

**Ireland's understudied flatfish:
Reproduction, age and growth of the
dab *Limanda limanda*(L.) in Irish
coastal waters**



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**Ireland's understudied flatfish:
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Laura M. Langan

Abstract

In Ireland, although flatfish form a valuable fishery, little is known about the smallest, the dab *Limanda limanda*. In this study, a variety of parameters of reproductive development, including ovarian phase description, gonadosomatic index (GSI), hepatosomatic index (HSI), relative condition (K_n) and oocyte size were analysed to provide information on the dab's reproductive cycle and spawning periods. Sampling were collected monthly over an 18-month period using bottom trawls of the Irish coastline.

A six phase macroscopic guide was developed for both sexes of dab, and verified using histology. In comparisons of macroscopic and microscopic phases, there was high agreement in the proposed female guide (86%), with males demonstratively lower (62%). No significant bias was observed between the the two reproductive methods. When the male macroscopic guide was examined, misclassification was high in phase 5 and phase 5 (41%), with 96% of misclassification occurring in adjacent phases. The sampled population was primarily composed of females, with ratios of females to males 1:0.6, although the predominance of females was less noticeable during the reproductive season. Oocyte growth in dab follows asynchronous development, and spawn over a protracted period indicating a batch spawning strategy. Spawning occurred mainly in early spring, with total regeneration of gonads by May. The length at which 50% of the population was reproductively mature was identified as 14cm and 17cm, for male and female dab, respectively.

Precision and bias in age determinations using whole otoliths to age dab was investigated using six age readers from various institutions. Low levels of precision were obtained (CV: 10-23%) inferring the need for an alternative methodology. Precision and bias was influence by the level of experience of the reader, with ageing error attributed to interpretative differences and difficulty in edge determination.

Sectioned otolith age determinations were subsequently compared to whole otolith age determinations using two age readers experienced in dab ageing. Although increased precision was observed in whole otoliths from previous

estimates (CV=0%, 0% APE), sectioned otoliths were used for growth models. This was based on multinomial logistic regression on age length keys developed using both ageing methods.

Biological data (length and age) for both sexes was applied to four growth models, where the Akaike criterion and Multi model Inference indicated the logistic model as having the best fit to the collected data. In general, female dab attained a longer length than males, with growth rates significantly different between the two sexes. Length weight relationships between the two sexes were also significantly different.

Declaration

I hereby declare that the work presented in this thesis is my own and that it has not been used to obtain a degree in this Institute of Technology or elsewhere.

Lucretia Logan
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21/09/2012
Date:

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Chapter 1

Literary Review

1.1 Fisheries: stock assessment and management

The effective management of fisheries draws on various biological characteristics attained through fisheries science in order to identify ways to protect fishery resources for sustainable exploitation. The basis for understanding changing fishery patterns and issues such as habitat destruction, predation and optimal harvest rates are identified through knowledge about the population dynamics of fisheries. There are a diverse range of management systems in use in the preservation and management of marine ecosystems, with most systems built upon a system of quotas called total allowable catch (TAC). These quotas are usually species specific and based on technical measures such as gear restrictions, minimum mesh sizes, seasons and closed areas.

1.1.1 Europe

The legal basis of fisheries management in the European Union (EU) is laid down in the Common Fisheries Policy (CFP), whose objective is the sustainable exploitation of renewable marine resources. One of the main instruments which assist in this objective is stock specific total allowable catches or TACs, which are agreed upon annually by the Council of Ministers. The scientific basis for TAC quotas is derived by the stock assessments and catch forecasts provided annually by the International Council for the Exploration of the Sea (ICES). The status of the stock is evaluated with reference to precautionary reference points for spawning stock biomass and fishing mortality (Bromley, 2003). This type of management relies heavily on reliable catch statistics, age compositions of fish landings and recruitment estimates based on research vessel surveys.

There are two distinct aspects in the exploitation of a commercial fishery, exploitation rate which covers fishing intensity and exploitation pattern which governs fishing selection (Vasilakopoulos et al., 2011). Currently, stock assessments are primarily based on the exploitation rate approach which is defined as the proportion of the population of fish removed each year. The aim of such assessments is to establish the status of the fish resource and determine the level at which it may be sustainably exploited. In order to carry out a stock assessment, it is necessary to build up a picture of how the fish population changes over time and examine the historical trends in biological traits

A wide array of biological information is collected from both recreational and commercial fisheries, in order to facilitate the assessment. Age estimation is

a fundamental component of these assessments, as age determined parameters such as age at first maturity, recruitment successes and growth are utilized in the population dynamics models necessary for stock assessments.

1.2 Management of *Limanda limanda*

Flatfish (Family Pleuronectidae) are among one of the most important marine fish resources in terms of economic value in the North Temperate zone (Florin, 2005). Many flatfish stocks are in decline due to overfishing, for example halibut, plaice and turbot. The dab *Limanda limanda* is unlike other flatfish in that an increase in fishing catch in European waters has been recorded in the past few years (ICES Resource Management Committee, 2005).

L. limanda is a common, low value marine flatfish, which is commercially fished in Iceland, the southern North Sea (Braber and De Groot, 1973) and specific European waters (Henderson, 1998). It is one of the most frequent occurring and commercially important species in the Baltic Sea (Sager and Berner, 1989).

Ortega-Salas (1987) first identified dab as having the potential to support a viable fishery in European waters, but it was not until the publication of an ICES working group report (ICES Resource Management Committee, 2005) that biological information about the various dab populations became of interest. Ortega-Salas (1987) estimated the minimum biomass of dab as between 82 and 98 million tonnes throughout its distribution. Until recently, the only information that was recorded for *L. limanda* was landing statistics (Casey et al., 2011), which is insufficient data to support management objectives and advice. Under the EU Data Collection Framework, ICES Advisory Committee (2010a) recognises five management units located in the Irish Sea, the North Sea and the Baltic Sea (ICES subdivisions II, V, VI, VII (excl. d), VIII, IX, X, XII, XIV; IIIa north; IIIa south, IIIb-d; IV, VIId and VIId) (Appendix: Figure 6.2). These stocks were identified through differences in meristic characteristics and migration patterns (Nissling et al., 2002; Jónsson, 1966). In 2012, ICES Advisory Committee further divided these management units into management regions to be known as the Celtic Seas Region, North Sea Region and the Bay of Biscay Region.

Currently, the European Union has set the total allowable catch (TAC) at 18,434 tonnes, for a combined fishery for flounder and dab in ICES areas IIa (Norwegian Sea) and area IV (North Sea), where a breakdown of the TAC per

country can be seen in Table 1.1.

Previously, dab was of economic importance in the Dutch Wadden sea, although this fishery closed in the 1950's (Florin, 2005). Dab are not easily caught in large quantities using longlines, although they are caught in large quantities using Danish seines (Florin, 2005). The catch rate of dab is influenced by many environmental factors (Bolle et al., 2001), but temperature plays an important role in their survival. For example, Bolle et al. (1994) identified an optimal range of environmental temperatures congruent with dab growth, where temperatures below this level impact on condition, growth, recruitment and feeding. These temperatures range from 15 – 18° for optimal growth in young dab, with temperatures above 18° showing rapid declines in growth and feeding and no discernible growth at temperatures of 22° and higher. Martin et al. (2010) previously observed that hydrological parameters such as temperature play an important component in the mortality of dab eggs and larvae.

In coastal and estuarine areas, the catch rate is considered variable. This is based on the known relationship between recruitment level in dab and the size of the nursery grounds (Bolle et al., 2001), where small nursery grounds limit the survival of large populations due to lack of adequate prey items.

Compared to other flatfish species, studies on *L. limanda* recruitment and population are relatively sparse (De Raedemaeker et al., 2011; Beggs and Nash, 2007; Lee et al., 2007, 2006; Stentiford and Feist, 2005; Nissling et al., 2002; Bolle et al., 2001; Folmer, 2000; Henderson, 1998; Rijnsdorp et al., 1992; Jónsson, 1966). The dab's greatest value in recent years in the scientific community is based on its use in ecotoxicological studies (Vethaak et al., 2009; Stentiford et al., 2009; Scott et al., 2007; Ward et al., 2006; Feist et al., 2004; Hutchinson et al., 2003). It has widely been used in the United Kingdom National Marine Monitoring Programme (NMMP) due to its abundance, ease of capture and tendency to express biomarkers of contaminant exposure (Feist et al., 2004). Stentiford and Feist (2005) reported the first case of ovotestis in dab in the North Sea and related it to the potential presence of endocrine disruption chemicals either at the sampling site or at the nursery grounds.

Table 1.1: Total allowable catch (TAC) of dab and flounder combined in ICES areas of IIa and IV. Table extracted from 57/2011 (2011).

Country	Tonnes
Belgium	503
Denmark	1,888
Germany	2,832
France	196
The Netherlands	11,421
Sweden	6
United Kingdom	1,588
EU Total	18,434
Precautionary TAC	18,434

1.3 Taxonomy and description

The dab *Limanda limanda* (Family Pleuronectidae, Class Actinopterygii) has an asymmetrical body form, with 16-18 caudal fins, 50-62 anal rays and 70-80 dorsal fin rays (Russell, 1976). The dab is identified primarily through a pronounced lateral line which has a distinctive semi-circular curve above the pectoral fin, which is also visible on the ventral surface of the fish (Figure 1.1). Colouring is not a good indication of species as some dab show similar colouration to plaice and flounder in certain situations. The presence of specific skin diseases which can alter the appearance of the skin in dab are well documented (Møllergaard et al., 1996). For example, oxygen deficiency in dab is known to cause a disease called lymphocystis which can cause nodules primarily on the fins of the fish (Møllergaard and Nielsen, 1995). The presence of adenovirus particles can also cause an alteration in the pigmentation of the epithelium called hyperplasia (Bloch et al., 1986) as documented in Figure 1.2.

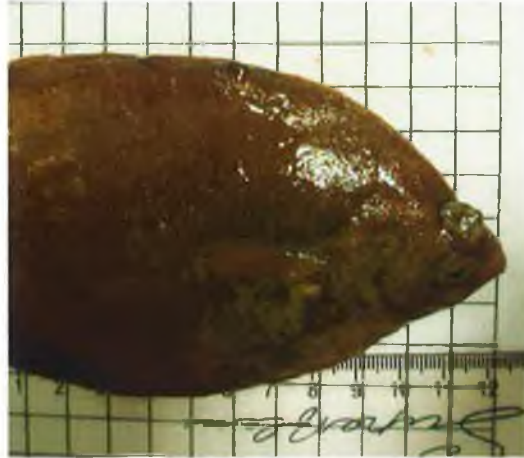


Figure 1.1: The common dab *Limanda limanda*. The pronounced curve in the lateral line present above the pectoral fin is considered an easily recognisable indicator in the identification of the dab.

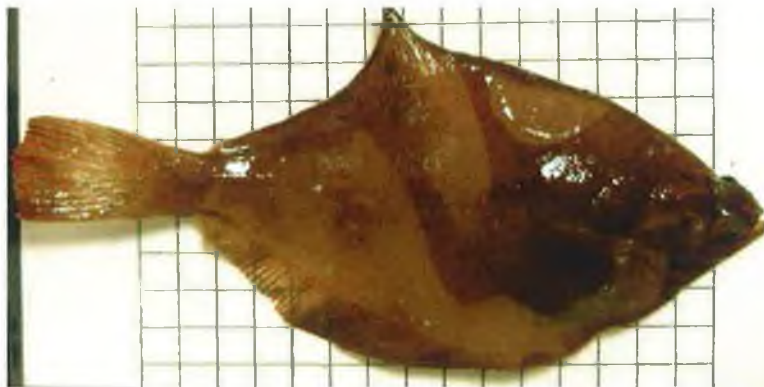


Figure 1.2: The appearance of the skin of *Limanda limanda* can change based on the presence of specific skins diseases such as hyperplasia as illustrated. This causes a pronounced alteration in the pigmentation of the fish, sometimes darkening the whole fish skin.

1.4 Habitat and distribution

Limanda limanda is abundant in depths of 20-40 meters, with areas of highest abundance within the 50 meter depth contour (Henderson, 1998; Bohl, 1957). The largest population of dab is thought to exist in the North Sea (Henderson, 1998), with important spawning grounds located in the southern North Sea (Campos et al., 1994).

Dab spawn offshore, typically at depths of 30-50 meters (Henderson, 1998). During the autumn, there is an inshore migration into shallow waters where there are richer feeding grounds (Ortega-Salas, 1987). The dab migrate to deeper waters as the temperature decreases to near 2.5°C (Rijnsdorp et al., 1992) for both adult and juvenile fish (Bolle et al., 2001). Dab of less than two years will occupy coastal waters until such time as they can join the adult population offshore (Henderson, 1998).

Over its wide geographical range, dab populations can be distinguished based on differences in meristic and migratory movements (Nissling et al., 2002), but also based on spawning locality, time of reproduction, growth rate and age-specific fecundity (Henderson, 1998).



Figure 1.3: Computer generated map of distribution of *Limanda limanda* generated by www.aquamaps.org. The colours on the map represent the relative probability of occurrence in this area, with the colour red indicating between an 80% and 100% probability of finding this species and the white colour representing a less than 2% probability.

1.5 Age and Growth

The growth and maturity of fish are traditionally measured using the von Bertalanffy equation describing size at age (Katsanevakis, 2006), with age usually attained through examination of otoliths (Armsworthy and Campana, 2010; Katsanevakis, 2006; Dwyer et al., 2003). Otoliths are hard, calcareous bodies in the paired labyrinth systems of teleosts, located in the cranial bones near the brain. Finfish have three pairs of otoliths, the sagittae, lapilla and asterisci, with the sagitta the largest and most commonly used in ageing studies. Age and subsequently growth studies assist with the understanding of timing and magnitude of spawning recruitment, habitat use, larval and juvenile duration, stock identification and population age structure in order that fisheries management may design appropriate management policies (Begg et al., 2005).

Limanda limanda grow rapidly and mature early, with females on average mature at 2-3 years (equivalent to 14cm) and males at 2 years (11cm) (Beggs and Nash, 2007; Jennings et al., 2001; Rijnsdorp et al., 1992). Life expectancy in *L. limanda* varies between 8 and 14 years dependent on environmental conditions and location (Florin, 2005; Ortega-Salas, 1987; Lee, 1972). Dab are primarily aged using whole otoliths (ICES Advisory Committee, 2010b; Folmer, 2000; Ortega-Salas, 1987; Jónsson, 1966) although this method has never been validated.

Dab can grow up to 42cm in total length (Jónsson, 1966), although most specimens will grow to no longer than 30cm. Previous studies have noted varying growth rates throughout the dab's range of distribution (Henderson, 1998; Ortega-Salas, 1988; Lee, 1972; Jónsson, 1966). Variabilities in growth rate have previously been associated with environmental water temperatures and length of growing season (Jónsson, 1966)(Table 1.2). For example, Beggs and Nash (2007) and Henderson (1998) identified adult dab as a species which experiences reduced growth rates and limited activity in the southern limits of its range, while Bolle et al. (1994) demonstrated that no discernible growth is visible in dab above temperatures of 22°C. Jónsson (1966) noted that dab sampled from Icelandic waters grew slower than those captured from the Baltic Sea, while identifying the North Sea as an area with the slowest growing dab population.

Differences in growth rate between populations is not simply a function of water temperature or the length of the growing season (Henderson, 1998). Lee (1972) linked variations in growth differences between populations located

in the North Sea to food quality and abundance. Adult dab are visual feeders with large eyes and fast acceleration (Hinz et al., 2005), with a diet confined to organisms found in or above the sea bed (Wyche and Shackley, 1986). There is evidence of considerable geographical and ontogenic variation in dab diet throughout its distribution (Hinz et al., 2005; Amara et al., 2001; Kaiser et al., 1997; Bels and Davenport, 1996), with males show a distinct seasonal feeding cycle reflecting distinct biological processes (Saborowski and Buchholz, 1996). In the presence of excess food, available through trawl disturbance, dab has proven to be an opportunistic feeder (Kaiser et al., 1997), and it is not unusual to find fish carrion in their stomachs.

Dab demonstrate isometric growth, where the body depth and head grow simultaneously with the body length (Folmer, 2000). Sexual dimorphism, as shown in Table 1.2, is common in dab, where female dab have a higher growth rate when compared to male dab (Folmer, 2000).

Table 1.2: Growth rates for the Irish Sea and North Sea populations of dab. K represents the growth rate within the dab, while L_{∞} and W_{∞} represent the asymptotic length and weight, respectively.

Location	Sex	Reference	L_{∞}	W_{∞}	K years ⁻¹
Irish Sea	Ortega-Salas (1987)	Female	-	398g	0.31
Irish Sea	Ortega-Salas (1987)	Male	-	110g	0.06
Irish Sea	Folmer (2000)	Female	31.53cm	-	0.40
North Sea	Lee (1972)	Female	-	328.5g	0.21
North Sea	Lee (1972)	Male	-	95.9g	0.44
Eastern North Sea	Rijnsdorp et al. (1992)	Female	30.5cm	-	0.28
Eastern North Sea	Rijnsdorp et al. (1992)	Male	25cm	-	0.26

1.6 Reproductive Biology

The age and length when a fish matures is species-specific and can vary between populations owing to a variety of intrinsic and extrinsic factors (Nash et al., 2010). In terms of management, maturity ogives (the proportion of a population mature by age) are an important measurement of fish populations and play a critical role in the formulation of fishing reference points such as spawning stock biomass (SSB) and back calculation of stock sizes from SSB estimates (Bromley, 2000). The aim of management policy is to conserve the spawning stock biomass in order to reduce the likelihood of having recruitment overfishing whereby young fish are not given the chance to contribute to

recruitment (Vasilakopoulos et al., 2011). Examples have shown that if young fish are allowed to contribute to recruitment, stocks may remain in good condition, despite being exploited at high rates. The Celtic sea whiting stocks is an example of this process, with an intrinsic part of this management the identification of what age/length the stock is immature at and management plans that encourage the survival of young fish such as larger mesh sizes in nets which allow the smaller fish to escape.

Limanda limanda experience a protracted spawning period from January to September throughout its distribution (Rijnsdorp et al., 1992). Each spawning ground has its own short period of peak productivity (Henderson, 1998). In the southern North Sea and the Irish Sea, peak spawning occurs from February to April (Beggs and Nash, 2007; Henderson, 1998; Bolle et al., 1994).

Female *L. limanda* enter the spawning grounds first, with older fish arriving before the younger fish (Bohl, 1957). Htun-Han (1978b) identified four distinct periods during the reproductive cycle for the dab (Table 1.3). Gonadosomatic index (**GSI**) calculations support the identification of peak spawning activity, with the annual GSI curve showing a pronounced peak in the spawning season (Htun-Han, 1978a).

Table 1.3: The four periods of reproductive activity proposed by (Htun-Han, 1978b) in respect of gonad weight, changes in liver weight and changes in condition factor.

Periods	Females	Males
Pre-spawning period	Oct-Jan	Sept-Nov
Spawning period	Feb-April	Dec-March
Post-spawning period	May-June	April-May
Resting period	July-Sept	June-August

Dab mature from minimum lengths of 10-14cm upwards (Htun-Han, 1978b) and are batch spawners, with the two ovaries maturing at different rates (Henderson, 1998; Htun-Han, 1978a). Larger, older individuals commence reproduction first (Ortega-Salas, 1987; Htun-Han, 1978b,a,c). Htun-Han (1978a) split oogenesis into six distinct stages based on easily recognizable anatomical structures and differences in size of oocytes.

Unusually, in female dab the ventral ovary is frequently heavier than the dorsal ovary, with assumed increased fecundity in the ventral ovary during spawning (Htun-Han, 1978b). Spawning is a rapid process, and lasts no longer than 6 weeks (Htun-Han, 1978a). Fecundity is size-dependent in female dab, with older females of the same size having more eggs than younger females

(Ortega-Salas, 1987).

The largest males are ready to spawn almost one month earlier than the younger fish (Htun-Han, 1978b). In the male *Limanda limanda* spermatogenesis comprises five distinct phases based on distinct anatomical structures identified through the process of histology Htun-Han (1978c). Spermiation in male *L. limanda* is often complete by December, although this is size-dependent (Htun-Han, 1978c,b).

Despite sustained fishing effort being applied to the dab fishery in the form of discarding, the fishes continued abundance has been attributed to its ability to reproduce before it becomes vulnerable to the demersal fishery (Pope et al., 2000). In Ireland, the current minimum landing size is set at 15cm (Power, 1980), although there are little commercial landings of this species.

Fecundity for *Limanda limanda* has been estimated to range from 64,000 to 943,000 dependent on the size of the spawning female (Jastania, 1995). *Limanda limanda* eggs are pelagic and are between 0.66-1.2mm in diameter (Nissling et al., 2002; Russell, 1976). The pelagic eggs hatch after 4.1 to 13.6 days, based on local environmental temperatures (Henderson, 1998; Bohl, 1957). The eggs are passively dispersed by local hydrodynamic processes (Henderson, 1998; Rijnsdorp et al., 1992; Bohl, 1957). Settling post-larvae become benthic at lengths of 12-20mm (Russell, 1976; Jónsson, 1966). Larval dab arrive in coastal and estuarine waters during the summer months in depths ranging from 3-10 meters, a depth deeper than larval plaice (Gibson et al., 2002). Maximum population densities are reported in depths of 3-5 meters (Bolle et al., 1994).

Juvenile dab (< 9cm) are primarily located in coastal waters in depths less than 10 meters, although they are not restricted to this location and have been caught at offshore stations (Rijnsdorp et al., 1992). Mean length of dab peaks at 6-7cm by the end of the first year (Bolle et al., 1994). Dab move offshore with increasing age (Bolle et al., 2001). Henderson (1998) suggests that at approximately 2 years, the juvenile dab become incorporated into the adult population.

1.7 The role of dab in the marine ecosystem

Limanda limanda play a crucial role in the marine food web, where they are considered prey items for numerous predators including seals (Hall et al., 1998), sharks, gurnards (Ellis and Gibson, 1995) and important demersal

species of fish such as cod and haddock (Ellis and Gibson, 1997). While dab plays an important prey item to a number of fish species, it is itself a broad spectrum opportunistic feeder (Besyst et al., 1999; Kaiser et al., 1997) and has been known to include heering eggs and sandeels in its diet (Høines and Bergstad, 2002). However, major prey items include molluscs, crustaceans and polychaetes.

