividing this by the mean fecundity for the whole region. The variance in SSB was estimated by raising the AEP variance as shown in EQUATION 5.7.

Reworking ichthyoplankton surveys from previous studies

The raw data of the winter 1948, 1949 and 1950 egg surveys carried out by Simpson (1959) and the 1987 and 1988 egg surveys carried out by Heessen and Rijnsdorp (1989) were used to re-estimate annual egg production and SSB using the above described method. Due to incomplete coverage in 1949 (FIGURE 5.2) it was not possible to compare data for the whole Southern North Sea in this year. Since plaice fecundity changes

over time (Rijnsdorp, 1991), rather than using the 2004 estimates, the fecundities of these periods (Simpson, 1959; Rijnsdorp, 1991) were used. In order to be able to compare data between periods all estimates were also calculated using the area division from Heessen and Rijnsdorp (1989; FIGURE 5.2).

FIGURE 5.2



Positions of recovered data from Simpson 1959 from December 1947 to March 1950 and survey area and divisions used by Heessen and Rijnsdorp (1989).

Age based SSB from stock assessments

The current estimates (XSA derived) were taken from the ICES Working Group on the Assessment of Demersal Stocks in the North Sea and Skagerrak (ICES 2007c). These include estimates of discard mortality (see introduction). The ICES stock assessment assumes a constant maturity ogive and is not sex specific. The time series from 1957 to 2006 is for the North Sea only (i.e. not the eastern English Channel). There are no reliable absolute SSB estimates available for plaice in the eastern English Channel at present (area A, FIGURE 5.1; ICES area VIId; see ICES 2007c), but an assessment indicative

of trends is available.

Another time series of the population dynamics of North Sea plaice was available to the current study. This was a newly developed female only XSA stock assessment (A. Rijnsdorp, IMARES and L. Kell, Cefas pers comm). This used sex specific catch matrices and adjusted for sexual dimorphism in growth and maturation (see Rijnsdorp and Ibelings, 1989). Whilst this did not use discard data, the estimation of SSB without discards is relatively robust (see Dickey-Collas *et al.*, 2007) since the majority of discarded fish are not mature

Results

Spatial and Temporal coverage of surveys in 2004

Areas B and C (see FIGURE 5.1) had sufficient surveys to carry out full AEP estimates for plaice. In these areas respectively 7 and 6 daily egg production estimates were available. The surveys in these areas had good temporal coverage so that the onset and decline in spawning was covered. Fewer surveys occurred in areas A and D, only 3 daily egg production estimates were available in area A and 5 in area D. The end of spawning was missed in area A (last estimate 21 January 2004) and the beginning was missed in area D (first estimate 15 January 2004). If spawning is assumed to be similar to previous years, as described in earlier studies, then dates for zero productions can be assumed to allow AEP estimates to be derived.

Estimation of fecundity of North Sea plaice in 2004

Fecundity in the Southern Bight and Dogger Bank was the same, but fecundity in the German Bight was lower, though not significantly (ANOVA, P=0.7; FIGURE 5.3). Relative fecundity was higher in 2004 than in the late 1940s (Simpson, 1959) and the 1980's (Rijnsdorp, 1991) (TABLE 5.5). For the Southern Bight and Dogger Bank areas temporal variation in sampling was large enough to allow estimation of down-regulation of fecundity. Mean oocyte diameter in December was 1.0 mm and 1.1 mm in January. The down-regulation in fecundity between December and January is 10% in both areas. The data show that even before the onset of spawning there is still a clear down-regulation of fecundity in North Sea plaice (FIGURE 5.4). Down-regulation was different in the two areas. Though the slopes did not differ significantly (ANOVA, P=0.4), there is a significant difference in the intercept between the two areas (ANOVA, $P<0.01$).

FIGURE 5.3

Plaice total fecundity for the three different areas sampled; ♦ Southern Bight (grey line), □ Dogger Bank (dotted line), ▲German Bight (grey dotted line). (See Figure 1 for areas.)

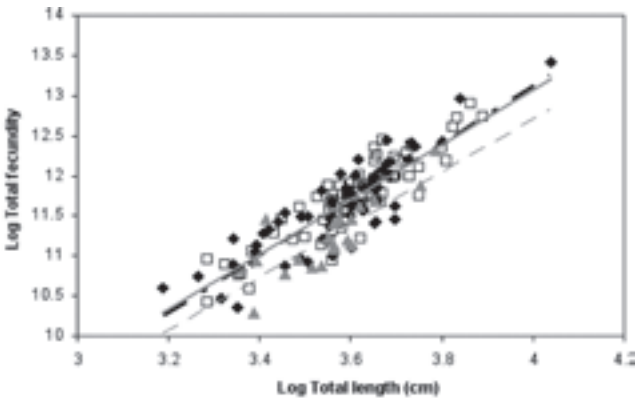


FIGURE 5.4

Down-regulation of fecundity in plaice for the different areas; ♦ Southern Bight (grey line), □ Dogger Bank (dotted line) (see Figure 1 for the areas) for samples taken in December 2003 and January 2004

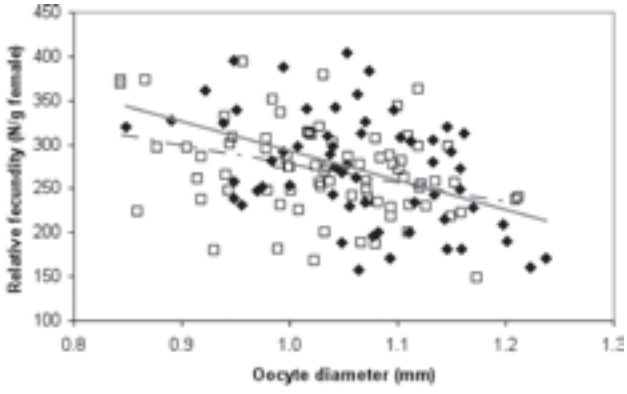


TABLE 5.5

Plaice relative fecundity estimates (oocytes per g female) for the three different areas sampled and total Southern North Sea, for comparison with Simpson (1959) and Rijnsdorp (1991). To be able to compare between the periods, the 2004 estimates are not corrected for down-regulation.

Area	Year	Relative fecundity	Standard deviation
Southern Bight (B)	2004	255	63
Dogger Bank (C)	2004	235	51
German Bight (D)	2004	185	43
Total Southern North Sea	2004	238	58
Southern Bight (Simpson, 1959)	1947-1949	163	
Total Southern North Sea (Rijnsdorp, 1991)	1982-1985	1711	

Effect of development rate

The study of Fox *et al.* (2003) was on the eggs of Irish Sea plaice but did use many more fish and different methods to investigate maternal or paternal effects compared to Ryland and Nichols (1975). It is clear that whilst the new relationships from Fox *et al.* (2003) do have an effect on the estimation of production, it is small. For example in area B, the Southern Bight, using the Fox *et al.* (2003) relationship production of stage IA at median age is estimated to be 89% of that using Ryland and Nichols (1975) relationship (TABLE 5.6). Due to the higher sea temperatures at the time of sampling in area A, this area shows the biggest difference. Since the Fox *et al.* (2003) was based on bigger sample size and takes maternal and paternal effect into account it was decided to use this method for the estimation of AEP.

TABLE 5.6

Area	Mean difference in estimate of daily egg production dependent on model choice	Mean sea temperature (°C)
A	0.72	9.57
B	0.89	7.38
C	0.97	6.90
D	0.98	6.37

Mean difference in the estimation of stage IA egg daily production at median age using Fox et al. (2003) and Ryland and Nichols (1975) by survey area. (Estimates were not weighted by egg abundance and based on the unweighted mean of cruises in each area.)

Effect of assumptions about egg mortality (Z)

To investigate the difference in the estimates of daily production at median age of stage 1A and at spawning (i.e. time = 0), a range of mortality rates were assumed and applied (see methods above). The estimated egg production at spawning increased by 16% in area A, 21% in area B and 24% in areas C and D if the assumed Z is increased from 0 to 0.29. When Z was assumed to be related to sea temperature following Dickey-Collas *et al.* (2003), the differences between the areas were larger (TABLE 5.7). The higher temperatures in area A resulted in a 37% increase of the production when back-calculated from stage 1A to spawning time. The back-calculated increase in production, and hence the impact of assuming Z to be related to temperature versus Z=0, was less in the other areas (FIGURE 5.5). The variations in estimated egg production at spawning caused

by different assumption on Z are well within the overall variability of the production estimates as reflected by the standard deviations in FIGURE 5.5. For the calculation of EAP and SSB, Z = 0 was used for a minimum estimate and compared to Z dependent on sea temperature.

TABLE 5.7

Assumed daily egg mortality rate (based on Dickey-Collas *et al.* 2003) and its impact on the estimate of egg production at spawning from back calculations of production at median age stage 1A. (Estimates are not weighted by egg abundance and based on the unweighted mean of cruises in each area.)

Area	Mean sea temperature (°C)	Mean assumed daily Z	Mean difference between median age stage 1A production and production at spawning
A	9.57	0.38	37%
B	7.38	0.16	21%
C	6.90	0.13	14%
D	6.37	0.11	13%
5	Southern Bight (B)	18-12-2003	23
6	Southern Bight (B)	07-01-2004	19
7	Dogger Bank (C)	06-01-2004	26

Trends in daily egg production by area

The seasonal trend in egg production varied by area (FIGURE 5.5). The smaller area A (see FIGURE 5.1) had equivalent maximum egg production per m^2 compared to area B. Areas C and D had lower productions per m^2. The coverage of area A ended at day 20, when egg production was still high (FIGURE 5.5). From the other areas it can be seen that egg production declines gradually. Another survey in area A around day 40 would have been very useful to allow a better estimate of the end of egg production in this area. Likewise a survey in early January in areas C and D would also have provided useful data to allow a better estimate of the start of the egg production. The peak in production appeared earlier in areas A and B compared to C and D (FIGURE 5.5) and A and B appeared to have a more marked peak compared to C and D. The dip in the egg production curve in area D (FIGURE 5.5) is probably due to sampling constraints. During cruise 5 and 8 only 4 stations could be sampled in this area.

FIGURE 5.5

Comparison of the seasonality in daily egg production in nos per m^2 per day of North Sea plaice in 2004 in survey areas A,B, C and D (see Figure 1). Day of year = 0 is 1st January 2004. Egg development is based on Fox *et al.* (2003). Daily production is estimated with daily mortality ♦ Z = T- the temperature to egg mortality relationship in Dickey-Collas *et al.* (2003) and □ at time of capture (i.e. Z = 0, median age stage 1A). Error bars denote 1 standard deviation, black for Z=0 and grey for Z temperature dependent.

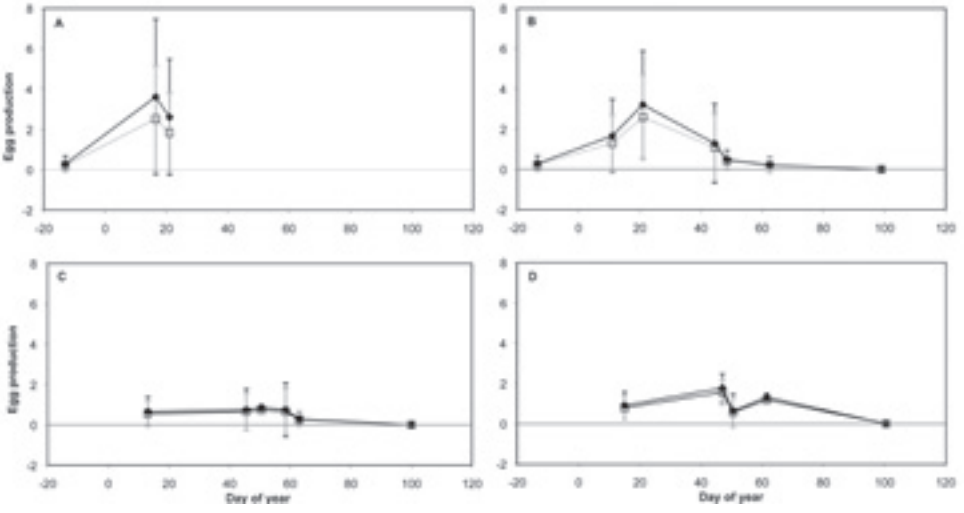


TABLE 5.8

Area	A	B	C	D	Total
AEP estimate Z=0 (*10^{12})	1.82 (0.85)	6.08 (0.53)	3.27 (0.63)	5.11 (0.23)	
AEP estimate Z=T (*10^{12})	2.58 (0.60)	7.50 (0.43)	3.76 (0.58)	5.78 (0.27)	
Fecundity (eggs g-1 female)	255*	255	235	185	
Female only SSB estimate Z=0 (tonnes)	7 126 (0.85)	23 832 (0.53)	13 917 (0.67)	27 615 (0.31)	72 489 (0.29)
Female only SSB estimate Z=T (tonnes)	10 103 (0.87)	29 395 (0.54)	16 006 (0.67)	31 268 (0.31)	86 771 (0.30)

Annual egg production (CV in brackets) and SSB estimates for North Sea plaice in 2004. Egg development to temperature relationship from Fox *et al.* (2003). Atresia during spawning assumed to be 0 although down-regulation was found in areas B and C

* taken from area B

Annual egg production and SSB estimates for 2004

The curves in egg daily production were summed and raised by area to determine the annual egg production (TABLE 5.8). The Southern Bight and German Bight (Areas B and D) appeared the most important for the production of plaice in the southern North Sea. Combining the AEP estimates with fecundity estimates resulted in a minimum female spawning biomass of North Sea plaice in 2004 of 72.5 Kt (CV= 0.29) based on the production of stage 1A eggs, and a maximum of 86.8 Kt (CV= 0.30) when accounting for egg mortality. When taking down-regulation of fecundity into account this resulted in a minimum female SSB estimate of 80.6 Kt. If a sex ratio of 1:1 is assumed this results in a SSB of 145-174 Kt.

