MATERNAL EFFECTS AND FECUNDITY OF PLAICE (*PLEURONECTES PLATESSA*) IN THE IRISH SEA

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

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ACKNOWLEDGMENTS

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As I am one of the final PhD students at the Port Erin Marine Laboratory which closes on the 30th June 2006 I feel I should say a great farewell to this outstanding Lab and wish all staff and students who passed through this lab all the best for the future.

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ABSTRACT

Kennedy, J. (2006). Maternal effects and fecundity of plaice (*Pleuronectes platessa*) in the Irish Sea. Ph.D. Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy.

The fecundity of Irish Sea plaice caught from Liverpool Bay, the Cumbrian coast and in the western Irish Sea was estimated in 2001, 2003 and 2004 and was compared with data previously collected for the years 1953, 1995 and 2000. The fecundity was also estimated for plaice caught during September 2004 and 2005. Temporal variation in fecundity was greatest in the western Irish Sea, followed by the Cumbrian coast and there were no significant differences between years in Liverpool Bay. Fecundity estimates from September did not differ between years or between areas but was higher than fecundities estimated during the spawning season. The maximum fecundity of an individual fish was determined by the weight of the fish at the end of follicle proliferation. This was then down regulated by atresia during the period between the cessation of follicle proliferation and spawning. The differences in fecundity between years and areas are hypothesised to be due to differences in the degree of down regulation.

To examine if the degree of down regulation was affected by the feeding level during late vitellogenesis, plaice were housed in individual pens and fed on either a high or low ration of food in late autumn. Biopsy samples were taken at the beginning, middle and end of the experiment and follicle size was determined using image analysis, the percentage of atretic follicles was noted and the change in ovary weight was monitored. Follicle growth rate increased with food level and the level of atresia was negatively correlated with change in condition factor. As food level decreased there was an increased dependence on stored reserves for metabolism and follicle growth.

The Total Egg Production (TEP) for plaice for the whole Irish Sea was back calculated using available fecundity estimates and the Virtual Population Analysis (VPA) data from 1964 to 2004 and compared with indices of recruitment at age 1. TEP was positively related to SSB. Recruitment at age 1 was not related to TEP or SSB. Mortality between the egg stage and recruitment was positively related to TEP which is believed to be due to density dependent processes occurring during the nursery ground phase. The estimates of TEP were approximately one third of the direct estimates of stage 1 egg production from plankton surveys in the eastern Irish Sea. This is hypothesised to be due to inaccuracies in the VPA data.

The effects of maternal size on various egg and larval characteristics were examined using plaice caught from coastal waters around the Isle of Man (Great Britain) and Bergen (Norway). Egg batches were incubated at 7°C with larvae from one batch being monitored at the individual level. Egg size increased with maternal size, with larger eggs producing larger larvae with a greater yolk sac volume. Eggs from earlier batches had greater incubation times than eggs from later times. A longer incubation time led to bigger larvae but with a smaller yolk sac volume. Growth during the two weeks after hatching was related to size at hatching and yolk sac volume, with smaller larvae with larger yolk sacs having the greatest growth. Larger larvae had no survival advantage under the present experimental conditions, which had a plentiful supply of food and no predators.

CHAPTER 1
GENERAL INTRODUCTION

Fisheries assessment models

Since the 1800's the exploitation of marine resources has been increasing due to a rise in the human population and advances in fishing technology (Cushing, 1988) which has resulted in fishing mortality exceeding natural mortality in most fish stocks. In recent years there have been decreases in the catches of many fish stocks throughout the world and in extreme cases, collapse of some stocks e.g. Atlantic cod (*Gadus morhua*) in Newfoundland (Myers *et al.* 1997). Fish are important as a food source with great economic value so there is great interest in preserving the sustainability of fisheries. To achieve this, mathematical models of the population dynamics of exploited populations have been developed which have now become the common tools to assess the status of stocks and to provide the biological basis of management advice (Beverton and Holt 1957; Pitcher and Hart 1982; Gulland 1983). During projections of future spawning stock biomass (SSB) there is an assumption that recruitment is positively correlated with SSB (Myers and Barrowman 1996). However, Marshall *et al.* (1998) showed that for Atlantic cod, spawner biomass estimated by Virtual Population Analysis (VPA) was not proportional to Total Egg Production (TEP). The main reason for this is that these models do not include the biological detail involved in the processes occurring between ovarian maturation and recruitment.

The maintenance of SSB above a set level is essential in fisheries management. At a decreased SSB, recruitment has been shown to be much more variable (Myers 2001) and there is an increase in sensitivity to environmental variability with respects to survival during the early life stages (Brander 2005). At decreased levels of SSB there is an increased dependence on small first time spawners, which produce fewer eggs per weight of fish and produce eggs of lower quality (Hislop 1988; Marteinsdottir and Steinarsson 1998; Trippel 1998). There is also a shorter spawning season and smaller spawning stocks may also occupy a reduced spawning area (Begg and Marteinsdottir 2002). These effects will decrease the distribution of early life stages in space and time reducing the chance that the production of eggs and larvae will coincide with optimal environmental conditions for progeny survival and recruitment success (Begg and Marteinsdottir 2000; Brander 2005). At low population sizes, Allee effects may also affect the

population. These are physiological and behavioural effects which result in decreased reproduction at low population sizes and are not included in assessment models. Examples of these include difficulty in finding a mate, a breakdown in social structure and migration patterns and difficulty in fending off predators or competitors (Frank and Brickman 2000).

Fisheries assessment models also do not consider fisheries-induced trends in reaction norms or possible evolutionary changes in a population. This is brought about by the size selective nature of fisheries. Fisheries generally select for larger sized individuals and fishing mortality can be as much as 4 times greater than natural mortality (Grift *et al.* 2003); this can lead to changes in age at maturation, increased reproductive investment and changes in growth rate (Law 2000; Grift *et al.* 2003; Yoneda and Wright 2004). The age or size at which most species mature is not fixed, but is described by a norm of reaction that is given by a well defined curve in age and size space (Stearns and Koella 1986). It is difficult to disentangle the effects of genetic response from the phenotypic response to concurrent changes in environmental conditions (Rijnsdorp 1993), thus, whether changes in the genetic structure of fish populations have occurred is still under debate. Attempts to disentangle the two responses have been made by several authors. Yoneda and Wright (2004) suggested that the fecundity differences of inshore North Sea cod over the past 30 years was more consistent with genotypic changes than phenotypic changes as there has been a decrease in body condition over the same period. Rijnsdorp (1993) found that the average length of four year old North Sea plaice (*Pleuronectes platessa*) (the age at which 50% of the females were mature) has decreased by 5.8 cm between the early (1904 to 1911) and late (1960 to 1990) 20th century and that phenotypic plasticity could explain about 2.7 cm of this decrease, the remaining was suggested to reflect fisheries induced evolution. In contrast to these studies, changes in the reaction norms for Norwegian spring-spawning herring (*Clupea harengus*) have been largely due to growth related phenotypic plasticity with very little evolutionary response (Engelhard and Heino 2004).

Reproductive ecology and fecundity

Fish must allocate their energy between maintenance, somatic growth, and reproduction (Calow 1985). Many animals store energy when food is plentiful and mobilize stored energy when food is scarce (Reznick and Braun 1987; Doughty and Shine 1997; Jonsson and Jonsson 1997). Often, energy reserves are severely depleted during breeding, when time and energy are diverted from procuring food and into tasks associated with reproduction (Wootton 1998). However, variable abiotic and biotic factors e.g. food availability, make it difficult for an individual to predict the amount of resources required to invest into reproduction. Life history theory suggests that the optimal level of reproductive effort depends on the degree to which additional current reproductive investment reduces future reproductive output (Shine and Schwarzkopf 1992). Future reproduction can be decreased in two ways, through (i) decreases in the organism's survival rate, and/or (ii) decreases in the organism's growth and hence subsequent reproduction (Shine and Schwarzkopf 1992). Therefore iteroparous species (species that reproduce more than once a lifetime) must balance energy allocation between maximising their reproductive output in the current season while maintaining a minimum condition for survival to breed in a subsequent season (Rijnsdorp 1990). Life history models generally predict that reproductive effort increases with age as the expectation of future reproduction decreases which results in reduced somatic growth with age (Gadgil and Bossert 1970; Schaffer 1974; Charlesworth and Leon 1976).

Reproductive effort in terms of fecundity has been shown to vary over temporal and spatial scales in many species including plaice (Bagenal 1966; Rijnsdorp 1991; Nash *et al.* 2000), cod (Kjesbu *et al.* 1998; Kraus *et al.* 2000), haddock (*Melanogrammus aeglefinus*) (Blanchard *et al.* 2003), Greenland halibut (*Reinhardtius hippoglossoides*) (Gundersen *et al.* 2000), sole (*Solea solea*) (Witthames *et al.* 1995), and orange roughy (*Hoplostethus atlanticus*) (Koslow *et al.* 1995). The cause of these variations have been linked to variations in food level (Kjesbu *et al.* 1998) and temperature (Tanasichuk and Ware 1987) and have been associated with reductions in stock size (Koslow *et al.* 1995). Fecundity differences in stocks of the same species may be adaptations to local conditions (Eldridge and Jarvis 1995; Milton *et al.* 1995) which affect the optimal energy allocation to egg

production (Stearns 1976). It has been shown that stocks with a higher recruitment variation generally have higher fecundities (Rickman *et al.* 2000).

Fish are generally considered to have either determinate or indeterminate fecundity. Determinate fecundity is where the potential maximum fecundity becomes fixed prior to the onset of spawning and decreases with each spawning because the standing stock of advanced yolked follicles are not replaced during the spawning season. Indeterminate fecundity is where the potential annual fecundity of a female is not fixed prior to the onset of spawning and un-yolked follicles continue to go through vitellogenesis and are spawned during the current spawning season (Hunter *et al.* 1992).

During vitellogenesis, vitellogenin is transferred into the developing follicles, which thereby are recruited into the maturing pool of follicles to become the current year's potential egg production (Tyler and Sumpter 1996). Fecundity is species specific, although varies within a species due to genetic variation, age, body size, environmental conditions, and nutrition (Bagenal 1969; Horwood *et al.* 1986; Tanasichuk and Ware 1987; Fleming and Gross 1990; Bromage *et al.* 1992; Kjesbu *et al.* 1998).

Fecundity can be down regulated by a process known as atresia which is the process by which follicles are reabsorbed and the material becomes available for other purposes. This process has been documented in many fish species including plaice (Armstrong *et al.* 2001), herring (Kurita *et al.* 2003), turbot (*Scophthalmus maximus*) (Bromley *et al.* 2000), cod (Kjesbu *et al.* 1991; Armstrong *et al.* 2001) and sole (Armstrong *et al.* 2001). This process is regarded as a mechanism by which fish can regulate their realised reproductive output in response to changing nutritional circumstances. This reduces the risk of females overly depleting body reserves and compromising their survival (Bromley *et al.* 2000), a "survive to spawn again strategy".

It is also known that some iteroparous fish will not spawn every year and may "skip spawning" for a particular year. This has been documented in both wild (Fedorov 1971; Ramsay and Witthames 1996; Rideout *et al.* 2000) and captive fish (Rijnsdorp 1990; Burton 1994; Bromley *et al.* 2000). This phenomenon has received very little attention and is not taken into consideration in fisheries assessment models. The failure to account for non-spawning adult fish may lead to an overestimation of the true number of spawners and expected recruitment

(Rideout *et al.* 2005a). The occurrence of skipped spawning can be hard to quantify, especially in fish which partake in spawning migrations where individuals which skip spawning do not migrate with the spawning population and so will not be present in the same location as spawning individuals (Bell *et al.* 1992). There are many reasons why individuals may skip spawning including a shortage of mates (Trippel and Harvey 1990), unsuitable temperature (Fedorov 1971; Pawson *et al.* 2000), low food availability (Hislop *et al.* 1978; Burton and Idler 1987; Rijnsdorp 1990) and recent models have suggested that skipped spawning could be an active component of the life history of fish (Jørgensen *et al.* 2006).

Effects of egg size

The allocated effort to reproduction is partitioned along a scale between a few large or many small offspring (Smith and Fretwell 1974). A negative relationship between egg size and egg number has been detected in several fish populations (LobonCervia *et al.* 1997; Johnston and Leggett 2002; Power *et al.* 2005). However, as maternal size increases there is an increase in both fecundity and egg size (Buckley *et al.* 1991; Kjesbu *et al.* 1996). Larger eggs produce larger larvae (Blaxter and Hempel 1963; Buckley *et al.* 1991; Rideout *et al.* 2005b), which are generally considered to be of a higher fitness, although this view is not universally supported (Miller *et al.* 1988) e.g. optimal egg size can vary with respect to offspring habitat quality (Johnston and Leggett 2002). As the abundance of phytoplankton (Scrope Howe and Jones 1985) and water temperature changes during the spawning season and larger individuals from a population have a tendency to spawn earlier or later in the season than their smaller counterparts (Jonnson 1982; Rijnsdorp 1989; Wright and Gibb 2005), larvae from smaller eggs will experience different environmental conditions than those from larger eggs.

There are various theories concerning the advantages that a larger egg size brings depending on the life history of the species. In sea urchins, where eggs and sperm are released in 'clouds' without paired matings, larger eggs are believed to be advantageous as they provide a larger target for sperm and so there is an increase in fertilisation success with egg size (Levitan 1993). The moor frog (*Rana arvalis*), has distinct populations living in different environments which have different sized

eggs. It was found that the populations with the larger eggs lived in more stressful (acidic) environments (Rasanen *et al.* 2005). This increased egg size was an adaptation to the acidic environment as the larger eggs had an increased chance of survival when in an acidic environment. Larger eggs do not always bring an advantage as seen in the frog, *Bombina orientalis*, where larvae from larger eggs in warm water pools are more susceptible to predation due to larger amounts of inert yolk which diminishes locomotory ability (Kaplan 1992). In fish, larger eggs produce larger larvae which have an increased resistance to starvation (Knutsen and Tilseth 1985; Marteinsdottir and Steinarsson 1998; Rideout *et al.* 2005b), faster swimming speed (Ojanguren *et al.* 1996) and greater escape ability from predators (Bailey 1984). However there are many questions that still remain unanswered including how long the initial size advantage of larvae hatched from larger eggs is maintained during subsequent growth (Springate and Bromage 1985). There is also little knowledge on how these size advantages apply in the wild and whether or not these advantages would be overwhelmed by environmental influences. It has been shown though that territory size is linked to fish size in sea-trout fry (*Salmo trutta*) with territory size being linked to survival (Elliott 1990). In captive fish there is a large variation in the durations of the initial size differential between species with values ranging from 20 days in Siberian sturgeon (*Acipenser baeri*) (Gisbert *et al.* 2000) to 8 months in Atlantic salmon (*Salmo salar*) (Glebe *et al.* 1979). There are conflicting views on the 'bigger is better' hypothesis concerning larval predation. The traditional view is that larger larvae are at a lower predation risk as larger larvae have a higher growth rate and so will spend less time within the vulnerability window of a specific predator, however, this is not necessarily beneficial as an individual may pass from one predator's window to another (Paradis *et al.* 1996). Larger larvae may also be selected preferentially as opposed to smaller larvae by visual predators (Litvak and Leggett 1992).

Estimation of Spawning Stock Biomass from egg production

The SSB of a population can be estimated using the annual egg production method (AEPM) which is based on a series of plankton surveys throughout the spawning season and an estimation of the annual fecundity of the stock (Lockwood

et al. 1981). The TEP is then divided by the average fecundity which gives the number of fish from which the SSB can be calculated. This provides an assessment which has the advantage that it is independent of commercial catch data (Armstrong *et al.* 2001) which can be inaccurate due to misreporting of catches and by-catch. However, experiences in comparing SSB estimates from AEPM with VPA based stock assessments have shown discrepancies that are not fully understood, as seen in the difference in the biomass estimates for cod, plaice and sole in the Irish Sea that were calculated by the AEPM and VPA (Armstrong *et al.* 2001). The reasons for these discrepancies are unknown but could be due to inaccuracies in fecundity estimation or due to poor understanding of the down regulation of fecundity by atresia.

The estimation of fecundity is a time-consuming and labour intensive process. However, due to advances in image analysis there have been many improvements in the methods with much more automation and an increase in information obtainable during the processing of samples (e.g. follicle size distributions, stage of ovary development) (Thorsen and Kjesbu 2001). This has led to a large increase in the knowledge concerning ovary development and factors affecting fecundity and egg production in fishes.

Plaice

Plaice is an abundant and commercially important flatfish occurring on the sandy bottoms of the European shelf. It feeds on polychaetes, crustaceans and bivalves and can found down to about 200 m (Wimpenny 1953). It is a determinate spawner in which fecundity is set before the onset of each spawning season (Urban 1991). Ovary development begins around late August to September (Dawson and Grimm 1980; Rijnsdorp 1989) with the spawning being from December to May. The eggs are released in batches every 3 to 5 days for approximately 1 month for an individual fish (Rijnsdorp 1989). The eggs hatch after approximately two weeks (Fox *et al.* 2003) and drift passively in the plankton. The larvae drift in the plankton but exhibit circatidal vertical swimming as they develop (Fox *et al.* In press). The larval plaice metamorphose after about 8 to 10 weeks (Ryland 1966),

dependent on temperature, at which time they settle in the inter-tidal zone of sandy beaches.

The main spawning grounds for plaice in the Irish Sea (ICES division VIIa) are off St. Bees Head, off Great Ormes head and in the western Irish Sea (along the central-eastern Irish coast) and Cardigan Bay (Simpson 1959). There is also a small spawning location on the west side of the Isle of Man (Ellis and Nash 1997) (Figure 1.1). There is very little mixing of adults between the western and eastern Irish Sea (Dunn and Pawson 2002) and there are differences in several biological traits between the two areas (Nash *et al.* 2000). However, new evidence suggests that some mixing between the east and the west occurs due to the drifting of eggs and larvae from the western Irish Sea into the east (Nash, pers. comm. IMR, Norway). Settlement in the eastern Irish Sea occurs from April to mid-June (Anonymous 2005) with the nursery grounds being generally close to the spawning grounds (Riley *et al.* 1986; Fox *et al.* In Press) and so the distances travelled by the eggs and larvae are relatively short in comparison to eggs and larvae spawned in the North Sea (Talbot 1977; van der Veer *et al.* 1998).

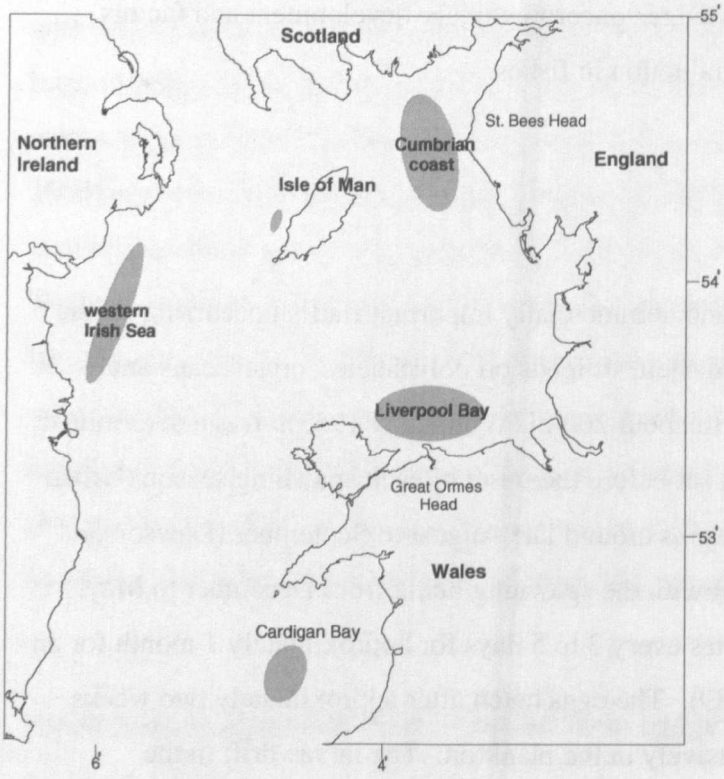


Fig. 1.1 Map of the Irish Sea showing the main plaice spawning areas (Shaded areas) in the Irish Sea: Liverpool Bay, the Cumbrian coast, Cardigan bay and the western Irish Sea. Also shows the small spawning area west of the Isle of Man.

Plaice in the Irish Sea are heavily exploited with over one third of the SSB being taken annually (ICES 2006). This stock is managed under the European Common Fisheries Policy (CFP) with quotas set each year for the total allowable catch (TAC) based on recommendations given by the ICES Working Group on the Assessment of Northern Shelf Demersal Stocks (WGNSDS). The population is currently managed as a single unit (ICES 2006), however it may consist of at least two components (Nash *et al.* 2000; Dunn and Pawson 2002). Population density is much higher in the western Irish Sea and fishing pressure on the adult population varies spatially with a greater pressure traditionally being on the eastern component (Nash *et al.* 2000). Results from egg surveys and research beam trawl surveys have shown that the plaice stock in the Irish Sea has been increasing in recent years, but standard assessments, based largely on commercial catch and effort data did not show this. The reason for this is believed to be due to substantial levels of unaccounted discarding of young plaice in the fishery (Anonymous 2005).

Aims

Several management issues have been mentioned in this introduction including the need for the effective assessment of SSB and the possible effects on population dynamics if there was a substantial reduction in SSB. During this study several aspects of plaice reproductive biology were addressed that could help fill in some of the knowledge gaps needed to improve the management of the Irish Sea stock. A fecundity study (chapter 2) was carried out in order to help understand factors that determine fecundity and also quantify the variations in fecundity between areas and years in the Irish Sea. The quantification of variability will help in the design of the methodology for future estimates of SSB using the AEPM. An alternative to Gilson's fluid for preservation of plaice ovaries and the application of an image analysis system as a tool for the estimation of fecundity in plaice was also assessed. This could give significant improvements in the methodology for fecundity studies in plaice.

It is hypothesised that feeding level during vitellogenesis affects plaice fecundity and differences in food availability result in the observed differences in fecundity in the Irish Sea. This hypothesis was addressed in chapter 3 by means of a laboratory experiment to evaluate the effect of food level during the late stage of

vitellogenesis on fecundity and intensity of atresia. The effects on ovary and follicle growth were also examined. The growth of the ovaries was assessed by the use of a new method for measuring ovary size in live plaice.

Using fecundity-weight relationships from chapter 2, the TEP for the Irish Sea was calculated for the years 1964 to 2004 (chapter 4). This was then compared with estimates of stage 1 egg production for the years 1995, 2000, 2001 and 2003 estimated from plankton surveys (Anonymous 1997; Anonymous 2005). The TEP was also compared with the recruitment at age 1 indices (ICES 2006) and the mortality between the egg stage and recruitment calculated.

With the selective removal of larger females by fishing it is essential to examine how this may affect egg and larval characteristics as these can influence larval survival and thus affect recruitment. Chapter 5 addresses this issue by examining how maternal size affects various egg and larval characteristics and also how these influence survival during the first two weeks after hatching. Batch averages of egg sizes are known to affect larval characteristics but it is unknown how egg size at the individual level affects larval traits. This was also examined during this chapter. Despite the large number of studies that have been carried out on larval fish, examination of egg size influence at the individual level appears to have only been carried out on one previous occasion in fish on a single fish species (capelin (*Mallotus villosus*)) (Chambers *et al.* 1989).

CHAPTER 2

DETERMINATION AND VARIATION IN FECUNDITY OF PLAICE IN THE IRISH SEA

INTRODUCTION

Spawning is a very costly activity with large investments of energy into egg production and the behaviour related to spawning (Rijnsdorp 1990; Smith *et al.* 1990). The total fecundity of a population represents the maximum number of potential recruits to the population and is affected by many factors. The maternal parent must balance resources between maximising reproductive output and also conservation of resources for survival after spawning. Resources must also be partitioned between growth and reproduction. A hypothetical model of the mechanism of surplus production (surplus energy after routine metabolism has been met) based on physiology was proposed by Rijnsdorp (1990). This postulated that surplus production is prioritised into building up reserves followed by reproduction and then somatic growth. This has been seen in plaice from the Irish Sea where sub-populations that had a higher surplus production had a higher reproductive investment (Nash *et al.* 2000). Variation in fecundity of plaice has been shown to occur on both spatial (Bagenal 1966; Nash *et al.* 2000) and temporal scales (Bagenal 1966; Horwood *et al.* 1986; Rijnsdorp 1991) and can be affected by food level (Horwood *et al.* 1989).

Plaice have a determinate spawning strategy, in which the annual fecundity of an individual female is determined before the onset of the spawning season (Urban 1991), with the development of the ovary beginning several months before spawning (Dawson and Grimm 1980; Rijnsdorp 1989). During spawning, eggs are released in batches which are recruited into final maturation at intervals of two to five days over a period of four to six weeks (Rijnsdorp 1989).

Fecundity estimates of plaice taken in 1995 from the Irish Sea exhibited spatial variation with fish from Liverpool Bay having the highest fecundity and fish from the western Irish Sea having the lowest (Nash *et al.* 2000). Nash *et al.* (2000) also showed that fecundity in Liverpool Bay and the Cumbrian coast in 1995 was not significantly different from estimates taken in 1953 (Simpson, unpublished data). This has also been the case in Cardigan Bay for the years 1953 and 1988 (Horwood 1990). No studies have looked at fecundity over a number of consecutive years in the Irish Sea.

Several species have been shown to recruit more oocytes than are taken to full development including herring (Kurita *et al.* 2003), turbot (Bromley *et al.* 2000), cod (Kjesbu *et al.* 1991; Armstrong *et al.* 2001) and sole (Armstrong *et al.* 2001). The fecundity is then down-regulated by atresia in relation to available energy reserves (Kurita *et al.* 2003). Atresia has also been shown to occur in pre-spawning plaice but this has been of low prevalence and intensity (Armstrong *et al.* 2001).

The SSB of a stock can be calculated using egg production estimates from plankton surveys (Lockwood *et al.* 1981); however these require accurate estimates of fecundity of the species in question. It is therefore important to have an understanding of the variability in fecundity of fish populations that are assessed by such methods; plaice in the Irish Sea being one of these populations with egg surveys planned to take place in 2006, 2008 and 2010 (Witthames, pers. comm. Cefas, UK).

This chapter aims to develop the application of image analysis for fecundity estimation of plaice in order to provide a more complete picture of follicle number and development and ovary maturation. The image analysis can be used for fecundity estimation using the Auto-diametric method which works on the principle that mean follicle diameter is negatively correlated to the number of follicles per gram of ovary tissue (follicle density). This has been demonstrated in cod (Thorsen and Kjesbu 2001) and is also true for plaice and mackerel (*Scomber scombrus*) (Witthames, pers. comm. Cefas, UK). By using this method, information on follicle diameter and size distributions can be collected which is indicative of the stage of ovary maturation (Kjesbu 1994).

The use of formaldehyde as an alternative to Gilson's fixative was investigated. Gilson's has been the traditional chemical used in fecundity estimation of several fish species however it is highly toxic and very costly for disposal. Tissue fixed in Gilson's is also unsuitable for histology. The use of formaldehyde in the fixation of ovary tissue for fecundity studies has not been applied to plaice previously. Formaldehyde has several advantages as it is less toxic than Gilson's and is less costly to dispose of.

The fecundity of plaice from the three main spawning areas of the Irish Sea (Liverpool Bay, Cumbrian coast and the western Irish Sea) over several years was estimated. The estimates were then tested for differences between years and

examined for an effect of whole body condition and muscle condition (muscle water content) on individual fecundity. Whole body condition was calculated as it is an indication of the level of stored energy available to the fish and a high water content in the muscle is an indication of protein depletion (Stirling 1976; Costopoulos and Fonds 1989). Fecundity estimates were also taken in September to investigate whether down regulation of fecundity occurs between the period of oocyte recruitment and spawning.

MATERIALS AND METHODS

Estimation of fecundity

The estimation of fecundity was carried out using a combination of the gravimetric (Hunter *et al.* 1989) and the Auto-diametric method (Thorsen and Kjesbu 2001). Follicle counts and measurements were performed using a PC based image analysis system Aphelion (ADCIS France) with commercially available software GFA (Pilkington Image Analysis Systems). The system consisted of a binocular microscope (magnification set at 24x), with a Pulnix TMC 1000 camera (resolution of 1 million pixels) that provided a live image of the sample on the PC monitor. Follicle samples were stained with Periodic acid and Schiffs reagent (see Appendix I) to improve the identification of follicles during image analysis. Follicles were identified by the computer program by analyses of the grey scale across the field of view as seen in Fig. 2.1. Illumination of the sample was standardised to a fixed grey scale for the 3 primary, red green and blue light colours to give a 1:1 relationship between follicles measured manually and those measured by image analysis (Appendix 1). Follicles that were not identified automatically were measured manually using point to point measurement with the distance calculated by the computer. The threshold between vitellogenic and pre-vitellogenic follicles was set at 200 μm as histological evidence shows that is the size in which yolk granules start to appear in the follicles (Witthames, pers. comm. Cefas, UK).

Gravimetric analysis

The pair of ovaries were dissected out of the dorsal and ventral sides of the body and preserved in 3.6 % formaldehyde buffered to pH 7.0. Prior to handling (after fixation) ovaries were rinsed in reverse osmosis water, to remove excess

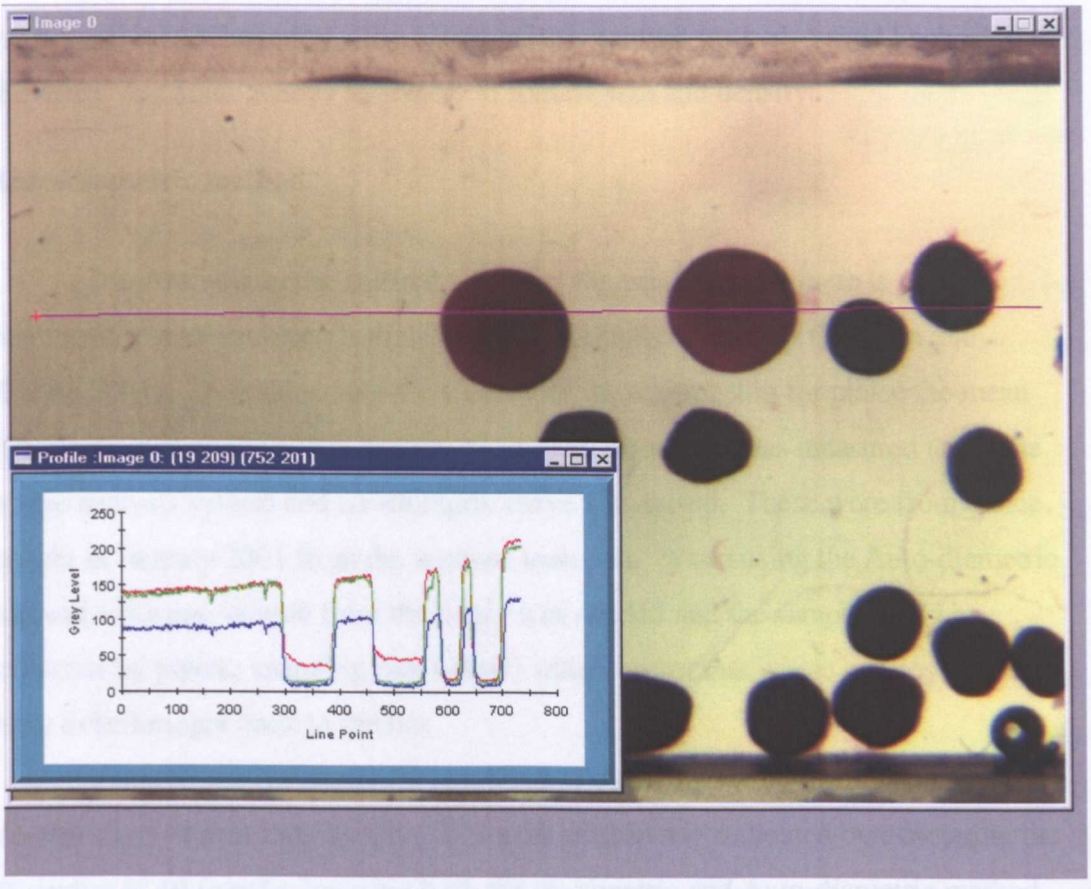


Fig. 2.1. View of plaice follicles in GFA with the graph showing changes along the transect line in measured grey level of the three primary colours.

formalin, blotted and each ovary weighed to the nearest 0.01 g. The ovary from the dorsal and ventral side was selected alternately for analysis and was cut into 3 pieces (anterior, middle and posterior) that were weighed individually. Approximately 100 mg of ovary tissue, weighed to the nearest 100 µg, was taken from each piece of the ovary and stained. The numbers of follicles in each sample were then counted using the image analysis system. The results were worked up to give the average follicle density, which was used in combination with ovary weight to give estimated fecundity. The results of this analyse were also used to check that the ovary was homogenous in respect to follicle size and density.

Auto-diametric method

The Auto-diametric method works on the principle that there is a relationship between mean follicle diameter and follicle density (Thorsen and Kjesbu 2001). To demonstrate the validity of the relationship for plaice the mean follicle diameter and follicle density of 28 pairs of ovaries was measured using the image analysis system and a calibration curve was set-up. These were from plaice caught in January 2001 from the western Irish Sea. When using the Auto-diametric method, only one sample from the ovary was needed and the sample could be collected by pipette sampling (see below) which meant that whole ovaries did not have to be brought back to the lab.

A linear regression model was fitted to the Log_e transformed mean follicle diameter and follicle density data. The relationship was validated by estimating the fecundity of 10 females by using both the gravimetric and Auto-diametric method and the results from both methods were compared.

The image analysis system was updated in 2002 to process a greater range of follicle sizes. The follicle diameter-follicle density relationship was re-calibrated with ovary samples from 57 fish caught in the western Irish Sea in January 2004 and 30 fish caught in Liverpool Bay in September 2004. A polynomial regression model was fitted to the Log_e transformed data.

Muscle tissue

Muscle tissue samples were taken from the dorsal surface of the fish for estimation of percentage water. The samples were stored in a 2 ml Eppendorf type

tube and frozen until analysis. The tissue and the tube were thawed, weighed to the nearest 100 µg and dried at 60°C until a constant weight was achieved. The microtube was then cleaned and re-weighed. The percentage water was then calculated using the following equation.

Percentage water = (OW-TW)-(FW-TW) / (OW-TW) * 100
TW = tube weight: FW = final weight (tissue plus tube):
OW = original weight (tissue plus tube)

Fecundity samples

The Irish Sea was divided into three areas, Liverpool Bay, Cumbrian coast and the western Irish Sea after Nash *et al.* (2000). Fish were sampled during the spawning seasons in the following years, 2001, 2003 and 2004 and during September in 2003 and 2004. Data was also available from fish sampled in 1953 (Simpson unpublished), 1995 (published in Nash *et al.* (2000)) and 2000 (Cefas, unpublished). The numbers of fish sampled in each area and year is shown in Table 2.1. The dissection and collection of ovaries/ovary samples was carried out by myself for the samples from Liverpool bay 2003, west of the Isle of Man 2004 and all samples collected in September. The samples from 2001, western Irish Sea 2003 and western Irish Sea 2004 were collected by workers at Cefas. Ovaries containing hydrated oocytes, which were easily identified in the whole mounts due to their large size, were excluded from the analysis to avoid including fish that may have previously spawned during the year in question.

The fish were dissected by one of the following two methods.

Partial – The fish length, total weight and ovary weight was measured

Full – As partial but liver, gut contents and empty gut were also weighed and a muscle sample was taken from the dorsal surface.

The method that was used for each group is indicated in Table 2.2.

The Gonadosomatic index (GSI) was calculated for each fish using the equation

GSI = (weight of gonad / total body weight) * 100

Condition was assessed for each fish using the relationship from Morgan (2004) rather than Fulton's condition factor which shows an increasing trend with length (Morgan 2004). Condition was assessed for total weight (whole body condition) and total weight minus ovary weight (carcass condition). A similar relationship was also used to calculate fecundity index

$K_r = W/W_p \quad F_r = F/F_p$

K_r = relative condition: F_r = fecundity index:

W_p = predicted body/carcass weight from the length - weight relationship from all stage 4 fish sampled in 2001: F_p = predicted from the length - fecundity relationship from all stage 4 fish sampled in 2001

Weight was a better predictor of fecundity. Despite this, length was chosen as the independent variable in comparisons of fecundity between areas and years as ovary weight is a significant component of total weight. Weight also varies throughout the year and changes during fasting in December, prior to spawning whereas length does not. Fecundity index was analysed against carcass condition as the ovary weight can be a significant proportion of the total weight and there is a strong relationship between fecundity and ovary weight.

Pipette sampling

Ovary samples obtained from fish caught in the western Irish Sea during the 2003 spawning season and all samples obtained from fish caught after September 2003 were taken using a pipette (200 µl pipette for fish in the spawning season, and a 50 µl pipette for fish sampled in September). The ovaries sampled for this method were dissected out and weighed to the nearest 0.1g at sea. Three replicate samples of ovary tissue were then extracted from the middle portion of the lighter ovary (there were no significant differences in follicle size or density throughout the ovary though it was decided that samples would be taken from the middle portion of the lighter ovary for consistency purposes) and stored in 2 ml Eppendorf type tubes containing 3.6% formalin. Samples taken with this method were analysed by the Auto-diametric method.