While flatfish species can occur in similar niches, they usually differ in the composition of their diet. This is not the case for larval plaice and dab, whose settlement has been reported to occur simultaneously in some areas (Bolle et al., 1994). Studies have shown that resources are not limited in these situations, and this is not likely to affect recruitment variability (De Raedemaeker et al., 2011).

1.8 Aim and Objectives

Information concerning the biology and ecology of dab *Limanda limanda* is lacking, especially from Ireland's coastal systems. Further, studies concerning the biology of dab is sparse. The information necessary to construct reproductive cycles or investigate age and growth of dab were last viewed in the late 1970's (Htun-Han, 1978b; Jónsson, 1966). In recent years, minimal effort has been directed towards evaluating the continued applicability of these early estimates of biological parameters. It was the goal of this study to provide information on the life history of the adult dab from Ireland's coastal waters, and to question the validity of certain assumptions regarding ageing methodology, and the static assumption of a similar maturity-at-length for all dab populations in different ecosystems. Data on adult dab populations was collected and analysed from Irish coastal waters in order to do the following

- Describe the reproductive behaviour of the dab *Limanda limanda* from the coast of Ireland, concentrating on the development of a macroscopic and microscopic guide for gonad phase identification, the age and size at sexual transition and the identification of the reproductive cycle.
- Describe the age distribution of this fish within Ireland's coastline, while evaluating the level of precision and bias when using whole otoliths in dab for age determination.
- Describe the growth of this species, with a focus on the development of an Age-Length-Key (ALK). Levels of precision between using whole otoliths for age determination and sectioned otoliths will be assessed to identify the more accurate method. Specific focus will be given to the identification of the most robust growth model to use for evaluation of growth of dab. This will be achieved through the use of four separate growth models for both sexes, with robustness assessed through Akaike criterion, and Multi-Model-Inference.

This information is vital as it forms an important component of fisheries assessments. This information allows the determination of the reproductive potential of a fish population and identifies whether the fish in question can sustain a viable fishery. This volume of work provides information concerning a small unvalued flatfish species whose role in the ecosystem of Irish waters is poorly understood, but nonetheless important.

Chapter 2

The reproductive biology of
Limanda limanda as assessed
macroscopically and
microscopically

Abstract

The current research analyses reproductive data for the dab (*Limanda limanda*), collected on a bi-monthly basis during the period of January 2009 to June 2010. Standardized macroscopic maturity ascription criteria was employed with descriptions of macroscopic phases of dab were verified via histological analysis of gonad slides. In comparisons of macroscopic and microscopic phases, there was high agreement in the proposed female guide (86%), with males demonstratively lower (62%). When the male macroscopic guide was examined, misclassification was high in phase 5 and phase 5 (41%), with 96% of misclassification occurring in adjacent phases. Hoenig's test of symmetry identified no significant bias between the two phase descriptions (macroscopic and microscopic), with corresponding coefficient of variation of 6% and 14% for both female and male dab.

Changes in Hepatosomatic index (HSI) and gonadosomatic index (GSI) followed a similar pattern during reproductive development, whereby gametogenesis initiates in the period of July/August and ends in May/June. Similarly, oocyte diameter measurements indicated that oocyte size peaked in January/February. While male dab may have reached peak GSI percentages prior to female dab, this is not an unusual occurrence and has been previously reported in the North Sea. Maturity ogives obtained with microscopic and macroscopic criteria identified 50% of the population to be mature at lengths of 14cm and 17cm for male and female dab, respectively. The smallest dab identified as mature occurred at a length of 12cm and 14cm for male and female dab respectively. The implications for the current reproductive status of dab throughout its distribution are discussed.

2.1 Introduction

Maturity estimates are an important component of fisheries assessments, allowing the determination of the reproductive potential of fish populations Williams (2007). These estimates allow for the monitoring of any changes in

biological and reproductive characteristics as a response to excessive exploitation. Many fish populations occupy species-specific temperature niches, where growth, condition and reproductive potential of the fish relies on this singular factor (Thorsen et al., 2010). The reproductive strategy that a fish employs is widely known to influence the potential fecundity, female size, ovary size, oocyte size and the quality and quantity of the eggs released (Thorsen et al., 2010; Lambert, 2008; West, 1990).

Microscopic examination of histological gonadal sections is recognised as the most accurate method of determining reproductive status of fish, and subsequently spawning strategy (synchronous, group synchronous, asynchronous)(Hunter et al., 1985). However, due to monetary constraints in most institutions, anatomical grading systems are more widely utilized. Grading systems, when used in conjunction with microscopic examination to routinely test the assigned phase, have been shown to provide an acceptable level of accuracy (Costa, 2009; Williams, 2007; Tomkiewicz et al., 2003).

Flatfish are considered one of the most valued species in terms of ecological and economical importance (Link et al., 2002). The dab *Limanda limanda* is considered one of the most common and widespread of the flatfish species. It is ubiquitous in the north eastern Atlantic (Henderson, 1998; Jónsson, 1966), with its European prevalence facilitating its use as a biological monitoring species (Freeman et al., 2009; Kammann et al., 2008; Feist et al., 2004). In spite of its use as a monitoring species, and the large reservoir of documented studies of its biological response to contaminants, little is known about the most basic of biological information, with no published information available on Ireland's population.

Studies containing biologically relevant information are restricted to research in the North Sea, Baltic Sea and Iceland (Nissling et al., 2002; Henderson, 1998; Jónsson, 1966). The studies identify *L. limanda* as a rapidly growing fish with early maturation (males mature at approximately 2 years while females mature at 3-4 years) (Beggs and Nash, 2007; Nissling et al., 2002; Folmer, 2000; Henderson, 1998; Kaiser et al., 1997; Rijnsdorp et al., 1992; Htun-Han, 1978a,b,c; Lee, 1972; Jónsson, 1966). Htun-Han (1978a,c) provides the earliest study of *L. limanda* reproductive biology, and identified it as a asynchronous indeterminate spawner. Spawning periods vary, with *L. limanda* population's located in the North Sea having distinct peaks of spawning activity (Henderson, 1998).

Recently, there has been renewed scientific interest in *L. limanda* due to its

recognition as a species with the potential to support a viable fisheries (ICES, 2006). Public interest in alternative edible fish, as opposed to heavily exploited fisheries, is increasing with dab considered a sustainable alternative (Marine Conservation Society, 2012). This increased interest has resulted in several workshops being set up through ICES on age (ICES Advisory Committee, 2010b) and reproductive biology of *L.limanda* (ICES, 2010b). In order to ensure effective management of any fish stock, the reproductive potential must be assessed, especially in multiple spawning fish, where there may be differences in egg viability between the first and second spawning (Trippel, 1999). The accurate assessment of maturity-at-length ensures that stock reproductive potential may be assessed, and in doing so ensure the most accurate measure of the reproductive potential.

This study aimed to describe the reproductive biology of the dab *Limanda limanda* captured of the north-west and south coast of Ireland. The first objective was to establish a macroscopic guide verified through histological sections of the gonads for both sexes. The second objective was to determine the spawning season of dab using a combination of condition indices. The final objective was to establish the length at which 50% of the population is mature for male and female dab.

2.2 Materials and methods

A total of 461 fish were collected in January and September of 2009 during demersal fishing surveys conducted aboard the "RV Celtic Voyager" and "RV Celtic Explorer" utilizing GOV nets in commercially important fishing areas off Ireland's coastline. Additionally, 2071 fish were sampled from commercial fishing vessels, from March 2009 to June 2010 on a monthly basis in two sampling localities, comprising the area off the north west coast of Ireland (51°44'N , and 8°11'W), and the second, off the south coast of Ireland (55°22'North and 8°15'W) (See Appendix, Figure 6.3).

All fish were measured to the nearest cm and weighed to the nearest gramme. The sex was determined and the fish gonads were macroscopically classified initially according to the written criteria of Htun-Han (1978a,c). Twenty five random samples of gonads and liver per trawl (sexes combined) were removed and weighed (g) and subsequently preserved in 4% formalin for histological analysis, and verification of macroscopic guide (Table 2.3). An overview of catch statistics for the duration of the study can be seen in Table 2.1. In

order to standardize the descriptive criteria in fish reproductive studies, the gonadal phase has replaced the common gonadal stage (Brown-Peterson et al., 2011; Murua et al., 2003).

Year	Month	Male	Female	Total
2009	February	181	145	326
2009	March	0	0	0
2009	April	96	156	252
2009	May	32	65	97
2009	June	76	108	184
2009	July	34	41	75
2009	August	52	63	115
2009	September	46	89	135
2009	October	0	0	0
2009	November	12	82	94
2009	December	111	241	352
2010	January	0	0	0
2010	February	30	91	121
2010	March	44	133	177
2010	April	55	157	212
2010	May	86	134	220
2010	June	75	97	172
Total		930	1602	2532

Table 2.1: Summary of sampled dab during the study period of February 2009 to June 2010. Note the pronounced differences in catch throughout the year of both male and female dab.

2.2.1 Histology

During the study period, a minimum of ten gonad samples of each maturity phase was taken for histological analysis. It was not possible to obtain this quantity in all maturity phases due to restrictions in time and locations during surveys. However, the phases collected are representative of phases routinely sampled during biological surveys. All samples were processed in the Analytical laboratory of the Marine and Freshwater Research Centre, Galway Mayo Institute of Technology. In total, 380 wax histological slides were created (Table 2.2). Samples were processed using an RVG1 automated tissue processor following a standardised protocol (See Appendix, sub-section 6.1). Tissues were subsequently embedded using a Histocentre2, and sectioned on a Slee Mainz CUT6062 at $4\mu m$, before staining. Standardized histological processing and staining (haematoxylin and eosin) were used following the protocol of Feist et al. (2004) with slight modifications (see Appendix, subsection 6.1).

Microscopic slides of gonadal sections were viewed on a PC using an Olympus SZX10 stage and microscope with a Retiga 2000R camera attachment. Gonadal sections were edited using imaging software Cell*, and subsequently classified by identifying the most advanced oocyte stage present. The microscopic slides were examined in conjunction with the macroscopic criteria in order to validate the macroscopic guide. Whole oocyte diameter was measured for all female gonadal sections using the computer software programme ImageJ (Thorsen and Kjesbu, 2001), where oocyte size was used as a proxy indicator for spawning time (Thorsen et al., 2010; West, 1990). Oocyte diameter was plotted against month in order to determine peaks in oocyte production.

Phase	Female	Male	Total
1	28	12	40
2	54	40	94
3	29	26	55
4	13	4	17
5	18	17	35
6	104	35	139
Total	246	134	380

Table 2.2: Sample sizes of dab by macroscopic/microscopic maturity phases during the study period of February 2009 to June 2010 off the Irish coastline. Samples for histological analysis were chosen at random .

In order to verify macroscopic guide, a bias plot of phase numbers (Campana

et al., 1995) were produced in order to visualize the deviation of the macroscopic phases from the microscopic phases according to a 1:1 equivalence line. The bias plots allowed the detection of non-linear bias, e.g. the relative underestimation of one methodology in one part of the phase range, and relative overestimation in another part of the phase range by the same method.

Although, average percent error (APE), was originally used to investigate the average percent error in age determinations (Beamish and McFarlane, 1983), a modified form was used to evaluate the percent error between the macroscopic phase and microscopic phases. The equation was modified as follows:

$$APE_j(\%) = 100 \times \frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} - X_j|}{X_j}$$

where R is the number of times each fish is assigned a certain phase, X_{ij} the i(th) phase determination of the j(th) fish, and X_j is the mean phase calculated for the j(th) fish. The coefficient of variation, which is expressed as the ratio of the standard deviation over the mean phase of the fish, was evaluated using the formula by Chang (1982),

$$CV_j(\%) = 100 \times \frac{\sqrt{\sum_{i=1}^R \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

where CV_j is the phase precision estimate of the jth fish.

In addition to these indices, the correlation coefficient of r^2 was calculated to evaluate the fraction of variation explained by the linear relationship between the two methods. Results from the present study were pooled to allow for an overall estimate of precision using the current methodologies. Based on the lack of acceptable levels of precision for reproductive methodology comparisons, we use precision levels for age comparisons as a baseline (Campana, 2001) ($CV = 8\%$, $APE = 6\%$). Hoenig's test (Hoenig et al., 1995) of symmetry (also referred to as Bowker's test) was used to indicate any systematic differences between the phase descriptions/methodologies.

2.2.2 Condition indices

In order to link resource allocations to the reproductive process and identify both the reproductive period and when the diversion of energy from somatic reserves occurs, Le Cren's relative condition (Le Cren, 1951) was calculated

on a bi-monthly basis for both sexes of dab. The equation was expressed as:

$$\text{RelativeCondition}(k_n) = \frac{W}{W^1}$$

where W represents the observed weight of the fish (g), and W^1 represents the standard weight for fish of the same length. Relative condition was calculated for each fish, and then an average ($\pm SD$) obtained per month .

Average Gonadosomatic index (GSI) ($\pm SD$) was estimated using the mean of the GSI values per month, for both male and female fish. GSI was estimated using the following formula

$$\text{GonadosomaticIndex(GSI)} = 100 \times \frac{GW}{TW}$$

where GW is equal to the weight of the dissected gonad measured in grammes(g) and TW is equal to the total weight of the fish minus all viscera and minus gonads(g).

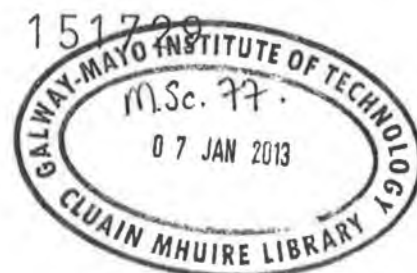
In fish, nutrients are stored in the liver, and are usually accumulated during the summer months, when prey is plenty. Unlike other flatfish, lipid reserves are not stored in the muscle of dab (Saborowski and Buchholz, 1996) and so the liver weight was chosen to represent lipid reserves. The energetic reserves of the fish can be expressed using the hepatosomatic index which relates liver weight to body weight using the following formula:

$$\text{HepatosomaticIndex(HSI)} = \frac{LW}{TW} \times 100$$

where LW is proportional to the weight of the dissected liver (g) and TW is equal to the total weight of the fish minus the viscera (g) and minus the gonads (g)

The modified six phase microscopic guide (Figure 2.4) was used as the basis for determining the length at which 50% of the sampled population was mature (L_{50}), by assigning phase 2 (Developing) to phase 6 (Regenerating) as mature and phase 1 (Immature) as immature. Periods of spawning were excluded as per Murua et al. (2003), to insure sufficient incorporation of immature/mature fish in the maturity estimate. The length at which 50% of the population was mature (L_{50}) was estimated separately for both male and female dab using the following a logistic regression

$$P_i = \frac{L}{L + e^{\alpha + \beta E}}$$



and then log transformed using the following formula

$$h \times \frac{P_i}{L - P_i} = \alpha + \beta E$$

where P_i equals the proportion mature at length (L) and α and β are the fitted parameters determined by using a general linear model. Fifty percent maturity was estimated for both sexes as the negative ratio of the parameters $-\alpha/\beta$.

2.2.3 Data Analysis

All data was tested for assumptions of normality and homogeneous variance (Levene's test) and analysed using r (R Development Core Team, 2011) and Excel 2007. Data is presented as mean values \pm standard deviation, with L_{50} maturity presented as mean values \pm standard error. L_{50} is defined as the length at which 50% of the dab population is mature. Rejection of normality assumptions resulted in the transformation of the data using the natural logarithm(Ln). However, when the data still did not conform to assumptions of normality, non-parametric statistics were used. The Mann-Whitney non-parametric statistics were used to test for differences between GSI, HSI and K and oocyte diameter between the two sampling areas. When no significant difference was observed, data was pooled for analysis.

2.3 Results

2.3.1 Gonadal Development

The timing of sexual maturation in Irish dab *Limanda limanda* of the north-west and south coast of Ireland is shown in Figure 2.1, for both male and female fish respectively. Gonadal activity commences around the period of July/August. By November/December, 35% of female gonads had progressed to phase 3 (Spawning Capable), while 71% of male gonads had attained this phase. Peak numbers of spawning capable gonads (Phase 3) were recorded in the period of November/December for males (Figure 2.1a)(71%), and in January/February for female dab (Figure 2.1a) (21%). Based on the proportion of running females (Phase 4) and increased oocyte diameter, spawning commenced in January/February, peaks in March/April and ends in May/June. By May/June, the majority of male and female dab are spent (Phase 6-Regenerating), 77% and 91% respectively.

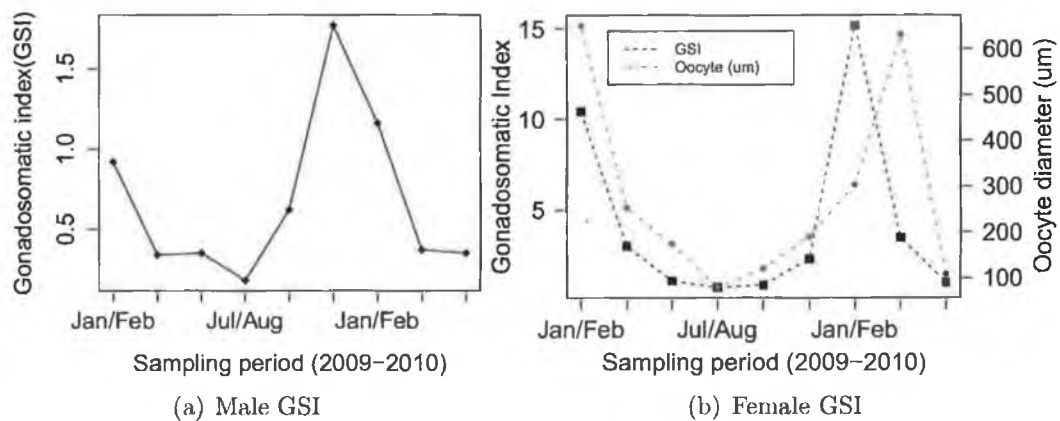


Figure 2.1: Indices are presented on a bi-monthly basis with (a) representing the increase in gonadosomatic percentage for male *L. limanda* throughout the sampling period of May/Jun 2009 to May/Jun 2010. No male samples were collected during the Jan/Feb and Mar/Apr period of 2009 (b) Represents changes in gonad condition index for females (GSI) with oocyte diameter also plotted on the graph.

2.3.2 Histological verification of macroscopic phase description

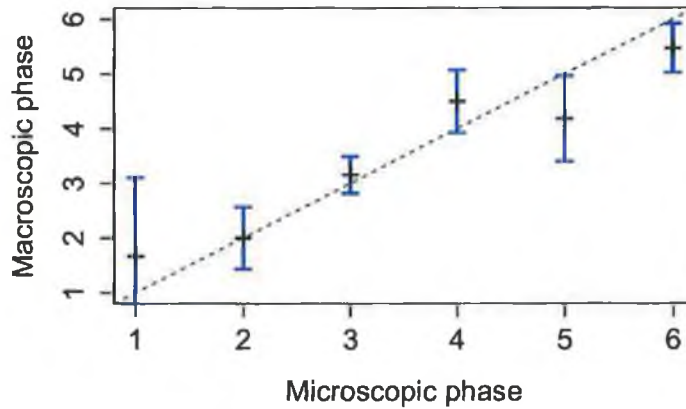
The distribution of oocytes stages in each phase observed in *L.limanda* typifies asynchronous indeterminate batch spawning. This is based on the presence of several batches of oocytes in various stages of development throughout the reproductive period, and the identification of post-ovulatory follicles (POF's) in phase 4 (Spawning) and phase 5 (Regressing) of female dab (Figure 2.3).

Based on the examination of 380 fish (Table 2.2), which ranged in size from 10-26cm for males and 12-35cm for females, the following six phase concise gonadal guide was determined (Table 2.3). The macroscopic guide was validated using histological slides of male and female *L.limanda* gonads (Figure 2.3) and microscopic phase descriptions (Table 2.4). Histological verification of the concise macroscopic guide resulted in an agreement of 86% for female *L.limanda*, while an agreement of 62% was determined for male fish. Phase 1 (Immature) and phase 2 (Developing) in male *L.limanda* were identified as being the most accurate (98-100%). In females, the most accurate phase identification corresponded to phase 1 (Immature) and phase 6 (Regenerating), 95% and 98% respectively. Histological examination of the gonads resulted in an overall correct classification rate (based on percent agreement between phases) of 62% and 86%, for male and female fish respectively. When misclassification of reproductive phase did occur (27%), observations were mostly reclassified into an adjacent phase (96%).

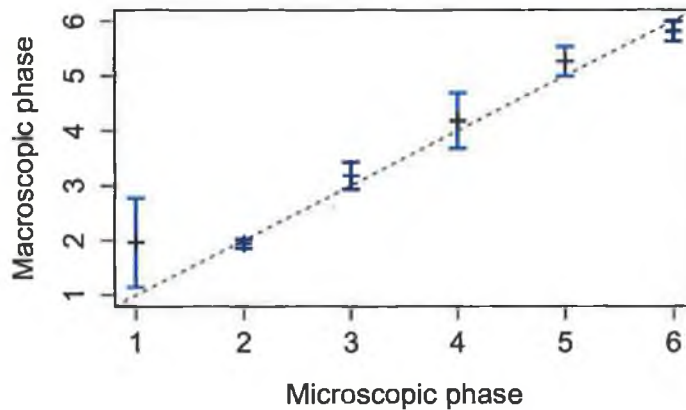
Hoenig's test of symmetry indicated no systematic differences in the assignment of phases between the two reproductive methodologies for either male ($p = 0.10$) or female dab ($p = 0.17$). Examination of the bias plots of both male and female reproductive phase assignment indicated non linear bias occurring in phase 1 and phase 5 of male dab comparisons (Figure 2.2(a)), and phase 1 in female dab (Figure 2.2(b)).

