Atlantic herring (*Clupea harengus*) in the Irish and Celtic Seas; tracing populations of the past and present

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A thesis submitted to the School of Science, Galway Mayo Institute of Technology in fulfilment for the degree of Doctor of Philosophy

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June 2008

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Abstract

Several herring stocks are found around Ireland, of which the Irish and Celtic Seas are two. Herring in the Irish Sea spawn in autumn and in the Celtic Sea spawning takes place in autumn and winter. During the first year of life Celtic Sea herring disperse into the Irish Sea. The two stocks mix at nursery grounds in the Irish Sea, hampering the production of juvenile abundance estimates for the Irish Sea stock.

In this study otolith shape was used to classify juvenile herring at Irish Sea nursery grounds as autumn spawned (most likely of Irish Sea origin) and winter spawned fish (most likely of Celtic Sea origin), with a success rate of 86-87%. Otolith shape analysis separated winter spawned juveniles that are retained in the Celtic Sea from those that disperse into the Irish Sea with a high degree of accuracy ($>95\%$). Shape analysis of the first winter ring in the otolith was then used to determine nursery ground of origin in two year old adult Celtic Sea herring, showing that approximately 42% of fish spent their nursery phase at Irish Sea nursery grounds. This provides strong evidence that Celtic Sea herring, which disperse into the Irish Sea as larvae, return to the Celtic Sea as adults by natal homing. The implications of these findings for herring population structure theories are discussed

Otolith microstructure analysis of historical otoliths, collected in the Irish Sea from 1993-2003, revealed interannual fluctuations in the relative proportions of autumn and winter spawned juveniles. The contribution of winter spawned fish (most likely of Celtic Sea origin) was removed from the estimate of juvenile abundance for the Irish Sea stock. This adjustment significantly improved the relationship between juvenile abundance at age-1 and the abundance of age-3 fish from the same year class ($r > 0.8$, $p < 0.05$). This separation method could be routinely used to generate recruitment indices for the Irish Sea stock.

The abundance of winter spawned juveniles in the Irish Sea was negatively correlated with the frequency of strong south westerly winds during the larval phase ($r = -0.96$, $p<0.05$). The underlying cause of this relationship can only be speculated; it may reflect the dispersal of Celtic Sea larvae out of the Irish Sea or increased larval or juvenile mortality during years of frequent strong south westerly winds.

Summary

Atlantic herring (*Clupea harengus* L.) is a valuable commercial species found in both the East and West Atlantic Ocean. Populations range from Georges Bank and the Gulf of Maine in the west to the Baltic and Barents Sea in the east. In the Northeast Atlantic there are approximately fourteen stocks, each with their own spawning time and location, and migratory routes. The factors that preserve this stock complexity have fuelled extensive research and a number of theories have been proposed to explain how population structure is maintained.

Several herring stocks are found around Ireland, of which the Irish and Celtic Seas are two. Herring in the Irish Sea spawn in autumn and in the Celtic Sea spawning takes place in autumn and winter. The two stocks are managed separately; however during the first year of life Celtic Sea herring disperse into the Irish Sea. As a result the two stocks mix at nursery grounds in the Irish Sea, hampering the production of juvenile abundance estimates for the Irish Sea stock.

Otoliths (earstones) provide a permanent record of growth rates in bony fish and can be used to separate stocks with different life histories. This study uses otolith microstructure and shape analysis to distinguish between herring from different spawning seasons and nursery areas. Otolith shape was used to classify juvenile herring at Irish Sea nursery grounds as autumn spawned (most likely of Irish Sea origin) and winter spawned fish (most likely of Celtic Sea origin). The method achieved a classification success rate of 86-87% when ground truthed using the already validated otolith microstructure method. Otolith shape analysis separated winter spawned juveniles that are retained in the Celtic Sea from those that disperse into the Irish Sea with a high degree of accuracy (>95%). Shape analysis of the first winter ring in the otolith was then used to determine nursery ground of origin in two year old adult Celtic Sea herring. The results indicate that approximately 42% of fish spent their nursery phase at Irish Sea nursery grounds. This provides strong evidence that Celtic Sea herring, which disperse into the Irish Sea as larvae, return to the Celtic Sea as adults by natal homing. These findings have important implications for herring population structure theories.

Otolith microstructure analysis of historical otoliths, collected in the Irish Sea over a ten period (1993-2003), revealed considerable annual fluctuations in the relative proportions of autumn and winter spawned juveniles. Separate abundance estimates

were produced for each component and the contribution of winter spawned fish (most likely of Celtic Sea origin) was removed from the estimate of juvenile abundance for the Irish Sea stock. This adjustment significantly improved the relationship between juvenile abundance at age-1 and the abundance of age-3 fish from the same year class estimated from commercial and acoustic survey data from the Irish Sea ($r > 0.8$, $p < 0.05$). This highlights the potential for using the separation method to generate recruitment indices more appropriate for the Irish Sea stock.