Table 2.1. The numbers of plaice sampled in each year, month and area of the Irish Sea.

	Liverpool Bay		Cumbrian Coast		Western Irish Sea		West of Isle of Man	
	Jan-Feb	Sep	Jan-Feb	Sep	Jan-Feb	Sep	Jan-Feb	Sep
1953	28		41					
1995	42		95		52			
2000	42		71		89			
2001	50		49		85			
2003	52	51		55	40	25		
2004		53			60		35	

Table 2.2. The vessel used to catch the sampled plaice, dissection method (P = partial, F = Full), type of sample taken at time of capture (WO = whole ovary, OS = three follicle samples) and method of analysis (G = gravimetric, A = Auto-diametric) for each area, year and month.

		Liverpool Bay		Cumbrian coast		Western Irish Sea		West of Isle of Man
		Jan-Feb	Sep	Jan-Feb	Sep	Jan-Feb	Sep	Jan-Feb
2001	Vessel	FV Resolute		FV Resolute		FV Resolute		
	Dissection	P		P		P		
	Sample	WO		WO		WO		
	Analysis	A		A		A		
2003	Vessel	RV Roagan	RV Corystes		RV Corystes	RV Lough Foyle	RV Corystes	
	Dissection	P	F		F	F	F	
	Sample	WO	OS		OS	OS	OS	
	Analysis	A	A		A	A	A	
2004	Vessel		RV Corystes			RV Lough Foyle		FV Elegant
	Dissection		F			F		F
	Sample		WO (30) + OS (23)			OS		OS
	Analysis		G+A			G+A		AD

Follicle size distributions

The change in follicle size distributions from the start of ovary maturation through to spawning was tracked by following the modal size of the follicle distributions which were used as an indicator of progression through ovary development. A size increment of 5 μm was used.

Statistical analysis

All statistical tests were carried out using Statistica 6.1 (StatSoft Inc. 2002). All length, weight and fecundity data was Log_e transformed to achieve normal

distribution of data. Differences in mean follicle diameter and follicle density between ovaries and between sampling sites within an ovary were tested using ANOVA. Differences between the two fecundity estimation methods were tested by students t-test. Linear regression models were fitted to the 'fecundity-length' and 'fecundity-weight' Log_e transformed data for all areas and years. Fecundity differences between areas and years were tested for using ANCOVA with length as the independent variable. Forward stepwise regression was used to find the best predictor of fecundity, with length weight and carcass condition as predictor variables.

RESULTS

Calibration of mean follicle diameter against follicle density

A linear regression model was fitted to follicle density and mean follicle diameter for 28 of the ovary samples collected in 2001 (Linear regression; R^2=0.87; N= 28; P<0.001) (Fig. 2.2). This relationship was used for calculation of follicle density from measured mean follicle diameter for samples collected in 2001 and the regression function was:

$$Log_e FD = -3.153 * Log_e MFD + 7.336$$

FD = follicle density

MFD = mean follicle diameter

Using data on follicle density and mean follicle diameter from the samples collected in the western Irish Sea in January 2004 and from Liverpool Bay in September 2004 a polynomial regression model best described the data (Polynomial regression; R^2=0.98; N=91; P<0.001) (Fig. 2.3). This was used for all fecundity samples collected after September 2003. The regression function was:

$$Log_e FD = -1.4036 * Log_e MFD^2 - 3.4952 * Log_e MFD + 7.5394$$

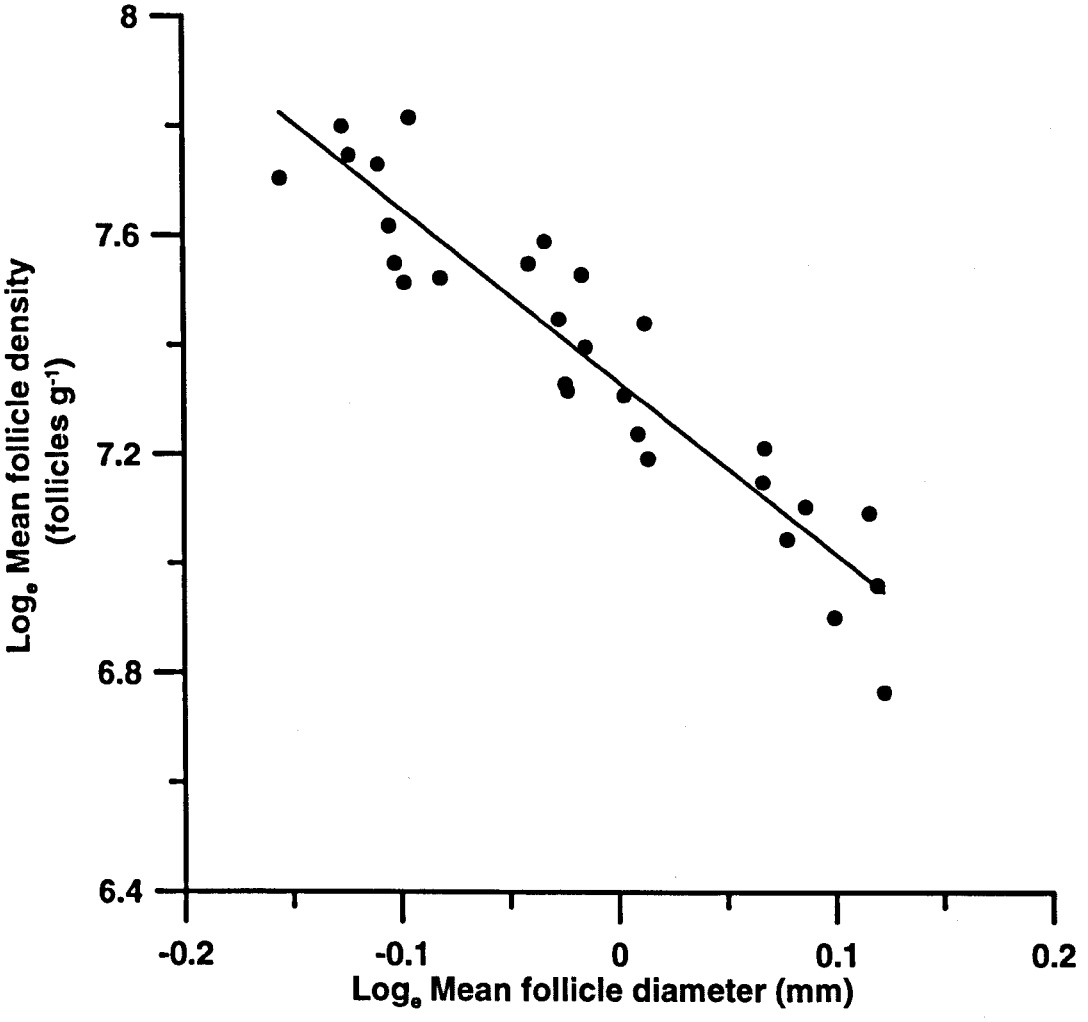


Fig. 2.2. The relationship between Log_e follicle density and Log_e mean follicle diameter for 28 plaice sampled from the western Irish Sea in 2001. Line shows linear regression line.

For validation of the Auto-diametric method, the fecundity of 10 plaice were estimated using both the gravimetric and Auto-diametric method (Fig. 2.4). The average difference in fecundity between the two methods was less than 1% and was not significant (t-test; t=0.07; n=10; p>0.05).

Homogeneity of ovary

There was no significant difference in the mean follicle diameter or follicle density in different parts of the ovaries (Two-way ANOVA; P value>0.05; Table 2.3). However, it was decided to sample from the middle of the smaller ovary for consistency.

Fecundity, fish size and condition

Linear regression models were fitted to the 'fecundity-length' and 'fecundity-total weight' Log_e transformed data for each area and year (Table 2.4). Total weight was the best predictor of fecundity for all groups except for fish in the western Irish Sea in September 2003 where length alone was the best predictor. Maternal condition had a small influence in 4 of the 17 groups and length exerted an influence in 8 groups (Table 2.5). There was an increase in fecundity index with carcass condition for fish in 2000 (Linear regression; R^2=0.03; n=202; p=0.004), 2001 (Linear regression; R^2=0.41; n=183; p<0.001), 2003 (Linear regression; R^2=0.17; N=26; p=0.02) and 2004 (Linear regression; R^2=0.07; n=93; p=0.005) but not for 1995 (the fish in 1953 were not tested for this as ovary weight was unavailable).

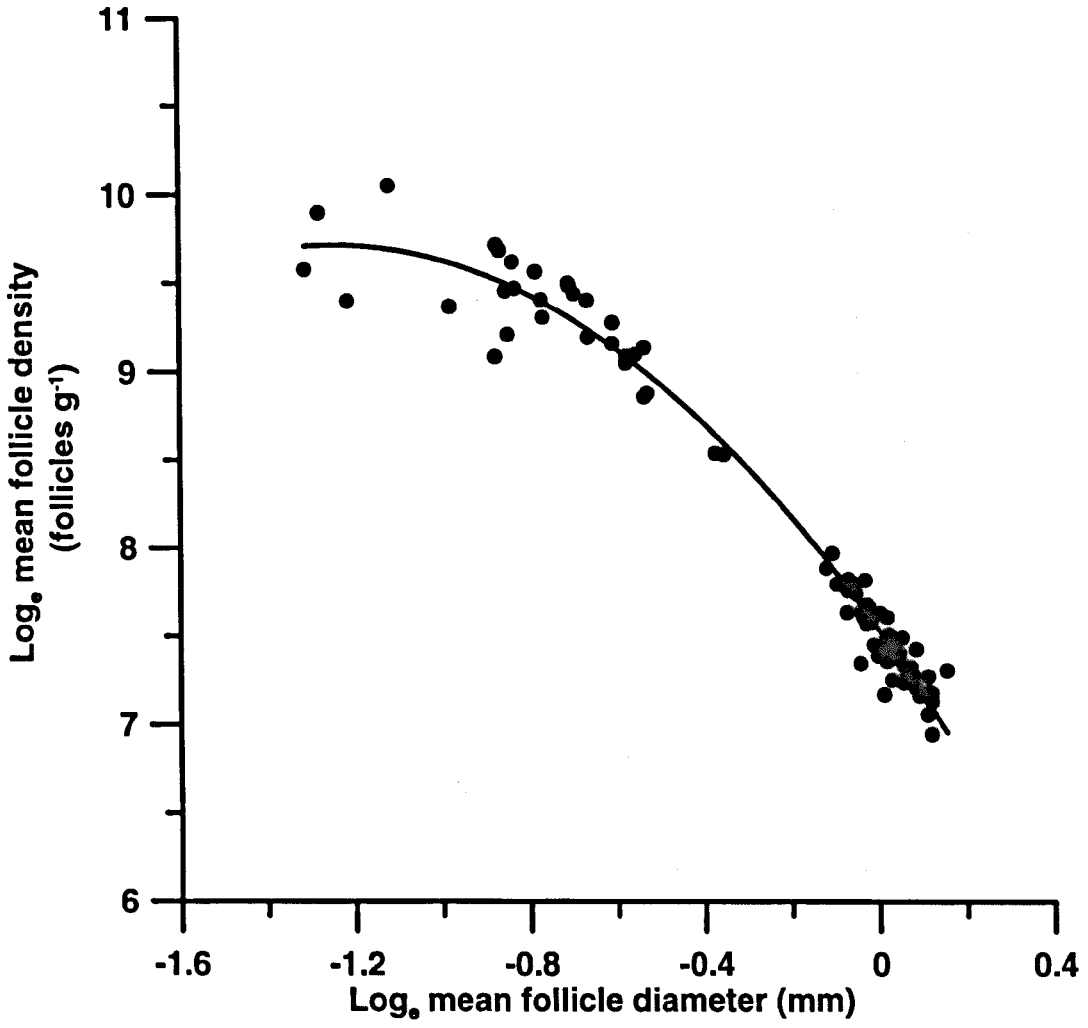


Fig. 2.3. The relationship between Log_e mean follicle density and Log_e mean follicle diameter for plaice sampled in the western Irish Sea in January 2004 and Liverpool Bay in September 2004. Line shows polynomial regression line.

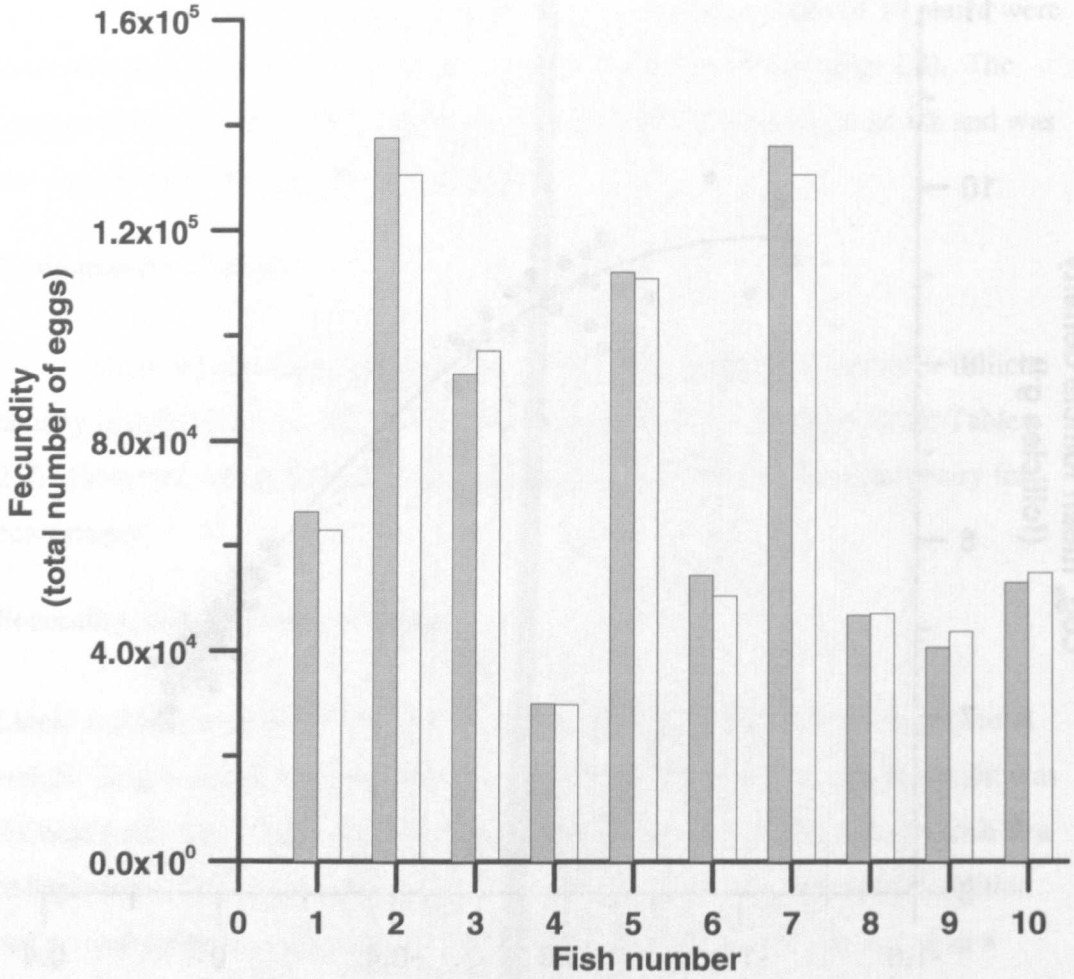


Fig. 2.4. Fecundity estimate for 10 plaice using gravimetric (grey) and Auto-diametric method (white).

Table 2.3. Two-way analysis of variance comparing differences of follicle diameter (top) and mean follicle density (bottom) between sampling sites in ovaries of plaice SS = sum of squares df = degrees of freedom MS = mean square.

	SS	df	MS	F	P-value
Follicle diameter					
Intercept	116.5607	1	116.5607	229631.1	0.000000
Sampling site	0.0004	2	0.0002	0.4	0.691828
Fish	0.0000	37	0.0000	0.0	1.000000
Error	0.0376	74	0.0005		
Follicle density					
Intercept	114.7072	1	114.7072	11010.18	0.000000
Sampling site	0.0364	2	0.0182	1.75	0.181405
Fish	0.2528	37	0.0068	0.66	0.919566
Error	0.7605	73	0.0104		

Table 2.4. . Summary of sample size after removal of fish containing hydrated oocytes (n), length range (min/max) (cm), Log_e fecundity–Log_e total length relationship (F-L), respective R^2, Log_e fecundity–Log_e total weight relationship (F-We) and respective R^2 for year, sampling time and area (LB = Liverpool Bay, CC = Cumbrian coast, W = western Irish Sea, WIOM = west of Isle of Man).

Year	Sampling month	Area	n	Length range	average	F-L Relationship	R^2	F-We relationship	R^2
1953	Jan-Feb	LB	28	22/35	28	ln F = -1.55 + 3.73 ln L	0.78		
		CC	95	22/49	32	ln F = -3.48 + 4.25 ln L	0.81		
1995	Jan – Feb	LB	42	22/41	27	ln F = -3.13 + 4.20 ln L	0.85	ln F = 3.98 + 1.26 ln W	0.90
		CC	95	22/49	32	ln F = -3.48 + 4.25 ln L	0.81	ln F = 4.42 + 1.18 ln W	0.81
		W	44	21/43	27	ln F = -4.32 + 4.43 ln L	0.84	ln F = 2.73 + 1.43 ln W	0.88
2000	Jan-Feb	LB	42	21/40	28	ln F = 0.09 + 3.28 ln L	0.55	ln F = 5.08 + 1.07 ln W	0.63
		CC	71	22/42	30	ln F = -1.59 + 3.80 ln L	0.84	ln F = 4.61 + 1.16 ln W	0.90
		W	89	20/48	30	ln F = -1.39 + 3.72 ln L	0.89	ln F = 4.75 + 1.15 ln W	0.91
2001	Jan-Feb	LB	52	21/43	29	ln F = -2.59 + 4.06 ln L	0.79	ln F = 3.92 + 1.27 ln W	0.84
		CC	46	21/45	31	ln F = 0.21 + 3.20 ln L	0.80	ln F = 5.14 + 1.04 ln W	0.84
		W	85	16/42	28	ln F =-2.43 + 3.95 ln L	0.84	ln F = 3.55 + 1.32 ln W	0.91
2003	Jan-Feb	LB	52	19/35	26	ln F = -2.59 + 4.06 ln L	0.79	ln F = 3.92 + 1.27 ln W	0.84
		W	13	23/45	30	ln F = -2.45 + 4.02 ln L	0.94	ln F = 4.20 + 1.23 ln W	0.96
	September	LB	50	23/43	31	ln F = 4.09 + 1.29 ln L	0.82	ln F = 4.09 + 1.29 ln W	0.87
		CC	54	26/49	32	ln F = -1.20 + 3.71 ln L	0.82	ln F = 4.13 + 1.29 ln W	0.89
		W	25	22/37	26	ln F = -4.18 + 4.58 ln L	0.88	ln F = 2.97 + 1.49 ln W	0.86
2004	Jan-Feb	W	58	20/50	31	ln F = -2.98 + 4.16 ln L	0.88	ln F = 4.09 + 1.25 ln W	0.91
		WIOM	35	25/44	31	ln F = -1.61 + 3.84 ln L	0.72	ln F = 4.58 + 1.22 ln W	0.84
	September	LB	53	23/43	32	ln F = 4.53+ 1.21 ln L	0.79	ln F = 4.53 +1.21 ln W	0.80

Table 2.5. Results from stepwise regression showing the variables included to give the best predictor of fecundity for each group of plaice (LB = Liverpool Bay, CC = Cumbrian coast, W = western Irish Sea, WIOM = west of Isle of Man), the variables included in the model (We = total weight, C = carcass condition, L = Length) and the variance explained by each variable (if any) and the total variation explained by the model (total R^2).

Year	Sampling month	Area	Variables included	C R^2	L R^2	Total R^2
1995	Jan-Feb	LB	We, L		0.03	0.92
		CC	We, C, L	0.04	0.04	0.89
		W	We			0.88
2000	Jan-Feb	LB	We, L		0.04	0.67
		CC	We			0.90
		W	We			0.91
2001	Jan-Feb	LB	We, C, L	0.02	0.01	0.87
		CC	We			0.84
		W	We, C, L		0.02	0.93
2003	Jan-Feb	LB	We			0.84
		W	We			0.96
	September	LB	We			0.87
		CC	We			0.89
		W	L		0.87	0.87
2004	Jan-Feb	W	We			0.91
	WIOM	We,	C, L	0.03	0.04	0.90
	September	LB	We, C, L	0.15	0.04	0.99

Carcass condition decreased with increases in follicle diameter for fish caught in the spawning season during 2000 (Linear regression; R^2=0.07; N=202; p<0.001) and 2001 (Linear regression; R^2=0.07; N=183; p<0.001) but not in 2003 or 2004 (Linear regression; p>0.05).

There was a significant effect of whole body condition on muscle water content for the fish caught in September 2003 (Linear regression; R^2=0.08; N=128, P<0.001) (Fig. 2.5), September 2004 (Linear regression; R^2=0.40; N=52; p<0.001) and fish caught during the spawning season in 2004 (Linear regression; R^2=0.05; N=91; P=0.017).

Gonadosomatic index (GSI) increased with follicle diameter for fish caught in September (Linear regression; R^2=0.72; N=184; P<0.001) and during the spawning season in 2000 (R^2=0.14; N=202; P<0.001), 2001 (Linear regression; R^2=0.19; N=183 p<0.001), 2003 (Linear regression; R^2=0.34; N=26; p<0.001) and 2004 (R^2=0.16; N=93; P<0.001). There was an increase in average follicle diameter with fish length in Cumbrian coast 2000 (R^2=0.10; N=71; p=0.004) and Liverpool Bay 2000 (R^2=0.10; N=42; p=0.02), in all 3 areas in 2001 (data combined) (R^2=0.03; N=182; p=0.007) and in the western Irish Sea and West of Isle of Man in 2003 (data combined) (R^2=0.16; N=26; P=0.02). This was not tested for data in 1953 and 1995 due to follicle sizes being unavailable.

Inter-annual and inter-area differences in fecundity

There were significant year effects on fecundity in the Cumbrian coast (ANCOVA; df=4; P<0.001) (Fig. 2.6a) and the western Irish Sea (ANCOVA; df=5; P<0.001) (Fig. 2.6b), however there was no inter-annual difference in fecundity in fish caught from Liverpool Bay (ANCOVA; df=5; P>0.05) (Fig. 2.6c).

There were significant area effects on fecundity in 1995 (previously reported in Nash *et al.* (2000)), 2000, 2001 and 2004. The rank in fecundity between areas changed from 2000 to 2001 (Fig. 2.7a) with fish from Liverpool Bay having the lowest fecundity of the three in 2000 to having the greatest in 2001. There was no difference in fecundity between Liverpool Bay and the western Irish Sea in 2003. There was a difference in fecundity between fish from the western Irish Sea and the Isle of Man population (Fig. 2.7b) with the latter having a higher fecundity than the

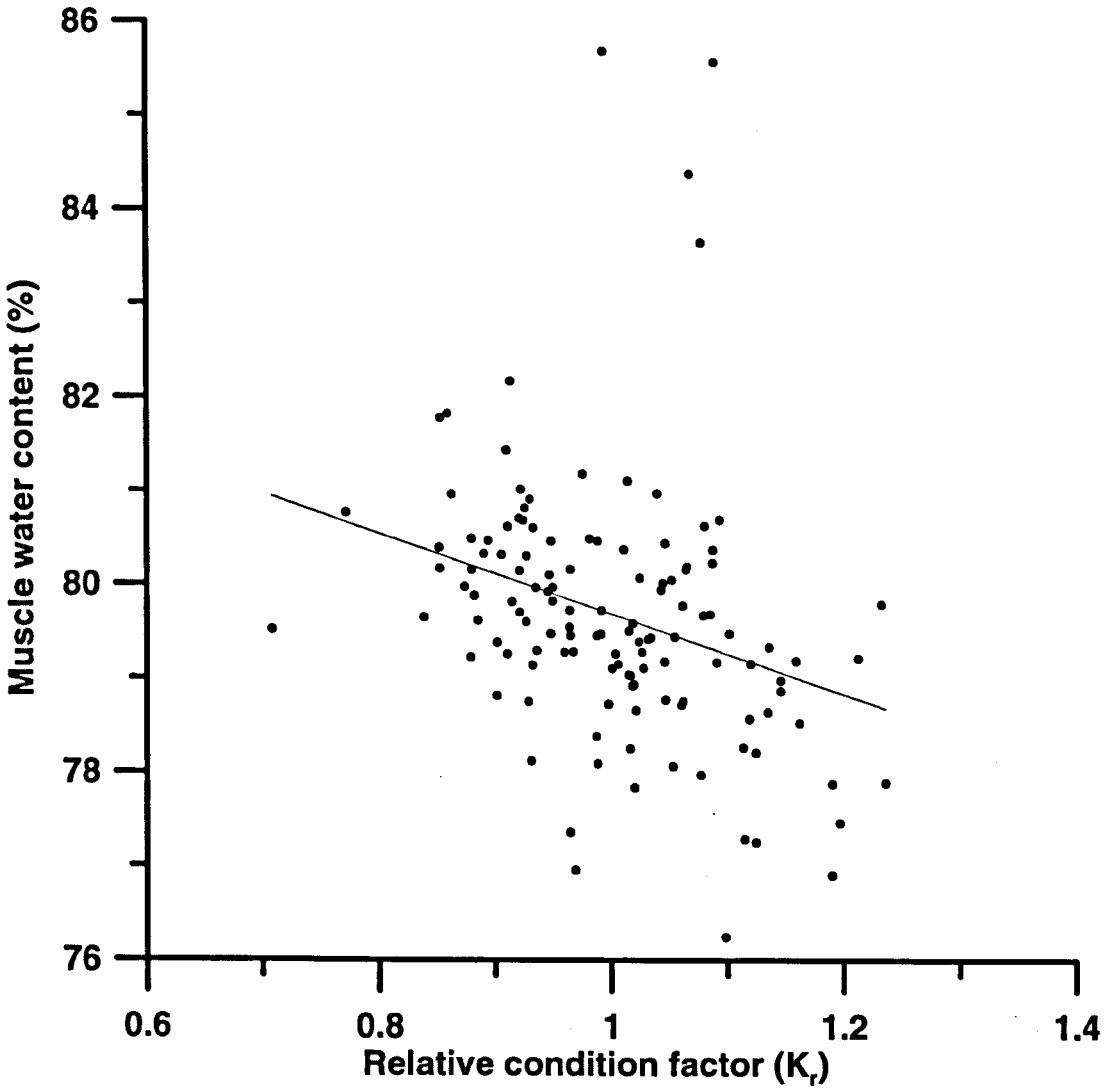
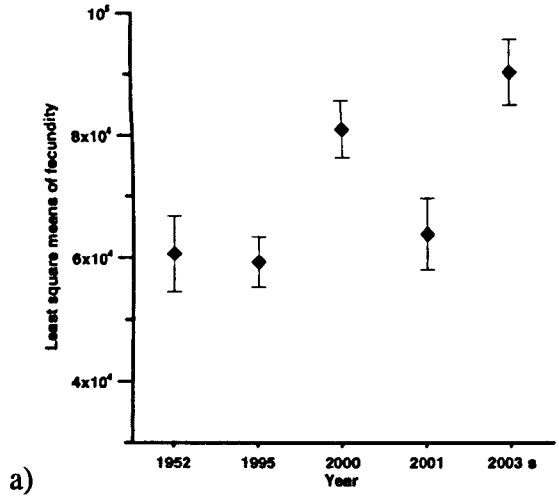
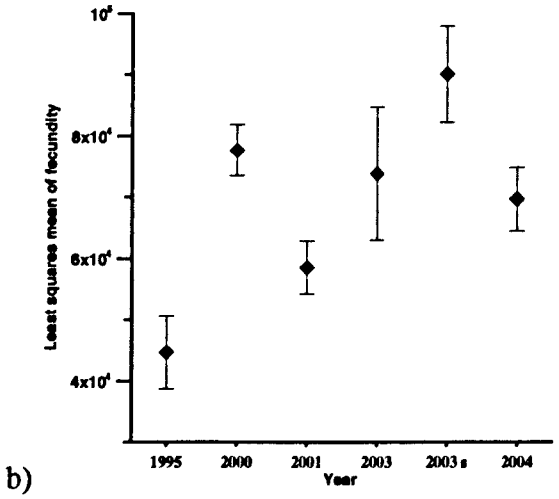


Fig. 2.5. The relationship relative condition factor and muscle water content in plaice sampled in September 2003. Line shows linear regression line.



a)



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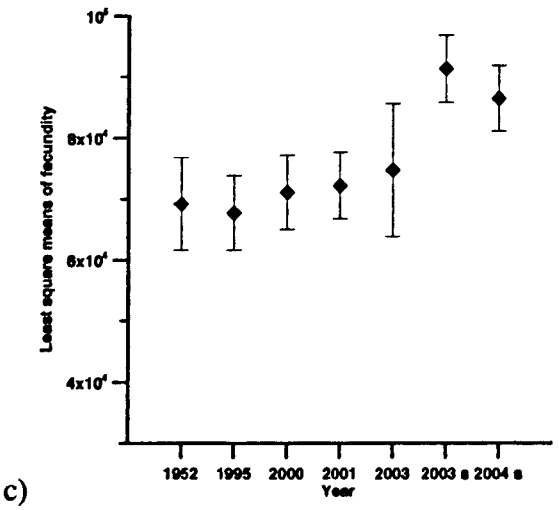


c)

Fig. 2.6. Least square means of Log_e transformed fecundity of plaice sampled from (a) Cumbrian coast, (b) western Irish Sea and (c) Liverpool Bay in 1952 to 2004 including estimates from September (s). Error bars show 1 standard error.

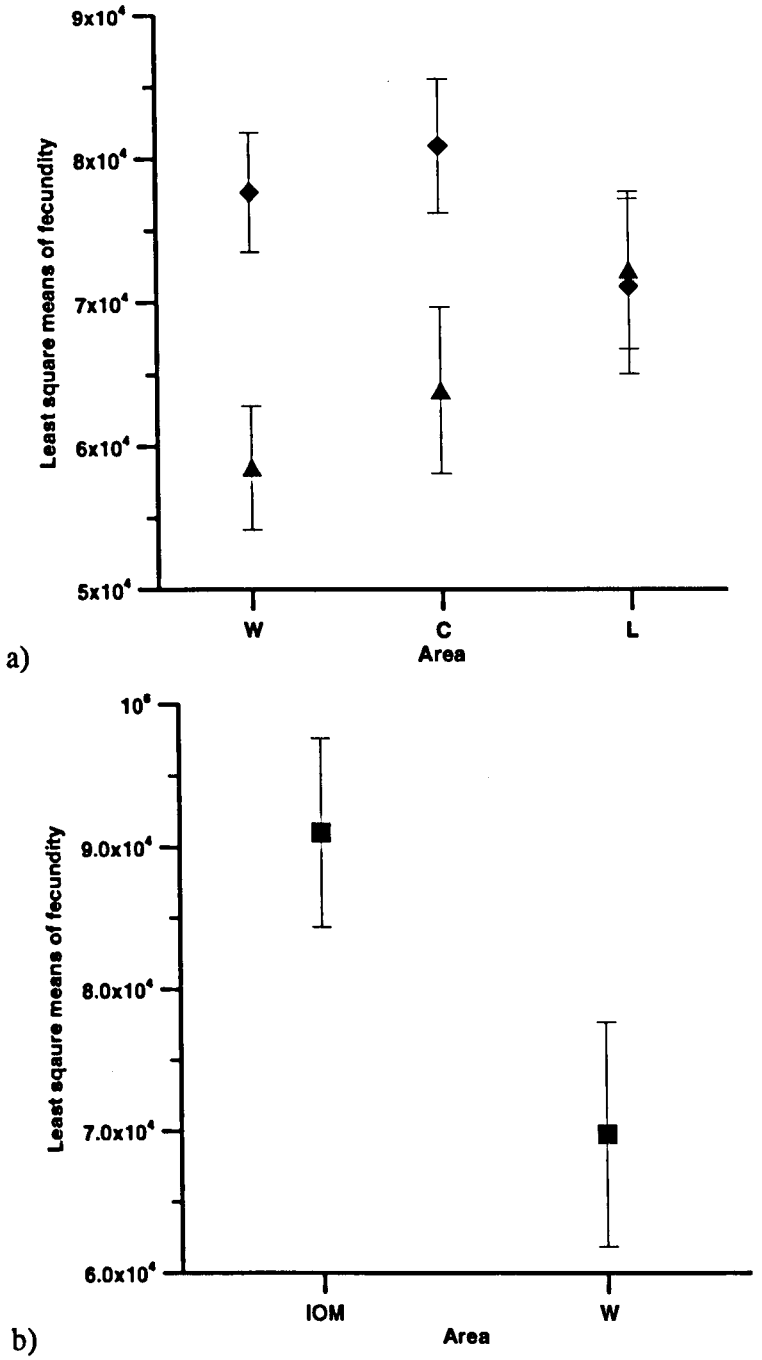


Fig. 2.7. Least square means of Log_e transformed fecundity of plaice sampled in (a) 2000 (◆) and 2001 (▲) and (b) 2004 (■) from the Cumbrian coast (C), Liverpool Bay (L), western Irish Sea (W) and west of the Isle of Man (WIOM). Error bars show 1 standard error.

western Irish Sea. The highest fecundity was in the Cumbrian coast in 2000 and the lowest was in the western Irish Sea in 1995. The fecundity differed in these two groups by 44, 39 and 33% for a fish of 30, 35 and 40 cm respectively.

Fecundity in September

The distribution of follicle diameters was generally tailed towards the smaller egg sizes with a continuum of follicle diameters existing between the pre-vitellogenic and vitellogenic follicles in many of the samples (Fig. 2.8).

The fecundity determined in September for fish in all 3 areas was significantly higher than all fecundity estimates taken in previous years during the spawning season (Fig. 2.6). There were no significant differences in the fecundity between the three areas in 2003 ($p>0.05$) or between 2003 and 2004 ($p>0.05$) in Liverpool Bay and so all fish sampled could be summarized with a common linear regression model for length (Linear regression; $R^2=0.871$; N=183; P=0.001) and weight (Linear regression; $R^2=0.873$; N=183; P=0.001) (Fig. 2.9). There was a positive correlation between fish length and follicle diameter (Linear regression; $R^2=0.14$; N=183; P>0.001) (Fig. 2.10) and also for follicle diameters less than 700 μm and relative fecundity (Linear regression; $R^2=0.18$; N=125; P>0.001) (Fig. 2.11). This became a negative correlation when average follicle size was greater than 700 μm (Linear regression; $R^2=0.087$; N=103; P=0.001) (Fig. 2.12). The relative fecundity appears to level off when the average follicle diameter reaches approximately 1000 μm.

Follicle size distributions

The follicle size distributions began as a uni-modal distribution then as the modal size increased a bi-modal distribution formed which then became a skewed distribution. As the largest modal value reached approximately 600 – 700 μm the spread started to decrease and the distribution became uni-modal. This modal distribution then increased until spawning where small batches began to increase in size which would represent the hydrating oocytes (Fig. 2.13).