Comparison with previous studies

As the current study (2004) had greater spatial coverage than that of Heessen and Rijnsdorp (1989) and Simpson (1959), the estimates from 2004 and around 1950 were reworked into the survey areas of Heessen and Rijnsdorp to allow direct comparisons (see FIGURE 5.2). The Fox *et al.* (2003) temperature to egg development relationships were also applied to all of the 20th century data to obtain comparable daily production estimates at median age stage 1A (FIGURE 5.6). Unfortunately the raw data from Land (1991) have been lost so for the results of this study no thorough comparisons with similar methods could be made, other than comparing the maps of production.

It is remarkably clear that in some of the sampling areas the production of plaice eggs appears not to have changed greatly between 1987, 1988 and 2004 (FIGURE 5.6). Heessen areas O and I show no marked changes in magnitude or timing of spawning, these areas correspond to the eastern Channel and Southern Bight. In the Dogger Bank and German Bight areas (Heessen areas II and III) the picture is less clear. The data appear more variable, and the lack of an earlier survey in area II in 2004 apparently prevent more robust comparisons on magnitude and timing of spawning in the Dogger Bank area, but the estimates of declining production in February appear similar. More obvious differences are seen when the 2004 data and the 1980s data are compared to that around 1950. Egg production in Heessen area I (the Southern Bight) was much higher around 1950 and the peak was later in 1948, though in 1950 the peak may have been slightly earlier compared to 2004. It also appears that spawning on or south of the Dogger Bank was earlier and more productive around 1950. There was no sampling by Simpson in the eastern Channel or the German Bight.

FIGURE 5.6

Seasonality in daily egg production in North Sea plaice in 1948, 1950, 1987, 1988 and 2004 in four Hessen areas. Estimates based on median stage IA egg production and Fox et al., (2003) egg development. Areas shown in Figure 2. Only a maximum three series per graph show error bars (denoting standard deviation) for ease of viewing

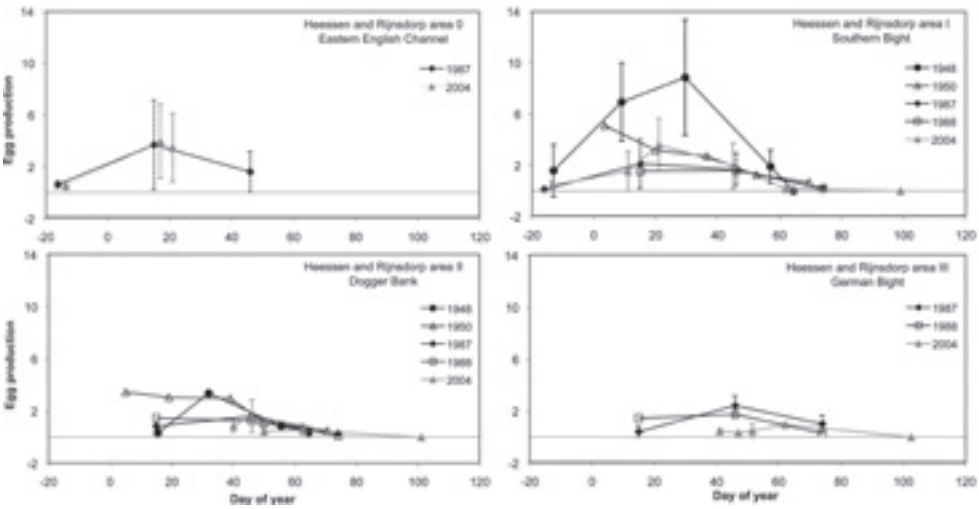
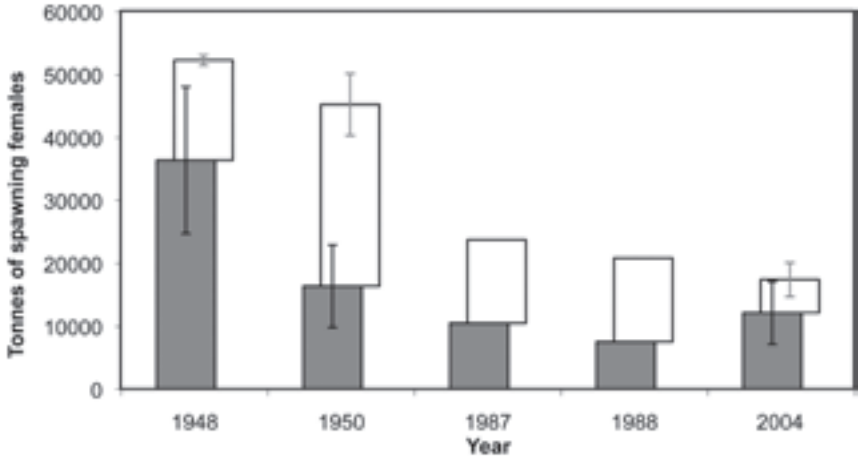


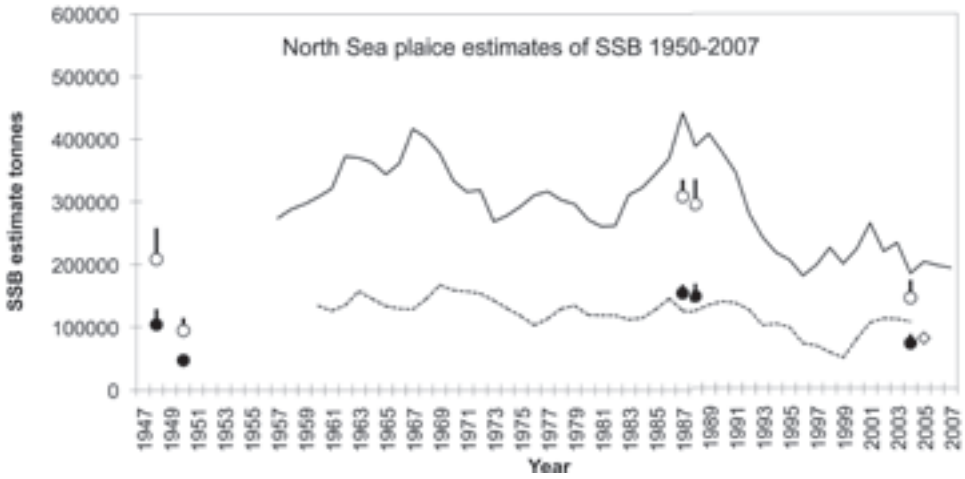
FIGURE 5.7



Estimated spawning biomass of females in two areas of the North Sea over 5 years. Areas shown in Figure 2. Fecundities applied described in table 9.■ Heessen area I, Southern Bight, ☐ Heessen area II, Dogger Bank. Error bars denote standard deviation

FIGURE 5.8

Comparison of AEP SSB with —— the ICES stock assessment using XSA (ICES, 2007) and ···· a "females only" assessment of SSB (from Rijnsdorp, IMARES pers comm). Circles denote the AEP estimate of SSB using egg production at the median age of egg stage 1A, whilst the top of the linked vertical bars denote the AEP estimate of SSB using the egg production at spawning (derived by mortalities varying due to temperature, Dickey-Collas *et al,* 2003). ● denote "females only" estimates, ○ denote total SSB using a sex ratio of females to males by weight, ◊ denotes the "females only" estimate with down-regulation of fecundity



The female only SSB associated to these AEP estimates shows a marked change over time (TABLE 5.9). Different estimates of fecundity were used for the 1950s, 1980s and 2004. These estimates of SSB are minimum estimates as the production at median age stage 1A was used. As apparent in the comparison of the seasonal production curves (FIGURE 5.6), the spawning in the Southern Bight declined from 1948 to recent years, however at the same level between the late 1980s and 2004. In the eastern Channel, the lower SSB estimate by the AEP in 2004 (45% lower) could be due to the failure to survey throughout the spawning season, and the large variability of estimation in this area in 2004 (TABLE 5.9). When Southern Bight is combined with the Dogger Bank estimates (FIGURE 5.7) a substantial decline in female spawning biomass is apparent, from approximately 48 Kt to 17 Kt, i.e. a decline to 35% of the post war spawning stock. In the German Bight the 2004 survey did not find the peak of spawning seen by Heessen and Rijnsdorp (1989) and this is reflected in an apparent decline in female SSB from approximately 16 Kt in the late 1980s to 5 Kt in 2004 (TABLE 5.9).

Comparison of AEP estimates of SSB and aged based stock assessments of North Sea plaice

The AEP derived estimate of SSB for plaice in the southern North Sea in 1987 and 1988 were similar to both the "females only" assessment and the SSB from ICES stock assessment. It is clear that the large proportion of males in the late 1980s in the stock led to the total SSB appearing more productive in terms of eggs than it was (FIGURE 5.8). The confidence interval of the AEP estimate of females (TABLE 5.9) in 2004 covers the "females only" assessment but the difference to the total stock assessment from ICES is somewhat larger (FIGURE 5.8). The Simpson data is from one region only, and when comparing to the later survey data covering the whole area, its inclusion shows the relative decline of the plaice SSB in the later half of the 20th century. Note that the temporal coverage was much less in 1950 compared to 1948. This is the first time that an XSA derived estimate of SSB for plaice has the same magnitude of an AEP (see Armstrong *et al.*, 2001; Kennedy, 2006). It should be noted that both of the assessment time series are for the North Sea only, whereas the AEP methods (≥ 1987) include the eastern English Channel.

TABLE 5.9

Comparison of AEP estimates of female SSB from 1948 to 2004 by Heessen area (see Figure 2). Production of median aged stage 1A eggs was estimated using egg development to temperature relationship of Fox *et al.* (2003). Rijnsdorp (1991) estimates of fecundity applied to 1987 and 1988. Area I fecundity applied to area 0 in 2004. (CV in brackets)

Area from Heessen and Rijnsdorp	0	I	II	III	Total
AEP estimate 1948 (*10^{12})		9.29 (0.32)	3.73 (0.05)		
AEP estimate 1950 (*10^{12})		4.18 (0.40)	6.79 (0.17)		
AEP estimate 1987 (*10^{12})	1.60 (0.68)	2.68 (0.58)	3.11 (0.34)	3.18 (0.27)	
AEP estimate 1988 (*10^{12})		1.93 (0.50)	3.13 (0.44)	2.83 (0.30)	
AEP estimate 2004 (*10^{12})	0.88 (0.55)	3.10 (0.41)	1.24 (0.51)	0.99 (0.15)	
Female only SSB 1948 (tonnes)		36 413	15 886		52 299
Female only SSB 1950 (tonnes)		16 382	28 879		45 261
Female only SSB 1987 (tonnes)	6 274	10 519	13 241	17 173	59 202
Female only SSB 1988 (tonnes)		7 565	13 306	15 299	54 352
Female only SSB 2004 (tonnes)	3 450	12 159	5 276	5 354	26 736

Discussion

The present study shows the ability of using the AEP method for estimating SSB and shows that the AEP method is a useful way to hindcast spatial dynamics of heavily exploited flatfish stocks. In 2004 the egg production was highest in the Southern Bight and the peak in production appeared earlier in the eastern Channel and Southern Bight. The lower fecundity in the German Bight resulted in the highest SSB estimate for this area. The egg production in 2004 is similar to the egg production in 1980s. However, in the 1940s egg production was higher in the Southern Bight and the Dogger Bank. There appears no major change in the timing of spawning since the 1940s. The total female SSB seems to decline over time. But the SSB estimates from AEP show the same magnitude as the VPA estimates.

