Age, growth and reproductive biology of Plaice, (*Pleuronectes platessa* L.) in Irish waters, 2003-2005.

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Abstract

The current study presents data on age and growth for plaice (*Pleuronectes platessa* L.) sampled between November 2003 and February 2005 in ICES areas VIa (northwest coast of Ireland), VIIa (Irish Sea), VIIg (Celtic Sea), VIIj (southwest coast of Ireland) and VIIb (west coast of Ireland), and data on the reproductive biology and maturity of plaice in ICES area VIIb (west coast of Ireland). This is the first detailed account of the biology of plaice for some of these areas. It is intended that this study will improve understanding of the life cycle of plaice and help fisheries scientists to better predict the effect of fishing effort on Irish plaice stocks. The overall length range found for plaice was 9-51.99cm TL, with a length range of 9-51cm TL for females and 9-40cm for males. In all ICES areas the length range for female fish was larger than for male fish. The age range of plaice sampled during this study was 1 to 16 years. In all ICES areas females had a greater range in ages and fish in the larger age groups. From analysis of length and age data it was concluded that there was a significant difference (P=0.000) in growth rate of males and females between ICES areas sampled in March 2004. The highest rate of fishing mortality was determined for ICES area VIa (F=1.06) and the lowest for ICES area VIIa (F=0.56). In each ICES area male and female plaice have fully recruited to the population by age 4, with the exception of females in ICES area VIa, for which a tr value of 5 years was determined. Length at first maturity (L_{50}%) was determined to be 23cm and 21cm for males and females respectively. Age at first maturity (A_{50}%) was determined to be 3 years for both males and females. It was found that males and females in ICES areas VIIb, VIIa and VIa are well above the length and age at first maturity when they are recruited to the fishery. In ICES area VIIb female plaice spawn from November to March, with peak spawning occurring in February, and male plaice spawn from November to April, with peak spawning occurring in November. Spawning females had an age range of 2 to 10 years and spawning males had an age range of 2 to 7 years. From the oocyte length frequency distributions, it was determined that the plaice is a determinate batch spawner.

During this investigation a total of 177 ovaries and 127 testes were staged using both macroscopic and histological criteria. The overall percentage of maturity stages which compared favorably between the two assessment methods was 22.03% for female plaice and 37.80% for male plaice. In general, the findings of this study indicate that there was a very poor match between the macroscopic and histological assessment methods. Given that the histological determination of these stages is based on the observation of a distinct set of developmental features, it is expected that it would be more accurate to use histologically assessed gonads to calculate the annual percentage maturity assessment. The biology of plaice in the areas studied is compared with previous studies of plaice in Irish and European waters.

Acknowledgements

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General Introduction

The plaice, *Pleuronectes platessa* (Linnaeus 1758) is a demersal marine flatfish of the family Pleuronectidae. Its distribution is widespread throughout the north east Atlantic, from Greenland and Norway south to Morocco, Spain and France in the Mediterranean, the White Sea (Nielsen, 1986; Kuznetsova et al., 2004) and Estonia (Nielsen, 1986), but the predominant concentration is in the southern and south-eastern North Sea (Wimpenny, 1953; Harding et al., 1978). Plaice is one of the best known and most economically important flatfish in Europe (Pastoors et al., 2000; Dunn and Pawson, 2002; Hoarau et al., 2002). Apart from its commercial potential (Pauly, 1994), plaice is an excellent angling fish. Its abundance, willingness to take suitable bait and above all its edible qualities make it a prized fish (Graham, 1956; Wheeler, 1969).

The plaice is easily identifiable by irregularly distributed bright red or orange spots called ocelli on a warm brown / greenish brown upper surface; the blind side is a clear pearly white and both eyes are on the right side of the head (Cole and Johnstone, 1901; Wheeler, 1969; Frimodt, 1995; Hayward and Ryland, 2002). It is oval in shape, the head and jaws are relatively small and the eyes are moderate in size (Wheeler, 1969, 1978; Hayward and Ryland, 2002). The small mouth is terminal but directed to the right of the eyes, the maxilla reaching to just below the right eye. The body is smooth with small scales and has a series of 4-7 distinctive bony knobs (tubercles) that run in a curved line from the interorbital ridge back to the lateral line, which is slightly curved above the pectoral fin. The pectoral fin is situated immediately below the posterior angle of the operculum. It usually has the same number of fin rays on both sides (about 10) but on the eyeless side one is small and may be overlooked (Cole and Johnstone, 1901).The pelvic fin is jugular in position, slightly in front of the pectoral fin and immediately in front of the anus (Cole and Johnstone, 1901). The dorsal fin commences vertically above the left eye, a short distance behind the left posterior nostril. It extends back to the root of the tail, and is highest about 2/3 of its length from the snout (Cole and Johnstone, 1901).The anal fin is preceded by a spine and the caudal peduncle measures about one third the length of the fin. The plaice has approximately 65-79 dorsal soft rays, 48-59 anal soft rays, 6 pelvic fin rays, 20 caudal fin rays and 42-43 vertebrae (Wheeler, 1969, 1978; Bagenal, 1973; Russel, 1976;

Nielsen, 1986; Hayward and Ryland, 2002; Froese and Pauly, 2008; Hayes et al., 2008). Hybrids have been reported with flounder and dab, and are intermediate in most characteristics between the parent species (Wheeler, 1969; Kijewska et al., 2009).

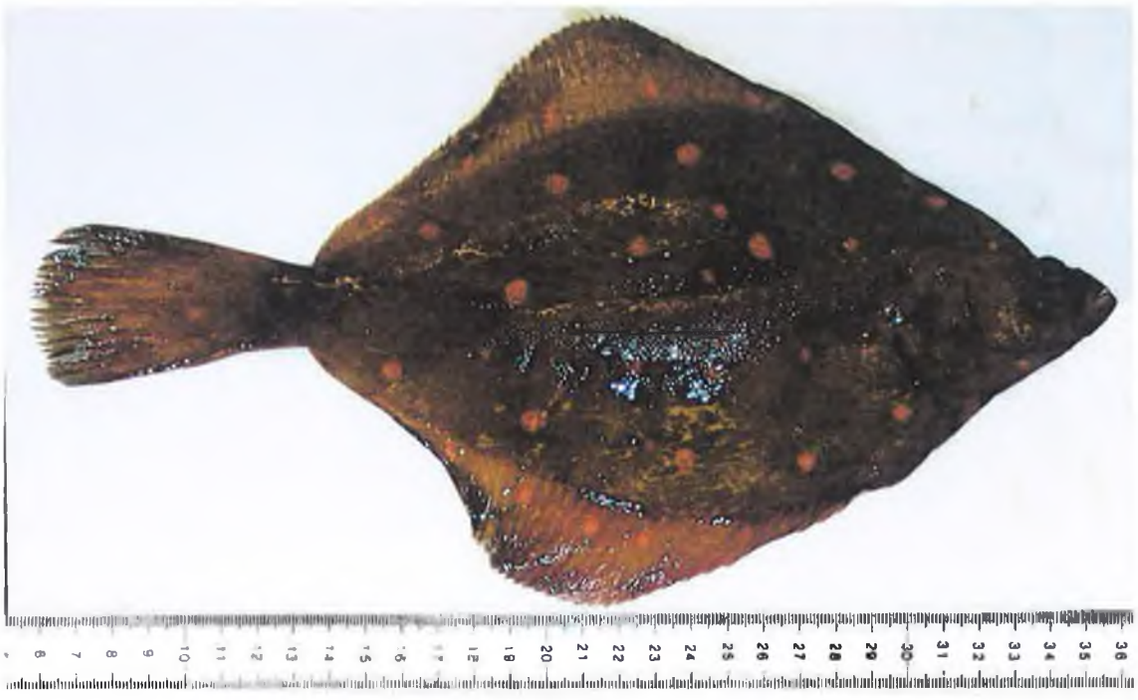

Plate 1. European plaice *Pleuronectes platessa* (Linnaeus, 1758) caught in ICES division VIIb off the west coast of Ireland during the present investigation. (Scale in centimetres).

A typical shelf species, the plaice is a bottom living fish, most abundant on sandy bottoms (Wimpenny, 1953; Graham, 1956), but is also found on muddy bottoms and gravel, in depths of 0-200m (Garstang, 1909; Heincke, 1913; Wimpenny, 1953; Wheeler, 1969; Nielsen, 1986; Rijnsdorp and Van Leewen, 1996). It is most common in 10-50m (Wheeler, 1978). Newly metamorphosed young plaice (c.2cm) are abundant in very shallow inshore waters, from the shoreline to 10m (Ryland, 1966; Wheeler, 1978; de Veen, 1978; Rijnsdorp, 1989; Rijnsdorp and Pastoors, 1995) and are reported to show homing behaviour (Harden-Jones, 1968; de Veen, 1978; Rijnsdorp and Pastoors, 1995; Cooper and Chapleau, 1998; Gibson, 1999; Dunn and Pawson, 2002; Hunter et al., 2003). It is not uncommon to find juveniles in sandy intertidal shore-pools (Wheeler, 1978; Frimodt, 1995).

An oceanadromous fish (Riede, 2004), the plaice is not as tolerant of fresh water as are flounders and it does not penetrate far up estuaries, but occasionally larger plaice may move up into the intertidal area with the flood tide to feed on sand and mud flats (Wheeler, 1969, 1978). It is more active at night or during dawn and dusk, while daytime is spent buried in the sand where it remains stationary for long periods of time (Harder and Hempel, 1954; Bregnballe, 1961; Breckling and Neudecker, 1994; Bos, 1999; Muus and Nielsen, 1999).

Plaice feed from March to December during which time gonad weight and body condition increases (Graham, 1956; Horwood et al., 1986). Spawning usually takes place off the bottom in depths of 20-40m (Wheeler, 1978), from December to March in the North Sea (Simpson, 1951; Wheeler, 1969), from January to April in the Irish Sea (Simpson, 1959; Ellis and Nash, 1997; Fox et al., 2000; Armstrong et al., 2001), between early January and early April in the Celtic sea (Simpson, 1959), from February to March in Danish waters (Wheeler, 1969), in March and April off the south west coast of Iceland (Wheeler, 1969) and as late as June off the Portuguese coasts (Anon, 2001). As with most fish, the breeding cycle of plaice is temperature dependant. The spawning season in plaice usually spans three months (Todd, 1911; Wegner et al., 2003), but spawning of individual fish takes less time (Rijnsdorp et al., 2005). The spawning duration in plaice was estimated as the time elapsed between 50% of females at stage V (oocytes transparent due to hydration, but not yet ovulated) to 50% at stage VII (spent) and stage II (recovering spent) (Iles, 1964).

Tagging experiments have shown that there are a few important spawning grounds on which mature fish congregate and migrate to over some distance (Wheeler, 1969; de Veen, 1978; Sigurdsson, 1989; Rijnsdorp and Pastoors, 1995; Cooper and Chapleau, 1998). One of the most important plaice spawning grounds is in the southern North Sea at depths of around 37m and here many of the vastly important North Sea plaice are spawned. Other important spawning grounds are in Danish waters, off south-west Iceland and in the Irish Sea (Wheeler, 1969). The main spawning areas for plaice in the Irish Sea are off the coast of North Wales between the Isle of Man and Cumbria, and off the north-east coast of Ireland between Dublin in the south and Dundrum Bay in the north (Scott, 1913, 1914 and 1915: Horwood, 1990).

A 35cm female will spawn on average 90,000 eggs (Rijnsdorp et al., 1983). At spawning they are 1.8 to 1.9 mm in diameter (Wheeler, 1969). The eggs float at first and then slowly sink as they mature. After spawning the pelagic eggs and larvae drift with the residual current in the open sea for about 3 to 4 months (Ryland, 1966; Harding et al., 1978; Pastoors et al., 2000). Hatching takes place from 10-30 days at around 6°C but the time varies with local temperature (Apstein, 1909; Wheeler, 1969; Ryland and Nichols, 1975; Harding et al., 1978; Nielsen, 1986; Hyder and Nash, 1998). At hatching the larva is approximately 6.5 mm in length (Wheeler, 1969). Metamorphosis is reached within approximately 40 days of hatching (Ryland, 1966; Nash, 1998; Allen et al., 2008), after which, with the eye now migrated to the right side of the body and other internal changes completed, it swims more to the left (Edwards and Steele, 1968; Zijlstra, 1972). The larvae and post-larvae are surface living for between 4 and 13 weeks (Wegner et al., 2003). At the end of the larval stage, plaice settle in shallow nursery grounds on sandy beaches (Graham, 1956; Wheeler, 1969; 1978; Pihl and Rosenberg, 1982; Van Beek et al., 1989; Modin and Pihl, 1994; Pastoors et al., 2000). Laboratory studies have shown that larvae and early benthic stages of plaice actively select a sediment substratum free of vegetation (Wennhage and Pihl, 1994).

In plaice, the first benthic stage is about 10-17 mm in length (Riley, 1966; Edwards and Steele, 1968; Zijlstra, 1972), and the fish will grow about 5 times in length during its first summer, remaining in shallow water (Pihl et al., 2005). Juvenile plaice may reach lengths of 6-8cm in their first winter, 10-13 cm in their second winter and by then they are living in slightly deeper water (Wheeler, 1969; Rijnsdorp and Pastoors, 1995; Wegner et al., 2003). Those fish that stay in inshore waters for a third winter average 15-20 cm in size (Wheeler, 1969). Almost all plaice in their fourth winter are in deeper water and their average length lies between 25 and 27 cm, a year later they average between 29 and 31 cm (Heincke, 1913; Wheeler, 1969; Hill, 1971; Rogers, 1993). They grow on average 3 or 4 cm a year, until 9 years of age, when the average length is just over 41 cm. Growth rate then typically slows and approximately 1.5 cm a year is added. Exceptionally plaice can grow to 91 cm in length (Wheeler, 1969; Nielsen, 1986), but are more usually around 50 cm (Hayward and Ryland, 2002), with a maximum published weight of 7 Kg (Muus and Dahlström, 1974). The growth rate

of plaice varies from area to area due to a number of factors including the available food, the temperature and the population density.

The plaice is a long lived flatfish, growing quickly early in life, then slowing down in growth rate. Plaice can live up to 30 years (Wheeler, 1978), and exceptionally up to 50 years (Cooper and Chapleau, 1998; Anon, 2001). Females reach sexual maturity at between 3 and 7 years and males between 2-6 years (Wimpenny, 1953; Wheeler, 1969, 1978; Rijnsdorp, 1989; Anon, 2001), depending on the water temperature.

After hatching the larvae do not eat, but once the yolk is absorbed they eat diatoms and minute planktonic larvae (Wheeler, 1969). Later, plaice larvae eat copepods almost exclusively (Wheeler, 1969). After metamorphosis, young plaice feed on small polychaete worms, harpacticoid copepods, amphipods, crab larvae and small molluscs (Mariani et al., 2011). The food organisms eaten by adult plaice are related to what is available on the bottom where it lives, and to the size and condition of the fish (Degel and Gislason, 1988; Piet et al., 1998). The diet of adult plaice is mainly composed of short-lived, highly productive benthic organisms like molluscs, crustaceans, worms (annelida), echinoderms and fish (Wheeler, 1969, 1978; Nielsen, 1986; Frimodt, 1995; Rijnsdorp and Vingerhoed, 2001).

Plaice is mainly caught by otter trawls, as part of a mixed whitefish fishery, and as a bycatch in the beam-trawl fishery for sole (Anon, 2008). It is also caught in bottom trawls, Danish seines, on lines and in set nets (Wheeler, 1969; 1978). The most important fishing ground for plaice is the southern North Sea, and the inshore regions of the central and northern North Sea (Hoarau et al., 2004). Substantial quantities are also taken in Danish waters, the Irish Sea, Icelandic grounds and the Barents Sea (Anon, 2005). Over the years evidence that plaice are overexploited has been accumulated and as the average size of landed plaice has decreased, the fishing effort to land a given unit has increased (Wheeler, 1969). However plaice is not in the IUCN Red List (Baillie et al., 2004) and is therefore not considered a threatened species.

In Ireland, plaice are caught in mixed species otter trawls, beam trawls and Scottish seines (Anon, 2008). To date, the state of plaice stocks off the west coast of Ireland (ICES area VIIb), south-west of Ireland (ICES area VIIj) and the north-west coast of

Ireland (ICES area VIa) are unknown (Anon, 2009), and given the mixed nature of the fisheries catching plaice it is unrealistic to develop a management plan for these stocks (Anon, 2009). Technical measures in force for all stocks include minimum mesh sizes, a minimum landing size, a precautionary TAC (total allowable catch) and restricted areas for certain classes of vessels (Anon, 2008). In ICES area VIIb, VIIj and VIa the minimum legal landing size is 22 cm (Anon, 2008). In ICES area VIIb the agreed TAC for 2009 was 94t with an Irish quota of 74t (Anon, 2008). The 2009 TAC for VIIj stocks was 258t with an Irish quota of 112t and the 2009 TAC for VIa stocks was 668t with an Irish quota of 243t (Anon, 2008). In the Celtic Sea, the minimum legal landing size is 27cm and the agreed TAC for 2009 was 420t with an Irish quota of 173t. Plaice stocks in the Celtic Sea (ICES areas VIIf and VIIg) are considered by ICES to be outside safe biological limits, over fished and classified as being at risk of suffering reduced reproductive capacity (Anon, 2008). ICES advise a 75% reduction in fishing mortality to aid an increase in spawning stock biomass by 2010. Plaice stocks in the Irish Sea (ICES area VIIa) are thought to be at full reproductive capacity and being harvested sustainably. There are no explicit management plans for this stock but the minimum legal landing size is 27cm and the TAC for 2009 was 1430t with an Irish quota of 935t (Anon 2008).

The current study presents data on age, growth, reproductive biology and maturity for plaice (*Pleuronectes platessa* L.) sampled between November 2003 and February 2005 from ICES areas VIa (north-west coast of Ireland), VIIa (Irish Sea), VIIg (Celtic Sea), VIIj (south-west coast of Ireland) and VIIb (west coast of Ireland), (Fig. 1, page 7). This is the first detailed account on the biology of plaice for some of these areas. It is intended that this study will improve understanding of the life cycle of plaice and help fisheries scientists to better predict the effect of fishing effort on Irish plaice stocks.

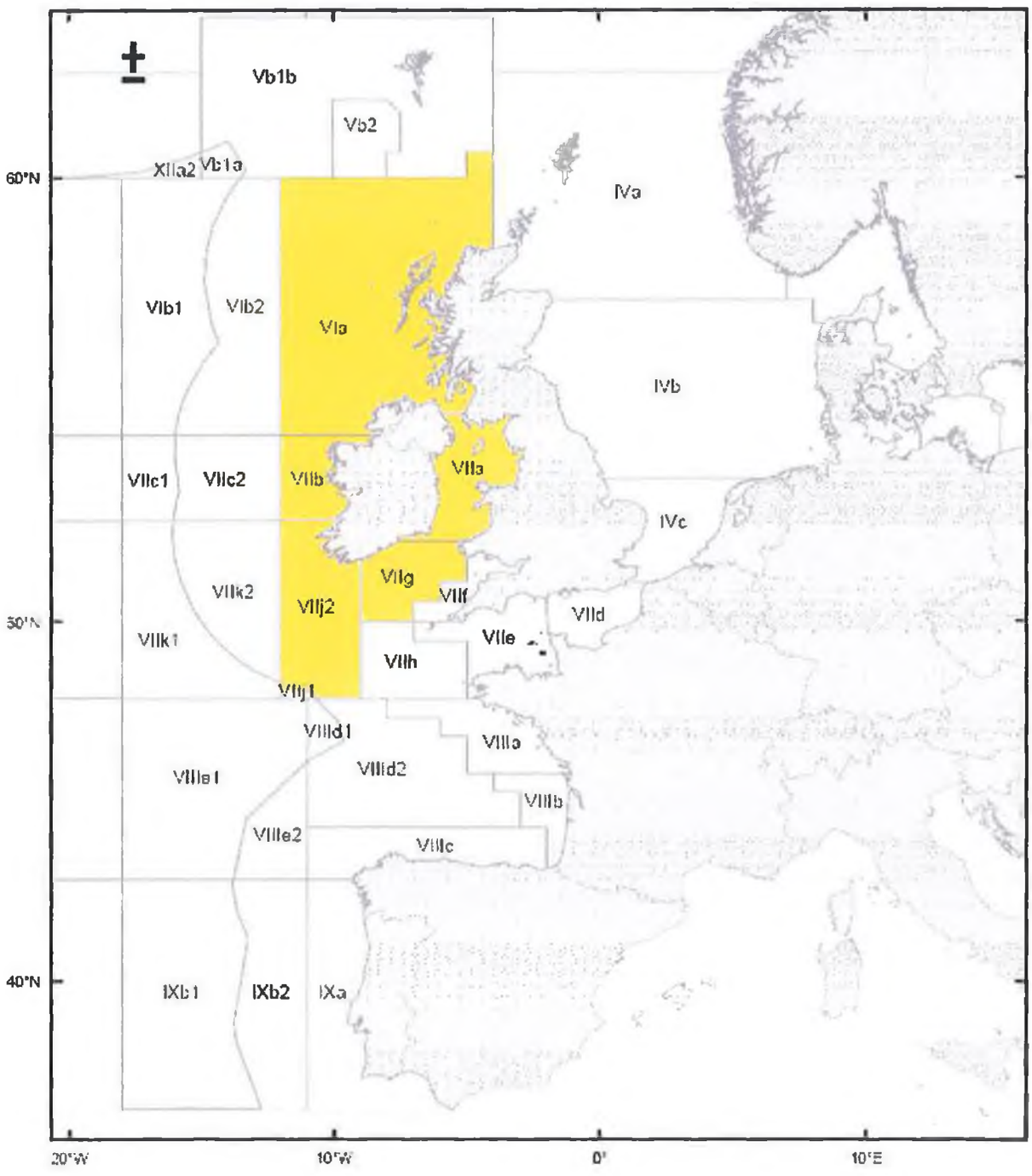

Figure 1. ICES fishing divisions around the Irish coast (ICES 2008). ICES divisions where samples were collected for the present study are highlighted in yellow.

Chapter 1

Age and growth of Plaice, Pleuronectes platessa in Irish waters, 2003-2005.

1.1 Introduction

Fisheries are managed because the consequences of uncontrolled fishing could include fishery collapse, economic inefficiency, loss of employment, habitat loss and decreases in abundance of rare species. The aim of fisheries management is to maximise yield (weight or revenue), maintain a particular level of stock in order to provide a buffer against poor recruitment years and maintain a minimum spawning stock (Jennings et al., 2001). Fish stock assessment is necessary in fisheries management for allocation of catch between competing fisheries, recognition and protection of nursery and spawning areas, and for development of optimal harvesting and monitoring strategies (Kutkuhn, 1981; Grimes et al., 1987; Smith et al., 1990; Hannah et al., 2002).

A fish stock can be defined as a population of a species living in a defined geographical area with similar life-history parameters (Ihssen et al., 1981; Hannah et al., 2002), which remain sufficiently spatially and temporally aggregated to form a self-perpetuating population unit (Dizon et al., 1992; Begg and Waldman, 1999; Metcalfe et al., 2002; Kell et al., 2004). Life history parameters include characteristics such as growth, survival, age at maturation, fecundity, distribution and abundance (Ihssen et al., 1981; Pawson and Jennings, 1996). Identifying stocks, discriminating among them and determining the stock composition of mixed stocks (Waldman, 1999) are important because different stocks of the same species may exhibit differences in one or more life-history parameters (Begg et al., 1999). A thorough understanding of the fisheries biology of any species is necessary to define these biological parameters (Bolle et al., 2004).

The ability to determine the age of a fish is an important tool in fisheries biology as knowledge about the age composition of a population is essential to sustainable resource

management (Bagenal, 1978). Correct age information is necessary for longevity predictions, to establish rates of growth (Caillet et al., 1986), age at maturity and to determine important stages in the life history of a fish population, i.e. age at recruitment and first capture to a fishery (Everhart and Youngs, 1981).

Fish may be aged by the study of seasonal ring formation. This involves counting marks that develop periodically in various hard parts of bony fish (Caillet et al., 1986). Several types of hard parts can be used, for example, scales, vertebrae, spines, opercula, fin rays and otoliths (Williams and Bedford 1974; Caillet et al., 1986). Otoliths and scales are the hard parts most often used in the study of age in bony fishes (Debrosses, 1948; Knudson, 1950; Bowers, 1954; Messtorff, 1959; Gambell and Messtorff, 1964). Otoliths or ear stones are hard calcareous structures in the paired labyrinth systems of teleost fish, located in the cranial bones near the brain (Cailliet et al. 1986), and function as organs of balance. Otoliths are composed of needle-shaped crystals of calcium carbonate radiating outwards in three dimensions from a nucleus and passing through a network of organic material (Williams and Bedford, 1974). The size and shape of the crystals and the angle at which they lie in relation to one another may vary within an otolith. The organic network consists of layers of concentric shells and is not uniformly distributed either throughout the whole otolith or within similar zones (Williams and Bedford, 1974).

There are three pairs of otoliths in each fish, namely the lapillus, sagitta and astericus (Pannella, 1980). As is the case in most teleosts, plaice are aged using the larger sagitta otoliths (Caillet et al., 1986). The plaice otolith is a calcareous and roughly oval body, rather more pointed at the posterior end. It is nearly flat on one side but somewhat convex on the side nearest the brain, where it is scored by a groove running along its axis (Wimpenny, 1953). Ageing by means of otoliths involves counting the annual zones, which are formed throughout the life span of the fish (Lux, 1971). In many species these marks represent seasonal variations (usually winter and summer) in somatic growth rates. An annulus is a concentric zone, band or mark that is a ridge, valley, translucent or opaque zone (Caillet et al., 1986). Opaque zones are laid down during the summer, and translucent / hyaline zones are formed during the winter months, indicating a period of

slow growth (Gambell and Messtorff, 1964; Williams and Bedford, 1974; Caillet et al, 1986). In general, the more extreme the temperature difference between summer and winter, the greater the differences will be in seasonal growth rates and hence the more obvious the annual marks will be. One opaque and one translucent zone correspond to one year and the date of birth is usually taken as January 1st (Blacker, 1974).

Knowledge of population dynamic processes such as growth, mortality and recruitment are important in creating a scientific basis for sustainable exploitation of fisheries resources (Landa et al., 2002). Growth can be defined as the increase in length and weight of a fish over a specific period of time or over an entire lifespan (Everhart and Youngs, 1981). Changes in growth have a profound impact on the productivity of a fish stock (Bolle et al., 2004), influence the sustainable catch weight that can be taken from a stock (King, 1995) and affect the reproductive output of a stock because both maturation and egg production are affected by it (Rothschild, 1986; Rijnsdorp, 1993a; Grift et al., 2003). Therefore, understanding the growth patterns of a stock of fish, in conjunction with age at maturity, mortality rates and reproductive studies, is essential for determining the management strategies for that stock.

Growth in marine fish is influenced by many factors including light, temperature, nutrients, salinity, oxygen, the amount and size of food available, the number of fish using the same food resource, and the size, age and sexual maturity of the fish (Brett and Groves, 1979; Weatherley and Gill, 1987; Rijnsdorp et al., 1991; Rijnsdorp, 1993b; Gibson, 1994; Neill et al., 1994; Van der Veer et al., 1994; Law, 2000; Grift et al., 2003). Temperature is the dominant environmental factor affecting growth rate of fish in the sea. The scope for growth increases up to the optimal temperature, but decreases beyond this optimum (Brett and Groves, 1979; Fonds et al., 1992). In addition growth rate may be under selective pressure from the fishery (Ricker, 1981; Nelson and Soulē, 1987), as removal of large individuals from a population by fishing selects for genotypes with a lower age and size at maturation (Grift et al., 2003).

Size compositions of plaice catches vary among areas (Wimpenny, 1953) and between seasons (Garstang, 1909; Heincke, 1913; Wimpenny, 1953; Rijnsdorp and Leeuwen, 1996) and therefore are not directly representative of the total population (Rijnsdorp et al., 1996). Spatial distribution in plaice is a function of size, with larger fish generally occupying deeper offshore waters and smaller fish preferring shallower inshore waters (Cole et al., 1901; Rijnsdorp and Van Leeuwen, 1992). Estimates of length and age may be biased if this is not taken into account when sampling (Van Leeuwen and Groeneveld, 1988). A reliable index with which to study long-term variations may be obtained from samples collected during the first quarter of the year when adults are congregated on the spawning grounds (de Veen, 1964; Rijnsdorp, 1989).

Most fisheries data are obtained from the analysis of commercial landings where the size of fish caught depends upon the mesh used and only fish above the legal minimum landing size are usually recorded (Horwood, 1993b). Therefore a mathematical model is used to give an estimate of growth. The most common method of assessing the growth patterns of marine fish is by the use of the von Bertalanffy growth equation (von Bertalanffy, 1938). This assumes that fish grow towards some theoretical maximum length (or weight) and that the closer the length (or weight) gets to the maximum size, the slower the rate of change is in size (Rijnsdorp and Pastoors, 1995).

Each year a proportion of the fish alive at the beginning of the year will die from predation, disease or other natural causes. This is natural mortality (M). It is very difficult to get an accurate estimate of natural mortality (M) (Barot et al., 2005), so some studies do not determine values for natural mortality (M), but use an arbitrary value. In this study a value of 0.12 for flatfish was used, as recommended by ICES (Anon, 2003). Although fishing mortality (F) may decrease or increase depending on the exploitation levels of the species, natural mortality (M) rates are likely to remain relatively similar over time. Once the fish reach a size where they can be taken by the fisheries, fishing mortality (F) becomes the major cause of death. Fishing mortality (F) is a measure of the proportion of the stock that is taken by fishing, and can be expressed as a percentage or as a fishing

mortality rate. The combined levels of natural and fishing mortality together lead to the total mortality (Z) (Anon, 1999).

In fisheries studies, recruitment refers to either the addition of new fish to the 'vulnerable' population by growth from smaller size categories (Ricker, 1975; King 1995; Wegner et al., 2003) or entrance of individuals to the area where fishing occurs (Beverton and Holt, 1957). The term is also used to refer to the number of fish recruiting to the stock in a particular year, reaching a certain age or entering the spawning population (Anon, 1999). The number of young fish produced each year varies widely and can have a pronounced effect on the abundance of spawning fish and on commercial catches in subsequent years. Variations in recruitment are caused partly by changes in egg production, particularly when the spawning biomass has fluctuated widely, but also by egg and larval survival, and environmental conditions (Anon, 1999). Recruitment often requires modeling to better understand the population dynamics, with forecasting of recruitment being an essential element of proper fisheries management (Anon, 2003).

The studies reviewed here are the principal age and growth investigations carried out for plaice in Irish and European waters.

Irish Sea plaice age and growth studies.

Daniel and Fleming (1933) undertook plaice marking experiments in the Irish Sea between 1929 and 1931. They reported on time and place of recaptures and discussed the size of fish recaptured and growth patterns between release sites. They found that female plaice released and recaptured, were bigger than the male plaice, although there was little difference in the actual range of lengths covered by each sex. Nash et al., (2000) studied regional variability in the dynamics of reproduction and growth of Irish Sea plaice. They looked at the differences in size-specific fecundity in relation to size/age at maturity and growth for female plaice caught in four regions of the Irish Sea. They found that plaice in the western Irish Sea grow slower, mature later and have lower fecundity than plaice in the eastern Irish Sea. Allen et al., (2008) examined the otolith microstructure of juvenile plaice from two beaches in Galway Bay on the west coast of Ireland. They determined

hatch date, larval duration, settlement date and larval growth rates for 85 fish. They found that hatching occurred from late January to late March, that mean larval duration was 33 days, and that fish settling earlier in the year had a longer larval life. They found that settlement occurred between early March and late April, and concluded that there was no difference in larval growth between nursery areas.

North Sea plaice age and growth studies

Rijnsdorp and Van Beek (1991) looked at changes in growth of plaice in the North Sea between 1957 and 1988 to study possible density dependant effects. They used age group data and Virtual Population Analysis from pre-recruit surveys and commercial fishery samples to estimate indices of potentially competitive biomasses based on Lloyds index of mean crowding. They found that growth increased in age groups 1 to 3 in the 1960's, and that growth of 1 year old plaice began to decrease in the 1980's. They concluded that growth did not show a negative correlation with mean crowding, except in age group 1 of plaice. Rijnsdorp and Van Leeuwen (1992) investigated changes in the somatic growth of female plaice between 1930 and 1985 by back-calculation of otoliths. They found that the growth rate in plaice was reduced at high density both in the juvenile phase and in the adult phase. Rijnsdorp and Pastoors (1995) modelled the spatial dynamics and fisheries of North Sea plaice based on tagging data. Their model provided a powerful tool for exploring the effects of fishing and growth on the spatial dynamics, and the effects of technical measures such as closed areas. Their simulations showed that exploitation substantially affected the spatial distribution patterns of age groups. Due to size dependant migration, exploitation was shown to be size-selective, leading to a lower perceived growth of the surviving population as compared with the true growth of the simulated, unexploited population. Rijnsdorp and Van Leeuwen (1996) examined changes in the growth of North Sea plaice since 1950 in relation to density, eutrophication, beam-trawl effort and temperature. They back-calculated annual length increments for female North Sea plaice, from distances between otolith rings. They found that eutrophication and beam trawling had both affected the growth rate of plaice.

Rijnsdorp and Millner (1996) looked at trends in population dynamics and exploitation of North Sea plaice from the late 1800's to 1993 by using data on catch per unit effort, total

international landings, size at age composition of landings, and growth rates. They concluded that observed changes in both growth rate and exploitation rate in young fish have been important. Wegner et al., (2003) did a literature review of the physical influences on stock dynamics of plaice in the North Sea. They concluded that temperature on the spawning grounds was inversely correlated with year class strength, and that growth depends primarily on temperature. Kell and Bromley (2004) looked at variability in the growth and sexual maturation of North Sea plaice, both through time and between the sexes using data on sexual dimorphism, trends in growth and recruitment, density dependant sexual maturation and discarding. They recorded a change in sex ratio following the introduction of plaice quotas in 1987 and postulated that as males grow more slowly than females they are more likely to be discarded and less likely to be included in the catch statistics. Bolle et al., (2004) investigated growth changes in plaice in the North Sea by comparing (post)-medieval and present day otolith measurements. They noted an increase in growth in the smaller size classes of plaice, and concluded that growth in juvenile plaice may be related to density-dependant processes.

Barents Sea plaice age and growth studies.

Kuznetsova et al., (2004) studied long term variability in the growth rate of Barents Sea plaice using data collected during the plaice fishery in the southern Barents Sea between 1999 and 2001, combined with the literature data for previous periods (Milinsky, 1938; Kovtsova 1976, 1982, 1985). They noted a marked decrease in the body length and age of fish in the commercial stock, a decrease in age at maturity and an increased growth rate, since the beginning of the plaice fishery in 1907.

English plaice age and growth studies.

Millner et al., (1996) examined trends in the growth of plaice stocks in different ICES areas over a period of 20 years by using published data on catch weight at age for these stocks. Analysis of variance was used to examine area, year and age effects. They found significant differences in catch weight-at-age within each area, and noted a decline in weight and growth of plaice over time. They suggest environmental factors and fishing pressures for the changes in growth.

In summary, most of the studies done to date on the age and growth of plaice have been undertaken on the very important North Sea stocks. Very little work has been done on plaice stocks in Irish waters, and the few studies that have been done, were mainly restricted to the Irish Sea. This study will look at plaice in five areas off the coast of Ireland, which correspond with ICES areas VIIb (off the west coast of Ireland), VIa (off the north-west coast of Ireland), VIIa (the Irish Sea), VIIg (the Celtic Sea) and VIIj (off the south-west coast of Ireland). The length, weight and age profiles of plaice in these areas, sampled between November 2003 and February 2005 will be examined and used to shed some light on the growth and mortality of the plaice populations in these areas.

1.2 Materials and Methods

1.2.1 Sampling Procedure

Samples were collected between November 2003 and February 2005 from ICES areas VIIb (west coast of Ireland), VIa (northwest coast of Ireland), VIIa (Irish Sea), VIIg (Celtic Sea) and VIIj (southwest coast of Ireland). Samples consisted of commercial and survey samples.

Commercial samples were taken from catches landed at the fishing ports of Rossaveal, Co. Galway by fishing trawlers operated by the Galway and Aran Fishermen's Co-Op, and the port of Castletownbere Co.Cork by trawlers operated by Castletownbere Fishermen's Co-Op. Commercial samples were caught in twin or single rig otterboard trawls, with a standard mesh size of 80mm at the cod end (Von Brandt, 1984; FAO, 2008). Plaice were captured as a bycatch of the prawn (*Nephrops norvegicus* L.*)* fishery off the west coast of Ireland in ICES division VIIb mainly on the fishing grounds west of the Aran Islands, or in mixed demersal catches as off the south coast (ICES Area VIIg). Fish were not frozen at auction halls, though they were packed in fish boxes which were covered in ice to retain freshness. All samples were dissected fresh.

Survey samples were collected on the Irish Ground Fish Survey (IGFS 2003) and other biological sampling surveys conducted by the Irish Marine Institute on board the R.V. Celtic Voyager and R.V. Celtic Explorer for the same period. Survey samples consisted of commercial size fish and discards (defined as part of the catch returned to the sea as a result of economic, legal or aesthetic considerations (Anon, 2008)). The fish were caught using GOV (*Grande Ouverture Verticale*) and BACA trawls; with a mesh size of 90mm and a 20mm internal liner at the cod end (FAO, 2008).

1.2.2 Sampling Protocol

Total length (TL) measurements (from the tip of the mouth to the extreme edge of the tail) were taken for all fish to the nearest 0.1cm below. Full body weight was measured to the nearest 0.1g on an *AND FA-2000®* top pan electronic balance in the laboratory and on a *POLS* portable marine electronic scales with a 13kg capacity aboard research vessels. Most fish were weighed whole, without surface drying, but some commercial samples were gutted (only viscera removed, gonads left intact). The gutted weight was multiplied by a factor of 1.05 to calculate the whole weight (FAO, 2000; ICES, 2000).

Each fish was sexed by macroscopic examination of the gonads *in situ*, and the maturity stage assessed (further details on this presented in Chapter Two: Reproductive biology and Maturity). Reproductive organs were dissected from the fish by cutting the surrounding membranes and connective tissue and lifting the entire gonad from the reproductive cavity. The gonad was then weighed (wet weight) to the nearest 0.01g using a top pan electronic balance and stored in a vial of 4% buffered formalin. Both gonads were weighed together. The liver was weighed and discarded. The gut was removed from the oesophagus to the anus, weighed to the nearest 0.01g and stored in 4% buffered formalin.

The otoliths were then removed. An incision was made parallel to the lower right eye, along the ridge of bony knobs (tubercles). The two otoliths were removed using a forceps, placed in a petri dish of water, and cleaned of any membranous material before drying. The otoliths were then stored in purpose made otolith boxes. Each box was labeled and stored in a dry, dark container pending further analysis.

1.2.3 Laboratory Analysis

Age readings were taken by examining the otoliths under a *Leica* zoom 2000® stereoscopic microscope using a varied combination of reflected overhead and transmitted lateral light sources, against a black background with the whole otoliths laid flat and immersed in water in a petri dish. Magnifications ranging from 7x to 30x were used on the stereoscopic microscope. Water was used as the immersion medium for all

ageing throughout the study. Leaving the otoliths to soak for several hours prior to ageing lessened the difficulty in ageing the larger otoliths. These represented older fish and normally have more concentric rings present.

Ageing of the otoliths was carried out by looking at the alternate opaque and hyaline zones (Bowers, 1954). These zones radiated out to the otolith edge from the nucleus (opaque zone). Age was determined by counting the number of hyaline bands. Though both the opaque and hyaline zones are used as annual marks, a completed annual ring is often defined as the interface between an inner hyaline and outer opaque zone (Cailliet et. al.1986). As the new opaque zone is just being formed, it can be quite difficult to see. This could lead to it being overlooked, and the fish assigned to an incorrect year class. For this reason an agreed birth date is often given for a species. This date generally coincides with the period when an annual band is formed. January 1st is widely used for flatfish / demersal species with annual rings (Williams and Bedford, 1974). The nature of the outermost edge and the date of capture were taken into consideration when ageing. If, for example, the outermost edge of the otolith was hyaline, it was included in the age count only if the fish was caught after January 1st and before the end of June. If however, the fish was caught between July and the end of December, the outermost hyaline edge would not be included in the ageing of that fish. Thus a fish with a hyaline edge caught in November would be aged as three years of age, whereas if that same fish were caught the following February, it would be aged as a four year old fish.

Quality Control: in order to check the accuracy of the age readings in the present project, an inter-calibration exercise in the ageing of plaice otoliths was carried out at the Fisheries Sciences Services Division of the Irish Marine Institute with technical staff experts. The latter are also active members of international workshops on ageing organized by ICES. The inter-calibration exercise was carried out on 18/06/04 at an early stage in the present investigation.

1.2.4 Statistical Methods

Sex Ratio

The sex ratio for plaice was calculated for each sample and each ICES area at different times of year. The sex ratio was examined in *Excel* using the Chi square equation as follows:

$$X^2 = \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}} + \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

Length

Length frequency distributions were constructed for the fish sampled in this study. The total number of fish present in each length class was determined and the percentages present were then plotted in a histogram or frequency distribution. This was carried out for all male and female fish combined as well as for males and females separately in each sample in each ICES area. From these length frequencies, it was possible to carry out comparisons of length between the sexes, at particular times of the year and in different ICES areas.

Length-Weight Relationship

The length-weight relationship was calculated for all samples combined in each ICES division. The length –weight relationship for plaice represented by the formula $W = q\ TL^b$ (Ricker, 1975) was log transformed into the following: $\ln W = \ln q + b\ (\ln TL)$, where W is the weight of the fish in grams (g), TL is the total length of the fish in centimeters (cm), q is a constant determined empirically and b is the slope of the line / an exponent with a value nearly always between 2 and 4 and often close to 3 (Ricker, 1975; King, 1995). If the value of b=3, it indicates that the fish grows isometrically while values other than 3 indicate allometric growth: if b>3, the fish becomes "heavier for its length"as it grows larger (Ricker, 1971).

Age

Age frequency distributions were calculated in a similar manner to that of the length frequencies, and for the same samples of fish as those used in the length frequencies. This made it is possible to carry out comparisons in age frequency between the sexes, at particular times of the year and in different ICES areas.

Growth

Growth was determined for the total number of males and females separately and the total number of males and females combined for each ICES area. Growth was determined in the form of the von Bertalanffy growth equation (von Bertalanffy, 1938).

$$L_t = L_\infty (1 - e^{-k(t-t0)})$$

Where: L_∞ is the maximum size that the fish would achieve if unaffected by fishing effort, predation, disease and natural mortality; k is the rate at which the fish reaches the limiting size, t is the age of a fish at time t and t_0 is the age at which the fish is theoretically 0mm long (Beverton and Holt, 1957; Ricker, 1975).

The parameters for the von Bertalanffy growth equation were determined by the Ford-Walford plot (Beverton and Holt, 1957) from Ford (1933) and Walford (1946) combined. The Ford-Walford plot was calculated by plotting mean length at age (Lt) against mean length at age plus one year's growth (Lt +1). The straight line fitting this data has a slope of $b = \exp[-k]$ and an intercept on the y axis of $a = L_\infty(1 - \exp[-k])$. These formulas were manipulated to estimate k and L_∞ as follows: $K = -\ln[b]$ and $L_\infty = a / (1-b)$. On the basis of the results of the Ford – Walford plot t_0 can be calculated using the formula:

$$t_0 = t + (1 - k)(\ln[(L_\infty - Lt) / L_\infty])$$

Where: Lt is the mean length at age. The value t_0 is normally small and negative.

Catch Curves

Catch curves (Edser, 1908; Baranov, 1918; Ricker, 1948; Gulland, 1985) were constructed for all fish combined in an ICES area. A plot of the natural logarithms of the number of fish against ages was constructed, resulting in a dome-shaped curve. From the catch curve a value of tr (age at full recruitment) was obtained, as one age group to the right of the peak of the dome (Gulland, 1985).

Mortality Determination

The mortality coefficients of Z (total mortality), M (natural mortality) and F (fishing mortality) as well as S (survivorship) were calculated for plaice in each ICES area. From the previously calculated catch curve, it was possible to estimate some of the mortality coefficients. Since the age group at the peak of the dome may or may not be totally vulnerable to the fishing gear, the portion of the descending leg used to estimate Z (the total mortality coefficient) is shifted one age group to the right of the dome. From the catch curve an estimate of Z is obtained, as the slope of the line. M (natural mortality) is an arbitrary value provided by ICES, and for flatfish it is 0.12, or can be calculated by using the following equation: - ln (0.01)/maximum age, where maximum age corresponds to the oldest fish recorded in the sample. The equation follows the theory that natural mortality (M) is the mortality rate that reduces an unexploited cohort to 1% of its initial size over an entire lifetime. Fishing mortality (F) was determined by taking the value obtained for natural mortality (M) from the value calculated for total mortality (Z), i.e. $Z = F + M$ (Beverton and Holt, 1957), so $F = Z - M$.