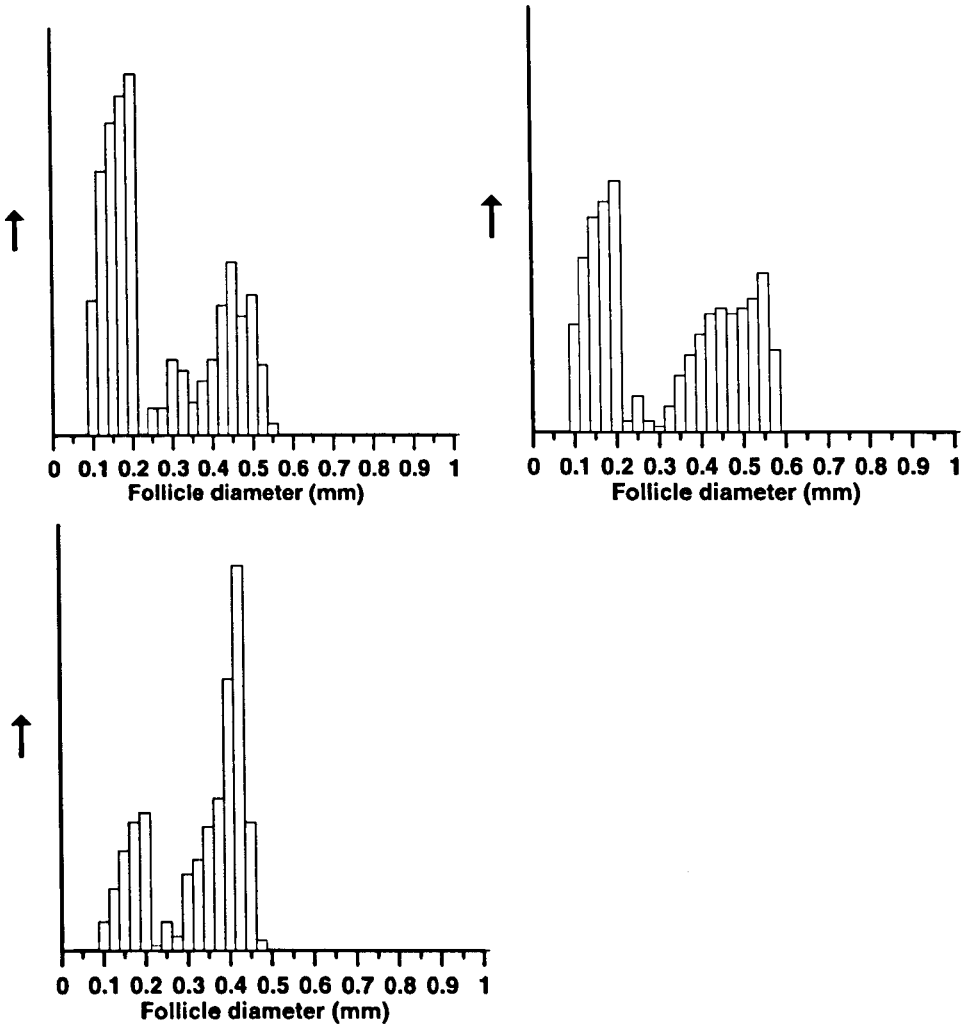


Fig. 2.8. Follicle size distributions of three plaice sampled in September 2003 showing the presence of the continuum of follicle sizes between vitellogenic (>0.2mm) and pre-vitellogenic (<0.2mm) follicles. Arrow on x-axis indicates increasing frequency.

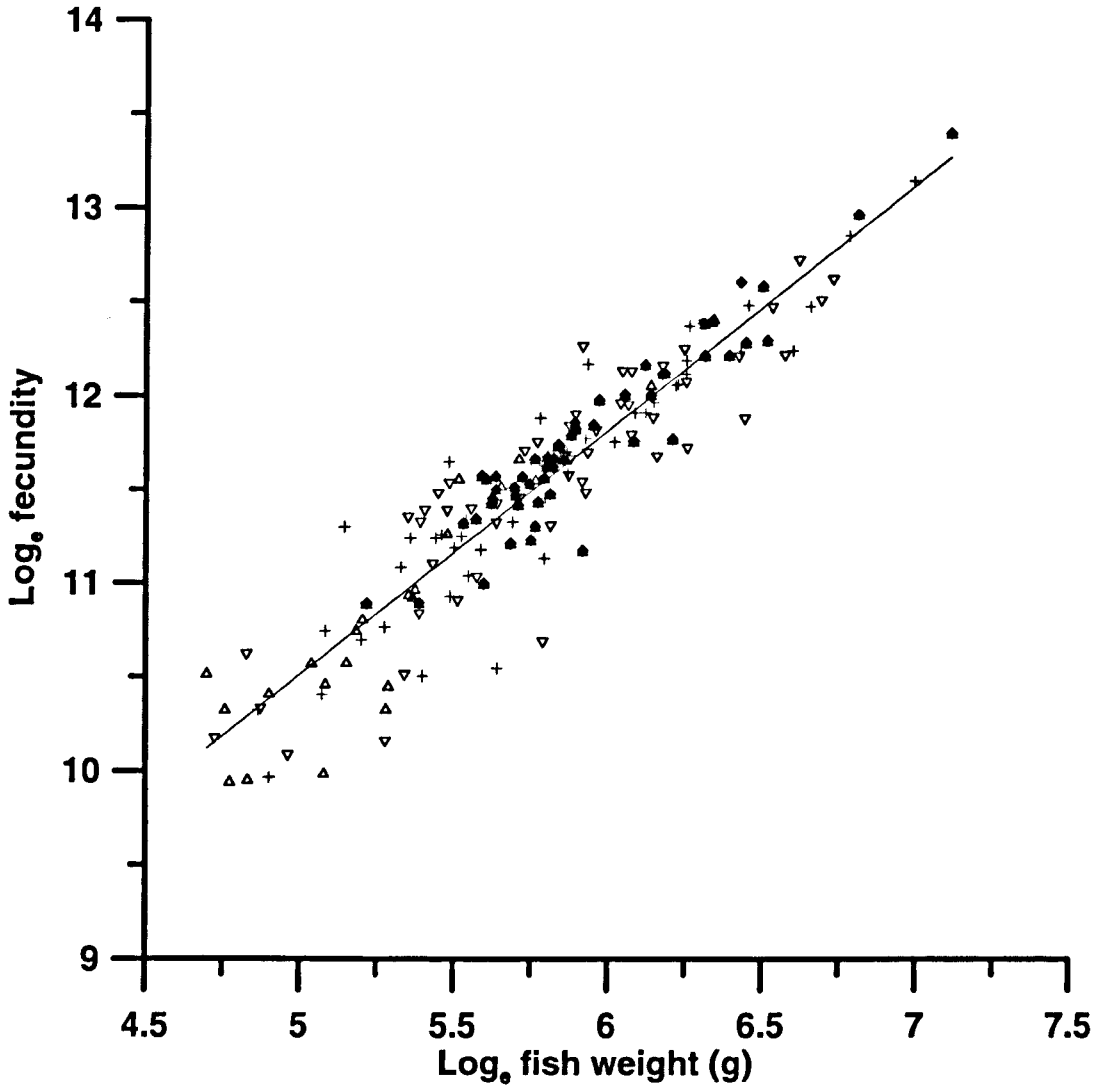


Fig. 2.9. Relationship between Log$_e$ weight and Log$_e$ fecundity for plaice sampled during September from Liverpool Bay in 2003 (+), Cumbrian coast in 2003 (◆), western Irish Sea in 2003 (△) and from Liverpool Bay in 2004 (▽). Line shows linear regression line for all data combined.

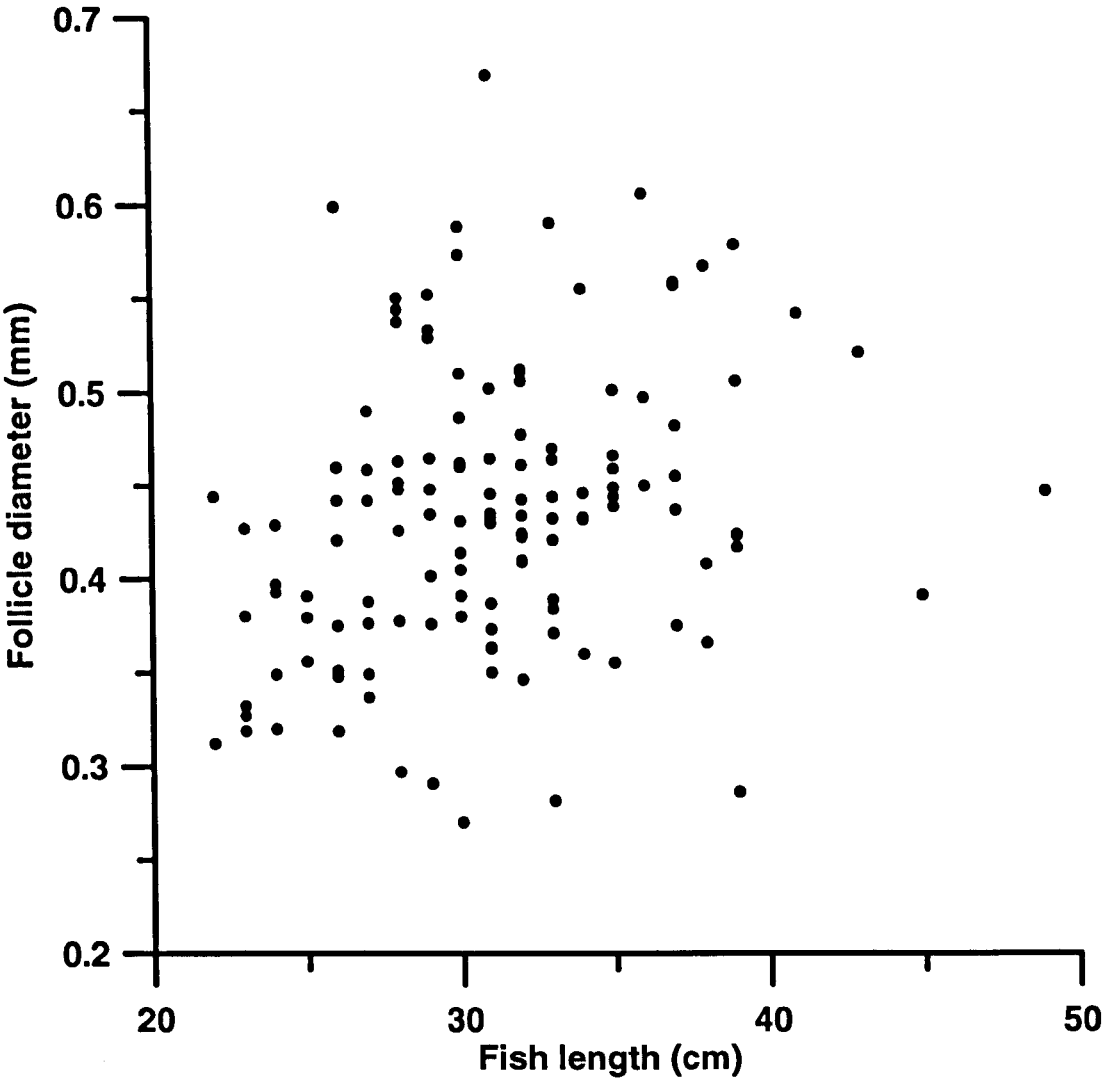


Fig. 2.10. Scatter plot of fish length versus mean follicle diameter for plaice sampled in the Irish Sea during September 2003.

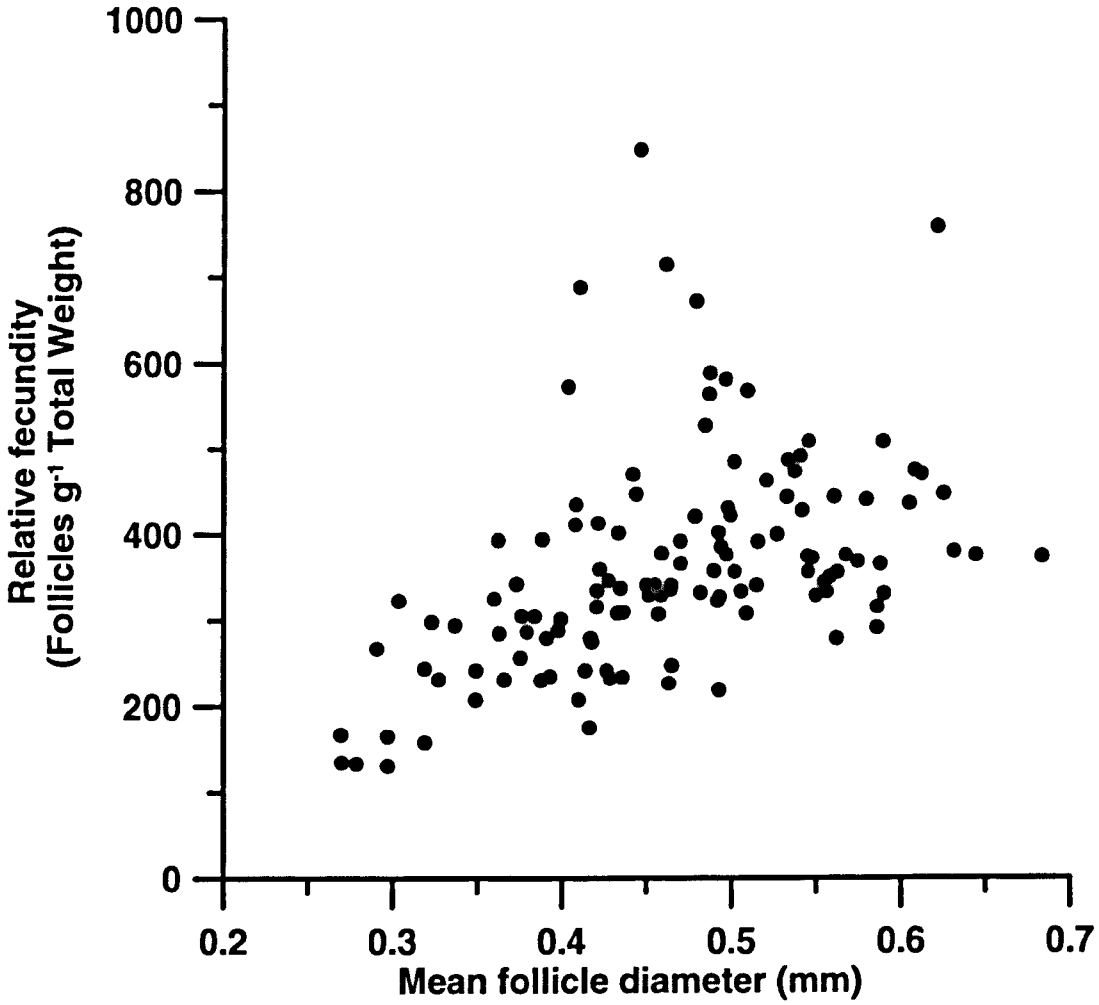


Fig. 2.11. Scatter plot of mean follicle diameter and relative fecundity (fecundity/total weight) for fish sampled from Liverpool Bay in January 2000.

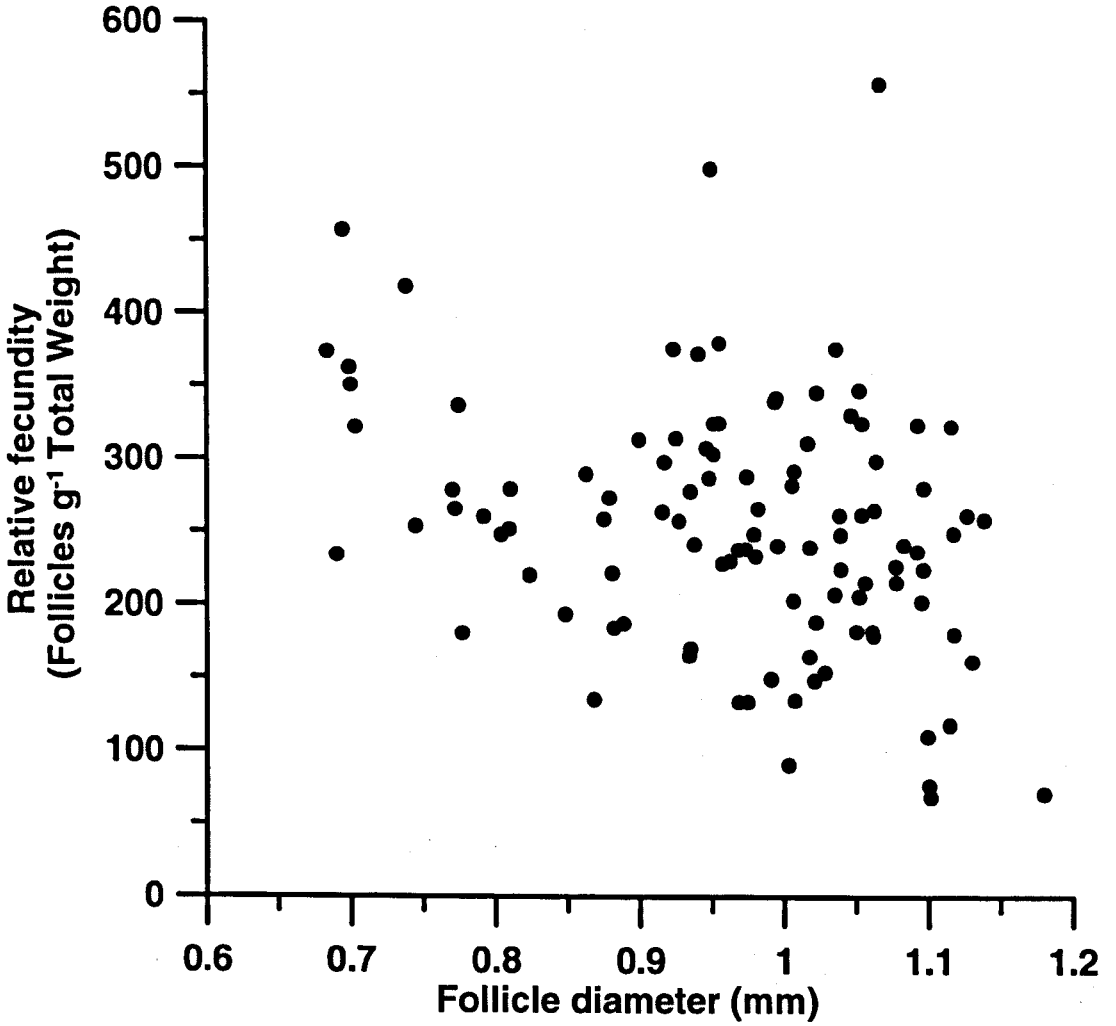


Fig. 2.12. Scatter plot of mean follicle diameter and relative fecundity (fecundity/total weight for fish sampled from Liverpool Bay in September 2003 and 2004.

DISCUSSION

As in cod (Thorsen and Kjesbu 2001), the Auto-diametric fecundity method was a very useful and efficient method for the estimation of plaice fecundity. The method gave results which were comparable to the gravimetric method and also gave information on follicle size distributions, easy identification of fish that had commenced spawning during the spawning season in question and also the same relationship between follicle diameter and mean follicle density could be used for fish from the start of follicle recruitment right through to spawning fish. This is in contrast with the conclusions made by Friedland *et al.* (2005) for American shad (*Alosa sapidissima*) who concluded that the relationship between oocyte size and density was too imprecise to provide useful fecundity estimates. They suggested that the poor precision may be due to their sample handling and preservation technique, but also may reflect species differences in ovarian development and ovarian anatomy. However, this method is deemed appropriate for the estimation of plaice fecundity as the relationship between follicle size and density had a high precision, it was also very time efficient and reduced the amounts of chemicals needed for the preservation of ovaries.

The use of formaldehyde rather than Gilson's for the fixation of ovary samples was highly beneficial. Formaldehyde is much less toxic and is less costly for disposal than Gilson's. The use of formaldehyde as a fixative rather than Gilson's was deemed to have no effect on the results as fecundity estimates from Liverpool Bay in the present study were not significantly different from fecundity estimates for the same area in Nash *et al.* (2000) where Gilson's fixative was used.

No differences were found in the mean follicle diameter or mean follicle density either between ovaries or within an ovary. This is similar to the results found by Nichol and Acuna (2001) who found no difference in mean oocyte diameter between ovaries or within ovaries of yellowfin sole (*Limanda aspera*). However they did find that oocyte density was greater in posterior areas of the ovary compared with the middle and anterior which was not the case in the present study.

Muscle moisture content was inversely related to condition which is in agreement with current knowledge. Condition factor is a good estimate of energy

content of plaice (Costopoulos and Fonds 1989) and it is known that increased white muscle water moisture is an indication of protein depletion (Stirling 1976).

Weight was the best predictor of fecundity with a small influence of length and condition independent of weight in some groups; this is in agreement with Koops *et al.* (2004) who found a similar result with cod and brook trout (*Salvelinus fontinalis*). However, weight is very variable as plaice do not feed during the spawning season and so decrease in weight in the period before spawning. Ovary weight can also be a significant proportion of the total weight and there is a very strong correlation between ovary weight and fecundity. There was a positive relation between carcass condition and fecundity index, i.e. fish with a higher condition factor had a higher fecundity than lower condition fish. Carcass condition index did not explain much of the variance in fecundity index, this may be because fecundity can decrease close to the spawning season but cannot increase. Therefore if a fish decreases in condition it will decrease its fecundity. However, it cannot increase its fecundity if its condition increases; this could lead to the high variance in the relationship. Carcass condition decreased with increasing follicle diameter for two out of the four years during the spawning season, this relationship had a high variance and may be why it was not detectable during 2003 and 2004 when sample numbers were much lower. This relationship indicates that fish are using stored resources for ovary development which is agreement with Rijnsdorp (1990) who estimated that up to 50% of the gonad growth in plaice is subsidised from body reserves built up during the growing period. Fish that have a higher intake of food during ovary development used less of their resources for ovary development (next chapter); this may explain the high variation in the relationship as individual fish may cease feeding at different times. As carcass condition decreases as ovary development proceeds and fecundity index decreases with carcass condition there will be the resultant decrease in fecundity with ovary development (see below). There was an increase in gonadosomatic index with follicle size which was expected as the ovary weight consists of the weight of the follicles therefore larger follicles will result in a heavier ovary.

This appears to be the first time inter-annual variations in plaice fecundity have been reported for the Irish Sea, however there appear to be only two studies on this in the literature, Nash *et al.* (2000) and Horwood (1990). These studies found no difference in the eastern Irish Sea (Nash *et al.* 2000) or Cardigan Bay (Horwood

1990) respectively from 1953 and their respective sampling dates. There were differences in fecundity between the three spawning areas and differences in the variance exhibited between years. Fish from the western Irish Sea showed the greatest variation between years and fish from Liverpool Bay showed no significant variation between years. The variations in the western Irish Sea are probably due to variations in food level after fecundity proliferation has ended (see below). This could indicate that food availability during autumn is much less variable between years in Liverpool Bay than in the western Irish Sea. Changes in population fecundity have been linked to changes in food level in Arcto-Norwegian cod (Kjesbu *et al.* 1998) and feeding level has been shown to affect fecundity in plaice (Horwood *et al.* 1989). Variations in fecundity have also been linked to changes in population density (which can indirectly affect food level) in plaice, witch (*Glyptocephalus cynoglossus*) and Norway pout (*Trisopterus esmarkii*) (Bagenal 1973).

At the time of sampling in September, plaice were at the stage of early vitellogenesis and were still recruiting follicles, as evident from the presence of a continuum of follicle sizes between the pre-vitellogenic and vitellogenic follicles and the increase in fecundity with mean follicle diameter (indication of progress through maturation). During the autumn months plaice are building up reserves and so gaining weight (Rijnsdorp 1990). As plaice are still recruiting follicles in September and there is a close correlation between weight and fecundity with no difference in the relationship between areas, it is clear that follicles are recruited over a period of time in line with increases in weight. The maximum fecundity is then determined by the fish's weight at the end of follicle recruitment. There is a good relationship between weight and fecundity during the spawning season as the weight of the ovary makes up a significant component of the total weight of the fish. This was not so for the fish sampled in September so a fish's fecundity must be a real reflection of an individuals resources rather than the result of an actual relationship between ovary weight, total weight and fecundity. The results are in agreement with the model proposed by Rijnsdorp (1990) which hypothesised that when surplus production was above a minimum level a fish will build up body reserves, which are necessary for winter metabolism, and will produce an amount of eggs proportional to its body size. A similar mechanism is present in cod, which is also a highly fecund, determinate spawner. For an individual cod, weight during the early period of vitellogenesis is the best predictor of fecundity (Skjæraasen *et al.* 2006).

As the fecundity estimates taken in September were generally higher than during the spawning season and there was a negative relationship between follicle size and relative fecundity, it is clear that down regulation of fecundity occurs and the level of down regulation is affected by the condition of the fish as indicated by the positive relationship between carcass condition and fecundity index. Atresia has been shown to occur in cod (Kjesbu *et al.* 1991), herring (Kurita *et al.* 2003) and turbot (Bromley *et al.* 2000) and is hypothesised to be a method of fine tuning fecundity in relation to available energy reserves (Kurita *et al.* 2003). Atresia in plaice is low during the spawning season (Armstrong *et al.* 2001) which means that the decrease in fecundity occurs before the spawning season commences. The recruitment of more follicles than are typically used for spawning will allow the fish to have an increased fecundity if feeding conditions are good. However, if food availability is low later in the season and all follicles cannot be sustained the fish can re-absorb by atresia without experiencing heavy energetic losses, since the follicles are reabsorbed and the nutrients are presumably available for recycling (Bromley *et al.* 2000). A similar method for the control of optimum egg production has been seen in captive Norwegian coastal cod whereby they produce too many follicles prior to spawning. This unsustainable production is subsequently re-absorbed during the spawning season with fish in good condition spawning more eggs than fish in poor condition (Kjesbu *et al.* 1998).

Plaice have been shown to segregate into discrete feeding aggregations during the summer non-breeding season (Hunter *et al.* 2004) and tagging studies in the Irish Sea have shown very limited movement of plaice between the eastern and western area (Dunn and Pawson 2002). Because of this segregation, plaice in the different areas will experience different feeding conditions. With differing food levels, the level of atresia will differ between populations, with populations with decreased feeding conditions having a greater incidence of atresia (Bagenal 1969; Wootton 1973; Kjesbu *et al.* 1991), resulting in the observed differences in fecundity between areas and years. Fish size will still remain a good proxy for fecundity within a population, as fish within a population will experience similar conditions during this period, therefore any changes in weight of individual fish within the population will be similar for fish across the whole population.

There is an effect of sampling date on fecundity estimates due to the down regulation that occurs in the period before spawning. The fecundity of fish from the

west of the Isle of Man was high compared to other areas. This is probably due to the fish being sampled in early January which was much earlier in the spawning season in comparison with the other areas and years. Fecundity appears to level off when the average follicle diameter reaches approximately 1000 μm. Many of the fish sampled from the west of Isle of Man had an average follicle diameter lower than this and so more down regulation of fecundity would maybe have occurred before the fish began spawning.

Mean follicle diameter increased with fish size when sampled in September and during the spawning season and this has been documented previously for plaice in Cardigan Bay (Horwood 1990). This suggests that larger fish started maturation at an earlier date and will probably be in spawning condition sooner and may begin spawning sooner which is known to occur in plaice from Cardigan Bay (Horwood 1990) and from the North Sea (Simpson 1951; Heessen and Rijnsdorp 1989). As fish increase in size they invest proportionally less in somatic growth and more into reproduction (Bromley 2000), therefore larger fish begin ovary development sooner than smaller fish as the larger fish will spend less time on somatic growth during the summer months and so can begin ovary development at an earlier time. By beginning ovary development at an earlier date larger fish will have a greater amount of time for follicle growth to take place and so may use this time to produce larger eggs; larger female plaice have been observed to produce larger eggs (Fox *et al.* 2003).

From the follicle size distributions it can be inferred that pre-vitellogenic follicles are recruited in batches into vitellogenic follicles over a period of time (Fig. 2.13a-i). Recruited batches then increase in size through vitellogenesis. As the first batch goes through vitellogenesis more batches are recruited resulting in the distribution of follicle diameters becoming skewed towards the smaller sizes (Fig. 2.13c-l). Oocyte recruitment appears to cease when the lead cohort reaches approximately 700-800 μm (Fig. 2.13j-l) (also evident from mean follicle diameter-relative fecundity relationship as it is at 700-800 μm that it changes from a positive to negative relationship). The later cohorts then begin to 'catch up' with the leading cohort until there is a single modal peak distribution (Fig. 2.13m). The follicles then increase in size until spawning, preserving the single modal distribution. During spawning, batches of oocytes are hydrated and increase in size (Fig. 2.13v-x). It

appears that a hydrated batch is not always spawned before the next batch begins hydration (Fig. 2.13w).

Horwood (1990) observed that several plaice had a bi-modal distribution in follicle sizes, which was the first time this had been documented in plaice, and suggested that this was due to a second burst in egg production. As these fish were caught in the autumn (Witthames pers. comm. Cefas, UK) the observed follicle distribution would have been the result of continuing follicle recruitment.

CONCLUSION

Fecundity varies on the temporal and spatial scale in the Irish Sea with fish from the western Irish Sea having the greatest inter-annual variability and Liverpool Bay showing no significant differences between present day estimates and estimates taken in 1953. The maximum plaice fecundity is determined by the weight of the fish at the end of follicle proliferation. This is then down regulated by atresia in the time between the end of proliferation and spawning. It is believed the degree of the down regulation is affected by the availability of food and is the cause of the observed differences in fecundity between years and areas. Larger fish are generally farther ahead in ovary development than smaller fish at a particular time during ovary development and are most likely to spawn earlier in the spawning season.

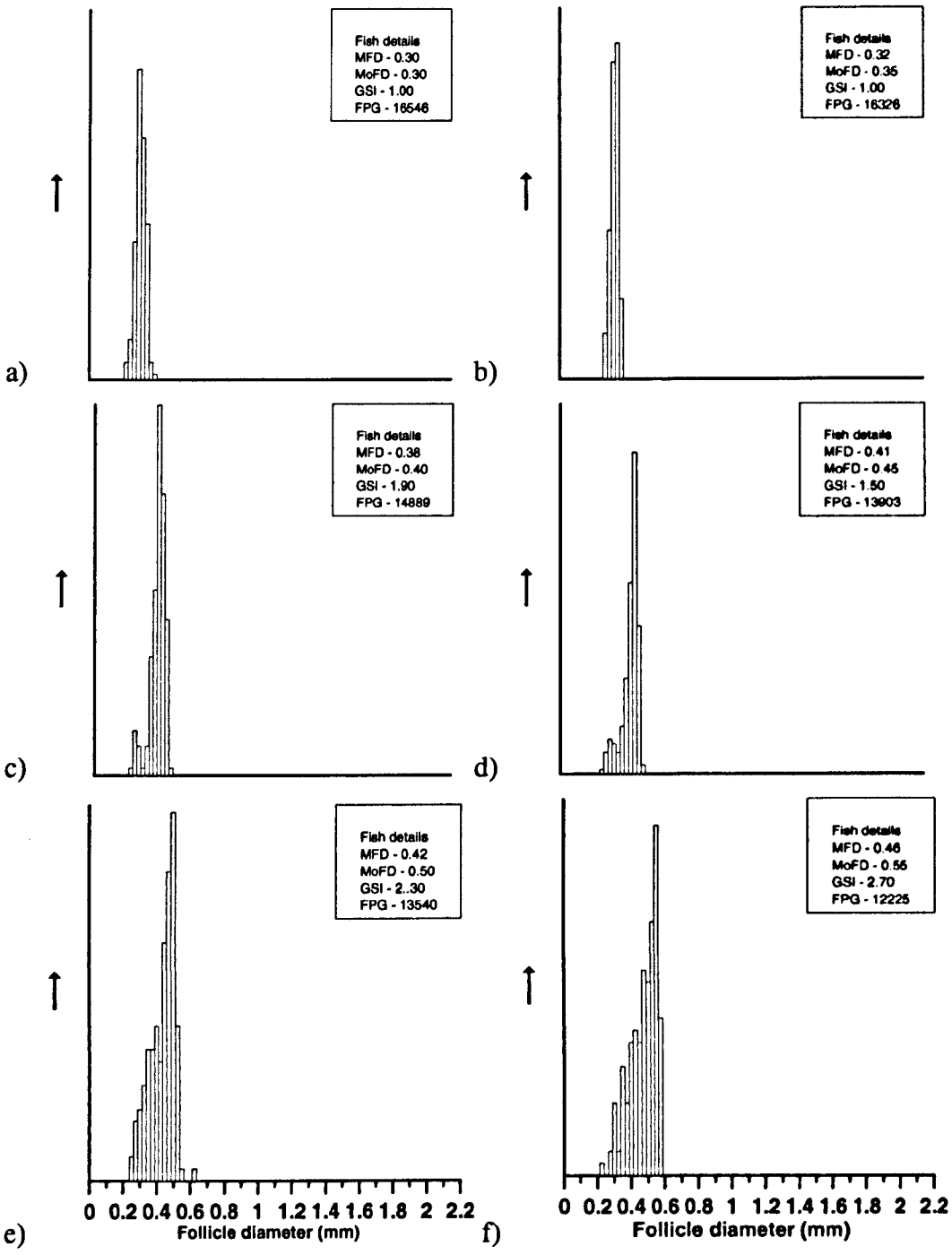

a)
Fish details
MFD - 0.30
MoFD - 0.30
GSI - 1.00
FPG - 16546
b)
Fish details
MFD - 0.32
MoFD - 0.35
GSI - 1.00
FPG - 16326
c)
Fish details
MFD - 0.38
MoFD - 0.40
GSI - 1.90
FPG - 14889
d)
Fish details
MFD - 0.41
MoFD - 0.45
GSI - 1.50
FPG - 13903
e)
Fish details
MFD - 0.42
MoFD - 0.50
GSI - 2..30
FPG - 13540
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2
Follicle diameter (mm)
f)
Fish details
MFD - 0.46
MoFD - 0.56
GSI - 2.70
FPG - 12225
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2
Follicle diameter (mm)