The AEP method is dependent on the spatial and temporal coverage of sampling, but despite the intensive sampling during all surveys reported here it was not possible to cover all the spawning areas (Simpson, 1959; Bagenal, 1966) and complete spawning season of plaice in the North Sea. Hence, we will expect some underestimation of the North Sea plaice annual egg production and SSB. Furthermore it was not possible to standardise the gear for the collection of the eggs (TABLE 5.1). The differences between Gulf III and Gulf VII are small and tested (Nash *et al.*, 1998) but with the bongo net the difference are bigger. The use of the different nets may lead to further bias of the estimates.

It is clear that there are still many assumptions in current methods of AEP which are still yet to be properly assessed Armstrong *et al.* (2001) and Hunter and Lo (1993). The current work did not investigate the empirical evidence for Z and errors introduced by poor staging of eggs. The "pseudo-synoptic" nature of the surveys was not accounted for, and no geostatistical or GAM methods were used (see Fox *et al.*, 2000). However as the methods broadly followed those of previous similar studies (Daan, 1981; Heessen and Rijnsdorp, 1989; Horwood, 1993a; b; Armstrong *et al.*, 2001), the estimates are felt to be robust to compare to previous studies. The 2004 ichthyoplankton survey of the North Sea provided a very good coverage of the Southern Bight, and allowed acceptable 5.comparisons with previous studies. As mentioned above, the temporal coverage was poor in the eastern English Channel and German Bight. This however did not prevent comparisons with previous studies.

The sensitivity analysis of using the Fox *et al.* (2003) egg development rates compared to Ryland and Nichols (1975) show that, as expected, when temperatures where

higher there was a greater influence in the choice of model, this was apparent in the eastern Channel. Thus the choice of model is important for future testing of climate change scenarios. Although Fox *et al.* (2003) argue there may be stock differences in development rate, comparison with the Ryland and Nichols (1975) development rates in this study only results in a minor differences. Since the Fox *et al.* (2003) study was based on more experiments and on many more crosses of parents, it is therefore considered to give the better relationship, despite the fact that it is based on Irish Sea material.

The fecundity of North Sea plaice has gradually increased since the 1940's. This study shows that down-regulation in North Sea plaice occurs prior to spawning. The sampling for fecundity could have been more extensive, and we do not known how representative the German Bight sample was of the whole population in that area, as a replicate sample could not be collected. However, if the fecundity was higher in the German Bight, then the estimates of SSB would be even lower than the current approach suggests. Although fecundity samples were only taken in December and January, just prior to spawning, the results showed a down-regulation of fecundity. In Atlantic herring it has been shown that down-regulation of fecundity increases towards the end of the maturation period (Kurita *et al.* 2003). The down-regulation is comparable to that found in Irish Sea plaice (Kennedy *et al.* 2007b). When accounting for down-regulation the SSB estimates increases 11%. This shows the importance of sampling for fecundity just prior to the onset of spawning or taking the stage of maturation and subsequent down-regulation into account.

For the back-calculation to the egg production at spawning, it was considered that since sampling was continuous throughout each day and night, the mean development duration of stage 1A eggs indicated the median of the 1A stage duration. This assumption is actually incorrect due to the exponential decline in fish eggs after spawning, which will result in an overestimation of egg production, depending on Z (Dickey-Collas *et al.*, 2003). However the median stage duration was used for simplicity.

The plaice SSB time series from 1957 to 2006 is for the North Sea only (i.e. not the eastern English Channel). There are no reliable absolute SSB estimates available for plaice in the eastern English Channel at present (area A, FIGURE 5.1; ICES area VIId; see ICES 2007c), but an assessment indicative of trends is available. This shows broadly the same trends as for plaice in the North Sea. So it is likely that any AEP estimate that includes the Channel, should have a positive bias when compared to the assessment of

the North Sea only population.

The results of this study were compared with a newly developed female only XSA stock assessment (A. Rijnsdorp, IMARES and L. Kell, Cefas pers comm). This used sex specific catch matrices and adjusted for sexual dimorphism in growth and maturation (see Rijnsdorp and Ibelings, 1989). Whilst this did not use discard data, the estimation of SSB without discards is relatively robust (see Dickey-Collas *et al.*, 2007) if the majority of discarded fish are not mature.

The current AEP estimate of North Sea plaice SSB, is in broad agreement with the current ICES standard XSA stock assessment. When the AEP method was used in other areas it was found that SSBs from empirical ichthyoplankton data are higher than the standard XSA results. In the Irish Sea this has consistently been by a factor of three (Armstrong *et al.*, 2001).This has been attributed to a range of possible factors including the selection pattern at age assumed in the assessment, the lack of discard estimates in the catch at age matrix of the assessment, changes in natural mortality and the spatial mismatch of the fishery and the spawning of plaice. None of these has been successfully proven to be the cause of the discrepancy.

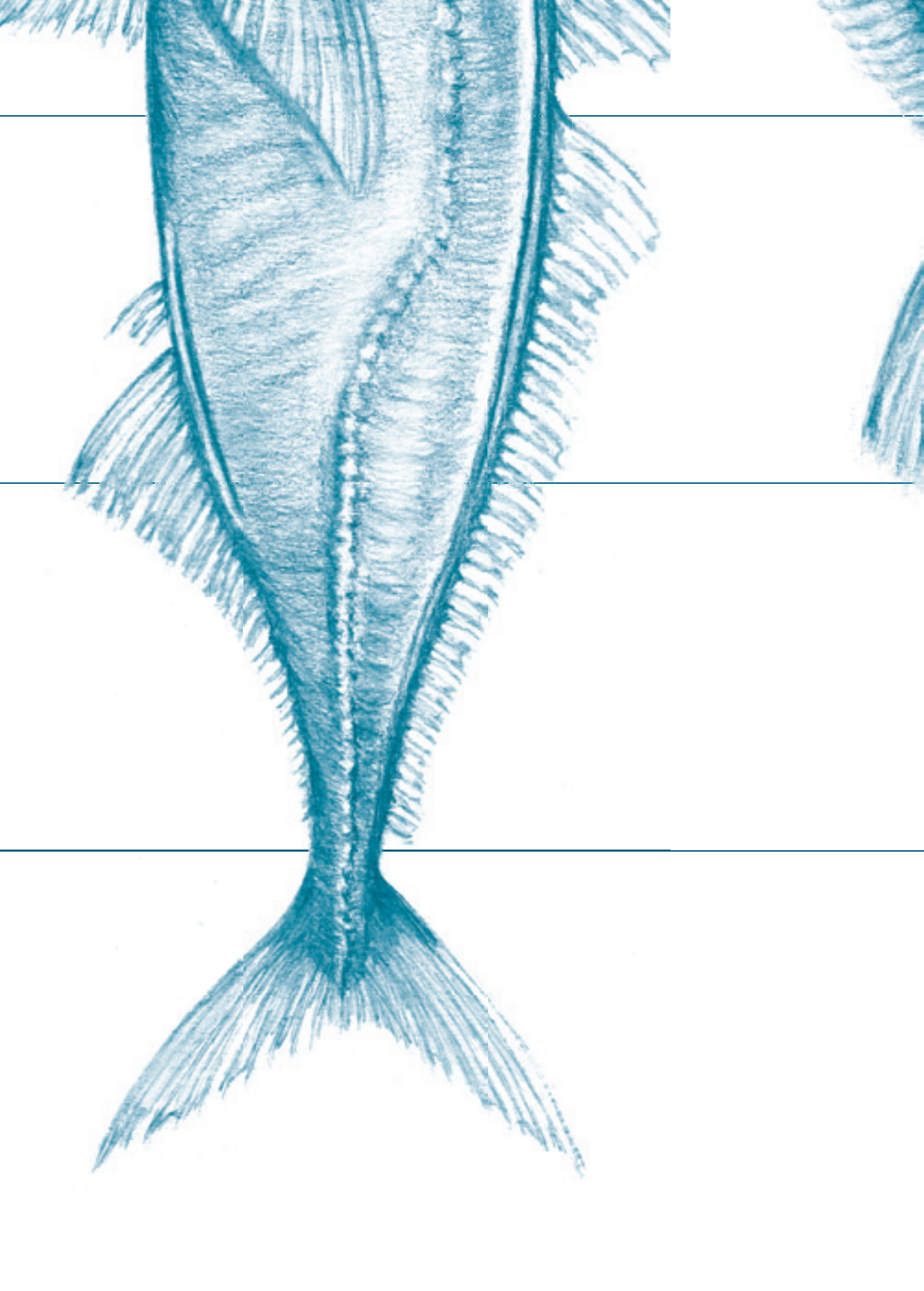
In the North Sea, the estimates from AEP and XSA appear similar. The decline in SSB from 1988 to 2004 was approximately 60% as estimated by XSA, and 50% as estimated by AEP. The AEP method supports the current ICES XSA stock assessment both in terms of the relative trend in SSB and the current absolute biomass. The AEP also suggests that most of this decline occurred in the Dogger Bank area and the German Bight. There appears to be no major change in timing of spawning of North Sea plaice, throughout the period of high exploitation. In the Southern Bight SSB appears to increase from the 1980s to 2004, but not yet up to the biomass of the 1940s. But the decline in the SSB on the Dogger Bank seems to suggest a switch from Dogger Bank to Southern Bight. During the last century changes have occurred in the North Sea plankton community due to climate changes (Fromentin and Planque, 1996; Beaugrand, 2003; Reid *et al.*, 2003), as well as changes in the benthic and fish community shown to be caused by fishing effects (Jennings *et al.*, 1999; Frid and Clark, 2000; Frid *et al.*, 2000; Jennings *et al.*, 2001; 2002; Daan *et al.*, 2005). These factors have probably also effected the change in North Sea plaice stock and spawning.

Conducting an AEP is expensive in terms of ship and staff time. However, it can provide a useful check on the trends from age-based methods. In addition, it can provide improved spatial resolution which tends to be lost when catch and effort data

are amalgamated across large management areas. Because of the high cost of egg based methods they are probably most useful when applied periodically to validate trends in age-based stock assessments or in cases, such as North Sea plaice, where there are particular uncertainties such as the level of un-recorded discarding.

Acknowledgements

The authors are grateful to Jos Buntsma, Anne van Duyn, Peter Groot, Kees Groeneveld, Arie Kraayenoord, Rick Mimpen, Betty van Os-Koomen and Simon Rijs from Wageningen IMARES for their help in collecting and working up of the samples. We would like to thank Richard Nash from IMR, Henk Heessen and Adriaan Rijnsdorp from Wageningen IMARES, and two anonymous referees for their helpful comments on this manuscript. This study was partly funded by EU 6th framework project UNCOVER.