% Survivorship

Survivorship (S) was calculated using the following equation; $S = e^{-z}$ where e is the exponential function and z is the value for total mortality.

Mean Life Span

Mean life span (t_{max}) (maximum age calculated) is defined as the time required for 95% of the fish to reach L_∞. This is estimated using the following formula: $t_{max} = 3/K$ (King, 1995).

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Statistical Tests

A range of statistical tests were carried out using the *Minitab 15* computer program on the data obtained during the present investigation. These included F-test for homogeneity of variances, ANOVA tests for analysis of variances, Mann-Whitney, Kruskal-Wallis test, Paired t-Tests, Fishers-Exact tests and General Linear Model analysis of variance to test for differences between samples. Other tests were undertaken in *Excel*, to analyze data such as percentage frequency distributions, mean values, modes, standard deviation, 95% confidence intervals and regression analysis.

1.3 Results

1.3.1 Descriptive Statistics

Over the duration of this investigation, a total of 2342 plaice were examined. Samples were collected between November 2003 and February 2005 from ICES areas VIIb (west coast of Ireland), VIa (northwest coast of Ireland), VIIa (Irish Sea), VIIg (Celtic Sea) and VIIj (southwest coast of Ireland). The samples (Table 1.3.1 (a)) consisted of commercial samples and research survey samples. The samples are not sequential and there were a number of months when samples were not acquired in the present investigation. Descriptive statistics for all plaice collected in Irish waters during the sampling period (2003-2005) are presented in Tables 1.3.1 (b-d).

Table 1.3.1 (a). The number of fish recorded per sample and per ICES division between November 2003 and February 2005.

	Month	Sample	VIa	VIIa	VIIb	VIIg	VIIj	Total
2003	Nov	Research Survey	151	375	109	91	32	758
	Dec							0
2004	Jan							0
	Feb	Commercial			119		106	225
	Mar	Research Survey	114	409	246	54	57	880
	Apr	Research Survey			82			82
	May	Commercial					147	147
	June							0
	July	Commercial			62			62
	Aug							0
	Sept							0
	Oct							0
	Nov	Commercial			43			43
	Dec	Commercial			43			43
2005	Jan	Commercial			48			48
	Feb	Commercial			54			54
		Total	265	784	806	145	342	2342

Table 1.3.1 (b). Descriptive statistics for all plaice and for females and males separately collected in November 2003 and March 2004 in ICES areas VIa (northwest coast of Ireland), VIIa (Irish Sea) and VIIg (Celtic Sea).

DATA	November 2003			March 2004		
	VIa	VIIa	VIIg	VIa	VIIa	VIIg
Sample	survey	survey	survey	survey	survey	survey
No. of fish	151	375	91	114	409	54
Length Range (cm)	15-42.99	11-44.99	19-44.99	9-40.99	11-44.99	14-38.99
Modal Length Group	20-24.99	25-29.99	25-29.99	20-24.99	20-24.99	26
Mean Lt. (cm)	26 ± 0.434	26 ± 0.299	29 ± 0.516	23 ± 0.63	24 ± 0.322	28 ± 0.626
Age Range (years)	1-8	1-12	2-7	1-10	1-12	2-7
Modal Age Group	3	2	3	3	3	3
Distribution	Normal	Normal	Normal	Normal	Not Normal	Normal
Sex ratio (M:F)	1:0.89	1:1.4	1:1.94	1:1.27	1:0.98	1:4.3
No. Female	71	220	60	64	204	44
Length Range (cm)	16-42.99	11-44.99	20-44.99	9-40.99	12-44.99	20-38.99
Modal Length Group	30-34.99	25-29.99	30-34.99	25-29.99	20-24.99	25-29.99
Mean Lt. (cm)	29	29	31	24	26	29
Age Range (years)	1-8	1-12	2-7	1-10	1-12	2-7
Modal Age Group	4	4	3	3	3	3
Mean Age Group	4	4	3	4	4	4
No. Male	80	155	31	50	205	10
Length Range (cm)	15-34.99	13-35.99	19-31.99	11-30.99	11-37.99	14-31.99
Modal Length Group	20-24.99	20-24.99	25-29.99	20-24.99	20-24.99	30-34.99
Mean Lt. (cm)	24	23	25	21	22	25
Age Range (years)	2-5	1-7	2-5	1-6	1-8	2-5
Modal Age Group	3	2	3	3	3	3
Mean Age Group	3	3	3	3	4	3

Table 1.3.1 (c). Descriptive statistics for all plaice and for female and male plaice separately collected over the sampling period in ICES area VIIb (west coast of Ireland).

	2003	2004						2005		
DATA	**NOV**	**FEB**	**MAR**	**APR**	**JULY**	**NOV**	**DEC**	**JAN**	**FEB**	**Total No. Fish**
Sample	survey	commercial	survey	Survey	commercial	commercial	commercial	commercial	commercial	
No. of fish	109	119	246	82	62	43	43	48	54	806
Length Range (cm)	17-40.99	18-47.99	9-37.99	12-41.99	23-38.99	27-36.99	20-34.99	24-36.99	30-35.99	
Modal Length Group	20-24.99	25-29.99	20-24.99	20-24.99	25-29.99	25-29.99	30-34.99	30-34.99	30-34.99	
Mean Lt. (cm)	26 ± 0.422	27 ± 0.475	21 ± 0.356	23 ± 0.585	29 ± 0.432	30 ± 0.298	30 ± 0.376	30 ± 0.453	32 ± 0.233	
Age Range (years)	1-9	2-11	1-8	1-10	2-7	2-10	2-6	2-7	3-7	
Modal Age Group	3	3	3	3	3	4	3	4	4	
Distribution	Not Normal	Not Normal	Normal	Normal	Normal	Not Normal	Not Normal	Normal	Normal	
Sex ratio (M:F)	1:0.59	1:1.6	1:1	1:0.75	1:3.8	1:2.6	1:2.33	1:2.0	1:1.1	
No. Female	40	74	123	35	49	31	30	32	35	449
Length Range (cm)	19-37.99	20-47.99	11-37.99	16-41.99	24-38.99	27-36.99	20-34.99	24-36.99	30-35.99	
Modal Length Group	20-24.99	25-29.99	25-29.99	20-24.99	25-29.99	25-29.99	30-34.99	30-34.99	30-34.99	
Mean Lt. (cm)	27	29	23	24	30	30	31	31	33	
Age Range (years)	1-7	2-11	1-8	1-10	2-7	2-10	2-6	2-7	3-7	
Modal Age Group	3	3	3	3	3	3	5	4	5	
Mean Age Group	4	4	3	3	4	4	5	4	4	
No. Male	69	45	123	47	13	12	13	16	19	357
Length Range (cm)	17-40.99	18-31.99	9-31.99	12-32.99	23-34.99	27-30.99	27-31.99	25-31.99	30-34.99	
Modal Length Group	20-24.99	20-24.99	20-24.99	25-29.99	25-29.99	25-29.99	25-29.99	25-29.99	30-34.99	
Mean Lt. (cm)	25	24	20	22	28	29	30	28	32	
Age Range (years)	2-9	2-5	1-8	1-10	2-5	2-5	2-6	3-7	3-7	
Modal Age Group	3	3	2	3	3	4	3	4	4	
Mean Age Group	4	3	3	3	4	4	4	3	5	

Table 1.3.1 (d). Descriptive statistics for all plaice and for females and males separately collected in November 2003 and March 2004 in ICES area VIIj (South-West coast of Ireland).

	2003	2004			
DATA	**NOV**	**FEB**	**MAR**	**MAY**	**Total No. Fish**
Sample	survey	commercial	survey	commercial	
No. of fish	32	106	57	147	342
Length Range (cm)	22-41.99	19-51.99	16-36.99	16-47.99	
Modal Length Group	30-34.99	25-29.99	25-29.99	20-24.99	
Mean Lt. (cm)	31 ± 0.684	28 ± 0.61	26 ± 0.59	25 ± 0.454	
Age Range (years)	2-6	1-14	2-5	2-16	
Modal Age Group	3	3	3	3	
Distribution	Normal	Not Normal	Normal	Not Normal	
Sex ratio (M:F)	1:3.5	1:0.6	1:1.1	1:1.3	
No. Female	25	40	30	83	178
Length Range (cm)	27-41.99	19-51.99	17-36.99	17-47.99	
Modal Length Group	30-34.99	25-29.99	25-29.99	25-29.99	
Mean Lt. (cm)	32	31	27	27	
Age Range (years)	2-6	3-14	3-5	2-16	
Modal Age Group	3	4	3	4	
Mean Age Group	3	5	4	4	
No. Male	7	66	27	64	164
Length Range (cm)	22-32.99	19-36.99	16-32.99	16-32.99	
Modal Length Group	25-29.99	25-29.99	25-29.99	20-24.99	
Mean Lt. (cm)	27	26	25	23	
Age Range (years)	3	1-7	2-5	2-6	
Modal Age Group	3	3	4	3	
Mean Age Group	3	4	3	3	

Table 1.3.1 (b) presents length and age data for plaice collected in November 2003 and March 2004 in ICES areas VIa (northwest coast of Ireland), VIIa (Irish Sea) and VIIg (Celtic Sea). These are the only months in which samples were collected in these areas, and all samples are research survey samples. In ICES areas VIIa and VIIg the November and the March samples had similar length ranges, but in ICES area VIa the March 2004 sample had a larger length range even though it was a smaller sample than the November 2003 sample. In all areas the mean lengths of males were smaller than females in both November 2003 and March 2004. The largest mean length (31cm) was recorded for females in ICES area VIIg, in the November 2003 sample, and the smallest mean length (21cm) was recorded for males in ICES area VIa in the March 2004 sample.

Table 1.3.1 (c) presents length and age data for plaice collected over the sampling period in ICES area VIIb (west coast of Ireland). The greatest length ranges were recorded in the February 2004 commercial sample (18-47.99 cm TL) and the April 2004 survey sample (12-41.99 cm TL). The smallest length range (30-35.99 cm TL) was recorded for the February 2005 commercial sample. The mean lengths of males were smaller than females in all samples. The largest mean length (33cm) was recorded for females in the February 2004 (commercial) sample, and the smallest mean length (20cm) was recorded for males in the March 2004 (survey) sample.

Table 1.3.1 (d) presents length and age data for plaice collected in November 2003 and February, March and May 2004 in ICES area VIIj (southwest coast of Ireland). These are the only months in which samples were collected in this area. The greatest length ranges were recorded in the February 2004 commercial sample (19-51.99 cm TL) and the May 2004 commercial sample (16-47.99 cm TL). The smallest length range (22-41.99 cm TL) was recorded for the November 2003 survey sample. The mean lengths of males were smaller than females in all samples. The largest mean length (32cm) was recorded for females in the November 2003 (survey) sample, and the smallest mean length (23cm) was recorded for males in the May 2004 (commercial) sample.

1.3.2 Sex Ratios

Using Chi Square (χ^2) analysis the sex ratio was tested for each sample in each ICES area and for all the samples combined within an ICES area, collected between November 2003 and February 2005 (Table 1.3.2 (b)). The sex ratios calculated for ICES areas VIa and VIIj showed no significant departure from a 1:1 sex ratio. The overall sex ratios calculated for ICES areas VIIa, VIIb and VIIg showed some departure from a 1:1 sex ratio. Overall, females were slightly the numerically dominant sex (Table 1.3.2 (a)). The results of Chi Square (χ^2) analysis testing the null hypothesis (H_0) of no departure from a 1:1 ratio, and the significance levels are shown in the same table.

Table 1.3.2 (a). The sex ratio of all plaice sampled in each ICES area during the sampling period, together with Chi Square (χ^2) values. (* = significant (P<0.05), ** = highly significant (P<0.01), N.S. = not significant (P>0.05)).

Area	No. Male	No. Female	χ^2 Value	P Value	Significance	Sex Ratio (M:F)
VIIb	353	443	7.68	0.010	*	1 : 1.2
VIa	130	135	0.13	0.900	N.S.	1 : 1.0
VIIa	360	424	5.22	0.050	*	1 : 1.2
VIIg	41	104	27.4	0.001	* *	1 : 2.6
VIIj	164	178	0.58	0.500	N.S.	1 : 1.1

Table 1.3.2 (b). Sex Ratio recorded per sample and per ICES area for Plaice collected between November 2003 and February 2005, together with Chi Square (X^2) values.
(*= significant (P<0.05), * * = highly significant (P <0.01), N.S = Not Significant (P >0.05))

Sample date	ICES area	Commercial / Survey	Number Females	Number Males	Total	Expected	X^2	Significance	Ratio M : F
November 2003	VIa	survey	71	80	151	75.5	0.54	N.S	1:0.89
March 2004	VIa	survey	64	50	114	57	1.72	N.S	1:1.27
Combined	**VIa**	**survey**	**135**	**130**	**265**	**133**	**0.13**	**N.S**	**1:1.04**
November 2003	VIIa	survey	220	155	375	187.5	11.2	* *	1:1.4
March 2004	VIIa	survey	204	205	409	204.5	0.002	N.S	1:0.98
Combined	**VIIa**	**survey**	**424**	**360**	**784**	**392**	**5.22**	*	**1:1.17**
November 2003	VIIb	survey	40	69	109	54.5	7.72	*	1:0.59
February 2004	VIIb	Commercial	74	45	119	59.5	7	*	1:1.6
March 2004	VIIb	survey	158	170	328	164	0.44	N.S	1:0.9
July 2004	VIIb	commercial	49	13	62	31	21	* *	1:3.8
November 2004	VIIb	commercial	31	12	43	21.5	8.4	* *	1:2.6
December 2004	VIIb	commercial	30	13	43	21.5	6.8	* *	1:2.33
January 2005	VIIb	commercial	32	16	48	24	5.4	*	1:2.0
February 2005	VIIb	commercial	21	19	40	20	0.1	N.S	1:1.1
Combined	**VIIb**	**C + S**	**435**	**357**	**792**	**396**	**7.68**	*	**1:1.22**
November 2003	VIIg	survey	60	31	91	45.5	9.2	* *	1:1.94
March 2004	VIIg	survey	44	10	54	27	21.4	* *	1:4.3
Combined	**VIIg**	**survey**	**104**	**41**	**145**	**72.5**	**27.4**	* *	**1:2.6**
November 2003	VIIj	survey	25	7	32	16	10.2	* *	1:3.5
February 2004	VIIj	Commercial	40	66	106	53	6.4	* *	1:0.6
March 2004	VIIj	survey	30	27	57	28.5	0.16	N.S	1:1.1
May 2004	VIIj	Commercial	83	64	147	73.5	2.46	N.S	1:1.3
Combined	**VIIj**	**C + S**	**178**	**164**	**342**	**171**	**0.58**	**N.S**	**1:1.1**

1.3.3 Length Frequency

The percentage length frequency distributions for male and female plaice sampled in November 2003 and March 2004 in ICES areas VIa, VIIb, a, g and j are presented in Fig 1.3.3 (a). The percentage length frequency distribution for male and female plaice sampled in ICES area VIIb throughout the sampling period is presented in Fig 1.3.3 (b).

There was a similar range of length (cm) classes for males and females. However a larger median length (cm) size was recorded for female fish when compared to males in each sample location. In order to choose a suitable statistical test to investigate whether the median length values of the sampled plaice differed between males and females, an investigation of the null hypothesis (H_0) that the lengths followed a normal distribution for both males and females was firstly carried out. The H_0 was accepted ($R=0.994$, $P>0.05$). A test for homogeneity of variances (F-test) was then carried out to determine if there was a difference in sample variances. It was determined that there was a difference in the sample variances ($F=1.46$, $P=0.000$). A Mann-Whitney test for samples with unequal variances was then calculated on the data. The null hypothesis (H_0) that there was no difference between median lengths of males and females was rejected as $P<0.05$. Therefore there was a significant difference ($P<0.05$) in median length values for male and female plaice in each ICES area.

Males sampled in ICES area VIIg had the largest median length (26cm) while males sampled in ICES area VIIa had the smallest median length (23cm). The male samples were determined to have unequal variances ($P<0.05$), so a Kruskal-Wallis test was calculated on the data and the null hypothesis (H_0) that there was no difference between median lengths of males between ICES areas was rejected ($H=28.00$, $P=0.000$, D.F. = 4).

A Fishers Exact test was then carried out to show how significantly different the median lengths of samples were from each other. The results are presented in Table 1.3.3 (a). The Fishers Exact test showed that samples collected in ICES areas VIa, VIIa and VIIb are significantly different from samples collected in ICES areas VIIg and VIIj. Samples collected in ICES areas VIIg and VIIj are not significantly different from each other. Samples collected in ICES area VIa are not significantly different from VIIa and VIIb. Samples collected in ICES areas VIIb and VIIa are significantly different from each other.

Table 1.3.3 (a). Results of the Fishers Exact test for male plaice between ICES areas. (S.D. = Significant difference, N.S. = No significant difference).

	VIa	VIIa	VIIb	VIIg
VIIa	N.S			
VIIb	N.S	S.D		
VIIg	S.D	S.D	S.D	
VIIj	S.D	S.D	S.D	N.S

Females sampled in ICES area VIIg had the largest median length (30cm) while females sampled in ICES area VIa had the smallest median length (27cm). The female samples were determined to have unequal variances ($P<0.05$), so a Kruskal-Wallis test was calculated on the data and the null hypothesis (H_0) that there was no difference between median lengths of females between ICES areas was rejected ($H = 24.27$, $P = 0.000$, D.F. = 4).

A Fishers Exact test was then carried out to show how significantly different the median lengths of samples were from each other. The results are presented in Table 1.3.3 (b). The Fishers Exact test showed that samples collected in ICES areas VIa, VIIa and VIIb are not significantly different from each other, but they are significantly different from samples collected in ICES areas VIIg and VIIj. Samples collected in ICES area VIIj are significantly different from samples collected in ICES areas VIIg, VIa and VIIb, but not VIIa.

Table 1.3.3 (b). Results of the Fishers Exact test for female plaice between ICES areas. (S.D. = Significant difference, N.S. = No significant difference).

	VIa	VIIa	VIIb	VIIg
VIIa	N.S			
VIIb	N.S	N.S		
VIIg	S.D	S.D	S.D	
VIIj	S.D	N.S	S.D	S.D

Figure 1.3.3 (a). Percentage length frequency distribution for male and female plaice in ICES areas VIa, VIIa, VIIb, VIIg and VIIj, sampled in November 2003 and March 2004. (N is the number of fish sampled).

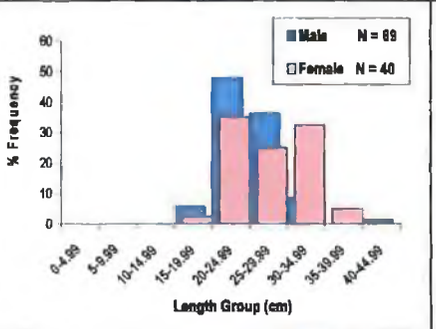
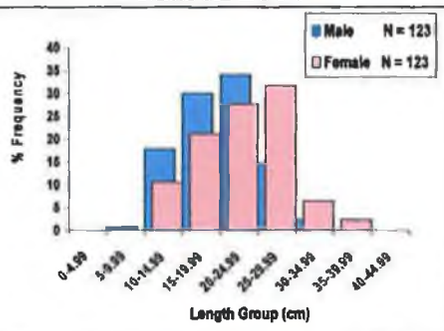
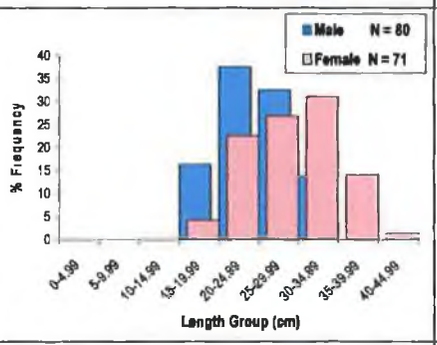
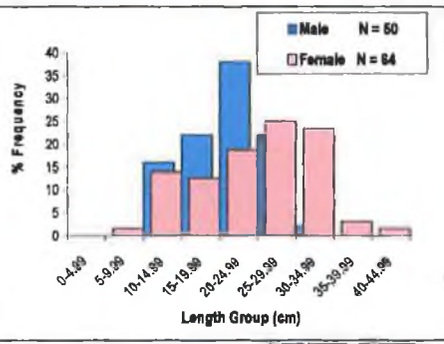
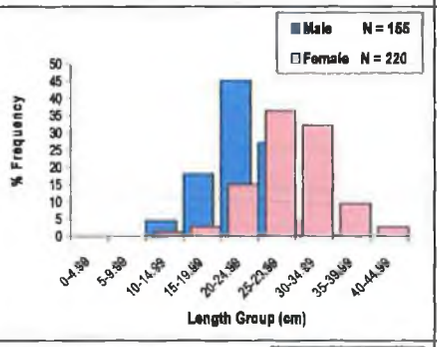
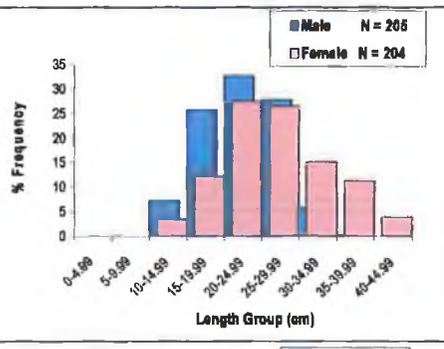
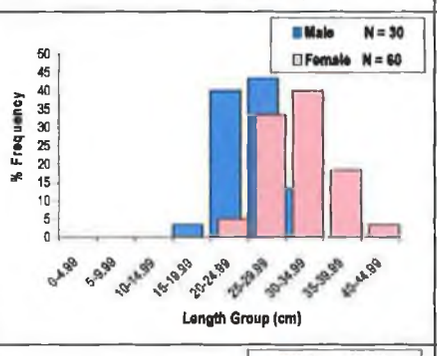
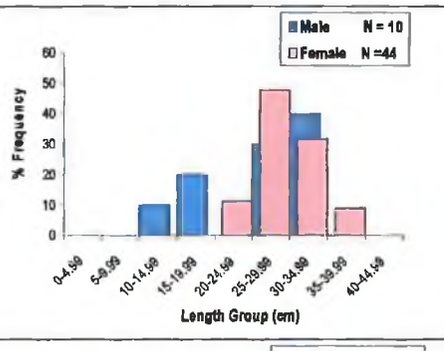
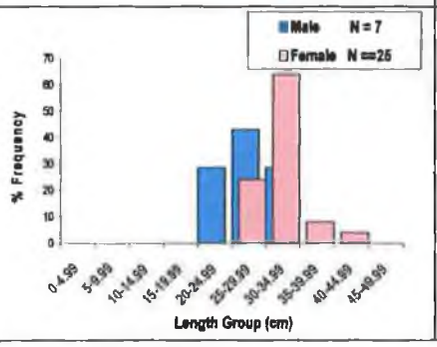
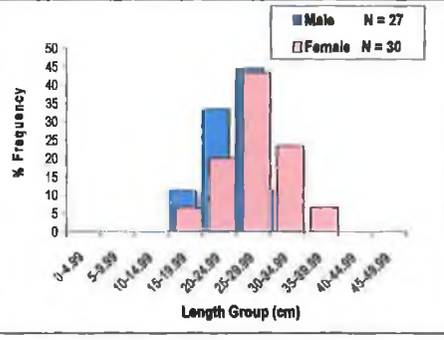
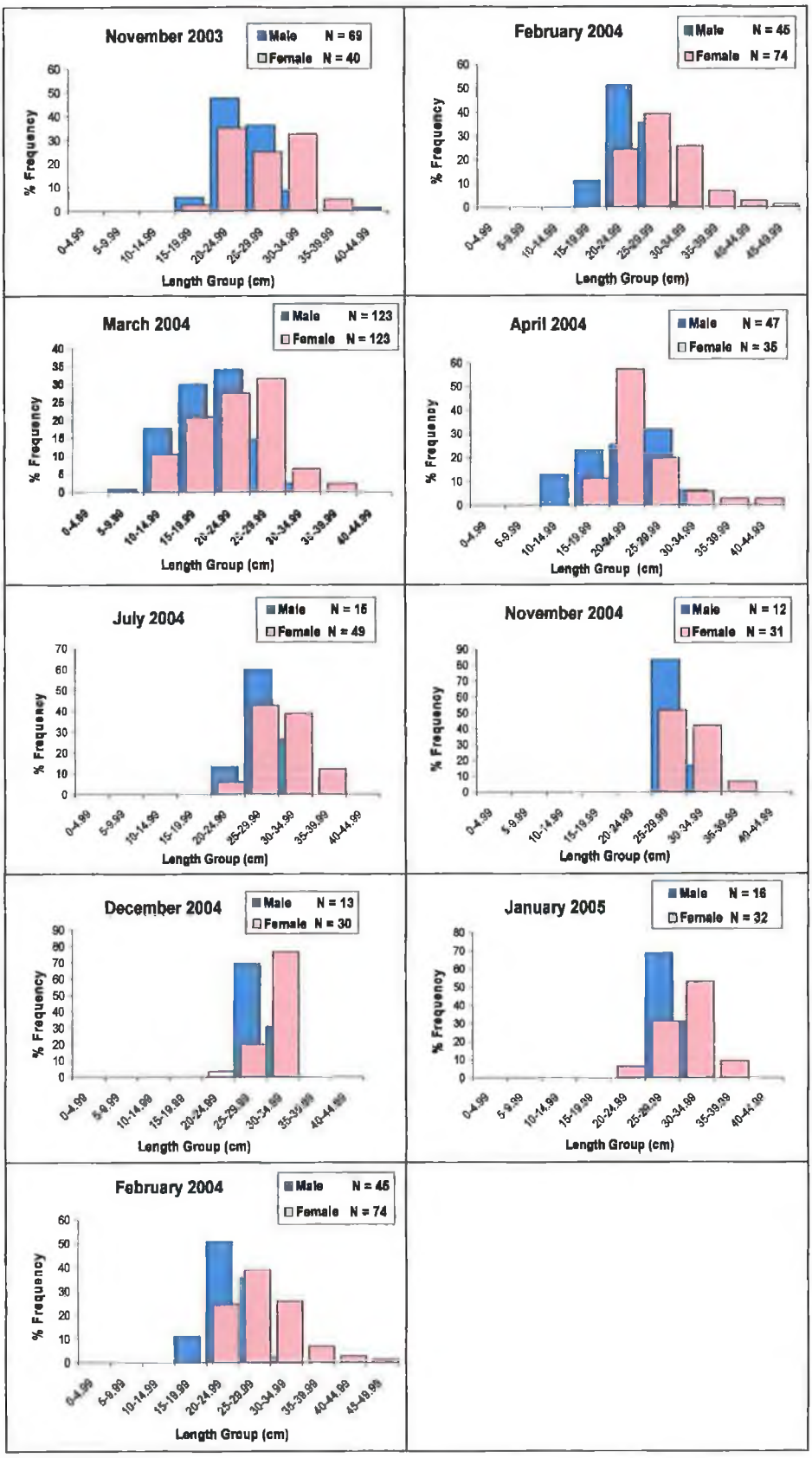
Month	November 2003	March 2004
VIIb **west coast of Ireland**		
VIa **northwest coast of Ireland**		
VIIa **The Irish Sea**		
VIIg **The Celtic Sea**		
VIIj **southwest coast of Ireland**		

Figure 1.3.3 (b). Percentage length frequency distribution for male and female plaice in each sample collected in ICES area VIIb during the sampling period. (N is the number of fish sampled).



1.3.4 Length - Weight Relationship

The linear relationship between $\log_e$ body weight (W) and total length (TL) was determined for the overall sampled population and for males and females separately in ICES areas VIa, VIIa, b, g and j using regression analysis, and is presented in Figures 1.3.4 (a-c). The length - weight relationship and R^2 value for each ICES area is presented in Table 1.3.4 (a). For all plaice and males and females separately collected in each ICES area the relationship between $\log_e$ total length and $\log_e$ body weight was highly significantly correlated. As the value for b was close to 3, growth was determined to be allometric for plaice, i.e. the fish becomes 'heavier for its length' as it grows larger. A General Linear Model analysis was calculated on the data to see if there was a significant difference in the length-weight relationship of males and females between ICES areas. The null hypothesis (H_0) that there was no difference in the length-weight relationship between ICES areas was rejected for males (P=0.000) and females (P=0.031).

Table 1.3.4 (a). Length weight relationship and equation of the line values for plaice collected in ICES areas VIa, VIIa, VIIb, VIIg and VIIj during the sampling period.

ICES area		Equation Of Line Values			Length-Weight Relationship
		a	b	R2	W(g)= qTLb
VIa	Male	4.7523	3.0395	0.976	$W(g)=0.0086TL\ (cm)^{3.040}$
	Female	4.7913	3.0523	0.989	$W(g)=0.0083TL\ (cm)^{3.052}$
	All plaice	4.7792	3.0484	0.985	$W(g)=0.0084TL\ (cm)^{3.048}$
VIIa	Male	4.4051	2.9201	0.968	$W(g)=0.0122TL\ (cm)^{2.920}$
	Female	4.9882	3.1261	0.971	$W(g)=0.0068TL\ (cm)^{3.126}$
	All plaice	4.9129	3.0951	0.973	$W(g)=0.0074TL\ (cm)^{3.095}$
VIIb	Male	4.9136	3.0846	0.975	$W(g)=0.0073TL\ (cm)^{3.085}$
	Female	5.2441	3.1889	0.977	$W(g)=0.0053TL\ (cm)^{3.189}$
	All plaice	5.5603	3.2633	0.974	$W(g)=0.0038TL\ (cm)^{3.263}$
VIIg	Male	4.8933	3.0874	0.978	$W(g)=0.0075TL\ (cm)^{3.087}$
	Female	5.1374	3.1543	0.953	$W(g)=0.0059TL\ (cm)^{3.154}$
	All plaice	4.9482	3.1001	0.969	$W(g)=0.0071TL\ (cm)^{3.100}$
VIIj	Male	5.3835	3.202	0.972	$W(g)=0.0046TL\ (cm)^{3.202}$
	Female	5.4956	3.249	0.969	$W(g)=0.0041TL\ (cm)^{3.249}$
	All plaice	5.5603	3.2633	0.974	$W(g)=0.0038TL\ (cm)^{3.263}$

Figure 1.3.4 (a). Log_e body weight versus log_e total length for all plaice sampled in ICES areas VIa, VIIa, b, g and j between November 2003 and February 2005. (N is the number of fish sampled).

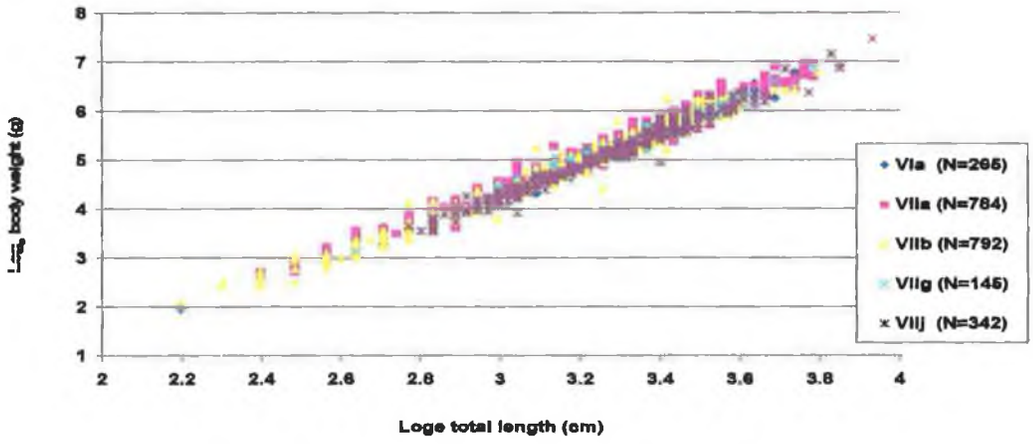


Figure 1.3.4 (b). Log_e body weight versus log_e total length for female plaice sampled in ICES areas VIa, VIIa, b, g and j between November 2003 and February 2005. (N is the number of fish sampled).

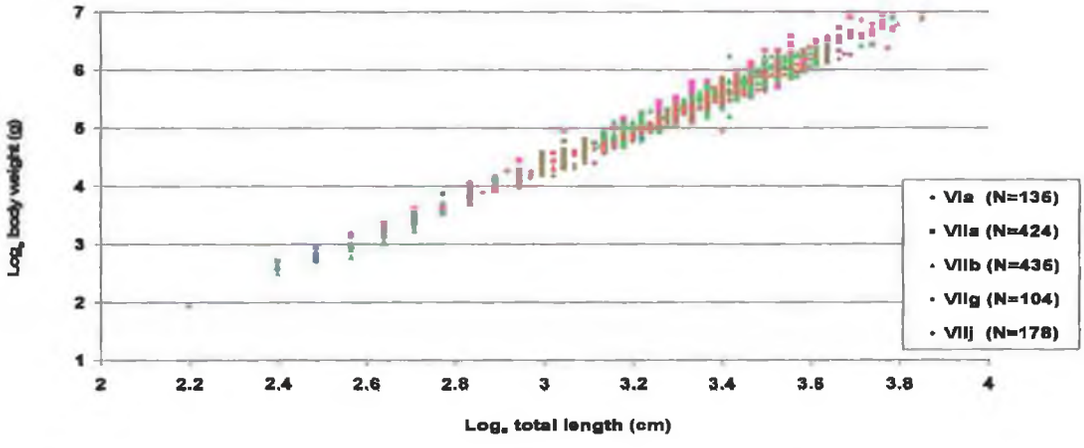
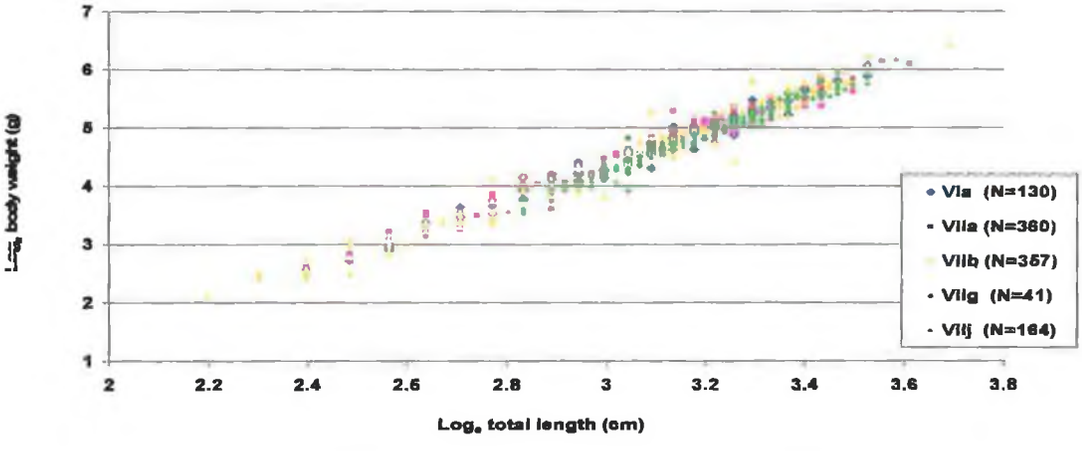


Figure 1.3.4 (c). Log_e body weight versus log_e total length for male plaice sampled in ICES areas VIa, VIIa, b, g and j between November 2003 and February 2005. (N is the number of fish sampled).



1.3.5 Length and Weight at Age

Age-Length keys for male and female plaice in each ICES area are presented in Appendix 1. Table 1.3.5 (a) presents mean length and weight at age data for males and females collected in each ICES area between November 2003 and February 2005. Mean length at age and mean weight at age graphs for male and female plaice sampled in each ICES area during the sampling period are presented in Figures 1.3.5 (a-d).

In ICES areas VIIb, VIIg and VIIj females showed a larger mean length and larger mean weight than males at all age groups. A General Linear Model analysis was calculated on the data and the null hypothesis (H_0) that the females were not significantly heavier or longer at a given age than males was rejected as $P<0.05$. Therefore females were significantly heavier and longer at a given age than males in these areas.

In ICES area VIa females showed a larger mean length at all ages except age 4, where males and females had a similar mean length at age. Males were heavier than females at ages 1 and 2 but after that females appear to be significantly heavier than males. A General Linear Model analysis was calculated on the data and the null hypothesis (H_0) that the females were not significantly heavier or longer at a given age than males was rejected ($P=0.020$).

In ICES area VIIa males showed a slightly greater mean length up to age 5, then the mean lengths were similar up until age 8, after which females appeared larger again. Males were heavier than females at age 1 but after that females appear to be significantly heavier than males. A General Linear Model analysis was calculated on the data and the null hypothesis (H_0) that the females were not significantly longer or heavier at a given age than males was rejected ($P=0.000$).

Table 1.3.5 (a). Mean length and weight at age data for male and female plaice in the populations sampled in ICES areas VIa, VIIa, b, g and j between November 2003 and February 2005.

ICES area	Sex	Age	1	2	3	4	5	6	7	8	9	10	11
VIIb	Female	Mean Lt. (cm)	16	21	26	28	30	32	33	32	44	39	47
		Mean Wt. (g)	48	103	193	253	287	355	364	352	887	504	0
	Male	Mean Lt. (cm)	12	18	24	26	28	28	31	31	28	0	0
		Mean Wt. (g)	16	72	144	182	222	245	289	313	214	0	0
VIa	Female	Mean Lt. (cm)	24	26	26	25	30	28	23	26	21	0	0
		Mean Wt. (g)	32	78	165	243	302	447	373	524	0	0	0
	Male	Mean Lt. (cm)	18	19	23	25	29	26	0	0	0	0	0
		Mean Wt. (g)	60	85	132	164	233	136	0	0	0	0	0
VIIa	Female	Mean Lt. (cm)	15	23	25	28	31	33	35	37	35	40	44
		Mean Wt. (g)	39	164	184	261	318	399	456	538	493	996	804
	Male	Mean Lt. (cm)	17	26	27	29	31	33	35	35	0	0	0
		Mean Wt. (g)	58	83	113	145	166	195	215	300	0	0	0
VIIg	Female	Mean Lt. (cm)	0	26	29	31	33	35	39	0	0	0	0
		Mean Wt. (g)	0	175	272	306	360	430	663	0	0	0	0
	Male	Mean Lt. (cm)	0	22	26	25	31	0	0	0	0	0	0
		Mean Wt. (g)	0	102	190	183	266	0	0	0	0	0	0
VIIj	Female	Mean Lt. (cm)	0	27	26	28	32	37	38	39	0	38	0
		Mean Wt. (g)	0	230	180	218	333	622	500	517	487	1752	1050
	Male	Mean Lt. (cm)	23	21	24	26	28	31	31	0	0	0	0
		Mean Wt. (g)	0	86	143	164	197	275	264	0	0	0	0

Figure. 1.3.5 (a). Mean length at age for male plaice sampled in ICES areas VIa, VIIa, VIIb, VIIg and VIIj between November 2003 and February 2005 (N is the number of fish sampled).

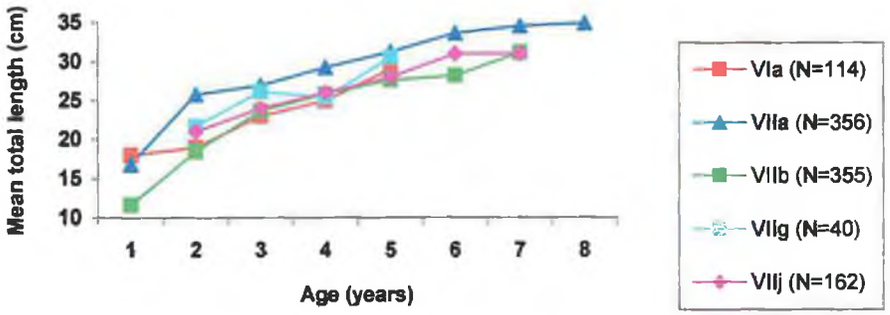


Figure. 1.3.5 (b). Mean length at age for female plaice sampled in ICES areas VIa, VIIa, VIIb, VIIg and VIIj between November 2003 and February 2005 (N is the number of fish sampled).

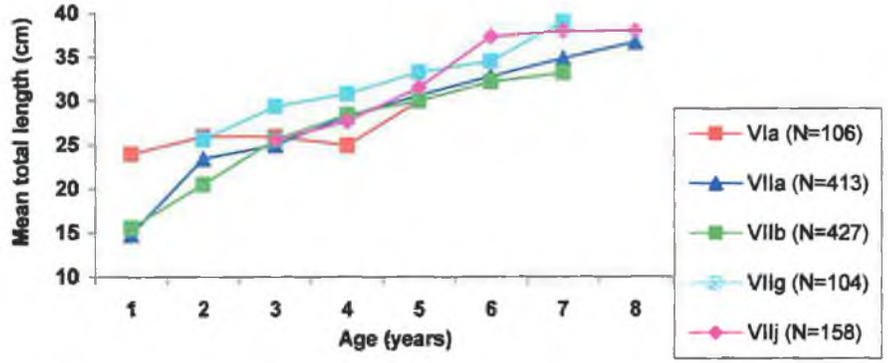


Figure. 1.3.5 (c).Mean weight at age for male plaice sampled in ICES areas VIa, VIIa, VIIb, VIIg and VIIj between November 2003 and February 2005 (N is the number of fish sampled).

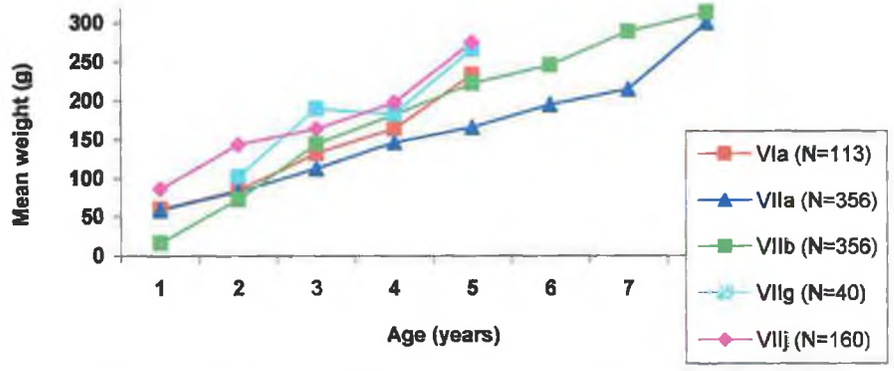
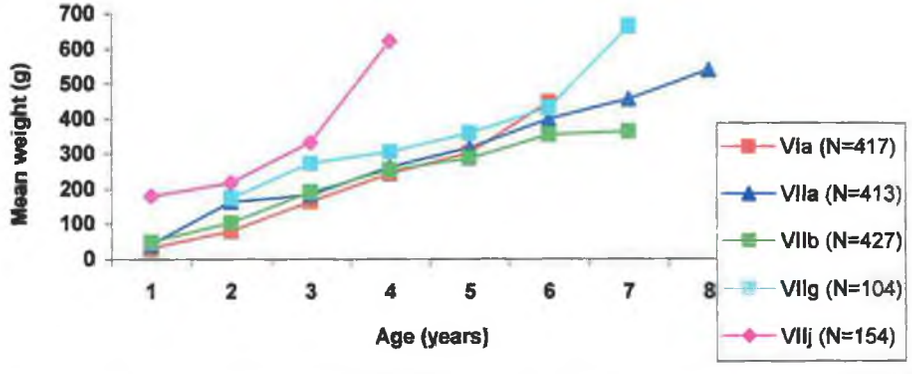


Figure. 1.3.5 (d). Mean weight at age for female plaice sampled in ICES areas VIa, VIIa, VIIb, VIIg and VIIj between November 2003 and February 2005 (N is the number of fish sampled).



1.3.6 Age

Paired sagittal otoliths were examined and aged for ICES areas VIIb (790 pairs), VIa (228 pairs), VIIa (777 pairs), VIIg (144 pairs) and VIIj (341 pairs). The range, median and modal age groups for male and female plaice in each sample in each ICES area are presented in Table 1.3.1(a-c). Figure 1.3.6 (a) presents the percentage age frequency distribution of male and female plaice for all areas sampled in November 2003 and March 2004. These months were selected for comparison because they were the only months for which samples from all ICES areas studied were available.

The oldest male was 10 years old and recorded in the April 2004 survey sample from ICES area VIIb. The oldest female was 16 years old and recorded in the May 2004 commercial sample from ICES area VIIj. In both November 2003 and March 2004 ICES area VIIa had the largest range in ages, ICES area VIIj had the smallest range in ages and females had a larger range of ages than males. In both sampling periods females in ICES area VIIa had the largest range of ages and females in ICES area VIIj had the smallest. In November 2003 males in ICES area VIIb had the largest range in ages and males in ICES areas VIIa and VIIg had the smallest. In March 2004 males in ICES areas VIIa and VIIb had the largest range and males in ICES areas VIIj and VIIg had the smallest range. All November 2003 and March 2004 samples were survey samples. In November 2003 and March 2004 in each ICES area, the modal age group varied from age three to age four for females, and from age two to age four for males.