Fig. 2.13

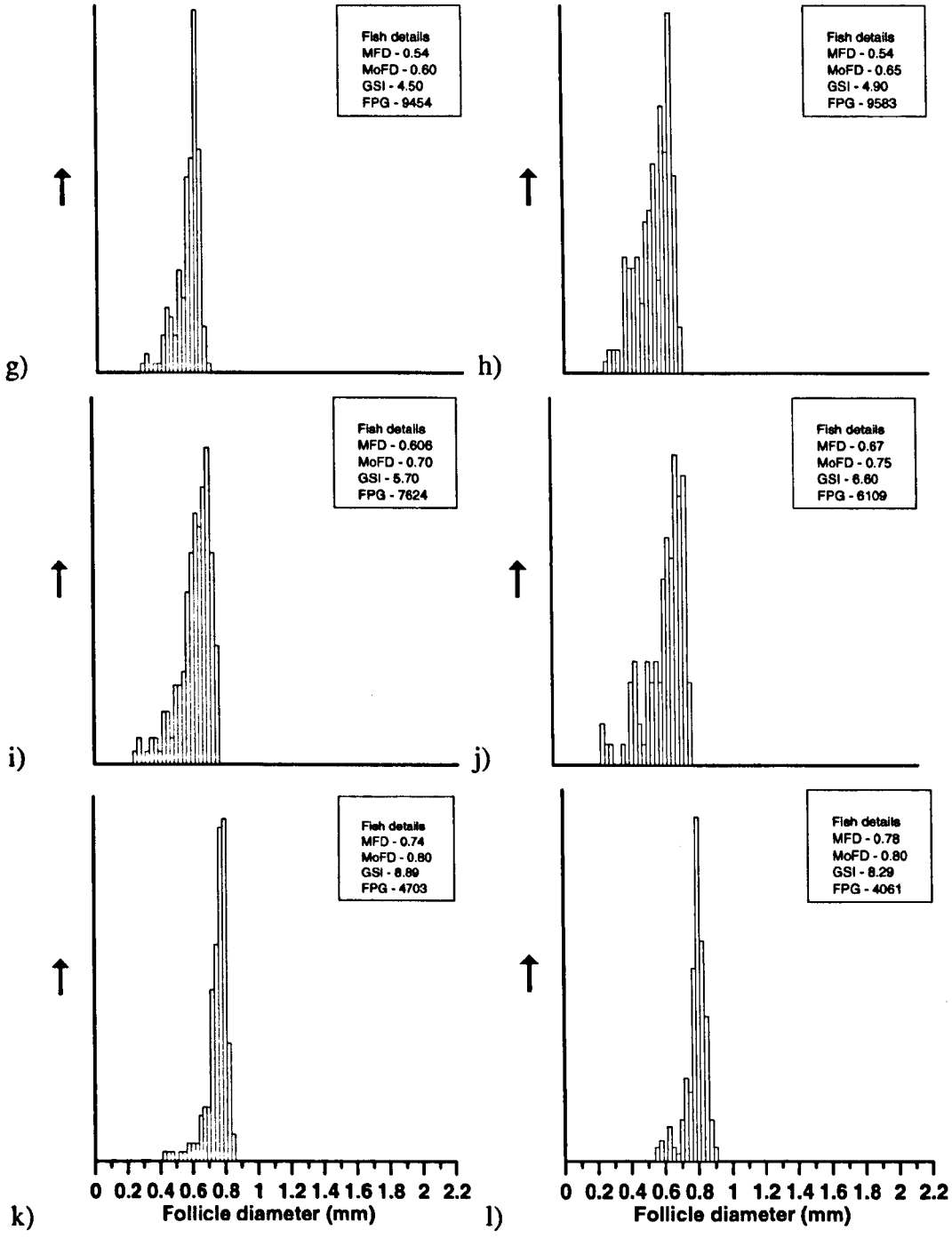


Fig. 2.13 continued

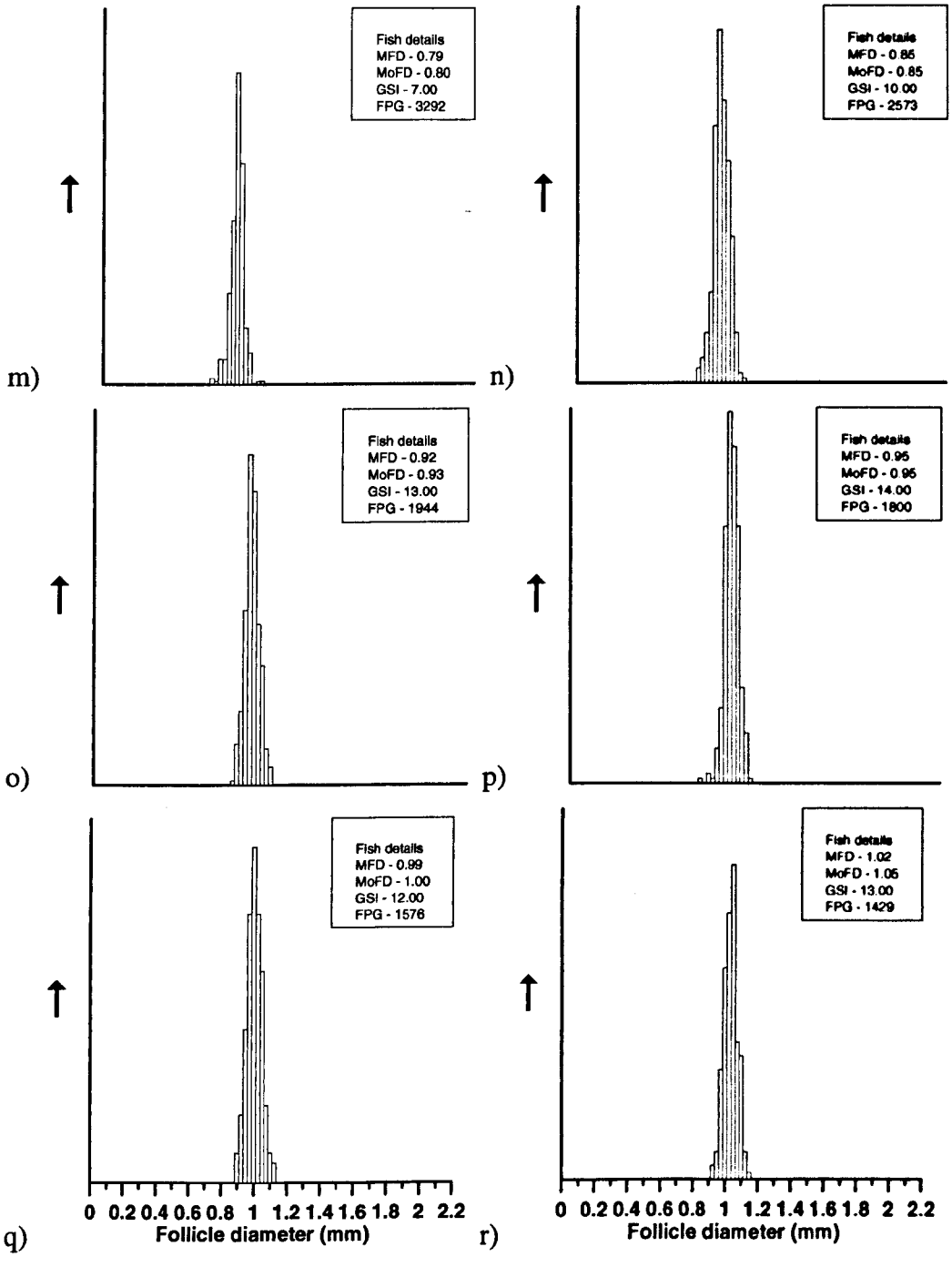


Fig. 2.13 continued

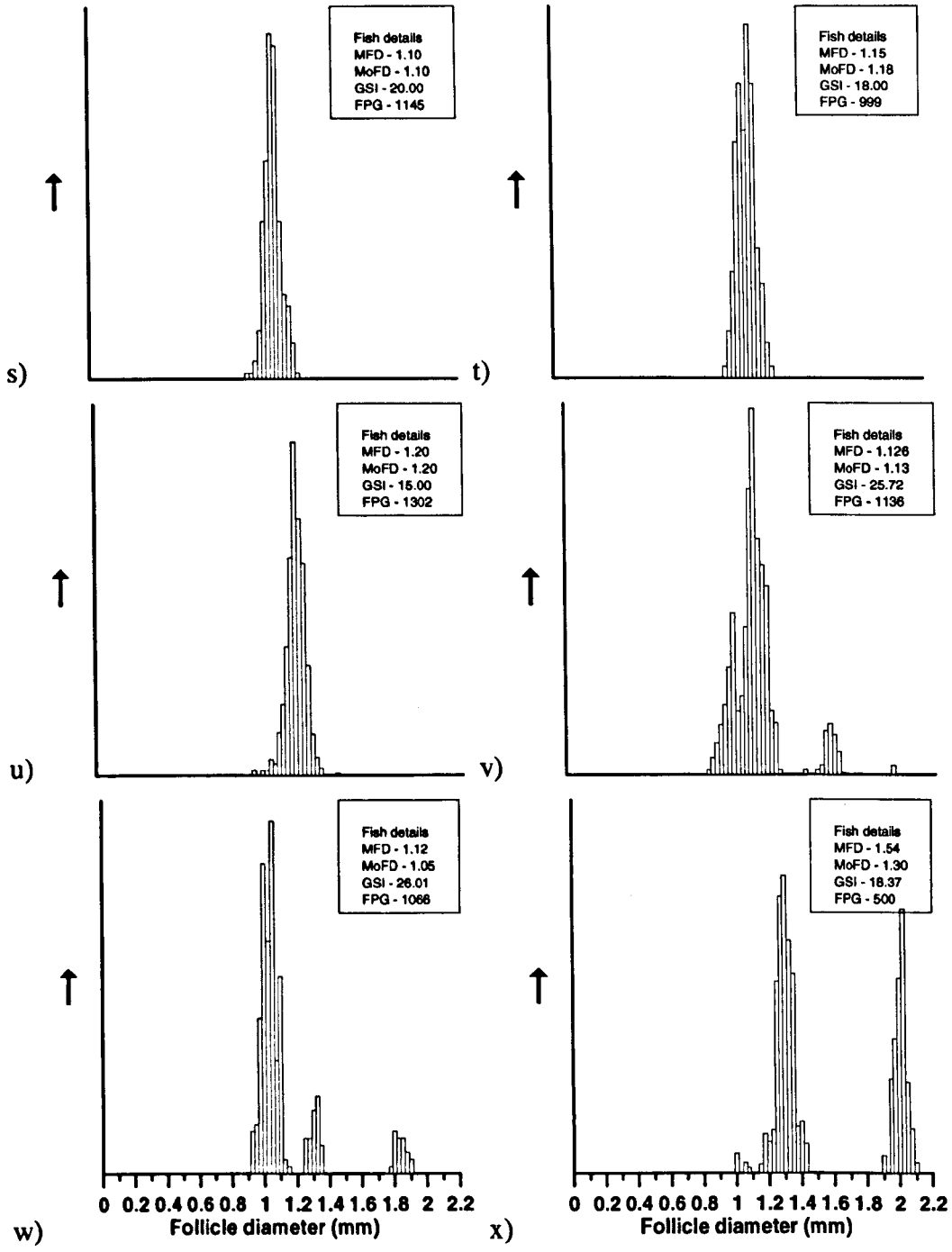


Fig. 2.13. Follicle size distributions of plaice (*Pleuronectes platessa*) through ovary development with panel showing information for each fish (MFD = Mean follicle diameter, MoFD = Modal follicle diameter, GSI = Gonadosomatic index and FPG = Follicles per gram ovary tissue).

CHAPTER 3

THE EFFECT OF AUTUMN FEEDING LEVEL ON FECUNDITY

INTRODUCTION

Fecundity in fishes has been shown to vary over temporal and spatial scales (Rijnsdorp 1991; Witthames *et al.* 1995; Nash *et al.* 2000). Annual changes in fecundity of Arcto-Norwegian cod have been linked to changes in environmental temperature and the availability of the main food item, capelin (Kjesbu *et al.* 1998). Several laboratory studies have shown that fecundity and maturation is affected by the level of food ration (Tyler and Dunn 1976; Horwood *et al.* 1989; Bromley *et al.* 2000) and in extreme cases low food level can result in abortive maturation (Burton 1994; Ramsay and Witthames 1996; Bromley *et al.* 2000). Plaice are an iteroparous species so must optimise their energy allocation between a minimum condition required to survive after spawning and an optimum amount of energy for reproduction (egg production and behaviour related to spawning) (Rijnsdorp 1990).

The effects of feeding regime on first-time spawning turbot were investigated by Bromley *et al.* (2000). It was found that low rations during vitellogenesis led to a drop of 70% in mean ovary weight, and was associated with poor growth of the vitellogenic oocytes or, in a third of cases, the absence of vitellogenic oocytes. Kjesbu *et al.* (1991) showed that the actual fecundity of cod deprived of food during the spawning season was between 20 and 80% of the potential fecundity, depending on the nutritional status of the fish.

Plaice are deemed to have a determinate spawning strategy, in which the annual fecundity of an individual female is determined before the onset of the spawning season (i.e. no more oocytes are recruited during spawning) (Urban 1991). An experiment carried out by Horwood *et al.* (1989) showed that fecundity differences in plaice could be generated in the laboratory by feeding different levels of ration. They also found that plaice on low rations may make an early decision not to proceed with gonad development. This was also found by Rijnsdorp (1990) where fish that showed poor growth between June and January did not proceed with gonad development.

In the previous chapter it became clear that the maximum potential fecundity of plaice is determined during early vitellogenesis which is in late September-October. During the period between follicle recruitment and spawning, fecundity is down-regulated by atresia. This has been witnessed in wild Atlantic

herring where the prevalence and intensity of atresia was highest in October and November, when fish were relying on accumulated body reserves. This resorption of follicles was deemed to be a method of optimising fecundity given available energetic reserves (Kurita *et al.* 2003). Atresia has been witnessed in many other species including cod (Kjesbu *et al.* 1991) Dover sole (*Microstomus pacificus*) (Hunter *et al.* 1992), sole (Horwood 1993; Witthames and Greer Walker 1995) and mackerel (Walker *et al.* 1994).

It has been estimated that up to 50% of the gonad growth in plaice is subsidised from body reserves built up during the growing period (Rijnsdorp 1990) with the major reserve store of lipid and protein being the carcass (Dawson and Grimm 1980) as opposed to the liver. Vitellogenin (VTG), the main precursor to yolk protein (Tyler and Sumpter 1996), is manufactured in the liver from these stored reserves and translocated to the ovary where it is incorporated as yolk granules in the vitellogenic follicles. For the optimisation of sexual reproduction in female turbot, a combination of adequate body reserves and a plentiful exogenous supply of food during the vitellogenic phase is required (Bromley *et al.* 2000).

In the previous chapter it was suggested that the differences in fecundity of plaice from different areas and years in the Irish Sea were due to differences in the degree of down-regulation in response to differences in the availability of food between areas and years. To test this hypothesis in the laboratory three techniques were devised to study fecundity regulation whilst feeding the fish with either high or low levels of food. Ovary growth was monitored in live fish by measuring the ovary silhouette whilst illuminated with strong light. Concurrently, follicle growth and regression were investigated by removing samples of follicles from the ovary by taking a biopsy from each fish whilst it was sedated before and during the experiment. The results will give a more complete picture of the movement of stored reserves from the muscle to the ovary in relation to food level which has never been followed during vitellogenesis in individual plaice. Trials were also done to investigate whether atretic follicles could be counted with equal efficiency in whole mounts (dispersed follicles in water) compared to using the more labour intensive histology.

MATERIALS AND METHODS

Experimental set-up

Feeding trials were undertaken on a total of 18 plaice. Six fish were caught from the French coast in the southern North Sea during the Cefas 2004 August beam trawl survey of the Channel. A further 12 fish were caught by beam trawl from the southern North Sea during a charter of a commercial fishing vessel in October 2004 (Fig. 1). The fish were kept in holding tanks at Cefas, Lowestoft from the date of capture until the start of the experiment and fed to satiation with live lugworm twice a week.

At the beginning and two weeks after the start of the experiment the fish were anaesthetised using phenoxy-ethanol (at a concentration 0.4 ml per litre), weighed to the nearest gram and measured to the nearest cm. The fish were then photographed over strong illumination for the assessment of ovary size, and an ovary sample was taken using a pipelle with an internal bore of 0.2 mm (Bromley *et al.* 2000). This was inserted through the oviduct into the ovary and approximately 0.15-0.3g of tissue was extracted from the ovary. The biopsy samples were preserved in 3.6% formalin and analysed using the image analysis system described in the previous chapter.

During the experiment the fish were housed individually in a compartmentalised tank with a flow-through system of filtered seawater at ambient temperature (range 9.5-13.7 °C average 12.1 °C). The fish were divided into a high and low feeding group by ranking the fish in order of increasing follicle diameter and putting alternate fish into each group; this would then eliminate any effect caused by the stage of ovary development. The feeding levels were set at 0.5 % and 1.5 % per day of the average fish's total weight for the low and high feeding group respectively. The fish were fed on live lugworm twice weekly with any uneaten worms removed and weighed the following day.

The fish were killed after four weeks and dissected. Each fish's length was measured and the liver, gut, ovaries and carcass were weighed to the nearest 0.01 g. Three follicle samples were taken from the middle part of the smaller ovary which were analysed using the image analysis system and histology.

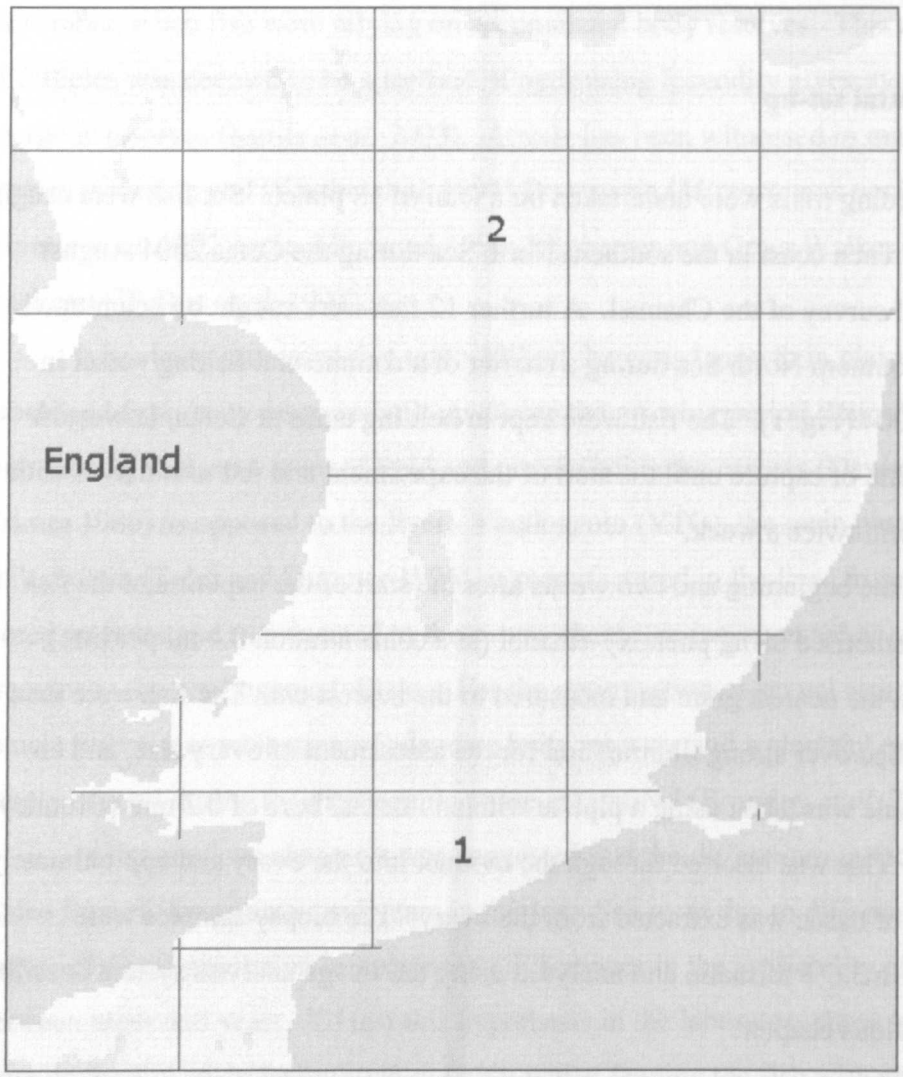


Fig. 3.1. Map of the southern North Sea with the numbers indicating the capture sights of plaice. 1 = Where 6 fish were caught from the French coast. 2 = Where 12 fish were caught from the southern North Sea.

Ovary area - ovary weight relationship

A relationship between ovary area and ovary weight was constructed using 40 fish. These consisted of;

- Four fish that were caught at the same time as the fish from the southern North Sea and kept in the same tank until the start of the experiment and killed at the start of the experiment.

-Eighteen frozen fish caught at the same time as the fish from the southern North Sea, which died before the start of the experiment.

-The eighteen fish that were used in the experiment

The area of the shadow created by the ovary, posterior to the body cavity, was measured in the fish by placing them over strong illumination and taking a photograph. The area of the ovary was then measured from the picture using image analysis software (Myrmica, Pilkington Image Analysis Systems) and plotted against the total ovary weight of the fish. The growth of the ovaries in the experimental fish throughout the experiment was then reconstructed from the ovary area measured at the beginning of the experiment.

Analysis of ovary samples

The ovary samples were analysed by the Auto-diametric method and the fecundity was estimated using the equation from the previous chapter:-

$$\text{Log}_e FD = (-1.4036 * \text{Log}_e MFD^2) - (3.4952 * \text{Log}_e MFD) + 7.5394$$

$$\text{Fecundity} = FD * OW$$

OW = ovary weight
FD = follicle density
MFD = mean follicle diameter.

Fish condition was calculated from the following equation.

$$\text{Expected weight} = 0.0063 * L^{3.1673}$$

$$\text{Condition} = \text{actual weight} / \text{expected weight} * 100$$

Where L is fish length and the expected weight is from a length-weight regression of 183 pre-spawning fish caught in the Irish Sea during January 2001.

During the analyses, the numbers of atretic follicles were counted which were distinguished in the whole mount from normal follicles by their non-round shape (Fig. 3.2). The final atretic counts were determined by identification in the whole mount and compared with a histological method where the follicles were dispersed into a layer one follicle deep and polymerized in hydroxymethyl

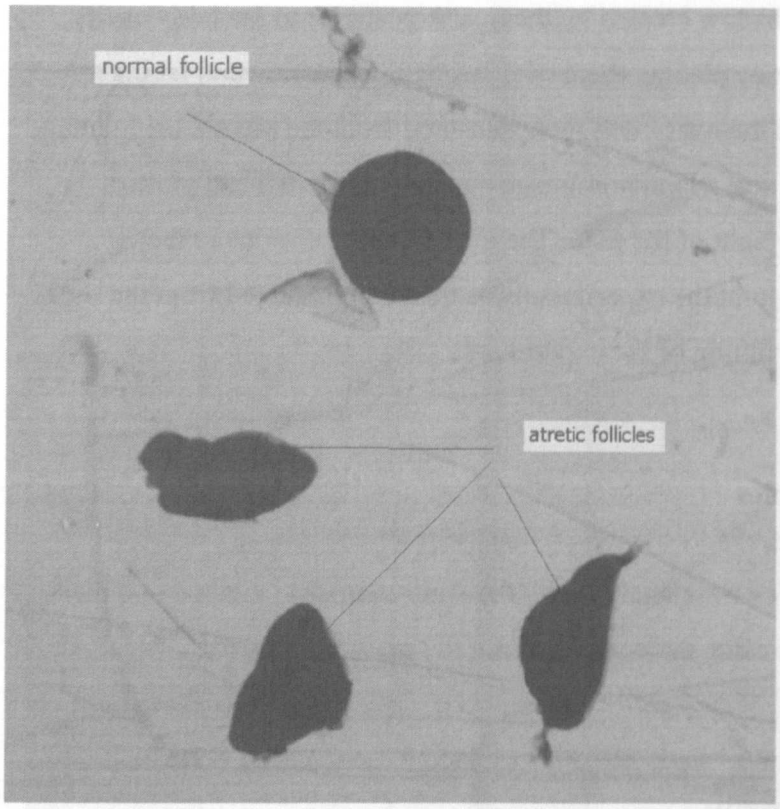


Fig. 3.2. View of plaice follicles as seen in GFA showing 1 normal follicle and 3 atretic follicles.

methacrylate. A section was then taken 250 μm from the block face and the numbers of atretic and normal follicles were counted (Fig. 3.3). The total atresia for a fish was calculated as the level in the second sampling date plus the level at termination of the experiment. The fecundity of the fish was adjusted for the level of atresia present in the ovary samples taken at the termination of the experiment.

Statistical analysis

All statistical tests were carried out using Statistica (StatSoft Inc. 2002). The food conversion efficiency among fish was assumed to be constant during the experiment. Measured variables were converted into relative change over the four week period. Carcass size at the start of the experiment was calculated from total weight minus estimated ovary weight and the weight of gut and liver at the end. The gut weight was assumed to be constant during the experiment and the effect of the change in liver weight on estimated carcass weight was assumed to be insignificant. Length, weight and fecundity measurements were normalised by a Log_e transform. Principal component analysis was used to find correlations between the different measured variables and to classify the variables into groups. The correlations that were detected were then further investigated using linear regression.

RESULTS

The details of the fish used in the experiment are shown in Table 3.1 including the success of the gonad biopsy at the start and middle of the experiment. The fish ranged in weight from 274 to 497 g with an average of 384 g. The weight of the plaice from the two locations were not significantly different (ANCOVA; $p>0.005$). Twelve of the fish were 3 year old with the other six being 4 years old. All fish re-established feeding after being moved to the individual pens although not all fish consumed all the food they were given. The fish from the high feed group consumed an average of 125 ± 35 g of food each throughout the experiment which amounted to an average of 35 ± 13.3 % of their total body weight. The fish

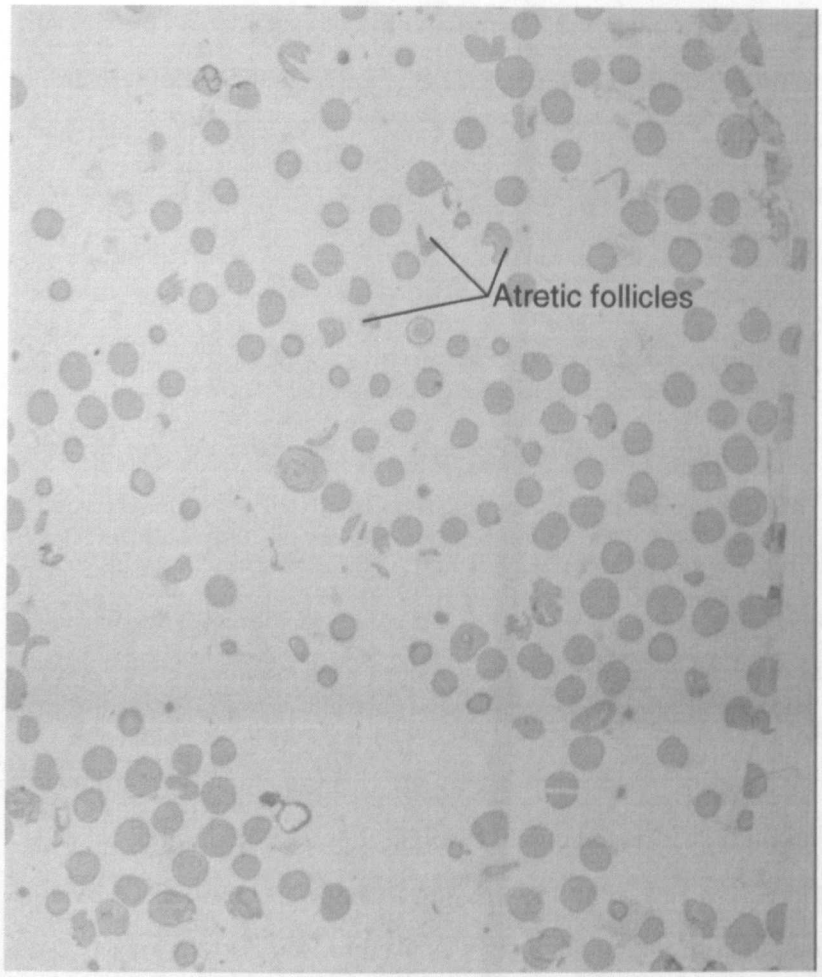


Fig. 3.3. Histological section of dispersed plaice follicles with label pointing to 3 atretic follicles.

Table 3.1. Details of the plaice used in the experiment with fish number, origin (N = North Sea, F = French coast), length (cm), ration level (RL) (H = high, L = low) and success of gonad sampling at the start and middle of the experiment (S = successful, N = non-successful).

Fish ID	Origin	Length	RL	1st Sampling	2nd Sampling
1	N	33	H	S	S
2	N	31	L	S	S
3	N	34	L	S	S
4	N	32	H	S	S
5	N	34	H	S	S
6	N	33	L	S	N
7	N	32	H	N	N
8	N	34	L	N	N
9	N	34	H	S	S
10	N	30	H	N	S
11	N	34	L	S	S
12	N	34	L	S	S
13	F	33	L	S	N
14	F	35	H	S	N
15	F	34	H	S	S
16	F	30	H	S	N
17	F	33	L	S	N
18	F	31	L	N	S

from the low ration group consumed an average of 47 ± 3.7 g each which amounted to an average of 12 ± 1.7 % of their body weight. One fish from the high feed group consumed only 33.6 g of food which was the lowest of all the fish

The total weight of the ovary was positively correlated with the area of the tail of the ovary (Linear regression; R^2=0.74; N=40; p<0.001) (Fig. 3.4). A total of 14 biopsy samples were successfully taken at the beginning of the experiment and 11 during the second sampling. A successful biopsy from a fish at the beginning of the experiment did not always result in a second successful biopsy and vice versa. All fish survived to the end of the experiment. One fish however went into mass atresia during the experiment. This fish was on the high food diet but had the lowest condition of all fish in the experiment.

Total weight gain/loss was related to the percentage of food each fish consumed relative to its body weight (Linear regression; R^2=0.35; N=18; p=0.015) (Fig. 3.5). Fecundity was positively correlated with length (Linear regression; R^2=0.45; N=17; P=0.002) (Fig. 3.6) and weight (Linear regression; R^2=0.55 N=17; P<0.001) with no influence of condition. The carcass of 13 fish decreased in weight (Fig. 3.7). Four of the fish that gained weight to their carcass were on the high food ration; the 5th was on the low food ration. The weight of the ovaries increased in 14 of the 18 fish with the average percentage change being a 10% increase. Sixteen of the eighteen fish exhibited an increase in average follicle diameter with the growth of follicles varying between 0.7 and 22 % with one fish exhibiting a decrease of 8 %.

The principal component analysis (PCA) yielded two axis explaining 45% and 24% (eigenvalues 3.60 and 1.95) of the data respectively. On axis one, the relative change in carcass weight, relative change in condition, hepatosomatic index (HSI), food consumed and the growth of follicles were all positively correlated with each other (Table 3.2). On the second axis percentage change in ovary weight, original weight and final adjusted fecundity were positively correlated.

Atresia levels estimated in the whole mount and by histological methods showed little difference between the two methods and were not significantly different (t-test; t=0.06; n=8; p>0.05) (Fig. 3.8). Atresia was present in all fish varying from 0 to 43 % (average 14%) at the start of the experiment, 2 to 25 % (average 8%) at the second sampling date and 2 to 19% (average 9%) at the end of the experiment (Fig. 3.9). Total atresia for each fish was negatively correlated

with the change in condition factor between the start of the experiment to the end. (Linear regression; R^2=0.76; N=11; p<0.001) (Fig. 3.10).

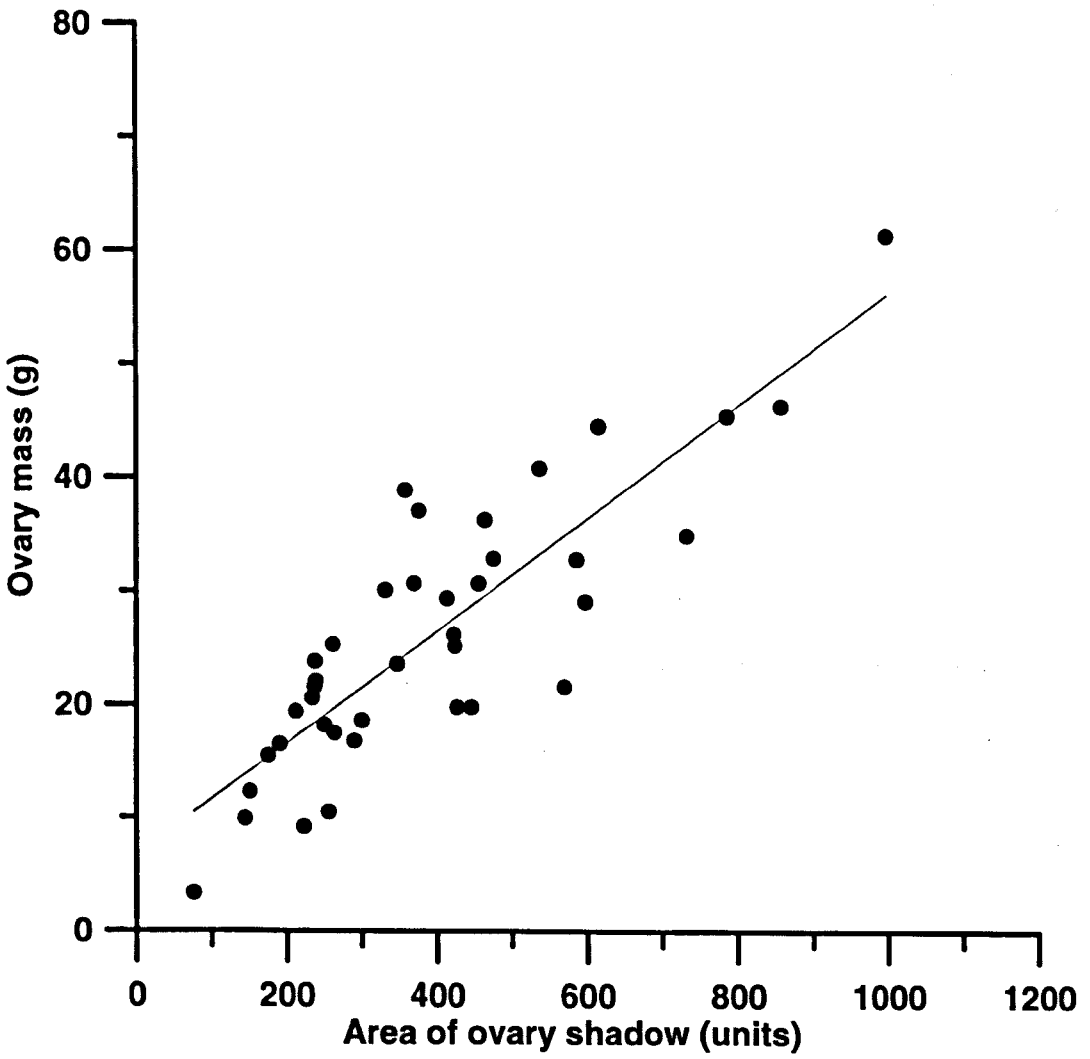


Fig. 3.4. Relationship between the area of plaice ovary shadow posterior to the body cavity and the total weight of both ovaries. Line shows fitted linear regression line.

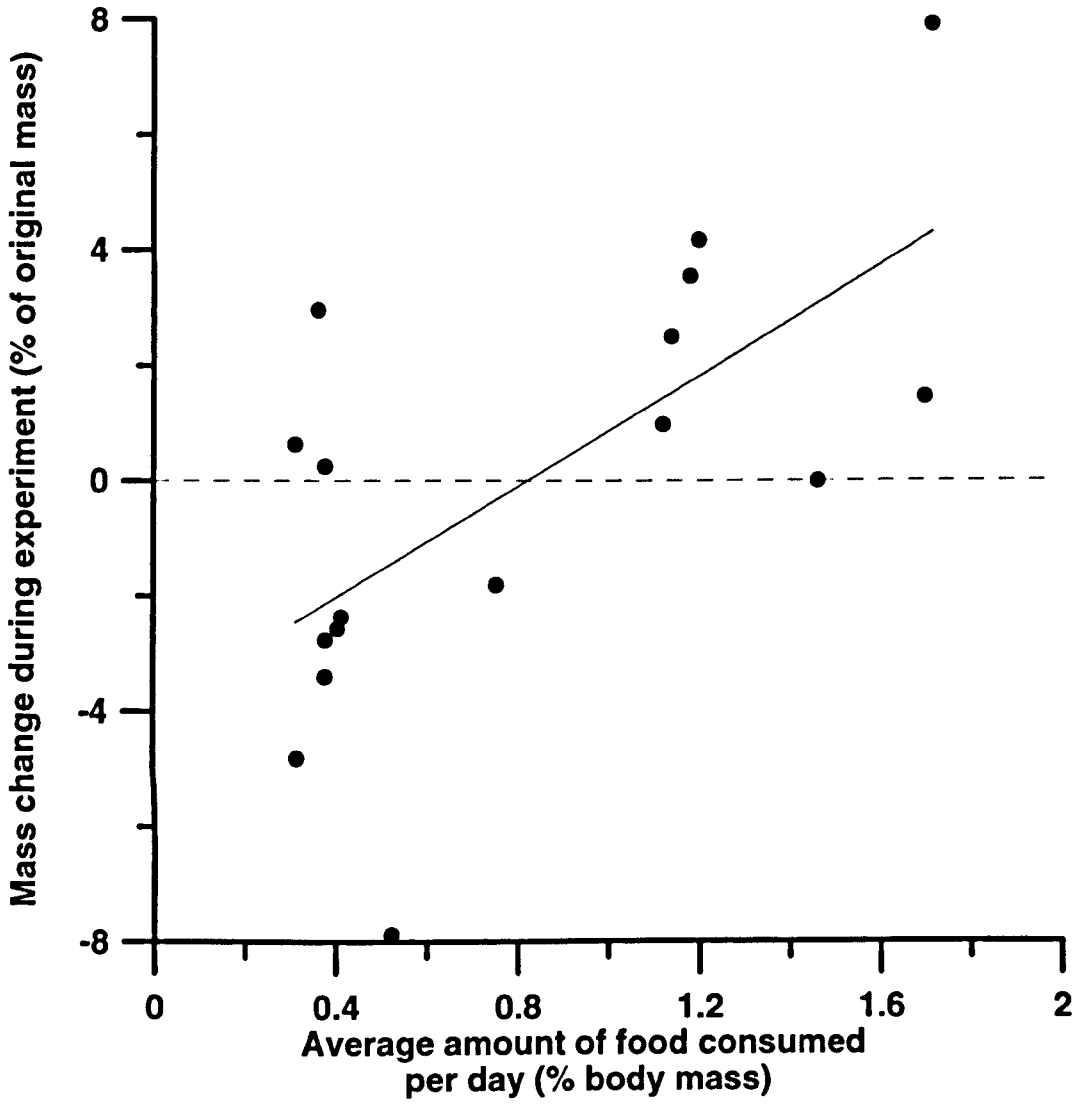


Fig. 3.5. The relationship between the average amounts of food consumed per day by each individual plaice expressed as a percentage of total body weight at the start of experiment and the percentage change in body weight during the experiment. Line shows fitted linear regression line.

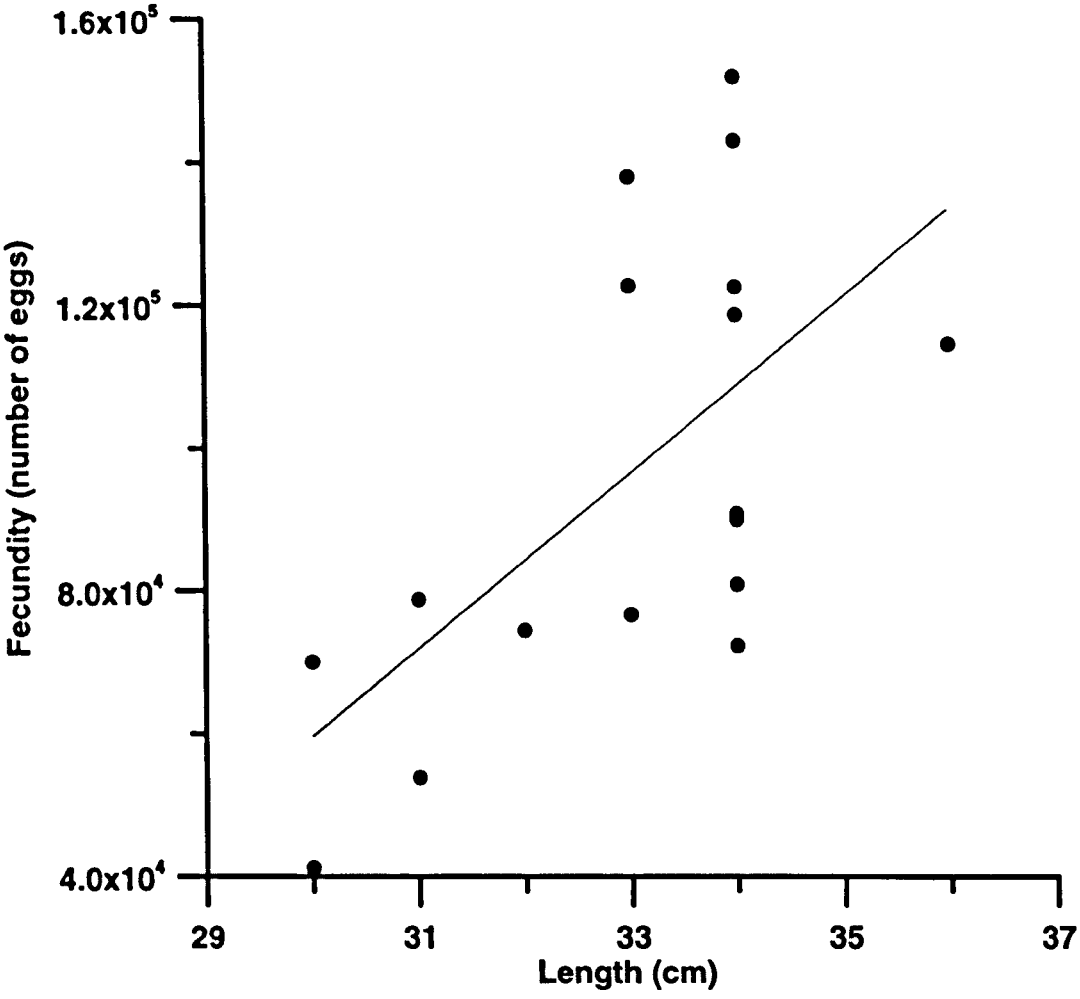


Fig. 3.6. The relationship between length and fecundity of the plaice at the termination of the experiment. Line shows fitted linear regression line.