There was a significant negative correlation between the abundance of winter spawned juveniles in the Irish Sea and the frequency of strong prevailing winds from a south westerly direction during the larval phase ($r = -0.96$, $p<0.05$). The underlying cause of this relationship can only be speculated; it may reflect the dispersal of Celtic Sea larvae out of the Irish Sea or increased larval or juvenile mortality during years of frequently strong south westerly winds.

Chapter 1

General Introduction

1.1 Herring biology and population structure

Herring are distributed throughout the Northern hemisphere, with populations occurring in the northeast and northwest, Pacific (Pacific herring, *Clupea pallasii* V.) and Atlantic Oceans (Atlantic herring, *Clupea harengus* L.). Within the northeast Atlantic, there are numerous stocks, each with their own spawning time and location, and migratory patterns, which are generally subject to their own distinct fishery (reviewed by Parrish and Savile, 1965). The Atlanto-Scandian complex of herring (Icelandic summer spawners and Norwegian spring spawners) are currently the largest group in the world (FAO, 2004). In 2004, global capture production was over 2 million tonnes, with Norway and Iceland being the countries with the largest catches (FAO, 2004). Their widespread commercial importance has led to extensive research during the last century, which has provided much information on their biology and ecology. Female herring lay their eggs on the seabed, usually in water 10-80m deep, on hard ground covered with small shells, stones or seaweed. The eggs are fertilized in the water by the male herring, which discharge their sperm at the same time as the females lay their eggs. The eggs incubate for between 10-30 days depending on water temperature until they hatch. By the time they reach about 40mm in length they are found at inshore nursery grounds. They can remain as larvae for between three and eleven months (typically seven). The metamorphosis to juvenile includes the development of scales and adult pigmentation. They remain at nursery grounds until they move out to more offshore feeding grounds when mature, usually around three years of age (Parrish and Saville, 1965) although this can vary depending on the stock. Adults migrate between feeding and spawning grounds throughout their lifecycle. These migratory patterns cause uncertainty for the assessment of the associated herring fisheries when fish from different management areas mix.

Herring in north-east Atlantic were historically considered to be one large biological unit (Parrish and Saville, 1965). However consistent differences in certain morphological features were recognized by Heinke (1898) and his co-workers towards the end of the 19th century and herring in northeast Atlantic were divided into

races. Since then a number of different theories have been developed, which try to explain herring population structure. Parrish and Saville (1965) separated the northeast Atlantic groups into shelf and oceanic populations, based on differences in morphology and physiology between the groups. The shelf population was distributed in the shelf waters of the North Sea and adjacent waters (including the Irish and Celtic Seas), while the oceanic population was widespread in oceanic waters, from the northern Norwegian and Barents Sea to the Atlantic seaboard of the British Isles. Shelf individuals were smaller in size to their oceanic counterparts, and also had a shorter lifespan. Many stocks are referred to by the spawning season in which they are productive, with winter spawners spawning between November and January, while autumn spawners spawn between August and October. Oceanic individuals are mostly winter – spring spawners, spawning between February to March, while shelf individuals spawned between August and December (Parrish and Saville, 1965). Cushing (1967) proposed the match/mismatch hypothesis, which suggested that herring spawn in time for peaks in plankton productivity. This resulted in herring larvae being dispersed during periods of high food availability.

While these theories try to predict how herring populations are structured, a number of other theories have looked at the mechanisms that maintain these structures. According to the discrete population concept (Iles and Sinclair, 1982), larval herring populations are kept separate from each other by oceanographic features and fish behaviour. This separation results in genetically distinct herring populations that spawn at distinct locations during seasonally fixed spawning periods. While herring populations maintain distinct spawning times and locations, juveniles from different spawning populations have been identified in mixed aggregations (Brophy and Danilowicz, 2002, Clausen *et al.*, 2007, Husebo *et al.*, 2005, Mosegaard and Madsen, 1996), suggesting that larval populations are not completely isolated from each other. Smith and Jamieson (1986) argued that herring populations expand and contract their range due to environmental and fishing pressures. According to their "dynamic balance" model, the observed lack of genetic variability between the proposed stocks of herring is the result of extensive gene flow between them (Smith and Jamieson, 1986). However this theory does not explain the consistent differences in certain morphological features in herring from different locations. An alternative theory put forward by McQuinn (1997) is based on the metapopulation concept (Levins, 1968).