ICES area VIIb (West coast of Ireland)

Fig 1.3.6 (b) presents the percentage age frequency distribution of male and female plaice for all samples collected during the sampling period in ICES area VIIb. Larger ages were grouped as the numbers of fish were very low. In ICES area VIIb the February 2004 commercial and April 2004 survey samples had the largest range in ages. The December 2004 and February 2005 commercial samples had the smallest range in ages. Females had a larger range in ages than males in all samples except the November 2003 survey sample. For female plaice sampled in ICES area VIIb the modal age group was 3 years throughout the sampling period, with the exception of samples collected in December 2004, January 2005 and February 2005 (modal age group = 5). These latter samples were obtained from Galway Bay Seafood's Ltd., and the range in ages for these samples was much smaller as the samples were pre-sorted by size. For males the modal age group for all samples ranged from two to four years.

Figure 1.3.6 (a). Percentage age frequency distribution for male and female plaice in ICES areas VIa, VIIa, b, g and j sampled in November 2003 and March 2004. (N is the number of fish sampled).

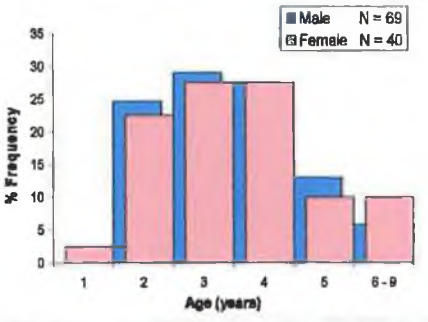
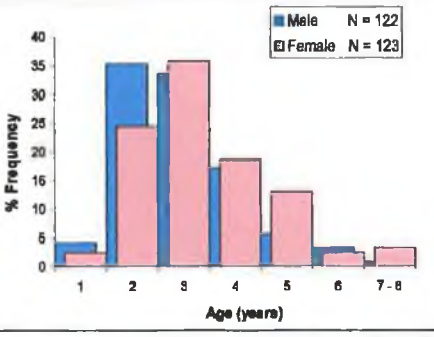
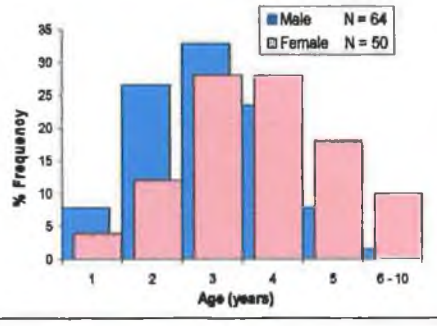
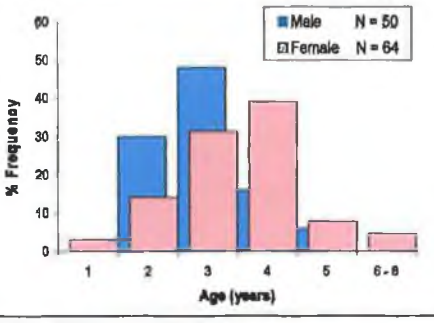
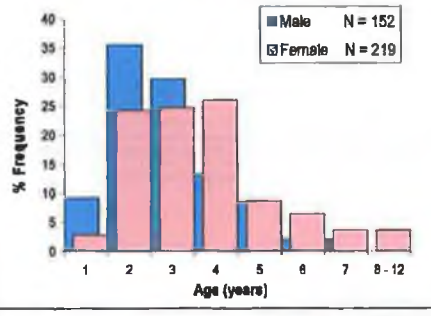
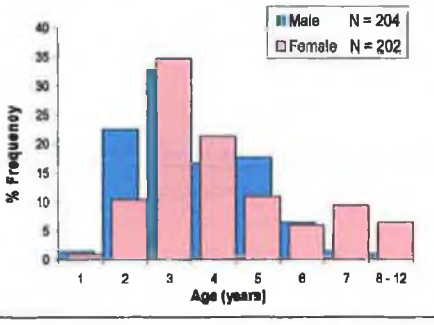
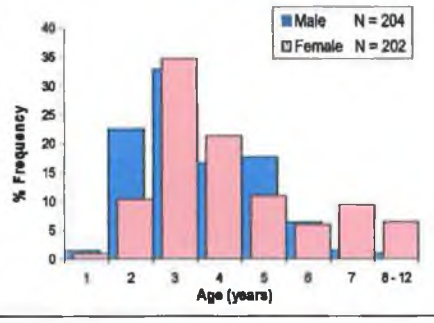
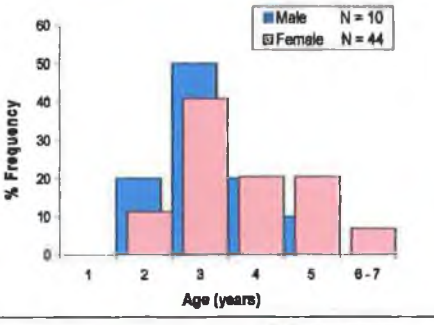
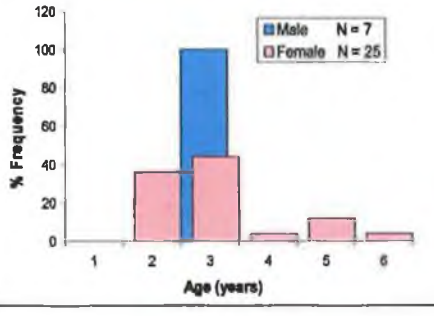
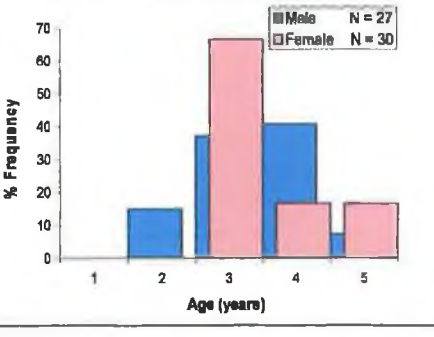
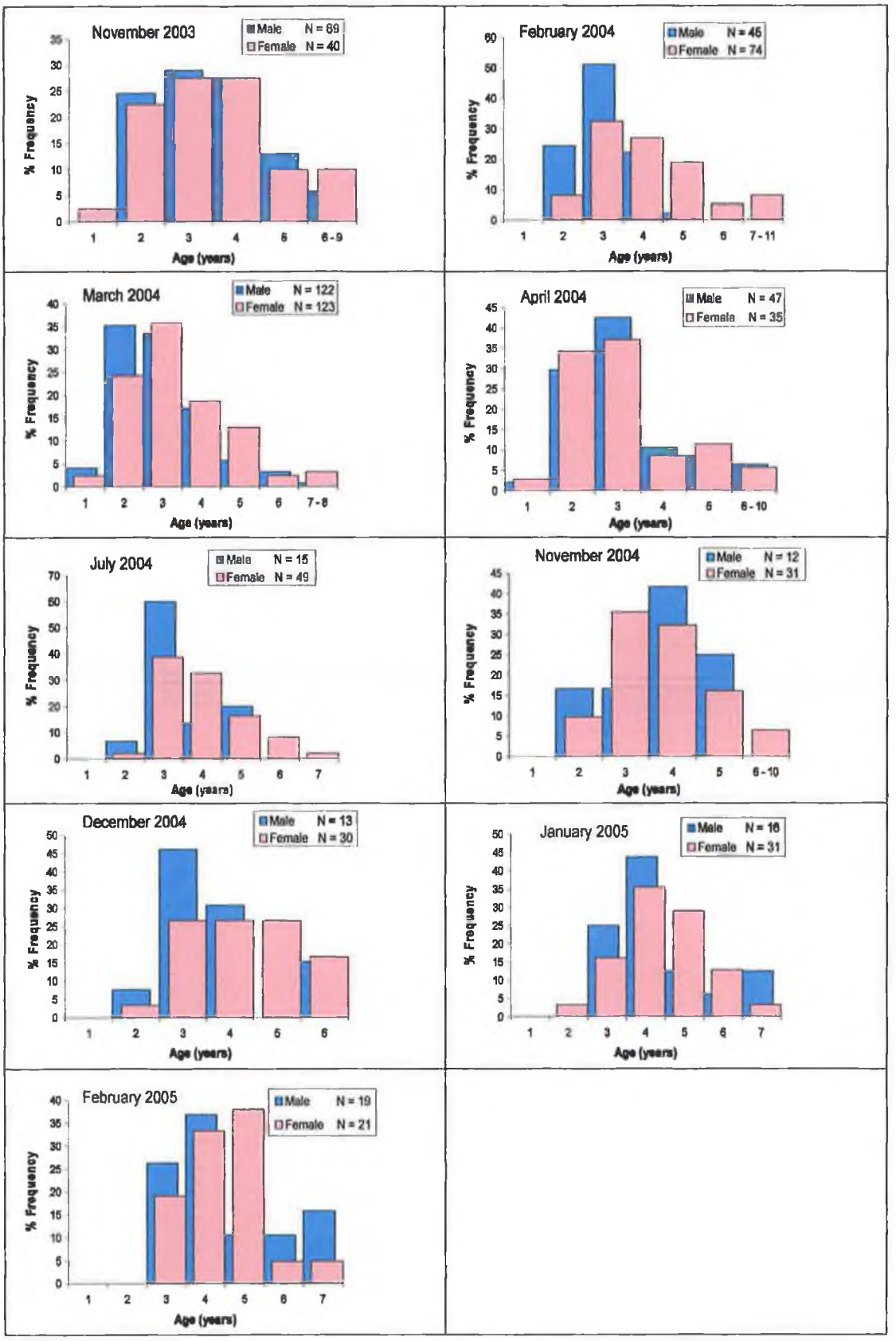
Month	November 2003	March 2004
VIIb **west coast of Ireland**		
VIa **northwest coast of Ireland**		
VIIa **The Irish Sea**		
VIIg **The Celtic Sea**		
VIIj **southwest coast of Ireland**		

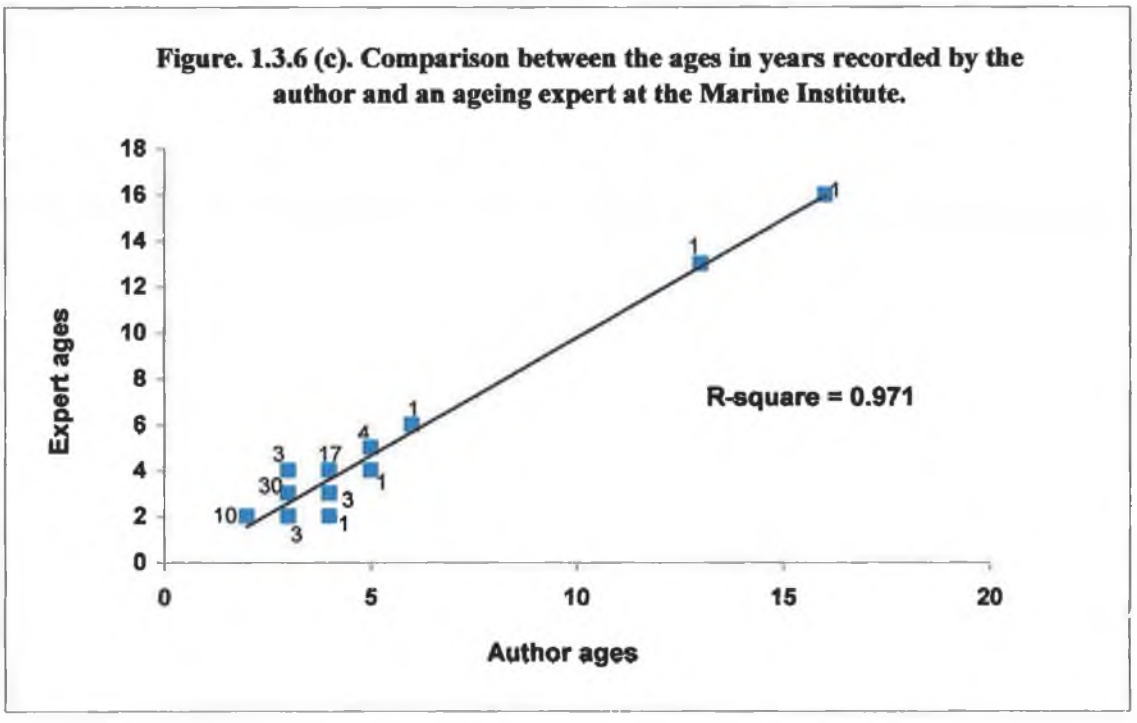
Figure 1.3.6 (b). Percentage age frequency distribution for male and female plaice in each sample collected in ICES area VIIb between November 2003 and February 2005. (N is the number of fish sampled).



Quality Control: Age Reading Comparison

Results from an inter-calibration exercise between the author and an ageing expert at the Marine Institute are presented in Fig. 1.3.6 (c). Seventy-five otoliths were compared for age. In 64 otoliths (85% of cases) there was agreement in age readings between the author and an expert in aging plaice. In 10 otoliths (13% of cases) there was disagreement by one year, and in 1 otolith (1% of cases) there was disagreement by two years. A Paired T-Test was calculated on the data and the null hypothesis (H_0) that there is no difference between the age readings of the author and the ageing expert was accepted at the 95% confidence interval (T=1.62, P=0.109), i.e. there is no significant difference between the age reading of the author and the aging expert. The main disagreement between the author and the ageing expert was at ages 3 and 4.

Figure. 1.3.6 (c). Comparison between the ages in years recorded by the author and an ageing expert at the Marine Institute.



1.3.7 Growth

Figures 1.3.7 (a-c) present Von Bertalanffy growth curves for all plaice, and male and female plaice separately in the populations sampled in ICES areas VIIb, VIa and VIIa between November 2003 and February 2005. ICES areas VIIj and VIIg did not fit the Von Bertalanffy equation due to abnormal sample sizes, and are not included in further analysis. The model illustrates a scatter plot of the length at age for all fish examined, together with the fitted Von Bertalanffy growth curve. Table 1.3.7 (a) presents the Von Bertalanffy coefficients for plaice sampled in ICES areas VIIb, VIa and VIIa between November 2003 and February 2005.

Table 1.3.7 (a). Von Bertalanffy Coefficients for all plaice and for male and female plaice separately in the populations sampled in ICES areas VIIb, VIa and VIIa between November 2003 and February 2005.

		$L\infty$ (cm)	K (yr^{-1})	t_0 (years)
VIIb west coast of Ireland	All Plaice	36.96	0.307	-0.47428
	Males	29.31	0.564	0.099373
	Females	33.56	0.401	-0.55907
VIa northwest coast of Ireland	All Plaice	41.7	0.187	-1.45034
	Males	27.3	0.461	-1.11258
	Females	36.34	0.184	-2.42012
VIIa The Irish Sea	All Plaice	48.57	0.139	-1.923684
	Males	44.35	0.103	-3.64837
	Females	35.87	0.411	-0.31056

Figure 1.3.7 (a). Von Bertalanffy growth curves for all plaice in the populations sampled in ICES areas VIIb (N=792), VIa (N=226) and VIIa(N=777) between November 2003 and February 2005.

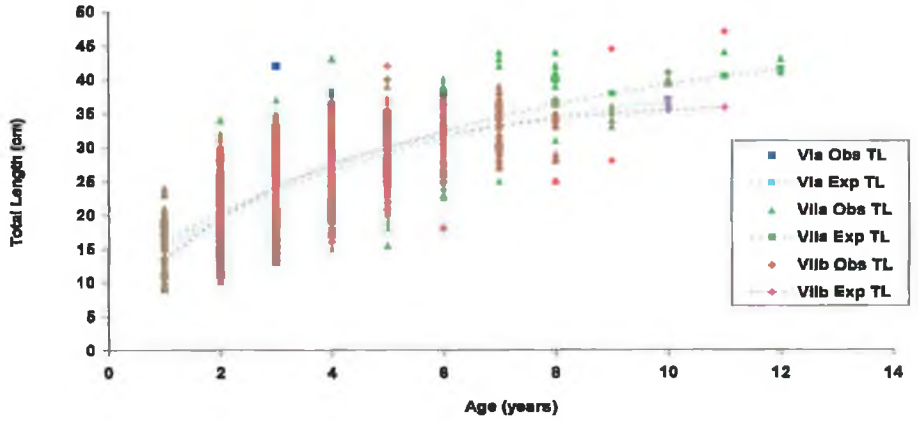


Figure. 1.3.7 (b). Von Bertalanffy growth curves for female plaice in the populations sampled in ICES areas VIIb (N=435), VIa (N=112) and VIIa (N=421) between November 2003 and February 2005.

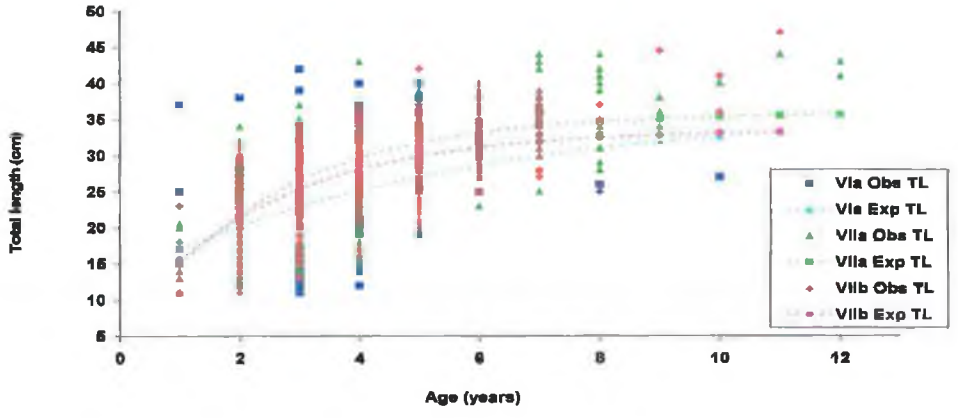
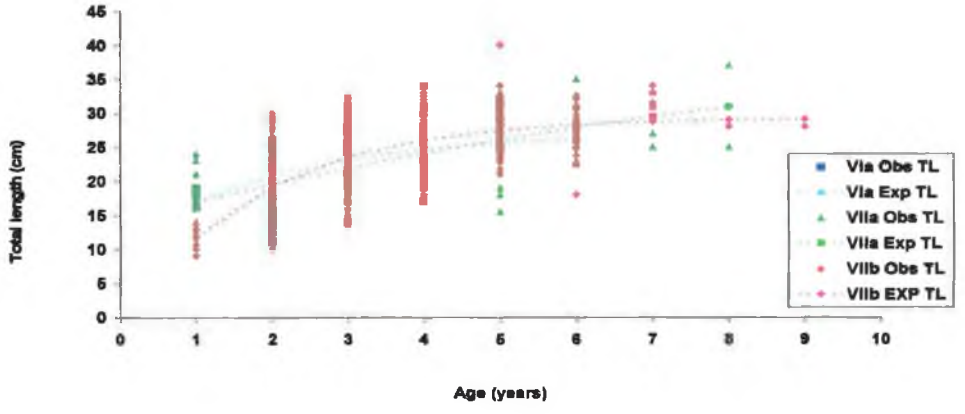


Figure. 1.3.7 (c). Von Bertalanffy growth curves for male plaice in the populations sampledin ICES areas VIIb (N=356), VIa (N=114) and VIIa (N=356) between November 2003 and February 2005.



In ICES area VIIb male plaice reached a length of 12cm at the end of their first year of growth, while in ICES areas VIa and VIIa male plaice reached 17cm at the end of their first year of growth. Males in ICES areas VIIb and VIa grew approximately 8cm in their second year and the growth rate slowed down by 2cm a year thereafter. In ICES area VIIa males only grew 3cm in their second year and maintained a growth rate of 2cm per annum for approximately 6 years and then the growth rate slowed down by 1cm a year. In ICES areas VIIb, VIa and VIIa female plaice reached an average length of 16cm in their first year, grew approximately 5cm in their second year, 3cm in their third, 2cm in their fourth and fifth and the growth rate slowed down by 1cm per annum after that. In ICES area VIIa where females had the smallest length at year one, they grew the most in their second, third and fourth years.

Plaice in ICES area VIIa (the Irish Sea) had the largest value of $L\infty$ for all fish (males and females combined), for males and for females, when compared with ICES areas VIIb (west coast of Ireland) and VIa (northwest coast of Ireland). ICES area VIIb (west coast of Ireland) had the smallest $L\infty$ value for all plaice and females while ICES area VIa (North-west coast of Ireland) had the smallest value of $L\infty$ for males. ICES area VIIa has the smallest value of K for all plaice and also for males. ICES area VIIb had the smallest value of K for females. ICES area VIIb had the largest value of K for all plaice and males while ICES area VIa had the largest value of K for females.

As an animal is unlikely to grow according to the Von Bertalanffy growth equation throughout its whole life-span, particularly in the pre-adult stages, the Von Bertalanffy curve often cuts the x axis at a value less than zero, hence (t_0), the theoretical age at zero length often has a small negative value (King, 1995). Negative values of t_0 show that juveniles grow quicker than the predicted growth curve for adults, and positive t_0 values show that juveniles grow slower than adults (King, 1995). ICES area VIa (northwest coast of Ireland) had the largest negative value of t_0 for all plaice, indicating that juveniles in VIa had the fastest growth rate. ICES area VIIa (Irish Sea) had the largest positive value of t_0 for all plaice indicating that juveniles in VIIa had the slowest growth rate. Males in ICES area VIIa grow fastest as juveniles, while males in ICES area VIIb grow slowest as juveniles. Females in ICES area VIIb grow fastest as juveniles while females in VIa grow slowest as juveniles. Overall, males in VIIa grow fastest as juveniles and females in VIa grow slowest as juveniles.

1.3.8 Comparison of plaice growth between ICES areas

Growth of plaice in the five sample locations (ICES areas) was compared. A General Linear Model analysis of variance was calculated on the data and the null hypothesis (H_0) that the growth rate of plaice did not differ between ICES areas was accepted (P=0.826).

Male and female data for each ICES area was analysed for two growth periods, i.e. November 2003 and March 2004. These periods were chosen as these were the only times when samples were collected in all ICES areas. Data was analysed using a General Linear Model analysis of variance and the null hypothesis (H_0) was that there was no difference in the growth rate of male and female plaice between ICES areas at these times. The null hypothesis was accepted for female (P=0.426), and male plaice (P=0.597) sampled in November 2003, but was rejected for female (P=0.000), and male plaice (P=0.001) sampled in March 2004. This means that male and female plaice sampled in November 2003 showed no significant difference in growth between ICES areas, but male and female plaice sampled in March 2004 showed a significant difference in growth between ICES areas.

When a General Linear Model analysis of variance was calculated on spawning fish (stages 5 and 6), the null hypothesis that there was no difference in growth between ICES areas was rejected (P=0.025). The results of the General Linear Model analysis of variance for comparison of growth between ICES areas are presented in Table 1.3.8 (a).

Table 1.3.8 (a). Results of General Linear Model analysis of variance for comparison of growth between ICES areas. (P< 0.05 = Significant, P> 0.05 = Not Significant).

Relationship	P-values					
Length x	November Male	March Male	November Female	March female	Spawning fish	All Plaice
Age (yr)	0.000	0.000	0.000	0.000	0.010	0.000
ICES area	0.597	0.001	0.426	0.000	0.025	0.826
ICES area x Age	0.423	0.076	0.414	0.000	0.304	0.403

1.3.9 Catch Curve

Estimates of total instantaneous mortality (Z), natural mortality (M), fishing mortality (F), percentage survivorship (%S), mean life span (t_{max}), and the age at full recruitment (tr) were determined using catch curves, for all plaice and male and female plaice separately in the populations sampled in ICES areas VIa, VIIa and VIIb between November 2003 and February 2005. Fig 1.3.9 (a) presents the age composition using a catch curve for all plaice, and for male and female plaice separately in the populations sampled in ICES areas VIIb, VIa and VIIa between November 2003 and February 2005, and Table 1.3.9 (a) presents the life history parameters for the same samples.

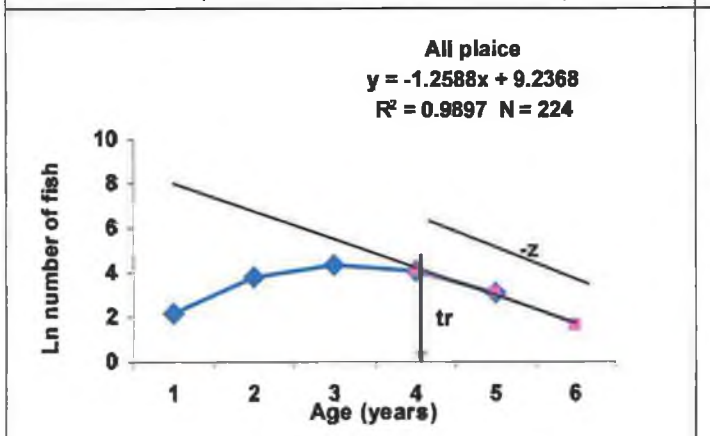
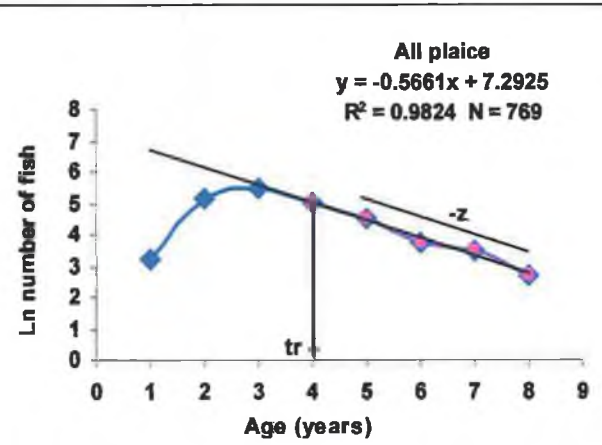
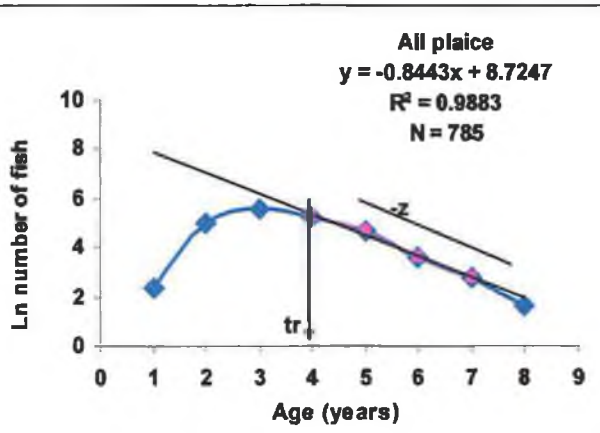
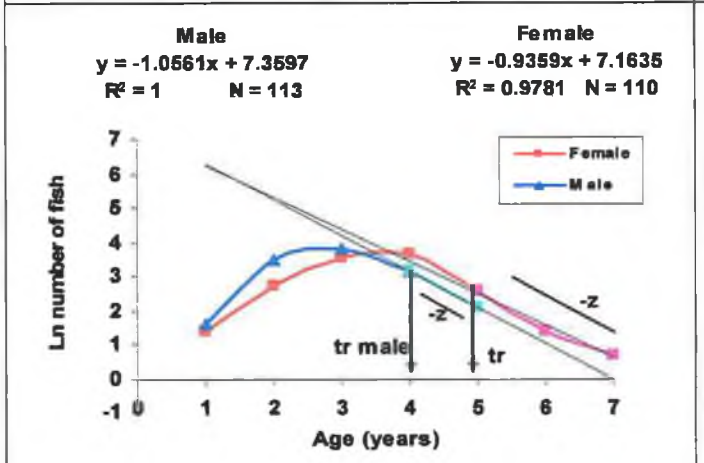
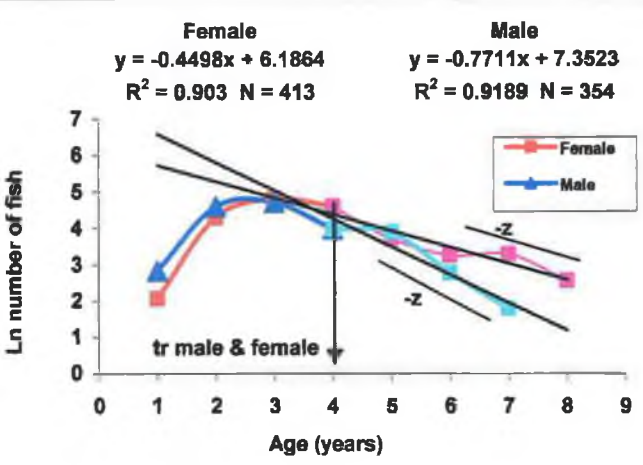
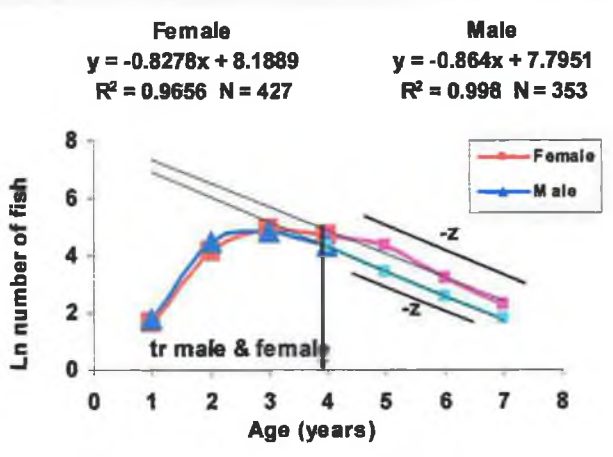
Table 1.3.9 (a). Life history parameters for all plaice and male and female plaice separately, in the populations sampled in ICES areas VIa, VIIa and VIIb between November 2003 and February 2005.

Overall population						
ICES area	**M (yr-1)**	**tr (yrs)**	**Z (yr-1)**	**F (yr-1)**	**% S**	**tmax (yrs)**
VIIb	0.12	4	0.92	0.80	0.40	9.77
VIa	0.12	4	1.18	1.06	0.31	16.04
VIIa	0.12	4	0.68	0.56	0.51	21.58
Males						
VIIb	0.12	4	0.86	0.74	0.42	5.32
VIa	0.12	4	1.06	0.94	0.35	6.51
VIIa	0.12	4	0.87	0.75	0.42	29.13
Females						
VIIb	0.12	4	0.92	0.80	0.40	7.48
VIa	0.12	5	0.94	0.82	0.39	16.27
VIIa	0.12	4	0.56	0.44	0.57	7.3

In ICES area VIIb males are six times and females are seven times more likely to die from fishing mortality (F) than from natural mortality (M). In ICES area VIa males are eight times and females seven times, while in ICES area VIIa males are six times and females four times more likely to die from fishing mortality (F) than natural mortality (M). Total mortality (Z) ranges from 0.56 yr^{-1} in VIIa to 1.18 yr^{-1} in VIa. ICES area VIa has the highest value for fishing mortality (F) while VIIa has the lowest. Males and females in ICES area VIIa have the highest

rate of survivorship (0.51%) while males and females in ICES area VIa have the lowest (0.31%). For all plaice (males and females combined), ICES area VIIa has the highest (21.58 yrs), and ICES area VIIb has the lowest (9.77 yrs) mean life span (t_{max}). Males in ICES area VIIa have the highest (29.13 yrs) t_{max} value, while males in ICES area VIIb have the lowest (5.32yrs).

Figure 1.3.9 (a). Catch curve for all plaice and male and female plaice separately, in the populations sampled in ICES areas VIa, VIIa and VIIb between November 2003 and February 2005. (N is the number of fish sampled).

VIa (northwest coast of Ireland)	VIIa (The Irish Sea)	VIIb (west coast of Ireland)
		
		

1.4 Discussion

The sampling procedures, length frequencies, length-weight regressions, mean lengths and weights at age, age frequencies, growth, mortality co-efficients and recruitment determined for *P. platessa* collected during this study are compared with similar works and assessments elsewhere in Ireland and Europe for the species.

Though it is assumed for the purposes of this study that the commercial samples were relatively representative of the population present, it would have been better to use fish caught on research surveys. A common problem in obtaining representative samples from commercial fishing gear is that the size selectivity of trawls can produce biased estimates of size frequency and size at age (Sampson and Al-Jufaily, 1999). Commercial samples were caught in twin or single rig otterboard trawls, with a standard mesh size of 80mm at the cod end, while the survey samples were caught using GOV and BACA trawls, with a mesh size of 90mm and a 20mm stretched mesh internal liner at the cod end. This retains fish well below the minimum landing size (Bromley et al., 2003). This means that survey samples will give a more representative picture of the population. It was not possible to acquire survey samples for the entire sampling period due to restricted and limited access to research vessels and surveys during the investigation. It would also have been desirable for samples to be attained monthly for each ICES area, so comparisons between ICES areas would be more accurate.

Sex ratio

Determining the sex ratio of a species is important in reproductive studies, as it gives an estimate of the structure of the population being examined. It also provides an insight into the exploitation levels of the fishery, due to the fact that females often grow faster than males and reach lengths where they become susceptible to the fishing gear before males do. In ICES areas VIIb and VIIa there was a significant departure from a 1:1 ratio ($P<0.05$) and in ICES area VIIg there was a highly significant departure from a 1:1 sex ratio ($P<0.01$). Female biased sex ratios are a commonly observed phenomenon in flatfish biology (Walsh, 1994). Possible reasons for a deviation from a 1:1 sex ratio include

differences in the distribution of the sexes according to depth (Boon, 1984), temperature and size (Simpson, 1951), and to selectivity of the fishing gear and also sampling time and location (Simpson, 1951). The most likely cause of the female biased sex ratio in plaice in ICES areas VIIb, VIIa and VIIg is that of net selectivity. During this investigation most of the samples were commercially obtained. Commercial samples were caught in twin or single rig otterboard trawls, with a standard mesh size of 80mm at the cod end. This large mesh size would have allowed the majority of the smaller males to escape during the fishing tows. Also due to their smaller size many male fish may have been discarded. When the sex ratio of plaice in each ICES area was examined by size class, it was observed that sex ratio is related to the size of the fish. Males were more abundant in the smaller length classes while females dominated the larger length classes.

When the sex ratio of each individual sample collected in each ICES area during this study was examined, it was found that in a number of samples the sex ratio was biased in favor of males. Male biased sex ratios were recorded in November 2003 in the samples collected in ICES areas VIa and VIIb. In ICES areas VIIa and VIIb in March 2004, and in ICES area VIIj in February 2004 there was a male biased sex ratio recorded. It is interesting to note that the November 2003 and March 2004 samples were research survey samples. The nets used on the research surveys had a mesh size of 90 mm with a 20mm stretched mesh internal liner at the cod end. These nets retain the smaller male fish (Bromley et al., 2003). The February 2004 sample collected in ICES area VIIj was provided by fishermen specifically for this project, and a lot of fish that would normally have been discarded from a commercial sample were retained. Hence, these samples may provide a more accurate representation of the sex ratio. It would have been preferable to only use fish caught on research surveys, but this was not possible during the present investigation due to restricted and limited access to research vessels and surveys.

It is also worth noting that all the samples with a male biased sex ratio were collected close to the spawning season. The existence of a high sex ratio in favor of males among demersal marine fish species on the spawning grounds is well known (Wallace, 1909; Mc Kenzie, 1940; Morgan and Trippel, 1996; Lawson and Rose, 2000), a phenomenon

observed for plaice, where males usually outnumber females (Wallace, 1909; Hefford, 1916; Simpson, 1959; de Veen, 1964; Solmundsson et al., 2003). Simpson (1959) also recorded a high sex ratio in favor of male plaice (10:4) on the spawning grounds in the Irish Sea. It has been suggested that this may be due to sexual differences in behavior (Beverton, 1964). Male plaice are known to occupy the spawning grounds for longer (Hefford, 1909; Arnold and Metcalfe, 1996), have a longer spawning duration and show more active behavior during spawning (Solmundsson et al., 2003). Beverton (1964) found the total fishing mortality coefficient of North Sea plaice to be 50-150% higher for males than females at spawning time. The swimming activity of males may increase their vulnerability, as a high level of swimming activity near the bottom is likely to cause flatfishes to be more readily caught than those lying on the bottom, perhaps partially buried (De Veen, 1964; Beamish, 1966; Woodhead, 1966, Morgan and Trippel, 1996; Solmundsson et al., 2003).

Simpson (1951) and Albert et al., (1998) observed that the sex ratio of plaice changed markedly with age. They observed that males dominated the younger age groups while the number of females increased sharply with age. In this investigation in all ICES areas males tended to dominate the younger age groups. Hefford (1916) suggested that the dominance of females among the older fish was the result of a higher mortality among the males during the fishing on the spawning stock. When each sample collected in ICES area VIIb was examined individually, the samples for January and February 2005 had more males in the older age groups. These samples were purchased from Galway Bay Seafood's, and were pre-sorted, so the percentage of males in the older age groups would be skewed and not representative of the actual population.

Length Frequencies

The length frequencies determined for each ICES area, and for two sampling periods (November 2003 and March 2004) in each ICES area are compared to each other and to those recorded elsewhere for the species.

In all ICES areas the range of female fish was larger than for the male fish. The largest range in lengths for males and females was in ICES area VIIb. The smallest range in lengths for both male and female fish was in ICES area VIIg. The difference observed in the size ranges is likely a factor of the size of the samples. ICES area VIIb had the largest sample size with 806 individuals, while ICES area VIIg had the smallest sample size (145 individuals). However ICES areas VIa and VIIa had very similar length ranges for both male and female fish, even though the sample size for ICES area VIIa was almost three times that of VIa. It is notable that the sample for ICES area VIIb consisted of both commercial and research survey samples, while the ICES areas with smaller length ranges consisted of just research survey samples. It is expected that survey samples would have a larger length range because the smaller mesh in the cod end should retain the smaller fish, but in this case the survey samples are just too small in comparison to the size of the ICES area VIIb sample. Though grading occurred, a considerable size range was recorded for most samples examined during the present investigation.

A significant difference in median length values of male and female plaice between ICES areas was recorded. Males and females sampled in ICES area VIIg had the largest median lengths. Males sampled in ICES area VIIa and females sampled in ICES area VIa had the smallest median lengths. Plaice stocks in the Celtic Sea (ICES areas VIIf and VIIg) are considered by ICES to be outside safe biological limits, over fished and classified as being at risk of suffering reduced reproductive capacity (Anon, 2008). This may account for the larger median lengths in VIIg as a response to fishing pressure. Bagenal (1966) and Rijnsdorp (1990) have suggested that in exploited populations with a high mortality rate, a reduction in population density, and hence in intra-specific competition, may result in an increase in growth. Another factor related to exploitation that may cause an increase in growth is an increase in per capita food availability (Rijnsdorp and Van Leeuwen, 1992, 1996; Rijnsdorp, 1994; Pastoors et al., 2000; Law, 2000: Van Marlen et al., 2005) due to environmental perturbations caused by beam trawl activity (Nikolsky, 1963: Weatherley, 1972; Trippel, 1995).

The percentage length frequency distributions indicate that in general males dominated the smaller length classes and the females the larger, with two exceptions. In ICES area VIa, in the March 2004 sample, females were found in the smallest length class. In ICES area VIIb in the November 2003 sample, males had a larger total length. This may be due to the fact that there were more males present in the sample. The fact that males dominated the smaller length classes and that females reach greater lengths is because male plaice grow more slowly than female plaice (Pauly, 1994a; Kell and Bromley, 2004). In all samples the distributions of male and female length frequency were unimodal, with a single length frequency class composing the largest percentage of fish present.

The months of November and March lie approximately at the beginning and end of the spawning season for plaice. While the length range in each ICES area is similar for these two sampling periods, it is notable that the mean length for both male and female plaice in each ICES area decreases from November to March. The November 2003 and March 2004 samples were not collected in exactly the same locations, in any of the ICES areas. It is not known how far apart the sampling locations were. This complicates any comparison of growth or median lengths, as it has been shown that the size and age compositions of plaice catches vary among areas (spatially) and between seasons (temporally) (Garstang, 1909; Heincke, 1913; Wimpenny, 1953). Small plaice are concentrated in shallow, inshore waters, and fish size increases with distance from the coast (Rijnsdorp et al., 1992).

However the samples were collected at the beginning and end of the spawning season, a period when mature plaice gather on the spawning grounds (de Veen, 1964; Rijnsdorp, 1989). This removes some of the uncertainty in comparing the samples collected in these time periods. If we then look at the decrease in mean lengths, we might assume it is caused by movement of larger fish away from the area after spawning. Horwood (1990) and Arnold and Metcalfe (1995) provided evidence that large female plaice tended to be the first to spawn and the first to move away from the spawning grounds.

Length - Weight Relationship

In each ICES area there was a high correlation between length and weight for males and females and the overall population (males and females combined). From the regression analysis of the relationship between total length and weight, it was concluded that the growth of *Pleuronectes platessa* was allometric, meaning that the fish become heavier for their length.

Length and weight at age

In ICES areas VIIb, VIIg and VIIj females showed a larger mean length and larger mean weight than males in all age groups. It was expected that females in the older age classes would have a larger mean length and weight at age than male plaice as sexual dimorphism in the growth of pleuronectiform fish is well acknowledged (Wimpenny, 1953; Landa et al., 1996; Bromley et al., 2000). However, in ICES area VIa, males were heavier than females at ages 1 and 2, and in ICES area VIIa males had a slightly greater mean length up to age 5. A larger mean length and weight at age is common in males in the younger age groups because they are faster growing and early maturing, however the mean length or weight of the younger age groups may be overestimated due to partial recruitment or discarding of undersized fish (Rijnsdorp, 1991).

In ICES area VIIa males appear to have a larger mean length up to age 5 but weigh significantly less than females at all ages except for age 1. This is unusual, and may indicate non-allometric growth. When the length-weight relationship was calculated for males in ICES area VIIa, a value for b of 2.9201 was attained. This value was very close to 3, so when rounded up it was assumed that male plaice displayed allometric growth. It is uncertain why male fish weigh less when they should weigh more than females given their greater length. Differential growth rate can be brought on by sexual maturity in fish. However, plaice do not reach sexual maturity until approximately 3 years of age, and even if this was the case it would not explain non-allometric growth. There must be some underlying behavioural difference that effects the feeding of male plaice in ICES area VIIa, but investigating this is beyond the scope of this project.

While data from other studies is limited, Albert et al. (1998) recorded the mean length of age 4 plaice off Norway to be 30cm, and Simpson (1959) recorded the mean length of age 4 plaice in the Irish Sea to be between 25 and 35cm. The findings of this study were similar, where it was found that at age 4, males had a mean length of between 25 and 29 cm, and females at age 4 had a mean length of between 25 and 31 cm.

Age frequencies

Within each ICES area, the age structure of plaice sampled in the months of November 2003 and March 2004 was examined. These months were chosen because they are the only months for which samples from all ICES areas studied were available. In each ICES area there was no significant difference in the age ranges of plaice sampled in November 2003 and March 2004. In both November 2003 and March 2004 ICES area VIIa had the largest range in ages and ICES area VIIj had the smallest range in ages. In all samples females had a larger range of ages than males. All November 2003 and March 2004 samples were survey samples. In November 2003 and March 2004 in each ICES area, the modal age group varied from age three to age four for females, and from age two to age four for males. Studies on North Sea plaice have shown that the age composition of mature plaice on spawning grounds changes between November and March. Simpson (1951) found that the mean age decreased from 7 to 4 years for males, and from 8 to 5 years for females and that catches at the end of spawning season were dominated by younger fish. He postulated that older fish arrived to the spawning grounds earlier and then moved off after spawning, to be replaced by younger fish.

At an early stage in this investigation, an age reading inter-calibration exercise was undertaken between the author and an ageing expert at the Marine Institute. It was found that the largest disagreement between the authors ageing and the experts occurred at ages 3 and 4. It was expected that the largest disagreement would occur when ageing the older fish. Older fish can be more difficult to age with accuracy as the annual rings are more numerous and closer together. However, in this case it was simply a case of numbers. The largest proportions of fish were in age groups 3 and 4. With more observations there is always a chance for more error.

Growth

ICES area VIa

The smallest value of L∞ for males was recorded in VIa. This means that male plaice in ICES area VIa (off the north west coast) would not be expected to grow as big as male plaice in either ICES areas VIIa (the Irish Sea) or VIIb (off the west coast). Female plaice in VIa grow faster (largest K value) than females in the other ICES areas. The largest negative value for t_0 was recorded for all plaice (males and females combined) in ICES area VIa. This indicates that juveniles in VIa had the fastest growth rate. However when this was broken down into the different sexes and compared with other ICES areas, it could be seen that females in VIa grow slowest as juveniles.

ICES area VIIa

The largest L∞ for all plaice and for male and female plaice separately was recorded in ICES area VIIa. This means that plaice in VIIa could potentially grow to a greater maximum length than plaice in other ICES areas. The smallest value of K was recorded for all plaice (males and females combined) and for males separately in ICES area VIIa. This means that plaice in VIIa grow slower than in other areas, and when broken down into the different sexes, males in VIIa have the slowest growth rate. The largest positive value of t_0 was recorded for all plaice in VIIa, indicating that juveniles in this area had the slowest growth rate. However, when this was broken down into the different sexes, it was found that male plaice in VIIa have the fastest growth rate as juveniles.