Based on the assumption that an acceptable level for precision of macroscopic guides is similar to those of age estimates, the female macroscopic guide presented in this study has a good agreement with the microscopic verified phases ($CV = 5.96\%$, $APE = 4.21\%$) ((Campana, 2001), $CV = 8\%$, $APE = 6\%$). The male macroscopic guide was apparently not as precise ($CV = 14.71$, $APE = 10.41$). The transitional phase of regressing (Phase 5), was proportionally the most commonly misclassified phase (41%), apparently sharing macroscopic appearance with phase 4 (spawning) in male dab. The incorrect assignment of phase 4 to a higher phase in male dab may be influ-

enced by the smaller sample collection of male gonads ($n=163$) in comparison to female gonads ($n=269$), and the small number of microscopically verified actively spawning (phase 4) males that were examined ($n=6$). Due to the importance of these phases in spawning boundary, the written description of macroscopic phase 5 in males appears to be inadequate to resolve it from similarity appearing phases (Figure 2.3).



(a) Male reproductive phase comparison ($n=163$)



(b) Female reproductive phase comparison ($n=269$)

Figure 2.2: Phase based bias plot of reproductive-phases from macroscopic examination and microscopic examination of male and female dab gonads. ($n=432$ gonads examined)

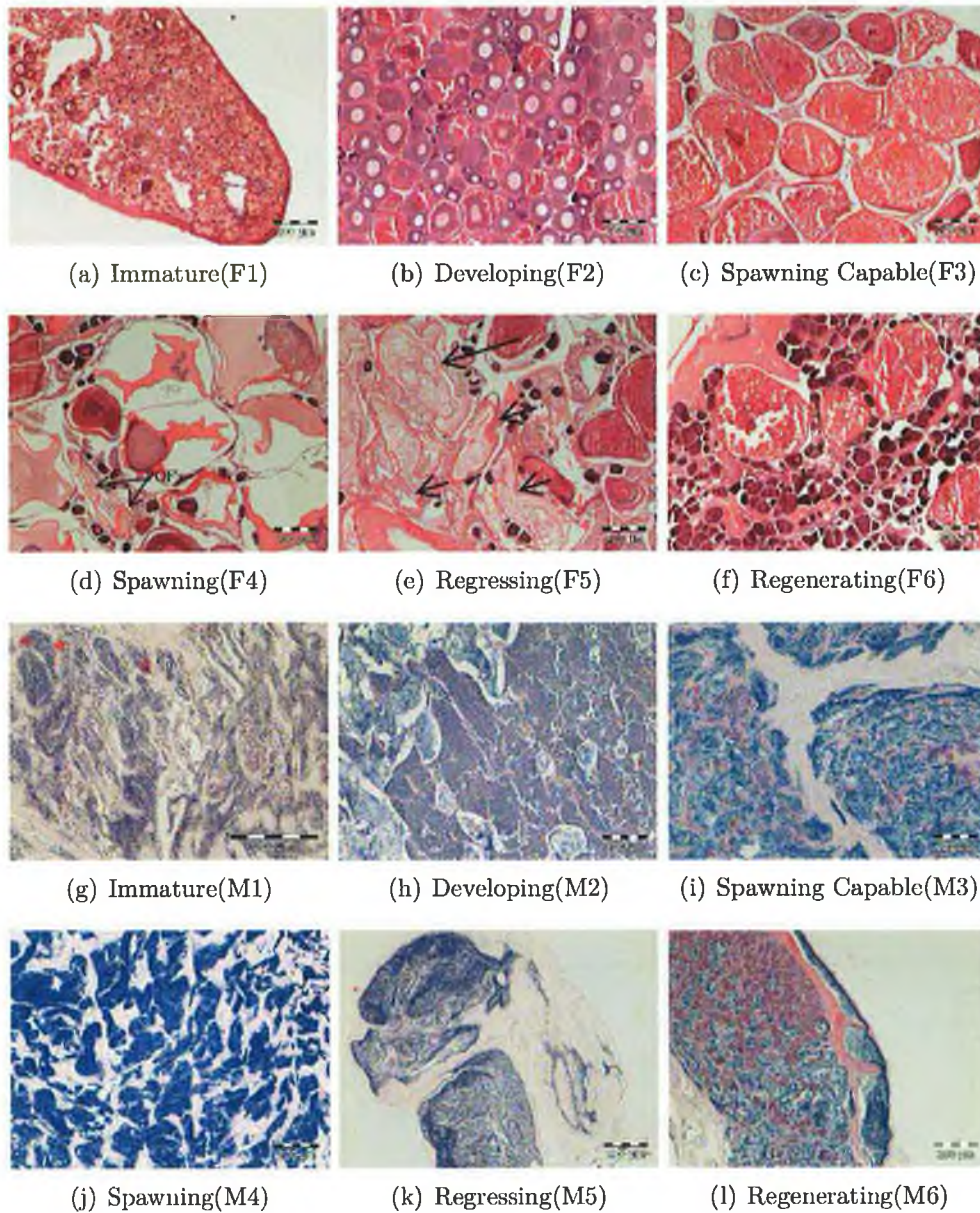


Figure 2.3: Photomicrographs of ovarian ($F1 - F6$) and testicular ($M1 - M6$) histology, corroborating the reproductive phases assigned to the concise macroscopic guide. Arrows on image (d)Spawning($F4$) and (e)Regressing($F5$) represent post ovulatory follicles (POF's). Scale bar is set at $200\mu m$. Descriptive criteria of the microscopic phases can be seen in Table.1.

Table 2.3: Concise guide of maturity phases for male and female *Limanda limanda* using macroscopic gonadal visualisation. The guide was validated using histological examination, with standardized reproductive terminology. Each square represents 1cm^2 . The images are orientated so that the gonad on the left is from the anterior of the fish while the gonad on the right represents the posterior.


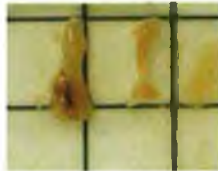
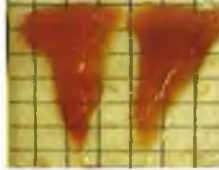




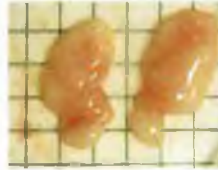
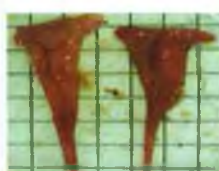



Terminology	Female	Male
<p>Phase 1-Immature Ovaries are small barely protruding out of the musculature, pale pink with no evidence of blood vessels on the dorsal and ventral ovary (F1). Testes are very small and translucent, located close to the cavity wall(M1).</p>		
<p>Phase 2- Developing Ovaries are small and easily distinguishable from other organs. Usually less than one quarter of the body length of the fish appearing flaccid and gently rounded in appearance (F2). Testes are beginning to enlarge, and are now noticeable in the body cavity. Light grey to white in colour (M2).</p>		
<p>Phase 3: Spawning Capable Ovaries are large, protruding into the musculature of the fish, occupying over half of the dab's body length. The gonads keep form when examine don a hard surface. The ovary is faint pink to dark orange in colouration with tiny oocytes visible towards the end of the cycle(F3). Testes filling and occupy three quarters of the body cavity of the fish. No milt is released on gentle pressure, although milt is visible when testes cut. White to pale pink in colouration (M3).</p>		
<p>Phase 4: Spawning Ovaries are large, occupying three quarters of the total length of the fish, with thin ovary walls and pronounced blood vessels. The ovaries are distended, soft and granulated in appearance with hydrated oocytes extruded upon light pressure (F4). Testes enlarged and run on light pressure (M4).</p>		
<p>Phase 5: Regressing Ovaries are pink to dark red in colouration with small opaque oocytes visible on close inspection. Reduced in size, with a flaccid appearance, and prominent blood vessels present on the dorsal portion of the ovary (F5). Testes shrinking with lobules becoming smaller. Sperm may still be seen in testes when cut, however will not be extruded upon gentle pressure. Testes commonly appear a light creamy grey in this phase. (M5).</p>		
<p>Phase 6: Regenerating Ovaries are small (occupying less than one quarter of the body length), pale organs usually glassy transparent to pink in colouration with red ovaries seen in larger specimens. Presence of previous blood vessels is visible in the ovarian wall, which is thickened and tough to the touch (F6). Testes thick and flabby often found close to the cavity wall. Testis lobule is pronounced in comparison to spawning capable phase however reduced. Pale cream in colour with no sperm visible when testes cut (M6).</p>		

Table 2.4: Concise descriptions of microscopic criteria for the determination of reproductive activity in male and female *L.limanda*.

Maturity Phase	Female Microscopic description	Male Microscopic description
Phase 1: Immature	Only primary oogonia present, including chromatin nucleolar and pernucleolar oocytes usually in finger like folds thickly packed. Cortical nucleolar diameter ranges from 21–134 μ m, with a centrally located nucleus which is strongly basophilic with H&E stain (<i>Fig.1a</i>)	The testes contain a small amount of cysts and are compromised of mostly immature spermatogonia and steroli cells (<i>Fig.1g</i>)
Phase 2: Developing	Oocytes in the following stages can be present in well organised folds: chromatin nucleolar, cortical alveoli, and early vitellogenic cells (no migrating nucleus). Oocytes are characterised by strong esinophilic germinal vesicle and a strongly basophilic cytoplasm when stained with H&E. Size ranges from 71–299 μ m. (<i>Fig.1b</i>)	Spermatocysts are present containing both primary and secondary spermatocytes. Spermatids can also be present. The germinal epithelium is continuous throughout the testes (<i>Fig.1h</i>)
Phase 3: Spawning Capable	Vitellogenic oocytes in all stages may be present however mid (migrating nucleus) and late (lipid rich globules) vitellogenic oocytes are prevalent. The ovary is usually interspersed with primary oocytes including both cortical alveoli although in lesser quantity than the previous stage. Vitellogenic oocytes in various stages range from 184–503 μ m (s.d. = 70.99) (<i>Fig.1c</i>)	Spermatogonia, spermatocytes, spermatids and spermatozoa can be present. Germinal epithelium can be continuous or discontinuous, dependent on the status of the cysts. As advanced cysts begin to show spermatozoa, the connecting cyst walls can be seen to break down allowing the release of spermatozoa into the lumen of the lumen. (<i>Fig.1i</i>)
Phase 4: Actively spawning	Spawning is known to have occurred by either germinal vesicle movement (GVM) or hydrated oocytes/germinal vesicle breakdown (GVBD). Less developed oocytes are common. Post ovulatory follicles (POFs) are present as an indication of recent spawning. Maturing oocytes range in size from 505–743 μ m (s.d. = 70.99) (<i>Fig.1d</i>)	This phase is very difficult to identify microscopically since histologically it is very similar to spawning capable with minute differences visible in the germinal epithelium (discontinuous in this phase). It is more accurately identified in studies based on macroscopic examination of "running" milt. (<i>Fig.1-j</i>)
Phase 5: Regressing	Alpha and beta atresia can be present with vitellogenic oocytes starting to be reabsorbed. Less developed oocytes such as cortical nucleolar or cortical alveoli can be common. (<i>Fig.1e</i>)	Residual spermatozoa present in lumen and sperm ducts with regeneration of germinal epithelium common. Cysts containing spermatids may also be present. (<i>Fig.1k</i>)
Phase 6: Regenerating	Primary growth oocytes including chromatin nucleolar and cortical alveoli are abundant. Muscle bundles are evident with enlarged blood vessels and distinct spaces present between cells. Vitellogenic oocytes undergoing atresia may also be present, in small quantities than regressing phase. (<i>Fig.1f</i>).	Residual spermatozoa may be present in the lumen, while regeneration of germinal epithelium is common on the periphery of the testes. Spermatids and spermatocytes (primary and secondary) are common with cysts containing residual spermatids sometimes present. (<i>Fig.1l</i>)

2.3.3 Maturity ogives

Microscopically validated maturity phases were used for the identification of the length at which 50% of the population attained maturity for both male and female *L. limanda*. The estimated length at which 50% of the population was mature corresponds to 14cm for male dab, with 100% of the population mature at an approximate length of 23cm. In contrast, 50% of the female dab population was mature at 16cm, with 100% of the sample mature at a length of 24cm. The smallest *L. limanda* identified as mature had a length of 12cm and 14cm, for male and female fish respectively.

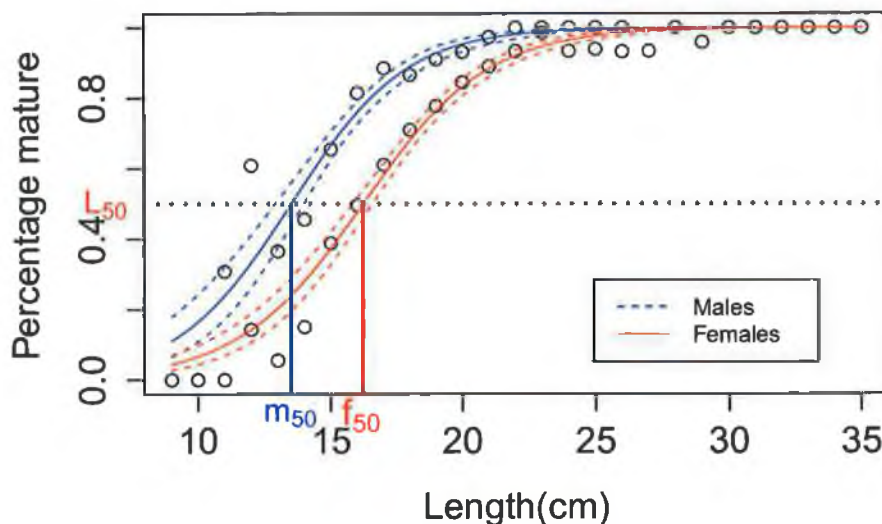


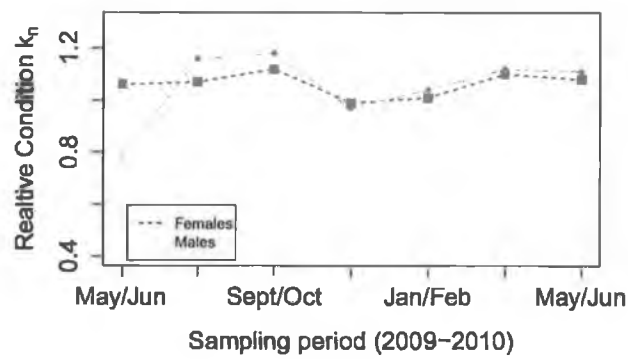
Figure 2.4: Maturity ogive of proportion mature at length of male and female dab derived from sampling off Ireland's coastline from January 2009 to June 2010. Periods of spawning were excluded to insure sufficient incorporation of immature/juvenile fish in the maturity estimate. The black dotted line represents the point at which 50% of the sampled population is mature. The blue line which drops from this point represents the male proportion mature (M_{50}), with a representative length of 13.5cm. Similarly, the female proportion mature is represented by the red line (f_{50}), with a representative length of 16.23cm.

2.3.4 Condition Indices

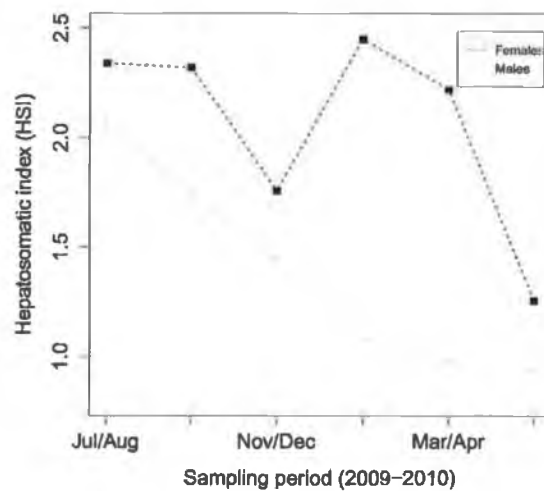
The average seasonal fluctuation in condition factor of dab is shown in Figure 2.5a. The relative condition (k_n) of the female and male dab followed an annual cycle, peaking prior to the initiation of reproductive activity as reproductive investment built up to a maximum level and then began to increase during spawning activity reflecting bias introduced through the increased size of the gonads. There was no marked difference in k_n between male and female dab, with values varying between 0.8 and 1.2%. Size ranges sampled during the study were 9-31cm and 9-35cm, for male and female dab respectively.

Hepatosomatic index (HSI)(Figure 2.5a) begins to decrease during the spawning period in both sexes of dab, reaching its lowest point in the post-spawning period, characterised by an abundance of Phase 6 fish in the period of May/June. Post-spawning (May/June) coincides with the time of the year when the fish are feeding heavily, with the storage of reserves in the liver of female dab clearly shown by the peak in hepatosomatic index percentage prior to the initiation of reproductive activity. Statistically, there is a difference between male and female HSI ($p=0.04$) and over the year ($p<0.05$), even though the difference between HSI percentage between the sexes is less than 1.5% (male=1% HSI, female=2.5%).

Female *L.limanda* experience a slight drop in liver condition upon the initiation of reproductive activity (November/December), with a substantial increase noted upon entry into the period of January-February where phase 3(Spawning Capable) are the most predominant reproductive phase (Figure 2.5b). The identification of periods of peak spawning and resting activity identified through calculations of the relative condition (k_n) and hepatosomatic index (HSI) were in complete agreement with estimates of these periods by the gonadosomatic index (GSI) (Figure 2.1).



(a) Relative Condition



(b) Hepatosomatic Index

Figure 2.5: Condition Indices are presented on a bi-monthly basis with (a) representing the variation in relative condition (k_n) for both sexes throughout a sampling year and (b) representing changes in hepatosomatic indice (HSI) for both male and female *L. limanda*.

2.4 Discussion

2.4.1 Macroscopic and microscopic criterion

Reports have suggested that experience in the macroscopic assignment of reproductive phases in any single flatfish species is sufficient to characterize gonadal phases in other flatfish species ICES (2010b), irrespective of the vast number of phase combinations that exist for one species. Phase descriptions in flatfish reproductive studies can range from 4 phases to ten phases dependent on the researcher/location (ICES, 2010b). By not establishing a standardized species-specific guide, you reduce the comparability of reproductive studies of a species within its range. The current study produced a standardized species-specific macroscopic guide (Table 2.3) which was verified via histological analysis of gonad sections.

Generally it was observed that for dab, it is possible to distinguish immature and resting male and female fish by the macroscopic guide presented in this study (Figure 2.2). Percent agreement between the macroscopic guide and microscopic gonadal slides for female dab were considered precise (%agr = 88%, CV=5.96 and APE= 4.21), when using as a baseline precision levels for age comparisons (Campana, 2001). In comparison, the criterion used to describe male reproductive phase was inadequate to resolve macroscopic phases from similarly appearing phases (%agr = 62%, CV=14.71 and APE= 10.4). Errors in maturity data can have a multitude of sources, such as incomplete or inaccurate phase description or experience level of reader. In the application of histological descriptors to macroscopic phases, the most advanced gonadal stage present is the primary indicator for histological staging (Williams, 2007). Macroscopic staging primarily relies on gonad characteristics (size, shape, colour and presence of "running") which are observable with the naked eye. Classification errors primarily involve boundary stages, with errors commonly occurring across the post spawning boundary during macroscopic staging (Ferreri et al., 2009; Tomkiewicz et al., 2003). Histologically verified macroscopic phase misclassification display a generally expected pattern of misclassifying adjacent phases (96%), potentially suggesting that at least some of the observed samples may have been in transition between phases, exhibiting characteristics of both adjacent phases. This has previously been noted by Williams (2007) for pollock *Theragra chalcogramma* and the european anchovy *Engraulis encrasicolus*(Ferreri et al., 2009). In some cases, macroscopic examination does not reveal all the characteristics of the maturity stages which are

apparent in histological sections. This is the case with phases 3 and 4 in male dab, where the primary separating character is a discontinuous germinal epithelium (Table 2.4), and phase 5, where the primary separating characteristic is the regeneration of the germinal epithelium. This similarity occurred due to the continued and rapid development of spawning capable fish (Phase 3) to actively spawning (Phase 4) in indeterminate multi-spawning fish. Despite the presence of misclassification in these phases, all the phases designate mature male dab making their misclassification of low overall importance in the assessment of reproductive maturity. It is recommended that phase 3 and phase 4 be collapsed into one phase in order to increase agreement between criteria, and reduce misclassification, where phase 3 will be considered the dominant phase, with phase 4 a sub-phase. This recommendation is in agreement with Brown-Peterson et al. (2011) who also noted that a five phase umbrella organisation of both macroscopic and microscopic reproductive criterion increases accuracy in fish reproductive studies. It should be noted that this is not the first study to identify difficulties with the accurate assignment of these particular phases. Previously, Ferreri et al. (2009) noted that while studying the European anchovy *Engraulis encrasicolus*, macroscopic misclassification was common in three spawning phases, imminent spawning, spawning and partial-post spawning and associated it with the macroscopic similarity of the phases. Similarly, Costa (2009) noted while studying horse mackerel *Trachurus trachurus*, difficulty in adequately describing distinct descriptive terminology for the macroscopic identification of each reproductive phase.

Analysis of the histological gonad sections of female dab highlight the presence of a combination of oocytes in all phases of development with no dominant population present. Therefore, the dab was identified as an asynchronous indeterminate batch spawner. This is based on the presence of several batches of oocytes in different phases of development, and the occurrence of POF's in phase 4 (Spawning) (Figure 2.3(d)). Results of the current study are in agreement with previous studies in the North sea on the reproductive biology of *L. limanda* (Htun-Han, 1978a,c,b). Concurrently, dab has also been identified as a group synchronous indeterminate spawner in the north Atlantic Ocean (Murua et al., 2003). Although Murua et al. (2003) did not validate the reproductive results with histology, it is not an unusual occurrence to have two different reproductive strategies for a single species. McMillan (2007) noted an increase in the occurrence of fish that exhibit multiple reproductive strategies based on location, food productivity and migratory patterns.

2.4.2 Maturity ogives

Predicting the age and size-at maturity of a fish species requires knowledge of the processes controlling maturation. These processes may include feeding conditions, pollution, light and photoperiod, diseases or hydrographical processes (Lambert, 2008). In the current study, the L_{50} was identified as 14 and 16cm for male and female dab, respectively. Previous studies in the North Sea have estimated that the minimum length at which maturation would occur would lie between 10 and 14cm for both male and female dab (Htun-Han, 1978b,a,c; Bohl, 1957). The differences in length at maturity between the two populations may be a result of temporal change or regional variation in reproductive development.