In his "Adopted Migrant Concept" McQuinn suggested that herring from a number of local populations form one metapopulation. The local populations retain morphological differences, although limited genetic exchange occurs. Population structure is maintained by repeat homing rather than natal homing, i.e. spawning behaviours are not imprinted during the larval phase, but are learned by juveniles from the adult herring with which they associate. This however has not been supported for herring in the Irish and Celtic Seas. Brophy and Danilowicz (2002) identified winter spawned juveniles of Celtic Sea origin in Irish Sea nursery grounds using otolith microstructure analysis. When adult spawning samples were classified to hatch type, only 2% were classified as winter spawned (Brophy *et al.*, 2006) suggesting that these Celtic Sea fish do not remain in the Irish Sea to spawn, and therefore do not learn their spawning behaviour from the adults they associate with in the Irish Sea.

There is little agreement on how herring populations are structured, with no one theory being useful for explaining the observed patterns in different populations. For example, Brophy and Danilowicz (2002) demonstrated that Celtic Sea juveniles that disperse into the Irish Sea do not switch spawning season and take on the spawning behaviour of the adult with which they associate (as predicted by the Adopted-Migrant hypothesis). In contrast McQuinn found sufficient evidence to say that the season of first spawning was not a fixed property in sympatric seasonal spawning herring populations in east and west Newfoundland and that juveniles can switch spawning season (McQuinn, 1997).

1.2 Herring fisheries around Ireland

Herring are one of the most important pelagic species exploited by the Irish fishing industry and important ports in the south and south-west include Cobh, Castletownbere, Dingle and Dunmore East (Marine, 2004).The preliminary total landing figures in 2002 amounted to 30,000 t and were valued at over €6.4 million (Marine, 2004).

Herring around Ireland have been divided into 4 different stocks for management and assessment purposes and are considered to be biologically separate (Marine, 2007). These stocks are as follows and are their ranges are shown in Fig 1.1: -

- West of Scotland – ICES Div. VIa (North)

- Irish Sea – ICES Div. VIIa (North)

- West and North of Ireland - ICES Div. VIa (South) and Div. VIIb

- Celtic Sea and South West of Ireland - ICES Div. VIIa (South); Div. VIIg and Div. VIIj

In 2003, the herring fishery was estimated to be worth more than €12 million per annum to the southwest region, supporting over 1000 jobs between the fishing vessels and the processing industry (Marine, 2004). During the 2003/2004 season, a maximum of 10 vessels were reported to be participating in herring fishing in the Celtic Sea area. While the number of vessels participating in the fishery has decreased in recent years, the efficiency of the larger boats has increased (Marine, 2007).

The main catches within the Irish Sea in recent years have been taken by a small number of Northern Irish trawlers. The UK were allocated 74% of the herring total allowable catch (TAC) quota in 2007, while Ireland was allocated the remaining 26% (Marine, 2007) in the Irish Sea. Within the Celtic Sea, Ireland was allocated 86.4% of the herring TAC Quota for 2007 (Marine, 2007). The fishery within the Celtic Sea was closed as a result of the stock collapse during the mid 1970's, which coincided with a period of high fishing mortality. The use of closed areas on a rotational basis and closed seasons is now used as a method to protect spawning shoals and was first implemented by fishermen in the southwest area in 2002. This closure was in response to increasing proportions of young fish (< 2 year old), which had dominated the catches in the area for a number of years. Historically, large catches were taken from the Celtic Sea area by the Dutch fleet but in recent years the reported catch has been negligible. Irish landings of herring from the Northwest have decreased sharply since 1999 due to decreasing demand for herring and the increasing availability of more valuable species, including mackerel, and horse mackerel (CSO, 2007). The absence of herring from traditional spawning grounds off Galway and Mayo illustrates the decline of the stock in this area although it may be stabilized at a low level. Although the number of boats involved in the herring fishery has declined in recent years it is still a valuable component in the Irish fishing sector (Marine, 2004).

Celtic Sea herring spawn from October to February with some reports of spawning in March. Fish are classified as autumn or winter spawners depending on their time of spawning (Breslin, 1998, Molloy, 1980a). Traditionally the stock of herring in the Irish Sea was viewed as two separate units, the Manx component and the Mourne component. Autumn spawners dominated both components, with the majority of herring spawning in a four-week period between September and October, however a small amount of spawning activity has been identified in areas north of the Isle of Man up to January (Dickey-Collas *et al.*, 2001). The Mourne contribution to the Irish Sea stock declined during the 1990's to approximately 3% of the total herring in the Irish Sea (Dickey-Collas *et al.*, 2001). However, in recent years, this stock has started to show signs of recovery and was reopened for fishing in 2005 (DARD, 2005). The North-West of Ireland stock spawns in winter and spring (Hatfield and Simmonds, 2002) and the fishery in this area exploit a mixture of autumn- and winter/spring-spawning fish. The Clyde west of Scotland stock is spring spawning (Hatfield and Simmonds, 2002) but its contribution to the overall number of herring around the Irish coast is uncertain.