ICES area VIIb

The smallest L∞ for all plaice (males and females combined) was recorded in ICES area VIIb. This means that plaice in VIIb would be expected to grow to a smaller maximum length than plaice in the other ICES areas. When this was broken down into the different sexes, females also had the smallest value of L∞, indicating that females in ICES area VIIb may not reach the sizes of female plaice in the other ICES areas. The largest value for K was recorded in VIIb for all plaice (males and females combined). This means that plaice in VIIb have the fastest growth rate when compared with plaice in the other ICES

areas examined. When this was broken down into the different sexes, males had the largest value for K and females the smallest, meaning that males in VIIb have the fastest growth rate and females the slowest when compared to the other ICES areas. When the growth rate of juveniles was looked at, it was found that juvenile male plaice in VIIb grow slowest, and female juvenile plaice in VIIb have the fastest growth rate.

The trend observed seems to be that if a fish has a fast growth rate as a juvenile, it will have a slow growth rate as an adult, and vice versa. Rijnsdorp (1993b) noted that the growth rates of mature and immature female plaice differ significantly. According to King (1995) it is unlikely that an animal will grow according to the Von Bertalanffy growth equation throughout its life, and it is not unusual for juveniles to grow quicker or slower than the predicted growth curve for adults.

Slower growth (small K value) should correspond to a lower $L\infty$ value, with the fish theoretically attaining a much smaller overall size. Some of the results of this study appear to contradict this assumption. In ICES area VIIa for example, the largest $L\infty$ value and the smallest K value were recorded for all plaice, and in ICES area VIIb the smallest $L\infty$ and the largest K value were recorded for all plaice. This means that fish with the slowest growth rates are reaching the largest maximum sizes, and that the fish with the fastest growth rates are not reaching the largest maximum sizes. A lowering of the $L\infty$ value may indicate a change in stock dynamics due to an increase in exploitation rate. It has been shown that there is a significant relationship between beam trawl intensity and growth rate of plaice (Kändler, 1932; Molander, 1955; de Veen, 1978b; Rijnsdorp and Van Beek, 1991; Rijnsdorp and Van Leeuwen, 1996), and many studies addressing the question of effects of exploitation on growth rate reported that growth increased after exploitation started (Rijnsdorp, 1994). Then, in a population experiencing large fishing mortalities the growth rate would be expected to increase, and equally, the fish may not live long enough to reach these largest maximum sizes (Rijnsdorp, 1992).

It should be noted that there was a significant difference in the sample sizes used to obtain the Von Bertalanffy growth parameters for each ICES area examined. For females

in ICES area VIa, there were very few individuals recorded in the upper size and age classes. Use of low numbers of fish may generate noise and make it more difficult to obtain accurate growth rate estimates or to draw precise conclusions.

Comparison of plaice growth between ICES areas

A General Linear Model analysis of variance was calculated on the data and the null hypothesis (H_0) that the growth rate of plaice did not differ between ICES areas was accepted (P=0.826). Male and female data for each ICES area was analyzed for two growth periods, i.e. November 2003 and March 2004. Data was analyzed using a General Linear Model analysis of variance and the null hypothesis (H_0) was that there was no difference in the growth rate of male and female plaice between ICES areas at these times. The null hypothesis was accepted for female and male plaice sampled in November 2003, but was rejected for female and male plaice sampled in March 2004. This means that male and female plaice sampled in November 2003 showed no significant difference in growth between ICES areas, but male and female plaice sampled in March 2004 showed a significant difference in growth between ICES areas. When a General Linear Model analysis of variance was calculated on spawning fish (stages 5 and 6), the null hypothesis that there was no difference in growth between ICES areas was rejected.

Adult fish undertake extensive migrations in the months prior to spawning (Saemundsson, 1926; Tåning, 1929; De Veen, 1978; Sigurdsson, 1989) and in general, fish of the same stock return to the same spawning grounds and during the spawning season discrete stocks are found in areas, i.e., no mixing of stocks (De Veen, 1962, 1964; Harden Jones, 1968; De Veen, 1978b; Rijnsdorp and Pastoors, 1995; Bailey, 1997; Dunn and Pawson, 2002; Hunter et al., 2003). This may be why a difference in growth rate between ICES areas was recorded for spawning fish. The fact that males and females in all ICES areas showed no significant difference in growth rates in November may reflect the fact that individuals had not migrated to their home spawning grounds at that time. While in March, as the spawning season was drawing to a close, the stocks were more separated, and hence the significant difference in growth rates between ICES areas.

Rijnsdorp (1991) noted that when samples are taken just before or early in the spawning season they may include both fish that will spawn locally and fish that are still migrating, so the time and place of sampling can affect the observed geographicial differences in reproduction- body size relationships.

Investigations on the European plaice in the Barents Sea and the North Sea show that plaice exhibit a large variability in growth rates (Milinsky, 1938; Kovtsova, 1976, 1985; Albert et al., 1998; Bromley, 2000) and that growth varies geographically with food abundance, predators (Rijnsdorp et al., 1991), and with temperature (Rijnsdorp, 1992; Fonds et al., 1992; Bolle et al., 2004)

Mortality

In ICES area VIIb similar values of fishing mortality (F) and total mortality (Z) were determined for males and females, though males suffer slightly more from each than females. They also had similar values for percentage survival (%S), with females having a slightly higher survival rate and mean life span. In ICES area VIa females are more susceptible to fishing mortality and total mortality than males, giving males a higher survival rate and a greater mean life span, almost four years greater than females. In ICES area VIIa, males suffer more from fishing mortality and total mortality than females and have a lower % survival rate, but a much greater mean life span.

It would be expected that females would suffer more from fishing mortality because their larger size makes them more susceptible to the fishing gear, as in ICES area VIa. However in ICES areas VIIb and VIIa males seem to have a higher rate of fishing mortality. This may be caused by differences in behavior. Wimpenny (1953) noted that male and female plaice can have a different reaction to fishing, with males being more susceptible in the deep-sea fishery, and females being more susceptible inshore. He postulated that these differing reactions were based on the different physiology, behavior and migration of the two sexes, and even went so far as to say that when plaice are being examined, the two sexes may sometimes have to be treated as if they were two separate species.

In ICES area VIIa males have a greater mortality rate (fishing and total) and a lower percentage survival, but a much greater mean life span than females. It would be expected that a greater mortality and lower survival rate would result in a smaller mean life span. It is unclear why this is not the case here.

The highest rate of fishing mortality (F) was determined for ICES area VIa, and the lowest in VIIa. The minimum legal landing size in ICES areas VIa and VIIb is 22cm, while in VIIa it is 27cm. This may be a factor in the difference in fishing mortality between the areas. Other factors known to affect fish catchability and CPUE data in commercial fisheries include different fishing power, non-random trawling, diurnal and seasonal variation in fish distribution (Garrod, 1964; Gulland, 1964).

Recruitment

Catch curves were used to provide an estimation of the age at recruitment (tr) of the fish into the fishery. In each ICES area male and female plaice have fully recruited to the population by age four, with the exception of females in ICES area VIa (northwest coast of Ireland) for which a tr value of 5 years was determined. It is unclear why females in ICES area VIa have a later age at recruitment (tr). However recruitment to the fishery is size-dependant rather than age dependant, meaning that at a higher growth rate, plaice will recruit to the fishery at a younger age (Rijnsdorp et al, 1996). Female plaice in VIa grow faster (largest K value) than females in the other ICES areas, so they should recruit at a younger age. Females in ICES area VIa also have the slowest growth as juveniles, and this may delay maturity. In this study the age at maturity was only calculated for female plaice sampled in ICES area VIIb (Chapter 2) and was determined to be 3 years. Maybe length and age at first maturity needs to be looked at for females in each ICES area separately. This was not possible during this investigation due to time constraints and low sample sizes. The catch curves for male and female plaice in each ICES area examined all have fairly broad domes, which may indicate that the fish are recruited over a number of age classes.

In general male plaice are smaller and lighter than females when recruited to the fishery, except for males in ICES area VIIa, which have a longer mean length than females, even though they are lighter by weight. It has been shown that males in ICES area VIIa have the fastest juvenile growth rate. This may be why newly recruited males to ICES area VIIa have larger mean lengths than females.

The length and age at first maturity was determined for all plaice sampled during this study (chapter 2). Length at first maturity ($L_{50\%}$) was determined to be 23cm and 21cm for females and males respectively. Age at first maturity ($A_{50\%}$) was determined to be 3 years for both males and females. Males and females in all ICES areas examined here (VIIb, VIIa and VIa) are well above the length and age at first maturity when they are recruited to the fishery. This should mean that plaice have a chance to mature and contribute to the spawning stock biomass before they become vulnerable to fishing. However, the minimum legal landing size in ICES areas VIa and VIIb is 22cm TL. This means that female and male plaice in ICES areas VIa and VIIb could potentially be removed from the stock before they have reached the length at first maturity.

The age at recruitment values recorded in this study for plaice are within the range recorded by other studies. Dunn and Pawson (2002) determined the age at recruitment for plaice in the Irish Sea to be 3 years, and Rijnsdorp and Ibelings (1989) determined the age at recruitment for North Sea plaice to be between 2 and 4 years.

Chapter 2

Reproductive biology and maturity in Plaice, Pleuronectes platessa off the West coast of Ireland, 2003-2005.

2.1 Introduction

Successful reproduction is vital for the continued existence of populations in their natural environment (Rijnsdorp et al., 2005), and reproductive potential is a measure of the capacity of a population to produce viable eggs and larvae (Murua and Saborido-Rey, 2003). The reproductive potential of a stock is influenced by many factors including the size of the spawning stock biomass (Bagenal, 1973; Myers and Barrowman, 1996), the age structure of the population (Alheit et al., 1983; Cardinale and Arrhenius, 2000), the proportion of repeat and first-time spawners (Evans et al., 1996; Trippel, 1998), the nutritional condition of the fish (Hislop et al., 1978; Hunter and Leong, 1981; Brooks et al., 1997; Rijnsdorp et al., 2003), the size and age at sexual maturity (Roff, 1981; Morgan and Hoening, 1997), the fecundity of the species and the sex ratio and state of maturity of individual fish (Rijnsdorp et al., 2005). Gonad development in teleost fish is a dynamic process that exhibits a diversity of patterns among species (Selman and Wallace, 1989) and within species (Fox, 1978; Urban, 1991; Witthames and Greer-Walker, 1995). Consequently, knowledge of gametogenesis and reproductive behaviour in commercially important fish species is essential to the management of any fishery (Cailliet et al., 1986; Trippel and Harvey, 1991; Garcia-Diaz et al., 2001; Tomkiewicz et al., 2003).

Studies on fish reproduction usually require knowledge of the stage of gonad development in individual fish. Gonad development is staged to allow seasonal patterns in the reproductive cycle to be identified by showing when fish begin to mature, spawn and recover (Jennings et al., 2001). Most frequently, only female ovaries are examined in reproductive studies, because these are larger and more easily examined than male teste and it is also assumed that development of both ovaries and teste is synchronous. The

most widely used method to determine the maturity stage of fish is visual or macroscopic examination of the gonads (West, 1990; Tomkiewicz et al., 2003). Macroscopic staging is based on the external appearance of the gonad and looks at the position and volume of the ovaries in the coelomic cavity, width, length, cross-section shape of the gonads, vascularisation, size and colour of the oocytes (Ricker, 1971; Brandao et al., 2003). Macroscopic assessment is a rapid and inexpensive method of determining maturity status and allows for a large number of specimens to be processed on board research and commercial vessels, and is especially useful where facilities to carry out extensive histological examinations are absent. Although macroscopic staging can enable detailed recording of the seasonal occurrence of differing reproductive stages, histological analysis of the gonads provide a more precise determination (West, 1990; Garcia-Diaz et al., 1997, 2002; Walsh et al., 2003).

Histological staging is based on a set of histological features representing progressive germ cell development (Barr, 1963c). Histological examination reveals certain characteristics that cannot be identified macroscopically (Dziewulska et al., 2002). These include cellular substructures in the developing follicles and ovarian tissue, and important physiological processes like the onset of vitellogenesis and the beginning of hydration (Maack and George, 1999). While both methods have their advantages and disadvantages, a combination of both methods of analysis has been suggested (Brandáo et al., 2003).

Macroscopic description of the plaice testes

In male plaice the testes are individual flattened masses lying one on each side of the first fin-ray (axonost) on the posterior wall of the body cavity (Wimpenny, 1953; Barr, 1963b). They project forward only a little way into the body cavity and have no extension backwards (Cole et al., 1901). They are attached to the body wall by mesenteries (Barr, 1963b) and are much smaller than the ovaries. They are indistinctly lobed, being often divided dorso-ventrally into three regions by deep grooves (Barr, 1963b). Towards the ventral and anterior extremity of the testes, the volume of the organ rapidly diminishes (Wimpenny, 1953).

True seminiferous tubules are not found in teleosts (Zuckerman, 1962) and in common with other fishes the plaice testis is composed of large numbers of ill defined seminiferous lobules or compartments (Grier, 1981) which are separated by thin connective tissue (Barr, 1963b), and orientated towards the central lumen of the gonad. The connective tissue of the lobule wall varies in thickness during the year, being only one cell thick during the period when the testis is swollen with sperm (Barr, 1963b). After spermiation, the lobules decrease and the consequent thickening of the connective tissue is thought to be due to contraction of the elastic fibres (Barr, 1963b).

The whole organ appears therefore as if divided up by a system of irregular tuberculae in the meshes of which are masses of cysts containing spermatogenic cells in different stages of spermatogenesis (spermatogonia, primary and secondary spermatocytes and spermatids). Spermatozoa are free in the lumen of the lobule. Each cyst is bounded by a thin layer of connective tissue and contains cells in the same stage of differentiation (Garcia-Diaz et al., 2002). The lobules radiate from the main collecting ducts which are situated on the anterior side of the testis (Barr, 1963b). These collecting ducts run longitudinally in the testis and unite to form the testis duct which arises near the ventral end of the testis, and opens into its terminal portion near the urinary papilla (Barr, 1963b). If the abdomen of a male fish close to spawning is gently pressed, the seminal fluid issues from the papilla (Barr, 1963b).

Spermatogenesis

The process of male germ cell development, from spermatogonial stem cells to mature spermatozoa, is termed spermatogenesis (Weltzein et al., 2004). Teleost fishes exhibit variation in testicular structure and spermatogenic patterns (Grier, 1981; Grier and Taylor, 1998), however the sequence of spermatogenesis can be broken down generally into the following; mitotic multiplication of spermatogonia, meiotic division of spermatogonia to form primary and secondary spermatocytes, spermiogenesis and spermiation. During spermatogenesis, the germ cells will go through several distinct stages of development. Most of these individual cell types are not visible in detail without

electron microscopy (Grier, 1981), but using light microscopy the associated structural changes in the testes can be identified.

In all testis types, the development of sperm takes place within cysts formed by sertoli cells (Grier et al., 1980b). Sertoli cells may also be present in the seminiferous lobules. A sertoli cell is a large cell of the epithelium lining of the testis, connected with a group of developing spermatogonia, it acts as a nurse cell. Sertoli cells are found surrounding either a single spermatogonium or cysts composed of spermatogonia, spermatocytes and spermatids. The central nucleus of the sertoli cell is irregular, and the chromatin of the nucleus is finely granular but some clumps are associated with the nuclear membrane. One or two nucleoli are located in the periphery of the nucleus (Garcia-Diaz et al., 2002).

Mitotic multiplication of spermatogonia

Spermatogonia are usually located in the periphery of the seminiferous lobules. They are either isolated or grouped into cysts with sertoli cells, and are intermingled with the interstitial tissue. Spermatogonial cells are relatively large cells, rounded in shape with a large central nucleus that contains a distinctive large nucleolus. Spermatogenesis starts with mitotic cell proliferation of spermatogonia which become aggregated in cysts which rapidly fill the lumen of the testes. In plaice mitotic divisions are first seen in June and their maximum production occurs in early autumn, as evidenced by the increased numbers and the increase in the size of the lobules (Barr, 1963b). Spermatogonia comprise the bulk of the testis during summer and early autumn, but are also present singly or in small groups at all other stages in the cycle (Barr, 1963b). The small nests of dormant spermatogonia found in the testis after spermiation appear to be responsible for its reconstitution.

Meiotic division of spermatogonia to form primary and secondary spermatocytes

In plaice the pre-spawning period begins fairly suddenly in October and is marked by an increased activity in conversion of spermatogonia to primary spermatocytes (Rijnsdorp et al., 2005). There is an increase in testis volume, height and width. This occurs because the lobules elongate as a result of both the larger volume of spermatocytes and the mitotic

divisions of peripheral spermatogonia (Grier et al., 1998). Primary spermatocytes are morphologically similar to spermatogonia though somewhat smaller. They have a nucleus that contains clumps of condensed chromatin that are associated with the membrane or are homogenously distributed along the periphery of the nucleus. The cytoplasm contains elongated mitochondria and free ribosomes. They lose their nucleolus and are most commonly seen in the prophase of the first meiotic division, the various stages of which cannot readily be distinguished (Barr, 1963b). They are absent in the spent testis.

Secondary spermatocytes are produced by the meiotic division of primary spermatocytes and are still located inside the lobules. They are small cells that possess a smaller nucleus, that is granular in appearance and surrounded by a sparse, clear cytoplasm that is highly electron dense (Garcia-Diaz et al., 2002). Both primary and secondary spermatocytes are always grouped into cysts and appear to be connected by intercellular bridges. Cell divisions are frequent within each cyst, but are slightly asynchronous. Although the two types of spermatocytes differ in size and nuclear morphology, they have similar features. The cells are oval in shape and have high nucleo-cytoplasmic ratios.

Spermiogenesis

In plaice, phases 2 and 3 overlap and together occupy the period between October and February in the population, although spermiogenesis is often complete in January (Barr, 1963b). Spermatids are produced by continuing meiotic divisions of the secondary spermatocytes. They are smaller than the secondary spermatocytes, irregular in shape and are increasingly numerous in the testis. Spermatids have a large rounded nucleus that contains electron dense granular chromatin. During differentiation, the nucleus becomes indented and a 'nuclear fossa' (Selman and Wallace, 1986) is formed where the centriolar complex resides (Garcia-Diaz et al., 2002).

Spermiation

Spermatids are transformed into spermatozoa during spermiation. The chromatin of the spermatid gathers at one side to form a cup-shaped mass which contracts to produce a

solid spermatozoa head (Grier, 1991). Tails are produced on the spermatids and spermatozoa are formed. There is a distinct invagination where the tail joins the sperm head. Unlike the large female gamete, spermatozoa are generally very small and less than 1μm in size (Rijnsdorp and Witthames, 2005). Its nucleus is similar in shape to that of a secondary spermatocyte and has very dense, homogenous chromatin.

Initially the spermatozoa are clustered with their heads attached to the lobules, often forming a ring around them. As they begin to mature, the lobular wall breaks down, leaving the spermatozoa unattached and lying free in the lumen (Htun-Han, 1978). Spermatozoa that are free in the lumen of seminiferous lobules flow towards the deferent duct where they will be expelled as sperm during spawning (Garcia-Diaz et al., 2002). In plaice, spermiation does not begin until March, but as spermiogenesis is complete by February, it is evident that there a period of 'potential maturity' during which ripe motile spermatozoa are present in the testis (Barr, 1963b).

Post spawning

After spawning physiologically the gonad is in the same stage as the immature testis, anatomically of course it is much larger and shows adult structure (De Sylva et al., 1997). A defining characteristic of the regressed stage is the presence of small nests of dormant spermatogonia within the testicular lobules (Taylor et al., 1998) which appear to be responsible for its reconstitution (Htun-Han, 1978). Cysts with secondary spermatocytes, spermatids or mature spermatozoa are absent (Grier et al., 1998). Residual spermatozoa that are present in the lumen of the testis, and sometimes in the vas deferens during the post-spawning and resting seasons, are few and noticeably inactive (Grier et al., 1998). This is evidenced by their tails being straight and rigid without the undulating characteristic of those found in the spawning period (Htun-Han, 1978). The occurrence of residual spermatozoa over a long period is fairly normal among temperate fishes (Barr, 1963b).

Macroscopic description of the plaice ovary

The ovaries are a pair of elongated conical shaped structures situated ventral to the kidney (Cole et al., 1901), and lying between the external surface of the vertebrae (haemal spines) and the muscles of the body (Wimpenny, 1953), one on each side of the fish. The anterior or base of each conical shaped ovary lies towards the head of the fish, and the posterior or cone extends backwards to the base of the tail (Cole et al., 1901; Wimpenny, 1953). The ovary of the ocular side of the fish is generally larger than that of the eyeless side (Cole et al., 1901; Wimpenny, 1953).

The ovary of the plaice, like that of the majority of teleostan fishes consists of a sac with an oviduct. The oviducts of the two ovaries join to form a common duct which opens during spawning in order to release the eggs, at other times however, this common duct is sealed up (Wimpenny, 1953). The internal walls of the ovaries have longitudinal folds (ovigerous lamellae) in which the ova are developed (Cole et al., 1901). As the eggs mature they burst out of the ovigerous lamellae and accumulate in the cavities of the ovaries and oviducts, and are expelled at intervals until the whole organ is exhausted (Wimpenny, 1953).

After spawning there is a marked change in the appearance of the ovary. The spent ovary is considerably retracted and is only visible on opening the body cavity. Its walls are soft and flaccid, and enclose a large cavity. The posterior extension still exists and is not much shorter than in the ripe specimen (Cole et al., 1901). The condition of the fish is indicated externally by a shallow groove running backwards on either side of the body, in the position formerly occupied by the full ovaries (Wimpenny, 1953).

Oogenesis

Oocytes develop within the ovary through different stages (Murua and Saborido-Rey, 2003). Although some differences occur among species, the sequence of oocyte developmental stages in teleost fish can broadly be classified into four main stages: the primary growth stage, cortical alveoli or yolk vesicle formation, vitellogenesis and maturation and ovulation (Wimpenny, 1953; Wallace and Selman, 1981; Brommage and Cumarantunga, 1988; Wallace and Selman, 1990; Tyler and Sumpter, 1996; Tyler et al.,

2000). Classification of these stages is based on physiological, biochemical, morphological and histological criteria (Tyler et al., 2000).

All ovaries, whether immature, developing or mature have several patches of oogonia (prefollicle or stem cells) present either singly or in small nests in the stroma of the ovigerous folds (Barr, 1963a; Wallace et al., 1987; Garcia-Diaz et al., 2002; Stequert et al, 2003). They are easily identified by the presence of a single, large nucleolus and are always found in association with one or more potential follicle cells (Barr, 1963a; Stequert et al., 2003). In plaice, oogonia are found all year round (Barr, 1963a) but they are most common under the epithelium lining the ovary in late Spring and Summer, during which time occasional mitotic divisions are seen (Barr, 1963a). They represent the initial stock of the oocytes which develop into the chromatin nucleolar stage of oocyte development (Wallace et al., 1987; Garcia-Diaz et al., 2002; Stequert et al., 2003).

The primary growth stage of oocyte development

Oocytes in the primary growth stage do not contain yolk and constitute a 'reserve fund' for future breeding seasons (Horwood, 1993; Murua and Saborido-Rey, 2003). The primary growth stage of oocyte development in teleosts has traditionally been divided into two phases, the chromatin nucleolar phase and the perinucleolar phase (Yamamoto, 1956; Wallace and Selman, 1981; Forberg, 1982).

The chromatin nucleolar stage is a little bigger than an oogonium, and is surrounded by a few squamous follicle cells. It has a centrally located large nucleus surrounded by a thin layer of cytoplasm (Barr, 1963a). The nucleus contains a single large strongly basophilic nucleolus (Khoo, 1979; West, 1990; Stequert et al., 2003). A distinctive feature of the chromatin nucleolus stage is the development of lampbrush chromosomes (Bara, 1960; Braeckevelt and McMillan, 1967; Lehri, 1968; Baummeister, 1973; Monaco et al, 1978), but these are not visible in histological analysis (Wallace and Selman, 1981).

The perinucleolar stage of oocyte growth can be divided into early and late perinucleolar stage oocytes. In the early stages of perinucleolar growth, the large central nucleus

(germinal vesicle) increases in size and several small basophilic nucleoli appear at its periphery (Barr, 1963a; West, 1990; Garcia-Diaz et al., 2002), apparently reflecting an amplification of ribosomal genes (Vincent et al., 1969; Vlad, 1976; Stequert et al., 2003). The size and morphology of the multiple nucleoli are variable among teleost species, but their presence throughout oocyte growth is ubiquitous (Wallace and Selman, 1981).

Late perinucleolar oocytes are slightly larger than early perinucleolar oocytes, with a circumnuclear ring and nucleus still with attached nucleoli. The oocytes begin to stain less basophilically (Selman and Wallace, 1986; Stequert et al., 2003) and vacuoles and lipid droplets begin to appear in the cytoplasm (Adrianov and Lisovenko, 1983; Casadevall et al., 1993). At the end of the perinucleolar stage the oocyte surface is extended into numerous microvilli around which the chorion precursor material begins to accumulate in patches (West, 1990; Garcia-Diaz, 2002).

The cortical alveoli or yolk vesicle formation stage of oocyte development

This stage in oocyte development can be broken down into two distinctive stages; the cortical alveolar stage and the late lipidogenic stage. In winter and spring spawning species like plaice, maturation starts in August when, through hormonal triggers, the late perinucleolar oocytes enter the cortical alveoli stage (Barr, 1963a; Horwood, 1993; Witthames and Greer-Walker, 1995; Murua and Saborido-Rey, 2003). The yolk vesicle or cortical alveolar stage is characterised by a vacuolated cytoplasm and the appearance of yolk vesicles near the nucleus, which migrate to the periphery of the cytoplasm (West, 1990). A membrane defines these spherical structures which appear empty in conventional Haematoxylin and Eosin preparations (Forberg, 1982). The yolk vesicles increase in size and number to form several peripheral rows in the cytoplasm just inside the cell membrane. Their size is variable and some may fuse with one another (Garcia-Diaz et al., 2002). The chorion (zona radiata, vitelline membrane, zona pellucida) appears at this stage, and some authors use the co occurrence of the zona radiata and vacuolated cytoplasm to distinguish between yolk vesicle and perinucleolar oocytes (Yamamoto, 1956; Yamamoto and Yamazaki, 1961; Pollard, 1972; Wu and Chang, 1979; Treasurer and Holiday, 1981; Adrianov and Lisovenko, 1983). However the appearance of the zona

radiata can vary between species, being reported in the late perinucleolar stage (Yamamoto and Yoshioka, 1964; Latif and Saady, 1973; Davis, 1977) and at the end of the yolk vesicle stage by Baglin (1982).

Late Lipidogenic oocytes have a central large nucleus and a vacuolated cytoplasm. Yolk vesicles increase in size and number and are visible throughout the cytoplasm, with the exception of a narrow zone at the periphery (Stequert et al., 2003). Late Lipidogenic oocytes are characterised by having an inner ring of lipid droplets, a growing ring of yolk globules, and a narrow slightly staining ring of cortical alveoli. In species in which the eggs contain an oil globule, oil droplets begin to accumulate in the cytoplasm at this stage (De Vlaming, 1983; Murua and Saborido-Rey, 2003). They are involved in the formation of the oil or lipid globule in fully developed eggs. The contents of these lipid droplets are dissolved during dehydration with alcohols and appear empty with conventional staining (West, 1990). The chorion is composed of two layers of different thickness and density, a clear and homogenous external layer that began its formation in the previous stage, and an inner layer that is electron dense and compact (Garcia-Diaz et al., 2002). Numerous canals through which microvilli extend traverse it, producing a characteristic striated appearance (Garcia-Diaz et al., 2002).

The vitellogenesis stage of oocyte development

The appearance of yolk proteins in fluid filled spheres (yolk spheres, granules or globules) is characteristic of vitellogenic or yolk-stage oocytes (Grodzinski, 1954, 1973; West, 1990). The oocytes increase considerably in size as yolk spheres fill the ooplasm (Murua and Saborido-Rey, 2003). The yolk spheres may maintain their integrity throughout oocyte growth (Yamamoto, 1957b) or fuse to eventually form a continuous mass of fluid yolk (Wallace and Selman, 1981). The fusion of yolk spheres may begin soon after their initial formation or as late as final maturation (Wallace and Selman, 1981). Other visible characteristics of this stage include an advanced chorion (consisting of two layers, the granulosa and the theca, each being one cell thick) and a central large nucleus, around which the oil droplets are concentrated. Vitellogenesis ceases once

oocytes reach their fully developed size and these eventually undergo maturation and ovulation after appropriate hormonal stimulation (Masui and Clarke, 1979).

The maturation and ovulation stages of oocyte development

In the maturation stage of oocyte development the oil droplets fuse into an oil drop that migrates towards the animal pole, together with the nucleus (Garcia-Diaz et al., 2002). When the nucleus has completed its migration, its membrane dissolves and the first meiotic division takes place (Foucher and Beamish, 1977; Wallace et al., 1987; Bromage and Cumarantunga, 1988; Murua and Saborido-Rey, 2003). At the same time, the yolk spheres coalesce and appear as a homogenous mass filling the interior of the oocyte (West, 1990). The oocytes appear transparent and the chorion is very thin (Barr, 1963a; Hunter and Macewicz, 1985). The oocyte increases in size due to a rapid uptake of fluid through its follicle (Fulton, 1898; Barr, 1963a; Hunter and Macewicz, 1985; Witthames and Greer-Walker, 1995). This process is especially pronounced in marine species that spawn pelagic eggs, and renders the spawned egg buoyant in seawater (Wallace and Selman, 1981; Craik and Harvey, 1987; Murua and Saborido-Rey, 2003). At this stage the oocyte is called a hydrated oocyte and is homogenous, finely granular and weakly basophilic in appearance (Hunter and Macewicz, 1985).

After maturation is complete, the oocyte bursts out of its follicle (Barr, 1963a; Witthames and Greer-Walker, 1995) and is ovulated into the ovarian lumen. After ovulation, the second meiotic division occurs and the oocyte becomes an egg (Murua and Saborido-Rey, 2003), surrounded by a tough chorion (Wallace and Selman, 1981). It is later expelled at sea during the spawning process (Stequert et al., 2003), where it awaits fertilisation (Horwood, 1993). Ovulation results in ruptured, empty oocyte envelopes or post-ovulatory follicles (West, 1990). These consist of a theca and granulosa layer of cells. They have an irregular arrangement due to shrinkage of the follicle, and remain in the epithelium lining of the ovarian cavity. About two months after spawning these follicles are present only as small accumulations of thecal cells which eventually disappear completely (Barr, 1963a).

Atretic oocytes

A small number of developing oocytes abort their development towards final maturation and become atretic (Wood and Van der Kraak, 1999). They are not of frequent occurrence and form only a small proportion of the total oocytes in the ovary (Wallace and Selman, 1981). Atretic oocytes (corpora atretica) are formed by the degeneration of stage V (vitellogenic) and VI (hydrated) oocytes inside the follicle membranes (Barr, 1963a). This atresia can be identified by hypertrophy in the follicular layer, which is accompanied by the degeneration of the chorion (outer boundary of the oocyte) and leakage of cytoplasm and yolk granules into the ovary lumen (Wood and Van der Kraak, 1999).

Previous plaice reproductive studies.

The studies reviewed here are the principal reproductive biology investigations carried out for plaice in Irish and European waters.

Irish Sea plaice reproductive biology and maturity studies.

Simpson (1959) looked at the spawning of plaice in the Irish Sea. He studied the timing and location of spawning through the use of systematic quantitative plaice egg surveys, and he examined the size and age composition of the spawning fish. Barr (1963a) studied the endocrine control of the sexual cycle in plaice. He described the cyclical changes in the ovary and the histology of the ovary in relation to oogenesis. Barr (1963b) examined the endocrine control of oogenesis in plaice, at different times during the reproductive cycle. He investigated the nature of the pituitary-gonad relationship, and the effects of hypophysectomy on the germ cells of immature plaice. Barr (1963c) studied the endocrine control of spermatogenesis in plaice, and the effects of gonadotropin withdrawal on males. He made a detailed study of spermatogenesis in the normal male in order to provide a basis for the comparison of experimental results. Horwood (1990) looked at the fecundity and maturity of plaice in the Irish Sea, and compared the size and age at maturity of plaice from Cardigan Bay with surrounding locations. Ellis and Nash (1997) looked at the spawning of plaice around the Isle of Man in the Irish Sea. The

locations and timing of plaice spawning were studied from distributions of recently spawned eggs recorded in plankton samples. Fox et al. (2000) examined patterns in the spawning of plaice in the Irish Sea. Generalised additive modelling was used to identify spawning localities and investigate temporal trends in egg production. Gerritsen and McGrath (2005) looked at variability in the assignment of maturity stages of plaice using macroscopic maturity criteria. They investigated if a macroscopic maturity scale could be applied consistently by examining the variability in the assignment of maturity stages to fresh plaice between and within 10 people who repeatedly assessed the sex and maturity stages of 80 plaice gonads collected off the north of Ireland and in the Celtic Sea.

North Sea plaice reproductive biology and maturity studies.

Horwood et al. (1986) looked at the fecundity of individual plaice in relation to the size and age of the fish, and examined fecundity differences between spawning area and year. Rijnsdorp (1989) investigated differences in maturation of male and female North Sea plaice. He examined changes in growth, the onset of maturity, fecundity, spawning duration, the relation between the reproductive state and the level of feeding, spatial distribution of immature and mature plaice, and the length and age at first maturity in different parts of the southern North Sea. Rijnsdorp and Ibelings (1989) looked at sexual dimorphism in the energetics of reproduction and growth in North Sea plaice. They analysed the chemical composition and energy content of mature and immature male and female fish during the spawning period, to study differences in the allocation of energy over reproduction and somatic growth between the sexes. Cushing (1990) described the hydrographic containment of a spawning group of plaice in the Southern Bight of the North Sea. Rijnsdorp (1990) examined the mechanism of energy allocation over reproduction and somatic growth in female North Sea plaice. He analysed the seasonal pattern in energy allocation to see whether processes of somatic growth and gonad growth are confined to different times of the year, and he also studied the relationship between somatic growth and reproductive investment in terms of fecundity, gonad weight and pre-spawning body reserves. Rijnsdorp (1991) reviewed the changes in size-specific fecundity, egg weight, ovary weight and fecundity – body length relationships of female North Sea plaice between three periods; 1900-1910, 1947-1949, and 1977-1985. Urban

(1991) used frequency distributions of oocyte diameters measured by means of semi-automatic computerised image analysis to determine the reproductive strategy of North Sea plaice caught during the spawning season. Rijnsdorp et al. (1991) examined inter-annual reproductive variability in North Sea plaice using time series of growth, maturation and fecundity, paying special attention to density dependent effects. Rijnsdorp (1993) attempted to disentangle the phenotypic and genetic components of the observed changes in maturation and reproductive investment in female North Sea plaice. Rijnsdorp (1993) explored the relationship between juvenile growth and the onset of sexual maturation in North Sea plaice on the individual and population level, by analysing individual growth curves back calculated from otoliths of immature and mature fish, and by analysing maturity-length ogives of individual cohorts. Bromley (2000) used a general linear model to investigate growth, sexual maturation and spawning in commercial and survey samples of central North Sea plaice. Grift et al. (2003) analysed how intensive exploitation may have caused evolutionary changes in the age and length at maturation in North Sea plaice. Nielsen et al. (2004) studied the spawning and maturity of plaice in the Kattegat to determine if plaice still spawned there, where the main spawning grounds were and whether the egg distribution is related to the local hydrogeography. Rijnsdorp et al. (2005) developed a model of the reaction norm for reproductive investment to disentangle fisheries-induced adaptive changes in reproductive investment from changes in growth in North Sea plaice.

English plaice reproductive biology and maturity studies.

Brule (1987) examined the reproductive biology and the pathological changes of the plaice after the 'Amoco Cadiz' oil spill along the North West coast of Brittany.

Icelandic plaice reproductive biology and maturity studies.

Solmundsson et al. (2003) examined sexual differences in the spawning behaviour and catchability of plaice west of Iceland. This was primarily accomplished by monitoring individual fish with DST's and conventional tags, supplemented by data on sex ratios and maturation obtained from commercial fisheries.

Baltic Sea plaice reproductive biology and maturity studies.

Nissling et al. (2002) studied the effect of salinity on the reproductive success of plaice in the Baltic Sea. Spermatozoa motility, fertilisation and egg development at different salinities was assessed, and the salinities at which eggs are neutrally buoyant were determined.

In summary, most of the studies done to date on the reproductive biology of plaice in European waters, have been undertaken on the very important North Sea stocks. Very little work has been done on plaice stocks in Irish waters, and the few studies that have been done, were mainly restricted to the Irish Sea. This study will examine the reproductive biology and maturity of plaice sampled in ICES areas VIIb (off the west coast of Ireland) between November 2003 and February 2005. A detailed study of the plaice reproductive cycle will be undertaken using both macroscopic and histological methods of assessment. A photographic record/key and a histological record of the reproductive developmental stages in male and female plaice will be presented. It is intended that, by successfully comparing the macroscopic and histological maturity assessments for plaice, and in conjunction with the determination of the gonadosomatic index (GSI), a concrete estimation of the spawning periodicity will be made for the species off the west coast of Ireland.

2.2 Materials and Methods

2.2.1 Laboratory Analysis

Macroscopic Maturity Assessment

In the body of the plaice the gonads lie lengthways, parallel to the spine. In the female plaice the gonads lie with the broader anterior end at the head of the fish and the apex extending backwards towards the tail (Plate 2). The male gonads are found in the same location as the females, but they have no extension backwards (Plate 3). A macroscopic examination of the maturity stages of the fish was made and fish were assigned maturity stages of I – VII for female fish and I – VI for male fish, according to the scale devised by Wimpenny (1953) and shown in Figure 2.2.1(a). The maturity stage of the ovaries or testes of a fish refers to the degree of ripeness or how close an individual is to spawning (Caillet et al., 1986). These maturity stages relate to the different development stages of immature, developing, ripening, mature, and spawning or spent (Table 2.2.1 (a)).

Table 2.2.1 (a). Development stages as they relate to female and male macroscopic maturity stages.

Stage of development	Female maturity stage	Male maturity stage
Immature / Virgin	I	I
Developing Virgin / Spent - Recovering	II	II
Ripening	III and IV	III
Mature	V	IV
Spawning	VI	V
Spent	VII	VI

The gonads were then removed from the body cavity of the fish and placed on a white surface under direct lighting. A ruler was placed along the length of the gonad to give scale to the picture. A stand held a camera in position above the gonad. The gonads were then photographed using a Concord 4060AF® camera with a 4 megapixel capacity, so that a photographic macroscopic key could be developed.

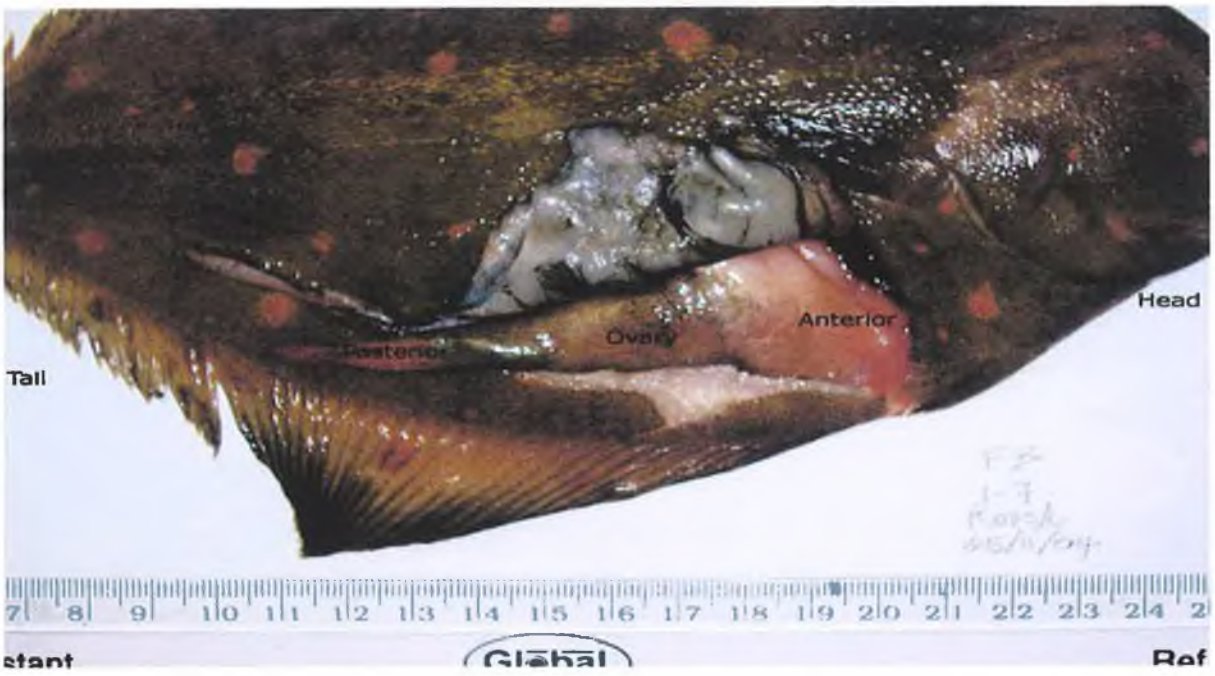


Plate 2. Dissection of a female plaice which was caught off the west coast of Ireland in November 2004. The ovary can be seen clearly. Scale in cm. (Photograph by author).

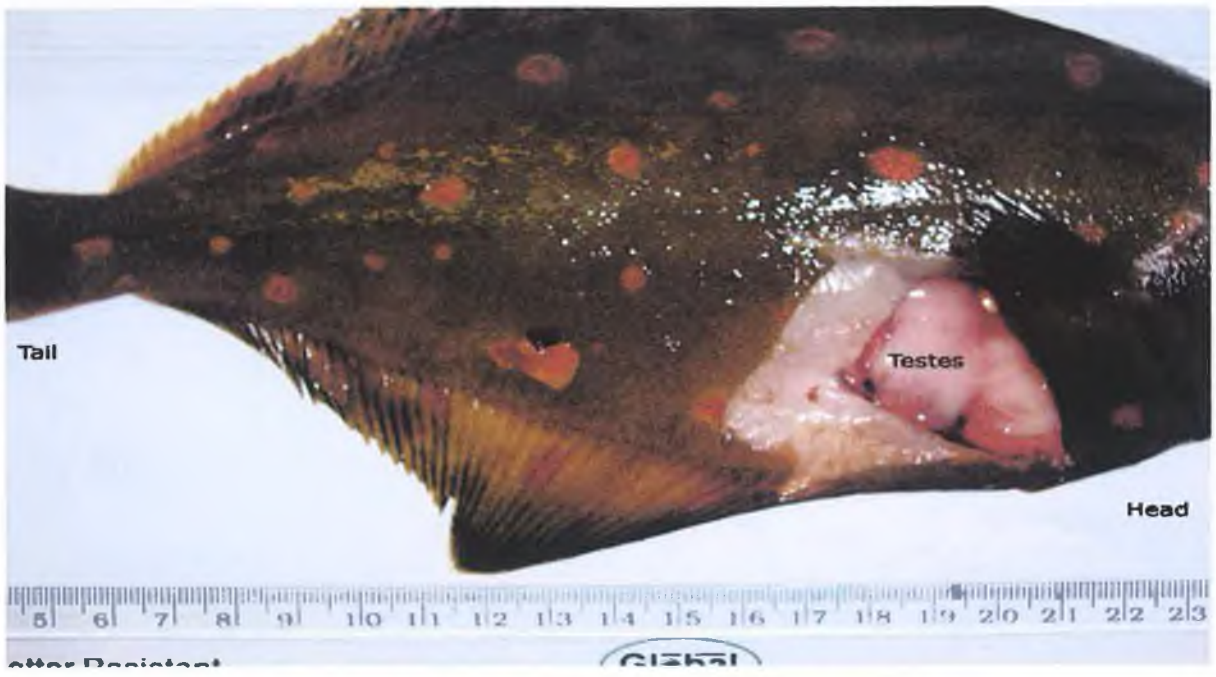
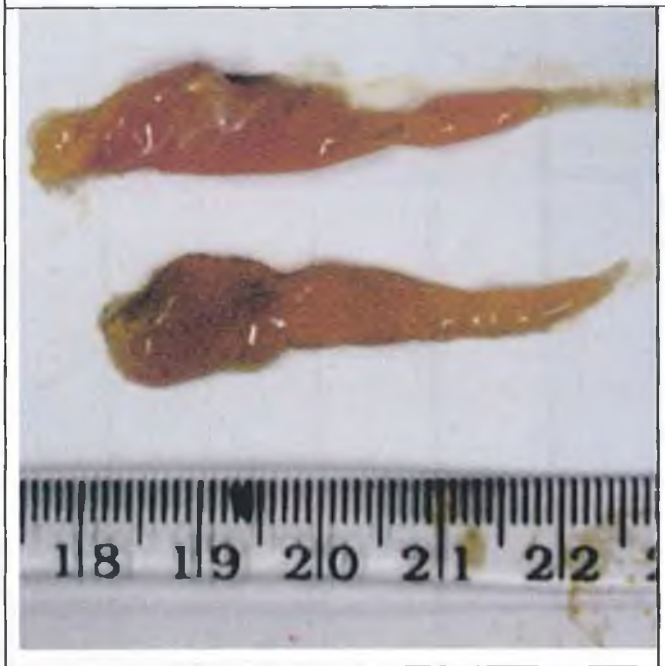


Plate 3. Dissection of a male plaice which was caught off the west coast of Ireland in November 2004. The testes can be seen clearly. Scale in cm. (Photograph by author).