Length and age at maturity has been known to vary within populations of the same species (Lambert, 2008), with *L. limanda* experiencing variable growth rates throughout its distribution (Henderson, 1998). Previous studies have attributed changes in biological parameters of dab as a response to shifts in apex predators (Kaiser et al., 1997; Ellis and Gibson, 1995), food availability (Hinz et al., 2005; Wyche and Shackley, 1986), reduced stock densities (Temming, 1989), temperature changes, geographical differences, increases in beam trawling and eutrophication (Florin, 2005). However, it should be noted that there is also evidence to suggest that delayed maturation may also arise as a result of a gain in fecundity or because it results in reduced juvenile mortality, or both (Lambert, 2008).

2.4.3 Gonadal Development

The gonadosomatic index (GSI) was used to indicate gonadal development and maturity in dab, and showed an annual process characterised by an increase in GSI of fish over the reproductive period and an abrupt decline after spawning in the period of May/June. Dab ovaries show a continuous distribution of oocyte diameters throughout the phases of mature gonad development. In the ovaries of active mature females, the oocytes gradually increase in size alongside ovarian development (Figure 2.1). The highest peak of GSI was observed in the period of January/February, while peak oocyte size was recorded in March/April. The findings of the current study are in agreement with previous identification of spawning periods for the North Sea dab population (Htun-Han, 1978a,c). This is despite the fact that samples in this study were taken on a bi-monthly sampling period, and in a different geographical location.

Peak spawning appears to occur for male and female dab at different time periods visible in Figure 2.1, however this is not unusual. Previously, Htun-Han (1978c) noted that male dab can be reproductively ready to spawn up to six weeks before females. It is plausible to suggest that this asynchrony in reproductive biology of the dab was previously utilized to allow for longer durations of spawning for larger fish. Although not reported in this study, it was observed that the larger females (>27cm) were ready to spawn during the same period as the male dab, while the smaller fish were still in the spawning capable phase. This phenomenon has also been noted in other flatfish reproductive studies (ICES, 2010b). Jónsson (1966) previously noted that in Icelandic waters, larger older *L. limanda* enter the spawning phase earlier than smaller younger dab, allowing for increased egg production through the larger gonads. This spawning strategy may account for their continued abundance throughout their distribution range, irrespective of fishing.

2.4.4 Condition indices

Condition, as a measure of energy reserves, is an important attribute of future population success because it has a large influence on growth, reproduction and survival. In the current study the lowest relative condition was recorded in the period of November/December, which may be connected with the fact that like other flatfish species, dab undergo an inshore migration into coastal spawning grounds (Henderson, 1998). Previous changes in the condition of dab have also been ascribed to a depletion of body reserves during gonadal maturation (Htun-Han, 1978b). Relative condition (k_n) improves after this period (Nov/Dec), which may be indicative of increased prey availability and unlike other flatfish, continued feeding throughout the period of gonadal maturation (Lee, 1972).

The hepatosomatic index is one of the most fundamental indices in the study of population dynamics and biology of fish species (Hunter et al., 1992). Previous studies have suggested the incorporation of individual condition, as assessed by lipid reserves, into estimates of spawning stock biomass (SSB), in order to prevent distortion of estimates by presenting a more accurate representation of mature fish that are conditionally prepared for spawning (Jørgensen et al., 2006). Previously, studies have used HSI as a measure of recent feeding activity (Saborowski and Buchholz, 1996), or to identify whether fish change feeding activity during reproduction (Jørgensen et al., 2006). The accumulation of energetic reserves, in terms of lipids and proteins, is vitally

important in reproduction, where maturation and spawning will not occur if energy reserves are too low (Lambert, 2008). In the current investigation, the seasonal course of the male HSI corresponds with that of relative condition, where a decrease is noted at the initiation of the reproductive period. A similar decrease is noted for female dab. However, unlike males, females liver weight increases through the winter period, until spawning begins in January/February. This is potentially due to continued feeding, as has previously been noted by Saborowski and Buchholz (1996). Similar results were also obtained for dab populations from the North sea (Htun-Han, 1978b; Lee, 1972). In contrast, male dab experience a constant decline in HSI throughout the reproductive period, with very little variation in relative condition. Female dab return to prey rich coastal areas earlier than males (Bohl, 1957). It is feasible to suggest that the subsequent continuous decrease of the HSI in males observed in this study may be related to decreasing food availability. As a result of this, storage products in the liver would be liberated to fuel metabolic processes.

2.4.5 Conclusion

In Ireland, present legislation stipulates a minimum landing size of 15cm for dab (Power, 1980), which was thought to allow for the fish to spawn (10-14cm (Htun-Han, 1978b)), before it became vulnerable to fisheries (Pope et al., 2000). Given the estimated length at first maturity presented in this study, 14cm and 17cm respectively, this regulation may no longer be appropriate and the minimum landing size should be re-examined. The change in length at maturity will have serious implications on the regenerative capabilities of *L. limanda* populations as the fishery has a doubling time of up to 4 years, dependent on the maximum length of the fish and assumed initial fecundity (Musick, 1999).

In the past thirty years there has been a substantial shift in water temperature, particularly noticeable in the North Sea (Simpson et al., 2011). These temperature increases (0.2-0.6° per decade) strongly influence egg maturation rates, growth and survival of fish, and also impact on the planktonic communities that underpin the food webs which support fisheries (ter Hofstede et al., 2010).

In light of the current investigation and the reproductive similarities documented between the North Sea *Limanda limanda* population studied in the 1970's and the population off of Ireland's coastline, it is recommended that a review be undertaken of the biological parameters of the North Sea in order

that an effective management strategy may be applied for a sustainable dab fishery.

Chapter 3

Consistency of age

determinations of the dab

***Limanda limanda* using whole**

otoliths

Abstract

The precision and bias of reader uncertainty in dab age determinations was assessed from whole otoliths obtained from fish caught of Ireland's coastline. Mean and systematic differences in age determinations were compared among six age readers with varying degrees of experience and between two age reading institutions following an International workshop on age reading of dab (WKARDAB). Precision of age reader comparisons was quantified through age bias plots, coefficient of variation and percent agreement. Significant linear bias was observed between readers, with ages differing, on average, by one year for age classes greater than four years, with coefficients of variation (CV) of 10-23% and average percent error(APE) of 7-16%.

Ageing precision and bias in the study was influenced by the level of experience in the age-readers, with experienced age readers producing more precise and less biased age estimates. Ageing error within the study was attributed to interpretation differences between readers and experience level. The interpretative differences between readers manifested in the form of differences in the identification of the first annuli and in the interpretation of annuli on the edge of the otolith of older fish. This study represents the first evaluation of precision and bias of whole otoliths age reading in dab between multiple readers and suggest that whole otoliths may not be the most appropriate structure for age estimation in dab. The differences in age estimates between the six readers in this study emphasize the importance of validating ageing structures to provide accurate age estimates for dab.

3.1 Introduction

Age determination is critical for estimating growth and mortality rates as well as productivity of a fish species (De Pontual et al., 2006). Age estimates are considered one of the most important biological variables sampled in fisheries management investigations (Campana, 2001). Precise age determinations (not necessarily accurate) form the basis for a number of parameters in fisheries management and research (Abecasis et al., 2008). Information garnered from

age determination investigations allow for the ecological sustainability of fisheries and aquatic ecosystems (Begg et al., 2005).

The term "accuracy" of age is reserved for comparisons of ages generated by age-readers with the "true" age of the fish. A thorough study of the literature has identified a lack of accurate age estimations for *L. limanda* otoliths, and so only precision will be discussed in this study. Studies exist that confuse precision and accuracy (Beamish and McFarlane, 1983). The term "precision" is commonly used as an assessment of the reproducibility of a repeated measurement (Campana, 2001), either between the same or different age-readers (Power et al., 2006) and does not imply accuracy (Campana, 2001).

Bias in age determination can have two forms, either relative or absolute. Relative bias occurs where ages are compared between two readers or when one reader is compared to a modal age determined where a fish is aged by several readers. Absolute bias represents the systematic over or under estimation of age compared to the true age. Assessment of bias and precision is carried out using a mixture of statistical tests (Hoenig et al., 1995; Kimura and Lyons, 1991). Campana et al. (1995) recommended the combination of CV and age bias plots in order to visualize significant variation in age assignment. The latter approach has been used in a number of recent investigations (Albert et al., 2009; Armsworthy and Campana, 2010; Power et al., 2006; Stransky et al., 2005; Dwyer et al., 2003).

The more precise the age determination of the fish, the more robust the population analysis is in terms of monitoring temporal variation in abundance and structure (Appelberg et al., 2005). Accurate age determinations are formulated from a number of methodologies such as release of known age and marked fish, mark-recapture of chemically tagged fish, bomb-radiocarbon and radio chemical dating (Campana, 2001). Determining the accuracy of age determinations, has historically rarely been achievable because of the cost of the validation techniques, however this is changing through the application of various techniques as demonstrated by Armsworthy and Campana (2010), Albert et al. (2009) and Dwyer et al. (2003).

The determination of the most suitable ageing structure to age fish is a common problem, with otoliths historically being the most utilized structure in age and growth studies (Begg et al., 2005). Flatfish are commonly observed to have distinguishable annuli on otoliths (Armsworthy and Campana, 2010; Katayama et al., 2010; Albert et al., 2009). Cope and Punt (2007) noted that the formation of ageing structures and the subsequent interpretation of

growth annuli in otoliths is not always consistent. It is further complicated by the presence of false annuli and discontinuous structures that correspond to non-seasonal events (Katayama et al., 2010). This has been known to lead to observational error in the assignment of ages (Campana, 2001), and can have huge implications in terms of stock assessment. For example, the systematic overestimation of age can result in growth underestimation, leading to inaccurate perceptions of stock status (De Pontual et al., 2006).

L.limanda are ubiquitous in coastal waters in Europe, and inhabit a wide variety of marine habitats at depths of 0-200 metres. Although the species sustains a growing commercial fishery in Icelandic waters and the Baltic Sea, a limited understanding of the biology of the species has hindered its management (ICES, 2010a; Nissling et al., 2002; Temming, 1989). Unusually, with no management strategy in place for dab, five management units have been recognised by ICES, located in the Irish Sea, North Sea and Baltic Sea (ICES Advisory Committee, 2010a). Recently ICES has designated *L.limanda* as a species of interest (ICES, 2006) with subsequent workshops held on its reproduction (ICES, 2010b) and age structure (ICES Advisory Committee, 2010b). Studies of *L.limanda* age using whole otoliths have been carried out previously in Iceland and the North Sea (Lee, 1972; Jónsson, 1966). However, no validation or assessment of the precision of this method has been established.

In the absence of known age populations or a validated reference collection, it is only possible to determine the consistency of the age estimates. The objective of this study was to determine the consistency of age determinations between readers using the modified approach of Power et al. (2006) which was based on the combined graphical and statistical approach of Campana et al. (1995).

3.2 Materials and Methods

Dab were sampled from January 2009 to June 2010 on a monthly basis over an 18 month sampling period using fish captured from commercial fishing vessels, and the Irish scientific research vessels "RV. Celtic Voyager" and "RV. Celtic Explorer". Trawl hauls were made in two locations, the first located off the north west coast of Ireland encompassing the area 51°44'N , and 8°11'W, and the second, off the south coast of Ireland encompassing the area of 55°22'North and 8°15'W (Appendix Figure 6.3). Saggittal otoliths were extracted, thoroughly cleaned in distilled water and stored in individual square

petri-dishes pending further investigation.

3.2.1 Marginal increment analysis

The periodicity of band formation was evaluated utilizing the mean monthly marginal increment ratio (MIR) and is presented as a proportional state of completion of a whole annuli (opaque and translucent zone)(Dwyer et al., 2003; Campana, 2001). Fish less than 12cm were used to represent immature and/or actively growing fish and were pooled into one group because of low sample sizes.

A subsection of otoliths (n=163) were embedded in saturate polyester resin (Crystic R115NTP), left to dry for 3-4 hours and then sectioned transversely through the nucleus (0.5mm thick) with a Pises high speed sectioning machine customised with Buehler diamond wafering blades. The sectioned otoliths were photographed using a compound stereoscope coupled to a digital camera under a combination of light sources to increase edge clarity. Image Pro Plus 6.2 (Media Cybernetics, Silver Spring, USA), was used to record the two measurements at 2.5X magnification. Annuli were counted along the dorsal edge of the otolith. The otoliths were subsequently aged twice under a combination of light sources, to derive an acceptable precision. The reader had no prior knowledge of length or sex. For this analysis, it was necessary to group fish according to season as samples were not obtained for each month of the year. The mean incremental gain was plotted as a function of the season of capture. Linear regression will be used to identify if any relationship exists between marginal increment gain and time.

3.2.2 Age determination

A sub-sample of 100 otoliths was chosen from the dab sampled, and utilized for estimates of precision and bias with samples pulled randomly from the two specified locations. Each otolith was examined once by each reader and read blind of any biological information excluding month of capture. All readers had experience in ageing of fish using whole otoliths. However only two readers were experienced in ageing of dab *L.limanda*, while the other four readers would be considered trainee dab age readers.

The method of age estimation employed was standardized as far as possible between the readers. Whole otoliths were submerged in distilled water to increase contrast between summer and winter growth zones, and examined

using a binocular dissecting scope with transmitted and reflected light. Age was estimated by counts of the otolith from the nucleus towards the proximal edge (Figure 3.1), with assignment of birth date in January (Williams and Bedford, 1974).

Six readers from four institutions at the workshop for dab age reading (ICES Advisory Committee, 2010b) agreed to read the otoliths. Two institutions were each represented by two readers (Reader1, Reader 2 and Reader 4 Reader 5). The age-readers involved in the study are henceforth referred to as age-readers 1-6. The two institutions represented by two age readers will henceforth also be referred to as Institute 1 and Institute 2. Modal age was attained through the identification of the mode of the six reader assigned ages, with bi-modal distributions (where two modes exist for overall age) being removed from the analysis.



Figure 3.1: Stereoscopic view of a pair of saggittal otoliths in water. Otolith's were immersed in water and examined under a combination of light (transmitted and reflected), with the otolith's positioned with the anti-sulcal face down. Markings on the first otolith denote typical interpretation of annuli (Summer + Winter growth), with age estimated to be 4 years. The second otolith has begun the formation of vaterite (crystalline formation) on the outer distal edge.

3.2.3 Data Analysis

Age determination data were analysed for precision and bias using a combination of age-bias plots (Campana et al., 1995) and parametric and non-parametric statistical analysis. Analysis of bias was carried out using linear regression on pair-wise comparisons using R software (R Development Core Team, 2011). Potential bias in the ageing was examined by producing a age bias plot of the paired age readings (modal age and individual reader, and

reader and reader comparisons). Bias plots of paired modal age and individual age reader are not graphically represented in the results.

Age bias plots (Campana et al., 1995) were constructed using the individual modal age of the sampled *L. limanda*. Modal age was determined using the most frequent value (mode) in the distribution of age assignments over the six readers for each of the sampled fish. Plots were constructed using the $\pm 95\%$ confidence intervals of ages assigned by one age-reader, for all fish estimated as belonging to a specific age category by a second age-reader.

The slope and intercept of linear regressions of reader age determinations were tested for significance ($p = 0.05$), from 1.0 and 0, respectively. The parametric paired t-test was used to detect significant differences from a paired difference of 0, while a one sample t-test was used to test for systematic bias between readers. Significance was set at $p < 0.05$. Age bias plots (Campana et al., 1995) were produced to visualize the deviation of the age readings of age readings from the 1:1 equivalence line. The bias plots allowed the detection of non-linear bias, e.g. the relative underestimation of age by one reader in one part of the age range, and relative overestimation in another part of the age range by the same reader.

Various estimators of precision exist in the ageing of fish, with one of the most commonly utilized being percentage agreement. This index however, does not allow for comparison between fish of different age categories (Stransky et al., 2005), and must be calculated for each individual age. Beamish and McFarlane (1983) previously had suggested the use of an average percent error (APE), which is dependent on the average age of the fish species investigated:

$$APE_j(\%) = 100 \times \frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} - X_j|}{X_j}$$

where R is the number of times each fish is aged, X_{ij} the i (th) age determination of the j (th) fish, and X_j is the mean age calculated for the j (th) fish. Chang (1982) subsequently modified this index to a coefficient of variation (CV), which is expressed as the ratio of the standard deviation over the mean age of the fish and is written as

$$CV_j(\%) = 100 \times \frac{\sqrt{\sum_{i=1}^R \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

where CV_j is the age precision estimate of the j th fish.

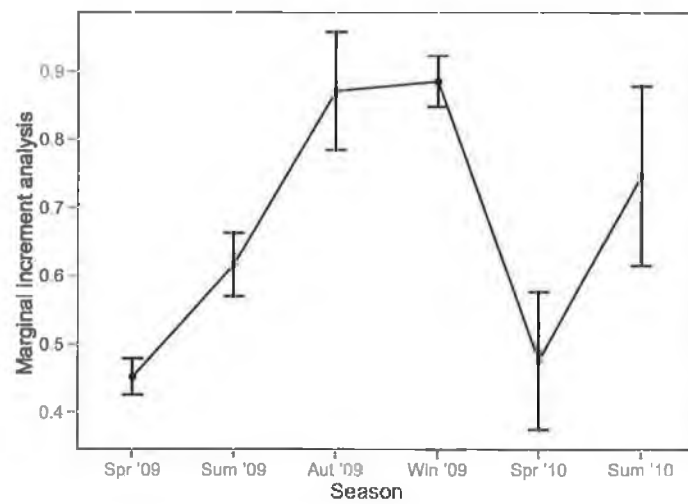
In addition to these indices, the correlation coefficient of r^2 was calculated

to evaluate the fraction of variation explained by the linear relationship between readers. Results from the present study were pooled to allow for an overall estimate of precision using the current methodology. For the analysis of precision, coefficients of variation (CV) were computed for comparative age determinations. Modal age was used to calculate APE, CV and percentage agreement in the initial comparison of readers against modal age, while subsequent comparisons of readers and institutes used the ages assigned during age reading. Hoenig's test (Hoenig et al., 1995) of symmetry (also referred to as Bowker's test) was used to indicate any systematic differences between the age-readers.

3.3 Results

3.3.1 Marginal Increment Analysis

Thin sectioned otoliths of 2-9 years old fish were sampled during the study period of February 2009 to June 2010, and showed an annual cycle in the monthly marginal increment (Figure 3.2). The increment decreased to a low value of 0.47 ± 0.21 during the spring (January, February and March), and rose during the summer and Autumn months, to peak at 0.89 ± 0.14 during the Winter period covering October, November and December. The marginal increment decreased to a similar low value in the spring of the second year. The cyclical pattern is consistent with the formation of one annulus per year, with the annulus appearing to be laid down in the spring (February, March and April). The parametric t-test showed a highly significant relationship exists between marginal increment formation and time ($P < 0.05$, $n = 163$). The slope of the interaction was assessed for both sexes combined where $time = 0.4496 + 0.047 * marginalincrement$.



	Season					
	Spring	Summer	Autumn	Winter	Spring	Summer
Sample number	49	42	20	22	19	25

Figure 3.2: Monthly changes in mean marginal increment for section otoliths of dab, with 95% confidence intervals. Number of observations per point can be seen in table below figure.

3.3.2 Comparison of readers against modal age

Individual age readings were first compared to modal age estimates, where bi-modal age distributions were removed from the study sample (individual fish which had two dominant age modes). These latter samples represented over 20% of the study sample. Modal age and individual reader estimates differed by up to three years. Modal age comparisons identified all but reader 6 as having an acceptable level of precision and bias in ageing *L. limanda* (Table 3.1). The mean percent agreement (%agr) and coefficient of variation (CV) between modal age and various age-readers was calculated as 72.1% and 7.8% respectively. For all age-reader comparisons to modal age, bias was found to be minimal (Hoenig's test, $p > 0.05$), with only one reader (Reader 6) indicating systematic differences in the assigned ages between the two age estimates. Age bias plots illustrate significant differences in reader behaviour, especially after age 4, where the reader begins to underestimate age relative to modal age. This trend is repeated in inter-reader comparisons (Figure 3.3).

In comparisons of intercept, significant deviations from the expected intercept of 0 ($p < 0.05$) indicated systematic bias, either under or over, in age determinations of dab. There was also significant deviation from the expected slope of 1 in all reader comparisons ($p < 0.05$), indicating inconsistency in the interpretation of annuli in each of the age readers.

Table 3.1: Coefficient of variation and percent agreement are presented for comparisons of modal age against reader age estimates. Pair wise comparisons, using Hoenig's test of symmetry are represented by the symbol p. Significant systematic differences in age estimates are highlight in gray. The significance level was set at $p < 0.05$. (n=79)

Reader	Reader	p	%agr	APE	CV
Modal Age	Reader 1	0.76	75.90	4.16	5.88
Modal Age	Reader 2	0.75	82.28	2.81	3.97
Modal Age	Reader 3	0.07	60.76	8.30	11.73
Modal Age	Reader 4	0.12	79.74	4.25	6.01
Modal Age	Reader 5	0.50	79.74	4.17	5.90
Modal Age	Reader 6	<0.01	53.16	10.17	14.39

3.3.3 Comparison of Readers

Bias was more obvious in individual age reader comparisons (Figure 3.3), where the deviation from the 1:1 equivalence line is non-linear for all reader pairs. The most pronounced bias was in comparisons involving age reader 6. Regression analysis indicated significant inconsistencies between all readers, with intercepts significantly different from the expected value of zero (Table 3.3). Mean age estimates for each specimen were significantly different between all readers, with mean paired differences varying from 0.62 to -0.68 years, respectively. The parametric paired t-test indicated high levels of significance differences in 42% of age reader comparisons, or 5 out of the 12 age reader comparisons, indicating significant deviations in age determinations between the age readers ($p < 0.05$).

In the age range of 4 years and older, all reader comparisons underestimated most of the ages relative to the other readers, resulting in regression slopes markedly less than 1, slopes of less than 0 and mean paired differences of up to 1 year (Table 3.3). Reader 3 and Reader 6, generally assigned higher ages than other age readers (Table 3.3).