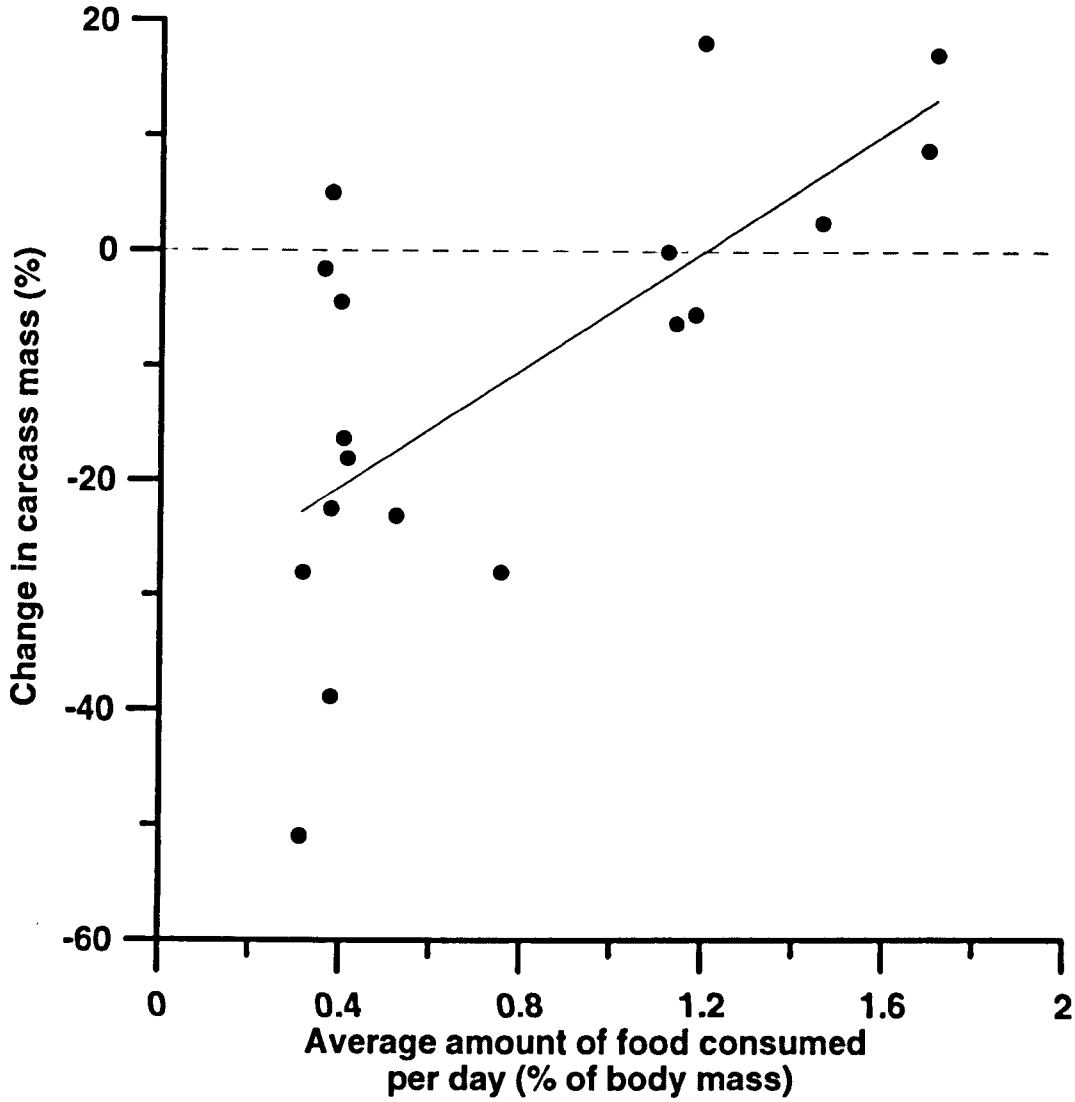


Fig. 3.7. The relationship between the average amount of food consumed per day by each individual plaice, expressed as a percentage of total body weight at the start of experiment and the percentage change in weight of the carcass. Line shows fitted linear regression line.

Table 3.2. Output from the principal component analysis. The loadings of the two axes, eigenvalues and percentage of variance explained by each axis are given. Significant correlations are shown in bold.

Characteristic	Factor 1	Factor 2
Relative change in carcass weight	**0.799643**	-0.377671
Relative change in condition factor	**0.858920**	-0.061203
Relative change in ovary weight	0.116849	**0.808011**
Food consumed	**0.648283**	-0.466932
Relative change in average follicle diameter	**0.642086**	-0.299604
Adjusted fecundity at end	-0.192470	**0.788113**
Hepatosomatic index	**0.772602**	0.338913
Log_e original weight	-0.190000	**0.901994**
Eigenvalue	3.600023	1.950035
% variance	45.00029	24.37543

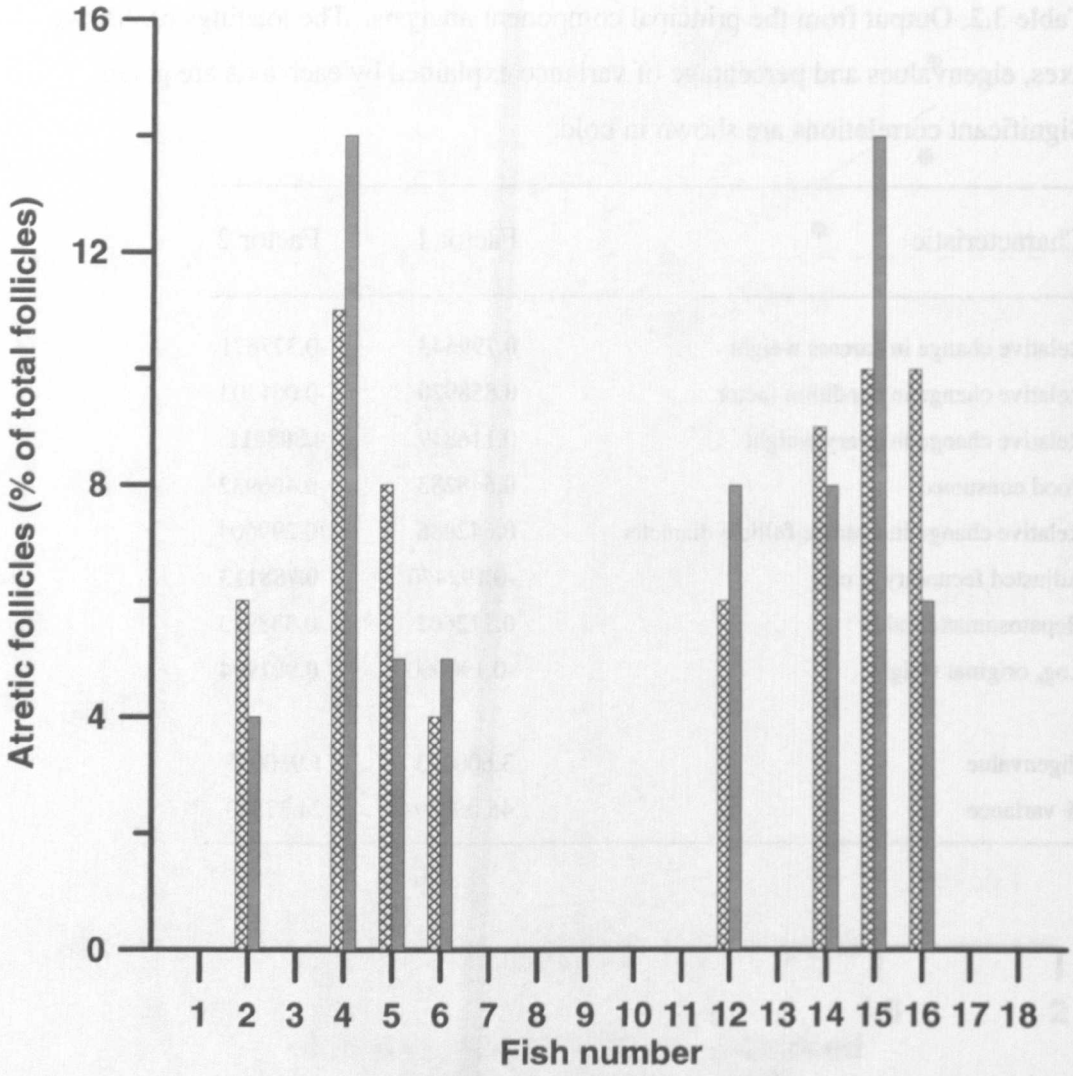


Fig. 3.8. Comparison between the level of atresia, for 8 plaice, estimated by whole mount (checked) and histology (grey).

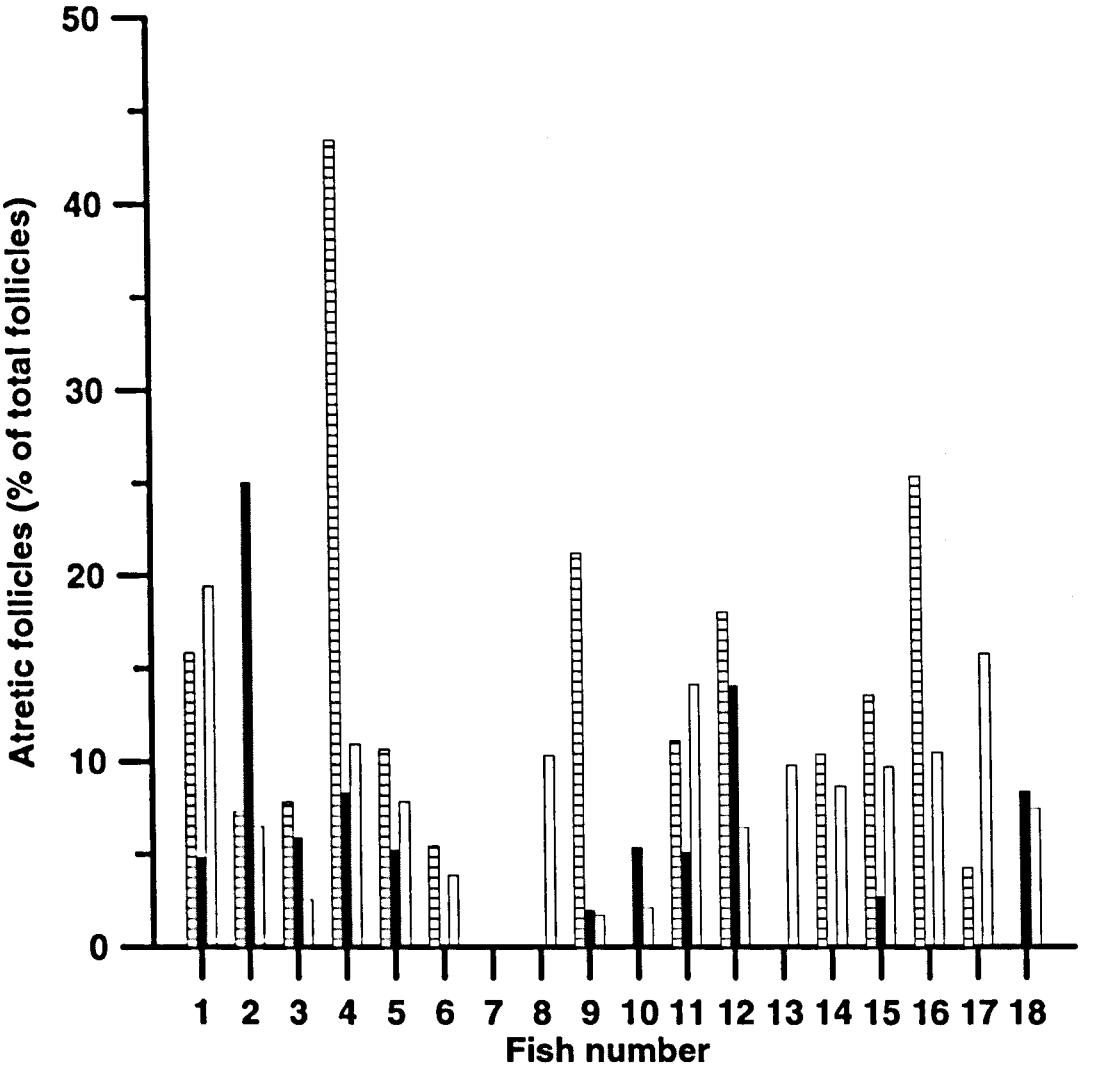


Fig. 3.9. Level of atresia of plaice, estimated by whole mount, on the three sampling dates, beginning of the experiment (striped), middle (black) and end (white). The absence of a bar indicates that atresia was not estimated due to unsuccessful sampling of the fish ovary.

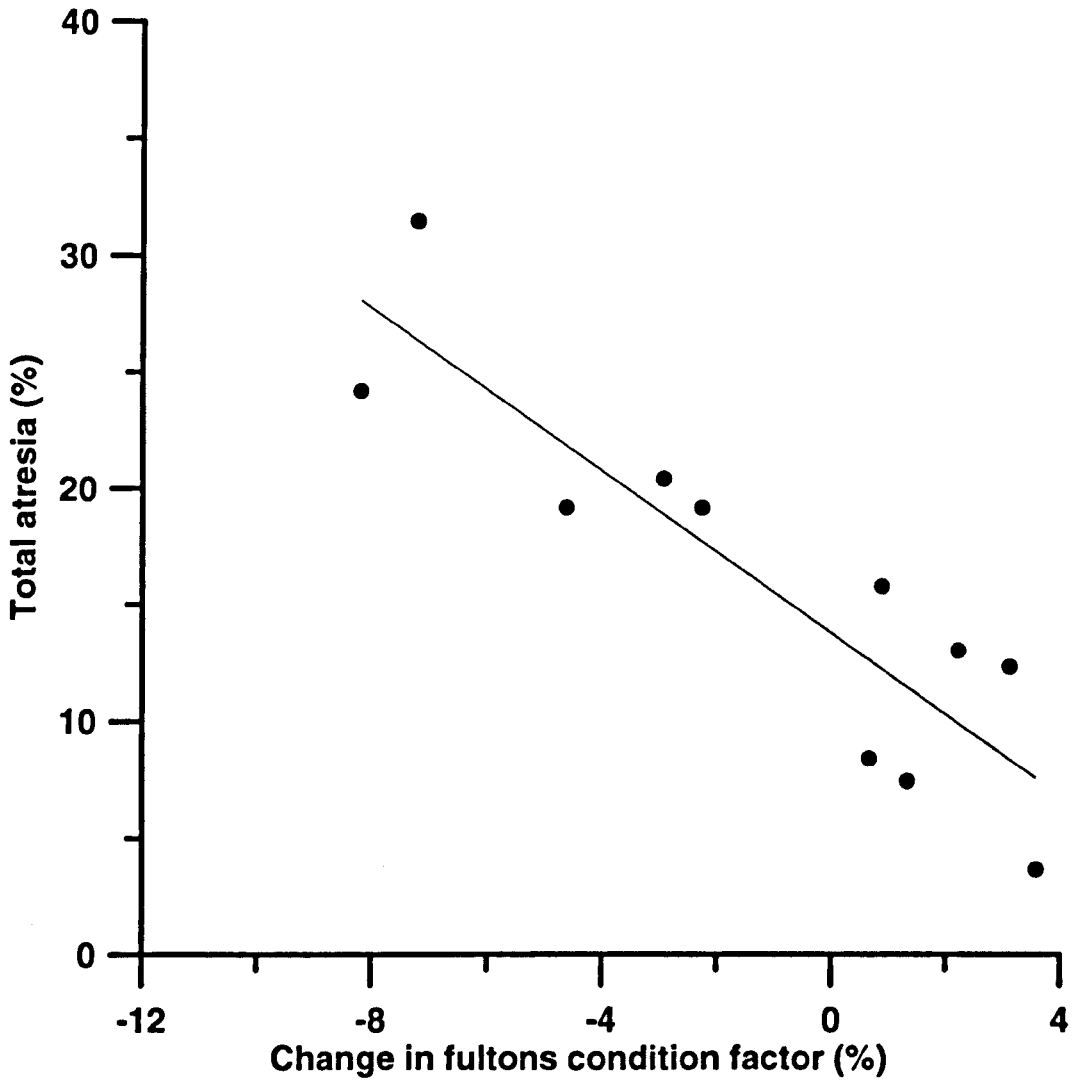


Fig. 3.10. The relationship between the change in condition factor of plaice from the beginning to the end of the experiment and total atresia. Line shows fitted linear regression line.

DISCUSSION

Ideally all fish would have been caught at the same time, however due to poor weather during the transport of fish from the capture site to the laboratory there was a high mortality of fish. The fish are most likely to be from the same stock due to the close location of the capture sites and are known to migrate between these two areas (Hunter *et al.* 2004). The starting weight of the two groups of fish were not significantly different and the numbers of fish from each area was distributed evenly between the high and low feed groups. Therefore the different

capture sites of the two groups of fish will not have affected the results. The gonad sampling was deemed to have no effect on final fecundity. Due to the small size of the fish gonad sampling was not always successful. The method for estimating ovary size was very successful and gave a reliable measurement of ovary growth although it was not accurate enough for the estimation of fecundity. It was not possible to estimate the area of the whole ovary as it was difficult to discern the anterior of the ovary as it was obscured by the organs of the abdominal cavity.

The estimation of atresia from whole mounts and by histological methods gave similar results, the average for all the fish estimated by both methods was not significantly different but there was a slight difference in individual fish. The histological method was not completely accurate as there is a tendency to underestimate the number of atretic follicles due to their smaller size. The dissector method (Mayhew 1992) removes this bias; however this was not possible with plaice eggs as they have a tendency to disperse into a mono layer and, due to their large size, very few show up in only one histological section. Estimation of atresia using the whole mounts was much more time efficient as it could be undertaken during the analysis of follicle diameter. These two methods were compared using only a small number of samples; therefore it is recommended that this method is investigated further.

There was a high variance in the relationship between food consumption and weight gain/loss which is probably due to differential absorption efficiency. This could not be confirmed as the energy in the faeces was not recorded, it could also be due to stress altering the metabolic rate of the fish.

The fecundity of the fish at the end of the experiment was positively correlated with the weight and length of the fish at the beginning and end of the experiment. Weight was the best predictor of fecundity as found by Koops *et al.* (2004). There was no effect of food level on relative fecundity which is in contrast to a previous study on plaice where feeding level had a significant effect on absolute and relative fecundity (Horwood *et al.* 1989). The fish from the previous study were much larger and most likely to be repeat spawners, and the period of the study encompassed both follicle proliferation and down regulation. The fish from the present study consisted of small fish that were either 3 or 4 years old and the majority were most likely to be first time spawners (Bromley 2000). During the present study follicle proliferation had ceased as evident from the presence of a

hiatus between the vitellogenic and pre-vitellogenic follicle distributions. It has been pointed out by Kjesbu and Holm (1994) that repeat spawners experience severe depletion of body reserves following spawning (Kjesbu *et al.* 1991) and are therefore more sensitive on subsequent feeding regimes. Further, repeat spawners typically show higher relative fecundities (Kjesbu *et al.* 1991) enhancing this dependence. A similar result to the present study has been seen in first-time spawning cod where fish fed on a high-high, high-low and low-high ration, during the follicle recruitment and vitellogenic phase respectively, over a year showed no difference in relative fecundity and fecundity differences were due to differences in the final weight of the fish (Kjesbu and Holm 1994).

Intensity of atresia was related to the nutritional condition of the fish with atresia being greatest in fish that had decreased in condition during the experiment so food level did have an affect on final fecundity. There was no effect of food level on relative fecundity due to both fecundity and weight changing during the experiment. During the previous study on feeding level and plaice fecundity (Horwood *et al.* 1989) there was sufficient time (406 and 30 days for the previous and present study respectively) for the fish to exhibit fluctuations in weight. This was likely as the fish were kept in communal tanks the ration each fish received would not be the same due to competition for food. As fecundity was seen to decrease with decreases in weight, but cannot increase with a weight increase after follicle proliferation has ended, the observed differences in relative fecundity would result. The level of atresia decreased from the start to the end of the experiment which is consistent with the idea that atresia is highest during the early part of maturation and decreases towards spawning (Witthames, pers. comm. Cefas, U.K.).

It is interesting to note that the fish with the highest fecundity exhibited a decrease in the average follicle diameter. This fish also had the highest intensity of atresia at the end of the experiment. There appears to be no previous record of a decrease in follicle size in the literature. It is hypothesised that the resorption of smaller follicles requires less energy than larger follicles (Kurita *et al.* 2003) thus smaller follicles should be selected preferentially before larger follicles for resorption, however in this particular fish this was not the case with the larger follicles becoming atretic. However by selecting the larger follicles they may receive more energy from each follicle and so can absorb fewer follicles for the required energy than if they had selected for smaller follicles. Another hypothesis

for the decrease is this could be a prelude to mass atresia which was seen in one other fish in the experiment. The fish that had mass atresia was on the high ration but had a low condition and for the successful completion of vitellogenesis a high exogenous food source and a high availability of stored reserves is believed to be essential (Bromley *et al.* 2000). Plaice and winter flounder (*Pseudopleuronectes americanus*) have previously been witnessed to make an early decision to skip spawning when under low food rations (Horwood *et al.* 1989; Burton 1994).

The positive correlation between follicle growth and HSI is consistent with the current knowledge that vitellogenin is manufactured in the liver from stored reserves and translocated to the ovary where it is incorporated as yolk granules in the vitellogenic follicles (Norberg and Kjesbu 1991). It was clear from the present results that the rate of vitellogenesis is controlled by the availability of an exogenous food source. When the daily amount of food fell below approximately 1.2 % of total body weight fish started to use reserves for the growth of follicles. Lower amounts of food consumed led to a greater utilisation of stored reserves and a lower rate in follicle growth. This is due to the greater amount of reserves that had to be utilised for basic metabolism and so there would have been a lower surplus available for vitellogenesis, resulting in lower follicle growth. The continuation of follicle development at low food levels was consistent with the findings of Rijnsdorp (1990) who estimated that 50% of gonad growth in plaice was subsidised from body reserves built up during the growing period.

Slowing the growth of the follicles during periods of low food availability may allow plaice the time to find the adequate amounts of food needed to complete vitellogenesis before reabsorbing follicles by atresia, which would decrease their reproductive output for that year. With the increase in the growth rate of follicles with increased feeding level, there will be a decrease in the time to reach spawning condition. This has been witnessed in cod where the timing of spawning can be predicted (for fish kept under constant conditions) from mean G1 oocyte diameter. Cod kept under lower rations took longer to bring their oocytes to spawning stage than fish fed with higher rations (Kjesbu 1994). However, there are contrasting results for this relationship. Kjesbu and Holm (1994) showed that high feeding in first-time maturing Atlantic cod led to earlier spawning, however this was not found in a later experiment (Karlsen *et al.* 1995). A study by Yoneda and Wright (2005), also on first-time spawning Atlantic cod, showed that faster growing individuals

entered into vitellogenesis earlier relative to the slower growing fish; however there was no effect of food level on follicle growth rate. It was suggested that these inconsistencies may be attributable to differences in food intake among individuals within a feeding group (Yoneda and Wright 2005).

The change in ovary weight was linked to final fecundity as the increase in ovary weight is the sum of the increase in all the follicles present, thus greater numbers of growing follicles, a greater increase in total ovary weight. These were both linked to the original weight of the fish which was a very strong predictor of fecundity. Growth in ovary weight was not linked to the follicle growth rate. The reason for this was un-clear but could be due to follicles being lost to atresia and so total growth of the ovary would be lower due to a smaller number of follicles growing and the weight of the atretic follicles being lost from the ovary.

CONCLUSION

Alteration of food level during the late stages of vitellogenesis affected the intensity of atresia thus affecting the final fecundity, however due to decreases in weight there was no effect on relative fecundity. The growth rates of follicles were affected by the food level with lower food levels resulting in slower growth of the follicles and greater utilisation of stored reserves for follicle growth. Thus, fish that feed more will be in spawning condition earlier than fish feeding less. Growth in the size of the ovary is linked to the fecundity due to a greater amount of growing follicles.

This work was performed to strict Home Office guidelines under the authority of license PPL80/1893.

CHAPTER 4

TOTAL EGG PRODUCTION IN THE IRISH SEA

INTRODUCTION

The assumption that SSB adequately represents stock reproductive potential has been prevalent in the fisheries literature for many years (Myers *et al.* 1994). In many instances, a given weight of adult biomass is presumed to have an equal likelihood of generating the same level of recruitment. This deduction occurs regardless of whether the SSB is comprised of scrawny, low condition fish, a large fraction of which may be skipping reproduction, or a well fed population of highly fecund fish (Trippel 1999). However, recent studies on Northeast Arctic cod have shown that SSB is not proportional to TEP (Marshall *et al.* 1998). It is now understood that the demography of the stock can have a great effect on the TEP and recruitment, which is usually greater with increased age diversity of the spawners (Marteinsdottir and Thorarinsson 1998; Marteinsdottir and Begg 2002) or with a higher proportion of older and larger fish in the population (Scott *et al.* 1999). This is due to SSB, when used as a proxy for fecundity, failing to take account of changes in relative fecundity and egg survival that may result from differences in age, size or condition of the spawning fish (Hislop 1988; Kjesbu *et al.* 1991; Trippel; 1998).

Condition indices reflect energy reserves in several species of fish (Costopoulos and Fonds 1989; Lambert and Dutil 1997) and condition has been show to affect egg production in individuals (Blanchard *et al.* 2003). The inter-annual variation in HSI and fecundity of Northeast Artic cod has been linked to variation in the biomass of the main prey item, capelin (Kjesbu *et al.* 1998; Marshal *et al.* 1998). The intensity of atresia in plaice was related to changes in condition of individual fish (see chapter 3) and differences in the intensity of atresia between areas and years was the cause of variations in fecundity in the Irish Sea (see chapter 2). These changes in fecundity and condition, when considered at the population level, can have large effects on the TEP of a population.

Research on plaice populations in the Southern Bight of the North Sea suggests that year-class strength is determined during the pelagic stages (van der Veer 1986; van der Veer *et al.* 1990). However, studies of plaice from the Irish Sea have shown that year class strength is largely determined during the nursery ground phase (Nash and Geffen 2000). Nash and Geffen (1999) found a direct relationship

between the numbers of stage 1 plaice eggs and the numbers that settle on the nursery grounds on the west side of the Isle of Man, suggesting that the initial variability in abundance or density on the nursery grounds originated from the spawning stock or from very early in the pelagic phase.

In this chapter the TEP for the whole of the Irish Sea was calculated using fecundity-weight relationships from chapter 2 and estimates of SSB from the VPA data (ICES 2006). This was then used to examine the relationship between SSB and TEP for Irish Sea plaice to test whether TEP is proportional to SSB and also whether the estimates calculated from VPA are equal to the estimates calculated from the results of plankton surveys (Anonymous 2005). Using estimates of recruitment at age 1 (ICES 2006) the mortality between the egg stage and recruitment will be examined for correlations between TEP, recruitment and mortality.

MATERIALS AND METHODS

Data on the age structure and weight-at-age was taken from the VPA data for Irish Sea plaice (ICES 2006; Appendices II and III). Differences in weight-at-age from the Cefas September beam trawl survey data and from the VPA were examined. The weight-at-age for ages 3+ were similar between the two data sets, but the weight-at-age was larger in the VPA data for ages 1 and 2. The VPA data covered a larger time period and only a very low proportion of ages 1 and 2 were mature according to the maturity ogive so the difference was considered insignificant.

The fecundity-weight relationships from chapter 2 were used to calculate the TEP for their respective areas and years. A common fecundity-weight relationship for each area was constructed by combining all fecundity estimates taken from that area (Table 4.1) these relationships were significantly different for each area (Table 4.1). These were used for their respective area when no fecundity-weight relationship was available for a particular year. A common fecundity-weight relationship for the entire Irish Sea encompassing all fecundity estimates from 1995-2004 was used to calculate the TEP for the whole Irish Sea (Table 4.2). This was compared with the TEP which was calculated from each area separately.

The Sex ratio was calculated for each age group using the Cefas September survey data and the same sex ratio was applied for the whole of the Irish Sea (Table 4.3). A female only maturity ogive, constructed from Cefas survey data, was used which was separated for the eastern and western Irish Sea but there was no evidence of a changing ogive over time (Table 4.4) (Scott unpublished data. Cefas, U.K.). The proportion of the population in each area was calculated from the results of two beam trawl surveys of population abundances, separated by area in 1995 (Table 4.5). This came from Cefas survey data and the proportion in each area was assumed to be constant over time.

TEP for each area was calculated from the following equation

$$TEP = \Sigma (ISPA_j * Prop_j * SR_j * M_j) * F_j$$

$$Log_e F = \alpha + \beta * Log_e W$$

ISPA = Total population abundance in the Irish Sea

Prop = Proportion of population in area

SR = Sex ratio

M = Proportion mature

F = Fecundity

$\alpha + \beta$ = constants from fecundity-weight relationship

W = Weight

j = Year class

The results were compared with estimates of TEP from Anonymous (2005) which were calculated from the sampling of stage 1 eggs from plankton surveys. Using VPA estimated numbers on recruitment at age 1 (R), the mortality between the egg stage and recruitment was calculated from the following equation:-

$$Mortality = Log_e (R/TEP)$$

RESULTS

TEP was positively correlated with spawning stock biomass (Linear regression; R^2=0.99; N=41; p<0.001) (Fig. 4.1). There was no significant

relationship between TEP and recruits at age 1 (Linear regression; P>0.05) (Fig. 4.2) or SSB and numbers of recruits at age 1 (Linear regression; P>0.05) (Fig. 4.3). The mortality between the egg stage and recruitment at age 1 varied between years and was positively related to TEP (Linear regression; R^2=0.38; N=40; p<0.001) (Fig. 4.4). Fig. 4.5 shows years of higher and lower than expected mortality between the egg stage and recruitment as predicted from the TEP and mortality relationship. The TEP for the whole of the Irish Sea and for Liverpool Bay, the Cumbrian coast and the Western Irish Sea are shown in Fig. 4.6. The TEP for Liverpool Bay and the Cumbrian coast calculated with the common area specific relationship and year specific relationships gave similar results, however the western Irish Sea showed large differences during years of low fecundity (1995 and 2000) (Table 4.6). The TEP for the whole of the Irish Sea calculated from the common relationship for the whole of the Irish Sea and calculated using area specific relationships gave similar results. The TEP estimates from the eastern Irish Sea from VPA were between 32% and 43% of the published estimates (calculated from stage 1 egg production) from 1995 to 2003 (Anonymous 2005; Table 4.7).

DISCUSSION

TEP was positively correlated with SSB; this is in contrast to the study by Marshall *et al.* (1998) who found that TEP was not proportional to SSB of Northeast Arctic cod. The difference may be due to the location of the main energy store in the different species. The main lipid energy store for cod is in the liver (Kjesbu *et al.* 1991) and total lipid energy (TLE) is proportional to TEP; HSI is a good measure of TLE (Marshall *et al.* 1999). However, the main store of lipids for plaice is the carcass (Dawson and Grimm 1980) therefore whole body weight would be a good predictor of fecundity, which was seen in Chapter 2. Comparisons of body weight in cod would not pick up differences in HSI and so would be less powerful as a proxy for fecundity when compared to plaice. Cod also grow much larger than plaice in terms of length and weight (Wimpenny 1953; Frimodt 1995) therefore, due to the effects of the power relationship between fish size and fecundity, there is much greater potential for large differences in relative fecundity between smaller and larger individuals and so result in a non-linear relationship

between SSB and TEP. There was no relationship between SSB and recruitment at age 1. This suggests that recruitment is affected mainly by processes acting on the egg and larval stages.

Mortality between the egg stage and recruitment at age 1 was positively correlated with TEP. This is probably due to density dependent processes' happening during the nursery ground phase as mortality during the pelagic phase is likely to be caused by density independent factors e.g. temperature, which is known to affect the survival rate of plaice eggs and larvae (van der Veer *et al.* 2000). The lack of density dependent factors in the pelagic phase is supported by previous studies of plaice in the Irish Sea where the numbers of settling larvae in Port Erin Bay were proportional to the TEP in the local area (Nash and Geffen 1999). Also, during years of high numbers of settling plaice, the population of juvenile plaice in July were similar to other years demonstrating that the numbers of settling plaice exceeded the carrying capacity resulting in density-dependent survival (Nash and Geffen 2000). Using the fecundity-weight relationships and VPA, information can be gleamed from the data revealing processes that can affect recruitment in fish populations as seen from the graphs showing TEP and mortality and the changes in higher and lower than expected recruitment through time as predicted by the TEP-mortality relationship. This remaining variance could then be linked to other factors e.g. temperature or predator abundance.

Estimation of TEP in an area using a common area specific fecundity-weight relationship gave similar results to using a year-area specific relationship for Liverpool Bay and the Cumbrian coast. However, estimates from the western Irish Sea overestimated the TEP by a large amount in 1995 and 2001, which were years of low population fecundity. This raises the question whether year specific fecundity measurements are worth the time and money for greater precision in understanding stock dynamics? For studies in the eastern Irish Sea, a common relationship using the present fecundity relationships would be adequate with periodic checks to make sure nothing has changed. For studies in the western Irish Sea where there are greater variations in fecundity, using a common fecundity relationship can obscure short term changes in TEP. However, these changes are not large enough to obscure any long term trend in TEP caused by large changes in SSB; as seen from the increase in TEP in recent years in both the present study and

Anonymous (2005). It will therefore depend on the needs of the study in question whether to reassess fecundity or use previous estimates.

Estimation of TEP from the VPA was approximately two thirds lower than the estimates made using plankton surveys. The value of the underestimation was similar in the different years suggesting that the historical trends in SSB are real but the results from the VPA may be offset due to an inappropriate scaling factor within the calculations used to estimate population numbers from the catch statistics. This underestimation given by these calculations may be due to a number of factors. The weight-at-age from the VPA are an underestimation as males have a lower weight at age and the weight-at-age estimates from VPA are not separated by sex. The weight-at-age was considered constant across the Irish Sea, whereas this is unlikely to be true as plaice have a slower growth rate in the western Irish Sea (Nash *et al.* 2003). The proportion of the whole population in each area was assumed to be constant over time which also may not be true. The main reason may be that the VPA data might not truly represent the real population as it is dependent on the completeness and accuracy of commercial catch at age data, the assumed relationship between VPA numbers and relative abundance indices used for tuning, and assumptions regarding natural mortality and selectivity characteristics of the fishery (Anonymous 2000). Discrepancies have been seen in SSB biomass estimates from VPA data and by the AEPM where the two assessments differed by a factor of 4.3 (Armstrong *et al.* 2000). The VPA data also failed to detect an increase in SSB between 1995 and 2000 which was detected by estimates of stage 1 egg production from plankton surveys. This is suspected to be due to unknown levels of discarding of young plaice (Anonymous 2005).

Table 4.1. The results for the analysis of covariance of Log_e fecundity of plaice versus area caught in the Irish Sea.

	SS	df	MS	F	P
Intercept	0.69	1	0.69	7.55	0.006
Log_e length	75.16	1	75.16	820.67	0,000
Area	0.57	2	0.29	3.13	0,046
Error	18.13	198	0.09		

Table 4.2. Common fecundity-weight relationships of plaice used for the three regions of the Irish Sea and the whole Irish Sea.