1.3 Stock structure of Celtic and Irish Sea herring

It is now widely accepted that a proportion of juveniles from the Celtic Sea area are found at nursery grounds in the Irish Sea due to dispersal during the first year of life. Evidence from larval drift studies (Özcan, 1974), length and vertebral count distributions (Bowers, 1964), tagging studies (Molloy *et al.*, 1993) and otolith increment widths (Brophy and Danilowicz, 2002) all show mixing of the two stocks in the Irish Sea during the early (larval/juvenile) life stages. Oceanographic evidence indicates that the water circulation patterns favour the dispersal of larvae from the Celtic Sea spawning grounds into the Irish Sea. Strong northerly flow rates through St. Georges channel drive water through the western Irish Sea before it exits the Irish Sea through the North Channel (Xing and Davies, 2001). Real wind-driven particle tracking studies have suggested that large wind events in February may force particles from the Celtic Sea into the west Irish Sea within 30 days (O' Brien and Little, 2006). Using otolith microstructure, Brophy and Danilowicz (2002) successfully separated juvenile herring collected from the Irish Sea, into autumn and winter spawned fish.

This verified that there are two components in the Celtic Sea stock, the juveniles that remain in the Celtic Sea (resident) and those that move into nursery grounds in the Irish Sea during the first year of life (migrant). Indirect evidence of this migrant component returning to join the Celtic Sea winter spawning stock as adults has been provided by tagging experiments (Molloy *et al.*, 1993) and by the absence of winter spawned fish from spawning assemblages in the Irish Sea (Brophy *et al.*, 2006). The coincidence of low recruitment in the Celtic Sea during the 1970's with the industrial fishery, which targeted juvenile herring in the Irish Sea from 1967 −1978, also suggests that the Irish Sea is an important source of Celtic Sea recruits (ICES, 2001). Environmental variables in particular may effect the transportation of juveniles into the Irish Sea and have important implications for the number of winter spawned juveniles found in the Irish Sea on an annual basis.

1.4 Herring assessments in the Irish and Celtic Seas

Accurate juvenile abundance estimates play an important role in stock assessments. Stock assessments are used to identify the current state of stock, to examine the future outcome for the stock given a number of alternative management strategies, to determine whether a stock is under- or over-exploited, or if its status will change in the future if subject to different levels of exploitation. Its ultimate goal is to provide reliable management advice to avoid long term economic or social problems associated with population crashes.

Herring are currently assessed as two single stocks in the Irish and Celtic Seas (ICES, 2007). TAC's are set annually and are based on assessments carried out seasonally in both areas. Assessments are based on survey and catch data which provides quantitative estimates of the numbers of fish expressed in tonnes (Marine, 2007). Much uncertainty surrounds the assessment of Irish and Celtic Sea herring due to mixing between the stocks outside of the spawning season (Brophy and Danilowicz, 2002, Molloy *et al.*, 1993) and juvenile mixing has resulted in the failure to produce an adequate recruitment index for the stocks (Marine, 2007). Estimates of Spawning Stock Biomass (SSB) and fishing mortality have been uncertain in the Celtic Sea and Irish Sea since 2000 (ICES, 2007).

The abundance estimates for age-1 fish (defined as individuals with one winter ring) provided by the Northern Ireland acoustic survey includes an unknown proportion of fish of Celtic Sea origin. Without the removal of this Celtic Sea fraction, juvenile abundance estimates from the Irish Sea are not appropriate for the calculation of Irish Sea recruitment indices, as they will include fish in the assessment who do not recruit to the Irish Sea spawning stock as adults. Similarly, if these adult fish return to join the Celtic Sea spawning stock, they may provide an appropriate recruitment index for that stock.

1.5 Otolith applications in fisheries science

Otoliths are ear stones which are found in the semi-circular canals of teleost fish from freshwater and marine habitats, from polar to tropical regions. They assist in detecting sound and are used for balance and orientation (Popper *et al.*, 2005, Campana and Neilson, 1985). There are three pairs, the lapilli, the sagittae and the asterisci of which the sagittae are the largest (Fay and Popper, 1980). Otoliths are easily stored and processed once extracted leading to their extensive use in fisheries research.