Chapter 6

Reproductive strategies and fecundity type regulation through food availability in marine fish

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Olav S. Kjesbu
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Adriaan D. Rijnsdorp

Abstract

Marine teleosts show a wide range of reproductive strategies. This study specifically compares reproductive strategies and fecundity types of different fish species in relation to their pattern of energy intake and allocation of energy over somatic growth and reproduction, using empirical information placed within a statistical framework (PCA). Generally capital spawners have a determinate fecundity (no de novo oocyte recruitment during spawning), whereas income spawners have an indeterminate fecundity (de novo oocyte recruitment during spawning). Hence, food availability of the adults is the most important factor regulating fecundity, although food availability for the larvae also constrains fecundity regulation, since larvae need to have enough yolk sac reserves to reach the nursery area before point of

Sammendrag

Marine teleoster viser et bredt spekter av reproduktive strategier. Ved hjelp av empirisk informasjon plassert innenfor en statistisk ramme (PCA) sammenligner denne studien spesielt hvilke reproduktive strategier og fekunditetstyper ulike fiskeslag har i forhold til deres mønster av energiinntak og allokering av energi til somatisk vekst og reproduksjon. Kapitalgytere har generelt en determinant fekunditet (ingen de novo oocyte rekruttering under gyting), mens inntektsgytere har en indeterminant fekunditet (de novo oocyte rekruttering under gyting). Mattilgjengeligheten for voksne individer er derfor den viktigste faktoren som regulerer fekunditeten, selv om tilgjengeligheten til mat for larvene også påvirker fekunditetsreguleringen, siden larvene må ha nok plommemassereserver til å nå oppvekstsområdet

Samenvatting

Mariene teleosten hebben een breed scala aan reproductie strategieën. Deze studie vergelijkt reproductie strategieën en fecunditeitstypen van verschillende vissoorten met betrekking tot de wijze van energie-opname en verdeling van energiereserves over somatische groei en reproductie, waarbij empirische informatie geplaatst wordt in een statistisch kader (PCA). Kapitale paaiers hebben over het algemeen een bepaalde fecunditeit (tijdens het paaien worden er geen nieuwe eicellen gerekruteerd), terwijl inkomende paaiers een onbepaalde fecunditeit (nieuwe eicellen worden gerekruteerd tijdens de paaiperiode) hebben. De beschikbaarheid van voedsel voor de adulten is de belangrijkste factor in de regulering van de fecunditeit, hoewel de beschikbaarheid van voedsel voor de larven ook restricties legt op de fecunditeit regulatie, aangezien de larven genoeg dooier moeten hebben om de kraamkamer te

no return. Other important factors affecting the determinate fecundity type are body condition and egg dry weight and the environmental parameter latitude. For the indeterminate fecundity type relative fecundity, spawning period and food availability and the environmental parameter temperature are most important. However, traditional determinate spawners may switch to a pseudo-indeterminate fecundity style at superfluous feeding condition, while indeterminate spawners stay with their fecundity style. In conclusion we show that fecundity type of marine fish females is not fixed at the species level but represents a plastic response to the environment through food availability and energy allocation.

før de dør av sult. Andre viktige faktorer som påvirker den determinante fekunditetstypen er kondisjonsfaktoren, tørrvekten på eggene og de miljømessige parameterne knyttet til breddegrad. Det viktigste for den indeterminante fekunditetstypens relative fekunditet er gyteperiode, tilgjengelighet av mat og den miljømessige parameteren - temperatur. Mens den tradisjonelle determinante gyteren kan bytte til en pseudo-indeterminant fekunditetstype når det er overflod av mat, holder den indeterminante gyteren fast på sin fekunditetstype. I konklusjonen viser vi at fekunditetstypen til marin hunnfisk ikke er fast på arts nivå, men kan endres som svar på miljøet gjennom mattilgjengelighet og energitildeling.

bereiken. Andere belangrijke factoren die het bepaalde fecunditeitstype bepalen zijn lichaamsconditie en eidrooggewicht en de omgevingsparameter breedtegraad. Voor het onbepaalde fecunditeitstype zijn relatieve fecunditeit, paaiperiode en voedselbeschikbaarheid en de omgevingsparameter temperatuur de belangrijkste factoren. Echter, vrouwtjes met een traditionele bepaalde fecunditeit kunnen overschakelen naar een pseudo-onbepaald fecunditeitstype op het moment dat er overvloedige voedselbeschikbaarheid is tijdens de paaiperiode, terwijl het onbepaalde fecunditeitstype bij dat type blijft. Tot slot laten we zien dat fecunditeitstype van mariene vissoorten niet vastligt op het soortniveau maar dat het een reactie is op de leefomgeving door middel van voedselbeschikbaarheid en allocatie van energiereserves.

Introduction

Reproduction is the most costly event for most organisms and the trade-off between survival and reproduction is an important topic in life history theory (Roff, 2000, Stearns, 2000). Capital and income breeding are the most extreme in the continuum of reproductive strategies (Bonnet *et al.*, 1998, Drent and Daan, 1980). The first type can cope with unpredictable 6.environmental conditions through long-term storage of energy (Calow, 1979), whereas the last type can utilise increases in food supply at or near spawning (Jönsson, 1997). It has been suggested that large organisms can cope with large energy storage and are capital breeders while small organisms cannot carry large energy reserves and are thus income breeders (Klaassen, 2002). Many organisms, when they grow, have access to an increasing spectrum of food types and sizes and may also be more effective at catching them (Ricker, 1958, Rose and Cowan, 1993).

Marine fish show a wide range of reproductive strategies (Murua and Saborido-Rey, 2003), with different reproductive traits (see TABLE 6.1 for definition of reproductive terminology). Depending on the environment, and thus geographical location, fish reproductive traits may differ between species and among populations within a species (Stearns, 2000). Are these variations in traits caused by genetic differences due to local adaptations or are they a result of phenotypic plasticity? The ability to adapt the reproductive traits within a species is genetically driven but whether variations between populations within a species are genetically driven or a plastic response to environmental conditions remains unclear (Wootton, 1992). In some populations of brown trout Salmo trutta, cod *Gadus morhua* and herring *Clupea harengus* plasticity in reproductive traits has been shown (Damme *et al.*, 2009b, Jonsson and Jonsson, 1999, Yoneda and Wright, 2004). It remains unclear which underlying mechanisms regulate this plasticity. In sole *Solea solea* populations in the northeast Atlantic fecundity, egg size and timing of spawning varies with latitude and temperature (Rijnsdorp and Vingerhoed, 1994). From north to south temperature increased and egg size increased and fecundity decreased and spawning occurs later in the year in the south. Rijnsdorp and Vingerhoed (1994) concluded that the difference in these reproductive traits were closely related and varied over latitude because of adaptations to local temperature and photoperiod and are results of phenotypic plasticity rather than genetic differences.

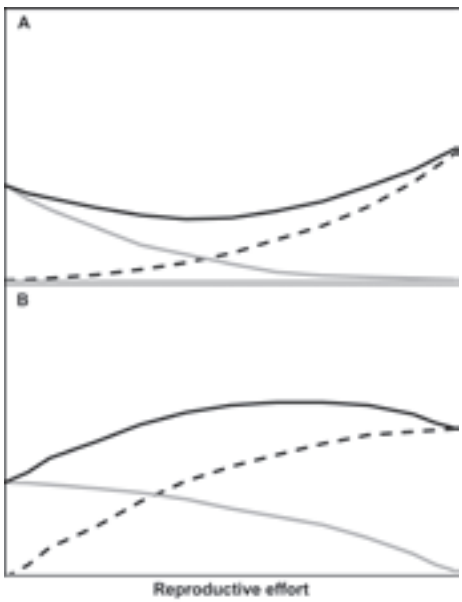
According to life history theory, reproductive strategies are aimed at maximizing the offspring production in the environment where the fish live. Hence, fish trade-off their survival probability with their future reproduction success (Stearns, 2000, Roff, 2000).

Definitions of reproductive terminology used in this paper

Term	Definition	Examples	References
Reproductive strategy	Expression of reproductive traits of individuals within a species over the full range of environmental situations	Semelparous, iteroparous	(Kjesbu, 2009, Wootton, 1998)
Reproductive trait	Distinct reproductive characteristics, genetically or environmentally determined	Fecundity (type), egg size, time of spawning, duration of oocyte maturation	(Kjesbu, 2009)
Spawning type	The manner in which eggs are spawned within one breeding season	Single batch (total) or multiple batches	(Murua and Saborido-Rey, 2003)
Spawning period	Length of spawning of an individual fish within one breeding season		(Kjesbu, 2009, Hickling and Rutenberg, 1936)
Fecundity	Number of oocytes produced in an individual ovary	Potential, relative	Multiple sources
Potential fecundity	Number of vitellogenic oocytes in an individual ovary prior to spawning		Multiple sources
Relative fecundity	Number of vitellogenic oocytes divided by wet body weight		Multiple sources
Fecundity type	Pattern of recruitment of new (de novo) oocytes to the vitellogenic stock within one maturation cycle	Determinate, indeterminate	(Kjesbu, 2009)
Determinate fecundity	No new pre-vitellogenic oocytes recruit to the vitellogenic stock during spawning		(Kjesbu, 2009)
Indeterminate fecundity	Pre-vitellogenic oocytes keep recruiting to the vitellogenic stock during spawning		(Kjesbu, 2009)
Final maturation	Migration of the nucleus and water uptake in the oocyte prior to ovulation		(Kjesbu *et al.*, 1996a)
Capital breeder	A fish that uses stored energy for reproduction		(Stearns, 1992)
Income breeder	The fish acquires energy for reproduction during the spawning period		(Stearns, 1992)
Body condition	Condition of the female at a certain point during the oocyte maturation cycle	Fulton's K, relative condition factor Kn, lipid content	Multiple sources

Basically this can be divided in two different trade-offs; 1) reproductive investment against adult growth and mortality (Roff, 2000, Stearns, 2000) and 2) egg size against egg number (Sargent *et al.*, 1987, Johnson *et al.*, 2010). These trade-offs are related to food availability and mortality risk during different life stages (adults, eggs and larvae). A third trade-off that can be identified, investment in somatic growth and present or future reproduction, will not be considered here. We review the female reproductive strategy of a variety of fish species and relate the fecundity type, egg size and fecundity with corresponding information on the food, survival and environment of the adult and larvae.

FIGURE 6.1



Fecundity curves; ···· current fecundity; — future fecundity, and — resulting fitness, favoring (a) semelparity or (b) iteroparity, after (Einum and Fleming, 2007).

Reproductive investment

Number of reproductive cycles

The optimal reproductive investment at a given spawning event is that which maximizes the current effective fecundity (i.e., number of offspring surviving to reproduce), and the future fecundity (Gadgil and Bossert, 1970, Schaffer, 1974). If both current and

future fecundity are accelerating than with increasing reproductive effort the fitness of the female will first decrease and later increase (FIGURE 6.1; Einum and Fleming, 2007), thus favouring semelparity. If both current and future fecundity are decelerating, the fitness will increase and maximise at an intermediate reproductive effort, favouring iteroparity (FIGURE 6.1; Einum and Fleming, 2007). In poor environmental conditions iteroparous fish may skip one spawning event in order to increase fitness and survival and probability of spawning in future events (Rijnsdorp, 1990, Rideout *et al.*, 2005).

Fecundity

The reproductive investment trade-offs affect different life stages. During the different life stages fish occur in specific time, and often space, windows. The first window is the oocyte maturation and relates to the oocyte or egg size and duration of the maturation of the oocytes. Fish in general have an entrainment, i.e. a complex network of regulation, in their reproductive biology (Migaud *et al.*, 2010). In their review Migaud *et al.* (2010) stress that photoperiod is an important cue for production of chemicals and hormones which trigger reproduction and oocyte maturation in fish. Increase or decrease in daylight length occurs when the sun crosses the equator at the spring and autumn equinox. This is suggested as the trigger for the start of oocyte maturation in some species (Kjesbu *et al.*, 2010, Damme *et al.*, in prepr). The duration of the oocyte maturation is dependent on the final oocyte or egg size, the development rate of the oocytes and is influenced by temperature that affects the development rate of oocytes and metabolic rate of the female. Oocytes undergo the same overall development pattern in all marine teleosts, irrespective of reproductive strategy (Tyler and Sumpter, 1996). The vitellogenic development stage is the main phase of oocyte growth, which can account for 95% of the final egg size. In capital breeders the vitellogenic growth phase may take up to 9 months, whereas in income breeders this growth phase maybe weeks or even days (Tyler and Sumpter, 1996).

The second is the spawning window which is related to favourable conditions for eggs and larvae. These time, and space, windows need to be matched carefully in order to maximize survival of eggs and larvae. The feeding conditions of the larvae are generally considered to be the critical phase (Cushing, 1990, Hjort, 1914), although it may also relate to a mismatch with predators (e.g. (Kuipers *et al.*, 1990, Bertram and Leggett, 1994, Taylor, 2003), or the retention in suitable 6.habitat (Iles and Sinclair, 1982). The third is the feeding window for the adults. Depending on the type of food consumed,

the feeding window of the adults will differ in time and space from the larval window. By building energy reserves and spawning migrations, fish can cope with differences in timing and spatial windows between life stages (Rijnsdorp and Witthames, 2004).

Fecundity and thus reproductive strategy is a consequence of the surplus energy, e.g. the total energy that is available for reproduction and somatic growth during a reproductive cycle (Rijnsdorp 1990). Further the mode in which energy is available is relevant because reproduction requires a specific ratio of biochemical compounds, mainly in terms of lipids and protein. In simplified terms, lipid storage are believed to be mostly used for maintenance of the individual while storage proteins are used for gonad development (Bradford, 1993). However, for the growth of oocytes females need access to both lipids and protein since the yolk of eggs consists of both chemical components (Kamler, 2008, Heming and Buddington, 1988), and larvae need both for growth and development (Rainuzzo *et al.*, 1997). Lipids are used for metabolism of the adults during spawning, although also proteins may be utilised (Bradford, 1993).

Female fish with a determinate fecundity type recruit all the oocytes of one maturation cycle to the vitellogenic stock before the onset of spawning (Murua and Saborido-Rey, 2003, Kjesbu, 2009). Indeterminate females keep recruiting new oocytes to the vitellogenic stock after spawning has commenced (Kjesbu, 2009, Murua and Saborido-Rey, 2003). The size of a female with an indeterminate fecundity type can be smaller because the ovary does not have to contain a high number of large vitellogenic oocytes at the same time. However this does require that the female gains enough energy during the spawning period to mature oocytes all the way from the cortical alveoli to the final maturation stage. The female must have either enough energy stored or a high nutritional-rich food supply during the spawning period (Hunter and Leong, 1981).