Figure 2.2.1 (a). Macroscopic maturity stages for plaice *P. platessa* L. (After Wimpenny, 1953).

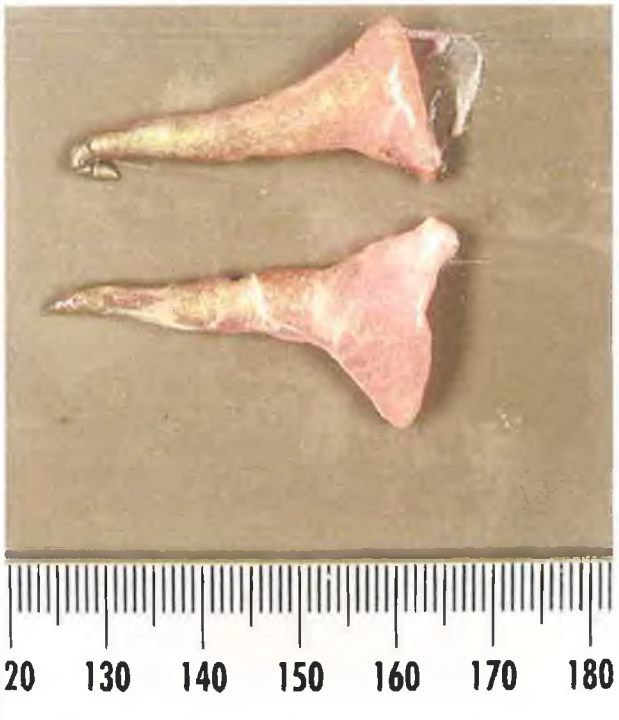
Females



Stage I. Immature / Virgin

Ovaries very small with posterior projections not more than three cm in length. Wall thin and easily broken. Internally, the lumen is transparent, and yellowish-orange in colour. Ovary 0.1-0.5 % of body weight. Present in the authors samples from October to March.

Pictured ovary from a stage I (immature or virgin) female *P. platessa*, measuring 28.5cm TL and aged 3 years old. Scale in cm. (Photograph by author).

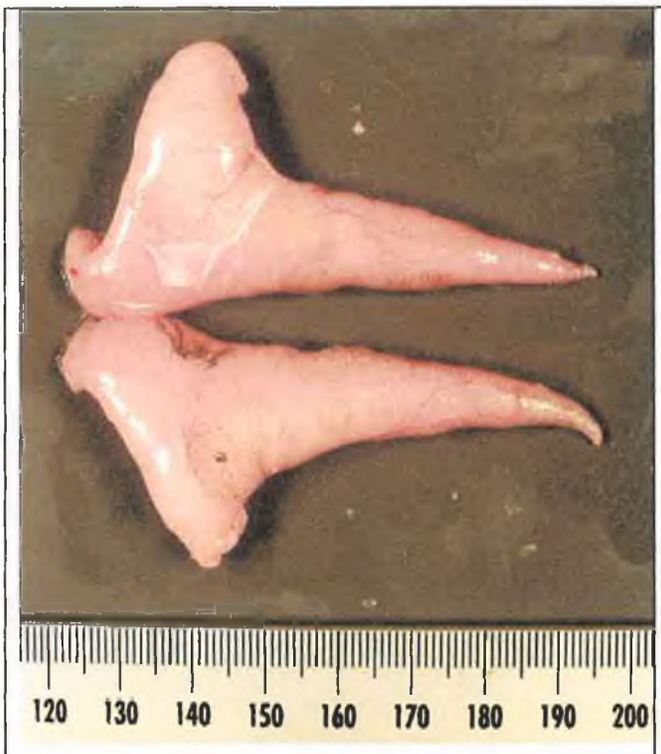


Stage II.

Developing Virgin / Spent - Recovering

Ovary increasing in size, flattish, firm, dull reddish-orange to pink in colour. Wall still thin. Little or no slime in ovaries, all eggs reabsorbed, ovary tissue somewhat translucent. Ovary 0.1-0.5 % of body weight. Present in the authors samples from late May to early September.

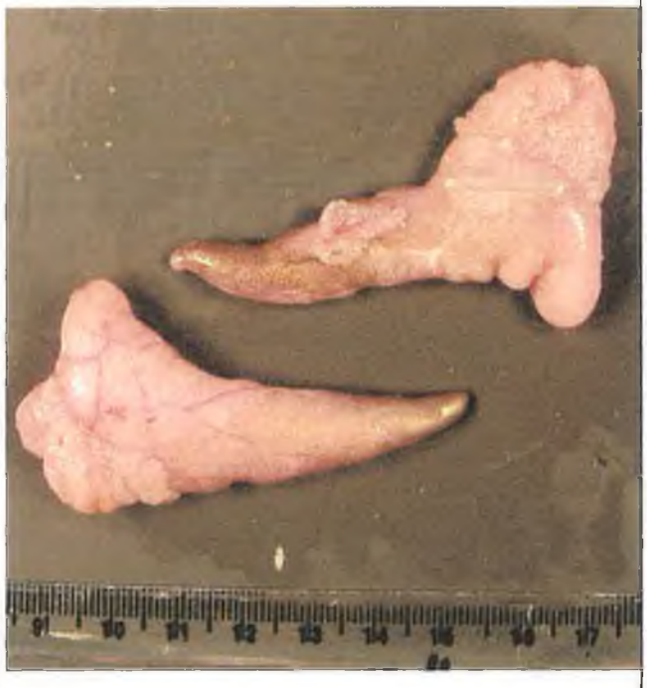
Pictured ovary from a stage II (developing virgin / spent-recovering) female *P. platessa*, measuring 33cm TL and aged 5 years old. Scale in cm. (Photograph by author).



Stage III. Early Ripening

Ovaries roughly half full with developing oocytes. Pinkish-white in colour. Opaque eggs just visible. Ovaries swollen to about 0.5-0.8 % of body weight. Spawning certain to take place in the same season. Present in the authors samples from late September to end of January.

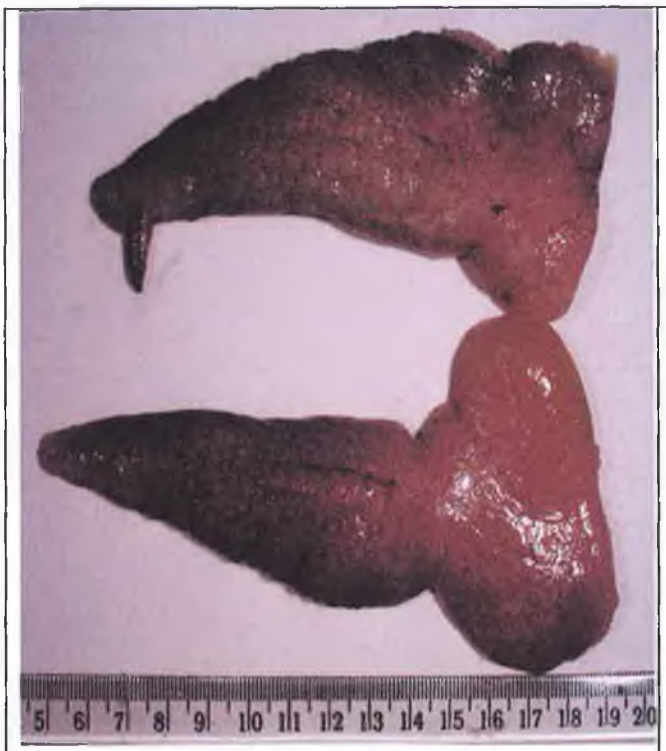
Pictured ovary from a stage III (early ripening) female *P. platessa*, measuring 29cm TL and aged 3 years old. Scale in cm. (Photograph by author).



Stage IV. Late Ripening

Ovaries large, firm and full with eggs, body distended. Orange to pinkish-white in colour. Opaque eggs clearly visible. No hyaline eggs. Ovary 8-15 % of body weight. Present in the authors samples from October to February.

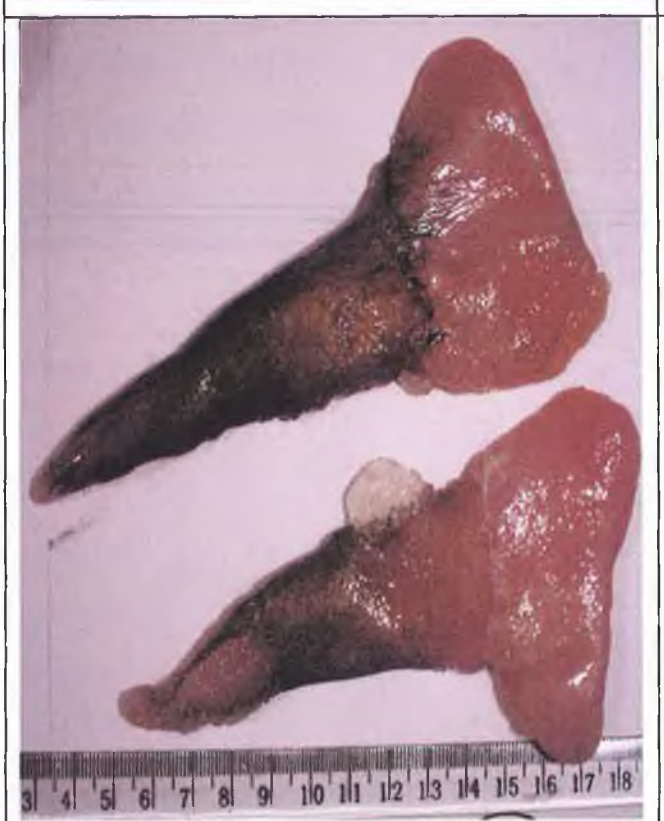
Pictured ovary from a stage IV (late ripening) female *P. platessa*, measuring 31cm TL and aged 2 years old. Scale in cm. (Photograph by author).



Stage V. Ripe / Pre Spawning

Gonad very swollen in body cavity. Ovaries contain from a few to many single glassy hyaline eggs which are clearly visible, but will not run under moderate pressure. Ovary 15-27 % of body weight. Present in the authors samples from January to March.

Pictured ovary from a stage V (ripe / pre-spawning) female *P. platessa* measuring 31cm TL and aged 4 years old. Scale in cm. (Photograph by author).



Stage VI. Running / Spawning

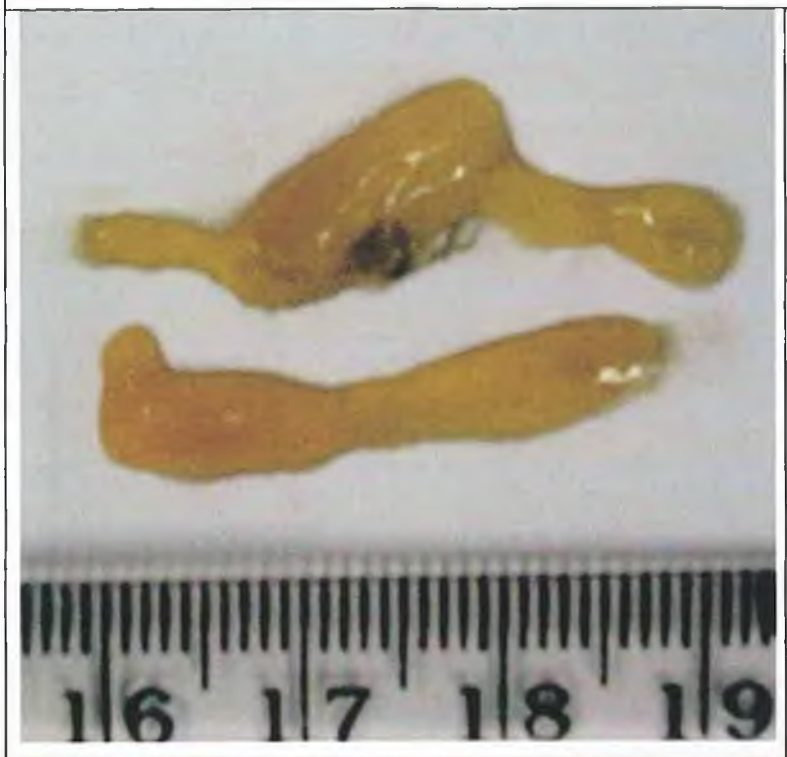
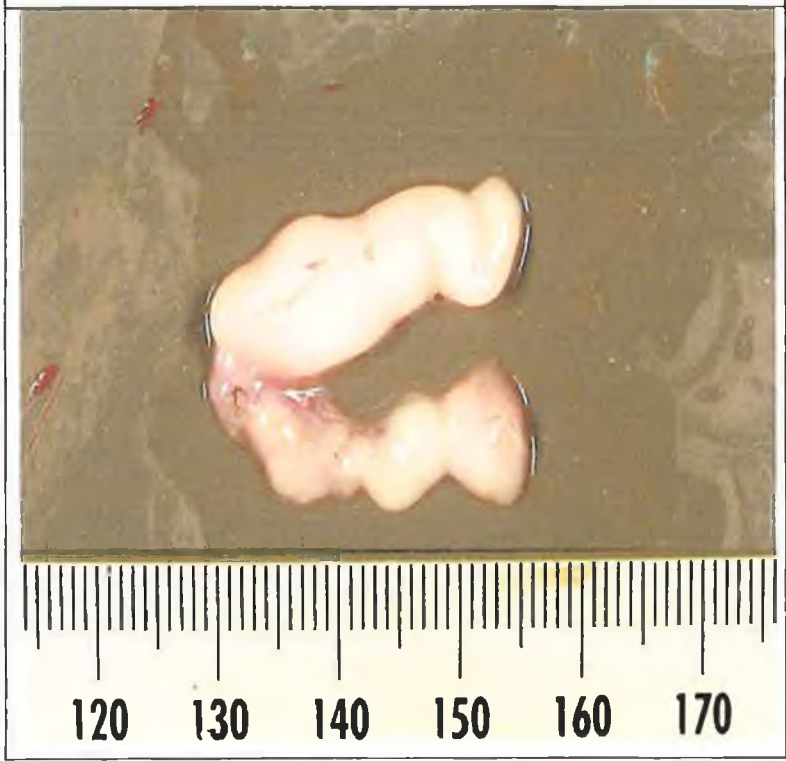
Gonad very swollen in body cavity. Ovary full with translucent hyaline eggs clearly visible under thinning ovary wall. Ovulated eggs are easily extruded by slight pressure and may be seen leaking from fish. Ovary is very fragile and difficult to remove from fish without rupturing. Ovary is 33 % of body weight. Eggs may be partly shed. Present in the authors samples from January to March.

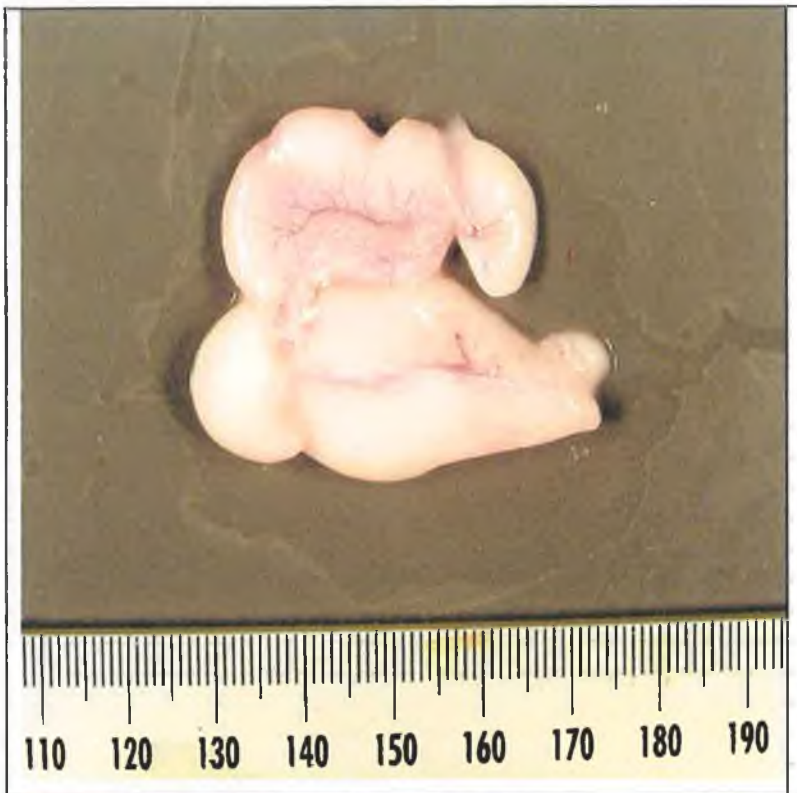
Pictured ovary from a stage VI (spawning) female *P. platessa* measuring 31cm TL and aged 4 years old. Scale in cm. (Photograph by author).

Stage VII. Spent / Post –Spawning

Ovary a small, flabby, flaccid empty bag with a wide lumen. Has a grey-silverish, bloodshot colour. Stringy in appearance. Much slime in ovaries. Few eggs remaining, some hyaline or atretic, mainly opaque, in a state of being reabsorbed. More and more frequent in spawning season until April, after which it gradually disappears. Ovary returns to a stage II.

Pictured ovary from a stage VII (spent) female *P. platessa* measuring 37cm TL and aged 6 years old. Scale in cm. (Photograph by author).

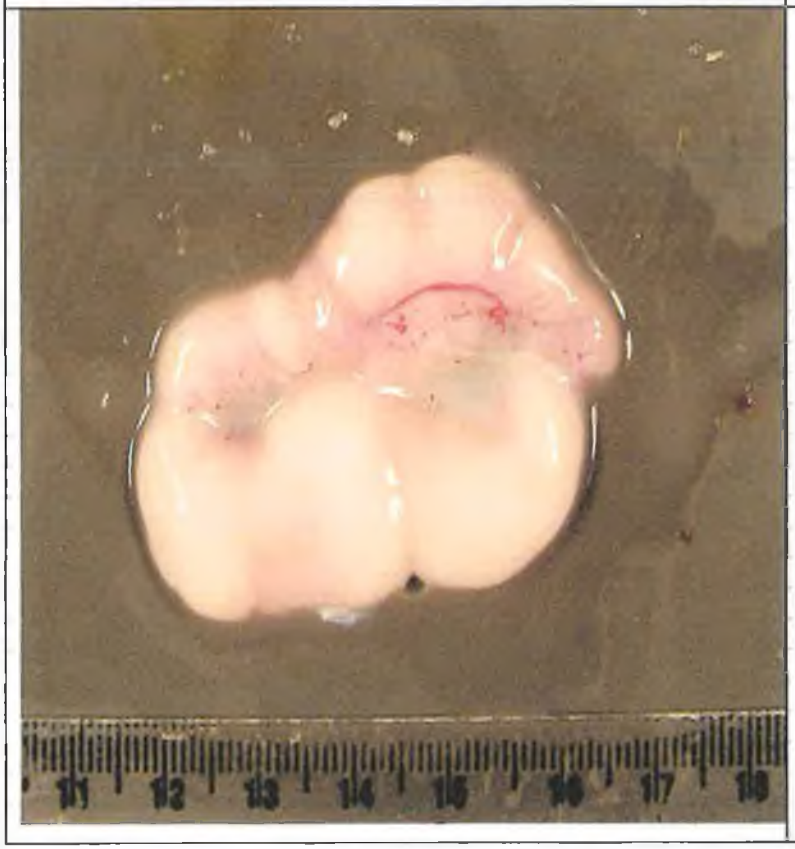
Males	
	**Stage I.** **Immature / Virgin** Testes tight against back of abdominal cavity and very small and thin. Stringy in appearance, translucent, white-pink in colour and difficult to remove from fish. These were present in the authors samples all year round. Pictured testes from a stage I (immature) male *P. platessa,* measuring 28cm TL and aged 3 years old. Scale in cm. (Photograph by author).
	**Stage II.** **Developing Virgin / Resting Spent** Testes more swollen but still quite thin and small. Still pinkish-white in colour. All sperm from ducts reabsorbed. These were present in the authors samples between July and September. Pictured testes from a stage II (developing virgin / resting spent) male *P. platessa,* measuring 19cm TL and aged 2 years old. Scale in cm. (Photograph by author).



Stage III. Ripening

Testes half full. Greyish-white in colour with some blood vessels visible. No sperm in ducts. Testes fully developed as to size, but milt not running and not squeezed out by moderate pressure. Recorded in the author's samples between October and February.

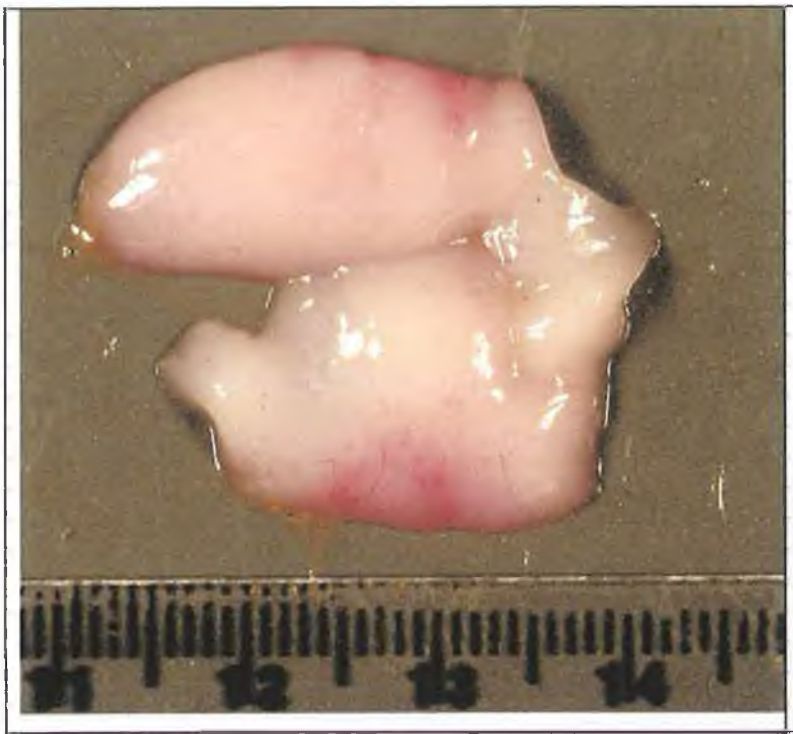
Pictured testes from a stage III (ripening) male *P. platessa,* measuring 31cm TL and aged 3 years old. Scale in cm. (Photograph by author).



Stage IV. Ripe

Testes fully swollen and nearly filling body cavity. Testes extremely difficult to remove from the body cavity due to the delicate nature of the tissue. Large and milky white in colour. Milt freely running or able to be forced out by slight pressure on the posterior ventral walls of the abdomen. Recorded in the author's samples between October and February.

Pictured testes from a stage IV (ripe) male *P. platessa*, measuring 32cm TL and aged 3 years old. Scale in cm. (Photograph by author).

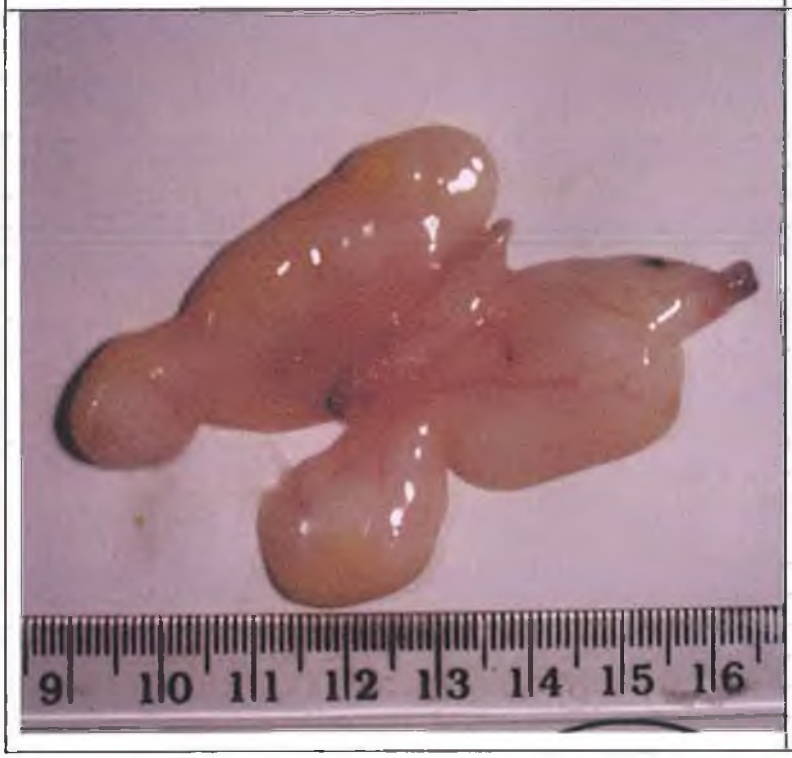


Stage V.

Running / Spawning

Testes becoming reduced and often somewhat discoloured. Milt expressible with difficulty. Present in the authors samples between February and April.

Pictured testes from a stage V (spawning) male *P. platessa,* measuring 32cm TL and aged 6 years old. Scale in cm. (Photograph by author).



Stage VI. Spent

Testes small, form of half moon, flaccid and shrunken, often going back to stage I in appearance. They can be yellow-white to pale brown in colour and often bloodshot. There may be residual traces of sperm present, which can be extruded with difficulty. Present in the authors samples between May and July.

Pictured testes from a stage VI (spent) male *P. platessa,* measuring 25cm TL and aged 4 years old. Scale in cm. (Photograph by author).

2.2.2 Histology

A transverse section (c. 1cm long) was taken from the dorsal gonad of each fish for histological analysis. The gross cut was made in approximately the same place on each gonad in order to maintain consistency. A maximum of 30 males and 35 females (approx. 5 individuals per maturity stage) were chosen at random from each monthly sample collected between October 2003 and February 2005. The sections were stored in carefully labelled vials of 4% buffered formalin prior to histological processing.

Tissue Processing

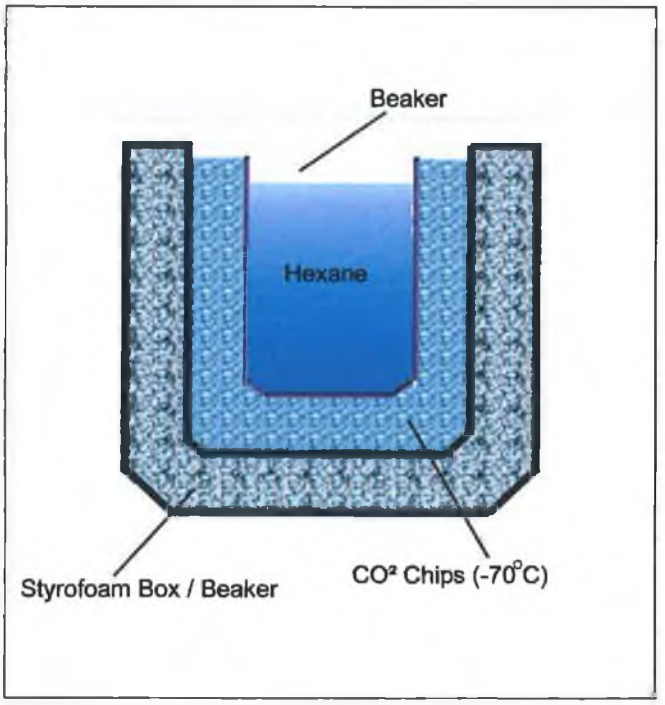
Cryohistology was used to fix samples in order for the microstructure to be studied. Cryohistology can eliminate many of the problems associated with standard practices of chemical fixation and wax embedding used in traditional wax histology (Donovan and Preston, 1994). It is also less labour intensive. The procedure was as follows: The selected gonad samples were placed in carefully labelled individual cells / trays and immersed in 4% buffered formalin for 1-2 hours to ensure that the fixative penetrated the entire sample. Then the samples were rinsed in PBS (Phosphate Buffered Saline) solution until there was no smell of formalin off the samples. They were then soaked in a series of cryoprotectants, 10%, 20% and 30% sucrose / DMSO (DMSO = Dimethylsulphoxide) solutions. Cryoprotectants are thought to increase viscosity at sub-zero temperatures, thereby decreasing the mobility of water molecules (Donovan and Preston, 1994). The constrained water molecules are prevented from forming ice crystal nuclei and ice crystal formation is inhibited (Donovan and Preston, 1994). The samples were left in each sucrose solution for 4 plus hours. The samples were then rinsed in PBS solution and blotted dry on tissue paper. Finally each sample was placed in a carefully labelled mould, covered in Cryo-M-Bed® embedding compound for frozen tissue specimens and left for up to 4 hours to mix well.

Cryofreezing

A cryobath (Fig. 2.2.2 (a)) was set up and the temperature monitored with an RS 206-3744® temperature meter. The cryobath was constructed by placing a glass beaker inside a larger Styrofoam® beaker and placing frozen CO_2 (dry ice) chips between the two beakers. The CO_2 chips were built up around the inner glass beaker. Hexane

($CH_3.(CH_2)_4.CH_3$) was poured into the inner beaker. CO_2 chips were added to the hexane until a constant temperature of between -75° and -80° C was achieved. Using forceps and wearing protective gloves, a sample in its mould was lowered into the cryobath and left for a few seconds. Then it was removed from the cryobath and from its mould and wrapped in aluminium foil. Finally it was stored in a labelled cell in a box of CO_2 chips. The above protocol was repeated for each sample, prior to storage at -80° C in a low temperature super freezer. Ovary samples were suspended on the surface of the cryobath using a forceps for a few seconds before full immersion in the hexane. This allowed the samples to be cooled at a slightly slower rate, thus preventing any large oocytes from cracking. The temperature of the cryobath was checked at regular intervals and modified by adding CO_2 chips to the hexane.

Figure. 2.2.2 (a). Cryobath diagram.



Sectioning

Slides were prepared for use by coating them in glycerin albumin and leaving them in a hot press to dry. The glycerin albumin coagulates on heating thus allowing the sections to adhere to the slide. Samples were sectioned on a Leica CM 1900® cryostat. Firstly, they were secured to a chuck in the cryostat using Bright Cryo-M-Bed®. This freezes in the low temperature of the cryostat. When the sample was secured to the chuck, the chuck was inserted in the cryostat above the blade. The samples were then sectioned at 4 and 7μm. When a satisfactory section was achieved, a slide was pressed onto a collection plate to pick up the section.

Staining

The slides with collected sections were left lying flat on a lab bench overnight to dry. The slides were then stained in a Leica ST4040 ® linear stainer using standard Haematoxylin and Eosin dye sequence after Bancroft and Stevens (1990). Haematoxylin stains nuclei, cartilage and RNA purple-blue while the counterstain Eosin stains only those structures not stained by the Haematoxylin an orangey-pink colour (Bancroft and Stevens, 1990).

Care was taken during staining to ensure that all 27 baths / compartments were full to the maximum level. The slides were left for 1 minute 10 seconds in each bath, with 5 seconds draining time between each bath. They were then automatically moved onto the next bath. The entire sequence took just over half an hour.

Directly following staining, excess histoclear was wiped from the slides without damaging the sections. The slides were then inverted onto large coverslips containing a couple of drops of DPX (Dibutyl phthalate). The DPX forms a bond with the histoclear, and thus permanently preserves and protects the tissue and seals the slide indefinitely. The completed slides were left flat in a fume cupboard for several days in order to allow the DPX to harden, and then stored in labelled slide boxes until examination.

2.2.3 Histological Maturity Assessment

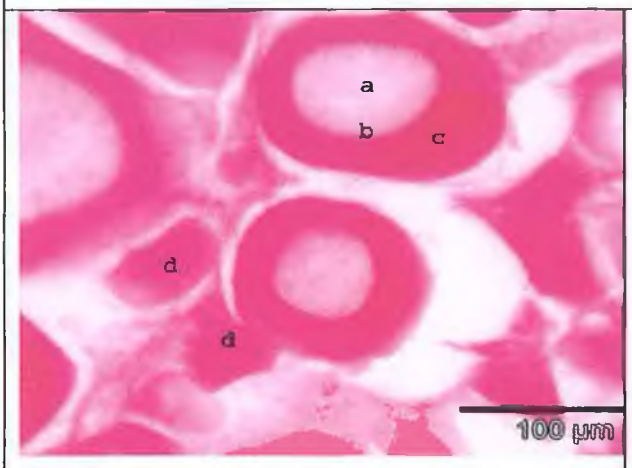
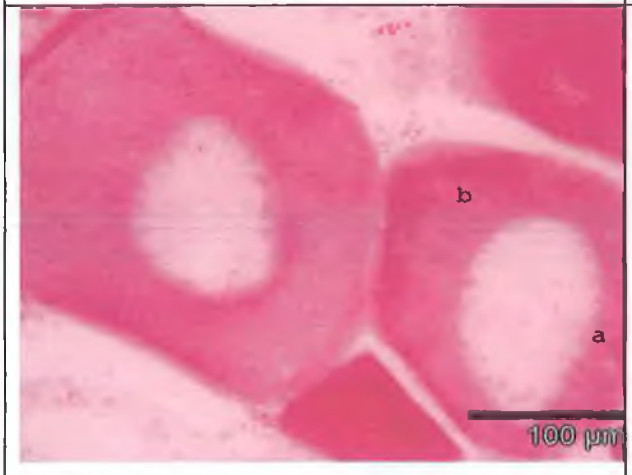
The prepared slides were examined microscopically using an Olympus CX41® compound microscope. For each histological section a number of developmental characteristics of oogenesis and spermatogenesis were observed and recorded, and used to determine the respective maturity stages. Photographs of selected sections were taken using an Olympus camedia C-3040 Zoom® digital camera with a 3.3 megapixel capacity. Light microscopy photomicrographs of the histological structure of male and female plaice gonads in different stages of maturation are shown in plates 4 to 17. Developmental stages have been marked with labels where appropriate.

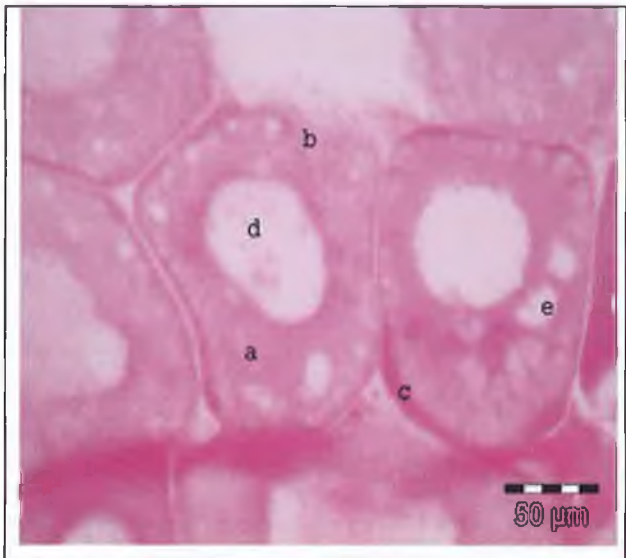
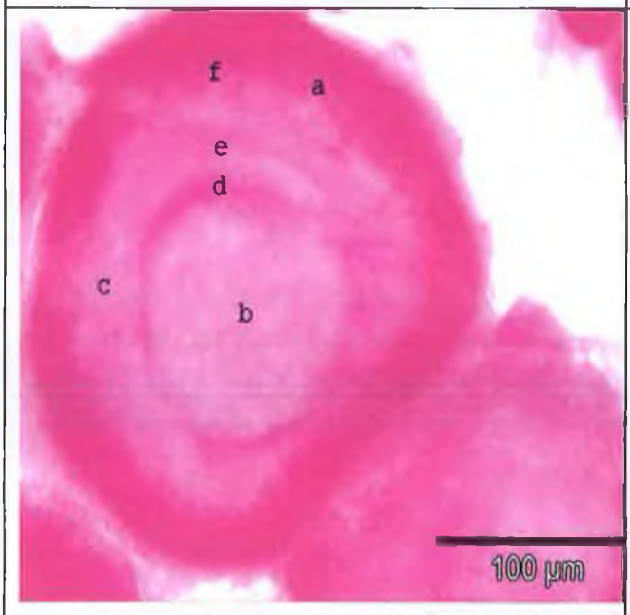
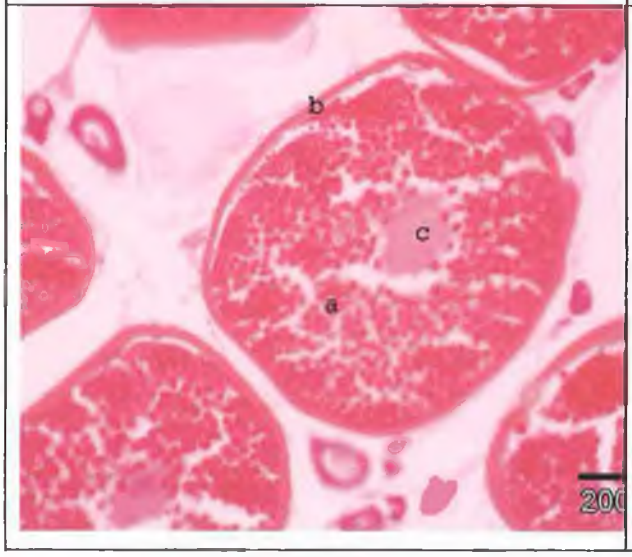
The maturity stages of plaice ovaries were characterised by a set of histological features representing progressive oocyte development, after Barr (1963a). These are described for female plaice in Table 2.2.3(c) and light microscopy photomicrographs of the various cell types seen in oogenesis are presented in Table 2.2.3(a). Most maturity stages in the ovary have more than one cell type present at any one time. The stage of maturity was therefore determined by the larger most advanced cell type present, after Tomkiewicz (2003). A summary of the cell types observed in each female plaice maturity stage is presented in Table 2.2.3(b).

The maturity stages of plaice testes were characterised by a set of histological features representing progressive germ cell development, after Barr (1963c). These are described for male plaice in Table 2.2.3(d). Light microscopy photomicrographs of the various cell types seen in spermatogenesis are not available due to the small size of the cells. Examination of male germ cell ultrastructure would require the use of scanning and transmission electron microscopy (Huang et al., 2002).

The reproductive stage of development as determined by histological examination was then compared to the original maturity stage that was visually assigned to the gonad. The accuracy of visual staging was assessed.

Table 2.2.3(a). Microscopic cell development stages for female plaice *P. platessa* L. (After Barr, 1963a). (Photographs taken by author).

	**Early Perinucleolar oocyte** This stage in development consists of a large circular nucleus **(a)** with peripheral nucleoli **(b)** surrounded by a thin layer of cytoplasm **(c)**. Early perinucleolar oocytes may be surrounded by a few squamous follicle or germ cells called chromatin nucleolar cells **(d)**.
	**Late Perinucleolar oocyte** These are slightly larger than early perinucleolar oocytes with a circumnuclear ring and the nucleus still with attached nucleoli **(a)**. Vacuoles and lipid droplets begin to appear in the cytoplasm **(b)**.

	**Cortical Alveolar stage oocytes** Oocytes with cortical alveoli/yolk vesicles **(a)** not entirely filling the cytoplasm. Cortical alveoli appear near the nucleus and migrate to the periphery **(b)** of the cytoplasm. Thin initial chorion/vitelline membrane **(c)**. Nucleus with atttached nucleoli **(d)** displaying early nucleus migration. Vacuolated cytoplasm **(e)**.
	**Late Lipidogenic stage oocytes** Yolk granules **(a)** starting to fill cytoplasm. Central large nucleus **(b)**. Vacuolated cytoplasm **(c)**. Oil droplets begin to accumulate in the cytoplasm. Oocyte characterised by having an inner ring of lipid droplets **(d)**, a growing ring of yolk globules **(e)** and a narrow lightly staining ring of cortical alveoli **(f)**.
	**Vitellogenic oocytes** Enlarged yolk granules entirely fill and expand the ooplasm **(a)**. Advanced chorion **(b)** and central large nucleus **(c)**. Yolk proteins contained in fluid filled spheres.

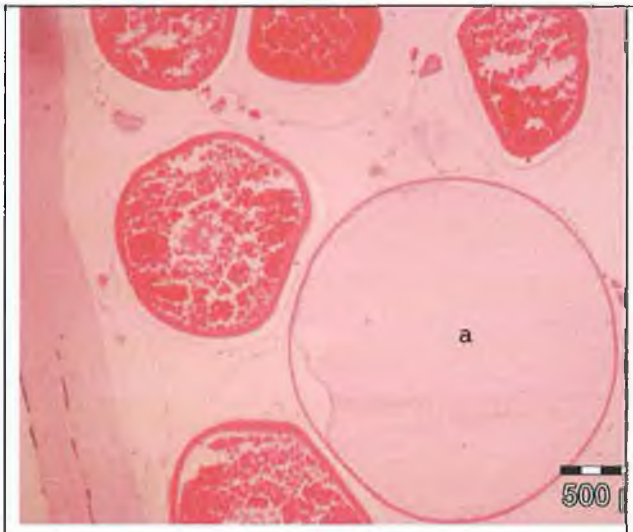
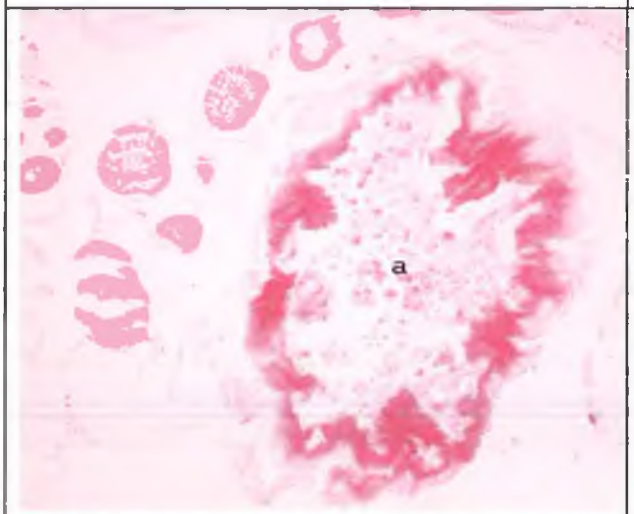
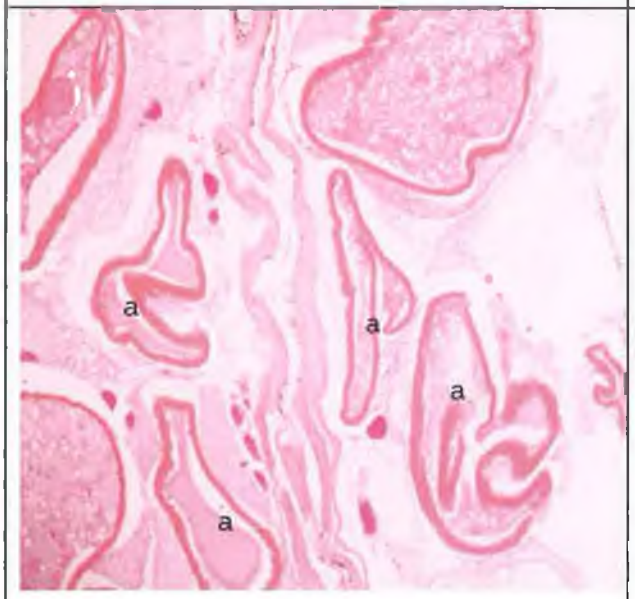
	**Hydrated oocytes** Yolk granules coalesce into a single droplet. Peripheral migration of nucleus and dissolution of its membrane. Oocytes become more transparent and increase in size **(a)**.
	**Atretic oocytes** Atretic oocytes **(a)** are formed by the degeneration of stage V (vitellogenic) and VI (hydrated) oocytes which remain in the ovary after ovulation has been completed (Barr, 1963a).
	**Post-Ovulatory follicles (P.O.F)** Post-ovulatory follicles (corpora lutea) **(a)** are the ruptured empty oocyte envelopes that are left in the ovary after ovulation of mature oocytes, indicating that the fish has spawned

Table 2.2.3(b). Cell types present in each female plaice maturity stage, as observed by the author.