Percent agreement and CV have been calculated for precision analysis on age determinations of six readers (Table 3.2). Between reader precision was shown to be quite poor in the majority of cases, with mean percent agreement calculated at 47%. The level of precision in each reader comparison is above the acceptable level of precision required for consistent age determination (CV 7.6%, APE 5.5%, (Campana, 2001)). This suggests that ageing of *L. limanda* is not wholly reproducible among the different age-readers. Mean CV was

calculated among the 6 readers as 12.7%.

The age bias plots illustrated significant differences in reader behaviour between individual paired comparisons. Reader pairs with no significant bias were generally age-readers trained in ageing *L. limanda* (reader 1, reader 2, reader 4 and reader 5). The slope of each reader comparison was significantly different from 1 (Table 3.3). Examination of the age bias plot (Figure 3.3) suggest that divergence from the 1:1 equivalence line begins at approximately age 5, indicating level of agreement for older fish age was considerably less precise than age determinations for the younger fish.

It is clear that among the various readers, reader 6 shows signs of non-linear bias. This is illustrated through the consistent over-estimating of the age of the younger fish (1-4 years) in comparison to the more experienced readers, while allocating lower ages in the older age-range (5-7 years) (Figure 3.3).

Table 3.2: Coefficient of variation and percent agreement presented for between age-readers comparisons. Mean % agreement and CV were calculated by averaging individuals CV across fish within the study sample. Pair wise comparisons, using Hoenig's test of symmetry are represented by the symbol p. Significant difference between two readers are highlighted in gray.

Reader	Reader	p	%agr	APE	CV
Reader1	Reader2	0.42	64.21	6.86	9.71
Reader1	Reader3	0.14	37.89	13.65	19.31
Reader1	Reader4	0.06	63.15	7.54	10.66
Reader1	Reader5	0.69	52.63	9.97	14.10
Reader1	Reader6	<0.01	39.79	13.21	18.69
Reader2	Reader3	0.20	45.26	10.88	15.39
Reader2	Reader4	0.09	56.84	9.15	12.94
Reader2	Reader5	0.09	56.84	9.15	12.94
Reader2	Reader6	0.09	56.84	9.15	12.94
Reader3	Reader4	0.01	47.67	12.53	17.72
Reader3	Reader5	0.26	50.52	10.34	14.63
Reader3	Reader6	0.03	42.11	10.85	15.33
Reader4	Reader5	0.06	57.89	8.80	12.44
Reader4	Reader6	<0.01	32.63	15.94	22.54
Reader5	Reader6	0.05	40.00	14.18	20.06

Table 3.3: Regression and paired t-test results for inter-reader comparisons. The paired t-test reports on significant deviation from a slope of 1 between inter-reader comparisons, while the t-test tests for significant deviation an intercept of 0.

Reader Comparisons	Regression Analysis			Paired T-test		T-test
	Intercept	R^2	p	t	Paired p	Slope
Read1 vs Read2	0.54 ± 0.26	0.57	< 0.05	-2.15	-0.20	< 0.05
Read1 vs Read3	1.12 ± 0.32	0.36	< 0.05	-4.04	-0.51	< 0.05
Read1 vs Read4	0.98 ± 0.23	0.56	< 0.05	1.18	0.12	0.24
Read1 vs Read5	1.11 ± 0.29	0.41	< 0.05	-1.36	-0.16	0.18
Read1 vs Read6	0.94 ± 0.32	0.40	< 0.05	-5.66	-0.68	< 0.05
Read2 vs Read3	1.22 ± 0.29	0.43	< 0.05	-2.68	-0.31	< 0.05
Read2 vs Read4	1.36 ± 0.23	0.51	< 0.05	3.10	0.31	< 0.05
Read2 vs Read5	1.02 ± 0.24	0.56	< 0.05	0.45	0.04	0.66
Read2 vs Read6	1.41 ± 0.32	0.34	< 0.05	-3.82	-0.48	< 0.05
Read3 vs Read4	1.68 ± 0.28	0.42	< 0.05	5.26	0.62	< 0.05
Read3 vs Read5	1.64 ± 0.32	0.36	< 0.05	2.77	0.35	< 0.05
Read3 vs Read6	1.37 ± 0.33	0.39	< 0.05	-1.42	-0.18	0.16
Read4 vs Read5	0.65 ± 0.27	0.51	< 0.05	-2.63	-0.27	< 0.05
Read4 vs Read6	1.01 ± 0.34	0.32	< 0.05	-1.43	-0.18	0.16
Read5 vs Read6	1.67 ± 0.36	0.23	< 0.05	-3.63	-0.53	< 0.05

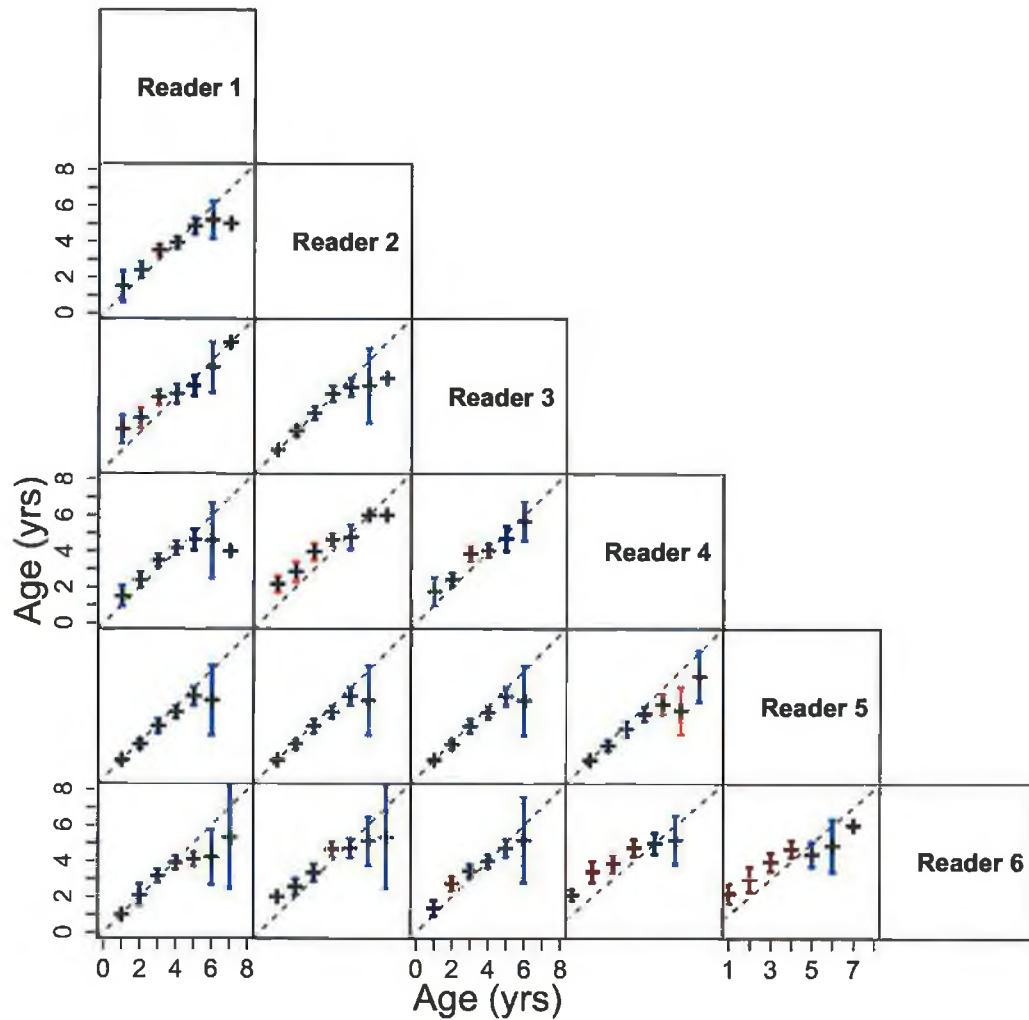


Figure 3.3: Age bias plots for the reader comparisons based on *L. limanda* whole otoliths from the Irish coast. The 1:1 equivalence line is indicated on the graph, while each tick mark represents 1 year of age, ranging from age 0 to age 8. 95% confidence intervals are indicated in blue. Ages at which there was significant bias (t-test) between the reader comparisons are highlighted in red ($p < 0.05$) (n=95).

3.3.4 Comparison of Institutes

Modal age comparisons of Institutional age reading identified both Institutes as having acceptable levels of precision and bias in ageing of *L. limanda* (Table 3.4) based on Campana (2001). This is not repeated in comparisons of age between the institutions visible in Table 3.4. Results of an ANOVA comparing slopes and intercepts of institute age determinations found no significant deviations from the expected slope of 1 and intercept of 0, indicating no systematic underestimating of age was occurring ($p = 0.82$). No significant bias using Hoenig's test of symmetry was identified in any of the comparisons ($p > 0.05$).

Table 3.4: Coefficient of variation and percent agreement presented for within and between Institutional age comparisons. Pair wise comparisons, using Hoenig's test of symmetry are represented by the symbol p. (n=75)

Institute	Institute	p	%agr	APE	CV
Institute 1	Modal Age	0.67	81.33	3.64	5.155
Institute 2	Modal Age	0.43	84.00	3.01	4.27
Institute 1	Institute 2	0.93	69.33	5.79	8.18

3.4 Discussion

The most common use of age data in fisheries is to attain an estimate of total mortality using catch curve analysis (Ochwada et al., 2008), hence knowledge of age in fish populations is crucial in stock assessment and management (Bermejo, 2007). It is essential that the method of estimating this parameter maximizes precision and reduces bias in order to obtain the best possible age estimate.

3.4.1 Marginal Increment Analysis

Marginal increment analysis supports the hypothesis that annuli formed once per year in dab *Limanda limanda*. Annual opaque zones were formed in the early spring (February, March and April), while annual translucent zones were formed during the winter period (October, November, December). Marginal increment analysis is considered one of the most abused age validation methods (Campana, 2001). It is only suitable as a validation increment in fast-growing fish (Dwyer et al., 2003). This is due to the subjective nature of ageing narrowing annuli at the margin of slow growing fish and the technical difficulties

in viewing of partial increments. For this reason, it was not used as an age validation method in dab *L. limanda*.

3.4.2 Bias

All between reader comparisons of age determinations for *Limanda limanda* identified considerable non-linear bias caused by relative under- or over estimation of up to 1 year mean paired difference, when compared to other readers. Bias in age determinations increases as a fish grows older, and the annuli at the outer edge of the otolith become more condensed. The maximum age observed in this study was 7 years, however a maximum age of up to 14 years are documented for dab located in the waters off Iceland (Jónsson, 1966) and greater than 10 years in the North Sea (Henderson, 1998; Rijnsdorp et al., 1992).

Due to a lack of known age dab samples with which to compare the current levels of precision and bias, two contrasting explanations for the non-linear bias are suggested, over-estimation of age or under-estimation of age, relative to other age readers. Assuming that over estimation of dab age was occurring, this can have serious implications for future stock assessment of the fish, in terms of the identification of biological reference points. Dab are known to mature at an age of 2-3 years and are fully recruited to the spawning stock at age 4 (Htun-Han, 1978b; Bohl, 1957). If over-estimation of age is occurring, this may potentially result in the removal of the fish stock before they are reproductively active as well as impact on the determination of growth in the species, as they are closely related. In European hake, De Pontual et al. (2006) clearly showed that age over-estimation resulted in growth underestimation of the species, and the same may be applicable for dab. The alternative is the assumption that under-estimation is occurring in dab age determinations within the current study. This would have a significant impact on annual assessments by ICES which require accurate age-length-keys by various regions/laboratories in order to accurately assess wild fish stocks (ICES, 2011, 2006). However, neither of these assumptions can be tested due to a lack of known age fish with which to compare the resultant ages to.

The current study used percent agreement to best visualize the prevalence of differences in paired age estimates. Percentage agreement between age-readers was found to be quite poor in paired age estimates of the present study (33-63%), based on the relatively few year classes represented. There was also considerable inconsistencies and systematic differences between age estimates

derived using different age readers. In some cases the choice of one age reader over another could cause bias in the estimation of age, especially when the two readers had been trained differently, with different ageing protocols. Age reader 6 demonstrated the lowest percent agreement and highest CV, 53.16% and 14.39 respectively, and was the least experienced of the six age readers. In contrast, age reader 2 demonstrated the highest percent agreement and the lowest CV, 82.28% and 3.97, and was the most experienced of the dab age readers. This is not unusual, in fact it is expected that new age readers will start at a high CV and gradually reduce it in line with experienced age readers over time (Walsh and Burnett, 2002).

Examination of age bias plots highlight the consistent non-linear bias in age determinations, whereby over-estimation occurred in the lower age groups and under-estimating of age in the older age groups relative to the other age readers. Significant non-linear bias in the age estimations may reflect a host of different factors, including difficulty in identification of the first annuli on whole otoliths, multiple banding which can also be referred to as checks or split rings, lack of adequate training/experience in dab age determinations or imprecise ageing structure choice. Difficulty in identification of the first annuli is a common problem in flatfish ageing, and was also reported by the age readers of the WKARDAB (ICES Advisory Committee, 2010b). Proceedings of the WKARDAB workshop identified the average size of the dab nucleus in order to reduce the impact this has on under- and overestimation of the species and will be used in future studies. The phenomenon of multiple banding is not restricted to flatfish otoliths (Power et al., 2006; Bergstad et al., 1998) and are assumed to be stress related reflecting environmental stress, maturation, spawning or metamorphosis (Dwyer et al., 2003).

3.4.3 Precision

In age determination studies, a small index of precision is thought to represent precise age determinations (Hoenig et al., 1995), with Campana (2001) setting the acceptable level of precision at 7.6%. However, good precision is open to interpretation. For example, a small measure of precision in long lived species is by its very nature different from short lived species, with studies demonstrating different levels of acceptable precision based on the age range of the species (Albert et al., 2009; Lee et al., 2009; Stransky et al., 2005; Fossen et al., 2003; Bergstad et al., 1998).

Precision/CV levels within the current study varied significantly between

reader comparisons to modal age, within reader comparisons and between institute comparisons. Based on the assumption that modal age will give a best corroboration of age with the currently available information, precision of readers to modal age is close to precise when using criteria developed by Campana (2001)(current study is 7.9%, Campana recommends 7.6%). This corroborates the idea that reasonable precision can be achieved with dab *L. limanda* whole otoliths when ageing methodology is standardized. However, many age reading laboratories have currently set a CV value of 5% to account for the various reading complexities and longevity of the species under investigation. When the current study investigated the two institutes available to them, precision estimates were within the acceptable level when compared to modal age (5% and 4%), but not when age determinations were compared between institutions (8%).

Between reader precision (compared to modal age) was found to be higher for this study, than previous studies (ICES Advisory Committee, 2010b), with both studies using whole otoliths for age determination and having a similar composition of fish length and age categories. An average CV value of 12% was achieved between twelve readers with a percent agreement of 79.3%. A precision level of 12% has previously been considered relatively precise for long lived species (Stransky et al., 2005), however this level is imprecise for short lived species. In contrast to ICES Advisory Committee (2010b), the current study attained an average CV of 8.7% and percent agreement of 72% with 6 readers from the same workshop used in the comparisons and compared to modal age. There appears to be an element of learning visible in the current results, with increased precision of age determination attained after the establishment of dab-specific ageing interpretation guidelines (WKARDAB=12% CV, current study=9%CV).

It should be noted that while good precision is always desired in age determination studies, it in no way validates the age determination. Albert et al. (2009) noted that precision in fish age determination can be improved by continuous species-specific exchange programmes and age interpretation guidelines. Two to three re-reads of a collection of ageing structures is thought to be sufficient to characterize ageing error (Cope and Punt, 2007), although this must be repeated occasionally as human interpretation of fish age can vary (Kimura et al., 2006). Good precision in general acts as corroborative evidence of age determination, but cannot guarantee ageing criteria (validating age) for a species until strong validation methods have been successfully applied

(Kimura et al., 2006). The assumption of the choice of the most precise ageing structure is experience dependent. However, previous studies have acknowledged that there is a tendency to underestimate the age of older fish using whole otoliths (Lee et al., 2009). This is due to growth increments not forming equally on all parts of the otolith (Chilton and Beamish, 1982). In a study of flatfish ageing, Abecasis et al. (2008) highlighted that the use of whole otoliths to determine fish age commonly leads to underestimation of older individuals, irrespective of the experience level of the reader (Walsh and Burnett, 2002). The current studies results may reflect the incorrect choice of a precise ageing structure and it is plausible to suggest that the application of a different ageing structure or structure preparation may increase the precision to an acceptable level.

3.5 Future direction

Based on the current study results, re-examination of dab otolith reading guidelines (ICES Advisory Committee, 2010b) may need to be further analysed in order to reduce the ageing bias to an acceptable level. This is essential in order to be able to accurately determine mortality in dab populations. If inter-reader variability remains high, this could potentially produce temporal and regional differences in the estimated parameters making it difficult to compare and manage stocks. Precision and accuracy of ageing may be improved through the identification of readable ageing structures, identification of the most precise reading axes, and standardization of preparation techniques throughout all age ranges for the species. The standardization of ageing methodologies across all ageing laboratories means that age determinations are reliable and comparable. This will subsequently result in spatial and temporal variation in growth rates between populations accurately identified and not falsely established as a result of the ageing methodology employed.

Abecasis et al. (2006) has indicated that the use of whole otoliths may result in under ageing in comparison to other ageing methodologies. Ageing studies previously identified improved precision between readers, when moving from whole otolith ageing methodology to sectioned otolith ageing methodology (Katayama et al., 2010; Stransky et al., 2005; Mc Dougall, 2004; Bergstad et al., 1998). It is proposed that in order to improve precision in ageing of dab, that the sectioned otoliths age estimation should be trialled. Sectioning of *L. limanda* otoliths as opposed to determining age from whole otoliths

may enhance the ability to differentiate opaque zones in otoliths due to the increased deposition of calcium carbonate material on the interior proximal surface of the otolith after a certain age. The presence of split rings/multiple banding in whole otoliths suggests that they may also be present in sectioned otoliths of dab and should be resolved before the development of a sectioned ageing protocol.

This study deals only with precision and relative bias of age reader interpretations of age and not with accuracy of age determinations. The development and standardisation of ageing protocols is an essential prerequisite for the accurate management of a fish stock. This should be supported by validation studies using daily increment counts on juvenile *L. limanda* as well as tag recapture studies to ensure that age determinations reflect the absolute age of the fish.

Chapter 4

The age and growth of *Limanda limanda* using whole and sectioned otoliths

Abstract

Age and growth of dab *Limanda limanda* was assessed by examination of annual growth increments in sectioned and whole sagittal otoliths from 524 fish sampled from the coastal waters of Ireland between February 2009 and June 2010. Marginal increment analysis suggest that dab deposit annual growth increments on a yearly basis. Age was therefore, estimated for all fish by counting assumed annual growth increments. Ages ranged from 1-9 years, with the majority of fish sampled between 3-9 years. Females were on average heavier than males. The estimated total length (cm)-weight(g) relationship was $W=0.014L^{2.89}$ for males and $W=0.009L^{3.04}$ for females. Both fish experience isometric growth.

Age determinations from an earlier ICES workshop showed bias in age estimation using whole otoliths, therefore whole otoliths were compared to sectioned otoliths as an alternative ageing methodology in order to improve estimates of growth. Precision and bias of the ageing methods was assessed against whole otolith age determinations using two experienced dab readers. Significant bias was identified in sectioned age determinations when compared to whole otoliths (CV=30%, APE=22%) using the modal age of both readers over the two methods. However, paired T-tests and multinomial logistic regression on age-length-keys identified the sectioned otolith as being a significant improvement on whole otolith age determinations, regardless of the presence of bias and low precision between readers. This method was subsequently used for growth modelling. A bias-corrected form of Akaike's Information Criterion (AIC) determined that the logistic model provided the best fit to the sectioned data, with model parameters estimated for male ($L_{\infty} = 21cm$, $t_0 = 0.14$, $k=0.71$) and female dab ($L_{\infty} = 23cm$, $t_0 = 0.73$, $k=-1.25$). There was significant differences in growth between sexes ($X^2 < 0.05$), with growth between sexes differing from age 4 onwards.

4.1 Introduction

Growth studies are considered an essential instrument in the management of fisheries resources since they contribute to estimates of production, stock size, recruitment and mortality of fish populations, and are a fundamental component of population dynamic models (Newman, 2002). Previous studies on growth of *Limanda limanda* have primarily focused on the growth of larval dab (Lee et al., 2007, 2006; Campos et al., 1994), although Folmer (2000) and Rijnsdorp et al. (1992) do provide an evaluation of growth rate for adult fish from the the Irish Sea and North Sea, utilizing the von Bertalanffy growth model. Although an age determination technique has not been validated for *L. limanda*, ageing of the species is typically carried out by enumerating concentric rings on otoliths, be they whole (Folmer, 2000; Jónsson, 1966) or burnt (Henderson, 1998), and was widely accepted as being precise although precision was never assessed. Recently, ICES Advisory Committee (2010b) highlighted the imprecision of utilizing whole otoliths in age estimation of *L. limanda* and lack of standardized ageing methodology between ageing institutions. It was suggested that other methodologies, such as sectioned otolith, should be investigated to improve the level of precision visible in older fish using whole otolith age determinations.

While aspects of *L. limanda* biology has previously been investigated in the North sea and Icelandic waters, this study represents the first detailed investigation of the age, length and growth characteristics of dab captured off the north-west and south coast of Ireland. These fundamental biological characteristics are recognised as an essential component of fisheries dynamics (Cope and Punt, 2007), specifically that of age data. Age data is used to attain estimates of total mortality using catch curve analysis (Ochwada et al., 2008; Newman, 2002) and so is crucial in identifying correct exploitation rates for sustainable management of a fishery (Bermejo, 2007). Dab was previously identified as a species which has the potential to become of economic importance (ICES, 2006), with the information contained in this study forming the basis for its eventual management.