Most of the oocyte maturation process in determinate spawners takes place before the spawning period and final maturation occurs just before spawning a batch of oocytes. For this mode of action the females need to have a large ovary to store a high number of vitellogenic oocytes at the same time. These females need a high energy storage or food supply during the oocyte maturation before the spawning period. Due to the high energy storage determinate spawners show a seasonally variable and high amplitude over time in body condition while this is less in indeterminate spawners (Rijnsdorp and Witthames, 2004).

Egg size

Food availability and larval survival are closely linked with latitude and temperature (Kamler, 1992). In contrast to fish living at lower latitudes, high latitude fish are confronted with highly seasonal production cycles (Cushing, 1990) due to the relatively large seasonal change in light and temperature. However, depending on cyclic weather patterns, river run off or upwelling events some seasonality in productions may also occur at lower latitudes. At high latitudes food can have higher nutritional values as compared to the lower latitudes (Conover, 1992).

Some fish species, mostly capital breeders, make extensive migrations without feeding between feeding and spawning grounds to reach the area with the optimal adult feeding conditions during the feeding season and the area with the optimal survival conditions for the larvae during the spawning season, e.g. eel Anguilla anguilla (Aoyama, 2009), herring (Zijlstra, 1969) and cod (Rose, 1993). Thus this creates seasonality in food availability by itself. While others species, usually income breeders, make limited migrations and ingest food during these migrations, e.g. anchovy *Engraulis encrasicolus* (Morais *et al.*) and sardine *Sardina pilchardus* (Carrera and Porteiro, 2003).

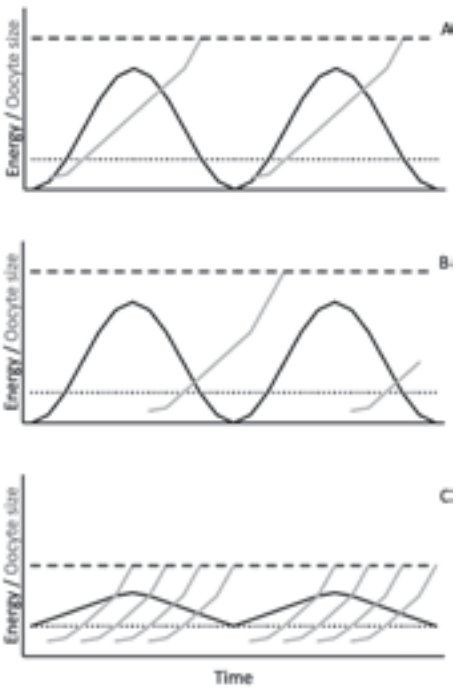
Seasonality in food availability influences the size of eggs produced. Females spawning in winter produce fewer but bigger eggs than those spawning in summer and autumn (Chambers, 1997, Damme *et al.*, 2009b, Rijnsdorp and Vingerhoed, 1994). Producing a single bigger egg probably also costs relatively more energy investment of the female compared to producing a smaller egg. However, it remains unclear if the production of a batch of a few big eggs requires more energy compared to producing a batch with many small eggs. Between species, larger eggs have a higher energy content compared to smaller eggs (Hislop and Bell, 1987). Within a species, between populations egg size varies (Rijnsdorp and Vingerhoed, 1994, Damme *et al.*, 2009b) and larger larvae hatch from the bigger eggs (Geffen, 2009), suggesting higher energy content of the bigger eggs. Within a population experiments showed bigger eggs did not contain a higher energy content and had the same lipid and protein content irrespective of size (Fletcher and Wootton, 1995, Lupatsch *et al.*, 2010).

Temperature has an indirect influence on spawning through the food availability of the females (Kjesbu and Holm, 1994), but there is also a direct influence on the metabolism and maturation of the oocytes (Van Der Kraak and Pankhurst, 1997, Thorsen *et al.*, 2010). Metabolism and development of the oocytes is faster in areas with higher temperatures. In warm areas the maturation of oocytes of a certain size takes less time

compared to the maturation of the same size oocytes in colder areas. Hence in warm areas more eggs of the certain size can be produced in a shorter time span (Kjesbu *et al.*, 2010).

Latitude affects the seasonality of food supply. The larvae require more yolk to increase their chance of survival in case of a low food supply at hatching. Body condition and egg dry weight are more important parameters for determinate fecundity type. Adults and larvae of indeterminate spawners will have a more continuous food supply. Hence the timing of larval window is less restricted compared to determinate spawners; the amount of yolk needed by the larvae of indeterminate spawners will be smaller compared to larvae of determinate spawners. Hence egg dry weight will be a less important factor for indeterminate spawners.

FIGURE 6.2



Energy content of a female and vitellogenic oocyte growth in two spawning seasons in an environment with a) highly seasonal food availability and a determinate fecundity type and start of the oocyte maturation at the spring equinox, b) highly seasonal food availability and a determinate fecundity type and start of the oocyte maturation at the autumn equinox, c) food available throughout the year and an indeterminate fecundity type;

···· metabolic energy level;

---- final oocyte size;

— energy content;

— oocyte growth.

Conceptual model of fecundity regulation

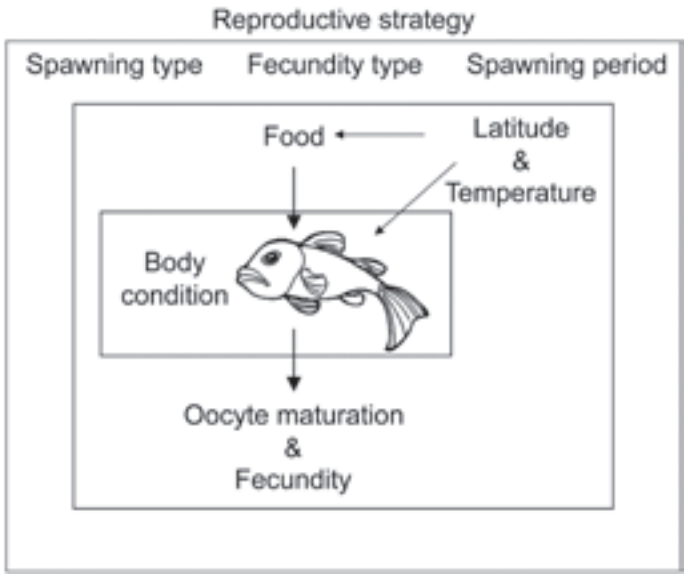
The two extreme situations fish experience are a high seasonality in food availability (FIGURE 6.2a) or a rather stable 6.food supply throughout the year (FIGURE 6.2c). When the energy content of the female is above the metabolic need, it can invest in oocyte maturation (Rijnsdorp, 1990). Oocyte maturation in females experiencing a seasonal food availability starts off before maximum surplus energy is gained at the spring equinox (FIGURE 6.2a) or after maximum surplus energy is gained at the autumn equinox (FIGURE 6.2b). The final maturation of the oocytes in these females occurs when the energy level is again at the metabolic rate (FIGURES 6.2a & b). No energy is available to mature other batches of vitellogenic oocytes (FIGURES 6.2a & b) and these females have a determinate fecundity type. In case of food availability throughout the year, the energy level does not reach a high level, but remains above the metabolic rate most of the year (FIGURE 6.2c). When the first batch of oocytes reach final maturation the females still have enough energy left to mature other batches of vitellogenic oocytes (FIGURE 6.2c). These females can have an indeterminate fecundity type.
In the conceptual model the reproductive strategy is defined as the combination of the above described reproductive traits: spawning type, fecundity type and spawning period (FIGURE 6.3). We test inferences from the conceptual fecundity type model in marine fish and relate this to fish condition and other biological and environmental factors influencing reproduction using Principal Component Analysis (PCA, (Grossman *et al.*, 1991)) in R (The R Project for Statistical Computing, http://www.r-project.org/). We test the hypothesis that food availability through the surplus energy, thus body condition, of the female is the main factor driving reproductive strategy. Females with high amounts of food available during the spawning period have an indeterminate reproductive strategy, while fish that have no food available during the spawning period are determinate spawners. In other words, the reproductive strategy for a species is not 'fixed', but 'flexible' and differs between populations within a species depending on the food availability and thus the environment these populations inhabit (Kjesbu, 2009).

Review of reproductive traits

Most reproductive studies have focussed on commercially important species. Therefore, this study focuses on different populations of these data rich species: anchovy, herring, hake *Merluccius merluccius*, cod, horse mackerel *Trachurus trachurus*, mackerel *Scomber scombrus*, Japanese flounder *Paralichthys olivaceus*, plaice *Pleuronectes*

platessa and sole (TABLES 6.2 & 6.3). Herring, cod and plaice are known determinate spawners (Damme *et al.*, 2009b, Kjesbu *et al.*, 1990, Urban, 1991) and anchovy and hake have an indeterminate fecundity (Murua *et al.*, 1998, Motos, 1996). Mackerel and sole are considered determinate spawners but during the spawning period oocytes of all development stages are found in the ovary (Greer Walker *et al.*, 1994, Witthames and Greer Walker, 1995) meaning that they are in 'grey zone' between 'determinacy' and 'indeterminacy'. Horse mackerel is considered an indeterminate type (Gordo *et al.*, 2008) but so far no direct evidence of this is available. Since the fecundity type for horse mackerel, mackerel and sole is unclear no fecundity type was set before the present exploratory analysis.

FIGURE 6.3



Conceptual model of parameters influencing reproductive traits which influence reproductive strategy in marine fish

Parameters as relative fecundity are length dependent. To be able to compare between the populations within a species the data were recalculated to standard fish length (TABLES 6.2 & 6.3). For determinate spawners the relative fecundity data were taken just prior to the onset of spawning. Relative fecundity of the indeterminate spawners was calculated from relative batch fecundity and the number of batches produced in a single spawning period. Temperature was calculated as the mean temperature at the

spawning ground during the spawning season. Latitude is the centre of the spawning area.

Multivariate analysis

A model including non-biological parameters, latitude and temperature (EQUATION 6.1) was tested (see TABLE 6.4 for explanation of the parameters).

$$Fecundity\ type = spawning\ type + spawning\ period + relative\ fecundity + food\ availability\ during\ spawning + body\ condition + egg\ dry\ weight + duration\ of\ oocyte\ maturation\ cycle + latitude + temperature \quad [6.1]$$

Food availability and latitude appear to be the most important parameters (TABLE 6.5) and explain 70% of the variance in the model. Food availability has a positive effect while latitude has a negative effect. There is a clear separation of the populations in fecundity type; determinate and indeterminate spawners and the group with uncertain fecundity type (FIGURE 6.4). Species with unclear fecundity type, horse mackerel, mackerel and sole, show up as a separate group (FIGURES 6.4 & 6.5), but they are on the indeterminate side of the graphs. This would suggest a more indeterminate fecundity type for these species.

Factors determining the determinate fecundity type are body condition and egg dry weight and the environmental parameter latitude (FIGURE 6.4). This corroborates the inferences made by Rijnsdorp and Witthames (2004). Determinate spawners have a highly fluctuating body condition during a reproductive cycle and a relatively low body condition after spawning. For indeterminate spawners seasonal variations in body condition are less.

Relative fecundity, spawning period and food availability and the environmental parameter temperature are important factors determining the indeterminate fecundity type. Both relative fecundity and spawning period are directly influenced by the food availability during spawning since indeterminate spawners take up food during the spawning season (TABLE 6.2). A shortage of food during the spawning season will have a direct negative effect on the relative and annual fecundity since the female will have no extra surplus energy available for the development of oocytes and will only be able to spawn a limited amount of batches for that spawning period.

TABLE 6.2

Reproductive traits of fish species, with known fecundity type, data are from standard length fish. Fecundity type: 1) determinate, 2) indeterminate; food availability 1) capital, 2) income. Temperature and latitude are the average for the spawning area. massa quis enim.