Maturity Stage	Chromatin Nucleolar	Early Perinucleolar	Late Perinucleolar	Cortical Alveoli	Late Lipidogenic	Vitellogenic	Hydrated	P.O.F. and Atretic
Female I	✓	✓						
Female II		✓	✓					
Female III		✓	✓	✓				
Female IV			✓	✓	✓			
Female V				✓	✓	✓		
Female VI					✓	✓	✓	✓
Female VII						✓	✓	✓

Table 2.2.3(c). Classification of histological maturity stages for female plaice *P. platessa* L. (After Barr, 1963a).	
Stage I **Immature / Virgin**	Characterised by the presence of chromatin nucleolar (germ cells) and early perinucleolar stage oocytes. Ovary contains much lumen. Oocyte diameter range is from 27 to 266 μm.
Stage II **Developing Virgin /** **Spent Recovering**	Early and late perinucleolar stage oocytes present. Oocyte diameter range is from 34 to 296 μm. Spent / Recovering → Characteristically a stage II ovary with atretic vitellogenic oocytes present.
Stage III **Early Ripening**	Cortical alveolar stage oocytes are the defining characteristic of this stage. Early and late perinucleolar oocytes are also present. Oocyte diameter range is from 28 to 260 μm.
Stage IV **Late Ripening**	Late lipidogenic stage oocytes are the defining characteristic of this stage. Cortical alveolar and late perinucleolar stage oocytes are also present. Oocyte diameter range is from 36 to 587 μm.
Stage V **Ripe / Pre-Spawning**	Vitellogenic oocytes are the defining characteristic of this stage. Cortical alveolar and late lipidogenic oocytes may also be present in this stage. Oocyte diameter range is from 43 to 1195 μm.
Stage VI **Running / Spawning**	Hydrated oocytes are the defining characteristic of this stage. This is the stage of final maturation. When the egg membranes rupture, ripe hydrated oocytes lie free in the lumen of the ovary (Barr, 1963a). This stage also contains late lipidogenic and vitellogenic oocytes. Oocyte diameter range is from 9 to 1398 μm.
Stage VII **Spent / Post –Spawning**	This stage of development is characterised by the presence of residual hydrated oocytes, atretic vitellogenic and hydrated oocytes and the presence of post-ovulatory follicles. Oocyte diameter range is from 18 to 1284 μm.

Table 2.2.3(d). Classification of histological maturity stages for male plaice *P.platessa* L. (After Barr, 1963c).	
Stage I **Immature /Virgin**	Well defined lobular organisation of the testes. Spermatogonia present in clusters at an early stage of development. Spermatocytes absent.
Stage II. **Developing Virgin /** **Resting Spent**	Mitotic multiplication of spermatogonia. Spermatocytes present, few spermatids.
Stage III **Ripening**	Spermatogonia and spermatocytes present only in the testes cortex. Meiotic division of spermatogonia to form spermatids. Large parts of the testes filled with spermatids. Few spermatozoa. Follicles appear which persist until the gonad is spent.
Stage IV **Ripe**	Spermiogenesis. Sperm hydration. Presence of sperm in vas deferens. Very few spermatogonia. Spermatozoa predominate and form into lamellar organisation.
Stage V **Running / Spawning**	Spermiation. Sperm present in ducts. Very few spermatogonia and spermatids. Spermatozoa still predominate.
Stage VI **Spent**	Few spermatogonia. Lobules contain residual spermatozoa and sperm. Empty seminiferal ducts. Empty/near-empty follicles and empty spaces in gonad.

Plates 4 to 11. Histological structures of female maturity stages I – VII.

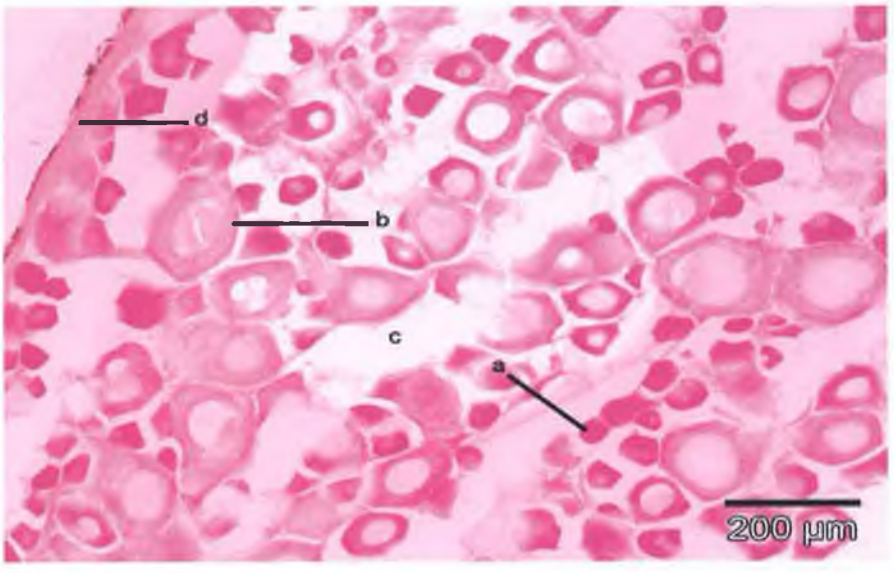


Plate 4. Stage I (Immature or Virgin) ovary.

(a) germ cells, (b) early perinucleolar stage oocytes (c) lumen and (d) ovary outer membrane.

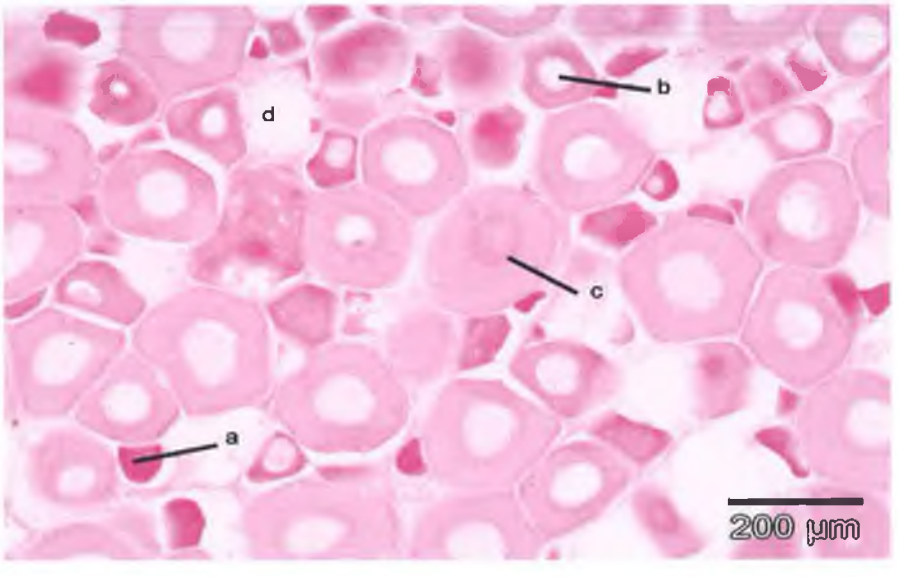


Plate 5. Stage II (Developing Virgin) ovary.

(a) germ cells, (b) early perinucleolar stage oocytes, (c) late perinucleolar stage oocytes (d) lumen.

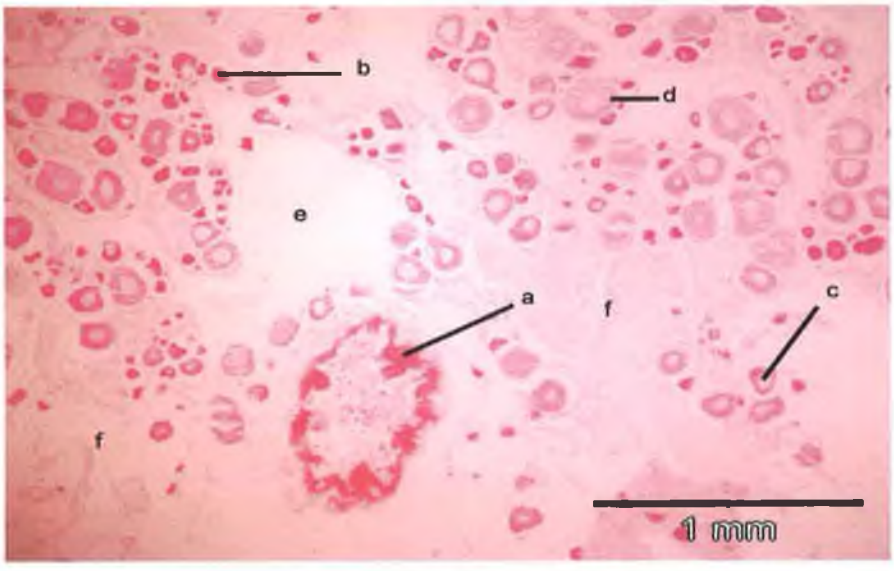


Plate 6. Stage II (Spent / Recovering) ovary.

(a) atretic oocyte, (b) germ cell, (c) early perinucleolar stage oocyte, (d) late perinucleolar stage oocyte, (e) lumen, and (f) undifferentiated ovary tissue.

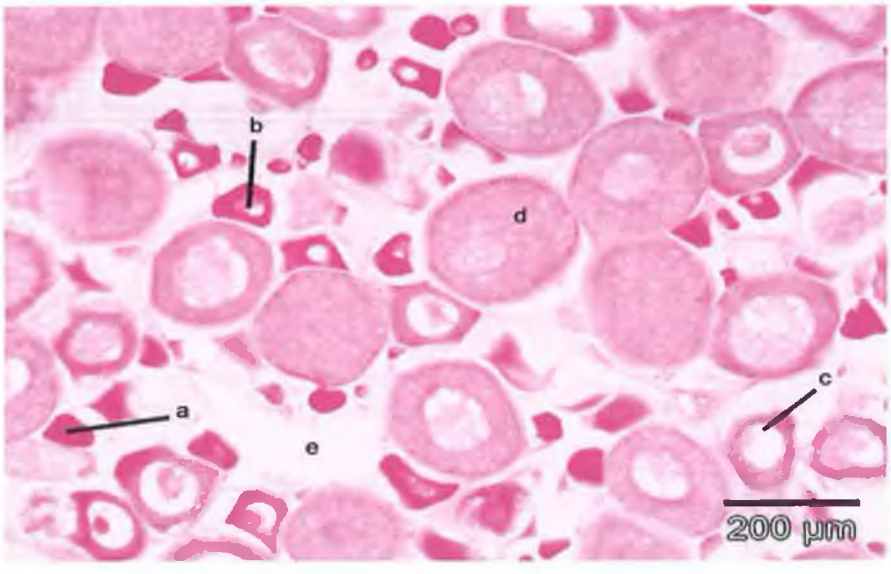


Plate 7. Stage III (Early Ripening) ovary.

(a) germ cells, (b) early perinucleolar stage oocytes, (c) late perinucleolar stage oocytes, (d) cortical alveolar stage oocytes and (e) lumen.

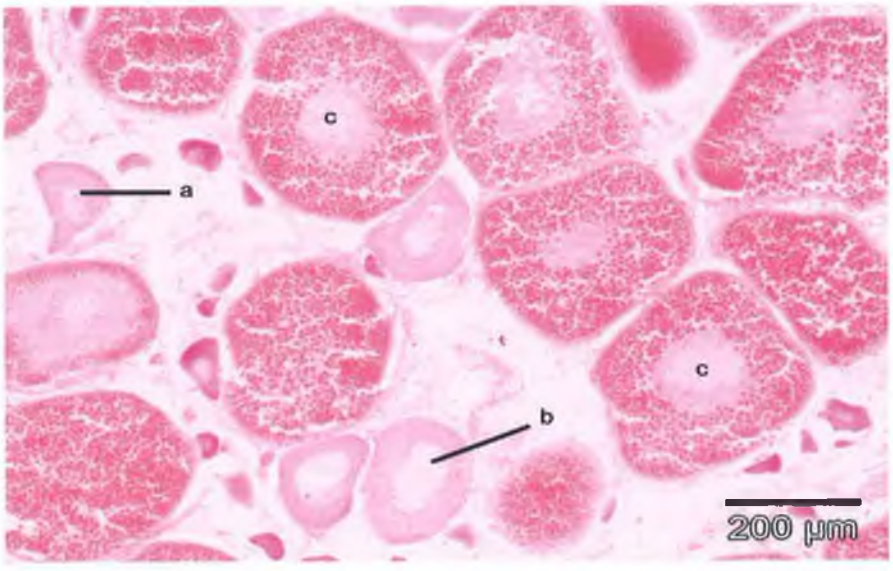


Plate 8. Stage IV (Late Ripening) ovary.

(a) late perinucleolar stage oocytes, (b) cortical alveolar stage oocytes, and (c) late lipidogenic stage oocytes.

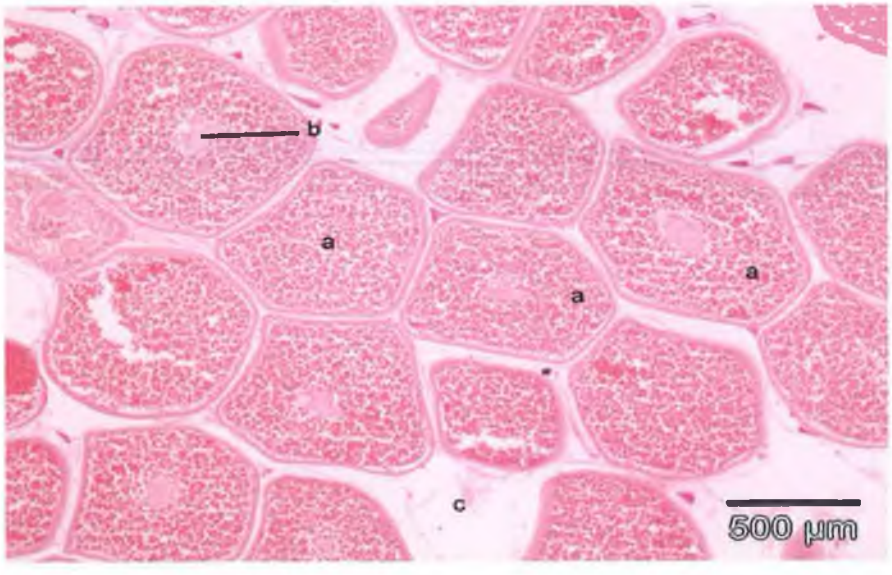


Plate 9. Stage V (Ripe / Pre Spawning) ovary.

(a) vitellogenic oocytes, (b) nucleus, and (c) lumen.

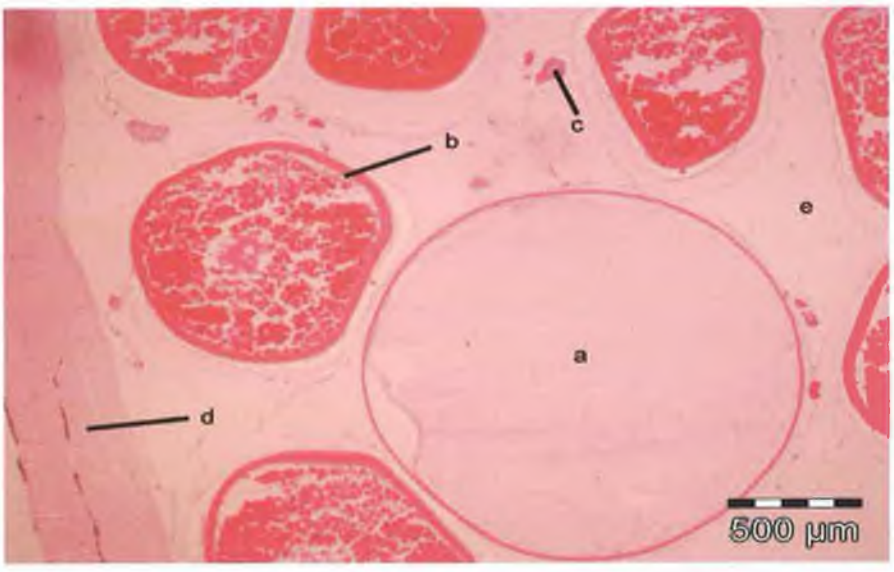


Plate 10. Stage VI (Running / Spawning) ovary.

(a) hydrated oocyte, (b) vitellogenic oocyte, (c) germ cells and (d) ovary wall, and (e) lumen.

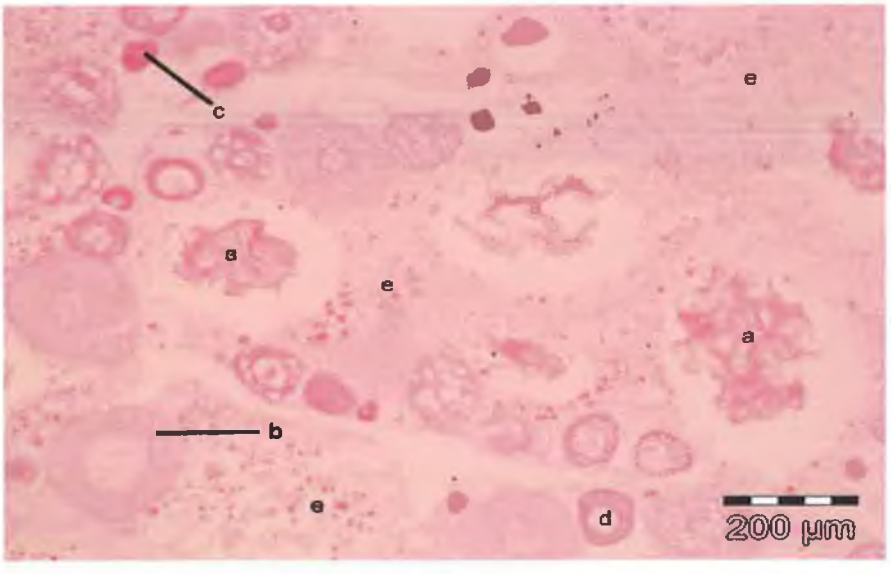


Plate 11. Stage VII (Spent / Post spawning) ovary.

(a) atretic oocytes, (b) cortical alveolar stage oocytes, (c) germ cells, (d) late perinucleolar stage oocyte, and (e) gonadal tissue.

Plates 12 to 17. Histological structures of male maturity stages I – VI.

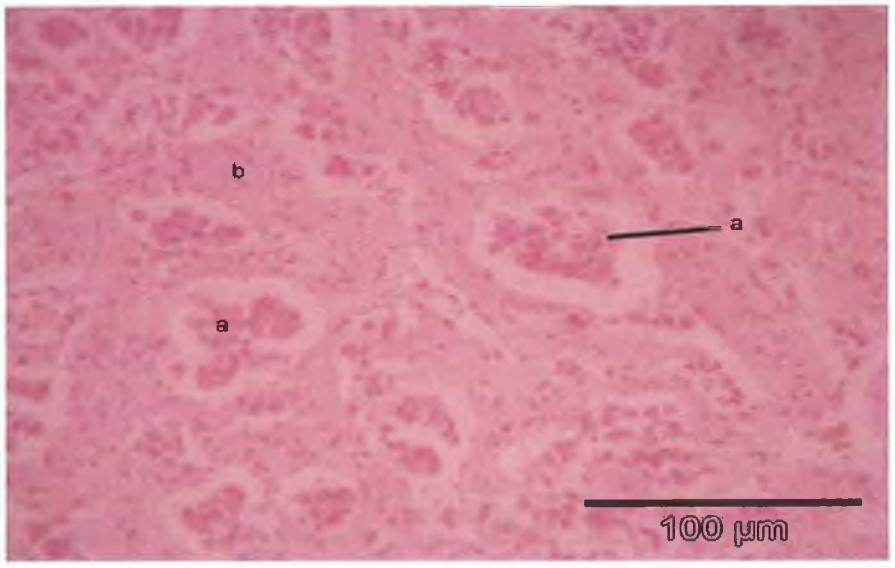


Plate 12. Stage I (Immature / Virgin) testis.

(a) spermatogonia, and (b) gonadal tissue.

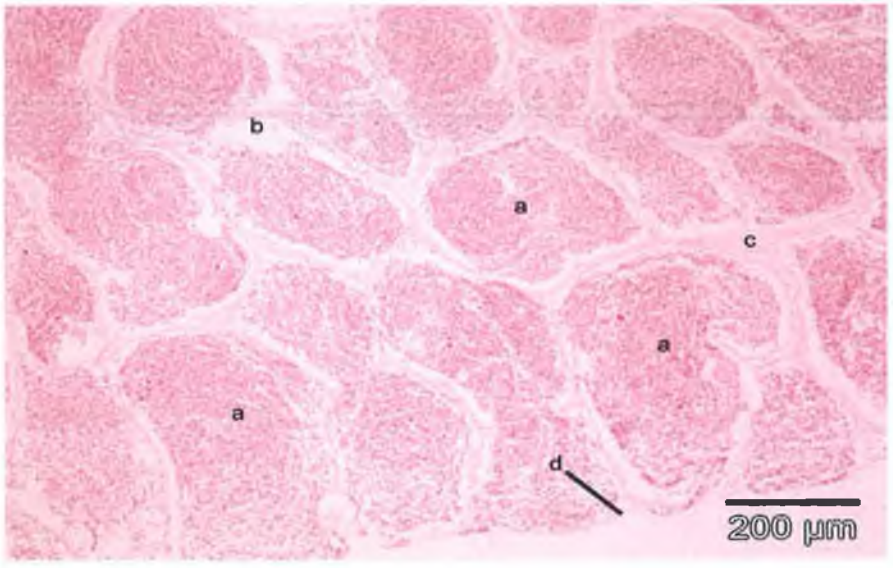


Plate 13. Stage II (Developing Virgin) testis.

(a) spermatocytes in follicles, (b) lumen, (c) blood vessel, (d) testes wall.

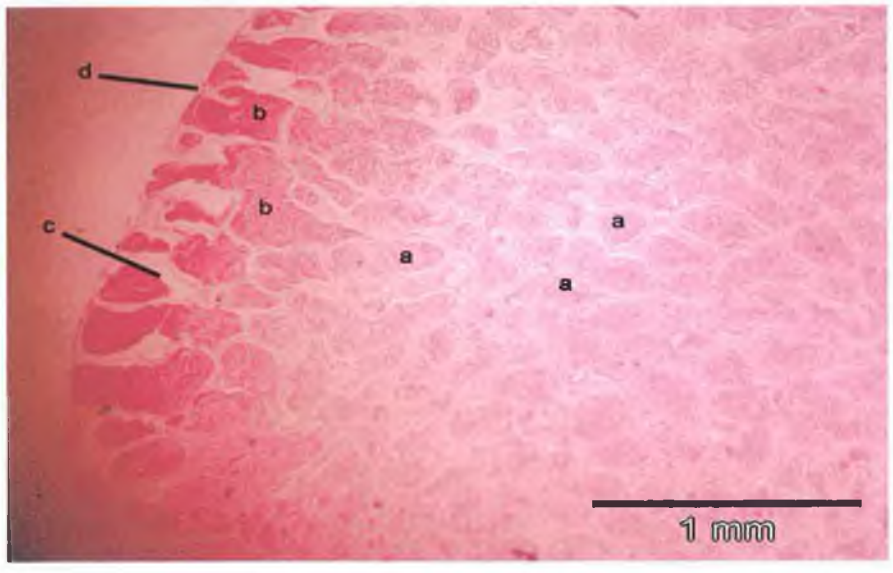


Plate 14. Stage III (Ripening) testis.

(a) spermatids, (b) spermatocytes, (c) follicle, and (d) testes wall.

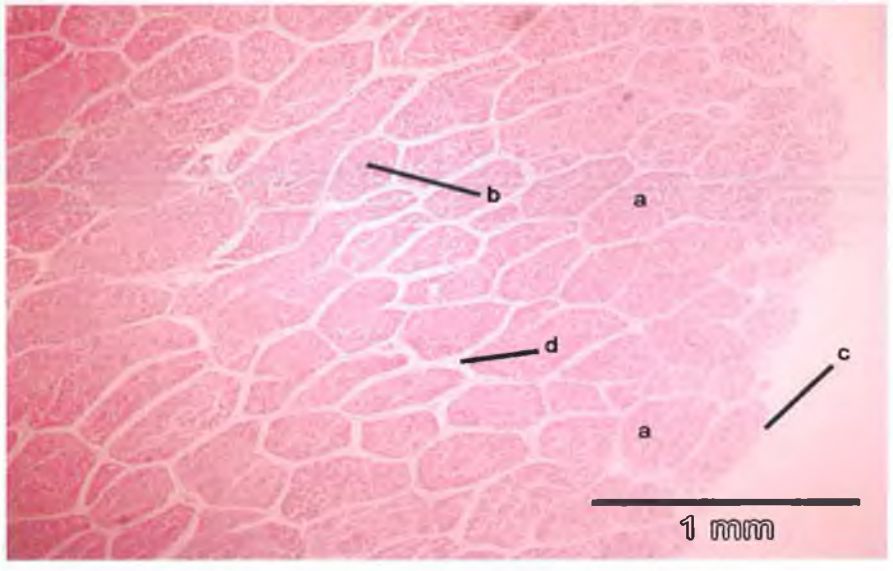


Plate 15. Stage IV (Ripe) testis.

(a) spermatozoa, (b) lamellae, (c) testes wall, and (d) vein.

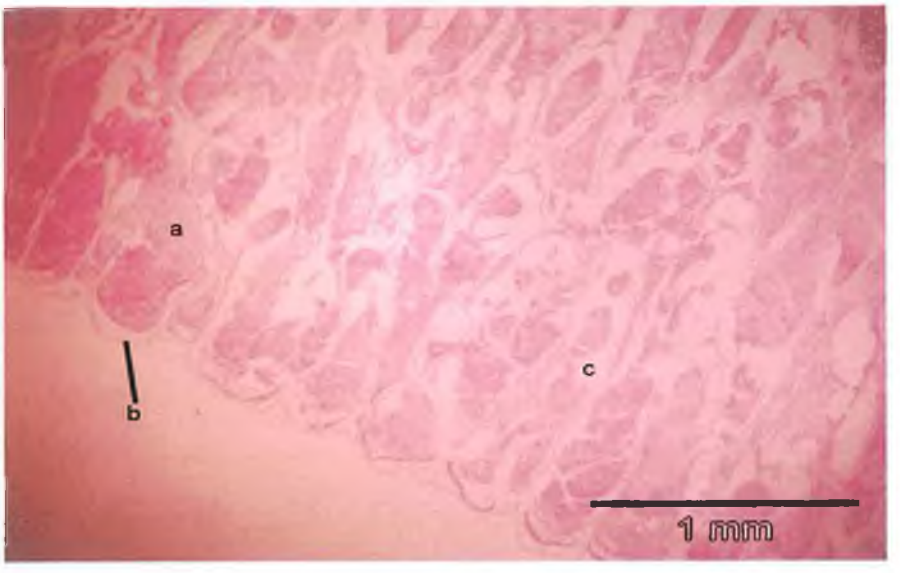


Plate 16. Stage V (Running / Spawning) testis.

(a) spermatozoa, (b) testes wall, and (c) near empty follicle.

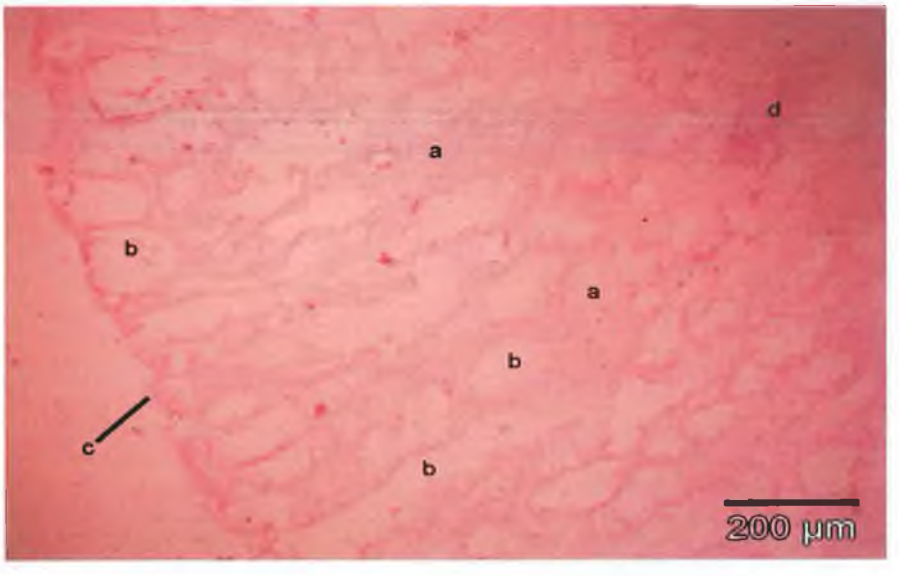


Plate 17. Stage VI (Post-Spawning / Spent) testis.

(a) residual spermatozoa and sperm in lobules, (b) near empty follicles, (c) thin testes wall, and (d) break down in gonadal tissue.

2.2.4 Statistical Methods

Maturity Ogive Determination

Maturity information is essential in deciding minimum landing sizes and mean age at first capture for a species, so as not to exploit fish that have not had a chance to sexually mature and reproduce. The mean age ($A_{50\%}$) and mean length ($L_{50\%}$) at first maturity may be defined as the age and length at which 50% of all individuals are sexually mature (Somerton, 1980; Rijnsdorp, 1993; King, 1995). This mean length and age at first maturity can be determined from the construction of maturity ogives (Ramsay and Witthames, 1996). In order to construct the maturity ogives, a definition of mature was required. The latter was defined according to the criteria used in previous studies of plaice maturity elsewhere, which stated that both macroscopic and histological stages 3-7 were mature whilst stages 1 and 2 were defined as immature (Rijnsdorp, 1989; Nielsen, 2004). The number of mature fish in each length and age class was determined. The percentage of mature male and female plaice in each length and age category collected during the spawning season was then plotted as maturity ogives. These provided an estimate of first maturity at age and at length for *P. platessa.* From these maturity ogives, estimates of $L_{50\%}$ and $A_{50\%}$ were calculated for fish examined macroscopically and histologically. These values represent the lengths and ages at which 50% of the examined fish are sexually mature respectively.

Oocyte Maturity Dynamics

Image analysis was carried out using an Olympus CX41® compound microscope connected to an Olympus camedia C-3040 Zoom® digital camera with a 3.3 megapixel capacity. Oocyte maturity dynamics were determined using the Olympus dp Soft analySIS® 3.1 software package. Oocyte maturity dynamics consisted of oocyte diameter length measurements. Using the image analysis software, a still image from each slide was captured and 100 oocytes per slide were measured along their longest axis. Only oocytes with a nucleus present were measured. Hydrated oocytes, post-ovulatory follicles and atretic cells were not measured due to a lack of a visible nucleus and distortion of

hydrated and atretic cells. Microscopic magnifications ranging from 40x to 400x were used, depending on the image being examined. A hundred oocytes were measured per slide and 10 slides per maturity stage were examined. A total of 7000 oocytes were measured and presented as oocyte length frequency distributions. The oocyte diameter range and the median oocyte diameter representing the different maturity stages were determined.

Gonadosomatic Index

Seasonal changes in gonad development were followed by calculating the gonadosomatic index (GSI). Monthly averages ± standard deviation were used to plot changes in this index. The GSI is the percentage of the fishes total body weight that is composed of gonad. This percentage increases as the fish nears spawning time and drops off dramatically following spawning. From this the period of spawning for the species can be determined (King, 1995). The GSI was determined for males and females separately and combined using the following formula:

$$GSI = \frac{Wgon}{W} * 100$$

Where, Wgon is the gonad weight (g) and W is the whole body weight of the fish in grams (Htun Han, 1978).

Condition Factor

The condition factor (C.F.) was calculated from the monthly samples, for female and male fish separately, using the equation: $C.F. = 1000*(W / TL^3)$ where W is the weight of the fish (g) and TL is the total length (cm) (King, 1995).

2.3 Results.

Presented in this section are the reproductive results recorded for *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

2.3.1 Macroscopic maturity assessments

Following macroscopic examination an annual percentage maturity assessment was determined for female and male plaice and the results are presented in Tables 2.3.1(a) and 2.3.1(b) respectively. The annual percentage occurrences of each macroscopic maturity stage for both female and male fish are presented in Figs. 2.3.1 (a-f). For both male and female plaice immature fish were recorded between January and July. For female plaice the percentage of fish in developing condition increases between March and November, while in male plaice the percentage increases from January to July, after which it decreases again. The percentage of female plaice in ripening condition increases between July and December, but decreases again in January and February. The percentage of male plaice in ripening condition increases from February to November, and declines again in December and February. For both male and female plaice the percentage of mature fish increases from July to November. The highest percentage of fish in spawning condition are found in January and February for female fish, and between December and February for male fish. For both male and female plaice fish in the spent condition are most common between February and April, but decrease significantly after that.

Table 2.3.1 (a). Macroscopic examination: Annual percentage occurrence of each maturity stage for female plaice.

Month	Immature	Developing	Ripening (stages III & IV)	Mature	Spawning	Spent
November 2003	9.09	20.45	56.82	11.36	0.00	2.27
February 2004	6.76	14.86	8.11	0.00	0.00	70.27
March 2004	53.54	7.87	2.36	3.94	0.00	32.28
April 2004	5.71	45.71	0.00	0.00	0.00	48.57
July 2004	30.61	40.82	24.49	0.00	0.00	4.08
November 2004	0.00	25.81	51.61	19.35	0.00	3.23
December 2004	3.45	0.00	93.10	0.00	0.00	3.45
January 2005	15.63	0.00	68.75	9.38	3.13	3.13
February 2005	0.00	0.00	31.82	36.36	9.09	22.73

Table 2.3.1 (b). Macroscopic examination: Annual percentage occurrence of each maturity stage for male plaice.

Month	Immature	Developing	Ripening	Mature	Spawning	Spent
November 2003	29.85	17.91	22.39	2.99	26.87	0.00
February 2004	0.00	18.18	63.64	6.82	9.09	2.27
March 2004	49.59	22.76	0.00	2.44	9.76	15.45
April 2004	34.78	0.00	0.00	0.00	2.17	63.04
July 2004	53.85	38.46	0.00	0.00	0.00	7.69
November 2004	0.00	8.33	66.67	16.67	8.33	0.00
December 2004	0.00	0.00	61.54	0.00	38.46	0.00
January 2005	0.00	18.75	50.00	0.00	18.75	12.50
February 2005	0.00	10.53	0.00	0.00	31.58	57.89

Fig. 2.3.1 (a). Macroscopic Examination: Annual percentage occurrence of immature fish (maturity stage I) for male and female plaice sampled off the west coast of Ireland.

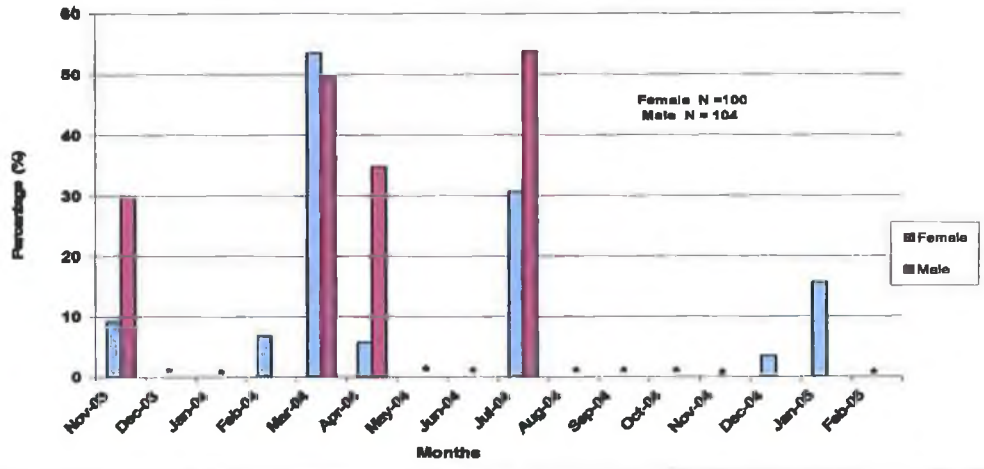


Fig. 2.3.1 (b). Macroscopic Examination: Annual percentage occurrence of developing virgin / resting-spent fish (maturity stage II) for male and female plaice sampled off the west coast of Ireland. (* = no sample available).

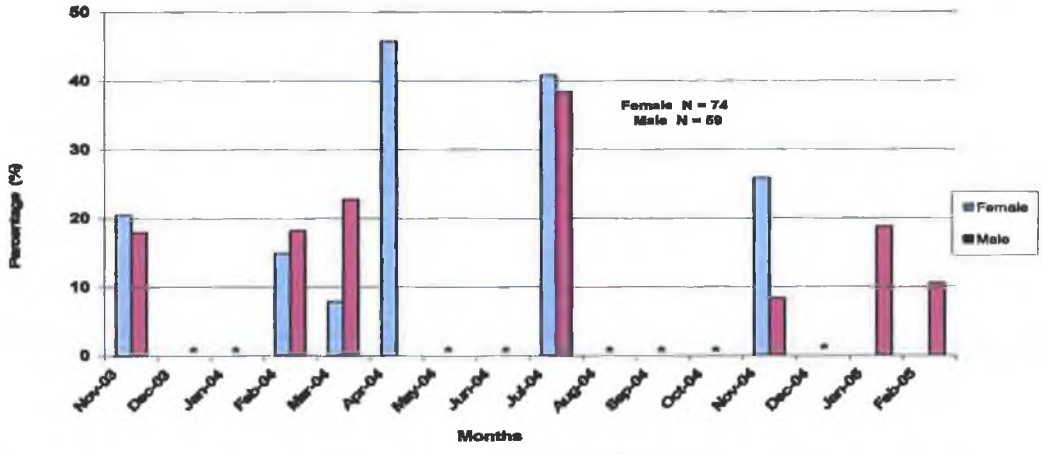


Fig. 2.3.1 (c). Macroscopic Examination: Annual percentage occurrence of ripening fish (male stage III and female stages III and IV) for male and female plaice sampled off the west coast of Ireland. (* = no sample available).

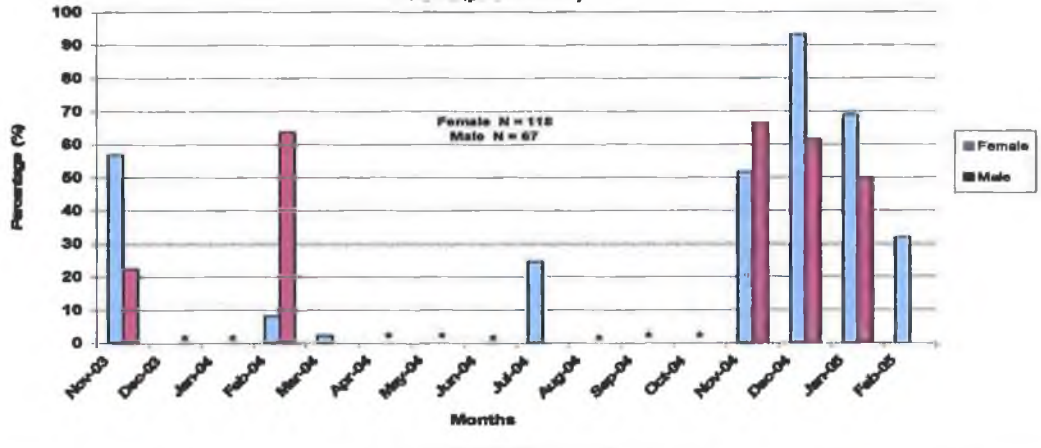


Fig. 2.3.1 (d). Macroscopic Examination: Annual percentage occurrence of mature fish (male stage IV and female stage V) for male and female plaice sampled off the west coast of Ireland.

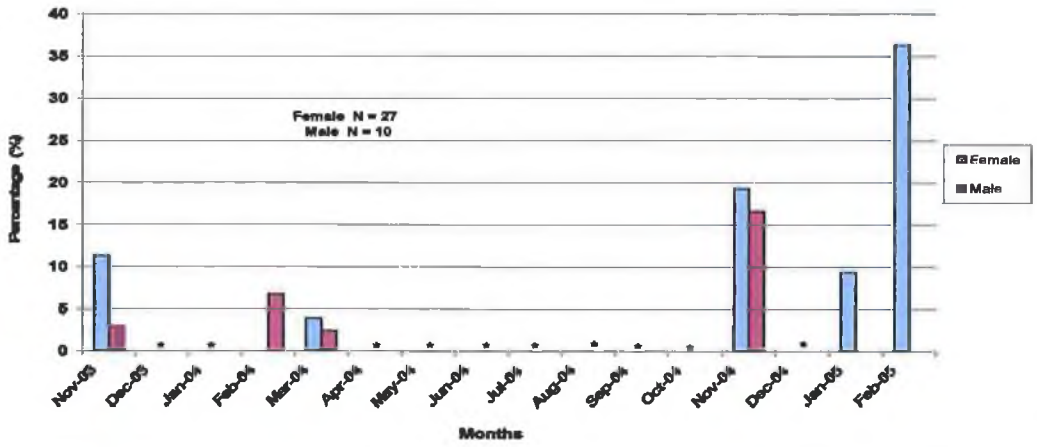


Fig. 2.3.1 (e). Macroscopic Examination: Annual percentage occurrence of spawning fish (male stage V and female stage VI) for male and female plaice sampled off the west coast of Ireland.

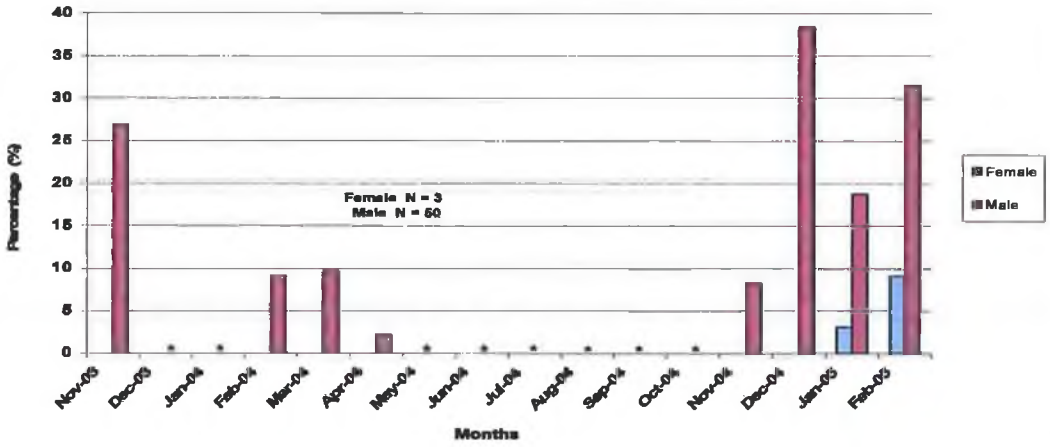
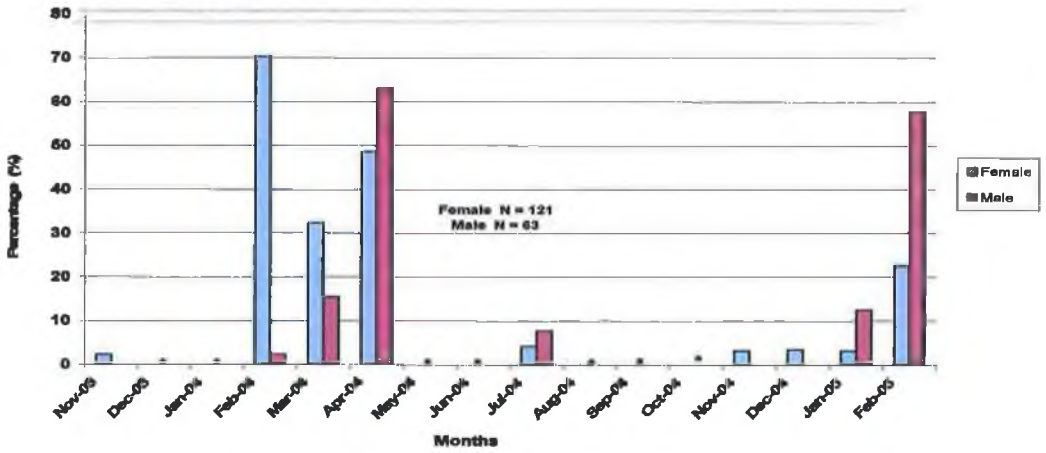


Fig. 2.3.1 (f). Macroscopic Examination: Annual percentage occurrence of spent fish (male stage VI and female stage VII) for male and female plaice sampled off the west coast of Ireland.



2.3.2 Histological maturity assessments

Following histological examination, an annual percentage maturity assessment was determined for female and male plaice in a similar manner as was carried out macroscopically and the results are presented Tables 2.3.2 (a) and 2.3.2 (b) respectively. The annual percentage occurrences of each histological maturity stage for both female and male fish are presented in Figs. 2.3.2 (a-f).

The percentage of immature female plaice increases between November and April, while the percentage of immature male plaice increases between March and April. For female plaice the percentage of fish in developing condition increases between November and April, and in male plaice the percentage increases from February to July. For female plaice, the percentage of fish in ripening condition increases between February and July, while the percentage of male plaice in ripening condition increases from February to November. For female plaice the percentage of mature fish increases between February and November, while in male plaice the percentage increases between April and January. The highest percentage of fish in spawning condition, are found in January and February for female fish, and between November and April for male fish. For male plaice, fish in the spent condition are most common between February and July. For female plaice fish in the spent condition are most common between November and July

Table 2.3.2 (a). Histological examination: Annual percentage occurrence of each maturity stage for female plaice.