This study aimed to describe the growth of adult dab *Limanda limanda* captured of the north-west and south coast of Ireland. The first objective was to explore the optimal method of age determination by comparing two different ageing methodologies using various estimates of precision and bias. The second objective was to identify which growth model best fit the Irish data, and determine the age, growth rate and size composition of the dab captured.

Model parameters will be compared with previous studies using a growth performance index (ϕ), in order to identify any similarity between populations. A secondary component of this objective will be to analyse the variability in the sex ratio. This information is considered an essential prerequisite to stock assessments of this species (age-structured assessments).

4.2 Materials and methods

A total of 461 fish were collected in January and September of 2009 during demersal fishing surveys conducted aboard the "RV Celtic Voyager" and "RV Celtic Explorer" utilizing GOV nets in commercially important fishing areas off Ireland's coastline. Additionally, 2392 fish were sampled from commercial fishing vessels, from February 2009 to June 2010 in two sampling localities, comprising the area off the north west coast of Ireland (51°44'N , and 8°11'W), and the second, off the south coast of Ireland (55°22'North and 8°15'W) (Figure 6.3).

Fish were measured to the nearest cm total length (L), assessed for sexual maturity using a macroscopic guide and weighed to the nearest gramme total weight (W) and gutted weight (GW). Gutted weight is defined as the total weight after removal of viscera. The sagittal otoliths were removed, washed in distilled water and stored dry in square petri dishes prior to sectioning. One-way analysis of variance was used to compare mean age, size and weight between sexes ($\alpha = 0.05$).

Length and weight data was transformed to a natural logarithm to satisfy assumptions of normality and homogeneity. The relationship between the length and weight of *L.limanda* was described by the power relationship:

$$W = aL^b$$

where W is total weight of fish (g) and L is length (cm), and subsequently back transformed to the above form. The significance of the recorded differences was tested by an analysis of covariance (ANCOVA) where $p = 0.05$. The deviation in slope and intercept was tested for significance using the following model, sex length+sex:length.

Growth type (isometric or allometric) was determined using the hoCoef function in R (R Development Core Team, 2011), where the growth model

parameters can be obtained using the following formula

$$t = \frac{\hat{\beta} - \beta_0}{SE_{\hat{\beta}}}$$

where $\hat{\beta}$, $SE_{\hat{\beta}}$ are from the linear regression of the length weight model, and β_0 is the specified value of 3, indicating isometric growth. A t-test is used to test for significant variation from this value. If the intercept of the model does not contain 3, then the growth is allometric, however if the intercept does contain 3, then growth is isometric.

Using a Chi Square test ($p = 0.05$), the sex ratio of dab was investigated to identify whether the sex ratio differed over time, and whether it significantly deviated from the expected 1:1 relationship.

An age-length-key (ALK) was estimated for both sexes from length stratified sampling of the catch and analysed for age by examining the annual ring formation in the otolith. Multinomial logistic models were used to compare ALK (Gerritsen et al., 2006) between sexes and between both ageing methods using the form of:

$$A \ M + S + M * S \quad (4.1)$$

where A is the predicted age distribution at length M, at sex S. M was fitted as a continuous variable, S as a factor, and M*S is an interaction term. The significance of the factor sex in the model was tested by comparing the initial model to a model without that factor. The difference in residual variance of these nested models was tested against difference in the alternative model using degrees of freedom from the Chi-square test. The minimum age in the sample was 1 due to catches of 0-group fish not being caught using the current sampling means.

4.2.1 Corroboration of correct ageing structure

The periodicity of band formation was evaluated utilizing the mean monthly marginal increment ratio (MIR) and is presented as a proportional state of completion of a whole annuli (opaque and translucent zone)(Dwyer et al., 2003; Campana, 2001) (See Chapter 3, subsection: Marginal Increment Analysis).

Growth rings were visible on whole otoliths as alternating opaque and translucent zones, and ages were assigned to fish based on their counts, where one year's growth was interpreted as a combination of one opaque and one translucent zone. The otolith of dab was found to grow in a cleavage formation

where the direction of the growth along the dorso-ventral axis curves to the internal side of the fish. As the dab otolith grows, the width of the growth margin (adjacent opaque and translucent band) becomes narrower, causing difficulties in ageing older fish using whole otoliths.

Subsequent to the initial examination of whole otoliths, a random sub-sample of transverse sections of saggittal otoliths were examined ($n = 534$). This was not length stratified, as the necessary length frequency distribution was not available. One otolith per fish, chosen based on the most central nucleus was embedded in saturate polyester resin (Crystic R115NTP), left to dry for 3-4 hours and then sectioned transversely through the nucleus (0.5mm thick) with a Pilses high speed sectioning machine customised with Buehler diamond wafering blades. Sections were brushed with water and photographed using a compound stereoscope coupled to a digital camera at magnification of 2.5X. The sectioned otoliths were photographed under a combination of light sources to increase edge clarity, and their image processed utilizing the computer programme Image Pro Plus 6.2 software (Media Cybernetics, Silver Spring, USA) (Figure 4.1).

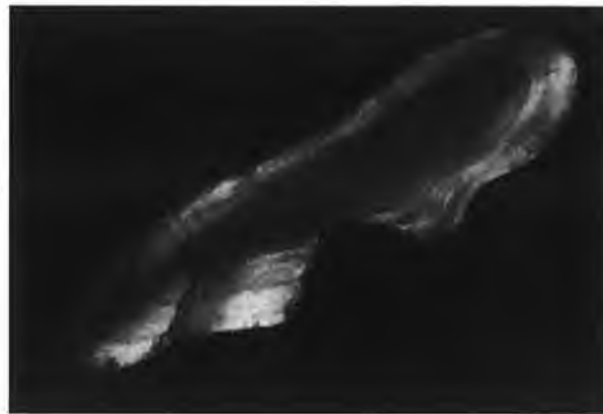
4.2.1.1 Precision and bias in ageing methodology

A sub-sample of sectioned and whole otoliths were analysed and counted twice by two readers, both experienced in age estimation using whole otoliths. Training of reader 1 was based in an academic institute, while reader 2 was trained in an fish age-reading laboratory.

First and second counts were performed with an interval of at least one week. Readers will henceforth be referred to as Reader 1 and Reader 2. The sections were examined under a dissecting microscope at 2.5X magnification with a combination of light (transmitted and reflected) on a neutral clear background. The number of summer-opaque zones (annuli) were recorded, without prior knowledge of length.

The precision of these age determinations (within reader and between reader) were quantified by the average percent error (APE) (Beamish and McFarlane, 1983), the coefficient of variation (Chang, 1982), and age-bias plots (Campana, 2001) (See Chapter:3: Data Analysis for more information). When counts significantly differed a third reading was preformed for consensus.

Modal age was determined using the most frequent value in the distribution of age assignment over the two age readers, two methodologies and time. Sectioned otoliths and whole otoliths were treated separately.



(a) Full sectioned otolith



(b) Close up of left side of the sucullus

Figure 4.1: Sectioned sagittal otolith of *Limanda limanda*. Age was determined to be 4 years. (a) Sectioned otolith (b) the same otolith with a close up of the left edge of the sucullus clearly showing cleavage formation.

Analysis of covariance (ANOVA) was used to determine if there was a significant difference between the two ageing methods, while a multivariate analysis of variance (MANOVA) was used to compare between readers and within readers to identify which variable contributed most of the observed differences among groups.

4.2.2 Growth

In order to identify which ageing method produces the best fit for growth modelling, the relationship between length and age was compared using a simple linear regression of both methods. The Akaike criterion (Haddon, 2001) was used to test for goodness of fit of the two methods, with the lowest AIC value indicating the better model fit.

A set of four candidate growth models were chosen to model fish growth:

von Bertalanffy growth model (VBGM), Schnute, Francis and Logistic model: $M_1 - M_4$, respectively. The VBGM is the most commonly utilized growth model and is based on the assumption that the growth rate of fish tends to decrease linearly with size. It is commonly formulated as the following equation,

$$L_t = L_\infty[1 - e^{-K(t-t_0)}]$$

where L_∞ is the asymptotic average length-at-age (but not the maximum length of the organism) and has the same biological meaning in the Logistic model. K is the Brody growth rate coefficient (units yr^{-1}), but not a growth rate and t_1 is interpreted as the time/age when the average length of the fish was zero.

The Schnute growth model (Schnute, 1981) is a parameterization of the original VBGM utilizing the average length of both the youngest and oldest fish in the sample and is delimited by the following formula:

$$E(L|t) = L_1 + (L_2 - L_1) \frac{1 - e^{-K(t-t_1)}}{1 - e^{-K(t_2-t_1)}}$$

where L_1 is the average length at the youngest age, t_1 , and L_2 is the average length at the oldest age, t_2 , in the example. It should be noted that $L_2 \neq L_\infty$ as L_∞ is the average size of the theoretical maximum age and L_2 is the average size at the maximum age in the sample.

The Gallucci-Quinn growth model (Gallucci and Quinn II, 1979) is another reparameterization of the VBGM, which is used to aid comparisons between two groups by introducing a new parameter,

$$\omega = KL_\infty$$

Gallucci and Quinn II (1979) state that ω can be thought of as a growth rate and is representative of the growth rate near t_0

$$E[L|T] = \frac{\omega}{k}(1 - e^{-K(t-t_0)})$$

Finally, the logistic model, given by the equation

$$L(t) = L_\infty(1 + e^{-k_3(t-t_3)})^{-1}$$

is an alternative sigmoidal curve, where the parameter k_3 is a relative growth parameter (with units year^{-1}), while t_3 corresponds to the inflection point of

the sigmoidal curve.

The parameters for all models were derived using sensitivity analysis on non-linear least squares using the Gauss-Newton algorithm (nls) performed on R (2.13.1) (R Development Core Team, 2011), with 95% confidence intervals estimated from bias corrected and accelerated bootstrap of the raw data (Bootstrapping =1,000). The sensitivity analysis was used to assess the robustness of the growth model to a set of initial parameters, where the models were fitted with a minimum of 35 combinations by crossing possible values for each of the three parameters. Fitting did not successfully converge on female whole otolith growth models.

The goodness of fit of the four growth functions were compared based on Akaike's information criterion (Haddon, 2001). AIC_c was used instead of AIC , as at large sizes, it approximates back to AIC values (Symonds and Moussalli, 2011). AIC_c was used to compare the goodness of fit of different growth functions based on the Kullback-Leibler divergence which identifies distance between two models:

$$AIC_c = AIC + \frac{2k(k+1)}{n-k-1}$$

where k is the number of parameters estimated in the growth function, n is equal to the sample size. The Akaike weight (w_i) was also used to demonstrate which model had the best fit to the data, using the following formula:

$$w_i = \frac{e^{-0.5 \times \Delta_i}}{\sum_{k=1}^5 e^{-0.5 \times \Delta_i}}$$

Based on multi-model inference (MMI) (Burnham and Anderson, 2004), when a model had an Akaike weight (w_i) of less than 90%, predicted values were estimated across models and substituted for average L_∞ and ($\hat{L}_{\infty,i}$) in

$$\hat{L}_{\infty,i} = \sum_{i=1}^2 w_i \times \hat{L}_{\infty,i}$$

The substitution occurs due to the lack of L_∞ in the logistic model.

Comparison of growth parameters (K, t_0, L_∞) for all models and between sexes was accomplished using likelihood ratio tests as per Kimura (1980), under the assumption of constant variance for all comparisons.

4.2.3 Comparison of growth between different regions

Given that sets of growth parameters for dab estimated by different authors was available, the reliability of these estimates was tested using the wide application phi-prime test (Φ) (Pauly and Munro, 1984; Munro and Pauly, 1983). ϕ is directly related to growth performance and indirectly related to metabolism and food consumption and so may be used to quantify the energetics of a given location (Munro and Pauly, 1983). According to Piñeiro and Sainza (2003), it also provides an indication of the reliability of age estimates and is calculated as follows

$$(\Phi = \log_{10}k + 2\log_{10}L_{\infty})$$

where k and L_{∞} are parameters extracted from growth models.

4.3 Results

4.3.1 Meristic Parameters

4.3.1.1 Length-weight Relationship

ANCOVA identified significant differences in length and weight between sexes of *Limanda limanda* ($p < 0.05$, $n=2,536$). Total length-weight relationships were assessed separately for both sexes ($n=2536$), where $\log W = -4.27 + 2.86 * \text{Log}(L)$ for male dab, and $\log W = -4.71 + 3.04 * \text{Log}(L)$ for female dab. On The transformed scale, this equates to $0.014L^{2.89}$ for male dab and $0.009L^{3.04}$ for female dab. There is significant deviation in the length weight relationships between sexes and also in the interaction of length with sex ($p < 0.05$), indicating there is a difference in condition of the fish. Consequently, weight at a given length may vary between sexes. Based on analysis of variance, there is a highly significant relationship between sex and length in dab ($p < 0.05$), where male fish are between 0.09 and 0.28 smaller on the log scale than female dab. Dab experience isometric growth ($p > 0.05$), where the exponential parameter of the growth lies between 2.94 and 3.01 with 95% confidence ($p > 0.05$).

The slope and intercept of the two regression lines (male and female length-weight relationship) are significantly different from 1 and 0 ($p < 0.05$) indicating that there is both a difference in the magnitude and rate of change of the length weight relationship.

4.3.1.2 Age-at-length

The study samples were dominated by specimens belonging to the age groups of 3-6 years. Age-length-keys (ALK) produced for female and male *Limanda limanda* using both whole otolith and sectioned otolith age determination methods are presented in Table 4.1, where a paired t-test identified significant differences between the two proposed age length keys for both sexes. Likelihood ratio tests between the two methods indicated that the sectioned model fits the data significant better than the whole age data with sex as an interaction term (Sectioned: $X^2 = 1$, $p = 0.64$, whole: $X^2 = 1$, $p = 0.07$). A highly significant sex effect was found for the ALK models that contained both sexes (whole: $p = 0.008$, $d.f. = 4$, $LR = 13.61$, Sectioned: $p = 0.01$, $d.f. = 4$, $LR = 12.86$). However, when the sex method interaction is added, this becomes non significant (whole: $p = 1$, $d.f. = 8$, $LR = 0.003$, Sectioned: $p = 0.42$, $d.f. = 4$, $LR = 3.87$). It was observed that if either of the methods is removed, the sex interaction remains high. This indicates that the ALK of both sexes is significantly different between the two methods (ageing).

The maximum age recorded was 9 years for both male and female dab determined using sectioned otoliths age determinations (Figure 4.3). The distribution of lengths for both male and female dab during the study period are presented in Figure 4.2, where female fish were most abundant in the 17 – 23cm length categories, with majority abundance seen in female dab after 27cm. Male dab were most abundant in the 14 – 20cm length category, with only one male recorded in the upper length categories.

Table 4.1: Length-at-age/ALK for dab sampled of the north-west and south coast of Ireland, with average length-at-age presented for age determinations by both whole and sectioned otoliths. Significant outliers were removed from analysis. Significant difference was tested using a paired t-test, with significant differences found between the length-at-age estimated using whole otoliths age determinations and sectioned otolith age determinations

Age	Whole		Sectioned		T-test Significance
	Male	Female	Male	Female	
1	16.00 ± 0.00	18.60 ± 4.70	-		
2	16.13 ± 2.21	17.82 ± 3.59	13.50 ± 1.73	13.67 ± 1.37	>0.05
3	16.82 ± 2.94	18.24 ± 4.36	16.55 ± 3.08	16.15 ± 3.11	>0.05
4	18.09 ± 1.96	21.19 ± 4.25	16.33 ± 2.25	19.08 ± 3.93	>0.05
5	17.80 ± 1.94	22.15 ± 3.15	17.22 ± 2.58	20.73 ± 4.39	>0.05
6	20.00 ± 0.00	23.88 ± 4.46	17.53 ± 2.74	21.54 ± 4.44	>0.05
7	19.00 ± 0.00	-	18.00 ± 2.35	22.80 ± 4.60	>0.05
8	-		18.14 ± 2.38	21.62 ± 2.96	
9	-	-	19.00 ± 0.00	28.00 ± 0.00	
Total	364	664	364	664	

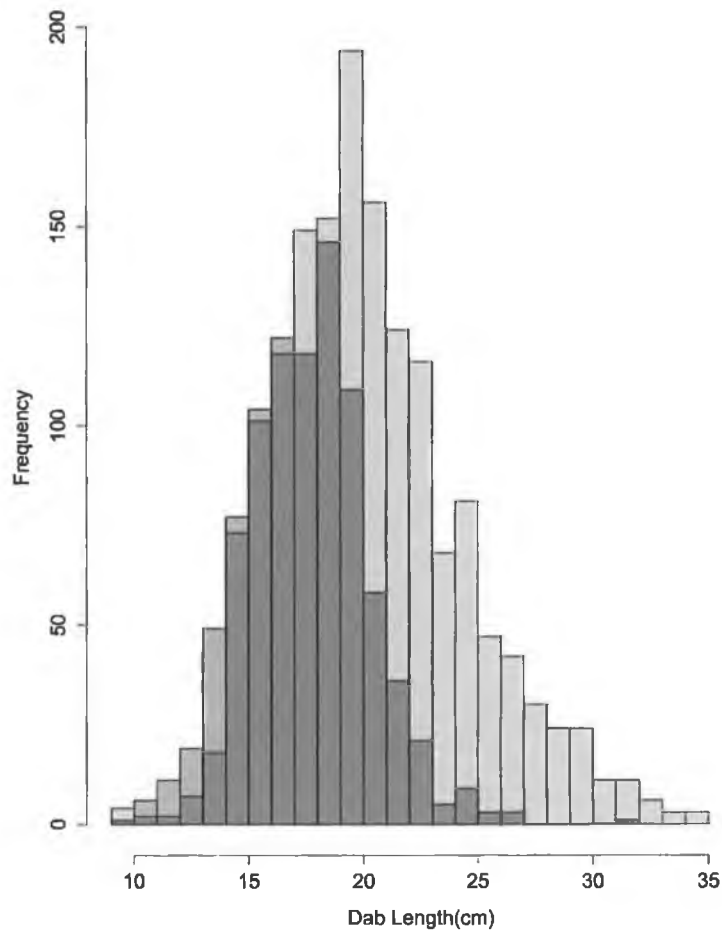


Figure 4.2: The length frequency distribution for both male and female dab ($n = 2464$). The light grey bars represent female length distribution, while the darker bars represent males.

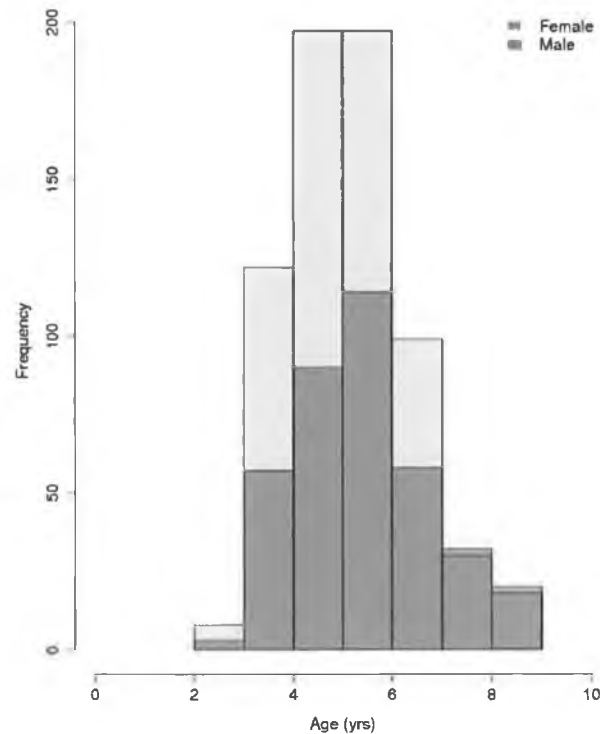


Figure 4.3: Age frequency plot for female and male dab ($n = 536$).

4.3.1.3 Sex-ratio

The chi-square test identified significant deviation from the expected 1:1 ratio of male to female dab during the sampling period ($X^2 = 119.76, p < 0.05, n=2534$), and significant differences in sex ratio over time ($X^2 = , p < 0.05$). The sex ratio was assessed on a bi-monthly basis, where a sex ratio of 1:0.6 (female:male) was observed. Males never outnumbered females during sampling, however the ratio of female to male did increase in favour of males during the months of June, July and August. During these months of reproductive rest, the ratio of female to male fish increased to 1:0.8 ($n=552$). Concurrently during periods of reproductive activity (February), the monthly ratio increased to 1:0.9 ($n=448$). The lowest ratio was identified in the months of November and December (1:0.3, $n=448$).

4.3.2 Comparison of ageing methodology

Comparison of individual reader against modal age identified an increase in precision over time based on comparisons of the first read and second read of each methodology over both age readers (Table 4.2). There was a significant

difference between sectioned and whole otoliths in age determinations of dab *Limanda limanda* ($p < 0.05$, $n=379$). MANOVA identified a significant difference in age determinations of dab between the first and second age determination of both whole and sectioned otoliths by reader 1 ($p = 0.04$). Pairwise comparisons using MANOVA identified reader identity to be of significant value in the age determination of sectioned otoliths ($p < 0.05$).

Table 4.2: Coefficient of variation (CV) and average percent error (APE) are presented for comparisons of modal age against reader age estimates over time. Hoenig's test of symmetry, represented by the symbol p , indicates significant variation between first and second reads of reader 1 in both whole otolith and sectioned otolith age determination. The significant values are highlighted in grey with the significance level set at $p < 0.05$.

Reader	Reader	Time	Method	p	APE	CV
Modal Age	Reader 1	1st Read	Whole otoliths	0.42	6.29	8.89
Modal Age	Reader 2	1st Read	Whole Otoliths	0.22	1.08	1.54
Modal Age	Reader 1	2nd Read	Whole Otoliths	0.04	9.17	12.96
Modal Age	Reader 2	2nd Read	Whole Otoliths	0.11	1.74	2.46
Modal Age	Reader 1	1st Read	Sectioned Otoliths	0.08	17.91	25.31
Modal Age	Reader 2	1st Read	Sectioned Otoliths	0.23	11.13	15.74
Modal Age	Reader 1	2nd Read	Sectioned Otoliths	0.04	13.16	18.62
Modal Age	Reader 2	2nd Read	Sectioned Otoliths	0.28	5.52	7.81

4.3.2.1 Within reader bias and precision

No systematic bias was identified within reader comparisons of whole otolith age determinations, with both first and second reads of whole otoliths demonstrating the exact same levels of precision ($CV=0$, $APE=0$ and percent-agreement=100%). However, Hoenig's test for bias identified significant systematic differences in the assignment of age in sectioned otoliths in reader 2 age comparisons ($p < 0.05$). In comparisons of precision within the reader comparisons, reader 2 was identified as having the higher level of precision and agreement in sectioned otolith age determinations (Table 4.3).