Species	Area	Standard length (cm)	Fecundity type	Spawning type (n batches)	Spawning period (months)	Relative fecundity (g-1)	Food availability
Anchovy	Aegean Sea (AS)	15	2	19	2.50	5700	2
Anchovy	Bay of Biscay (BoB)	15	2	19	2.50	9528	2
Herring	North Sea Autumn spawners (NSA)	34	1	1	0.25	343	1
Herring	North Sea Winter spawners (NSW)	34	1	1	0.25	178	1
Herring	Norwegian Spring Spawners (NSS)	34	1	1	0.25	195	1
Herring	Icelandic Summer Spawners (ISS)	34	1	1	0.25	530	1
Hake	Bay of Biscay (BoB)	60	2	20	3.00	2260	2
Hake	Galician Shelf (GS)	60	2	20	3.00	4360	2
Cod	North Sea (NS)	90	1	15	1.50	711	1
Cod	Irish Sea (IS)	90	1	15	2.00	892	1
Cod	Barents Sea (BS)	90	1	15	1.00	497	1
Cod	Gulf of St Lawrence (GSL)	90	1	9	2.50	629	1
Japanese flounder	Pacific Ocean	60	2	67	2.50	5000	2
Plaice	North Sea (NS)	35	1	5	1.50	238	1
Plaice	Irish Sea (IS)	35	1	5	1.50	250	1

TABLE 6.2 CONTINUED

K	Egg dry weight (mg)	Oocyte maturation (months)	Latitude	Temperature (°C)	References
0.3	0.06	9	38.5	21	(Somarakis *et al.*, 2002, Krautz *et al.*, 2010)
0.7	0.06	9	43.5	22	(Somarakis *et al.*, 2002, Motos, 1996, Sanz and Uriarte, 1989, Krautz *et al.*, 2010)
0.9	0.16	5	58.5	13	(Haegele and Schweigert, 1985, Hempel and Blaxter, 1967, Zijlstra, 1973, Damme *et al.*, 2009b)
0.9	0.38	8	50.0	10	(Haegele and Schweigert, 1985, Hempel and Blaxter, 1967, Zijlstra, 1973, Damme *et al.*, 2009b)
0.9	0.30	10	65.0	6	(Haegele and Schweigert, 1985, Hempel and Blaxter, 1967, Ndjaula *et al.*, 2010, Óskarsson *et al.*, 2002, Prokopchuk, 2009, Kurita *et al.*, 2003)
0.9	0.16	5	65.0	7	(Haegele and Schweigert, 1985, Hempel and Blaxter, 1967, Óskarsson, 2008, Óskarsson and Taggart, 2006, Oskarsson and Taggart, 2009, Óskarsson and Taggart, 2010)
0.6	0.03	10	45.0	12	(Korta *et al.*, 2010, Murua *et al.*, 2006, Domínguez-Petit *et al.*, 2008, Alvarez and Cotano, 2005, Murua *et al.*, 1998, Macchi *et al.*, 2006)
0.6	0.03	10	43.0	15	(Korta *et al.*, 2010, Murua and Motos, 2006, Murua *et al.*, 1998, Domínguez-Petit *et al.*, 2008, Domínguez-Petit *et al.*, 2010, Macchi *et al.*, 2006)
1.1	0.75	5	55.0	8	(Thorsen *et al.*, 2010, Fox *et al.*, 2008, Oosthuizen and Daan, 1974)
1.2	0.75	5	53.0	10	(Thorsen *et al.*, 2010, Fox *et al.*, 2000)
0.9	0.32	5	67.0	4	(Thorsen *et al.*, 2010, Kjesbu *et al.*, 1996b, Kjesbu *et al.*, 1998, Kjesbu *et al.*, 2010)
0.8	0.15	6	48.5	5	(Lambert, 2008, Méthot *et al.*, 2005, Ouellet *et al.*, 2001, Ouellet *et al.*, 1997)
0.02	0.04	1.5	37.5	14	(Kurita and Kjesbu, in prep)
1.0	0.84	10	52.0	8	(Damme *et al.*, 2009a, Rijnsdorp and Vingerhoed, 2001, Rijnsdorp, 1991, Rijnsdorp, 1989, McGurk, 1986, Damme *et al.*, in prepr)
1.0	0.84	10	54.0	8	(McGurk, 1986, Kennedy *et al.*, 2007a, Kennedy *et al.*, 2007b, Rijnsdorp, 1989)

TABLE 6.3

Reproductive traits of fish species, with unclear fecundity type, data are from standard length fish. Food availability 1) seasonal, 2) during the spawning season. Temperature and latitude are the average for the spawning area.

Species	Area	Standard length (cm)	Fecundity type	Spawning type (n batches)	Spawning period (months)	Relative fecundity (g-1)	Food availability
Horse mackerel	Western Atlantic (W)	25	?	16	2.50	1040	2
Mackerel	Western Atlantic (W)	35	?	30	2.50	1100	2
Mackerel	Southern stock (S)	35	?	30	2.50	1100	2
Sole	Portugal (Por)	35	?	30	2.00	205	2
Sole	English Channel (Chan)	35	?	30	2.00	324	2
Sole	North Sea (NS)	35	?	30	2.00	440	2

TABLE 6.4

Description of the parameters in the fecundity type model (Eq. 6.1).

Parameter	Description	Type
Fecundity type	Determinate or indeterminate	Categorical
Spawning type	Number of batches produced	Continuous
Spawning period	Duration of spawning	Continuous
Relative fecundity	Fecundity per gram body weight	Continuous
Food availability	Outside the spawning season or during the spawning season	Categorical
Condition	Fulton's K	Continuous
Egg dry weight	Dry weight of a single egg	Continuous
Oocyte maturation	Duration of oocyte development from previtellogenic to ovulation	Continuous
Latitude	Latitude at the centre of the spawning area	Continuous
Temperature	Average temperature during the spawning period	Continuous

TABLE 6.3 CONTINUED

K	Egg dry weight (mg)	Oocyte maturation (months)	Latitude	Temperature (°C)	References
0.8	0.14	6	52	12	(ICES, 2008b, Abaunza *et al.*, 2003, Damme *et al.*, 2005, Damme *et al.*, in prep)
1.0	0.14	6	52	12	(McGurk, 1986, ICES, 2008b, Watson *et al.*, 1992, Greer Walker *et al.*, 1994)
1.0	0.14	6	39	12	(McGurk, 1986, ICES, 2008b, Watson *et al.*, 1992)
1.0	0.23	7	41	14	(Witthames *et al.*, 1995, Vinagre *et al.*, 2008, McGurk, 1986)
1.0	0.23	7	50	7	(Witthames *et al.*, 1995, McGurk, 1986, Witthames and Greer Walker, 1995)
1.0	0.23	7	54	7	(Witthames *et al.*, 1995, McGurk, 1986, Witthames and Greer Walker, 1995)

To test if food availability is the main factor influencing fecundity type, within the available dataset (TABLES 6.3 & 6.4) this parameter was changed from capital to income for the determinate cod and plaice populations and the PCA was rerun. The cod and plaice populations moved from the determinate spawner group side of the graph closer to the indeterminate fecundity type group (FIGURE 6.5). Based on this, when food is available during the spawning season, determinate spawners will be able to show a more indeterminate fecundity type. Kjesbu *et al.* 1996b showed that cod in captivity which are extremely fed the whole length of time up spawning have a clear overproduction of vitellogenic oocytes. North Sea plaice is able to up-regulate fecundity after the summer feeding season (Damme *et al.*, in prep.). Also sole are known to show a more indeterminate fecundity type in situations with high food availability during spawning (Witthames and Greer Walker, 1995, Kjesbu, 2009, Rijnsdorp and Vingerhoed, 1994).

Capital and income breeding strategies have been recognised in insects (e.g. (Wessels *et al.*, 2010)) and many vertebrates: reptiles (e.g. (Bonnet *et al.*, 1998)), amphibians (e.g.

(Bull and Shine, 1979, Elmberg, 1991, Jönsson *et al.*, 2009)) and birds (e.g.(Drent and Daan, 1980, Meijer and Drent, 1999)). However, recent studies show there is continuum between the two extreme reproductive strategies and animals are flexible in reproductive strategies. Insects use stored energy for the production of the first batch, but are able to utilise available food resources for producing following batches (Wessels *et al.*, 2010). Marine zooplankton produce a first batch of eggs before the feeding season and increasing the reproductive output with later income breeding (Varpe *et al.*, 2009). Recently it has been shown that reptiles and birds that have a capital breeding strategy utilise income energy to add extra nutrients and lipids to their eggs (Warner *et al.*, 2008, Bond and Diamond, 2010, Rendón *et al.*, 2011). Hence, most organisms optimise their reproductive strategies to food supplies available and are flexible to cope with changing circumstances.

FIGURE 6.4

Results of the PCA for fecundity type model (Eq. 6.1) and loadings of the different parameters.

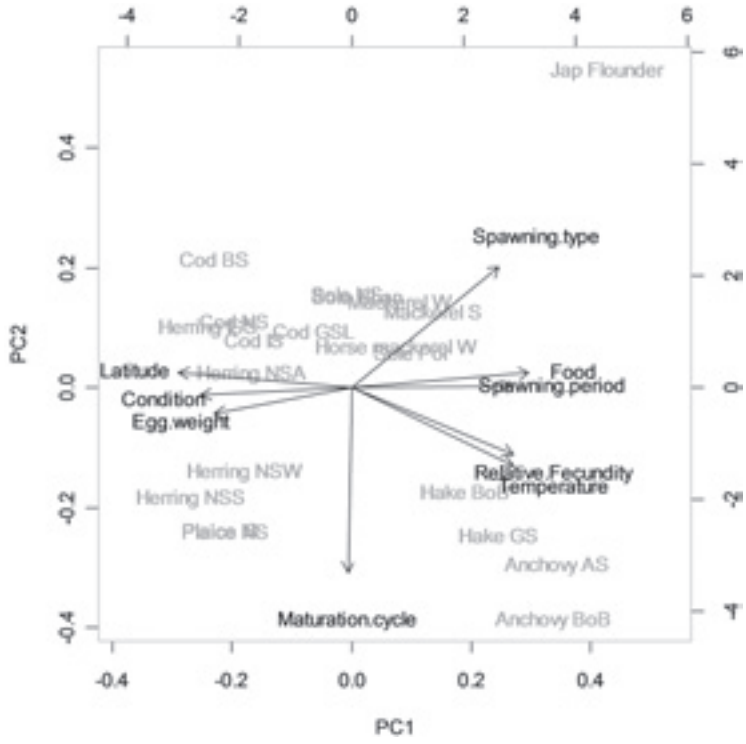


FIGURE 6.5

Effect of the change in food availability during the spawning season on cod and plaice.

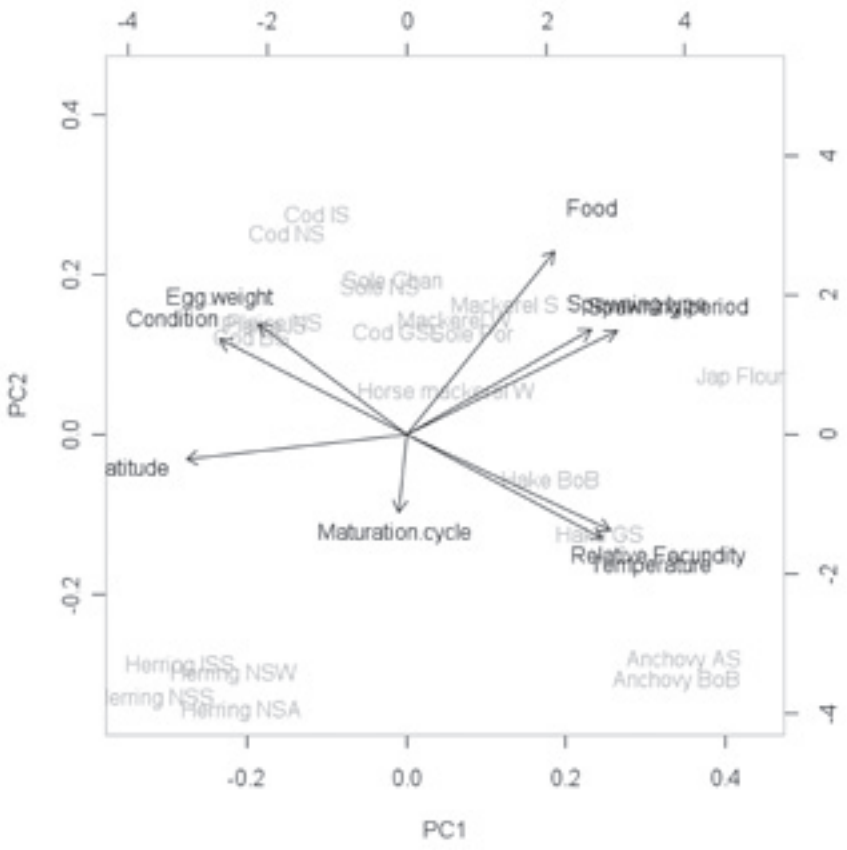


TABLE 6.5

Importance of parameters in fecundity type model (Eq. 6.1)

	Spawning type (n batches)	Spawning period (months)	Relative fecundity (g-1)	Food availa-bility	K	Egg dry weight (mg	Oocyte matu-ration (months	Latitude	Temperature (°C)
Weight	0.33	0.36	0.36	0.39	-0.34	-0.30	-0.01	-0.39	0.36

Conclusions

Generally, food availability during the spawning season and latitude are the most important factor explaining fecundity type (FIGURE 6.4 & TABLE 6.5). Body condition, egg dry weight and latitude stand out as the most important parameters for the determinate spawners, while relative fecundity, spawning period, food availability and temperature appear important for the indeterminate spawners (FIGURE 6.4). The model shows a more indeterminate fecundity type for species with unclear fecundity as horse mackerel, mackerel and sole.