Year	Maturity Stage	Immature	Developing	Ripening (stages III & IV)	Mature	Spawning	Spent
2003	November	6.45	16.13	22.58	51.61	0.00	3.23
2004	February	14.29	21.43	4.76	19.05	28.57	11.90
2004	March	10.71	25.00	14.29	17.86	21.43	10.71
2004	April	11.11	66.67	22.22	0.00	0.00	0.00
2004	July	0.00	52.94	41.18	0.00	0.00	5.88
2004	November	0.00	9.52	9.52	80.95	0.00	0.00
2004	December	0.00	10.00	10.00	80.00	0.00	0.00
2005	January	0.00	17.65	17.65	58.82	5.88	0.00
2005	February						

Table 2.3.2 (b). Histological examination: Annual percentage occurrence of each maturity stage for male plaice.

Year	Maturity Stage	Immature	Developing	Ripening	Mature	Spawning	Spent
2003	November	0.00	0.00	66.67	26.67	6.67	0.00
2004	February	0.00	9.52	38.10	21.43	28.57	2.38
2004	March	25.00	50.00	25.00	0.00	0.00	0.00
2004	April	11.11	11.11	22.22	11.11	11.11	33.33
2004	July	0.00	30.00	20.00	20.00	10.00	20.00
2004	November	0.00	0.00	33.33	66.67	0.00	0.00
2004	December	0.00	0.00	33.33	66.67	0.00	0.00
2005	January	0.00	0.00	21.43	71.43	7.14	0.00
2005	February						

Fig. 2.3.2 (a). Histological Examination: Annual percentage occurrence of immature fish (maturity stage I) for male and female plaice sampled off the west coast of Ireland.

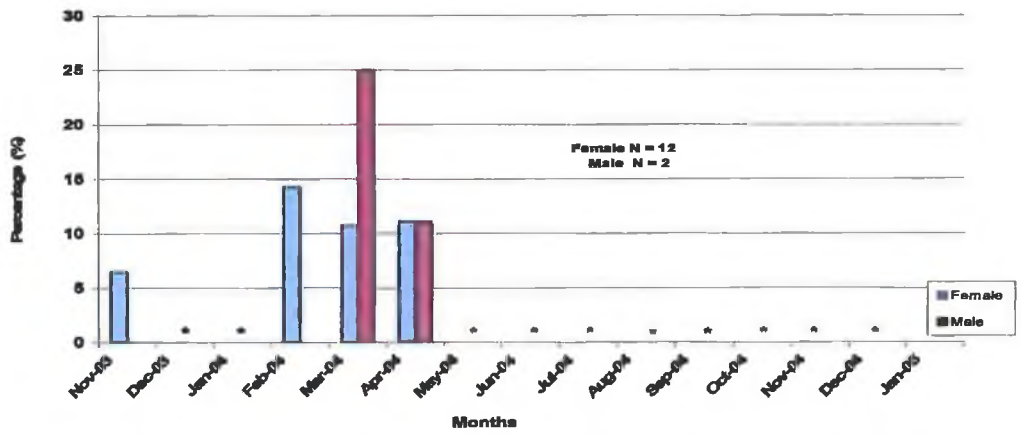


Fig. 2.3.2 (b). Histological Examination: Annual percentage occurrence of developing virgin / resting -apent fish (maturity stage II) for male and female plaice sampled off the west coast of Ireland.

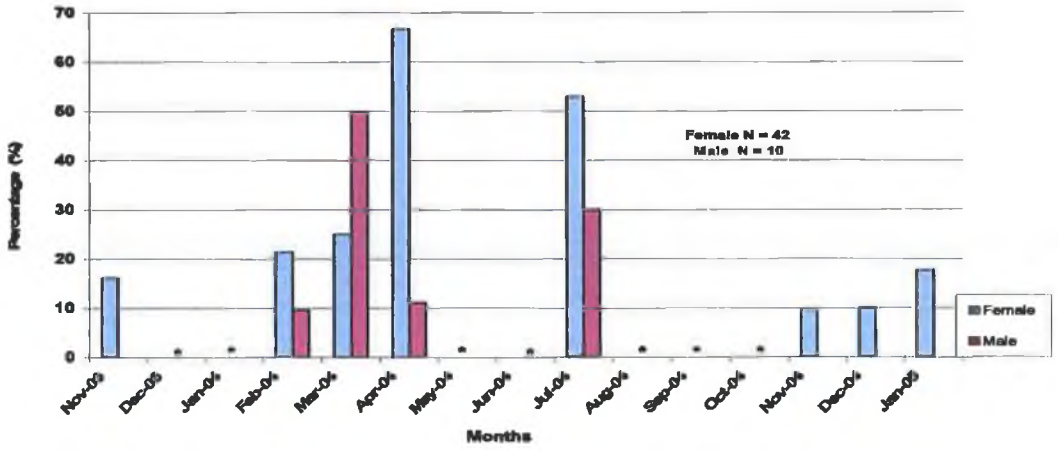


Fig. 2.3.2 (c). Histological Examination: Annual percentage occurrence of ripening fish (male stage III and female stages III and IV) for male and female plaice sampled off the west coast of Ireland.

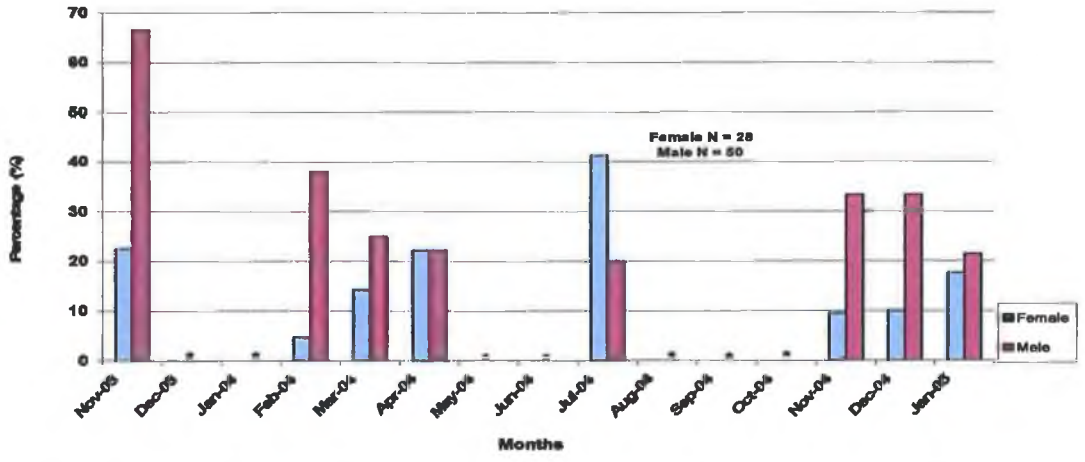


Fig. 2.3.2 (d). Histological Examination: Annual percentage occurrence of mature fish (male stage IV and female stage V) for male and female plaice sampled off the west coast of Ireland.

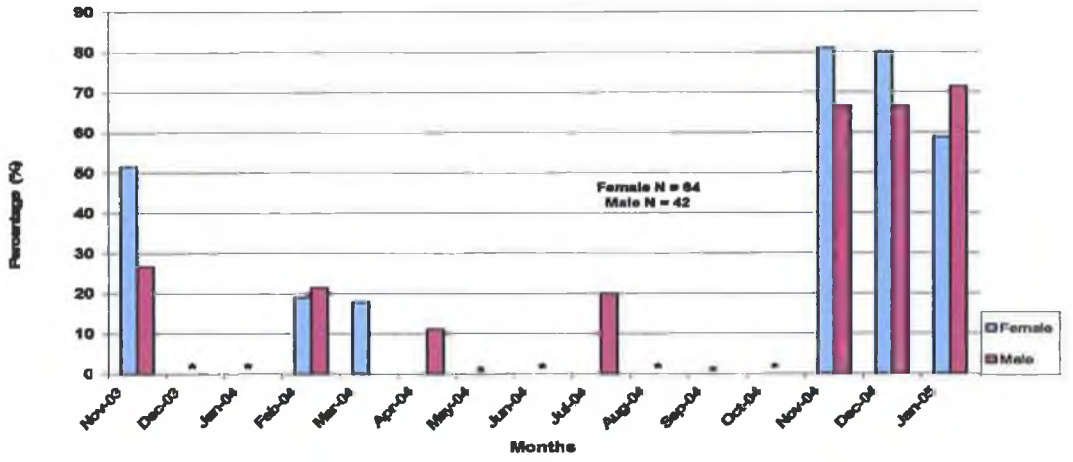


Fig. 2.3.2 (e). Histological Examination: Annual percentage occurrence of spawning fish (male stage V and female stage VI) for male and female plaice sampled off the west coast of Ireland.

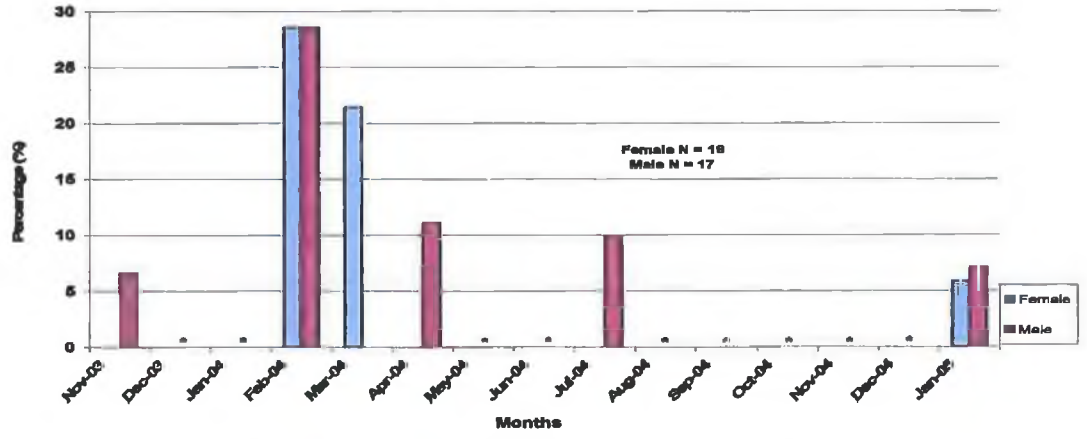
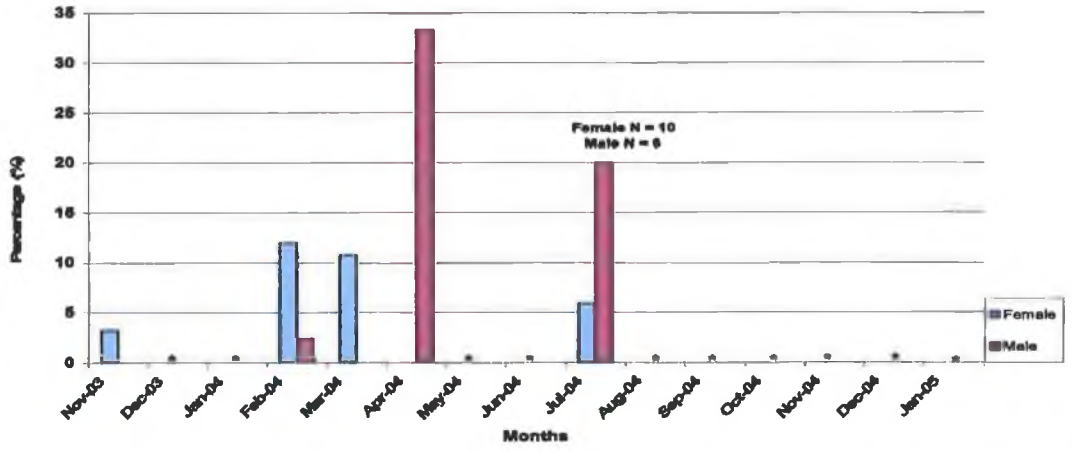


Fig. 2.3.2 (f). Histological Examination: Annual percentage occurrence of spent fish (male stage VI and female stage VII) for male and female plaice sampled off the west coast of Ireland.



2.3.3 Comparisons between macroscopic and histological maturity assessments

A comparison between the macroscopic and histological maturity assessments was made to establish the validity of carrying out visual or macroscopic assessments. This was carried out for both female and male plaice. The results of these comparisons are given below.

Females

Ovaries which were staged using histological criteria were compared with the original macroscopic maturity stage that had been given to them at the time of dissection. A total of 177 ovaries were staged using both macroscopic and histological criteria. The results are presented in Table 2.3.3 (a). There was a poor match between the macroscopic and histological maturity assessments. The maturity stage which compared most favourably was that of stage I (immature) followed closely by stage VI (spawning). Stages III and IV (early and late ripening) had the least favourable comparison between methods. No stage IV (late ripening) was found to be correctly assessed using macroscopic maturity assessment. The overall percentage of maturity stages which compared favourably between the two assessment methods was 22.03%.

Table 2.3.3 (a). Percentage matches between macroscopic and histological maturity assessments for female plaice.

Females	**Histological Maturity Stages**							
Macroscopic Maturity Stages	**Maturity Stage**	**Stage I**	**Stage II**	**Stage III**	**Stage IV**	**Stage V**	**Stage VI**	**Stage VII**
	Stage I	38	50	44	0	0	0	10
	Stage II	15	31	22	10	9	0	0
	Stage III	0	0	6	60	25	0	20
	Stage IV	0	0	0	0	48	0	10
	Stage V	0	0	0	0	15	63	0
	Stage VI	8	2	0	0	0	37	30
	Stage VII	38	17	28	30	3	0	30
	Total	100	100	100	100	100	100	100

Males

A comparison was also made between the macroscopic and histological maturity assessments for the male testis. A total of 127 testes were used for comparative purposes. The results of these comparisons are presented in Table 2.3.3 (b). There was a relatively poor match between the macroscopic and histological maturity assessments for male plaice. The maturity stage which compared most favourably with both assessment methods was stage I (immature), with 100% of the testis having the same assessed maturity stage. This corresponds to the most favourable maturity stage of the female fish which was also stage I. As in the females, stage VI (spent) had the second highest most favourable comparison of assessment methods. Stage IV (mature) had the lowest correspondence between macroscopic and histological assessment methods. The overall percentage of maturity stages for males which compared favourably between the two assessment methods was 37.80%, which is slightly higher than the overall percentage for females.

Table 2.3.3 (b). Percentage matches between macroscopic and histological maturity assessments for male plaice.

Males	**Histological Maturity Stages**						
Macroscopic Maturity Stages	**Maturity Stage**	**Stage I**	**Stage II**	**Stage III**	**Stage IV**	**Stage V**	**Stage VI**
	Stage I	100	50	6	2	6	17
	Stage II	0	30	22	12	12	17
	Stage III	0	10	44	26	23	0
	Stage IV	0	10	12	24	6	0
	Stage V	0	0	16	31	41	0
	Stage VI	0	0	0	5	12	66
	Total	100	100	100	100	100	100

2.3.4 Maturity at length keys

Following macroscopic and histological maturity assessment the number of fish at each maturity stage (females I-VII, and males I-VI) were matched with their corresponding total lengths, giving an indication of what length a fish should be, at a certain maturity stage. For comparative purposes all maturity at length keys were constructed using the same length range. Macroscopic maturity at length is presented for female plaice in Table 2.3.4(a) and male plaice in Table 2.3.4(b). Histological maturity at length is presented for female plaice in Table 2.3.4(c) and male plaice in Table 2.3.4(d).

Male and female macroscopic maturity at length

The smallest stage I male and female were 11cm and 9cm in length respectively, and the largest stage I male and female were 32cm and 40cm respectively. The smallest stage II male and female were 16 and 11cm in length respectively, and the largest stage II male and female were 32 and 37cm respectively. The smallest stage III male and female were 18 and 22cm in length respectively, and the largest stage III male and female were 31 and 38cm respectively. The smallest stage IV male and female were 14 and 24cm in length respectively, and the largest stage IV male and female were 34 and 37cm respectively. The smallest stage V male and female were 14 and 26cm in length respectively, and the largest stage V male and female were 33 and 37cm respectively. The smallest stage VI male and female were 17 and 27cm in length respectively, and the largest stage II male and female were 34 and 35cm respectively. The smallest stage VII female was 14cm and the largest was 47cm in length.

Male and female histological maturity at length

The smallest stage I male and female were 18cm and 19cm in length respectively, and the largest stage I male and female were 25cm and 35 cm respectively. The smallest stage II male and female were 21 and 14cm in length respectively, and the largest stage II male and female were 32 and 39cm respectively. The smallest stage III male and female was 20cm in length, and the largest stage III male and female was 34cm. The smallest stage IV male and female were 22 and 27cm in length respectively, and the largest stage IV male and female were 36 and 44cm respectively. The smallest stage V male and female were 21 and 23cm in length respectively, and the largest stage V male and female were 35 and 46cm respectively. The smallest stage VI

male and female were 22 and 20cm in length respectively, and the largest stage VI male and female were 31 and 43cm respectively. The smallest stage VII female was 20cm and the largest was 38cm in length.

Table 2.3.4 (a). Macroscopic maturity at length for female *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

Length Class (cm)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII	Total
9-9.99								
10-10.99								
11-11.99	2	1						3
12-12.99								0
13-13.99	5							5
14-14.99	4						1	5
15-15.99	5							5
16-16.99	4	1						5
17-17.99	3	1					1	5
18-18.99	4	1						5
19-19.99	7	6						13
20-20.99	6	3					1	10
21-21.99	6	6					6	18
22-22.99	5	6	1				6	18
23-23.99	8	5	1				6	20
24-24.99	7	3	1	2			10	23
25-25.99	11	5	2	1			8	27
26-26.99	7	3	2	1	1		8	22
27-27.99	6	3	3	3	2	1	11	29
28-28.99	4	3	5	2	1		10	25
29-29.99	2	8	5	5	1		11	32
30-30.99	2	7	9	13	3		6	40
31-31.99	1	4	4	11	7	1	9	37
32-32.99	1	1	5	9	2		4	22
33-33.99		2	4	10	4		6	26
34-34.99		3	1	7	1		2	14
35-35.99		1	3	2	2	1	5	14
36-36.99			1		1		4	6
37-37.99		1	2	2	2		2	9
38-38.99			1					1
39-39.99								0
40-40.99								0
41-41.99							1	1
42-42.99							1	1
43-43.99								0
44-44.99								0
45-45.99							1	1
46-46.99								0
47-47.99							1	1
48-48.99								0
Total	100	74	50	68	27	3	121	443

Table 2.3.4 (b). Macroscopic maturity at length for male *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

Length Class (cm)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Total
9-9.99	1						1
10-10.99	2						2
11-11.99	4						4
12-12.99	4						4
13-13.99	10						10
14-14.99	6			1	1		8
15-15.99	7			1			8
16-16.99	7	2			1		10
17-17.99	9	2				1	12
18-18.99	6		2		2	2	12
19-19.99	8		3		1	3	15
20-20.99	6	5	4		2	1	18
21-21.99	6	3	5		2	3	19
22-22.99	4	9	5		6	4	28
23-23.99	3	8	2	1	6	5	25
24-24.99	3	3	7		2	6	21
25-25.99	4	3	6	1	7	5	26
26-26.99	3	3	3	1	3	6	19
27-27.99	1	5	6		1	6	19
28-28.99	3	6	10	2	4	3	28
29-29.99	2	2	10	1	1	1	17
30-30.99	1	4	3	1	2	3	14
31-31.99	3	3	1		3	6	16
32-32.99		1			5	4	10
33-33.99					1	2	3
34-34.99				1		2	3
35-35.99							0
36-36.99							0
37-37.99							0
38-38.99							0
39-39.99							0
40-40.99	1						1
41-41.99							0
42-42.99							0
43-43.99							0
44-44.99							0
45-45.99							0
46-46.99							0
47-47.99							0
48-48.99							0
Total	104	59	67	10	50	63	353

Table 2.3.4 (c). Histological maturity at length for female *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

Length Class (cm)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage V I	Stage VII	Total
9-9.99								
10-10.99								
11-11.99								
12-12.99								
13-13.99								
14-14.99		1						1
15-15.99								0
16-16.99								0
17-17.99								0
18-18.99								0
19-19.99	3	1						4
20-20.99		2	1			1	1	5
21-21.99		1	1				1	3
22-22.99		2						2
23-23.99		2	1		1			4
24-24.99	1		1		2		2	6
25-25.99		5	1		1		1	8
26-26.99	1	3	3		2		1	10
27-27.99	1	5		1	3	3	1	14
28-28.99	2	4	1		3	1		11
29-29.99		4	4		7	1		16
30-30.99	1	3	2	1	6	1		14
31-31.99	1	2	1	3	13	1		21
32-32.99		2			5	2		9
33-33.99		3		1	9	1	1	15
34-34.99	1		2	1	4	1		9
35-35.99	1				2	1	1	5
36-36.99		1		1	2	1		5
37-37.99					1	1		2
38-38.99				1	1	1	1	4
39-39.99		1			1	1		3
40-40.99						1		1
41-41.99								0
42-42.99								0
43-43.99						1		1
44-44.99				1				1
45-45.99								0
46-46.99					1			1
47-47.99								0
48-48.99								0
Total	12	42	18	10	64	19	10	175

Table 2.3.4 (d). Histological maturity at length for male *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

Length Class (cm)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage V I	Total
9-9.99							
10-10.99							
11-11.99							
12-12.99							
13-13.99							
14-14.99							0
15-15.99							0
16-16.99							0
17-17.99							0
18-18.99	1						1
19-19.99							0
20-20.99			2				2
21-21.99		1	2		1		4
22-22.99			3	1	1	1	6
23-23.99			3	1	2		6
24-24.99			2	1	1	1	5
25-25.99	1	1	3	4	1		10
26-26.99		1	11		2		14
27-27.99		1	6	3	2		12
28-28.99		3	4	11	1	2	21
29-29.99			6	7		1	14
30-30.99			4	5	2		11
31-31.99		2	1	3		1	7
32-32.99		1	2	4	1		8
33-33.99				1			1
34-34.99			1		1		2
35-35.99					2		2
36-36.99				1			1
37-37.99							0
38-38.99							0
39-39.99							0
40-40.99							0
41-41.99							0
42-42.99							0
43-43.99							0
44-44.99							0
45-45.99							0
46-46.99							0
47-47.99							0
48-48.99							0
Total	2	10	50	42	17	6	127

2.3.5 Maturity at age keys

Macroscopic and histological maturity at age was determined for female and male plaice. For these, the number of fish at each maturity stage (females I-VII, and males I-VI) were matched with their corresponding age, giving an indication of what age a fish should be at a certain maturity stage. For comparative purposes all maturity at age keys were constructed using the same age range. Macroscopic maturity at age is presented for female plaice in Table 2.3.5(a) and male plaice in Table 2.3.5(b). Histological maturity at age is presented for female plaice in Table 2.3.5(c) and male plaice in Table 2.3.5(d).

Male and female macroscopic maturity at age

The age range of stage I fish was from 1 to 9 years old for female fish, and from 1-7 years old for male fish. The age range of stage II fish was from 1 to 6 years old for female fish, and from 2-8 years old for male fish. The age range of stage III fish was from 2-8 years old for female fish, and from 2-5 years old for male fish. The age range of stage IV fish was from 3-7 years old for female fish, and from 2-8 years old for male fish. The age range of stage V fish was from 2-5 years old for female fish, and from 2-7 years old for male fish. The age range of stage VI fish was from 4-5 years old for female fish, and from 2-7 years old for male fish. The age range of stage VII female fish was from 2 to 11 years old.

Male and female histological maturity at age

The age range of stage I fish was from 1 to 8 years old for female fish, and from 3-5 years old for male fish. The age range of stage II fish was from 1 to 7 years old for female fish, and from 1-6 years old for male fish. The age range of stage III fish was from 1-5 years old for female fish, and from 2-7 years old for male fish. The age range of stage IV fish was from 2-9 years old for female fish, and from 1-7 years old for male fish. The age range of stage V fish was from 2-10 years old for female fish, and from 2-5 years old for male fish. The age range of stage VI fish was from 2-10 years old for female fish, and from 2-3 years old for male fish. The age range of stage VII female fish was from 3-8 years old.

Table 2.3.5 (a). Macroscopic maturity at age for female *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

Age Class (Years)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII	Total
1	4	1						5
2	32	22	3		1		8	66
3	36	31	15	14	8		34	138
4	18	11	18	24	9	1	27	108
5	8	6	7	16	9	2	31	79
6		3	4	11			9	27
7	1		2	3			5	11
8			1				3	4
9	1						1	2
10							2	2
11							1	1
Total	100	74	50	68	27	3	121	443

Table 2.3.5 (b). Macroscopic maturity at age for male *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

Age Class (Years)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Total
1	6	0					6
2	51	13	14	3	3	6	90
3	36	21	32	3	18	20	130
4	7	18	15	3	19	17	79
5	2	5	6		4	10	27
6	1	0			5	7	13
7	1	1			1	3	6
8		1		1			2
9							
10							
11							0
Total	104	59	67	10	50	63	353

Table 2.3.5 (c). Histological maturity at age for female *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

Age Class (Years)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII	Total
1	1	3	1					5
2	2	8	3	2	4	2		21
3	2	16	7	5	19	2	4	55
4	2	9	5	1	18	6	4	45
5	3	4	2		15	5		29
6		1			5	1		7
7		1		1	1	2	1	6
8	2						1	3
9				1	1			2
10					1	1		2
11								0
Total	12	42	18	10	64	19	10	175

Table 2.3.5 (d). Histological maturity at age for male *Pleuronectes platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

Age Class (Years)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Total
1		1		1			2
2		2	4	2	2	1	11
3	1	3	26	10	8	5	53
4		1	13	17	6		37
5	1	1	4	5	1		12
6		2	2	4			8
7			1	3			4
8							0
9							0
10							0
11							0
Total	2	10	50	42	17	6	127

2.3.6 Maturity Ogives

Maturity ogives were constructed for male and female plaice and from these, macroscopic and histological estimates of first maturity at length and age were determined (Figs. 2.3.6 (a-d)). The estimates from the maturity ogive determinations are presented in Table 2.3.6(a).

Table 2.3.6 (a). Maturity ogive estimates for female and male *P.platessa* sampled off the west coast of Ireland between November 2003 and February 2005.

	L_{50}% (cm)		A_{60}% (years)	
	Macroscopic	**Histological**	**Macroscopic**	**Histological**
Female	23	24	3	1
Male	21	20.5	3	2

There was a difference of 1cm in the $L_{50\%}$ values determined for female plaice using the macroscopic and histological maturity at length ogives. For male plaice the difference in the $L_{50\%}$ values was 0.5cm. There was a difference of 2 years in the $A_{50\%}$ values determined for female plaice using the macroscopic and histological maturity at age ogives, and a difference of 1 year for the male plaice.

Fig. 2.3.6 (a). Macroscopic and histological maturity at length ogives for female plaice sampled off the west coast of Ireland between November 2003 and February 2005.

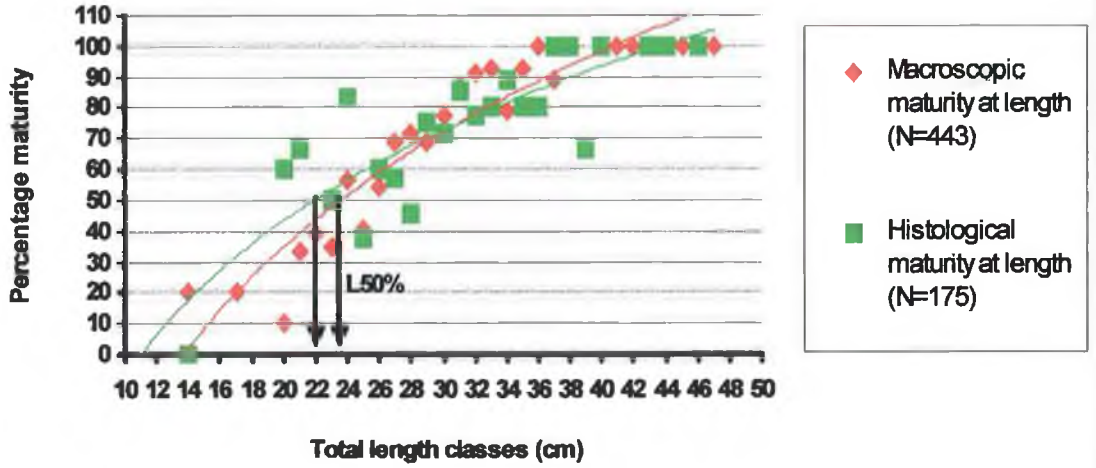


Fig. 2.3.6 (b). Macroscopic and histological maturity at length ogives for male plaice sampled off the west coast of Ireland between November 2003 and February 2005.

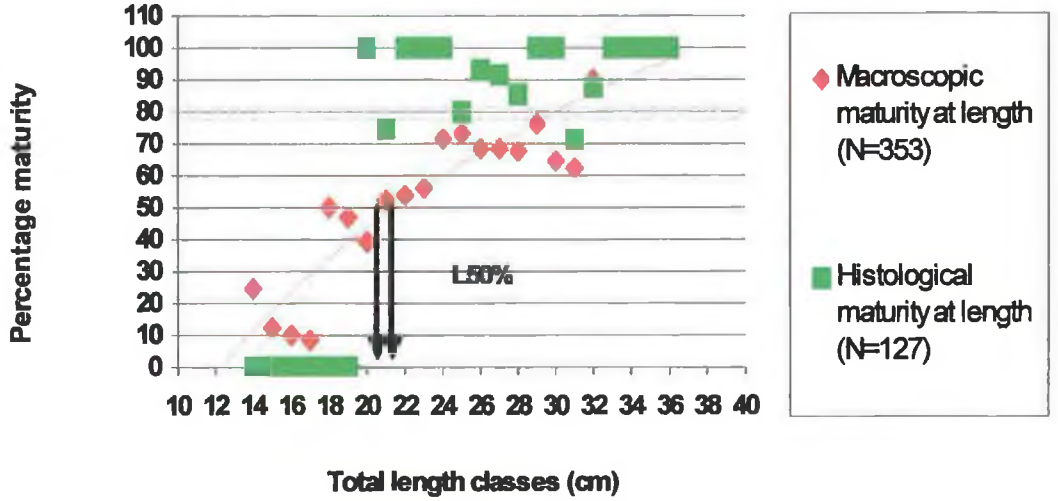


Fig. 2.3.6 (c). Macroscopic and histological maturity at age ogives for female plaice sampled off the west coast of Ireland between November 2003 and February 2005.

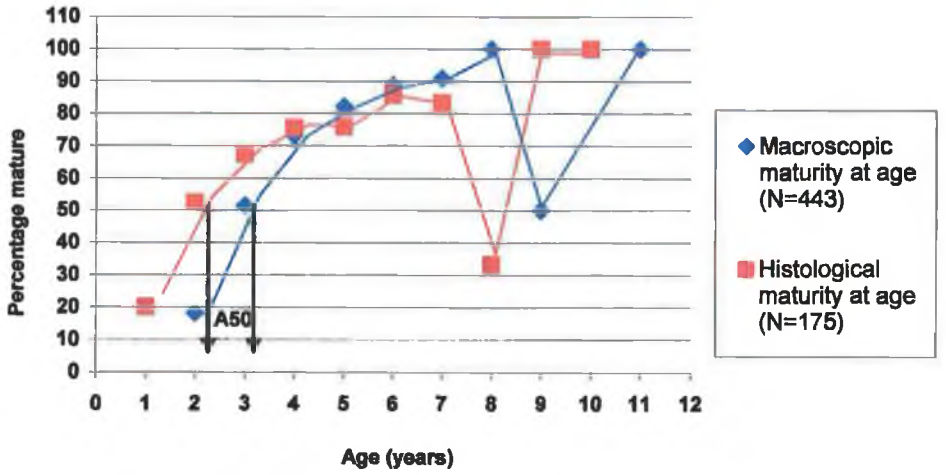
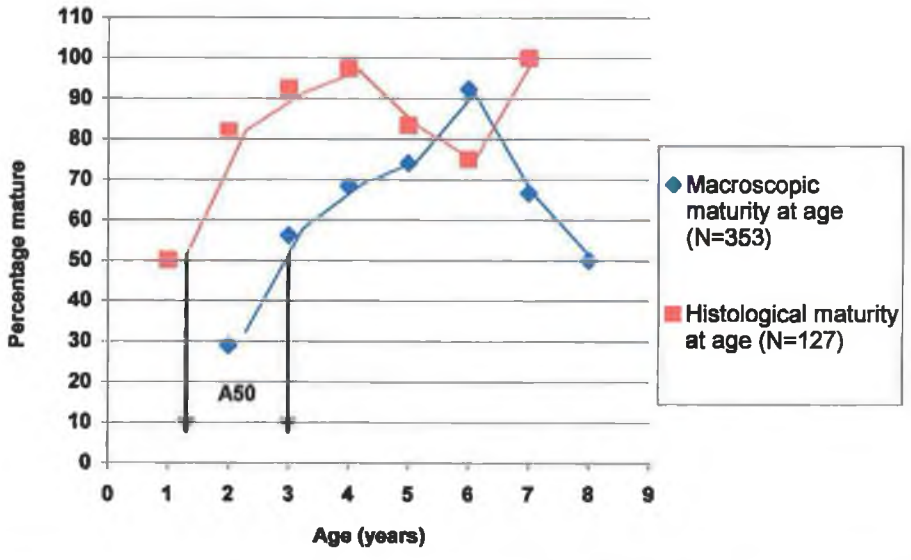


Fig. 2.3.6 (d). Macroscopic and histological maturity at age ogives for male plaice sampled off the west coast of Ireland between November 2003 and February 2005.



2.3.7 Histological image analysis

Image analysis was carried out on the histologically prepared slides of the female and male reproductive tissues. Histological developmental structures observed on the prepared slides of the ovaries and testes of the fish were identified and presented in annotated images in Plates 4 to 17 respectively. During the histological process there was in some cases failure to get complete sucrose impregnation for some of the samples, particularly for the cells of the middle or inner areas of gonad tissue. This resulted in tissue damage from the cryofreezing and also made it very difficult to get good sections on the cryostat. It should be noted that only small pieces of gonad tissue could be processed and mounted in the cryostat. This is because above a critical specimen size optimal freezing will only occur to a certain depth, and the cooling rate in the deeper parts of the sample will be slow enough to allow the formation of hexagonal ice crystals with subsequent tissue damage (Donovan and Preston, 1994). Plaice gonads are simply too big to carry out image analysis on a complete transverse section of ovary or testis (Tomkiewicz et al., 2003). It was also observed that many oocytes were ruptured or not intact following the histological process, meaning that developmental structures could not always be successfully identified.

Oocyte length frequencies

One thousand oocytes were measured for each of the female maturity stages I – VII, making a total of 7000 oocyte diameters recorded. Only oocytes with an identifiable nucleus were measured during the present study. Percentage oocyte length frequency distributions were then constructed for the female maturity stages. Oocyte length parameters are presented in Table 2.3.7(a) and Table 2.3.7(b). Percentage oocyte length frequency distributions are presented in Figs. 2.3.7(a-h). Figure 2.3.7 (i). presents a boxplot of oocyte lengths for female maturity stages I – VII.

For stage I female plaice an oocyte diameter range of 27 to 266 µm was recorded. Only three diameter classes were constructed for the stage I oocytes, indicating the similarity in oocyte size throughout Stage I ovaries. For stage II females, oocytes ranged in diameter from 34 to 296 µm. For stage III females oocytes ranged in diameter from 28 to 260 µm. For stage IV females an oocyte diameter range of 36 to 587 µm was recorded. Stage V oocytes ranged in diameter from 44 to 1196 µm. For stage VI females an oocyte diameter range of 9 to 1399 µm. For stage VII females oocytes ranged in diameter from 18 to 1284 µm.

From the percentage oocyte length frequency distributions it can be seen that plaice show only one mode of vitellogenic oocytes (unimodal type) that is carried through vitellogenesis and increases in size throughout the development period. Just before spawning the vitellogenic oocytes hydrate asynchronously and are released in several successive batches (Rijnsdorp and Witthames, 2005). In the histograms of stages I to III a reservoir of pre-vitellogenic oocytes can be seen. In the histogram of stage IV the diameter range of oocytes is increasing as oocytes are beginning to mature. In the histogram of stage V it can be seen that a batch of hydrating oocytes is separating from the reservoir. In stage VI it can be seen that just before ovulation the batch is fully separated. In stage VII it can be seen that after spawning, the ovary contains mainly pre-vitellogenic oocytes again.

Table 2.3.7 (a). Oocyte length parameters for female maturity stages I - VII.

Maturity Stage	Oocyte Diameter Range (µm)	Mean Oocyte Diameter (µm)	Standard Deviation
Stage I	27 - 266	102.88	± 39.3942
Stage II	34 - 296	132.54	± 47.2652
Stage III	28 - 260	131.32	± 46.8479
Stage IV	36 - 587	215.14	± 128.5556
Stage V	44 - 1196	572.25	± 287.8042
Stage VI	9 - 1399	802.51	± 322.023
Stage VII	18 - 1284	102.69	± 92.3799

Fig 2.3.7 (i). Boxplot of oocyte length parameters for female maturity stages I – VII.

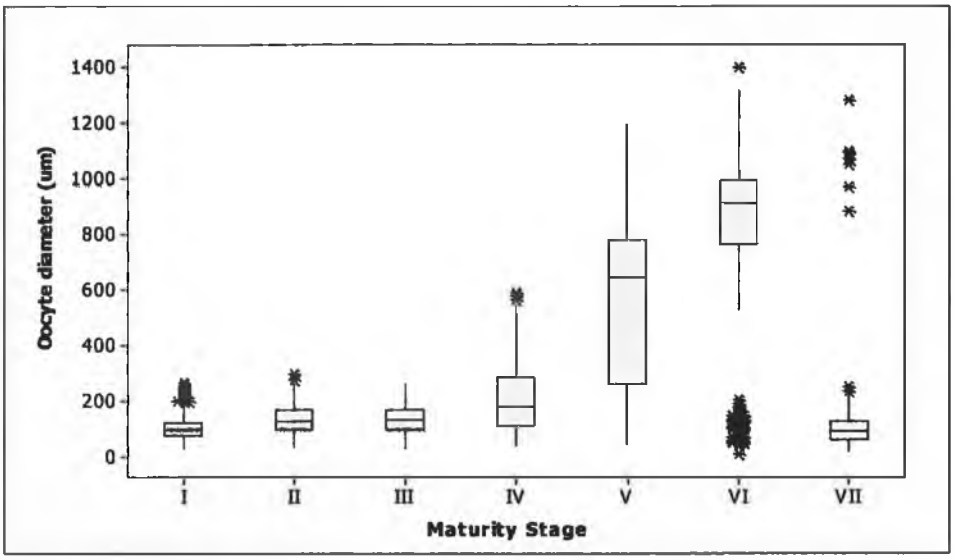
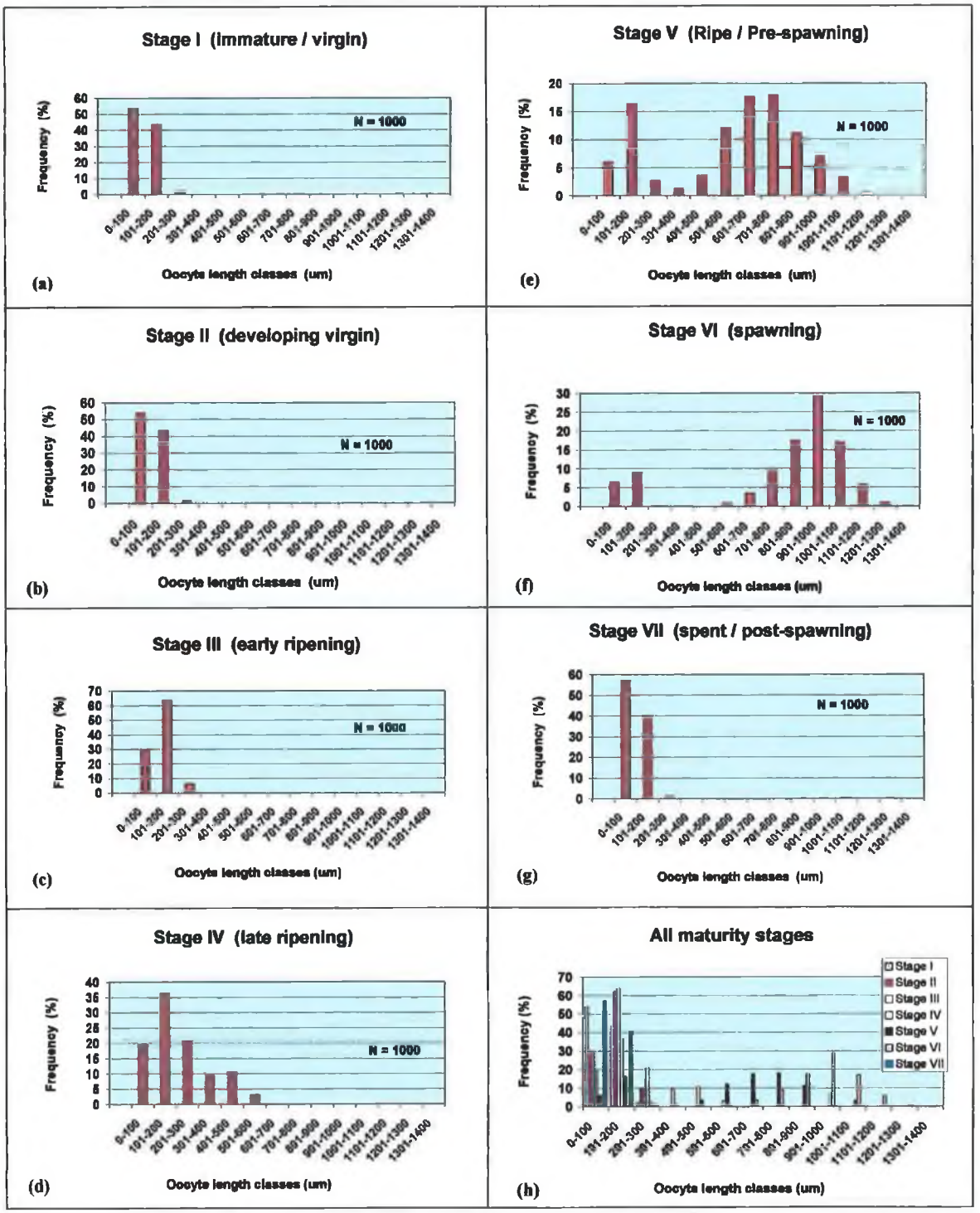


Table 2.3.7 (b). Number and percentage of oocyte diameter range per female maturity stage.

	Stage I		Stage II		Stage III		Stage IV		Stage V		Stage VI		Stage VII	
length group (µm)	number	%	number	%	number	%	number	%	number	%	number	%	number	%
1-100	542	54.2	278	27.8	297	29.7	196	19.6	61	6.1	65	6.5	572	57.2
101-200	436	43.6	625	62.5	637	63.7	364	36.4	165	16.5	91	9.1	408	40.8
201-300	22	2.2	97	9.7	66	6.6	208	20.8	27	2.7	1	0.1	13	1.3
301-400							95	9.5	13	1.3		0		0
401-500							106	10.6	37	3.7		0		0
501-600							31	3.1	121	12.1	9	0.9		0
601-700									177	17.7	35	3.5		0
701-800									179	17.9	91	9.1		0
801-900									112	11.2	175	17.5	1	0.1
901-1000									71	7.1	290	29	1	0.1
1001-1100									33	3.3	171	17.1	4	0.4
1101-1200									4	0.4	58	5.8		0
1201-1300										0	11	1.1	1	0.1
1301-1400										0	3	0.3		0
Total	1000		1000		1000		1000		1000		1000		1000	

Figure 2.3.7 (a-h). Percentage oocyte length frequency distribution for stages I-VII in female plaice sampled off the west coast of Ireland.



2.3.8 Gonadosomatic index and condition factor

The gonadosomatic index (GSI) and condition factor (CF) were calculated for male and female plaice separately for all samples collected during the sampling period in ICES area VIIb. The results are presented in Table 2.3.8(a) and in Figures 2.3.8(a) and (b).

Female GSI and condition factor

The GSI value began to increase in November 2003 and peaked in February 2004. In March 2004 it decreased dramatically. The GSI began to gradually increase again in July 2004 and climbed steadily to a peak in February 2005. There were no samples collected in the months following February 2005, so it can only be inferred that the GSI drops again in March as in the previous year. These peak spawning times correspond with the majority of females being at maturity stage VII (February 2004) and V (February 2005). Immediately following the peak spawning time in February 2004, there was a dramatic drop in the mean GSI value which indicated that the fish had spawned, and as a result had a lower percentage of the gonad comprising the total body weight. The highest values for condition factor were observed in the months just prior to spawning, e.g. November 2003 and January 2005. There was a relatively high peak in condition factor in July 2004. The lowest values for condition factor were recorded in February, March and April 2004.