Table 4.3: Within reader comparisons of ageing precision using age determinations by the sectioned otolith method. Honeig's test of symmetry is represented by the symbol p . Significance level was set at $p < 0.05$. (n=96)

Reader	Reader	Method	p	APE	CV	%agr
Reader 1	Reader 1	Sectioned	0.07	5.19	7.35	64
Reader 2	Reader 2	Sectioned	0.00	5.00	7.07	78

4.3.2.2 Between reader bias and precision

Hoening's test of bias in age determinations identified no systematic differences in the assignment of age between the two readers using the whole otolith ageing method for *Limanda limanda*. Systematic difference in the assignment of age for sectioned otolith age determinations varied significantly between readers over time ($p = 0.00$, n=96). Mean percentage agreement for the sectioned otolith method was 12% for between readers, as compared to 100% for whole otolith age determinations. Within reader precision on the second age determination read showed an increase in precision (Table 4.4). The coefficient of variation (CV) identified higher levels of precision within age readers, than between age readers, but also identified higher levels of precision in terms of CV and APE in age determination using whole otoliths (CV=0%, APE=0%). Mean percentage agreement for whole otoliths was calculated at 100% for between readers and within readers. In comparisons, sectioned otoliths demonstrated a mean CV of 7% for within reader comparisons, and 35% for between reader comparisons.

Table 4.4: Between reader comparisons of ageing precision using age determinations by the sectioned otolith method. Honeig's test of symmetry is represented by the symbol p . Significance level was set at $p < 0.05$. (n=96)

Reader	Reader	Method	Time	p	APE	CV	%agr
Reader 1	Reader 2	Sectioned	1st read	<0.05	28.17	39.84	9.38
Reader 1	Reader 2	Sectioned	2nd read	<0.05	21.89	29.89	13.54

4.3.3 Growth of *Limanda limanda*

Growth analysis of dab was based on sectioned otolith age determinations due to lower AIC values (AIC, whole=883.50, sectioned=654.54). The maximum likelihood ratio test indicated that the growth functions between sexes were significantly different ($p < 0.05$). The estimated growth curves using sectioned otolith age determinations were plotted for female *L. limanda* in Figure 4.4, with the estimated growth parameters for both sexes presented in Table 4.5. The theoretical asymptotic mean lengths (L_{∞}) based on age determinations from whole otoliths (M=19.3cm, F=16.74) and sectioned otoliths (M=22.17cm, F=23.24cm) were less than the maximum observed lengths (M=26cm, F=33cm) suggesting an underestimation of L_{∞} .

Growth performance indexes (ϕ) and von Bertalanffy parameters obtained by other authors are summarized in Table 4.7. Previous studies on *L. limanda* elsewhere (Table 4.7) have reported L_{∞} of between 28-30cm for females and 21 – 25cm for males, with k values of 0.26 – 0.40. Markedly slower growth of male dab was indicated by a lower k value in male sectioned otolith age determinations (0.09). The t_0 value for this study varied between method and between sex, and indicated erratic estimates of down to -10.82 years in the worst case scenario (Table 4.7).

Table 4.5: Growth parameters estimated by different models for male and female dab. Age data used for models was taken from sectioned otolith age determinations, due to non-convergence of whole otolith age determinations when applied to nls model. Values in parentheses indicate the standard error. Results from likelihood ratio tests to choose among the models are also shown, indicating the difficulty in choosing of a single model to describe growth of dab in the study. L_{∞} =asymptotic mean length, k=growth rate, t_0 =theoretical age at zero length, LL: log likelihood value, λ :likelihood ratio, p: significance for the chi-square test (d.f.=4 in all cases)

Growth Function	Sex	Model Estimates			Likelihood Ratio Tests		
		L_{∞}	k	t_0	LL	$-2\ln\lambda$	p
von Bertalanffy	Female	23.24(01.27)	0.49(0.17)	00.52(00.66)	-942.57		
von Bertalanffy	Male	22.17(19.6)	0.09(0.37)	-10.82(29.27)	-437.12	1010.87	1
Schnute	Female	12.04(01.55)	22.88(0.88)	00.17(02.91)	-942.57		
Schnute	Male	15.51(00.88)	18.38(0.68)	00.09(00.37)	-437.12	1010.87	1
Galucci Quinn	Female	11.47(03.36)	0.493(0.17)	00.52(00.66)	-942.57		
Galucci Quinn	Male	2.08(06.26)	0.09(0.37)	-10.82(29.27)	-437.12	1010.87	1
Logistic	Female	22.63(00.92)	-1.25(0.54)	00.73(00.20)	-942.56		
Logistic	Male	21.23(12.84)	0.72(1.39)	00.14(00.38)	-437.13	1010.33	1

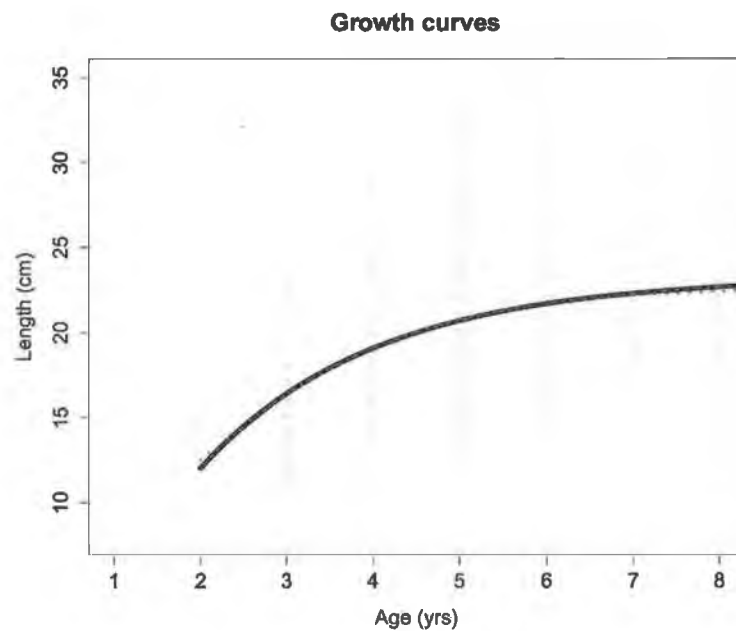


Figure 4.4: Estimated growth curves for female dab *Limanda limanda*, off Ireland's coastline; black line= VBGM, grey line= Schnute model, dotted grey line= Galucci Quinn mode and dashed purple line= Logistic model. Circular symbols represent observed length-at-age data ($n = 337$). Note that all growth model lines overlap on one another with very little variation. This overlap is further evident in the growth model choice using the Akaike criterion.

Likelihood ratio tests showed no significant differences between growth obtained for male and female dab comparisons, except in the final hypothesis where $L_{\infty 1}=L_{\infty 2}, k_1=k_2, t_0 1= t_0 2$ (Table 4.6) which appears realistic, reflecting the significant correlation between these parameters in growth modelling.

Table 4.6: Likelihood ratio tests comparing von Bertalanffy growth parameters for both sexes combined according to (Kimura, 1980). Likelihood ratios were calculated for each model comparison by using residual sum of squares and tested against chi-square statistics with the appropriate degrees of freedom. Blanks indicate parameters which were not estimated.

Hypothesis	Restriction	L_{∞}	k	t_0	$-2\ln\lambda$	d.f.	p
1	Null	40.28	0.08	-3.13			
1	$L_{\infty 1}=L_{\infty 2}$	37.36	0.09	-2.78	1.53	1	0.22
2	$k_1=k_2$	33.81	0.13	-2.23	0.99	1	0.32
3	$t_0 1= t_0 2$	35.79	0.11	-2.51	0.22	1	0.64
4	$L_{\infty 1}=L_{\infty 2}, k_1=k_2, t_0 1= t_0 2$	25.57	0.17	-2.60	26.09	3	0.00

The highest second order information criterion or AIC_c value was estimated using the Logistic model (1884.58) which was considered the best model for describing the growth of dab with an Akaike weight of 30%. In both comparisons of models, the VBGM model was considered the second best and achieved in both cases similar AIC_c values, with mean Akaike weights of 28% and 25% for female and male dab respectively. The first three models are mathematically similar, as they are all re-parametrizations of the same original model (VBGM), but differ in covariance structure.

The "average" model was determined by averaging the predicted model values as a consensus parameter between the model with substantial statistical support (Logistic and the Schnute). The von Bertalanffy, Galluci-Quinn and Schnute growth models are mathematically equivalent, so for both sexes of dab the logistic model was compared against the Schnute model. Thus, for female dab, there was a difference of 0.53 in Akaike weight estimates in favour of the logistic model, while for male dab there was a difference of 0.005.

Growth performance indexes/phi-prime test (Φ) on the von Bertalanffy parameters are summarised in Table 4.7, with sectioned otoliths used for comparison. Φ was used to compare the growth parameters L_{∞} and K estimated for the sexes separately with those obtained in other studies.

Table 4.7: Parameters of the von Bertalanffy growth equation (L_{∞}, k, t_0) obtained by different authors, differentiated by sex and area. The various size ranges and ages covered in the studies are presented in the footnotes. Age determination in previous studies were obtained using whole otolith age determination methods, with this study presented data for sectioned otoliths. Male VBGM for whole otoliths are presented, although females are not due to non-convergence in nls model.

Author	Method	Sex	Area	L_{∞}	k	t_0	Φ
This study ¹	Whole	Female	Ireland Waters	16.74	-0.31	-1.52	N/A
This study ²	Whole	Male	Ireland Waters	19.29	0.35	-2.41	4.86
This study	Sectioned	Female	Ireland Waters	23.24	0.49	0.52	5.59
This study	Sectioned	Male	Ireland Waters	22.17	0.09	-10.82	3.78
Folner (2000) ³	Whole	Female	Irish Sea	31.03	0.40	-0.28	5.97
Rijnsdorp et al. (1992) ⁴	Burnt sectioned	Female	Eastern North Sea	30.50	0.28	-0.07	5.56
Rijnsdorp et al. (1992) ⁵	Burnt sectioned	Male	Eastern North Sea	25.00	0.26	-0.34	5.90
Ortega-Salas (1988) ⁶	whole	Female	Irish Sea	33.00	0.32	0.24	5.85
Ortega-Salas (1988) ⁷	Whole	Male	Irish Sea	22.00	0.60	0.43	5.68
Bakhsh (1982)	Whole	Female	Anglesey Waters	28.00	0.30	-0.88	5.46
Bakhsh (1982)	Whole	Male	Anglesey Waters	21.00	0.42	-1.25	5.22

¹Size range 14-32cm, 1-7 years.

²Size range 14-24cm, 1-6 years.

³Size range 8-28cm, 0-6 years.

⁴Size range 11-32cm, 2-11 years.

⁵Size range 9-23cm, 1-11 years.

⁶Size range 4-36cm, 0-8 years.

⁷Size range 3-27cm, 0-7 years.

4.4 Discussion

4.4.1 Meristic Parameters

The length distributions of dab in the current study were dominated by the size groups 15-21cm and few dab below 10cm were caught. In general, the estimates of length-weight relationships obtained here are similar to those reported in Ortega-Salas (1988). Differences in sample size at specific length margins, specifically in the larger sizes, can explain the differences in values reported in other studies.

In the current study, the length range of the specimens was similar to those studied by (Folmer, 2000) in the Irish Sea. However, while similar growth rate values (k) were obtained for female dab, and similar growth performance indexes were recorded, that is where the similarities end. When parameters obtained in the North Sea (Rijnsdorp et al., 1992) are compared to the present study, the growth rates estimated for Ireland's population are substantially higher for female dab and lower for male dab. The higher growth rate for North Sea (Rijnsdorp et al., 1992) in males may reflect differences in sampling, where dab in the most shallow waters of the North Sea grow faster in comparison to dab caught offshore. This has previously been demonstrated by Bohl (1957) and Jónsson (1966) for dab. There was a marked difference in length-at-age and length frequency distribution between sexes consistent with previous studies (Folmer, 2000; Rijnsdorp et al., 1992; Lozan et al., 1989; Bakhsh, 1982) and indicative of sexual dimorphism, where females attain a larger size-at-age than males. Difficulties in the attainment of an age length key for the age categories of 7 years onwards may reflect a small sample size, or length sizes in this age category which were significantly shorter than the predicted length.

The observed length-at-age data of *L. limanda* from Ireland's coastline when compared to existing length-at-age data for dab from the North Sea (Rijnsdorp et al., 1992) identify significant differences ($p < 0.05$) in length-at-age data from age group 3 onwards. This potentially reflects differences in growth patterns between populations dependent on reproductive activity. As Lee (1972) noted, dab becomes reproductively active at an approximate age of 2, equivalent to a length of 14cm. In the current study, differences in lengths between the two sexes becomes more prominent after age 3, a finding which is similar to results obtained in Chapter 2. This difference in growth between the two sexes is in agreement with a study by Rijnsdorp et al. (1992) for the

North Sea. Jónsson (1966) also noted a shift in growth rate in females at 2-3 years in waters off of Iceland, whereby the female gained an average of 2-3cm in length on the male dab.

Peak abundance and dominance of male dab sampled during the reproductive rest period (June, July and August) from Ireland's coastline fit a pattern previously reported by Bohl (1957) for the North Sea. Female dab return to the coastal feeding grounds earlier than the males, artificially inflating the observed sex ratio. Previous studies of dab have reported ratios in the range of 1.5:1 in favour of female dab from populations sampled in the Irish Sea (Ortega-Salas, 1988). In contrast, dab sampled from the coast of Iceland (Jónsson, 1966) reported a reverse in sex ratio, with males being more dominant than females. Based on the sampling grounds off the coast of Ireland, the observed sex rates may be an artefact of the sampling location, as has previously been noted in a study by Jónsson (1966). Female dab in this study are more common in shallow coastal waters of Iceland. Females often predominate populations from stable environments (Lambert, 2008). The sex ratio can vary between populations, year-classes and even within a single year class. This variability in sex ratios emphasize the importance of estimating reproductive potential annually.

4.4.2 Age and Growth

Studies of fish age and growth arguably provide some of the most useful biological information for stock assessment modelling, with fish age data used as an estimation of the age structure of a population. Dab have previously been aged by interpreting annual growth marks on otoliths (Folmer, 2000; Rijnsdorp et al., 1992; Ortega-Salas, 1988; Bakhsh, 1982). However, to date the use of otoliths to age dab has not been validated. During a workshop by ICES, the working group on age reading of dab (ICES Advisory Committee, 2010b) proposed a standardized ageing methodology using whole otoliths, where age ranged from 1 to 5 years. Evaluations of precision of this method indicated that it was not precise according to criteria of (Campana, 2001). Based on the difficulty in assigning ages to the older age range during this study, ICES Advisory Committee (2010b) recommended that otoliths be sectioned in order to investigate whether precision could be improved.

Ideally, age and growth studies of fishes are conducted with multiple age "readers", so that individual biases can be identified and an assessment of ageing precision be conducted (Appelberg et al., 2005; Campana, 2001). However,

an inherent problem with the determination of precision of an ageing method lacking in any validation is the knowledge that human interpretation, regardless of training, varies over time (Kimura et al., 2006). The accuracy of a fish age can only be determined through age corroboration studies and validation studies (Campana, 2001), with the effect of inaccurate age determinations well documented (Melvin and Campana, 2010; Cope and Punt, 2007; De Pontual et al., 2006). Age validation is always necessary in age studies, however rarely achieved. Age corroboration is the next best thing, with the present study presenting the best information available given current knowledge.

In the current study, no significant bias and good precision was identified between and within readers using the whole otolith ageing method. This is in direct contrast to results of sectioned otoliths, which identified significant bias and lower precision than expected. For both within reader and between reader comparisons of sectioned otolith age determinations, precision was improved over the duration of the study. Although, within reader comparison showed good agreement, with good precision as determined by Campana (2001), between reader precision was almost triple that of within reader comparisons. Differences between readers were based largely on the experience each had in examining otoliths. While both readers had sufficient experience in consistently estimating the age of whole otoliths as demonstrated by the 100% agreement in modal age comparisons and between and within reader comparison, Reader 1 had no experience in reading sectioned otoliths. Where readers may not have agreed, using sectioned otoliths, difference may have been caused by several factors: the location of the first annuli, sections not cut through the otolith nucleus/core and split rings (false annuli). This collection of factors affecting the inter-reader precision in sectioned otoliths has been previously noted in angelfish (Steward et al., 2009). Campana (2001) acknowledges that the ageing of whole otoliths, also referred to as surface reading, becomes less reliable at older ages. Sectioned otoliths offer an alternative to the common whole otolith ageing methodology for dab. The current study demonstrated that within reader comparisons and comparisons to modal age had acceptable levels of precision for short lived species (Campana, 2001). A disadvantage of the sectioned otolith ageing method for *Limanda limanda* is the lack of clear ageing criteria for this method in comparison to whole otoliths. Studies on various fish species have shown improved precision in ageing when using sectioned otoliths (Katayama et al., 2010; Stransky et al., 2005; Mc Dougall, 2004).

Growth dynamics in fisheries are complicated, with existing growth patterns of heavily fish stocks substantially different from pristine or light exploited conditions. Moreover, distributions of ages and lengths are quantitatively related to specific catch-ability of the gear in use, with factors such as sample size and sampling duration also playing an important component in the applicability of this data to growth models. Estimations of growth parameters for this study were based on the use of different growth models (Burnham and Anderson, 2004) and included the statistical weight for each model using the Akaike information criterion (*AIC*) as indicative of the robustness of the growth model to the fish data (Katsanevakis and Maravelias, 2008). Accordingly the lowest *AIC* is interpreted as identifying the model that best describes the growth of the species. Candidate models were chosen according to the criteria of Katsanevakis and Maravelias (2008), where a VBGM, a sigmoid curve model and a non-asymptotic model were included in the study design. However, the choice of the best growth model is purely subjective, and in some cases is based on the decision of the researcher and their level of experience in reading otoliths from the species in question.

Among the four growth models employed in the present study, The Logistic model proved more statistically appropriate for describing dab from Ireland's coastline, based on Akaike criterion. The von Bertalanffy growth model was chosen for both female and male dab, as the second model that best fitted the data.

Regarding the Akaike weight, when no model has an Akaike weight of greater than 90%, Multi-Model Inference (MMI) should be performed for agreement among plausible models (Katsanevakis, 2006). Both the VBGM, Galucci-Quinn and Schnute models all achieved similar Akaike weights (30%), indicating the applicability of the consensus estimate of multi-model Inference between them. Thus, when there was no clear model choice, the choice of the second best model is dependent on the first model (Katsanevakis and Maravelias, 2008). Based on the current study, the logistic model was identified as yielding the best fit followed by the VBGM, with the other two models placing joint third. The choice of the Logistic model over the commonly applied VBGM in this study, may reflect differences in age composition of the dab population. Previous studies on dab growth have demonstrated different age and length ranges in their samples (Table 4.7) when compared to the current study. The current investigations falls in the middle of previous studies age and length frequencies, thus making the asymptotic L_{∞} calculated using

the maximum likelihood lower than expected.

Although there is uncertainty in the choice of these two growth models based on Akaike weights, model selection is at best an approximation of the biological growth being investigated (Symonds and Moussalli, 2011). The "concept" of a true and universal law of fish growth is of very little use in wild populations, where growth is a complex process mediated by many small effects and generally unknown interactions (Katsanevakis and Maravelias, 2008). According to some authors, although the von Bertalanffy is one of the most used growth functions in fisheries sciences (Haddon, 2001) and allows for comparability between studies, it is not necessarily the best descriptor of individual growth (Katsanevakis and Maravelias, 2008; Cailliet et al., 2006). Previous studies on growth of dab have used the von Bertalanffy growth model as the model that best fits the growth of dab (Folmer, 2000; Rijnsdorp et al., 1992), with Ortega-Salas (1988) reporting that the von Bertalanffy adequately described growth of dab in the Irish Sea, after comparison with the Gompertz model. Comparisons of L_{∞} obtained in this study, using the VBGM, with those obtained by other authors throughout the distribution of the dab, identified large differences between the various study areas (Table 4.7) (Folmer, 2000; Rijnsdorp et al., 1992; Lozan et al., 1989; Bakhsh, 1982).

In the majority of growth studies of dab, the L_{∞} is reported in the upper 25cm in total length for females. However, in the current study, a value of 23cm was recorded, with only a small difference in L_{∞} identified between the two sexes. Estimated values of L_{∞} for the dab growth model were inconsistent with maximum observed total lengths for both sexes during the study (M=26cm, F=33cm). Growth models that emerged in this study were much flatter than expected (Figure 4.4) and may reflect the lack of younger individuals in the samples (Minimum age in the sample is 1, but not validated). Cailliet et al. (2004) previously reported that growth model estimates may be largely impacted by the lack of very young or very old individuals, which can result in the overall variance of the growth model becoming inflated. Unfortunately this can also result in dramatically different estimates of K , and t_0 as is seen in the growth model of male dab using sectioned otoliths (Table 4.7).

Lack of young fish often results in an age-at-length plot which is primarily linear which makes it difficult to accurately portray the "early" curvature of the growth model. Conversely, lack of very old individuals in the sample can also impact the model, due to not enough old fish to accurately characterize the asymptote. In order to solve these problems in the data set, it is suggested

that more young fish are added to the sample, or that the t_0 is fixed at a constant in order to anchor the young portion of the model. The lack of young fish in our sample is primarily due to the use of commercial trawls during the study, which do not cover inshore dab nursery areas. This has previously been seen in Icelandic dab populations, where in shallow waters of less than 40 meters, sizes 14-20cm were most common, with juvenile dab (<13cm) captured in depths of less than 2 meters (Jónsson, 1966). We would suggest that the lack of old fish in the sample could be related to a mixture of factors including size selective fish gears, under ageing of older fish or the mortality rate in the fish being high enough that the fish do not live long enough to approach the asymptotic length. In order to improve the model, we feel it is essential that the age of dab be thoroughly investigated including validation of the age of the fish, and the preparation of a calibration set of otoliths in order to increase precision and reduce bias, thereby improving the estimations of the growth parameters of the fish.