Area	Fecundity-weight relationship
Liverpool Bay	$Log_e F = 1.195 + 4.3547 * Log_e W$
Cumbrian coast	$Log_e F = 1.1541 + 4.3547 * Log_e W$
Western Irish Sea	$Log_e F = 1.3146 + 3.6314 * Log_e W$
Whole Irish Sea	$Log_e F = 1.2713 + 3.8935 * Log_e W$

Table 4.3. The sex ratio of plaice applied to the whole Irish Sea population.

Age	Proportion female
1	0.51
2	0.56
3	0.58
4	0.58
5	0.54
6	0.58
7	0.57
8	0.48
9+	0.80

Table 4.4. The maturity ogive of plaice for the eastern and western Irish Sea.

Age	Proportion mature	
	Eastern Irish Sea	**Western Irish Sea**
1	0.10	0.27
2	0.21	0.46
3	0.39	0.67
4	0.60	0.83
5	0.78	0.92
6	0.89	0.92
7	0.95	0.98
8	0.98	0.99
9+	0.99	1.00

Table 4.5. The proportion of the Irish Sea plaice population in each area. The missing proportion is for Cardigan Bay which is not considered during this study.

Area	**Proportion in area**
Liverpool Bay	23.7%
Cumbrian coast	21.7%
Western Irish Sea	41.1%

Table 4.6. TEP for plaice in each area and year using the fecundity-weight relationship a) that was constructed using data from that area and year b) that was constructed using all years combined for that area c) the difference between the two estimations (b/a).

a)

	1995	2000	2001	2003	2004
LB	1.04×10^{11}	1.38×10^{11}	1.65×10^{11}	2.37×10^{11}	
CC	7.44×10^{10}	1.04×10^{11}	1.83×10^{11}		
W	1.95×10^{11}	3.89×10^{11}	3.54×10^{11}	6.29×10^{11}	7.22×10^{11}

b)

	1995	2000	2001	2003	2004
LB	1.04×10^{11}	1.38×10^{11}	1.64×10^{11}	2.24×10^{11}	
CC	7.65×10^{10}	9.93×10^{10}	1.67×10^{11}		
W	2.73×10^{11}	3.55×10^{11}	5.85×10^{11}	6.32×10^{11}	7.22×10^{11}

c)

	1995	2000	2001	2003	2004
LB	1.00	1.00	1.00	0.94	
CC	1.03	0.95	0.91		
W	1.40	0.91	1.65	1.01	1.00

Table 4.7. TEP estimates for the eastern Irish Sea from the present study, calculated from Virtual Population Analysis (VPA) and results from Anonymous (2005) calculated from stage 1 egg production using plankton surveys, and the estimated Total Egg Production (TEP) calculated from VPA as a percentage of TEP calculated from plankton surveys.

Year	**Present study**	**Published**	**Percentage**
1995	2.11×10^{11}	6.11×10^{11}	35%
2000	2.75×10^{11}	8.55×10^{11}	32%
2001	3.41×10^{11}	7.97×10^{11}	43%
2003	5.04×10^{11}	1.38×10^{12}	37%

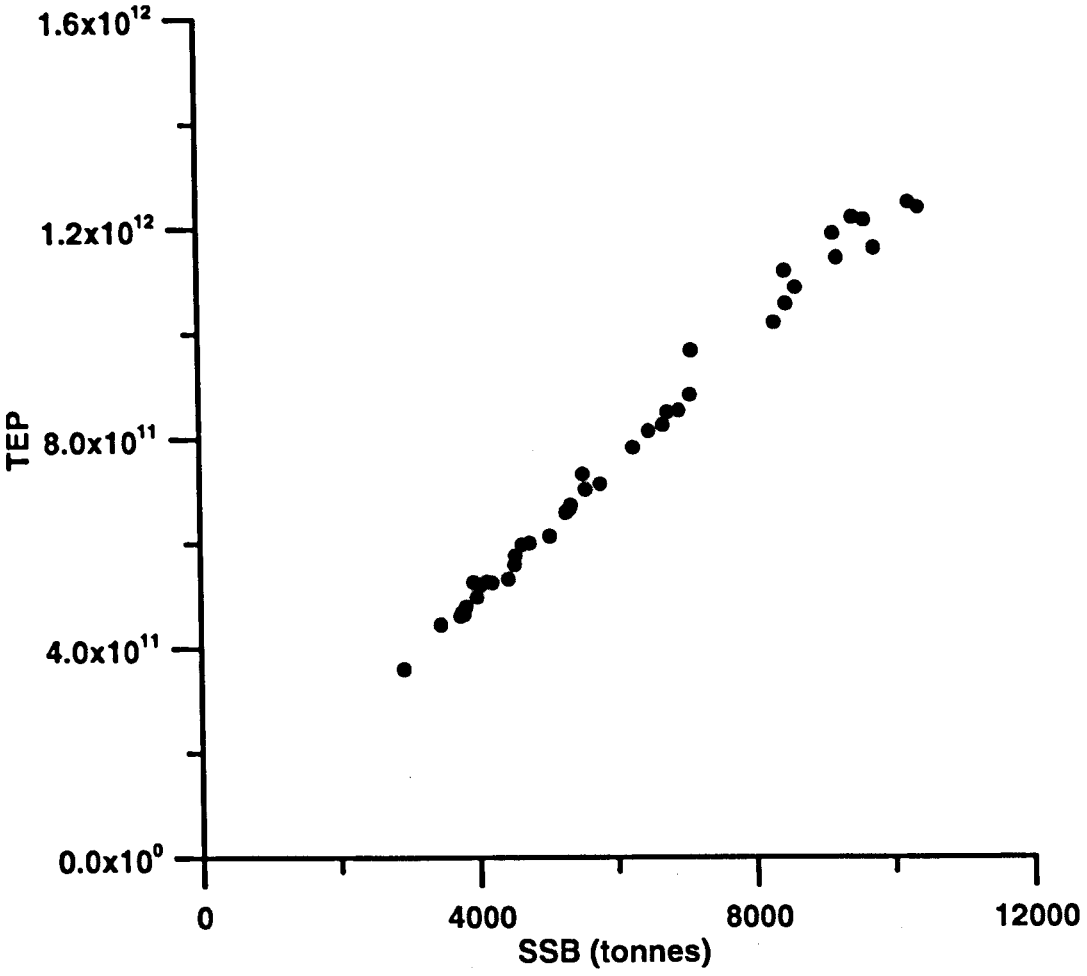


Fig. 4.1. The relationship between the Spawning Stock Biomass (SSB) and Total Egg Production (TEP) of plaice for the whole Irish Sea.

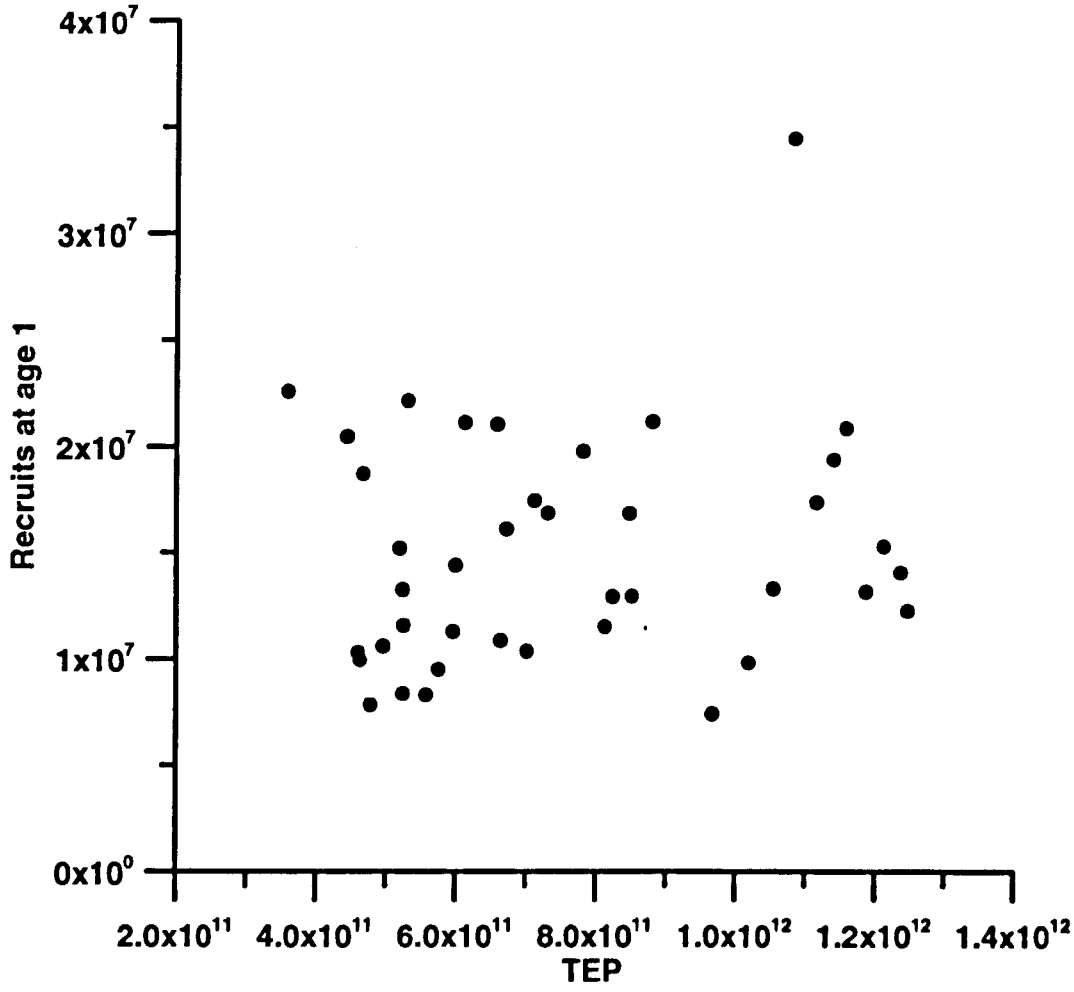


Fig. 4.2. Scatterplot of Total Egg Production (TEP) versus recruitment at age 1 for plaice in the Irish Sea.

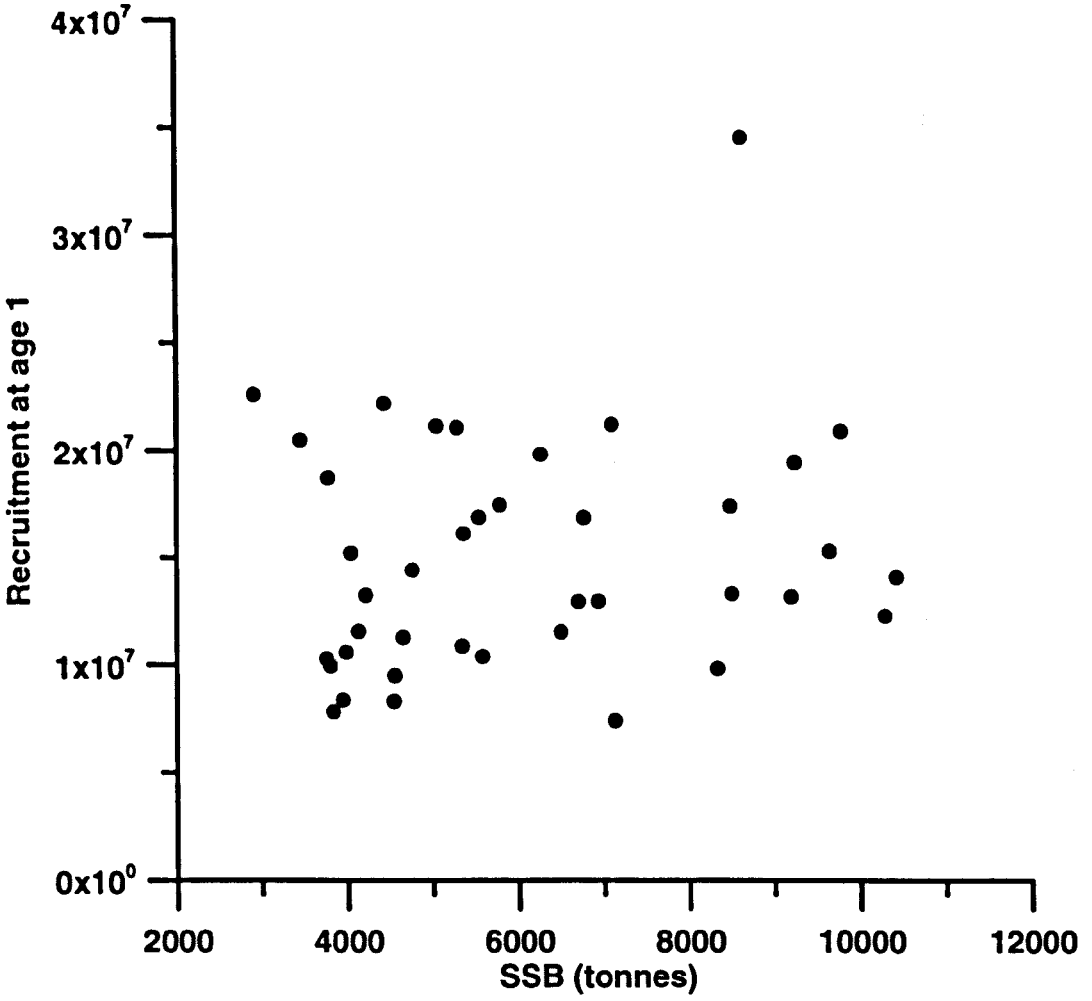


Fig. 4.3. Scatterplot of Spawning Stock Biomass (SSB) versus recruitment at age 1 for plaice in the Irish Sea.

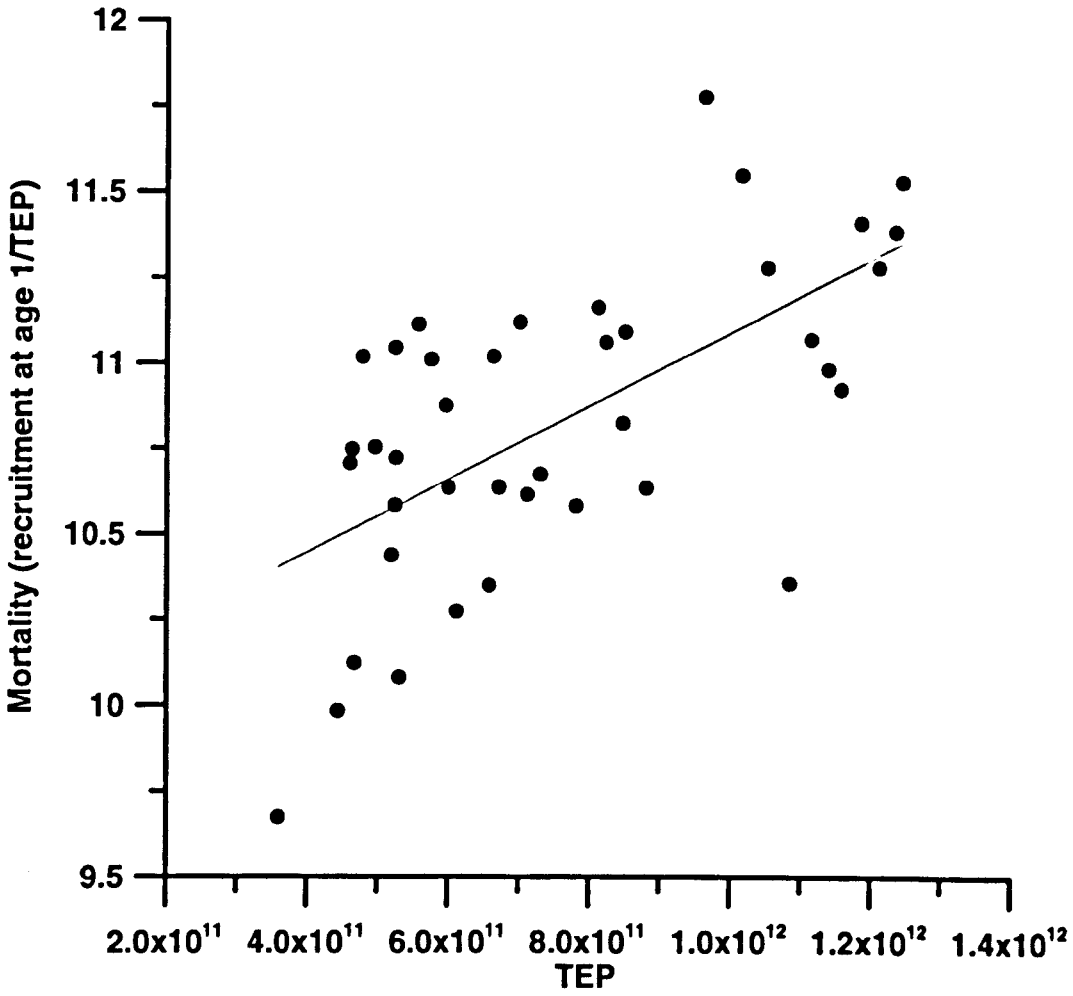


Fig. 4.4. Relationship between Total Egg Production (TEP) and mortality between egg stage and recruitment at age 1 for plaice in the Irish Sea. Line shows fitted linear regression line.

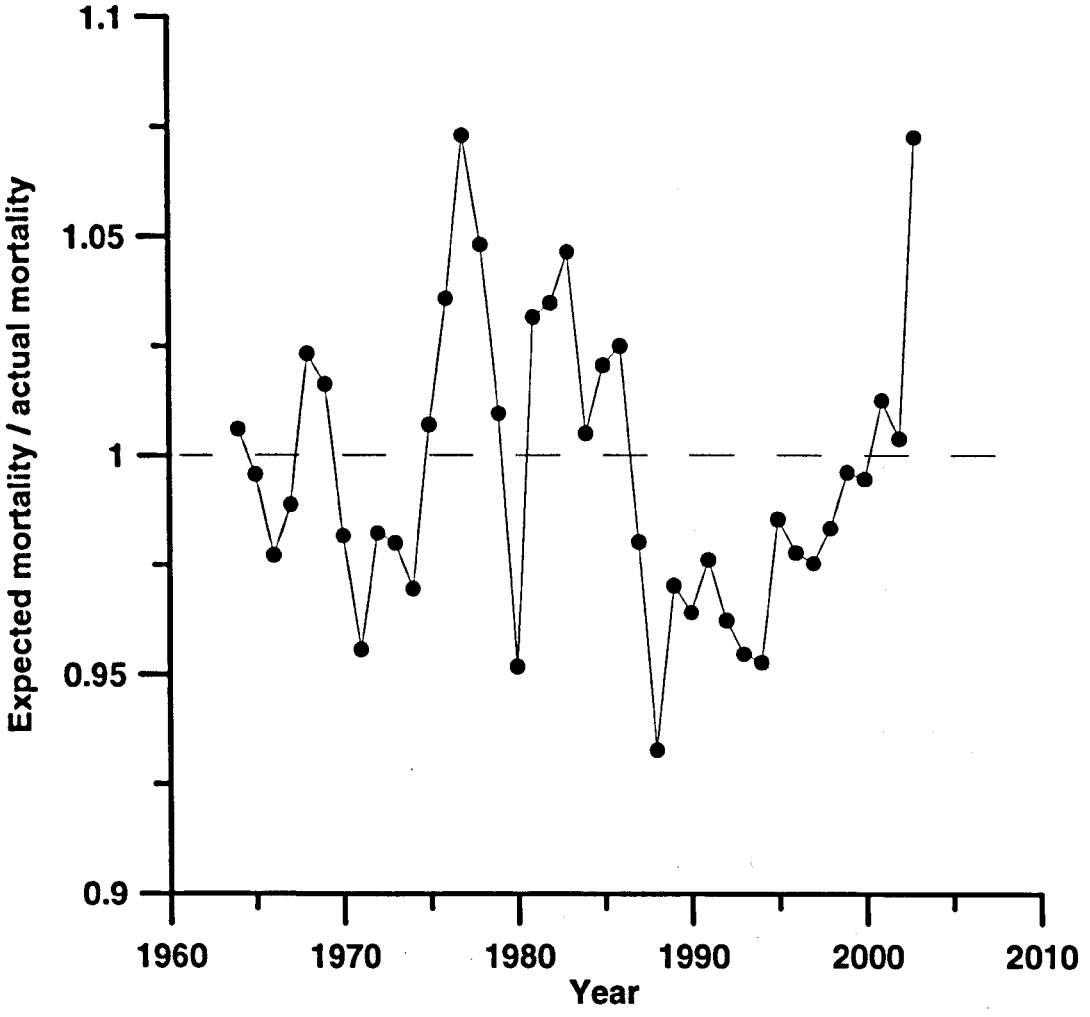


Fig. 4.5. Line plot showing expected mortality / actual mortality for Irish Sea plaice between spawning and recruitment at age 1. Predicted mortality from relationship between Total Egg Production (TEP) and recruitment at age 1. Values above 1 show years of lower than expected mortality.

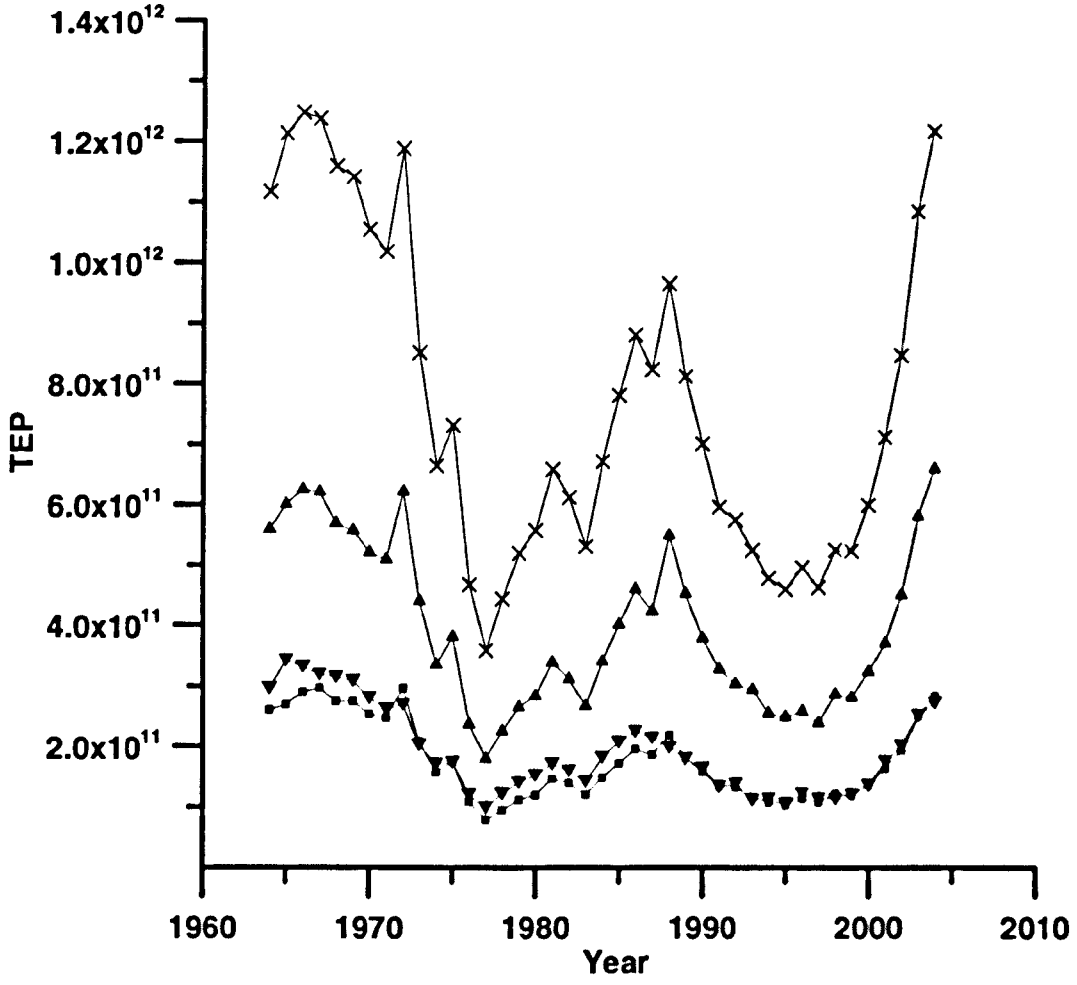


Fig. 4.6. The Total Egg Production (TEP) for the whole Irish Sea (X), the western Irish Sea (▲) the Cumbrian coast (▼) and Liverpool Bay (■).

CHAPTER 5
MATERNAL EFFECTS ON EGGS AND LARVAE

INTRODUCTION

Current fisheries assessment models include only limited biological details for the processes occurring between spawning and recruitment. Instead they traditionally assume that TEP (reproductive potential) and recruitment is proportional to the SSB (Marshall *et al.* 1998). However, SSB is not often an accurate measure of reproductive potential (Marshall *et al.* 1998; Marteinsdottir and Steinarsson 1998) because the reproductive potential of fish stocks varies with the age, size, spawning experience and condition of spawning fish (Marteinsdottir and Begg 2002).

Maternal effects are defined from the perspective of the offspring as the contributions of the mother, other than her genes, that influence offspring phenotype (Johnston and Leggett 2002). In many fish species larger females often produce larger eggs and egg size for each female decreases with each progressive batch through spawning (Kjesbu *et al.* 1996; Marteinsdottir and Begg 2002; Rideout *et al.* 2005b). This has also been demonstrated in plaice (Rijnsdorp 1991; Schmidt 2001)

The constituents of an egg, genetic and nutritive, determines its quality since fish eggs take up little, if any, nutrients once ovulated (Brooks *et al.* 1997). Thus the nutritive investment in an egg must provide for the larvae through to first feeding. Egg size has been shown to be an important factor in the early life history of fish as larger eggs provide more energy for development (Hempel 1979) and egg size is positively correlated with many larval traits including larval length at hatching (Blaxter and Hempel 1963; Buckley *et al.* 1991; Rideout *et al.* 2005b), incubation period (Dannevig 1894; Chambers *et al.* 1989; Huang *et al.* 1999), yolk sac volume (Blaxter and Hempel 1963; Beacham *et al.* 1985; Gisbert *et al.* 2000), first feeding age (Gisbert *et al.* 2000) and survival potential and resistance to starvation (Knutsen and Tilseth 1985; Marteinsdottir and Steinarsson 1998; Rideout *et al.* 2005b).

Larvae with a greater size-at-age have been shown to have longer swimming endurance (Ojanguren *et al.* 1996) and a greater burst swimming speed, both of which can help in the capture of prey items and escape from predators (Miller *et al.*

1988). In plaice, larger larvae have a greater escape swimming speed and are less vulnerable to planktonic invertebrate predators (Bailey and Batty 1984).

All of the previous studies on maternal effects are based on batch averages of larvae except for one (Chambers *et al.* 1989). This study examined the egg size and female effects on early life history traits of capelin. It was found that neither initial yolk volume, length at hatching, nor yolk volume at hatching was related to post-hatching lifespan when individuals were evaluated. This contrasted with both the correlations within the same study (when based on family averages) and with previous generalisations (from group averages) that large initial egg size leads to hatchlings that survive longer in the absence of food (Blaxter 1988).

Larger adult plaice begin spawning earlier in the season (Simpson 1959; Rijnsdorp 1989; Horwood 1990), during which time the availability of food items for the hatched larvae is much lower in comparison to later in the season (Scrope Howe and Jones 1985). Since larger fish produce larger eggs, earlier hatched larvae may be better equipped to withstand starvation than later hatched individuals. Fishing pressure on the adult population can lead to the selective removal of larger fish thus changing the proportion of old and young spawners. To understand the implications of shifts in the structure of the spawning population it is critical to evaluate maternal influences on the characteristics of the eggs and larvae.

The aims of this chapter are to examine how maternal size and the seasonal progression of spawning affects egg size, and how the egg size influences batch averages of various egg and larval characteristics. Individuals within a batch were also monitored to examine egg size influence at the individual level as the results from Chambers *et al.* (1989) showed that results from individual larvae can be different from batch averages.

MATERIALS AND METHODS

Fish capture and housing

Twenty mature adult plaice were collected by trawl in February 2004, in the Irish Sea, from inshore areas to the east of the Isle of Man and transferred to the Port Erin Marine Laboratory (PEML). The fish were kept in 2 metre diameter

circular tanks with an inflow of filtered seawater at ambient temperature (6-10°C). Each fish was measured (Total Length) and weighed and implanted with a passive integrated transponder tag (pit tag). Milt was obtained from males that were part of the broodstock of the Larval Rearing Centre in PEML.

In February 2005, 15 mature adult plaice were obtained from local Bergen (Norway) fisherman and transported live to the Department of Biology, University of Bergen where they were kept in $1m^2$ circular tanks at 7°C. The fish were measured, weighed and tagged. All fish were visually checked daily for signs of ovulation (high degree of swelling in abdominal area and reddening around the genital opening (Panagiotaki 1992)). Milt was obtained from males that were caught at the same time as the females and housed in a similar tank.

Fish that showed signs of ovulation were 'stripped' of their eggs by applying gentle pressure along the ovary. Egg batches that were chosen for incubation were fertilised with pooled milt from three males which was mixed with seawater then mixed in with the eggs. The eggs were then allowed to stand in a controlled temperature (CT) room at 7°C. After 30 minutes, using a fine mesh hand net, the eggs were dipped in seawater to rinse off excess sperm and transferred to a larval rearing tank.

Egg batch experiment

In 2004 the rearing tanks were 30 l black cylindrical tanks with an inflow of UV sterilised filtered seawater with a mesh covered outflow at the top of the tank. The water was at ambient temperature (6-10°C) but had a very low inflow rate. The tank also had a central air stone to circulate and aerate the water.

In 2005 the larval rearing tanks consisted of 15 l black cylindrical tanks which had a gentle inflow of seawater at 7°C. The tanks had a mesh bottom where water flowed out to a larger holding tank. Each larval tank had a central air stone to circulate the water.

In all batches produced, the egg diameter of a sample of unfertilised eggs (10 in 2004, 30 in 2005) was measured to the 0.01 mm using a dissecting microscope and graticule. Egg dry weight (EDW) was measured for a sample of fifty unfertilised eggs which were rinsed with sea water, dried at 60°C, and weighed daily to the nearest 0.01 microgram until a constant weight was achieved.

Throughout the rest of the text, EDW refers to the average egg dry weight for a batch of eggs and ED refers to the average egg diameter of a batch of eggs.

Unfertilised and dead fertilised eggs were removed every two days and counted. On day 12 after the eggs were spawned, the numbers of surviving eggs were estimated volumetrically by mixing the water and taking 3 samples of known volume and counting the eggs; an average number per ml was calculated which was used to estimate the total number of eggs in the tank. This was repeated daily until the eggs hatched. When the eggs hatched the same method was used to estimate the number of larvae.

Peak hatching was defined as the period in which the largest proportion of the eggs hatched. The incubation period was defined as time between fertilisation and the morning following peak hatching (most eggs hatched during the night). The hatching period was defined as the number of days between the first and last eggs hatching. The day following peak hatching was termed 1 day after hatching on which the standard length (SL) and the length and height of the yolk sac of 30 larvae were measured, to the nearest 0.01 mm, using a dissecting microscope and graticule (larval length and remaining yolk sac for 30 larvae in each batch was measured at 6 days after hatching for the Norwegian larvae). On day 4 after hatching, food was introduced in the form of newly hatched *Artemia*, which were added daily at a concentration of 500 l^{-1}. The larvae were allowed to feed for at least 1 hour before estimating the number of larvae with food in their gut. On day 14 after hatching, the numbers of surviving larvae were estimated, the tank was drained and the SL of 30 larvae measured to the nearest 0.01 mm using a dissecting microscope and graticule. In 2005 all larvae that were measured were photographed under the microscope and measured using 'Image-J' image analysis software (Rasband 1997-2005). The yolk sac was assumed to be a spheroid structure and the volume was calculated using the equation

$$YSV = 4/3\pi * (L/2) * (H/2)^2$$

YSV = Yolk sac volume

L = Length of yolk sac

H = Height of yolk sac

The specific growth rate (SGR) of the larvae was calculated using the following equation

SGR = ((Log_e final length - Log_e starting length) / time in days) * 100 (Busacker *et al.* 1990).

Experiment with individuals

Forty eggs were taken from a batch of eggs, spawned by a 43cm female from the Norwegian experiments, on the day before peak hatching. The eggs were photographed and placed in individual static water tanks measuring 79x55x47 mm in a temperature controlled room at 7°C. The morning after hatching, each larva was photographed under the dissecting microscope. Half the volume of water was changed every other day. Newly hatched *Artemia* were introduced daily after day 4 after hatching and the age at which each larvae started feeding was noted. Each larva was photographed at 7, 14 and 21 days after hatching. Individual egg diameter, larval SL, yolk sac length and height (from which the YSV was calculated) and larval area was measured, to the nearest 0.01 mm, from the images using Image-J. Larval area rather than standard length was used to examine the relationship between individual egg diameter and larval size to give better resolution due to the restricted range of individual egg diameters.

Statistical analysis

All statistical analyses were done using Statistica 6.1 (StatSoft Inc. 2002). Single factor regression was used to analyse the relationship between EDW and ED for the Irish Sea fish and the Norwegian coastal fish and the slopes of the regression for the two areas were compared using ANCOVA. The mean of the EDW of all egg batches produced by each female was analysed by Log_e transformed maternal weight in single factor regression. All larval traits were tested for normality with YSV and SGR being Log_e transformed. The relationships among the different larval traits were analysed by principal components analysis. Forward stepwise regression was carried out on incubation time with batch number and EDW as

predictor variables. Forward stepwise regression was carried out with larval SL and Log_e transformed YSV as dependent variables and EDW, incubation time and batch number as predictor variables. Due to the different ages in relation to days after fertilisation, larval SL at 14 days after hatching was analysed against EDW and days after fertilisation using multiple regression. The yolk sac volume at 7 days after hatching was analysed against EDW by single factor regression. The change in the standard deviation of larval sizes was analysed with age at first feeding using single factor regression.

For the individuals' experiment, YSV was normalised by a Log_e transform. Single factor regressions were carried out on individual egg diameter and the measured larval traits at two and three weeks after hatching. SGR was analysed against YSV and larval SL at hatching using multiple regression.

RESULTS

Spawning of fish

The Irish Sea plaice began spawning on the 23rd February and continued until the 8th April 2004. A total of 24 batches of eggs were incubated from 11 females ranging in size from 27- 39 cm, average 29 cm. The fish from the Norwegian coastal waters spawned from 24th February until the 2nd April 2005. A total of 15 batches were incubated (but only 8 were raised until two weeks post hatch due to problems with experimental setup) from 8 females ranging in size from 42 – 54 cm, average 46 cm. The number of batches produced and the batch number of the batches that were incubated for each female are shown in Fig. 5.1. Eggs were obtained from an additional 5 females from the Irish Sea that were only used in the analysis of EDW in relation to maternal weight.

Egg characteristics

EDW was positively correlated with ED, however the slope of the relationship was significantly different for the two populations with Irish Sea fish

having a lower EDW for an equivalent ED than the Norwegian coastal fish (ANCOVA, F=70.8, 79.7; p<0.001) (Fig. 5.2). The overall average EDW of all the batches produced by a single female was positively correlated with Log_e maternal weight (linear regression, R^2=0.44; N=25; P<0.001) (Fig. 5.3) which was a better predictor of average EDW compared to maternal length. There was no effect of maternal origin, independent of maternal size, on EDW (Table 5.1). There was an average decrease of 12% in EDW for each individual female as the spawning season progressed, with a decrease from the first to the last batch varying from 3 to 30% between females. The magnitude of the decline did not vary significantly (p>0.05) with any of the measured maternal characteristics.

	fish ID	ML (cm)	MWt (g)	Batch number													
				1	2	3	4	5	6	7	8	9	10	11	12	13	total batches
Irish Sea	5114	27	197														6
	E090	27	190														5
	9172	32	376														7
	F18D	32	365														3
	FD2D	33	360														6
	9A39	39	721														7
	8AAC	32	387														5
	8ACE	34	405														5
	9A7C	35	479														7
	F23A	37	630														5
	7AC7	35	499														8
Norwegian coast	0054	54	2205														12
	93D2	41	900														7
	7570	44	957														10
	F911	48	1598														12
	2E0F	42	884														8
	99D9	43	920														8
	7F02	49	1603														13
	FC21	45	1043														9

Fig. 5.1. Length and weight of each individual plaice in the experiment and the total batches produced over the spawning season (indicated by grey boxes). Incubated batches are shown by black boxes.