Male GSI and condition factor

The GSI value began to decrease in November 2003 to a low in April 2004. In July 2004 it began to increase again to a peak in November 2004. The GSI began to gradually decrease again in December 2004. These peak spawning times correspond with the majority of males being at maturity stage I (November 2003) and III (November 2004). Immediately following the peak spawning times in November 2003 and 2004 there was a dramatic drop in the mean GSI value which indicated that the fish had spawned, and as a result had a lower percentage of the gonad comprising the total body weight. The highest values for condition factor were observed in the months just prior to spawning, e.g. November 2003 and December 2004. The lowest values for condition factor were recorded in February and March 2004.

Table 2.3.8 (a). Summary of gonadosomatic index (GSI) and condition factor (CF) for male and female plaice sampled off the west coast of Ireland between November 2003 and February 2005.

		Males					Females				
Year	Month	No. of Fish	Mean GSI	Standard Deviation	Mean C.F.	Standard Deviation	No. of Fish	Mean GSI	Standard Deviation	Mean C.F.	Standard Deviation
2003	November	67	1.5981	± 1.7582	10.3472	± 1.4642	44	5.02	± 3.3024	10.9687	± 0.9808
2003	December										
2004	January	No Samples					No Samples				
2004	February	44	0.7982	± 0.3644	8.9596	± 0.6730	74	6.9989	± 7.0708	9.1822	± 1.4348
2004	March	123	No Data		9.1416	± 1.2960	127	1.3153	± 1.5083	9.2560	± 1.5131
2004	April	46	0.1084	± 0.1391	9.5135	± 1.6942	35	0.5959	± 0.6065	9.1355	± 1.6360
2004	May										
2004	June	No Samples					No Samples				
2004	July	13	0.1745	± 0.0845	10.5716	± 0.9091	49	0.8846	± 0.6118	10.6142	± 1.6804
2004	August										
2004	September										
2004	October	No Samples					No Samples				
2004	November	12	1.2571	± 0.3794	9.7213	± 0.6112	31	4.3783	± 2.4131	9.9597	± 0.7138
2004	December	13	1.2168	± 0.4043	10.0128	± 0.9147	29	10.6086	± 17.5215	10.7652	± 3.5443
2005	January	16	0.9284	± 0.2686	9.5330	± 1.0015	32	12.7333	± 9.1431	11.2690	± 6.0208
2005	February	19	0.9406	± 0.4417	9.4637	± 0.8363	22	18.8155	± 24.5978	9.0911	± 2.2069
	Total	353					443				

Fig. 2.3.8 (a). Gonadosomatic index (GSI) and condition factor (C.F.) for female plaice sampled off the west coast of Ireland between November 2003 and February 2005.

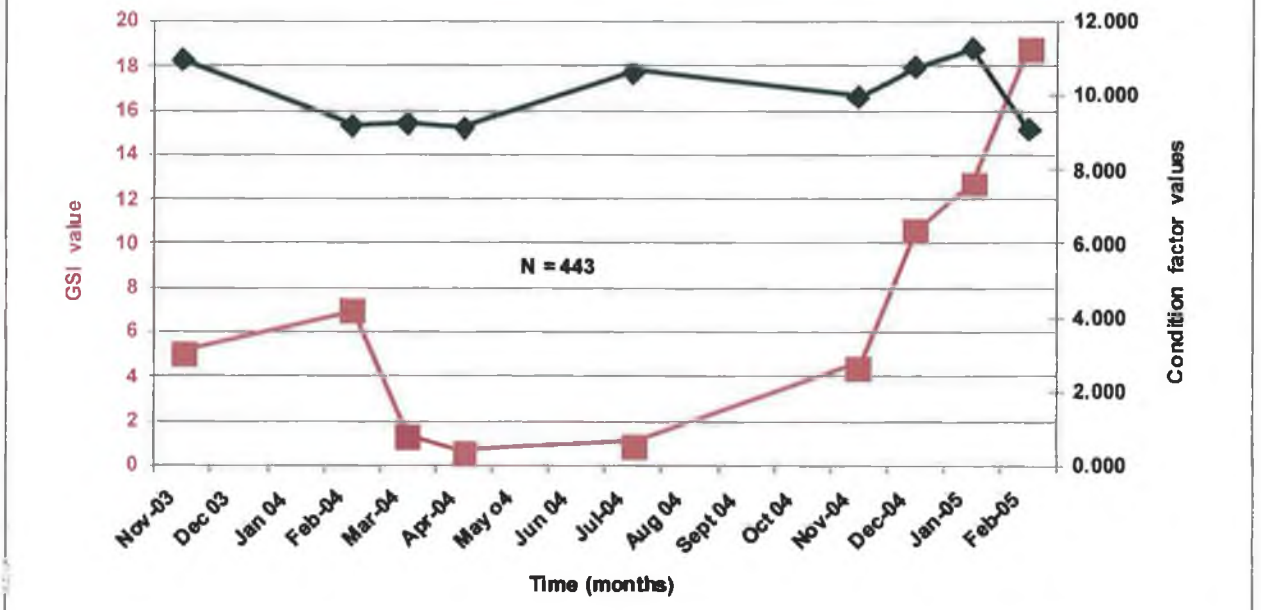
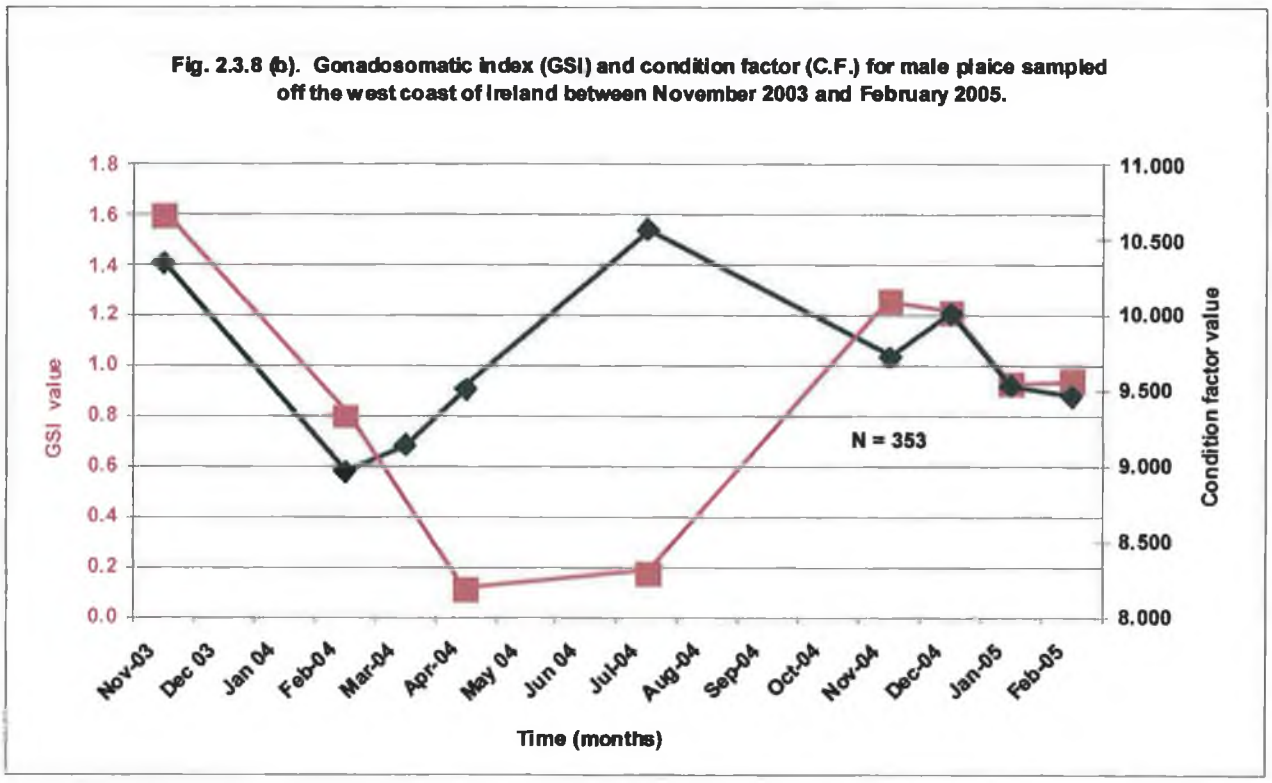


Fig. 2.3.8 (b). Gonadosomatic index (GSI) and condition factor (C.F.) for male plaice sampled off the west coast of Ireland between November 2003 and February 2005.



2.4 Discussion

The reproductive results determined for *Pleuronectes platessa* off the west coast of Ireland during this study are discussed and compared with similar studies elsewhere in Europe for the species.

Macroscopic and histological maturity assessments

Following macroscopic and histological examination of the gonads, an annual percentage maturity assessment was determined for female and male plaice. While samples were not available for all months during this investigation it was possible to track the percentage occurrences of each maturity stage for male and female plaice through the year. The results of the macroscopic and histological methods of assessment were then compared.

For female plaice (Table 2.3.1 (a)) the highest percentage of immature fish were recorded in March, the highest percentage of developing fish were recorded in April and July, the highest percentage of ripening fish were recorded between July and January, the highest percentage of mature fish were recorded in November and February, and the highest percentage of spent fish were recorded in February, March and April. The timing of the occurrence of each development stage is similar to that observed by Rijnsdorp (1989) for North Sea plaice and by Albert et al., (2001) for Greenland halibut (*Reinhardtius hippoglossoides*). From Table 2.3.1 (a) it can be seen that there were not many fish recorded as mature or spawning, but there were large numbers of fish recorded as ripening between November and February. It seems likely that some of these may have been mature, and were assigned the wrong maturity stage. Following histological examination (Table 2.3.2 (a)) the annual percentage occurrences of each maturity stage followed a similar pattern to the macroscopic assessment except for ripening and mature fish. The highest percentages of ripening fish were recorded between March and July, as compared to the macroscopic assessment, where the highest percentages of ripening fish were recorded between July and January. Also there was a much higher percentage of mature fish recorded, and these were recorded between November and January. It seems likely that the histological examination determined many fish as mature that had been classes as ripening using macroscopic assessment.

For male plaice (Table 2.3.1(b)) the highest percentages of immature and developing fish were recorded between March and July, the highest percentage of ripening fish were recorded between November and January, the highest percentage of mature fish were recorded in November, the highest percentage of spawning fish were recorded between December and February, and the highest percentage of spent fish were recorded between February and April. From Table 2.3.1 (b) it can be seen that there were not many fish recorded as mature but there were large numbers of fish recorded as ripening between November and February. Again, it seems likely that some of these may have been mature and were assigned the wrong maturity stage. Following histological examination (Table 2.3.2 (b)) the annual percentage occurrences of each maturity stage followed a similar pattern to the macroscopic assessment except for mature and spawning fish. In the macroscopic percentage maturity assessment there were low numbers of fish determined as mature and high numbers of fish determined to be in the spawning condition. This situation was reversed in the histological percentage maturity assessment. It seems likely that the histological examination determined many fish as mature that had been classed as spawning using macroscopic assessment.

Comparison between macroscopic and histological maturity assessments

During this investigation a total of 177 ovaries were staged using both macroscopic and histological criteria. The maturity stage which compared most favorably was that of stage I (immature) with 38% of ovaries having the same maturity stage following use of both assessment methods. Stage VI (spawning) came in second with a match value of 36.84%. Stages III and IV (early and late ripening) had the least favorable comparison between methods, with a 6% match for stage III and zero matches for stage IV.

In most cases where there was not a match between assessment methods, the methods differed only by one stage of development. Ovaries which had been identified as stage I using histology were identified as stages II or VII using macroscopic methods. Ovaries which had been histologically identified as stage II were identified as stages I or VII, stage III were identified as stages I or VII, Stage IV were identified as stages III or VII,

stage V were identified as stages III or IV, stage VI were identified as stage V and stage VII were identified as stages VI or I using macroscopic methods.

It is clear that the majority of difficulties in macroscopic assessment occurred with identifying stage III (early ripening) and stage IV (late ripening) ovaries. The majority of ovaries which had been identified as stage III using histology, were identified as stage I or stage VII using macroscopic methods. The majority of ovaries which had been identified as stage IV using histology were identified as stage III using macroscopic methods. While it is possible for a small stage III ovary to be mistaken for a stage I ovary, it seems unusual that a stage III ovary would be identified as a stage VII (spent) ovary, because the condition of a spent ovary makes them very distinctive. However this happened in 44% of cases. The fact that many histologically staged IV ovaries were identified as stage III ovaries using macroscopic assessment is not so surprising. Stages III and IV are visually quite similar. However, when these stages are examined histologically, they are quite distinctive.

Fifty percent of ovaries which had been assigned stage I using macroscopic assessment were assigned stage II using histological assessment. When using macroscopic assessment, distinguishing between stage I and stage II ovaries can be difficult, because they are visually quite similar. Ketchen and Forrester (1966) commented on the difficulty in determining visually between ovaries of immature fish and those of fish in the resting state, particularly in the summer when mature and immature plaice are well mixed. Many authors suggest that macroscopic assessment is more accurate for samples collected around spawning season, as maturity status is more easily determined visually (Halliday, 1987; De Martini et al., 1999; Hannah et al., 2002; Hunter and Macewicz, 2003). However problems can occur with histological assessment also. Grift et al. (2003) noted that in females, once any post ovulatory follicles disappear, it became increasingly difficult to distinguish histologically between mature and immature ovaries.

During this investigation a total of 127 testes were staged using both macroscopic and histological criteria. The maturity stage which compared most favourably with both assessment methods was stage I (immature), with 100% of the testis having the same assessed maturity stage. This corresponds to the most favourable maturity stage of the

female fish which was also stage I. Stage VI (spent) had the second highest most favourable comparison of assessment methods, with 66.67% of testis having the same maturity stage. Stage IV (mature) had the lowest correspondence between macroscopic and histological assessment methods. In males where there was not a match between assessment methods, the methods differed by many stages of development. Male fish that were determined to be one particular stage using histology were assigned many different stages macroscopically. This is probably because plaice testes are very difficult to stage macroscopically, changing only slightly in appearance throughout development (Bromley, 2000). However it should be noted that during this investigation, a number of problems were encountered in the histological analysis of the testes (see histological structure discussion later), so it would be unreasonable to say that all the inconsistencies were solely due to macroscopic staging. Stage I (immature) testes and stage VI (spent) testes are the most recognizable macroscopically. This would account for them having the greatest match between assessment methods. However many authors have noted extreme difficulties in distinguishing macroscopically between the immature, early maturing and spent male fish (Welcomme, 1967; Siddiqui, 1979; Hunter and Macewicz, 1985; Halliday, 1987; Beacham, 1987; Rijnsdorp, 1989; Albert et al., 2001; Gerritsen et al., 2003).

The overall percentage of maturity stages which compared favorably between the two assessment methods was 22.03% for female plaice and 37.80% for male plaice. Both these values are quite low. In general, the findings of this study indicate that there was a very poor match between the macroscopic and histological assessment methods. The macroscopic determination of maturity stages in male and female fish can be very subjective (Godinho et al., 1974; Narahara, 1983; West, 1990; Ramsay and Witthames, 1996; Dias et al., 1998; Albert et al., 2001). Many stages are quite similar in appearance, and many gonads may appear and actually be midway between two stages. Hunter and Macewicz (2003) and Gerritsen and McGrath (2006) suggested that maturity scales could be improved by reducing the number of classes and focusing on the most reliable characteristics. Given that the histological determination of these stages is based on the observation of a distinct set of developmental features, it is expected that it would be more accurate to use histologically assessed gonads to calculate the annual percentage

maturity assessment. The percentage match between the two assessment methods might also be improved by restricting samples to closer to the spawning season.

Maturity at length keys

The macroscopic and histological maturity at length keys gave slightly different results. This probably reflects the poor match between the macroscopic and histological assessment methods as discussed above. For females the largest difference in mean length between the two assessment methods occurs at stage I. For males the largest difference occurs at stage II. According to the table, the mean length class does not increase with maturity stage. For example in macroscopically staged females, the mean length of stage V is 32cm and stage VI is 31cm. In histologically staged females, the mean length of stage IV is 32cm and stage V is 31cm. This is likely due to differences in the size of the samples in each maturity stage. For example there were only 10 fish recorded as stage IV while there was 64 fish recorded as stage V.

In general females have a larger mean length than males at each maturity stage, with the exception of stage II. The larger mean length of males at stage II might be accounted for by resting-spent individuals being classed as stage II developing / virgin.

Maturity at age keys

The macroscopic and histological maturity at age keys gave slightly different results. Again this probably reflects the poor match between the macroscopic and histological assessment methods as discussed above. For females the largest difference in modal age between the two assessment methods occurs at stage I and stage V. For males the largest difference occurs at stage I and stage IV. The histological assessment of maturity at age gives a modal age class of 5 years for stage I males and females. This is very different from the modal age class determined for stage I using macroscopic assessment, which was 3 years for females and 2 years for males. As male and female plaice recruit to the population at approximately 4 years and the histological $A_{50\%}$ was determined as age 2 and 1, for males and females respectively, it seems probable that some of the fish determined to be stage I were actually stage VII fish. There is also the possibility that plaice older than the $A_{50\%}$ estimate may not necessarily be mature. As with any

population of fish, a proportion of plaice may reach maturity later in life than the normal age at maturity.

Maturity ogives

The maturity at length and age keys provided an initial profile of at which age and length plaice reach sexual maturity off the west coast of Ireland. The $L_{50\%}$ for female plaice was estimated as 23cm from the macroscopic maturity ogive and as 24cm from the histological maturity ogive. The $L_{50\%}$ for male plaice was estimated as 21cm from the macroscopic maturity ogive and as 20.5cm from the histological maturity ogive. When both macroscopic and histological estimates of $L_{50\%}$ were compared between the sexes, it was observed that male fish reached a length at which 50% of fish were sexually mature before that of females. The minimum landing size for plaice in ICES area VIIb is 22cm. This means that females can be caught before they have reached maturity, and males may have just started to mature.

For both sexes the macroscopic and histological maturity at age ogives gave different results for the $A_{50\%}$. A difference of 1 and 2 years was recorded between them for males and females respectively. According to the macroscopic maturity at age ogive males and females reach $A_{50\%}$ at the same age, but according to the histological maturity at age ogive females reach $A_{50\%}$ a year younger than males. This contradicts the findings of previous studies on plaice, where it has been well documented that males mature younger (Kyle, 1900; Wallace, 1909, 1915; Mastermann, 1911; Hefford, 1916; Wimpenny, 1953; Simpson, 1959; Bannister, 1978; Harding et al., 1978; Rijnsdorp, 1989; Kell and Bromley, 2004) and at a smaller length than females (Bromley, 2003; Grift et al., 2003; Nielsen, 2004; Rijnsdorp et al., 2005).

According to the macroscopic maturity at age ogive, males and females reach $A_{50\%}$ at the same age of 3 years. The mean length of age 3 males and females in ICES area VIIb was determined to be 24cm and 26cm respectively. This would mean that even if the $A_{50\%}$ was the same for both male and female plaice, the males are still maturing at a smaller length. The histological maturity at age ogive determined $A_{50\%}$ as 2 years for males and 1 year for females. In ICES area VIIb the mean length of age 1 females was determined to

be 16cm, and the mean length of 2 year old males was determined to be 18cm. Then an $A_{50\%}$ of 2 years would mean that males are maturing at a slightly larger length. This contradicts the results for the $L_{50\%}$. Females reaching an age at first maturity before that of males, coincides with the mean length at age, where female fish were consistently larger than males observed at the same age. It is generally assumed that the onset of sexual maturity is determined by a constant threshold length rather than a specific age (Roff, 1982; Rijnsdorp, 1989, 1993), which can be affected by the growth rate of the fish (Alm, 1958; Nikolski, 1969). Females may have matured at a younger age because they had reached a threshold length for maturity, which males did not reach until 2 years of age.

The age at recruitment for both male and female plaice in ICES area VIIb was estimated as 4 years (chapter 1, age and growth), indicating that plaice are reaching maturity before they become vulnerable to the fishing gear. However, the mean length of age 3 males and females in ICES area VIIb was determined to be 24cm and 26cm respectively. This would put both male and female plaice at $A_{50\%}$ well above the minimum legal landing size of 22 cm. The histological maturity at age ogive determined $A_{50\%}$ as 2 years for males and 1 year for females. In ICES area VIIb the mean length of age 1 females was determined to be 16cm, and the mean length of 2 year old males was determined to be is 18cm. This means that according to the histological maturity at age ogive, males and females are reaching $A_{50\%}$ well before they become vulnerable to the fishing gear.

The estimates of first maturity at length and age determined during the present investigation are well within the ranges of those observed elsewhere for the species. However, because the distinction between mature and immature males is often not easy, data on the onset of first maturation in male flatfishes is less well documented than in females (Rijnsdorp et al., 2005). This made it difficult to compare the results of this study to other studies. In this study $L_{50\%}$ was determined as 23-24 cm for females and as 21 cm for males. For female plaice $L_{50\%}$ values ranging from 24 to 43 cm have been recorded in the North Sea (Simpson, 1959; Rijnsdorp, 1989; Bromley, 2000; Nielsen, 2004), values of 38 to 40 cm have been recorded in the English Channel (Brule, 1987), and values of 22cm have been recorded in the Irish Sea (Horwood, 1990). For male plaice, $L_{50\%}$

values ranging from 14 to 37 cm have been recorded in the North Sea (Simpson, 1959; Rijnsdorp, 1989; Bromley, 2000; Nielsen, 2004).

In this study $A_{50\%}$ was determined as between 1-3 years for females and as 2 or 3 years for males. For female plaice $A_{50\%}$ values ranging between 4 and 7 years have been recorded in the North Sea (Wimpenny, 1953; Simpson, 1951, 1959; Rijnsdorp, 1989; Bromley, 2000; Nielsen, 2004), an $A_{50\%}$ value of 3 years was recorded in the English Channel (Denial, 1984; Brule, 1987) and $A_{50\%}$ values ranging between 2 and 4 years were recorded in the Irish Sea (Horwood, 1990; Nash et al., 2000). For male plaice $A_{50\%}$ values ranging between 2 and 6 years have been recorded in the North Sea (Wimpenny, 1953; Simpson, 1959; Rijnsdorp, 1989; Bromley, 2000; Nielsen, 2004). The $A_{50\%}$ values determined in this study are most similar to the values recorded for plaice in the English Channel and the Irish Sea. This may indicate some latitudinal effect on age at maturity.

The length and age at first maturity varies with environmental conditions, from area to area and from year to year (Simpson, 1959; Beacham, 1983; Bowering, 1987; Rijnsdorp, 1989; Bowering and Brodie, 1991; Rochet, 2000; Bromley, 2000; Nielsen, 2004). Wimpenny (1953) recorded different lengths and age at maturity for plaice in up to four different areas in the North Sea. Nash et al., (2000) recorded small differences in $L_{50\%}$ and large differences in $A_{50\%}$ for female plaice in different regions of the Irish Sea, with females in Liverpool Bay maturing for the first time nearly two years earlier than females in the Western Irish Sea. Differences in age at maturation are expected when the growth rate differs between stocks of the same species (Alm, 1959). Rijnsdorp (1993) found that the size at maturity in North Sea plaice depends on growth rate prior to first maturity which occurs around 2-3 years of age, and Kovtsova (1982) found that faster growth in Barents Sea plaice lead to earlier maturation. Most studies concluded that female plaice grow faster and reach sexual maturation at an older age and larger size than males (Bromley, 2000; Kell, 2004). Estimates of first maturity have been presented for plaice in the Irish Sea by Horwood (1990) and Nash et al., (2000). However those determined during the present investigation provide the first specific maturity estimation for the population of plaice inhabiting the waters off the west coast of Ireland in ICES area VIIb.

Histological image analysis

Oocyte length frequencies

The largest percentage of stage I oocytes were recorded in the diameter class of 1-100 µm. Only three diameter classes were constructed for the stage I oocytes, indicating the similarity in oocyte size throughout Stage I ovaries. The largest percentage of stage II oocytes were found in the diameter class of 101-200 µm and the largest percentage of stage III oocytes were found in the diameter class of 101-200 µm. The largest percentage of stage IV oocytes were recorded in the diameter class of 101-200 µm and the largest percentage of stage V oocytes were found in the diameter class of 701-800 µm. The largest percentage of stage VI oocytes were recorded in the diameter class of 901-1000 µm. The largest percentage of stage VII oocytes were found in the diameter class of 1-100 µm. It was observed that in general the mean oocyte diameters increased with each maturity stage. The oocyte length frequency distributions determined for plaice during the present investigation were similar to those presented for North Sea plaice in a study by Urban (1991), and the mean oocyte diameter is similar to that recorded by Caputo et al., (2001) for brill (*Scophthalmus rhombus*).

Measuring the oocytes within ripe ovaries prior to spawning gives an estimate of what type of spawner a species is. When ova of greatly differing diameters (and stage) are observed, the species of fish are usually multiple spawners, ie, the fish spawn on more than one occasion annually. Ovaries which contain oocytes which vary little in diameter are normally total spawners, ie, fish that spawn once a year (Cailliet et al., 1986). The oocyte length frequency distributions presented here show gaps between the small unyolked oocytes and the maturing and mature oocytes. This indicates that plaice has a discontinuous oocyte development. In conclusion the plaice is a determinate batch spawner, meaning it spawns once annually, releasing its eggs in batches over a protracted spawning season. This supports the findings of Barr (1963c) and Rijnsdorp (1989).

In this study, the maturity stage of ovaries was determined by the most advanced oocyte stage present, after Tomkiewicz (2003). In retrospective, with further reading and practical experience, the author would stage ovaries by proportion of oocyte type present, as was done by Hunter et al., (1992) for Dover sole (*Microstomus pacificus*).

Histological structures

The developmental structures identified and recorded for both sexes were consistent with the developmental stages observed for plaice (Barr, 1963; Brule,1987) and in other flatfish reproductive studies, such as that described by Htun-Han (1978) for dab (*Limanda limanda*), Abdel-Aziz (1994) for Egyptian sole (*Solea aegyptiaca*), Ramsay and Witthames (1996) for sole (*Solea solea*), Maddock and Burton (1999) for American plaice (*Hippoglossoides platessoides*), Rideout et al. (1999) for Greenland halibut (*Reinhardtius hippoglossoides*), Bromley et al. (2000) for turbot (*Scophthalmus maximus*) and Merson et al. (2000) for summer flounder (*Paralichthys dentatus*).

For female plaice, histological examination of the gonads was straight forward, and all the defining developmental characteristics of each maturity stage were clearly observed. However, for male plaice, the individual cell types representing the various stages of development are not visible in detail without electron microscopy (Grier, 1981). Using light microscopy the associated structural changes in the testes were identified and recorded, but were difficult to see, and therefore may be considered unreliable.

Gonadosomatic index (GSI) and condition factor (CF)

The highest values for condition factor for female plaice were observed in the months just prior to spawning, e.g. November 2003 and January 2005. There was a relatively high peak in condition factor in July 2004, which was likely due to optimal feeding conditions. The lowest values for condition factor were recorded in February, March and April 2004. These months occur at the peak of spawning and just after spawning. Fish would probably have just resumed feeding and have not started to recover from the spawning.

For female plaice the GSI value began to increase in November 2003 and peaked in February 2004. In March 2004 it decreased dramatically. The GSI began to gradually increase again in July 2004 and climbed steadily to a peak in February 2005. There were no samples collected in the months following February 2005, so it can only be inferred that the GSI drops again in March as in the previous year. The drop in the GSI between

February and March indicates that the female plaice spawned at this time. From the examination of the annual percentage occurrence of each maturity stage, it could be seen that in February the majority of female plaice were recorded in the spawning condition (histological assessment) or in spent condition (macroscopic examination).

The highest values for condition factor in male plaice were observed in the months of November 2003, July 2004 and December 2004. The lowest values for condition factor were recorded in February and March 2004. The high values correspond with the months just prior to spawning, and the summer months. The low values correspond with the spawning period and just after spawning.

For male plaice the GSI value began to decrease in November 2003 to a low in April 2004. In July 2004 it began to increase again to a peak in November 2004. The GSI began to gradually decrease again in December 2004. The drop in the GSI between November 2003 and April 2004 and November and December 2004 indicates that male plaice were spawning at this time and as a result had a lower percentage of the gonad comprising the total body weight. The examination of the annual percentage occurrence of each maturity stage gives differing results for male plaice. The macroscopic annual percentage maturity assessment puts the majority of males in November 2003 as immature, and in November 2004 as in ripening condition. The histological annual percentage maturity assessment puts the majority of males in November 2003 as ripening and males in November 2004 as in mature condition. These differences between assessment methods probably reflect the difficulties encountered in staging the male gonad. However they do indicate that males were ready to spawn.

During the present investigation, it was observed that from July each year, there was a general trend of increasing GSI values for plaice, with GSI for female plaice reaching a peak in February, and GSI for male plaice reaching a peak in November. This was followed by a dramatic decrease in GSI indicating that the fish spawned at this time (Caputo et al., 2001). Values were at very low levels through the remaining spring and summer months, until just prior to the start of the next spawning season when GSI values

began to increase once again. A similar cycle in GSI was recorded for North Sea plaice (Rijnsdorp et al., 2005) and for plaice in the English Channel (La Haye, 1972; Houghton and Harding, 1976; Denial, 1981; Brule, 1987). Between the months of November and February the GSI for male plaice was decreasing, while the GSI of female plaice was climbing. The difference in the peak of the GSI between males and females reflects the fact that males reached peak spawning slightly before females. It was also observed that male plaice started spawning earlier and had a more protracted spawning season. Rijnsdorp (1989) observed a similar trend in North Sea plaice, where spawning females were recorded from January to April with a peak in February, and spawning males were observed from November to May with a peak in December.

When the condition factor and GSI values for plaice recorded during the present investigation were compared, a distinct pattern emerged. It was noted that the peak or maximum values for condition factor occurred just before those of the GSI, indicating that plaice were in their best condition immediately prior to spawning. After spawning the condition of the fish declined as did the GSI, but then started to recover over the following months. Rijnsdorp et al. (2005) and Dawson and Grimm (1980) found a similar pattern in North Sea plaice where condition steadily increased during the growing period and reached a maximum in November and December, just before spawning.

Spawning period

From analysis of the macroscopic and histological maturity assessments and the GSI, it was determined that in ICES area VIIb female plaice spawn from November to March, with peak spawning occurring in February, and male plaice spawn from November to April, with peak spawning occurring in November. In the present study spawning females had an age range of 2 to 10 years and spawning males had an age range of 2 to 7 years. The smallest spawning female was 20cm and the smallest spawning male was 14 cm. Male plaice showed an earlier peak in spawning and had a longer spawning period.

The spawning period of plaice in Irish waters is poorly documented compared with the profusion of studies on plaice in the North Sea. Studies in the Irish Sea found that the

duration and timing of the spawning season varies with location around the British Isles and Ireland (Cole et al., 1901). Spawning of plaice off the west coast of Ireland has not been studied to date. A comparison of spawning times for the present study and other studies in different areas are presented in Table 2.4 (a). The timing of spawning in the present study was most similar to that observed by Lockwood (1984) for plaice in the North Sea. Spawning in plaice however, changes with temperature and latitude, with each region having its own distinctive spawning period. There is a delayed start and end of plaice spawning at lower water temperatures (Rijnsdorp and Vethaak, 1997; Nash et al., 2000), and earlier peak spawning in the warmer years (Van der Land, 1991). It is thought that the timing of spawning is synchronized with the productivity of the pelagic system (Cushing, 1969; Bagenal, 1971; Rijnsdorp et al., 2005).

Table 2.4 (a). Spawning period as recorded for the present study and by other authors for plaice in European waters.

	Study	September	October	November	December	January	February	March	April	May	June	July	August
North Sea	Bromley (2000)												
	Rijnsdorp et al. (2005)												
	Wegner et al. (2003)												
	Hoarau et al. (2002)												
	Rijnsdorp (1989)												
	Lockwood (1984)												
	Harding et al. (1978)												
	Barr (1963b)												
	Simpson (1951)												
	Todd (1911)												
	Simpson (1959)												
Irish Sea	Armstrong et al. (2001)												
	Ellis and Nash (1997b)												
	Daan et al. (1985)												
	Pommerantz (1981)												
	Daniel and Fleming (1933)												
English Channel	Brule (1987)												
	Deniel (1981)												
	Houghton & Harding (1976)												
ICES area VIIb	Present study												

Conclusions

The present study finds the following conclusions, which contribute to the knowledge of population dynamics and biological characteristics of P.platessa, and which may be used to better manage plaice stocks in Irish waters.

- Males were more abundant in the smaller length classes while females dominated the larger length classes. A significant difference in median length values of male and female plaice between ICES areas was recorded.

- Growth of plaice did not differ between ICES areas. However, a significant difference in growth rate of male and female plaice between ICES areas was recorded when plaice were sampled in March. This needs to be considered when sampling plaice for comparison of growth or median lengths.

- The recorded mean length and weight at age for plaice in Irish waters during this study is in line with values recorded for the species elsewhere in Europe.

- The highest rate of fishing mortality was recorded for ICES area VIa, and the lowest for VIIa. In each ICES area male and female plaice have fully recruited to the population by age 4, with the exception of females in ICES area VIa which had an age of recruitment of 5 years.

- The length at first maturity (L_{50}%) was determined to be 23cm and 21 cm for females and males respectively. Age at first maturity (A_{50}%) was determined to be 3 years for both males and females. Males and females In ICES areas VIIb, VIIa and VIa are well above the length and age at first maturity when they are recruited to the fishery.

- There was a very poor match recorded between the macroscopic and histological assessment methods. The overall percentage of maturity stages that compared favorably between the two methods was 22% for female plaice and 37% for male plaice. Given that the histological determination of these stages is based on the observation of a distinct set of developmental features, it is concluded that it would be more accurate to use histologically staged gonads for percentage maturity assessments.

- Female plaice in ICES area VIIb spawn from November to March, with peak spawning occurring in February, and male plaice spawn from November to April, with peak spawning occurring in November. Spawning females had an age range of 2-10 years and spawning males had an age range of 2-7 years. Male plaice showed an earlier peak in spawning and had a longer spawning period.

- Plaice demonstrated a discontinuous oocyte development, indicating that it is a determinate batch spawner. This finding concurs with studies for the species elsewhere in Europe.

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Table 1.3.4 (b). Age Length Key – Numbers of female plaice at each age in each length class sampled in ICES area VIa (north west coast of Ireland) between November 2003 and February 2005. (N = 114).

	Age (years)										
length (cm)	1	2	3	4	5	6	7	8	9	10	Total
9							1				1
10			1								1
11											0
12		1	1	3							5
13			1								1
14			1	1							2
15			1	1							2
16			2			1					3
17	2										2
18					1						1
19				3							3
20				1							1
21				3					1		4
22		1	2	2							5
23		1	1	4	1						7
24		2	2	2							6
25	1		2	2	1						6
26		1	3	1	1			1			7
27		3	2	1							6
28		3	1	3		1					8
29		1	2								3
30		1	2	3	2						8
31			2		1						3
32			2	2	3	1					8
33			3	4	1						8
34			1	1		1					3
35					1						1
36				1	2		1				4
37	1										1
38		1	1								2
39											0
40				1							1
41											0
42			1								1
Total	4	15	34	39	14	4	2	1	1	0	114
Mean Length (cm)	24	26	26	25	30	28	23	26	21	0	26

Table 1.3.4 (c). Age Length Key – Numbers of male plaice at each age in each length class sampled in ICES area VIa (north west coast of Ireland) between November 2003 and February 2005. (N = 114).							
	Age (years)						
length (cm)	1	2	3	4	5	6	Total
11		1					1
12		1					1
13		4					4
14		1	1				2
15		2					2
16	1	2					3
17	1	1	2	1			5
18	2	1					3
19	1	4	4	1			10
20		1	2	1			4
21		2	6	1			9
22		3	4	4			11
23		2	6	1			9
24		3	4	2			9
25		1	3	3	1		8
26		2	4	1	1	1	9
27			5	3			8
28			1	1	1		3
29		1	1		2		4
30			1	1	1		3
31			1	1	1		3
32					1		1
33							0
34				2			2
Total	5	32	45	23	8	1	114
Mean length (cm)	18	19	23	25	29	26	23

Table 1.3.4 (d). Age Length Key – Numbers of female plaice at each age in each length class sampled in ICES area VIIa (Irish Sea) between November 2003 and February 2005. (N = 421).

	Age (years)												
length (cm)	1	2	3	4	5	6	7	8	9	10	11	12	Total
11	1												1
12		1											1
13	3												3
14	1	1	3										5
15	1	5	1	1									8
16		3	2										5
17		3	1	1									5
18		2	3	1									6
19		3	3	1									7
20	2	4	7	3									16
21		4	10	1	1								16
22		4	7	4									15
23		4	11	4	1	1							21
24		6	8	4	1								19
25		3	13	4	1		1						22
26		5	10	9	1								25
27		8	8	6	2	3							27
28		5	8	12	2	1	1	1					30
29		5	5	10	6	2		1					29
30		5	8	6	5	1	1						26
31			3	7	4	1	3	1					19
32		2	4	7	3	2	3						21
33			5	6	4	1	2	1	1				20
34		1	1	6	3	3		1	1				16
35			2	2	6	2	6	1					19
36				3		5			1				9
37			1	1		2	3						7
38							3		1				4
39					1	1	1	2					5
40						1		1		1			3
41								1				1	2
42							1	2					3
43				1			1					1	3
44							1	1			1		3
Total	8	74	124	100	41	26	27	13	4	1	1	2	421
Mean length (cm)	15	23	25	28	31	33	35	37	35	40	44	42	28

Table 1.3.4 (e). Age Length Key – Numbers of male plaice at each age in each length class sampled in ICES area VIIa (Irish Sea) between November 2003 and February 2005. (N = 356).

	Age (years)								
Length (cm)	1	2	3	4	5	6	7	8	Total
11	1								1
12		2							2
13	2	3							5
14	5	6	2						13
15		9	2		1				12
16	2	8	6						16
17		11	5						16
18	1	4	8	1	1				15
19	2	5	10	2	1				20
20		12	8	2					22
21	1	7	12	4					24
22		9	11	6	3	1			30
23	2	7	6	5	4	1			25
24	1	9	13	6	6	1			36
25		4	10	6	5	1	1	1	28
26		2	6	6	6				20
27		2	2	7	7	1	2		21
28			6	1	4	6			17
29			2	3	3	3	1		12
30			1	2	6	1			10
31			2	2	1		1		6
32				1					1
33					1		1		2
34									0
35						1			1
36									0
37								1	1
Total	17	100	112	54	49	16	6	2	356
Mean length (cm)	17	26	27	29	31	34	35	35	22

Table 1.3.4 (f). Age Length Key – Numbers of female plaice at each age in each length class sampled in ICES area VIIb (west coast of Ireland) between November 2003 and February 2005. (N = 434).

	Age (years											
Length (cm)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Total**
11	2	1										**3**
12												**0**
13		4	1									**5**
14		5										**5**
15	1	3	1									**5**
16		2	2	1								**5**
17		2	1	2								**5**
18	1	3	1									**5**
19		3	8									**11**
20		9	2		2							**13**
21		5	9	1								**15**
22		4	11	1	1							**17**
23	1	7	8	4	3							**23**
24		3	11	8	2							**24**
25		2	12	5	1	1		1				**22**
26		2	10	10	5							**27**
27		2	10	5	6	2	1					**26**
28		1	8	11	5	1	2					**28**
29		3	14	11	5	1						**34**
30		1	11	15	9	3						**39**
31		1	7	14	11	2						**35**
32			3	9	6							**18**
33			6	6	7	4	1	1				**25**
34			3	3	2	5	1					**14**
35				2	7	3	2					**14**
36				1	1	2				1		**5**
37					2	1	2	1				**6**
38							1					**1**
39												**0**
40												**0**
41										1		**1**
42					1							**1**
43												**0**
44									1			**1**
45												**0**
46												**0**
47											1	**1**
Total	**5**	**63**	**139**	**109**	**76**	**25**	**10**	**3**	**1**	**2**	**1**	**434**
Mean length (cm)	**16**	**21**	**26**	**28**	**30**	**32**	**33**	**32**	**44**	**39**	**47**	**27**

Table 1.3.4 (g). Age Length Key – Numbers of male plaice at each age in each length class sampled in ICES area VIIb (west coast of Ireland) between November 2003 and February 2005. (N=356)

	Age (years)									
Length (cm)	1	2	3	4	5	6	7	8	9	Total
9	1									1
10	1	1								2
11		4								4
12	1	3								4
13	3	6	1							10
14		7	1							8
15		4	4							8
16		8	2							10
17		9	2	1						12
18		6	4	1		1				12
19		7	6	1						14
20		7	8	3						18
21		4	10	3	2					19
22		7	15	7						29
23		2	12	11						25
24		4	9	4	4					21
25		2	12	10	2	1				27
26		2	9	4	4	1				20
27		1	9	5	2	3				20
28		2	9	9	6			1	1	28
29		2	5	5	2	1	2			17
30		1	4	5	3	1				14
31			6	5	2	2	2			17
32			2	4	1	3				10
33				2			1			3
34							1	1		2
35										0
36										0
37										0
38										0
39										0
40					1					1
Total	6	89	130	80	29	13	6	2	1	356
Mean length (cm)	12	18	24	26	28	28	31	31	28	23

Table 1.3.4(h). Age Length Key – Numbers of female plaice at each age in each length class sampled in ICES area VIIg (Celtic Sea) between November 2003 and February 2005. (N = 104).

	Age (years)							
Length (cm)	1	2	3	4	5	6	7	Total
20		2						2
21		1						1
22								0
23								0
24		2	3					5
25		4	3	1				8
26		2	5	1				8
27		1	4	3				8
28		2	3	1				6
29			7	2	2			11
30		1	3	2	1			7
31		2	4	2	3			11
32			5	4		1		10
33			2	3	2			7
34					1	1	1	3
35			2	3	2			7
36			1			2		3
37			1	1	1			3
38			1		1			2
39								0
40					1			1
41								0
42								0
43								0
44							1	1
Total	0	17	44	23	14	4	2	104
mean length (cm)	0	26	29	31	33	35	39	30

Table 1.3.4(i). Age Length Key – Numbers of male plaice at each age in each length class sampled in ICES area VIIg (Celtic Sea) between November 2003 and February 2005. (N = 40).

	Age (years)					
Length	1	2	3	4	5	Total
14			1			1
15						0
16						0
17				1		1
18						0
19		1	1			2
20		2				2
21		1				1
22		3	1			4
23				3		3
24		1	1			2
25		1	1			2
26			5	1		6
27			2	2		4
28			2	1		3
29				1		1
30			2		1	3
31			3	1	1	5
Total	0	9	19	10	2	40
mean length (cm)	0	22	26	25	31	25

Table 1.3.4 (j). Age Length Key – Numbers of female plaice at each age in each length class sampled in ICES area VIIj (south west coast of Ireland) between November 2003 and February 2005. (N = 177).

	Age (years)																
Length (cm)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
17			2	1													3
18			2														2
19		3	4	1													8
20		1	2	1													4
21			4	1													5
22			4	2													6
23			9	2	1												12
24			5	2													7
25			4	5	1												10
26			6	4													10
27		2	2	6	2												12
28			2	6	3												11
29			7	5	2												14
30		3	7	5	3												18
31		4	4	1	3												12
32			1	1	2												4
33			2	1	2	2											7
34			2	4	1												7
35				1		1		1		1							4
36			1		4	1											6
37					2												2
38				1			1	1									3
39				1						2							3
40																	0
41						1											1
42					1												1
43								1									1
44																	0
45																	0
46						1											1
47														1		1	2
48																	0
49																	0
50																	0
51													1				1
Total	0	13	70	51	27	6	1	3	0	3	0	0	1	1	0	1	177
Mean Length (cm)	0	27	26	28	32	37	38	39	0	38	0	0	51	47	0	47	28

Table 1.3.4 (k). Age Length Key – Numbers of male plaice at each age in each length class sampled in ICES area VIIj (south west coast of Ireland) between November 2003 and February 2005. (N=164)								
	Age (years)							
Length (cm)	1	2	3	4	5	6	7	Total
16		2						2
17		1	4					5
18		6	2					8
19	1	4	1	1				7
20		3	8	1				12
21		3	6	4				13
22		1	10	3				14
23		2	4	3	1			10
24		3	8	2				13
25		1	9	4	2			16
26		1	5	4	2			12
27	1		8			1		10
28		1	3	5				9
29		1	6	1	2	2		12
30			1	1		1	1	4
31			2	1	1			4
32			3	3	1		1	8
33					1			1
34			1			1		2
35				1				1
36						1		1
Total	2	29	81	34	10	6	2	164
Mean Length (cm)	23	21	24	26	28	31	31	24

Figure 2.4 (a). Percentage age frequency distribution for male and female plaice in ICES area VIIb collected between November 2003 and February 2005. (females n= 434, males n=358).