Within the wide geographic area that dab inhabit, Φ values obtained from our growth parameters and those published elsewhere showed similar results to those of Rijnsdorp et al. (1992) and Bakhsh (1982) for female dab, while Folmer (2000) gave a lower growth performance (Table 4.7). Values of k and t_0 estimated from male sectioned otoliths were particularly low, while the value of L_∞ was similar to other studies (Table 4.7). Comparing the growth performance results obtained with sectioned otoliths growth models, it can be observed that the values obtained for age and growth are quite similar. The t_0 value had a relatively small absolute value, and is probably explained by the absence of 0-age specimens in the study sample. In the study comparisons, it is suggested that the variability in growth performance may be due to differences in ageing methodologies or sampling strategy. However, it should be noted that there is evidence to suggest that similar stocks living in different environments may evolve hidden genetic adaptation resulting in higher growth performance to overcome a shortened growing phase (Jobling, 2002), and this may have implications in terms of management of the Irish stock.

4.4.3 Conclusion

Based on the observations made in this study, expansion of the ageing guidelines for sectioned otoliths needs to be investigated, potentially in a future ICES dab age reading workshop. In the current investigation, although the differences between readings of whole and sectioned otoliths were significantly different

($p < 0.05$), it is recommended that sectioned otoliths should be implemented as the standard means of preparation. Our findings indicate that while guidelines proposed by ICES Advisory Committee (2010b) allow for consistent age estimation for dab using whole otoliths, sectioned otoliths are more representative of growth based on the age length key (ALK) and multinomial logistic regression.

Results of the current study indicated that dab experience lower growth rates than those of dab populations elsewhere, and are only slightly similar to results obtained from the Irish Sea (Table 4.7). These results must be taken with caution. As previously discussed, a lack of young fish in the sample and small number of larger at age fish may have influenced both the growth coefficient and the asymptotic length, two central components of the estimate of growth performance. In order to verify if there is a significant difference between the growth of these two locations, further sampling would need to be undertaken, specifically targeting the inner coastal areas where younger immature fish are located. This would need to occur to ascertain whether another management unit for dab is necessary.

This study represents the first detailed estimate of age and growth in dab from Ireland's coastline, which can be used to enhance the sustainable management of a dab fishery in future stock assessments.

Chapter 5

General Conclusions and Future Research

The work presented in this study demonstrated the impracticality of assuming that biological information collected from fish in one ecosystem within its geographical range is similar to that of another ecosystem, especially within a constantly changing marine environment. Changes in reproductive cycles, age and growth of fish is not an uncommon in recent years. Changes in environmental conditions are forcing fish to adapt their biological processes in order to facilitate their continued survival (ter Hofstede et al., 2010). In fact, changes in fish distribution and ecosystem assemblage compositions are now common, where the assumption of similar biological characteristics found in previous studies of similar species is implausible.

Although dab is not currently managed as a commercial fishing stock in Ireland, due to relatively small commercial landings and low value, it is included with flounder in a combined TAC (Table 1.1). The necessity for sustainable fishing is increasing, with detailed life history information crucial for the successful management of a potential dab fishery off Ireland's coastline, as well as facilitating the increased understanding of Ireland's coastal ecosystem.

The current study was successful in providing a visual guide for determination of reproductive phases, verified through histological analysis. The identification of the spawning period in early spring will allow for the future investigation of spawning stock biomass which will support any future management objectives for sustainable fishing. An accurate description of size and age at maturity is not only considered critical for understanding a species' life history (Dieckmann and Heino, 2007), but is also essential in fisheries management. The calculations of proportion mature at age and L_{50} presented in the current study contrast with previous assessments (14cm and 16cm for male and female dab respectively). Htun-Han (1978b) previously states that maturity occurs in this fish at a length of 10-14cm. Results of the current study indicated that at this combined length category, only 31% of the population (both sexes) was reproductively mature. The higher proportion mature at age and increased L_{50} suggest that dab in Ireland mature at larger sizes than previously estimated, which will have implications for its future management.

Although the precision of the age determinations is not as high as would be expected, given the accepted use of whole otoliths as the ageing methodology for this fish (Folmer, 2000; Henderson, 1998; Ortega-Salas, 1988, 1987; Jónsson, 1966; Bohl, 1957), the identification of a workable age length Key (ALK) using sectioned otolith and verified by multinomial logistic regression, allows for the possibility of increased precision. This is under the provision that strict

ageing methodologies/protocols are applied for use between all institutions which age dab. The preliminary determination of the logistic growth model as the model which best fits the growth data of this species is questionable. The lack of young of year within the sample, and length categories below 9cm, may have biased the results, in terms of estimates of the growth coefficient (k) and asymptotic length (L_{∞}) (Chapter 4:Discussion). Previous studies, while not having a similar range of sizes in comparison to the current study, have used the von Bertalanffy growth model (VBGM) with young of the year included in the sampled data. A common practice among research of fish growth is to adopt the von Bertalanffy growth model (VBGM) (Katsanevakis and Maravelias, 2008), as it is the most used and ubiquitous equation to describe the growth of fish and other animals (Russo et al., 2009). Although the logistic model was chosen as having the best fit to the data, the VBGM runs a close second for both sexes. The inclusion of younger length categories, or the adoption of a static t_0 within these models may change the outcome of the best growth model to the data, and warrants further investigation.

5.1 Implications of the study on similar populations elsewhere

Climate variability and climate changes have led to marked changes in the north east Atlantic marine conditions over the last century, with comparable changes elsewhere. For example, sea surface temperatures in the north Atlantic (including the English Channel, North Sea and Baltic Sea) and Irish Sea have warmed by 0.2 – 0.6°C every decade, equivalent to a 1.3°C over the last three decades (Simpson et al., 2011; ter Hofstede et al., 2010).

Previous studies have demonstrated that increases in water temperatures have significant impacts on the growth of dab (Bolle et al., 1994), with associated studies of flatfish reproduction demonstrating a trend in spawning both later in the year and later in the life cycle (Rijnsdorp and Witthames, 2005). These factors will have significant impacts on assessment of recruitment potential and growth estimates of the species, further delaying any possibility of developing a management strategy for a sustainable fishery for the dab.

The impact of changing environmental conditions on dab's biological parameters is visible in studies of the North Sea population. No two geographical regions will have exactly the same reproductive cycle for dab, with Henderson (1998) noting that different dab population units had peak spawning periods

spanning the months of January to September, based on the necessary environmental cues. However, in comparisons of the current studies, the dab population located off the coastline of Ireland, and a single population located in the North Sea studies by Htun-Han (1978b), the exact same reproductive period was identified for the two regions. Without a recent parallel study in the North Sea for comparison, this observation may simply be a coincidence.

However, it is more plausible to speculate that changing environmental conditions may have altered the reproductive cycle of the North Sea dab population, and potentially populations located elsewhere. It is acknowledged that the North Sea has been warming significantly over the last decade, with many fish experiencing more northerly distributions (ter Hofstede et al., 2010; Dulvy et al., 2008). Previously, Drinkwater (2002) noted a strong correlation between increasing water temperature and changes in reproductive cycle in North Sea cod stocks. Similarly, Teal et al. (2008) noted that timing of spawning has been influenced by environmental temperature during the spawning period of other flatfish species in the North Sea over the past thirty years (1970-2004). Temperature has a strong influence on egg maturation rates, growth and survival of fish larvae and impacts on the planktonic communities that support food webs which sustain commercial fisheries. In fact, although Ortega-Salas (1987) indicated that the dab located off the coast of the United Kingdom could support a fishery, this may no longer be accurate. Further studies would need to be initiated throughout the range of dab distribution in-order to ascertain the scale of the impact that changes in reproduction would have on the future potential management of a dab fishery.

5.2 Recommended Future Work

While this study provides valuable biological information for the dab *Limanda limanda*, it also provides valuable information on the ecosystem off of Ireland's coastline. The results of this study are valuable in that they create a baseline for future comparisons and parallel studies with other dab management units. However, more research is needed before we can fully understand the life history of dab and attempt to manage the stock sustainably.

5.2.1 Estimates of Fecundity

Given the substantial impact of fishing on the demography of fish stocks (Planque et al., 2010; Wright and Trippel, 2009; Pope et al., 2000), estimation

of stock reproductive potential and recruitment is a top priority of future research for dab. Estimates of dab fecundity have previously been published based on small sample size ($n=13$) (Jastania, 1995). The effects of adult age and body size on stock reproductive output are a critical component of this research. Although Jastania (1995) describes fecundity for the majority of length categories of 19-33cm, only one fish is represented for each of these length categories. The smaller reproductively active length categories are not represented at all. Maternal size has an important influence on fecundity as it contributes both to the quantity and quality of the eggs produced (Lambert, 2008). For example, the larger the fish, the more eggs it can produce. Naturally if the fish is in good condition, the eggs may be larger as a survival mechanism. Ideally, the fish fecundity should be measured at a time when a stock is being reduced (fished), has different feeding conditions (different prey items) or varying water temperatures. This would enable the production of a set of predictive equations of fecundity which could be used to convert spawning stock biomass into stock egg production. Time of spawning should be noted concurrently as early life survival is influenced not only by egg size, but by the environmental conditions that the parental reproduction occurs in.

5.2.2 Validation of age determinations

The current study has shown that the use of whole otoliths for the age determination of dab is not precise enough for management purposes, with coefficient of variation ranges far above the standard (standard=5%, whole otoliths =9–23%). The use of sectioned otoliths provides a suitable alternative to whole otolith ageing criteria, and has been shown to give a more accurate portrayal of growth (Figure 4.1, Table 4.7). Regardless, it is crucial that age estimates of dab are validated. The use of the mark and recapture methodology is suggested as a means to validate age estimates.

5.2.3 Improved knowledge about how dab stocks react to external factors, both abiotic and biotic

Climate warming and the combined effects of overexploitation has resulted in a steady decline in northern winter spawning flatfish in recent years (Millner et al., 2005). In 2000, catch statistics for dab fishing from Irish waters estimated that over 39 tonnes was caught by fishing vessels. In contrast, estimated catches of 2009 were 3,000 tonnes. It is believed that this decrease in catch statistics is

primarily due to unreported catches by the Netherlands, Norway and Spain.

In Ireland, dab is not specifically targeted in fisheries. Instead, they are taken as by-catch by demersal trawlers targeting other flatfish, shrimp and demersal species. Discard rates of dab in these fishing vessels varies between 50 – 98% depending on the fishing fleet. Discarding in Ireland alone, may be a driving force of the apparent decline in dab biomass based on these discarding practices. Doubling time of a population of dab can be up to 4 years (Froese, R. and Pauly, D., 2004). Investigations into the viability of increasing mesh sizes to reduce by-catch in dab is necessary if a sustainable fishery is to be established. This will potentially have a knock on effect on spawning biomass, potentially strengthening the year class strengths and allowing the dab population to retain equilibrium.

5.2.4 Improved monitoring of biological parameters

Long term monitoring of key biological characteristics of *Limanda limanda* throughout its distribution is critical for survival of this species and many other flatfish species which share similar niches, as well as commercial predator species such as cod and haddock (Ellis and Gibson, 1997). Growth in adult dab is strongly correlated with environmental temperatures, where reduced growth, increased mortality and decreases in condition is common at temperatures above 18°C (Bolle et al., 1994). At the current rate of increase in water temperatures in European waters, it is proposed that the temperature at which these changes in growth will occur will happen within the next two to three decades. Plausible results of this include changes in species distribution, collapses of existing stocks, adaptive changes in reproduction and possibly increased susceptibility to diseases or viruses. Constant monitoring of biological parameters may ensure that a sustainable fishery may be developed and stock collapses due to reduced spawning biomass or overfishing potentially avoided. The vast distribution of dab, over a variety of ecosystems may indicate that these changes may be visible even now. If constant monitoring is employed, modelling of these changes may provide a means of managing the fishery sustainably without the assumed collapse of stocks due to improper application of TAC's and overexploitation.

Chapter 6

Appendix

Study Equipment, consumables and software utilised

Histology Equipment	Model	Manufacturer
Automated Tissue Processor	RVG1	Serosep
Tissue Embedder	Histocentre2	Shandon
Microtome	Slee Mainz CUT6062	Medical Supply
Tissue Stainer	LECIA514040	Lecia
Otolith microtome	Pilses sectioning machine	Pilses
Otolith blade	Diamond wafering blades	Buehler
Microscope and stage	BX41 Olympus stage and microscope	Olympus
Camera	Cannon EOS 200	Cannon
Microscope and stage	Olympus SZX10	Olympus
Camera	Retiga 2000R	Q-Imaging

Histology Consumables	Supplier
4% buffered Formalin	Lennox
Histoclear	Reagecon
Absolute Ethanol	Lennox
Hydrochloric Acid (37%)	Sigma Aldrich
Eosin Y (Alcoholic Solution)	Surgipath
Pertex	Surgipath
Saturate polyester resin (Crystic R115NTP)	Scott Bader

Software Usage	Programme	Supplier
Data collection	Excel(2007)	Microsoft
Imaging software	ImageJ	http://rsbweb.nih.gov/ij/
Imaging software	Image Pro Plus 6.2	Media Cybernetics
Imaging software	Cell*	Olympus Image Software
Statistical Analysis	R	http://www.r-project.org/
Statistical Editor	R-Studio	http://rstudio.org/
Referencing	Qiqqa	http://www.qiqqa.com/
Document composition	Latex	http://www.latex-project.org/ftp.html
Document editor	TexMaker	http://www.xmlmath.net/texmaker/

6.1 Protocols:

Tissue processing

Processing stage	Reagent	Time (hr:min)	Temperature (°)
1	4% buffered Formalin	00:05	Ambient
2	Ethanol (70%)	01:00	Ambient
3	Ethanol (80%)	01:00	Ambient
4	Ethanol (90%)	00:45	Ambient
5	Ethanol (100%)	00:45	Ambient
6	Ethanol (100%)	00:45	Ambient
7	Ethanol (100%)	01:00	Ambient
8	Histo-Clear ¹	00:45	Ambient
9	Histo-Clear	00:50	Ambient
10	Histo-Clear	00:50	Ambient
11	Paraffin	01:00	60
12	Paraffin	01:30	60
13	Paraffin	00:30	60

¹histo-clear is a non-toxic derivative of Xylene, which is a clearing reagent. It can also be found under the name of Histolene/neo-clear.

Staining Protocol

Processing stage	Reagent	Time (min:sec)
1	Histo-Clear	01:20
2	Histo-Clear	01:20
3	Histo-Clear	01:20
4	Ethanol (100%)	01:20
5	Ethanol (90%)	01:20
6	Ethanol (90%)	01:20
7	Running water	01:20
8	Ethanol (70%)	01:20
9	Distilled water	01:20
10	Haematoxylin	01:50
11	Running Water	01:50
12	Running Water	01:20
13	Haematoxylin	01:20
14	Distilled water	01:20
15	1% Acid alcohol ²	00:06
16	Ethanol(70%)	01:20
17	Alcoholic Eosine	01:20
18	Alcoholic Eosine	01:20
19	Ethanol (90%)	01:20
20	Ethanol (100%)	01:20
21	Ethanol (100%)	01:20
22	Ethanol (100%)	01:20
23	Histo-Clear	01:20
24	Histo-Clear	01:20
25	Histo-Clear	01:20

²Acid alcohol is made using a sufficient percentage of hydrochloric acid and diluting with 70% Ethanol.

6.2 ICES Sub divisions

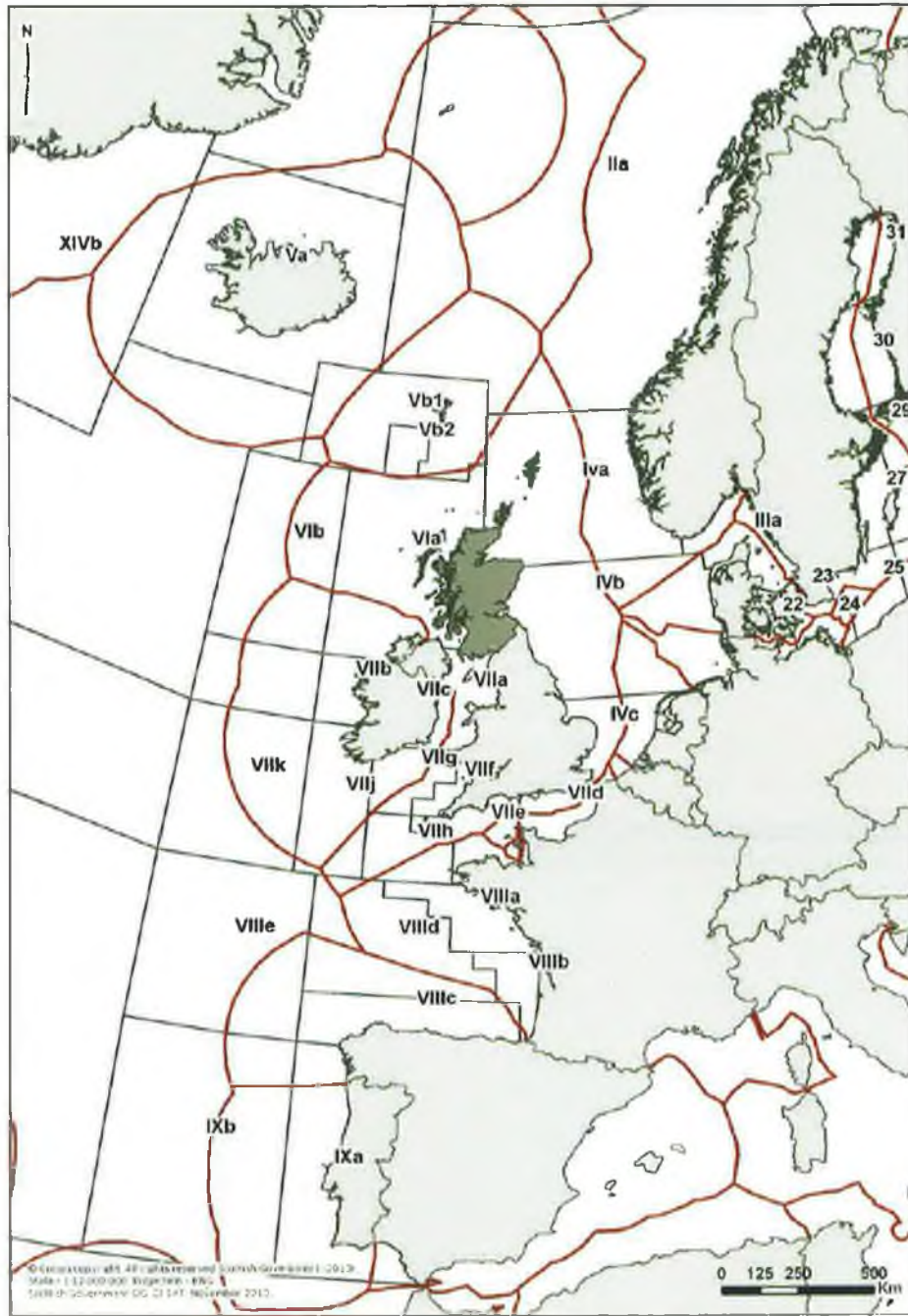


Figure 6.1: ICES Sub-division Boundaries

6.3 Sampling Locations

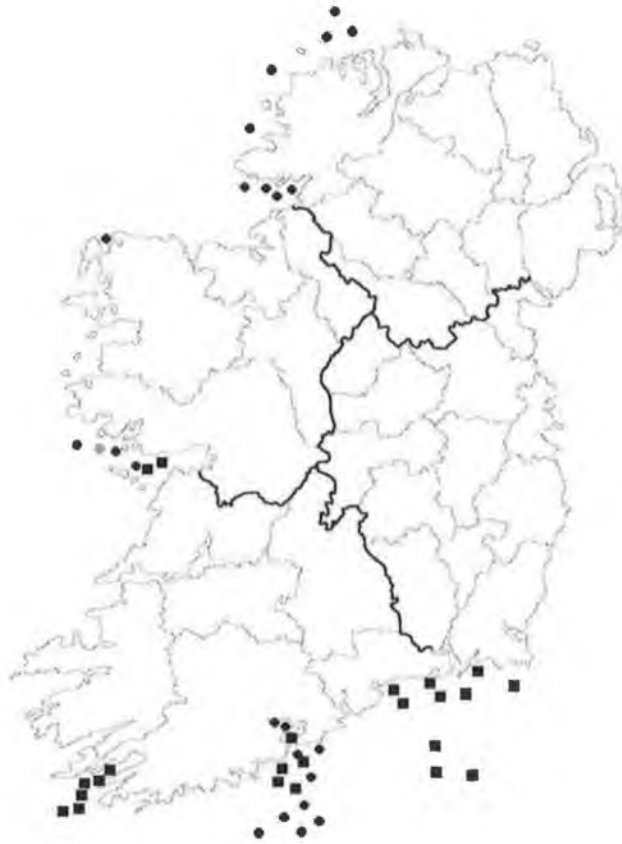


Figure 6.2: Ireland and surrounding fishing grounds. The squares are the locations where samples were caught during scientific sampling surveys carried out by the Marine Institute during the year of 2009. The circles represent dab samples collected using commercial fishing vessels from February 2009 to June 2010.

6.4 Converting model parameters for growth model comparisons

By solving the following growth model specific formulas, the researcher is able to compare between the study growth models:

6.4.1 Schnute Parameterization

$$L_{\infty} = \frac{L_2 - L_1 e^{-K(t_2 - t_1)}}{1 - e^{-K(t_2 - t_1)}}$$

$$t_0 = t_1 + \frac{1}{K} \ln\left(\frac{L_2 - L_1}{L_2 - L_1 e^{-K(t_2 - t_1)}}\right)$$

6.4.2 Gallucci and Quinn Parameterization

$$\omega = KL_{\infty}$$

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