Taken together, this review clarifies that food availability during the spawning season is the primary factor influencing fecundity type of a marine fish. If food is available during the spawning season a determinate spawner could in theory switch to a more indeterminate fecundity type, but will not become a definite indeterminate spawner. Thus, fecundity type of marine fish females is not 'fixed' but 'flexible'.

Acknowledgements

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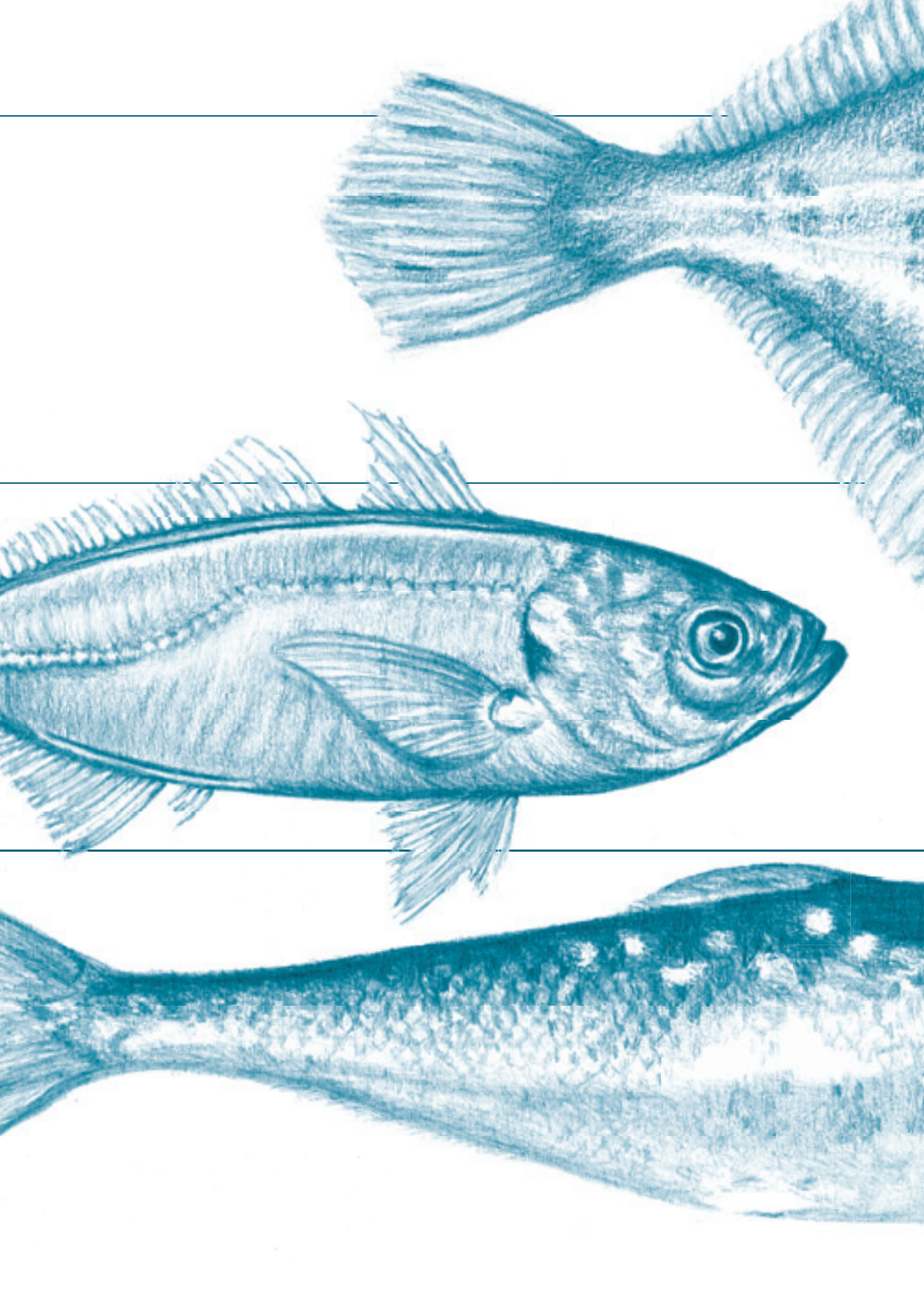
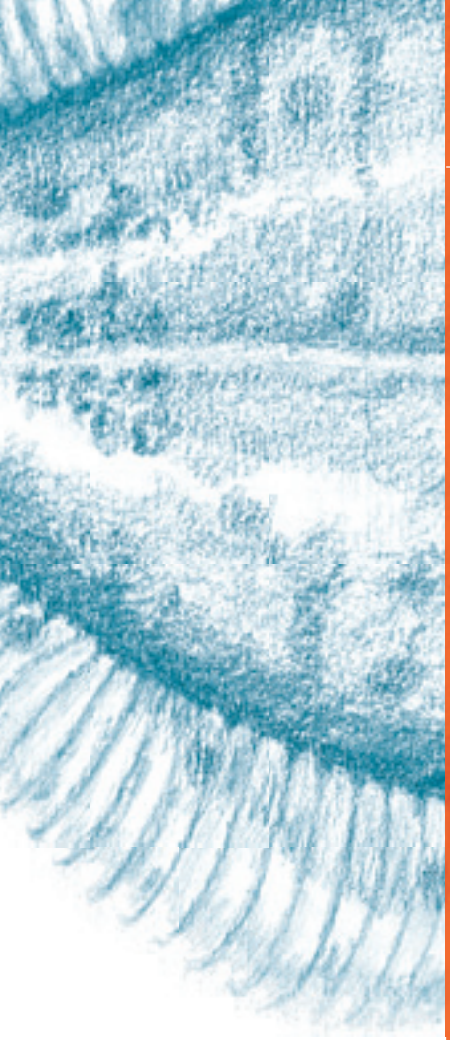
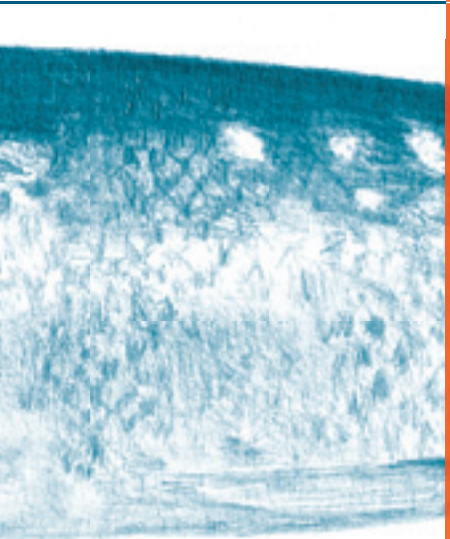




Chapter 7

Synthesis



Cindy J.G. van Damme

Fisheries management and reproduction biology

The biomass of the adult population of exploited species – the spawning stock biomass SSB - is an important indicator of the status of the stock. The SSB can be estimated from fisheries dependent and fisheries independent methods (Egg Production Methods). Knowledge on the reproductive biology of the commercial species is of paramount importance to assess the accuracy of the estimates of SSB and the credibility of the assumptions made in the estimation.

In this thesis female reproductive strategies of a variety of fish species and populations within a species are investigated. The thesis focuses on the mechanisms regulating the reproductive strategies and fecundity and the consequences for the estimation of spawning stock biomass and its application in the management of fish stocks. Fecundity type, egg size and fecundity are related to information on food availability and body condition. It also shows and discusses the possibilities for the use of reproductive strategy and fecundity regulation fisheries management.

The main questions are:

- What are the underlying mechanisms regulating fecundity and reproductive strategy?
- What are the implications of the different fecundity types and reproductive strategies on the inter-annual variation in population egg production?
- What are the implications of the different fecundity types and reproductive strategies for the use in fisheries management?

The oocyte maturation cycle and fecundity development is studied from the first stage, cortical alveoli, until the final maturation of the oocytes. Oocyte maturation and fecundity in first time or recruit spawners is compared to repeat spawners within a single stock and between stocks within a species. Oocyte maturation and fecundity development is related to fish body conditions and environmental factors and how the fecundity type and reproductive strategy of a female is determined by these factors.

Fisheries dependent estimate of SSB

To assess the size of SSB it is necessary to collect data on the maturation of fish and egg production. Maturation is assessed from the development stage of the gonads, ovary or

testis, of the fish throughout the year, thus in various stages of oocyte or sperm development. It is important to know the reproductive strategy and the way oocytes develop through the year in order to assess if the female is juvenile, a first time spawner or repeat spawner and if she will be able to spawn her eggs in the spawning period. In plaice *Pleuronectes platessa* recruit spawners start oocyte development at the same time as repeat spawners but spawning of repeat spawners occurs earlier that recruit spawners (CHAPTER 2). Autumn and winter spawning herring *Clupea harengus* populations start oocyte development at the same time. But winter spawners continue to develop the oocytes during autumn (CHAPTER 3). When assessing maturity of the ovary before the spawning period, oocyte and ovary development stage will be the same for recruit and repeat spawners as well as winter and autumn spawners. But during the spawning period repeat spawners can have spawned while recruit spawners still haven't started spawning. In autumn, the autumn spawning herring will have started spawning while the winter spawners are still developing oocytes.

Fecundity (total number of oocytes produced by a female) is needed to for a reliable estimate of the egg production of the spawning stock. Ideally fecundity should be measured just prior to spawning, but this is not always possible. Most fecundity studies focus on a certain point in time before spawning or during spawning, thereby ignoring the possibility of regulation processes affecting the ultimate fecundity at the time of spawning (CHAPTERS 2, 3 & 5).

Egg production methods

Egg production methods (EPM) are an important tool in fisheries management to provide information for and assist in the management and recovery of intensively fished stocks (Armstrong and Witthames, 2012, Bernal *et al.*, 2012, Kraus *et al.*, 2012). EPM deliver fisheries independent estimates of Spawning Stock Biomass (SSB) and can be used in recovery situations where fishing effort must be reduced to rebuild the SSB and thus no biological sampling of the fish can be carried out.

Although the underlying principle is simple (if you know how many eggs are spawned in a spawning season and if you know how many eggs are produced by a single female in that spawning season you can calculate the size of the spawning stock (Bernal *et al.*, 2012)), EPM is not as straight forward as it sounds because the method is sensitive to changes in fecundity (numbers of eggs produced by a female) (Stratoudakis

et al., 2006, Somarakis *et al.*, 2004, Damme *et al.*, 2009). In order to get a reliable fecundity estimate one needs to have a good insight in the fecundity regulation of the female.

Oocyte development and fecundity regulation sampling

To study fecundity regulation in commercially exploited fish species, ideally one would carry out tank experiments and follow the ovary development in individual fish. Tank experiments with fish are costly and pose a number of practical difficulties. It is often difficult to get fish in captivity to spawn. Although, tank experiments have the possibility to control the environment of the fish, this will not necessarily lead to comparable circumstances as in the field. In addition, not all fish species can be handled easily and taking successive biopsies of ovaries during the developmental process is not always feasible making it difficult to follow the oocyte development and fecundity regulation of individuals. Alternatively, in a field study it is not possible to follow the oocyte development and fecundity regulation of individual fish, although it does give the possibility to follow and compare different populations within a species.

This thesis combined the experimental approach with field studies to study the development of the ovary from the start of oocyte development until the end of spawning. The samples were collected from running survey and market sampling programmes.

TABLE 7.1

Oocyte development period and final oocyte size in herring and plaice.

Species	Spawners	Start of oocyte development	Oocyte size at start of development (µm)	Final oocyte size (µm)	Timing of spawning
Herring	Autumn	April	185	965	September
	Winter	April	185	1345	December-January
Plaice	Recruit	March	185	1190	December
	Repeat	March	185	1190	January

Oocyte maturation and fecundity regulation

The first stage of oocyte maturation, the cortical alveoli stage, is triggered by the production of chemicals and hormones in the female. Photoperiod is an important cue for production of these chemicals and hormones (Migaud *et al.*, 2010). Increase or decrease in daylight length and light intensity occurs when the sun crosses the equator, tropic of Capricorn or tropic of Cancer at the spring and autumn equinox. This is suggested as the trigger for the start of oocyte maturation in some species (Kjesbu *et al.*, 2010; CHAPTER 2). The duration of the oocyte maturation, from cortical alveoli stage to final maturation, is dependent on the final oocyte or egg size and the development rate of the oocytes. The development rate is influenced by temperature and metabolic rate of the female.