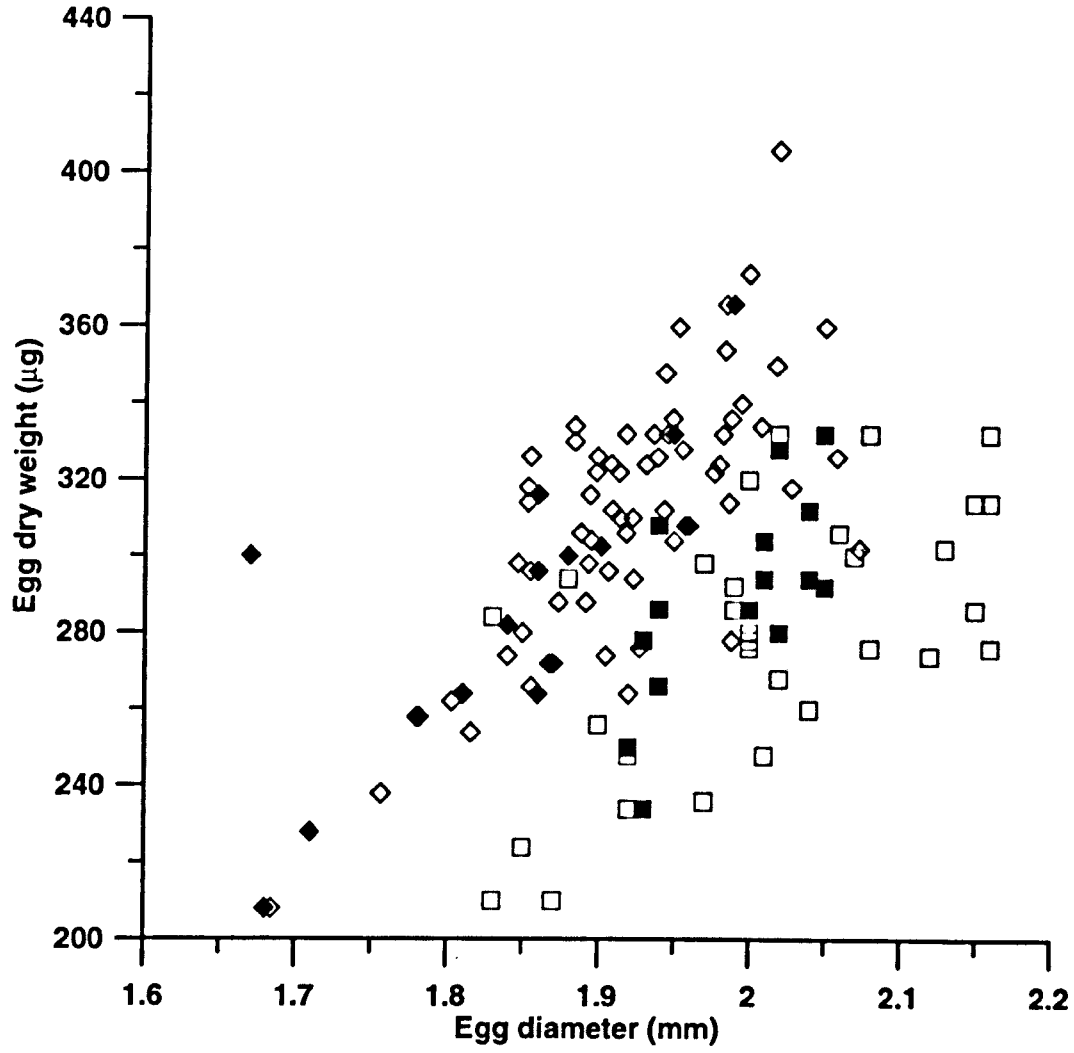


Fig. 5.2. Scatter plot of the average egg diameter (ED) and average egg dry weight (EDW) for each batch of eggs produced by the Irish Sea (+) and Norwegian coastal plaice (×) (incubated egg batches are shown in bold).

Egg batch experiment

The ranges and means for EDW, ED, larval SL at hatching, YSV and SGR (from hatch to two weeks post hatch) of the egg batches that were incubated are shown in Table 5.2. The incubation period varied from 13 to 17 days with an average of 16 days. The SGR was higher in the first week (measured only in Norwegian fish) than in the second.

Principal component analysis (PCA) yielded two axis explaining 38% and 24% (eigenvalues 3.49 and 2.25) of variability in the data respectively. Axis 1 contrasted mainly factors relating to physical characteristics of the larvae (egg size, larval length, and incubation period) while axis 2 contrasted mainly characteristics that were related to energy reserves (yolk sac volume, SL at hatching, SGR) (Table 5.3).

The stepwise regressions showed that batch rank was the best predictor of incubation time (R^2=0.40; F=27.2; P<0.001) and that larval SL at hatching and YSV were best predicted by EDW and incubation time (R^2=0.46; F=17.8; P<0.001). Larval SL at hatching increased with EDW and incubation time while YSV increased with EDW but decreased with incubation time.

The yolk sac reserve remaining at 1 week post hatch was positively correlated with EDW (Fig. 5.4) (this was from the average EDW of the batch and the standard deviation of the of the yolk sac volume for the 30 larvae measured in each batch). The multiple regression of larval SL at 14 days after hatching against EDW and time from fertilisation showed that SL at 14 days after hatching was significantly correlated with time from fertilisation but not significantly correlated with EDW (Table 5.4). The change in standard deviation of larval sizes between

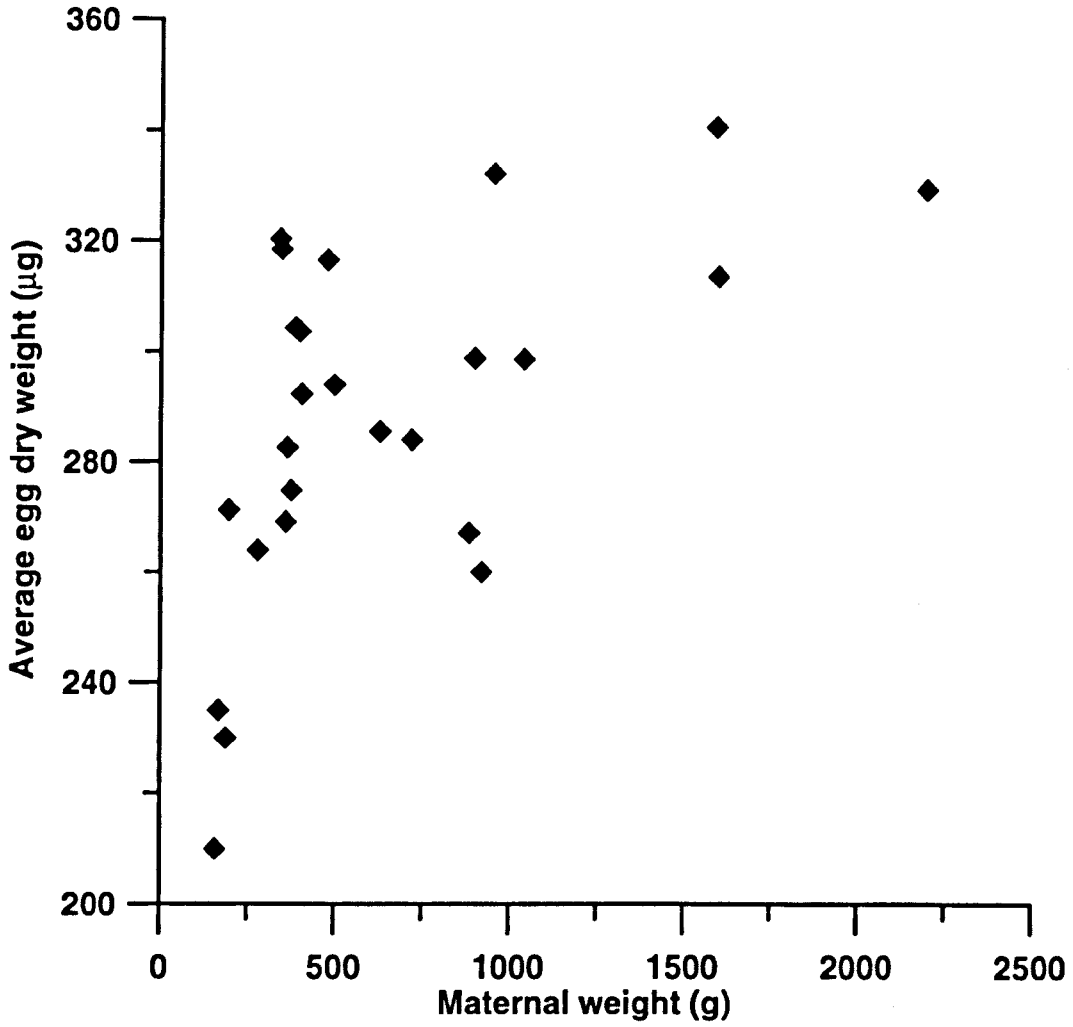


Fig. 5.3. Scatter plot of maternal weight and overall average egg dry weight (EDW) of plaice, based on all egg batches produced by each fish.

Table 5.1. Results of ANCOVA (separate slopes model) of plaice (*Pleuronectes platessa*) maternal weight and origin (Norwegian coast and Irish Sea) on average egg dry weight of all batches. SS = sum of squares df = degress of freedom MS = mean squares.

	SS	df	MS	F	p-level
Intercept	0.016	1	0.016	0.297	0.591
Weight * Origin	1.056	2	0.528	9.603	0.001
Origin	0.007	1	0.007	0.133	0.718
Error	1.154	21	0.055		

Table 5.2. The minimum, maximum and mean values of the characteristics of the egg batches that were incubated from the Irish Sea plaice and Norwegian coastal plaice (SL=Standard length).

Year	**Irish Sea**			**Norwegian coastal**		
	Min	Max	Mean	Min	Max	Mean
Egg dry weight (μg)	234	363	295	208	366	282
Egg diameter (mm)	1.92	2.09	2.00	1.67	1.99	1.84
Larval SL at hatching (mm)	6.15	7.59	6.97	5.99	6.93	6.62
Yolk sac volume (mm^3)	0.35	2.21	0.89	0.15	2.60	1.42
Specific growth rate	0.11	1.54	0.55	0.32	1.08	0.69

Table 5.3. Results from the Principal Component Analysis showing the loadings of the two axes. Eigenvalues and percentage of variance are given. Significant correlations are shown in bold.

Characteristic	Factor 1	Factor 2
Egg dry weight	**0.628**	0.194
Egg diameter	**0.893**	0.095
Larval SL at hatching	**0.703**	**-0.535**
Log_e transformed Yolk sac volume	-0.057	**0.736**
Larval SL at end	**0.720**	0.387
Time to Peak hatching	**0.783**	-0.395
Log_e transformed growth rate	0.046	**0.860**
Duration of Hatching period	-0.003	**-0.626**
Batch rank	**-0.751**	0.199
Eigenvalue	3.494	2.245
% variance	38	24

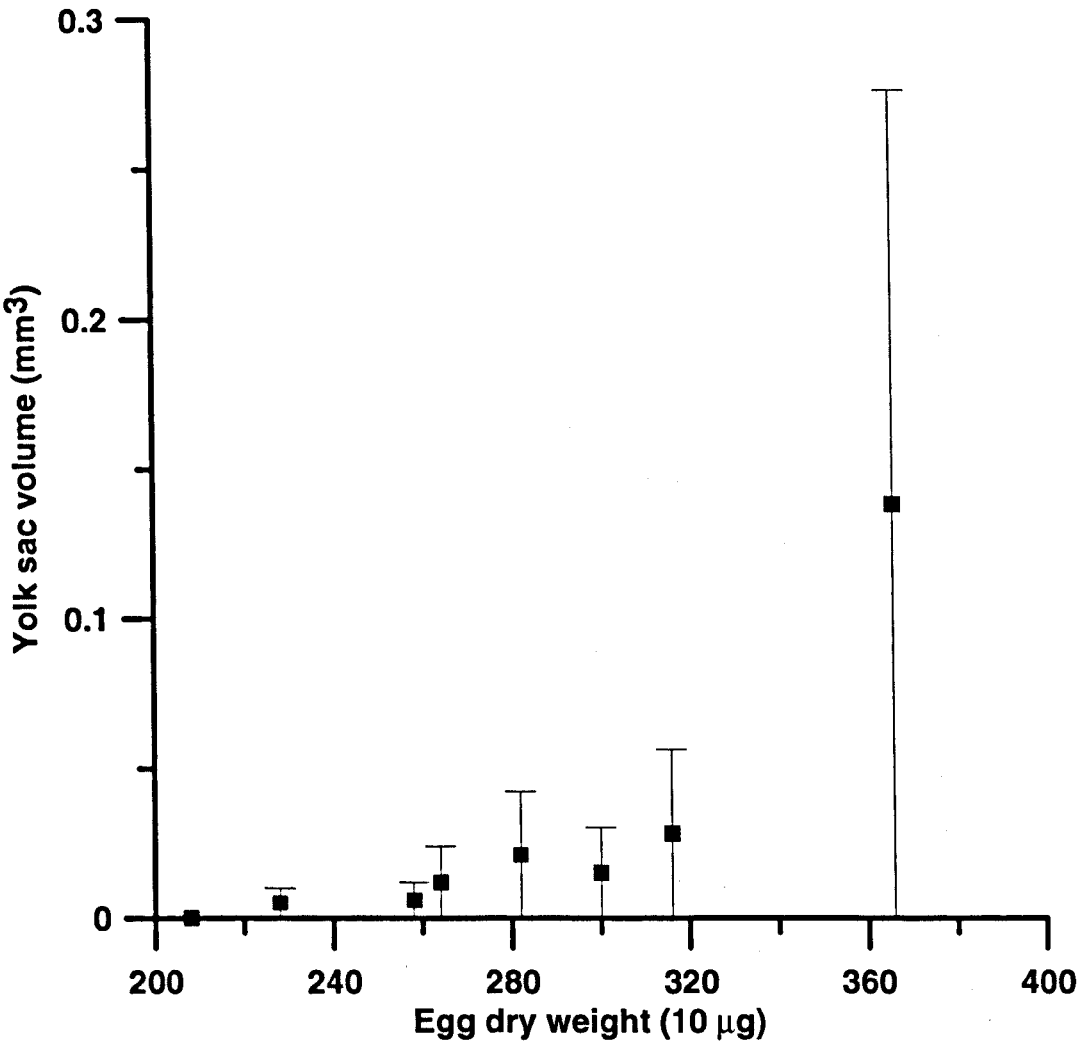


Fig. 5.4. Scatter plot of egg dry weight (EDW) and yolk sac volume remaining after one week post hatch for Norwegian coastal plaice. Error bars show standard deviation.

Table 5.4. Regression summary of larval SL at 14 days after hatching against EDW and days after fertilisation for Irish Sea plaice. Adjusted R^2=0.228, F(2,29)=5.5749 p<0.00894 Std.Error of estimate: 0.226

	Beta	**Std.Err. of Beta**	**B**	**Std.Err. of B**	**t(29)**	**p-level**
Intercept			3.768	1.251	3.010	0.005
Egg dry weight	0.303	0.161	0.237	0.126	1.883	0.070
Days after fertilization	0.375	0.161	0.102	0.044	2.327	0.027

hatching and 14 days after hatching was negatively correlated with age at first feeding, with the standard deviation generally increasing with batches that fed before 9 days after hatching and the standard deviation generally decreasing with batches that fed after 9 days after hatching (Fig. 5.5).

Experiment with individuals

The eggs used in the experiment with individuals ranged from 1.65 – 1.80 mm in diameter (mean ± 1 sd=1.72 ± 0.03). In order to back calculate the diameter of the eggs before fertilisation the measured values were decreased by 1% in accordance with van der Wateren (1990) to account for the increase in egg size during incubation. The size range and mean of the samples of the egg diameters taken from the entire batch before fertilisation and those taken for the individuals' experiment were significantly different (t-test, t=-2.14; n=70; p=0.04) (Fig. 5.6). All eggs taken for the individual experiment hatched during the night after being transferred to individual beakers. Three larvae died after hatching. The larval SL at hatching ranged from 6.32 to 6.88 mm, larval area at hatching ranged from 1.76 to 2.18 mm^2 and YSV ranged from 0.09 to 0.40 mm^3. The SGR of larvae in the first week ranged from 0.06 to 0.73 with an average of 0.18.

Individual ED was positively correlated with individual YSV (Linear regression; R^2=0.08; n=37; p=0.04) (Fig. 5.7a) and individual larval area at hatching (Linear regression; R^2=0.05; n=37; p=0.05) (Fig. 5.7b). There was no significant correlation between egg diameter and larval SL at hatching (p>0.05). SGR in the first week after hatching was a function of both YSV and larval SL at hatching as it was positively correlated with YSV and negatively correlated with larval SL at hatching (Table 5.5, Fig. 5.8). There was no correlation between larval SL at hatching and SGR or YSV and SGR for the period of hatch to 14 days after hatching or from 6 days after hatching to 14 days after hatching. The yolk reserves were completely used up in all larvae by the end of the first week. The relationship between egg diameter and larval area was gone by 7 days after hatching

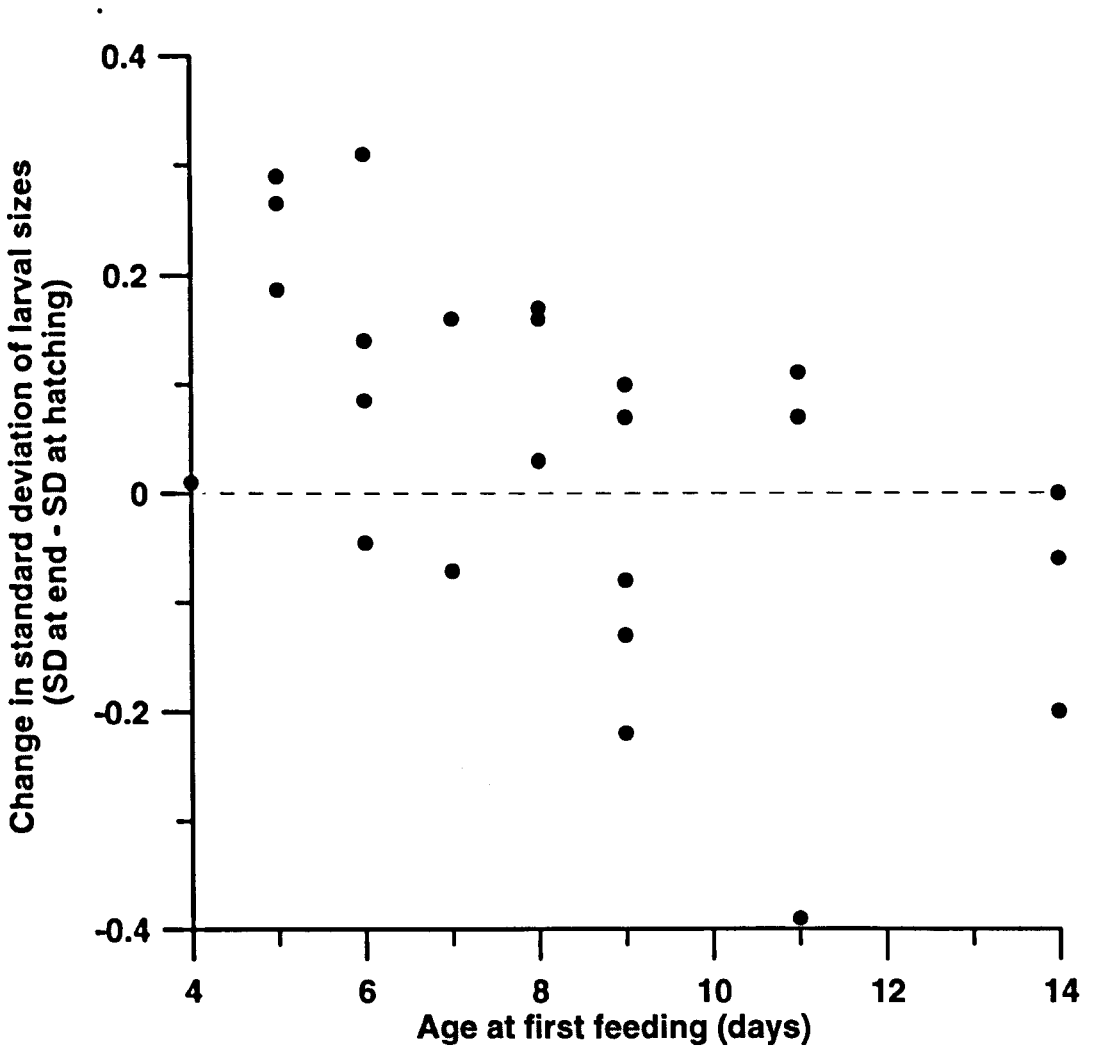


Fig. 5.5. Correlation between age at first feeding of larval plaice and the change in standard deviation of larval sizes within a batch from hatching to two weeks post hatch (end).

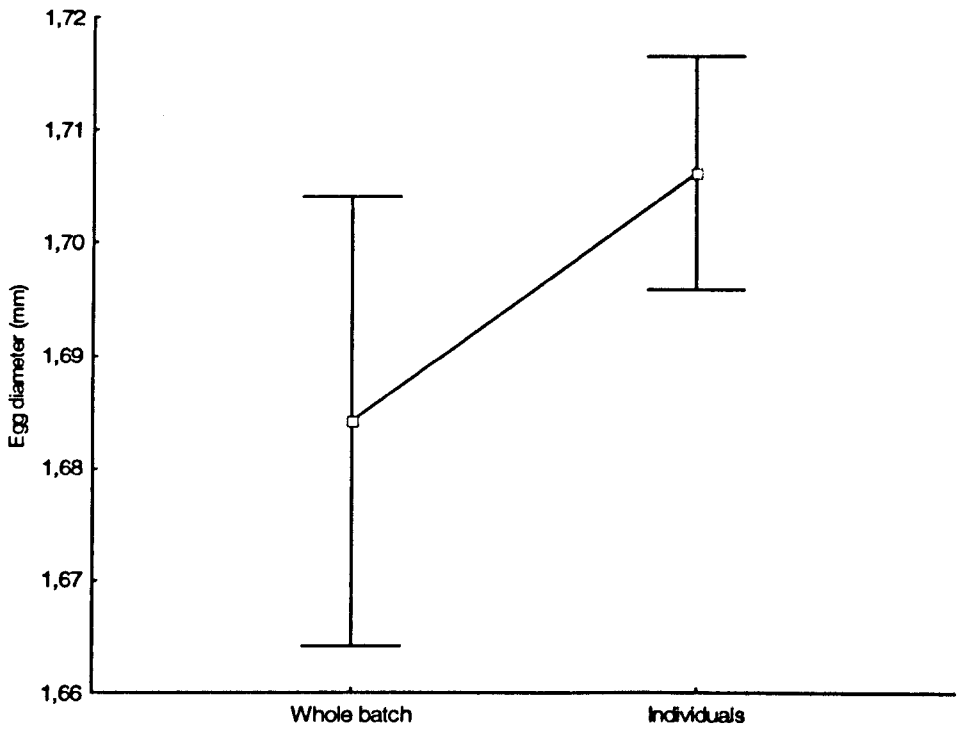
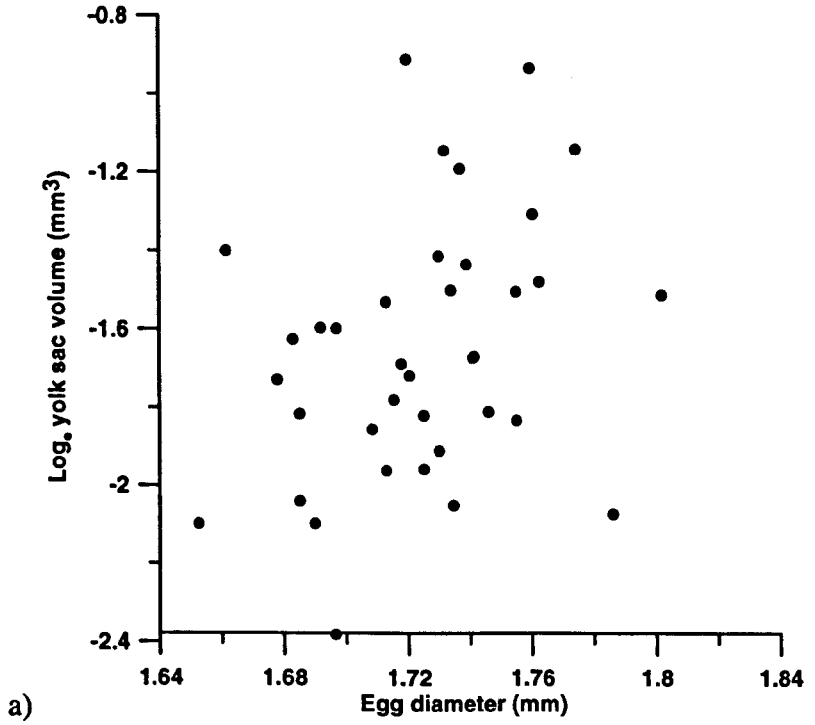
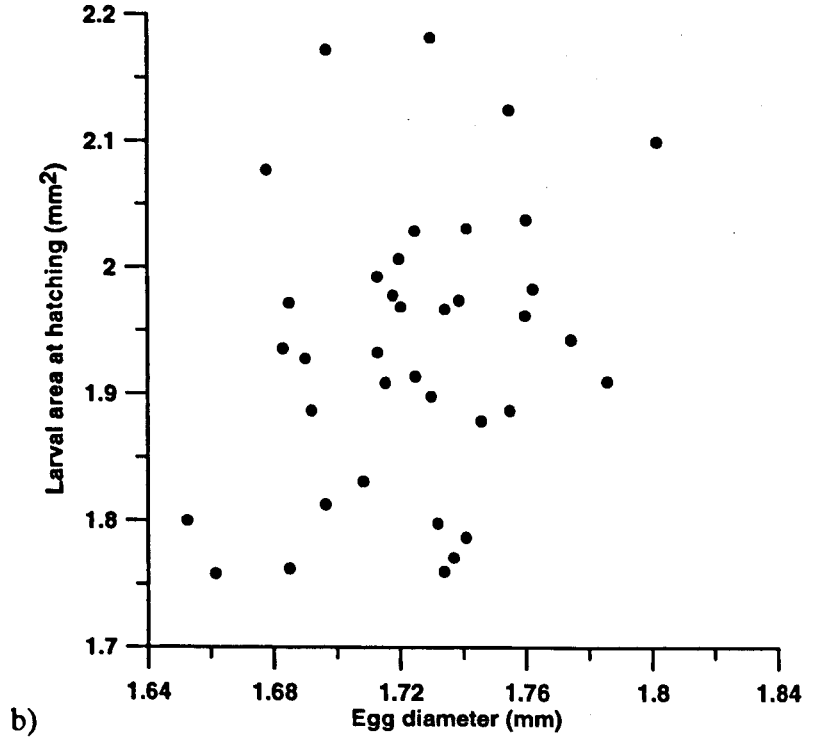


Fig. 5.6. Egg diameter (ED) of entire batch of eggs before fertilisation and eggs that were used in the individual experiment (egg sizes for individuals were adjusted for increase in egg size due to development). Values shown are means ± 1.96 S.E.



a)



b)

Fig. 5.7. Scatter plot of egg diameter and a) Log$_e$ transformed yolk sac volume and b) larval area at hatching for individually followed plaice larvae.

Table 5.5. Regression summary for SGR against Log_e yolk sac volume and larval SL at hatching of plaice larvae.

N=30	R= 0.7312
R^2=0.5354	Adjusted R^2=0.5010
F(2,27)=15.557	p<.0001
Std. Error of estimate: 0.1301	

	Beta	Std.Err. of Beta	B	Std.Err. of B	t(27)	p-level
Intercept			5.116	1.049	4.875	0.000
LLH	-0.542	0.131	-0.655	0.158	-4.130	0.000
ln YSV	0.477	0.131	0.251	0.069	3.633	
0.001						

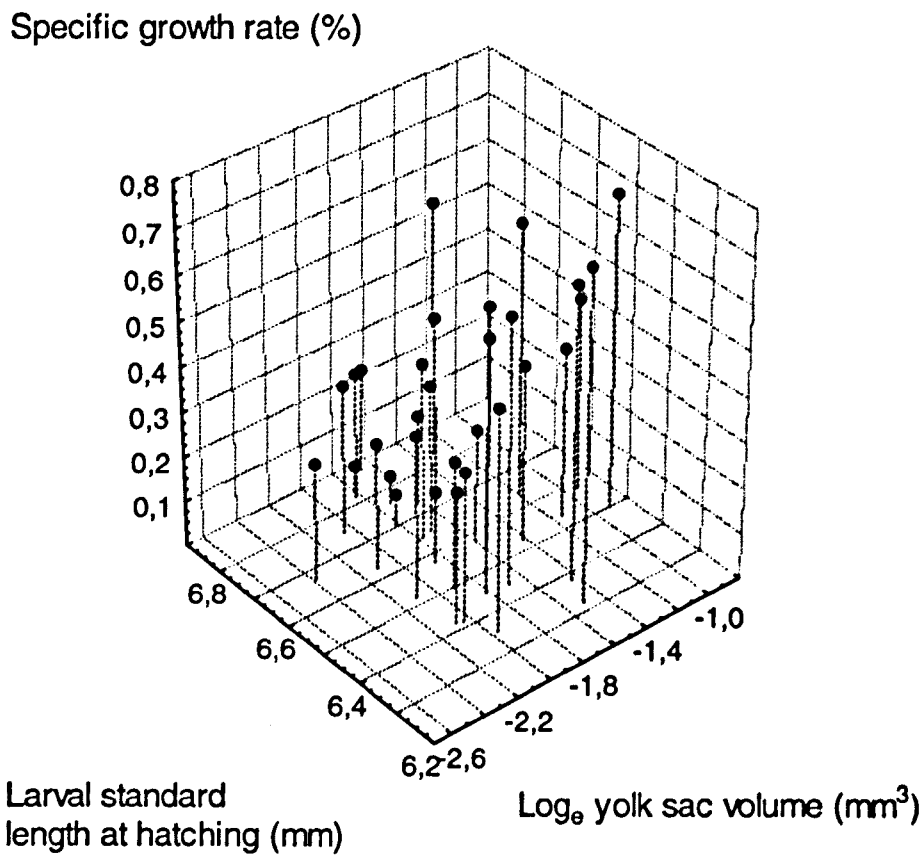


Fig. 5.8. Three dimension scatter plot of relationship between specific growth rate, Log_e transformed yolk sac volume and larval standard length at hatching for individually followed plaice larvae.

DISCUSSION

Although this experiment was conducted in two different locations, with two different plaice populations, location and origin may have had some influence, but did not obscure the main observed results. Paternal effects, which are known to be an issue in early life history studies (Trippel et al. 2005), were not controlled because it was not possible to use the same males in the Norwegian and Irish Sea study neither was it possible to set up replicates to test for such effects. The selection of males for fertilisation was random but paternal effects on the eggs and larvae are a possible source of error in the results. Maternal origin is also another potential source of error, and the effect was examined. The relationship between EDW and ED was the main difference due to maternal origin; because of this EDW was used as the indicator of egg size in the analyses as it is a measure of the total constituents of on an egg whereas ED can be affected by the degree of hydration of the egg. The water temperature in the adult tanks also differed between the Norwegian and Irish Sea experiment. It is known that rearing temperature can affect egg size in some species (Hotta et al. 2001; Funamoto and Aoki 2002). However, temperature did not appear to affect the egg sizes in the present experiment as there was no effect of maternal origin on egg size independent of maternal weight as maternal origin included both place of capture and temperature in the tank. The experiment with individuals was done with eggs from one female; this was a preliminary investigation and the results should be viewed with caution due to the lack of replicates.

There was a consistent link between maternal size and egg size in these experiments, as noted previously by Fox *et al.* (2003). No such link was found by Rijnsdorp (1991) but this was probably because only one batch of eggs was sampled for each female. The best fit relationship was logarithmic. Three fish had a much lower EDW than the others (EDW below 2.4). These are most likely to be recruit spawners (inferred from their size (Nash et al. 2000)); it has been found previously in other species that there is a large increase in EDW between recruit and repeat spawners (Hislop 1988; Kjesbu et al. 1996) which may be due to recruit spawners having a lower amount of energy available for reproduction. The relationship between maternal size and egg size has been demonstrated in many other species including brown trout (*Salmo trutta*) (Ojanguren *et al.* 1996), cod

(Marteinsdottir and Steinarsson 1998; Trippel 1998; Marteinsdottir and Begg 2002), haddock (Trippel and Neil 2004) and winter flounder (Buckley *et al.* 1991).

As shown in previous studies (Rijnsdorp 1991; Schmidt 2001; Fox *et al.* 2003), eggs from an individual female decreased in size as spawning progressed, whether measured as ED or EDW. This has also been documented in two related species; yellowtail flounder (*Pleuronectes ferrugineus)* (Manning and Crim 1998) and winter flounder (Buckley *et al.* 1991). The average decrease in EDW of 12% in the present study is similar to that found by Rijnsdorp (1991) in wild North Sea plaice and by Schmidt (2001) in captive Irish Sea plaice. Several reasons have been suggested for this decrease in egg size including temperature (Marsh 1984; Houghton *et al.* 1985; Imai and Tanaka 1987), depletion of body reserves (Kjesbu *et al.* 1991; Hsiao *et al.* 1994; Chambers and Waiwood 1996) or endogenously influenced hormonal profiles (Kjesbu *et al.* 1996). The decline in egg size in 2005 occurred at a constant temperature as also noted by Chambers and Waiwood (1996) who found that the decline in egg size in cod occurred despite a constant temperature being used during the study. They concluded from their study that the decline was related to deteriorating female condition. Female condition was not assessed during spawning in the present study so it was not possible to link the decline in egg size to a decline in fish condition. The reason for the difference in the relationship between EDW and ED of fish from the Irish Sea and Norwegian coast is unclear thought the sample size was small and no firm conclusions can be drawn from it.

There was a consistent link between egg size and larval SL at hatching, both between and within batches. This has been reported in many other species including cod (Knutsen and Tilseth 1985; Marteinsdottir and Steinarsson 1998), haddock (Rideout *et al.* 2005b), herring (Blaxter and Hempel 1963), winter flounder (Buckley *et al.* 1991), striped bass (*Morone saxatilis*) (Monteleone and Houde 1990), dace (*Leuciscus leuciscus*) (Mann and Mills 1985) and Siberian sturgeon (Gisbert *et al.* 2000).

There was also a consistent link between egg size and YSV, both between and within batches. In the batch experiment there was an inverse relationship between larval SL at hatching and YSV which was not seen in the experiment with individuals. This can be explained by differences in incubation times with a difference of up to 3 days between the later and earlier hatched batches. A longer

incubation time results in the larvae using a greater amount of reserves before hatching than earlier hatching batches. This was observed in capelin where hatching later exacted a cost on yolk reserves (Chambers *et al.* 1989). An increase in YSV with egg size has been observed in other species including cod (Trippel 1998), herring (Blaxter and Hempel 1963), capelin (Chambers *et al.* 1989), brown trout (Ojanguren *et al.* 1996), haddock (Rideout *et al.* 2005b), black porgy (*Acanthopagrus schlegeli*) (Huang *et al.* 1999), and Siberian sturgeon (Gisbert *et al.* 2000).

Batch number had an effect independent of egg size with eggs from later batches hatching earlier, resulting in the larvae hatching at a smaller size and with more yolk. This is a disadvantage to the larvae as yolk sac utilisation is much greater in hatched larvae compared to un-hatched larvae (Kamler 1992) and so the yolk will be used up quicker. However, they will be able to avoid predators sooner which they cannot do before hatching which is a greater advantage to larvae from later batches as there will be greater amounts of predators in the plankton later in the season.

The difference in the mean size of the eggs between those used in the experiment with individuals and of the mean of the entire batch which they came from (which was measured before fertilisation) could be due to size selective mortality during the incubation period or could also be due to the smaller eggs hatching first. Smaller eggs have been seen to hatch earlier in European flounder (*Platichthys flesus*) (Dannevig 1894), black porgy (Huang *et al.* 1999), dace (Mann and Mills 1985), and capelin (Chambers *et al.* 1989).

The duration of the hatching period was greater for larvae with a greater SL at hatching. No direct relationship has been documented in the literature with respect to this correlation. However a study by Ryland *et al.* (1975) showed there was an inverse relationship between temperature and the size of larvae produced and the duration of the hatching period of plaice. A similar relationship was found for Pacific cod (*Gadus macrocephalus*) by Forrester and Alderdice (1966) in respect to both temperature and salinity. Whether the increase in the duration of the hatching period was directly caused by the abiotic conditions experienced by the egg or due to the increased larval size produced by the different abiotic conditions is unclear.

SGR was influenced by larval SL at hatching and YSV, with smaller larvae with larger yolk sacs having the highest growth rate. The increase in SGR with lower SL at hatching and higher YSV was due to the larvae endogenously feeding for a greater period (larval growth was greater during endogenous feeding compared to exogenous feeding). This was evident from the SGR being lower in the second week after hatching compared to the first. The relationship between SGR, YSV and larval SL at hatching disappeared after 1 week in the experiment with individuals but was still apparent after two weeks in the batch experiment. This was probably the result of the larvae in the experiment with individuals having a very low YSV at hatching (in comparison with the averages of the batches in the batch experiment) so had used up their yolk sac reserves by the end of the first week. The yolk sac reserves of the larvae in the batch experiment persisted for a longer period so the relationship between larval SL at hatching and SGR was still evident at the end of the second week.