Oocytes undergo the same overall development pattern in all marine teleosts, irrespective of reproductive strategy (Tyler and Sumpter, 1996). The rate of oocyte development and the final oocyte size differ between species, as well as within species (TABLE 7.1). Recruit spawning plaice start oocyte development at the same time as repeat spawners (CHAPTER 2). But repeat spawners exhibit faster oocyte growth and are therefore ready to start spawning earlier, while recruit spawners reach maximum oocyte size later and as a consequence spawn later. In Atlantic herring the oocyte maturation period differs between populations (CHAPTER 3). Both autumn and winter spawning herring start oocyte maturation in April. Oocyte development and growth is the same for both spawning types until September when the autumn spawners oocytes undergo final maturation. Winter spawners continue to grow their oocytes until they are ready to be spawned in December-January. This shows a high plasticity in herring spawning strategy.

Income breeding (which utilise income energy) horse mackerel has an indeterminate spawning strategy (de novo oocyte recruitment during spawning) and oocyte development starts just prior to spawning (CHAPTER 4), in contrast to the capital breeders (which use stored energy) with determinate (no de novo oocyte recruitment during spawning) spawning strategy, such as plaice and herring.

At the start of oocyte maturation in determinate spawners, generally 6-10 months prior to spawning, fecundity is high. During the oocyte maturation period fecundity is down-regulated through follicular atresia according to the surplus energy available (CHAPTERS 2 & 3). Females start developing a high number of oocytes at the start of the oocyte maturation period in order to end up with the maximum number of eggs that

she can spawn during the spawning period. During the oocyte maturation period the female gains energy for the oocyte development. The number of developing oocytes is reduced or down-regulated according to the energy the female has gained at the point of highest body condition. The oocytes which will not be spawned are resorbed by the female (follicular atresia). In herring, fecundity is down-regulated over the whole oocyte maturation period (CHAPTER 3; Kurita *et al.*, 2003). Plaice has a summer feeding period and highest body condition level is reached at the end of summer. After the initial down-regulation of fecundity until summer (CHAPTER 2; Kennedy *et al.*, 2007), plaice females are able to up-regulate their fecundity after the summer feeding period by recruiting new oocytes to the vitellogenic phase (CHAPTER 2). This shows the high flexibility in regulation of fecundity. Also in indeterminate spawners down-regulation of fecundity is observed, e.g. horse mackerel (CHAPTER 4).

Independent of reproductive strategy, fecundity increases with size of the female as shown in plaice, herring and horse mackerel (Kurita *et al.*, 2003, Kennedy *et al.*, 2007; CHAPTERS 2, 3 & 4).

Reproductive strategies and fecundity type

Fecundity and thus reproductive strategy is a consequence of the surplus energy, e.g. the total energy that is available for reproduction and somatic growth during a reproductive cycle (Rijnsdorp, 1990). In determinate spawners there is a positive relationship between body condition and fecundity (CHAPTERS 2 & 3). In indeterminate spawning horse mackerel a positive but weak relation is found (CHAPTER 4). Determinate spawners feed and reach highest body condition well before spawning and most species stop feeding during the spawning period. Hence, they use stored energy for the development of their oocytes. Body condition of horse mackerel dips just prior to spawning indicating that the first batch of oocytes is partly develop from stored energy (CHAPTER 4). This has also been shown for income breeding insects and zooplankton (Wessels *et al.*, 2010, Varpe *et al.*, 2009). Body condition in horse mackerel increases again after the onset of spawning, showing continued feeding while spawning. In the conceptual model of fecundity regulation, fecundity is regulated by the body condition of the female, while the body condition itself is regulated by the food that is available to the female, prior to and during spawning (CHAPTER 6). The conceptual model clarifies that food availability during the spawning season is the primary factor influencing

fecundity type of a marine fish (CHAPTER 6). This also indicates plasticity in fecundity type. If food is available during the spawning season a determinate spawner could in theory switch to a more indeterminate fecundity type. This is also shown by the up-regulation in plaice (CHAPTER 2), where the females utilise income energy to increase their fecundity after the summer feeding period. Thus, fecundity type of marine fish females is not 'fixed' but 'flexible'.

At the start of this thesis work the common believe in fish reproductive biology was that despite different reproductive strategies a female could be classified as either a capital breeder with determinate fecundity type or an income breeder with an indeterminate fecundity type (Murua and Saborido-Rey, 2003, Kjesbu, 2009). Capital and income breeding is recognised in insects (e.g. (Wessels *et al.*, 2010)) and many vertebrates: reptiles (e.g. (Bonnet *et al.*, 1998)), amphibians (e.g. (Bull and Shine, 1979, Elmberg, 1991, Jönsson *et al.*, 2009)) and birds (e.g.(Drent and Daan, 1980, Meijer and Drent, 1999)). However, it is also shown that there is continuum between the two extreme reproductive strategies and that there is flexibility in reproductive strategies. Insects use stored energy for the production of the first batch, but are able to utilise available food resources for producing successive batches (Wessels *et al.*, 2010). Marine zooplankton produce a first batch of eggs before the feeding season and increasing the reproductive output with later income breeding (Varpe *et al.*, 2009). Recently it has been shown that reptiles and birds that have a capital breeding strategy utilise income energy to add extra nutrients and lipids to their eggs (Warner *et al.*, 2008, Bond and Diamond, 2010, Rendón *et al.*, 2011). Even in marine mammals capital and income breeding is recognised and the flexibility of utilising capital or income energy during the weaning period influencing the length of the pregnancy and weaning periods (Houston *et al.*, 2007).

Hence, most organisms optimise their reproductive strategies to food supplies available and are flexible to cope with changing circumstances. It is thus no surprise that fish are also flexible in their fecundity type.

Implications for fisheries management

Maturity stage is used in SSB assessments. When assessing gonad development for maturity staging one should take into consideration at which time of the oocyte development cycle the maturity staging is determined and which part of the population is

sampled. Fecundity can be used reliably in SSB assessments and through EPM (Parker, 1980, Lasker, 1985, Hunter and Lo, 1993, Armstrong *et al.*, 2001; CHAPTER 5). However, as fecundity is down-regulated or up-regulated according to the surplus energy available, for a reliable SSB estimate fecundity should be estimated just prior to spawning.

The Annual Egg Production Method (AEPM) has been used successful for determinate spawners spawning fish (Parker, 1980, Lasker, 1985, Armstrong *et al.*, 1988, Hunter and Lo, 1993, Armstrong *et al.*, 2001) and the Daily Egg Production Method (DEPM) has been used successful for indeterminate spawners (Lasker, 1985, Macewicz and Hunter, 1993, Stratoudakis *et al.*, 2006, Ward *et al.*, 2011). However, it has been shown that the DEPM is sensitive to changes in fecundity (Stratoudakis *et al.*, 2006, Somarakis *et al.*, 2004). In horse mackerel batch fecundity varies over time and within the peak spawning period over latitude (CHAPTER 4). The samples collected for the horse mackerel chapter in this thesis could not be used to estimate spawning fraction thus an actual SSB estimation using the DEPM could not be carried out. But the reliability of an SSB estimate from the DEPM for horse mackerel can be questioned. The species for which the DEPM has been used successfully have a relatively small spawning area, while the western stock horse mackerel has a much larger spawning area extending from the south of Portugal up to west of Scotland. The females in this area will experience different environmental and feeding conditions. Since the flexibility in fecundity type and fecundity regulation is driven by food availability, the question arises if all females in a stock covering a large spawning area exhibit the same reproductive strategy? Or if it is wise to manage such a large stock as one unit? The decision to manage western horse mackerel as one stock is based on genetics (Abaunza, 2008, Abaunza *et al.*, 2008c, Abaunza *et al.*, 2008b). The western horse mackerel are genetically the same but is the reproductive strategy of the females within this stock also the same?

A comparison of the AEPM, DEPM and Daily Fecundity Reduction Method in Baltic cod *Gadus morhua* showed the robustness of all methods in comparison to catch based assessments and only the variance of the methods differed (Kraus *et al.*, 2012). Because the current survey design for horse mackerel is based on an AEPM for mackerel *Scomber scombrus* (ICES, 2011), and the huge variance in the batch fecundity over latitude it might be worth while carrying out an investigation to check the reliability of the AEPM and DEPM for horse mackerel with the current survey restrictions.

Currently only in a few stock assessments reproduction parameters are used through EPM. Although recruitment is recognized as an important parameter in stock

assessment modelling reproductive biology parameter are currently not included. Recent advances in reproductive biology studies have increased the knowledge on fish reproduction and management of fish stocks should take advantage and incorporate this. However, currently most reproductive studies focus on females, while of the effect of males on stock reproductive potential relatively little is known.

Concluding remarks

What are the underlying mechanisms regulating fecundity and reproductive strategy?
Food availability through body condition is the most important factor regulating reproductive strategy of marine teleosts. Fecundity is down-regulated or up-regulated according to the surplus energy the female has available. Females that can take up food during the spawning are able to recruit and develop oocytes during the spawning period and have an indeterminate fecundity type. Females which have no food available during the spawning season need to develop the oocytes prior to the spawning period. These females are capital breeders and have a determinate fecundity type.

What are the implications of the different fecundity types and reproductive strategies on the inter-annual variation in population egg production?
Fish, like other vertebrates and insects, show a high plasticity in reproductive strategy. Depending on the food availability during the spawning female fish can have a determinate or indeterminate fecundity type and theoretically determinate females can exhibit indeterminacy when they have food available during the spawning season and thus can increase the population egg production. The amount of food a female has available not only regulates the reproductive strategy but also down-regulates or up-regulates fecundity and thus the population egg production.

What are the implications of the different fecundity types and reproductive strategies for the use in fisheries management?
For a reliable SSB estimate flexibility in reproductive strategy and fecundity should be taken into account. Due to this flexibility fecundity will not be a constant between or within years. Fecundity is down- or up-regulated according to the surplus energy available and for a reliable SSB estimation one should take into account at which oocyte development stage fecundity and maturation stage are estimated.

Fecundity has been shown to be a reliably tool in fisheries management through EPM. However, for large stocks covering a vast spawning area, changes in fecundity within the spawning area are likely and, the reliability of EPM should be investigated.

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Training and Supervision Plan

Name PhD student	Cindy van Damme	
Project title	Reproductive biology of fish with different spawning strategies	
Group	IMARES	
Daily supervisor(s)	Adriaan Rijnsdorp, Mark Dickey-Collas	
Supervisor(s)	Olav Kjesbu	
Project term	from 01-01-2006	until 23-08-2013
Submitted	23-08-2013	certificate

EDUCATION AND TRAINING (minimum 30, maximum 60 credits)	year	credits *
The Basic Package (minimum 3 credits)		
Subtotal Basic Package	2010	2
Scientific Exposure (conferences, seminars and presentations, minimum 8 credits)		
International conferences (minimum 3 credits)	2007-2011	8,6
Seminars and workshops	2009	1,1
Presentations (minimum 4 original presentations of which at least 1 oral, 1 credit each)	2007-2011	6,0
Subtotal Scientific Exposure		15,7
In-Depth Studies (minimum 6 credits, of which minimum 4 at PhD level)		
Disciplinary and interdisciplinary courses	2007-2010	4,3
Advanced statistics courses (optional)	2009-2013	6,0
Subtotal In-Depth Studies		10,3
Statutory Courses		
Safety at Sea	2008-2012	0,6
Subtotal Statutory Courses		0,6
Professional Skills Support Courses (minimum 3 credits)		
Subtotal Professional Skills Support Courses	2004-2013	7,1
Research Skills Training (optional)		
Subtotal Research Skills Training	2006-2009	5
Didactic Skills Training (optional)		
Supervising theses (max 2 credits per MSc major, 1.5 c MSc minor, 1 c BSc thesis)	2007-2012	4,0
Tutorship (real time)	2012	1,0
Preparing course material (real time)	2009-2010	7,1
Subtotal Didactic Skills Training		12
Management Skills Training (optional)		
Organisation of seminars and courses	2009-2011	4,3
Membership of boards and committees	2007-2013	2,9
Subtotal Management Skills Training		7
EDUCATION AND TRAINING TOTAL (minimum 30, maximum 60 credits)		**60**

* one ECTS credit equals a study load of approximately 28 hours

Colofon

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