Smaller larvae had a greater SGR because they have a lower respiration rate which rises isometrically with body weight in larval fishes (Giguere *et al.* 1988; Nelson and Wilkins 1994). This implies that to meet metabolic needs, larger larvae need a greater amount of food (Giguere *et al.* 1988). Therefore smaller larvae would utilise their yolk reserves at a lower rate than larger larvae, therefore carrying out endogenous feeding for a greater period of time resulting in a higher SGR. This was seen by Panagiotaki (1992), where smaller, earlier hatched larvae had a lower mean daily yolk sac utilisation rate when compared with their larger, later hatched siblings.

At 14 days after hatching, the relationship between larval SL and EDW in the batch experiment was gone with the main influence on larval SL being time from fertilisation. This influence of larval SL and EDW was nearly significant with a p-value of 0.07. Egg size no longer had an influence on larval size after one week in the experiment with individuals. Larvae from eggs with a greater EDW had a greater amount of yolk reserves remaining 1 week after hatching. This means that larvae from larger eggs have a longer time to find food before the 'point of no return'. In zebrafish (*Danio rerio*), where the yolk sac volume was manipulated experimentally, yolk sac volume was positively correlated with the time to yolk sac absorption and size at complete yolk sac absorption (Jardine and Litvak 2003). The duration of the influence of egg size on larval size in the present experiment was

low when in comparison with other species. Glebe *et al.* (1979), working with Atlantic salmon reported that the length of the fry was still correlated with original egg diameter some 8 months later. Wallace and Aasjord (1984) found that the effect of egg size on the length of Arctic charr (*Salvelinus alpinus*) alevins was still evident 140 days after hatching. The maternal influence on larval size of striped bass was still evident at 25 days post hatch (Monteleone and Houde 1990). Larval size still remained correlated with egg size in tilapia (*Oreochromis mossambicus*) at 22 days post hatch (Rana 1985) and the effect of egg size on Siberian sturgeon larval size was still evident at 20 days post hatch (Gisbert *et al.* 2000). The reason for these differences across species is unknown but could be due to what advantages come with being larger. If the advantages bring about a greater ability to gain food either through predation (Marteinsdottir and Steinarsson 1998) or holding territory (Elliott 1990) the advantages should last longer in a form of cascading effect i.e. larger larvae get more food so become bigger and therefore better foragers. However, if the size advantage brings about only a better ability to avoid predators then in situations without predators, size differences may persist for only short periods.

The change in standard deviations of larval SL is related to age at first feeding. There was a decrease in standard deviation of larval sizes within a batch if the larvae did not feed until after approximately day 9 and an increase if the larvae started before 9 days. The decrease in standard deviation within a batch of larvae not feeding before day 9 could be due to smaller larvae 'catching up' with their larger siblings. This has been noted previously by Panagiotaki (1992) who showed there was a decrease in size variation of plaice larvae between hatching and the end of the yolk sac stage when no food is offered. The decrease in the present study could also be due to size selective mortality.

Implications for larvae

There was no link between any of the measured maternal, egg or larval traits on survival of the larvae from hatch to two weeks post hatch. This has been found in other species kept under high prey densities (McEvoy and McEvoy 1991; Gisbert *et al.* 2000; Jonsson and Svavarsson 2000; Rideout *et al.* 2005b). Larger plaice

invest greater amounts into each individual larvae. This infers there is an advantage to hatching at a greater size which was not apparent in the present study where there were no predators and abundant evenly dispersed food. Larger larvae have been shown to have greater swimming endurance times (Ojanguren *et al.* 1996) and a greater burst swimming speed, both of which can help in the capture of prey items and escape from predators (Miller *et al.* 1988). Bailey (1984) showed that larger plaice larvae have a greater escape swimming speed and are less vulnerable to planktonic invertebrate predators. However, it has been noted that there are conflicting studies on the matter of the 'bigger is better' hypothesis concerning larval predation (Litvak and Leggett 1992) and the vulnerability of larval fish to predation is dependent on many different variables (Paradis *et al.* 1996). The larvae from the larger eggs did have a greater amount of yolk reserve after one week post hatch and so could live by endogenous feeding for a longer period and so have a greater amount of time to find food. In batch averages, larger larvae have been shown to have a greater ability to withstand periods of starvation (Blaxter and Hempel 1963; Imai and Tanaka 1998; Rideout *et al.* 2005b). With this greater ability to survive starvation, larvae from larger females which spawn earlier in the season than smaller females (Rijnsdorp 1989), and the larvae from early season batches, both of which have a greater EDW are more able to deal with the low zooplankton density at the beginning of the season (Scrope Howe and Jones 1985) than larvae from later batches and smaller fish.

CONCLUSION

Larger maternal size leads to eggs of a greater EDW which give rise to larvae with a greater SL at hatching and greater yolk reserves, however the maternal influence on size had began to disappear by two weeks after hatching. With progression through spawning there was a decrease in egg size and also a decrease in incubation time which led to a decrease in larval SL at hatching and to larvae having a greater YSV at hatching. Larger larvae had no apparent survival advantage under the present experimental conditions. There was also great variation within a batch of eggs and the growth of an individual larva is wholly dependent on its individual resources.

CHAPTER 6
GENERAL DISCUSSION

In order to manage a fishery as a sustainable resource it is essential to monitor the SSB during exploitation hence accurate methods of estimating its size are required. Assessment models with increased biological detail are required as present models calculate SSB from relative changes in biomass and have the underlying assumption that the reproductive potential of fish stocks is proportional to the SSB. This was shown to be untrue for Northeast Artic cod (Marshall *et al.* 1998), and as stocks reach low levels the consequences of inaccuracies in model assumptions become more serious as other factors such as Allee effects and climate change can have an effect on recruitment when population is low thus limiting recovery (Frank and Brickman 2000; Brander 2005). By including information on the age composition of the stock, the stock recruitment relationship was shown to be improved for Icelandic cod thus demonstrating the need for a broad range in the age of spawners for increased reproductive potential (Marteinsdottir and Thorarinsson 1998). The results from the present study showed that there was a positive linear relationship between TEP and SSB which is in contrast with the study by Marshall *et al.* (1998). This is believed to be due to the differences in the main store of lipids in the different species and the maximum size of the different species.

The results of the present study showed that the fecundity of plaice varied between years in the western Irish Sea and in the Cumbrian coast, however there were no significant differences in the fecundity between years in Liverpool Bay. Fecundity also varied across the Irish Sea and variations have been seen in other life history traits of Irish Sea plaice (Nash *et al.* 2000). Variations in many life history traits including fecundity, egg size, growth and longevity are seen in many other species of fish (McQuinn 1997; Begg *et al.* 1999; Nash *et al.* 2000). These life history traits are phenotypic expressions of the interaction between genotypic and environmental influences. Although these differences are not conclusive evidence of non-mixing between stocks, they do provide baseline information that typically assists with the initial recognition and delineation of geographical areas that are representative of individual fish stocks (Pawson and Jennings 1996). There is evidence that mixing of plaice between the east and west occurs at the egg and larval stage (Nash, pers. comm. IMR, Norway) however there is very little mixing of these fish at the adult stage (Dunn and Pawson 2002) therefore their phenotypic differences in life-history traits will be a reflection of the environmental conditions

in the area they inhabit. This has been witnessed in Atlantic cod, haddock, and yellowtail flounder in the Northwest Atlantic where there is evidence of mixing between stocks during early life stages but there are distinct differences in life history traits between stocks (Begg *et al.* 1999).

Fecundity has been shown to vary over the temporal and spatial scales in many other species of fish including cod (Kjesbu *et al.* 1998; Kraus *et al.* 2000), haddock (Blanchard *et al.* 2003), Greenland halibut (Gundersen *et al.* 2000), sole (Witthames *et al.* 1995), and orange roughy (Koslow *et al.* 1995). The causes of the variations in fecundity of cod from the North-east Arctic have been linked to the availability of capelin prey (Kjesbu *et al.* 1998; Marshall *et al.* 1998)); prey availability is hypothesised to be the reason for the fecundity differences in plaice in the present study. As plaice are an iteroparous species they must balance energy allocation between reproduction which should be maximised while maintaining adequate resources for survival to the following breeding season (Rijnsdorp 1990). Species which use such a strategy must be capable of adjusting their reproductive effort in response to available food resources as they have a limited supply of energy which must be allocated amongst maintenance, somatic growth, and reproduction (egg production and behaviour related to spawning) (Calow 1985). This energy, in a long lived species, will be prioritised into maintenance and metabolic needs, then to build up stored reserves before allocation goes into reproduction and then somatic growth, though this does depend on the life history situation (Rijnsdorp 1990; Abrams 2004), therefore if food levels fall below a specific level then the allocation into reproduction could decrease (Jørgensen and Fiksen 2006).

It was seen that plaice can alter their fecundity both during and after follicle proliferation. During follicle proliferation fecundity was determined by the weight of the fish with an optimum level as a proportion of total body weight. As the fish increased in weight during this time, fecundity increased. This occurs in September to October when the material for ovary growth is supplied mainly from energy intake that is surplus to routine metabolism and maintenance. This surplus production is first prioritised into building up reserves and then to ovary growth. Plaice produce an amount of eggs proportional to their body size (Rijnsdorp 1990); therefore as the fish builds up reserves and increases in weight more follicles are recruited for vitellogenesis.

There was a positive relationship between fish condition and fecundity (see chapter 2). The intensity of atresia in the fish, as shown in chapter 3, was related to the decrease in condition. Fish of higher condition have a greater amount of reserves which can be used for reproduction, therefore a greater fecundity. This has been documented in many other species including haddock where condition increased the amount of explained variance in fecundity-length relationships (Blanchard *et al.* 2003) and cod in good condition spawned a greater proportion of their eggs than cod of lower condition (Kjesbu *et al.* 1991).

Atresia is the degeneration and resorption of ovarian follicles. The material from the resorption can then be re-used by the body for other purposes. This mechanism was used by plaice to down regulate fecundity after follicle proliferation has ceased. Down regulation occurs when food availability or stored reserves are insufficient to bring all follicles to full development. Such down regulation has been witnessed in many other species including herring (Kurita *et al.* 2003), turbot (Bromley *et al.* 2000), cod (Kjesbu *et al.* 1991; Armstrong *et al.* 2001) and sole (Armstrong *et al.* 2001). During the autumn and winter months, plaice generally feed less, due to a combination of lower temperatures and lower food availability, and rely more on stored reserves during this time. The results from the feeding experiment in chapter 3 showed that fish which ate more at this time lost less weight during late vitellogenesis than fish which ate less. The level of atresia was positively correlated to the decrease in condition. It was hypothesised in chapter 2 that the temporal and spatial variations in fecundity of plaice populations in the Irish Sea are due to differences in the degree of the down regulation of fecundity which was a result of different feeding conditions experienced by the fish in the different areas. This is supported by the results of chapter 3 where it was demonstrated that food levels affected the levels of atresia in captive plaice.

It is known that some iteroparous fish will not spawn every year and may 'skip spawning'. This has been documented in several species of both wild (Fedorov 1971; Ramsay and Witthames 1996; Rideout *et al.* 2000) and captive fishes (Rijnsdorp 1990; Burton 1994; Bromley *et al.* 2000) and was witnessed in one fish during this research (see chapter 3). This fish had a very low condition which indicated it's stored reserves were low so if it had commenced spawning it may have not have survived the spawning season. By aborting ovary development early there was a higher probability that it could survive and reproduce in a future

spawning season. Very few non-mature plaice that were above the size expected to be mature were caught during sampling (2 fish from 1995-2004). Skipped spawning in Irish Sea plaice thus may be rare or plaice that have skipped spawning may not migrate to the spawning grounds as seen in orange roughy (Bell *et al.* 1992) and herring (Engelhard and Heino 2005).

This phenotypical plasticity in fecundity is an important strategy amongst long-lived species to maximise the numbers of offspring produced during their life. To do this they must maximise their reproductive output over their entire lifetime, rather than over a single spawning season. By balancing available resources between reproductive output during the current season and having adequate resources for survival after spawning, they ensure they have a greater chance of surviving to breed again in the following season. Being able to adjust fecundity to available resources allows a species to adapt to new environments as they can adjust their fecundity in response to the conditions in the new environments and also survive in environments where food supply is variable.

As larger fish allocate a lower proportion of energy into somatic growth (Bromley 2000) they start ovary development much sooner than smaller fish. Fish with a higher food availability may also begin vitellogenesis sooner (Yoneda and Wright 2005). The results from this thesis showed that fish which ate more had higher growth rates of follicles during vitellogenesis. This would result in larger, better fed fish being in spawning condition earlier than their smaller, less well fed counterparts. With each individual fish spawning over a period of time there will be a higher chance that its own eggs and larvae will be produced during a period of optimal conditions for the larvae. Larger fish will also spawn earlier in the season as they have more batches to spawn and, due to spending less time on somatic growth, they can develop their gonads sooner, and therefore spawn earlier so give their offspring a longer growing season.

With different sized fish spawning at different times during the spawning season, there is a greater chance that the production of eggs and larvae will coincide with optimal environmental conditions for larval survival. Fishing pressure on the adult population can lead to a reduction in the abundance of older age groups (Myers and Cadigan 1995) and change the proportion of young and old spawners (Trippel *et al.* 1997). This could lead to a contracted spawning season which may result in reduced recruitment due to a lower chance that the production of eggs and

larvae will coincide with optimal conditions. This has already been documented in the Irish Sea where during times of decreased SSB the duration of the spawning season was shorter than during times of an increased SSB (Anonymous 2005). As larger fish have larger eggs, reductions in older age groups could affect the size range of eggs produced by the population. It is un-clear how egg size affects larval plaice survival in the wild (see below); however, under laboratory conditions, egg size has been shown to have an affect on larval characteristics and survival in several other species (Marteinsdottir and Steinarsson 1998; Berkeley *et al.* 2004; Rideout *et al.* 2005b). Fishing pressure can also skew sex ratios due to the higher catchability of males (for plaice) (Beverton 1964) and the consequences of sexual dimorphism, whereby females grow to a larger size and so are under greater fishing pressure from a size selective fishery.

Several studies have shown that eggs and larvae from particular parts of the spawning season can be 'selected for' and the part that is selected can vary between years (van der Veer *et al.* 2000; Wright and Gibb 2005). This could be due to optimum conditions for the larvae at this time in the spawning season of the surviving portion or could be due to the eggs produced at this time being of a higher quality due to older fish spawning at this time (van der Veer *et al.* 2000; Wright and Gibb 2005).

As maternal size increased, both fecundity and investment into each egg increased. In marine invertebrates, larger eggs are believed to be advantageous as they provide a larger target for sperm and so there is an increase in fertilisation success with egg size (Levitan 1993). This did not appear to be the case for plaice eggs as there was no effect of egg size on fertilisation success. A larger egg size is not always an advantageous as seen in the frog, *Bombina orientalis*, where larvae from larger eggs in warm water pools were more susceptible to predation due to larger amounts of inert yolk which diminished locomotory ability (Kaplan 1992). The bigger is better hypothesis is still under debate for larval fish (Litvak and Leggett 1992).

Plaice have relatively large eggs in comparison to other marine fish species in the eastern Atlantic. It is believed that it is this larger size that results in plaice eggs having a lower mortality than cod eggs (which are smaller) (Heessen and Rijnsdorp 1989; Rijnsdorp and Jaworski 1990) as there are very few predators in the zooplankton that can handle large plaice eggs (Nash pers. comm.. IMR,

Norway). However, plaice eggs are preferentially selected for by sprat and herring (Daan *et al.* 1985; Ellis and Nash 1997) but total mortality is the cumulative mortality from all predators and it is believed that the majority of predators of plaice and cod eggs are organisms other than herring. It has also been noted that larger plaice eggs suffer a lower mortality than smaller plaice eggs (Rijnsdorp and Jaworski 1990) therefore eggs produced at the start of the spawning season may suffer a lower mortality than eggs produced later in the spawning season due to larger females, which have larger eggs, spawning earlier and egg size decreasing with an individuals progression through spawning.

Another possible advantage brought about by a larger egg size is that larger eggs gave rise to larger larvae with greater yolk reserves. Larger plaice larvae had no survival advantage under the experimental conditions used during this study but it is known that larger larvae with larger yolk sacs are more resistant to starvation under experimental conditions (Blaxter and Hempel 1963; Huang *et al.* 1999). Early in the spawning season, during which time larger eggs are produced, there is a much lower zooplankton density (Scrope Howe and Jones 1985), therefore, by having larger eggs the larvae of larger fish may be better equipped to deal with this low food abundance and survive until the spring bloom.

With the splitting of reproductive capacity into numbers and size of eggs, there must be an optimum balance between egg size and numbers (brought about by probability of survival of the offspring) which will be an adaptation to the local environment (Power et al. 2005). From the current results and results from many other species of fish it can be inferred that the status of the maternal parent may influence this balance between optimum egg size versus egg number. Large females have an increased capacity for producing eggs but this extra capacity is used to increase egg size as well as numbers. It therefore may be more advantageous in terms of numbers of offspring surviving to increase the chances of survival of each egg and egg numbers rather than only egg numbers as reproductive capacity increases. This may be in density-dependent situations where resources for the offspring are limited; the offspring from larger females may have an increased survival chance through competition with others of the same species (Elliot 1990). It could also be through density independent factors where larger larvae have better predator escape abilities (Bailey 1984).

The egg incubation period was shown to be affected by the stage of spawning of the maternal parent with batches from the start of an individuals spawning having a longer incubation period than batches produced later in the season. This was an unexpected result and has not been reported previously in the literature. A relationship has been found previously with egg size and incubation period in plaice, this was a non-linear relationship at 6°C (Fox *et al.* 2003) If predation risk varies throughout time and/or among sites it would be expected that selection would favour plasticity in hatching strategies (Vonesh 2005). There was also an effect of larval size on the hatching period (time between the first eggs hatching and when all the eggs have hatched) with the length of the hatching period increasing with larval size which also has not been reported previously in the literature.

Egg size affected larval characteristics both at the batch and individual level with larger eggs giving rise to larger larvae with larger yolk sacs. Growth rate was highest in smaller larvae with larger yolk sacs. The duration of the effect of egg size on larval size was affected by the size of the yolk sac. This was seen in the batch experiment versus the experiment with individuals where the effect of egg size on larval size was still evident after one week in the batch experiment, whereas it was gone after 1 week in the experiment following individuals. The larvae are therefore affected by their individual resources and if a larger size at hatching does bring about an advantage then larvae within a batch may not have an equal chance of survival.

It has been shown that the plaice population in the Irish Sea has increased in recent years (ICES 2006). Changes in population density have been linked to changes in fecundity with a negative relationship between the two, possibly due to changes in food availability with changes in population density (Bagenal 1966; Koslow *et al.* 1995). This does not appear to be the case for plaice in the Irish Sea with no decrease in fecundity with the observed increase in population size. This could be due to an expansion of the feeding grounds inhabited, and so no effective increase in density. With the increase in population size there was no expansion of the spawning grounds (Anonymous 2005) however it is known that plaice in the North Sea exhibit aggregations during the breeding season and disperse over a much larger area during the summer non-breeding season (De Veen 1978; Hunter *et al.* 2004). Comparison of growth rates between North Sea plaice from before pre-

industrial fishing and current times, a period in which there has been a great reduction in stock size, has shown that for plaice above 30cm, the growth is similar. This suggests that density dependent growth does not play a major role in North Sea plaice (Bolle *et al.* 2004) but it is not known if this is true for plaice in the Irish Sea. However, if food was unlimited for the adult part then there would be no need to down regulate fecundity, thus fecundity just prior to the spawning season would be expected to be non-different from the fecundity in September and thus not different between years.

The AEPM can be used for the estimation of SSB. This method requires a series of plankton surveys throughout the spawning season in which the stage 1eggs of the species in question are identified and counted, this is then used to calculate the TEP for the entire area surveyed (Lockwood *et al.* 1981). The total egg production is then divided by the weighted average fecundity of the stock to give total biomass of spawning females. This provides an assessment that is independent of commercial catch data (Armstrong *et al.* 2001) which can be inaccurate due to discarding and mis-reporting of catches. However, experiences in comparing SSB estimates from AEPM with VPA based stock assessments have shown discrepancies that are not yet understood. These are seen in the difference in the biomass estimates for cod, plaice and sole in the Irish Sea that were calculated by the AEPM and VPA (Horwood 1993; Armstrong *et al.* 2001).

To achieve accurate results from the AEPM accurate estimates of fecundity are required. For this, accurate knowledge of the time window between full recruitment of oocytes to the vitellogenic stock and the commencement of spawning and it necessary to be able to identify fish that have started spawning (Macewicz and Hunter 1994). Using the method described in this thesis and previous knowledge on plaice reproduction fish could be selected for inclusion in the fecundity estimates. It is know that for some species, potential fecundity is not equal to realised fecundity due to down regulation by atresia in the period before and during fecundity (Kjesbu *et al.* 1991; Armstrong *et al.* 2000) it is therefore necessary to estimate and control for this. From the results it was seen that plaice fecundity appears to level of when follicle diameters reached 1000 μm therefore by excluding fish with follicle sizes lower than this, more accurate estimates of fecundity can be achieved. It is also assumed that 100% of the eggs produced are fertilised, this was never the case in the laboratory experiments but it is unknown if

this happens in the wild. The gathering of fecundity estimates can be expensive and time consuming, however by assuming an unchanging fecundity large, changes in the population abundance can be detected, however this will obscure small fluctuations in population abundance.

The discrepancies seen in the SSB estimates from VPA and AEPM, and in the TEP estimates from plankton surveys and from VPA may be due to errors in the VPA estimates. VPA data cannot be considered absolute, as it is dependent on the completeness and accuracy of commercial catch at age data, the assumed relationship between VPA numbers and relative abundance indices used for tuning, and assumptions regarding natural mortality and selectivity characteristics of the fishery. Further, the analyses are not carried out separately for males and females which differ in the age at maturity and growth rate (Anonymous 2000).

CONCLUSIONS

The fecundity of plaice varied between areas and years in the Irish Sea with fish from the western Irish Sea having the greatest variability between years and fish from Liverpool Bay showing no significant variability between years. Fecundity at the individual level was determined by fish size at the end of follicle recruitment and was then down regulated by atresia in relation to available energy resources. The variation exhibited in the fecundity estimates between areas and years was caused by differences in the degree of down regulation suggesting that food availability varies sufficiently over space and time to influence reproductive investment. In the feeding experiment fish that received higher rations relied less upon stored reserves for vitellogenesis than fish which received lower rations. Fish fed with higher rations also exhibited a higher growth rate of follicles and lower levels of atresia.

These results suggested several improvements that could be made to the methodology of the AEPM for estimating biomass. Estimates of fecundity are currently taken from stage 4 fish, however this should be further limited to fish with follicle diameters greater than 1000 μm as this will bring estimates closer to the realised fecundity. As fecundity varies between years, concurrent estimates must be made each year to detect small fluctuations in population abundance, however

large changes in population abundance can be detected when assuming an unchanging fecundity.

TEP was positively correlated with SSB, however there was no relationship between recruitment and SSB or TEP. Mortality between the egg stage and recruitment was positively correlated with the TEP and was hypothesised to be due to density dependent processes occurring in the nursery ground phase. There were differences in the estimated TEP from VPA data and direct estimates of stage 1 egg production, this is hypothesised to be inaccuracies in the VPA data.

Egg size increased with maternal size and egg size decreased with progression through the spawning season. Larger eggs resulted in larger larvae with larger yolk sacs. Eggs from earlier batches had longer incubation times than eggs from later batches. A longer incubation time led to a larger size at hatching but with a smaller yolk sac volume. Growth during the two weeks after hatching was related to size at hatching and yolk sac volume, with smaller larvae with larger yolk sacs having the greatest growth. Larger larvae did not show an enhanced survival under the experimental conditions used where there was a plentiful supply of food and no predators.

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Appendix I

Protocol for the staining of oocytes and calibration of image-analysis system

Solutions
0.1% Periodic acid (Taab email [sales@taab.co.uk] code P005 99.5% periodic acid).
Schiffs Reagent product number J/7300/PB08
Dilute to 15% for 'working Schiffs'

Equipment
Net Well Carriers 74 μm net mesh - Costar Scientific Corporation
Supplied in UK by Fisher Cat. ref. TKN –540 – 010L
Zeis stereo microscope with f 1.2 objective
JIA colour camera
Resolution 0.01938 mm / pixel
Schott light source 3300K filament temperature subsequently replaced with a Jessops Light panel for very much flatter illumination.

Tissue in 3.6% neutral buffered formaldehyde.

- Place up to 100 mg ovary tissue into a Netwell suspended in a multiwell plate containing approximately 15 ml purified water per well for a minimum of 15 minutes.
- Remove the Netwell, blot its base and rinse the sample with a jet from a wash bottle containing RO water. Blot the base.
- Suspend the Netwell in a multiwell plate containing approximately 15 ml 0.1%* periodic acid for 15 minutes.
- Remove the Netwell, blot the base and suspend the Newell in a multiwell plate containing approximately 15 ml purified water for 5 minutes.
- Remove the Netwell, blot its base and rinse with a jet from a wash bottle containing RO water. Blot the base.
- Suspend the Newell in a multiwell plate containing approximately 15 ml working (diluted to 15%*) Schiffs.
- Remove the Netwell, blot the base and suspend the Newell in a multiwell plate containing approximately 15 ml purified water for 10 minutes.
- Remove the Netwell, blot the base and rinse further with a jet from a wash bottle containing RO water and resuspend in water.

The size of oocytes measured by image analysis after staining by this protocol is stable for at least 4 days storing them at 0-5° C.

Calibration method
Disperse samples of ovary tissue containing hydrated and developing oocytes from several species e.g cod, hake, mackerel and plaice.) in purified water.
Select 4 oocytes of different sizes and stage of maturation covering the calibration range from 0.15 mm to 2 mm per well and place them in one of the wells in a multi well plate. Replicate 12 times until all the wells of the multiwell plate are filled.

Push the oocytes to the centre of the well and measure each group manually and then follow the staining protocol (in the well) before re-measuring each group by image analysis. Pair the two sets of measurements and compare oocyte size (Figure 1).

The illumination RGB grey level (Table 1) can be adjusted to give a 1 to 1 relationship between manual and image analysis. Record the statistics of RGB grey levels and use these conditions to determine the relationship between Log_e transformed mean oocyte diameter and log transformed Oocytes g^{-1} ovary. Use this relationship to determine the number of eggs per g ovary in unknown samples following the Autodiameteric method (Thorsen and Kjesbu 2001).

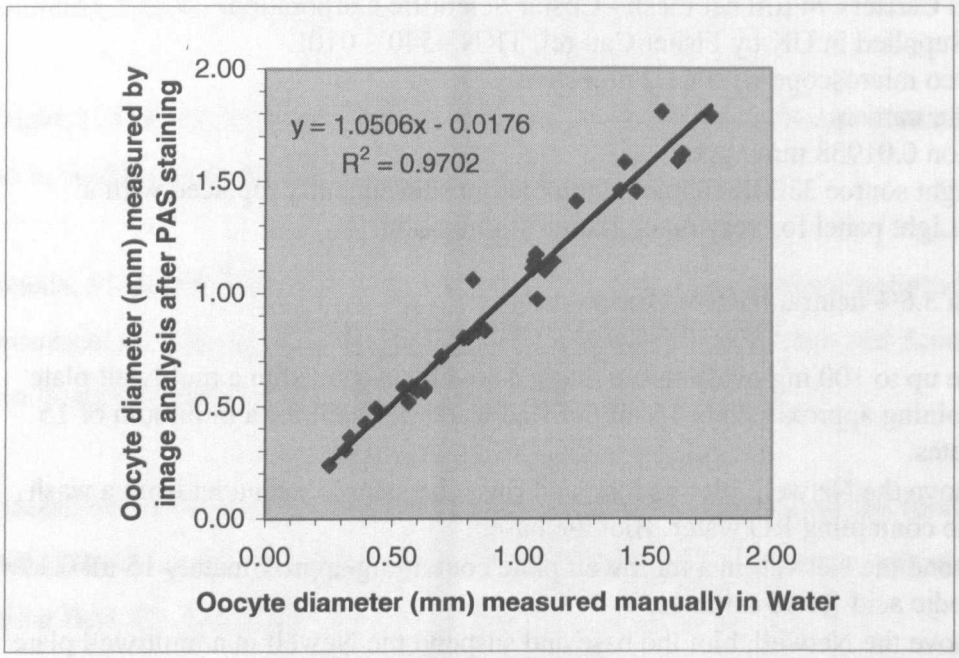

Figure 1. Comparison of oocyte diameter measured manually in water and by image analysis after PAS staining

Table 1. Example of grey scale statistics measured by Aphelion

Parameter	Red	green	Blue
Min grey level	75	92	59
Max grey level	208	206	216
Mean	173	172	173

In 2004 due to the purchase of a new system by Cefas a greater degree of staining was required. This was done increasing the concentration of periodic acid to 0.5% and working schiffs was changed to 30%. This had no effect on egg size.

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Thorsen, A. and Kjesbu, O.S. 2001. A rapid method for the estimation of oocyte size and potential fecundity in Atlantic cod using computer-aided particle analysis system. *Journal of Sea Research* 46:295-308.

Appendix II Irish Sea plaice population abundance on the 1st January (numbers in thousands)

AGE	1964	1965	1966	1967	1968	1969	1970	1971
1	33022.	17398.	15304.	12271.	14070.	20875.	19419.	13304.
2	21735.	29287.	15405.	13572.	10882.	12478.	18459.	17215.
3	11276.	18339.	24643.	13550.	11883.	9491.	10663.	15616.
4	5452.	8206.	13302.	17815.	10629.	8697.	6243.	7318.
5	2918.	3261.	4616.	8416.	10557.	6231.	4966.	3496.
6	2839.	2169.	1847.	2055.	3525.	5022.	3577.	2647.
7	1672.	1720.	1403.	1057.	892.	1620.	2371.	2208.
8	507.	1033.	1235.	694.	340.	493.	709.	1261.
9+	814.	162.	758.	1349.	556.	734.	655.	1081.

AGE	1972	1973	1974	1975	1976	1977	1978	1979
1	9823.	13176.	12961.	10885.	16898.	18734.	22615.	20485.
2	11799.	8712.	11685.	11489.	9637.	14966.	16084.	20037.
3	14866.	10331.	6857.	9236.	8902.	6152.	9405.	11388.
4	10666.	10131.	5333.	3712.	4159.	3846.	2869.	3518.
5	2867.	4659.	4098.	2213.	1516.	1428.	1112.	1112.
6	1528.	1178.	1631.	1650.	882.	503.	484.	480.
7	1067.	652.	445.	474.	593.	258.	226.	240.
8	1498.	428.	256.	216.	278.	162.	89.	109.
9+	1639.	1137.	466.	603.	394.	185.	204.	310.

AGE	1980	1981	1982	1983	1984	1985	1986	1987
1	15210.	8315.	21053.	21134.	22184.	16138.	19811.	21190.
2	18157.	13487.	7354.	18647.	18696.	19637.	14309.	17541.
3	14596.	13489.	10325.	5850.	13791.	13615.	15201.	11138.
4	4795.	6645.	6409.	6075.	2855.	7381.	6322.	8535.
5	1407.	1863.	3103.	2803.	2390.	1426.	3459.	2857.
6	646.	644.	869.	1482.	1067.	1134.	690.	1863.
7	250.	281.	363.	462.	708.	517.	604.	291.
8	111.	105.	151.	193.	212.	348.	214.	190.
9+	215.	363.	315.	250.	261.	306.	374.	341.

AGE	1988	1989	1990	1991	1992	1993	1994	1995
1	12945.	7410.	11522.	10399.	11290.	9516.	8375.	7844.
2	18736.	11438.	6550.	10205.	9054.	9871.	8414.	7335.
3	12068.	13871.	8515.	4852.	7166.	6192.	7899.	6386.
4	4909.	5987.	6740.	5007.	2891.	3354.	3945.	4968.
5	2677.	1971.	2812.	3233.	2634.	1224.	1705.	2272.
6	1280.	1067.	949.	1434.	1739.	1068.	641.	909.
7	652.	436.	538.	407.	836.	699.	428.	272.
8	143.	283.	187.	235.	191.	379.	268.	151.
9+	546.	391.	238.	200.	285.	260.	258.	190.

AGE	1996	1997	1998	1999	2000	2001	2002	2003
1	10302.	10605.	9968.	11572.	13253.	14415.	17462.	16878.
2	6937.	9102.	9380.	8836.	10200.	11752.	12782.	15484.
3	5603.	5348.	7293.	7669.	7082.	8729.	10098.	11036.
4	4066.	3707.	3252.	4836.	5389.	5246.	6600.	7822.
5	2593.	2485.	1872.	1924.	3075.	3503.	3532.	4634.
6	1299.	1417.	1265.	1087.	1054.	1941.	2297.	2425.
7	508.	805.	805.	676.	701.	694.	1322.	1629.
8	112.	331.	447.	337.	416.	449.	461.	917.
9+	232.	281.	523.	360.	400.	338.	381.	591.

AGE	2004	2005
1	34512.	24676.
2	14967.	30606.
3	13449.	13116.
4	8808.	11227.
5	5782.	7036.
6	3366.	4573.
7	1807.	2701.
8	1193.	1436.
9+	1025.	1772.

Appendix III Catch weight at ages 1 to 9+ for Irish Sea plaice

Age	1964
1	0.000
2	0.190
3	0.292
4	0.413
5	0.463
6	0.597
7	0.831
8	1.042
9+	0.791

AGE	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974
1	0.07	0.000	0.000	0.000	0.056	0.058	0.000	0.000	0.000	0.063
2	0.177	0.152	0.133	0.149	0.146	0.149	0.14	0.143	0.143	0.158
3	0.269	0.223	0.218	0.213	0.215	0.219	0.207	0.235	0.218	0.246
4	0.388	0.316	0.299	0.313	0.311	0.324	0.295	0.332	0.316	0.334
5	0.556	0.418	0.382	0.413	0.405	0.417	0.396	0.432	0.415	0.445
6	0.653	0.532	0.516	0.509	0.541	0.523	0.489	0.56	0.491	0.514
7	0.69	0.697	0.518	0.584	0.643	0.648	0.595	0.737	0.645	0.686
8	0.719	0.691	0.759	0.777	0.787	0.685	0.753	0.712	0.694	0.847
9+	1.063	0.979	0.743	0.922	0.822	0.871	0.755	1.052	0.894	1.072

AGE	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984
1	0.072	0.06	0.059	0.071	0.069	0.066	0.069	0.201	0.232	0.26
2	0.185	0.15	0.153	0.185	0.176	0.177	0.176	0.274	0.261	0.29
3	0.275	0.228	0.226	0.268	0.262	0.255	0.267	0.284	0.29	0.33
4	0.398	0.323	0.34	0.391	0.376	0.365	0.376	0.348	0.319	0.38
5	0.531	0.419	0.43	0.525	0.557	0.483	0.512	0.421	0.368	0.47
6	0.644	0.525	0.51	0.672	0.668	0.517	0.592	0.545	0.426	0.56
7	0.749	0.59	0.592	0.72	0.794	0.671	0.678	0.65	0.484	0.66
8	0.924	0.719	0.738	0.91	0.915	0.884	0.863	0.651	0.552	0.76
9+	1.278	0.959	0.971	1.121	1.068	1.164	1.149	1.003	0.813	1.129

AGE	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994
1	0.29	0.27	0.26	0.23	0.227	0.2	0.247	0.169	0.26	0.156
2	0.31	0.28	0.29	0.26	0.272	0.257	0.267	0.218	0.27	0.207
3	0.34	0.34	0.315	0.3	0.321	0.316	0.295	0.274	0.292	0.268
4	0.39	0.42	0.37	0.37	0.374	0.376	0.332	0.337	0.328	0.338
5	0.47	0.5	0.44	0.46	0.43	0.439	0.377	0.407	0.375	0.416
6	0.54	0.54	0.52	0.55	0.491	0.504	0.431	0.484	0.436	0.504
7	0.63	0.63	0.61	0.68	0.555	0.57	0.494	0.568	0.508	0.6
8	0.73	0.83	0.72	0.82	0.623	0.639	0.566	0.658	0.594	0.706
9+	0.988	1.065	1.025	1.353	0.86	0.832	0.753	0.893	0.829	0.929

AGE	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
1	0.201	0.144	0.134	0.202	0.174	0.000	0.142	0.185	0.204	0.207
2	0.229	0.203	0.184	0.222	0.213	0.222	0.205	0.225	0.244	0.23
3	0.266	0.268	0.239	0.252	0.257	0.257	0.269	0.271	0.289	0.261
4	0.312	0.338	0.299	0.294	0.309	0.302	0.337	0.324	0.34	0.3
5	0.366	0.414	0.362	0.346	0.366	0.357	0.407	0.383	0.395	0.348
6	0.429	0.496	0.43	0.41	0.43	0.422	0.479	0.449	0.455	0.404
7	0.501	0.584	0.502	0.484	0.501	0.497	0.554	0.521	0.52	0.468
8	0.581	0.677	0.579	0.569	0.577	0.581	0.632	0.6	0.59	0.542
9+	0.767	0.925	0.748	0.804	0.719	0.787	0.772	0.731	0.743	0.662