The shape and size of the sagittal varies between species, and therefore can be used in taxonomy (Harkonen, 1986). Many factors have been cited as influencing the size and shape of the sagittal otolith. They include the physical packing of the otolith within the skull (Smith, 1992), and environmental factors such as depth, water temperature, mineral and food availability (Lombarte and Cruz, 2007, Lombarte and Lleonart, 1993, Wilson, 1985, Fey, 2001). Gauldie (1988) proposed the function of hearing itself controlled the size and shape of the otolith. Cruz and Lombarte (2004) found that fish that belonged to groups considered as being specialists in sound production had larger otoliths than those which used visual communication and bright body colours. Another suggested reason for otolith size differences, is related to balance and swimming. Popper *et al*. (2005) outlined a number of observations to support this hypothesis including the fact that some of the largest ocean fish, such as tuna and swordfish have some of the smallest otoliths relative to body size, whereas many shallow water reef fish have considerable large otoliths. They suggest that large otoliths would be a disadvantage in fish that are fast, agile swimmers, resulting in over sensitivity of the fish to changes in angular motion (Popper *et al.*, 2005).

Otoliths consist of layers of protein and calcium carbonate, which are deposited throughout the life of the fish. These depositions form microscopic increments, which are visually similar to the growth rings of a tree and provide a permanent record of daily and annual growth rates. Daily increments were first discovered in 1971 (Panella, 1971). When growing conditions are less favourable, the rate of deposition slows and a protein poor translucent ring is laid down on the otolith. In temperate regions, this period of slower growth usually occurs in winter. These macroscopic annual structures can be used to age a fish and have been used since 1899 when Reibisch first observed annual ring formation in plaice (*Pleuronectes platessa* L.) (Ricker, 1975).

Determinations of fish age and growth rates are important techniques in the study of fish biology. Of the structures which encode age information in fishes (bones; fin rays, vertebrate, cleithra, opercular bones; scales and otoliths), otoliths provide the most reliable indication of age for most species (Campana and Jones, 1992). Otoliths grow throughout the life of a fish and, unlike scales and bones, are metabolically inert; once deposited, otolith material is unlikely to be reabsorbed or altered (Campana and Neilson, 1985). Otolith growth rate is proportional to fish growth (Campana and Neilson, 1985) and measurements of otolith length and annual bands can provide us with knowledge of age structure, growth rates and life history events within a population. Otolith growth is influenced by a number of environmental factors including temperature (Fitzhugh and Nixon, 1997, Fey, 2001), prey density (Folkvord *et al.*, 1997, Feet *et al.*, 2002) and photoperiod (Dowd and Houde, 1980) and changes in environmental conditions can cause variations in otolith structure. These variations have been used to identify individuals from different stocks and spawning aggregations which have experienced different environmental conditions (Berg *et al.*, 2005, Moksness and Fossum, 1991, Mosegaard and Madsen, 1996).

1.6 Otolith microstructure

Otolith daily increments form as a result of a circadian rhythm (once per 24hrs) and by the action of environmental conditions (often more frequent than one per 24hrs). Sub-daily increments are a potential error in otolith interpretation (Campana and Neilson, 1985). The assumptions that there is only one increment deposited per day,

and that otolith growth is proportional to fish growth, should be validated before microstructure analysis can be used to estimate exact daily ages and hatch dates of individual fish (Geffen, 1992). Methods of validation include using fish of known age in the laboratory (Neilson and Geen, 1981) and the incorporating of time/date markers, such as thermal marking (Volk et al., 1999).

Otolith microstructure can also be used to differentiate between stocks (Campana and Neilson, 1985), by looking at differences in growth patterns. When otolith microstructure is used as a population marker, exact daily age does not need to be known and deviation from regular daily increment formation should not change the interpretation of the results. Thorrold and Williams (1989) found significant variation in otolith growth patterns between different cohorts of larval tropical herring and Munk et al. (1991) showed that geographical location influenced otolith growth in larval herring from the North Sea. Otolith microstructure has been used to differentiate between seasonal herring populations in the Irish and Celtic Seas (Brophy and Danilowicz, 2002), the Norwegian Sea (Moksness and Fossum, 1992), and the North Sea (Mosegaard and Madsen, 1996). Autumn spawned fish generally experience slower growth than winter and spring spawned resulting in narrower increment widths and smaller fish sizes.

1.7 Otolith morphometrics and shape analysis

Otolith shape would appear to be an ideal marker for fish populations. It is distinctly species specific (L'Abee-Lund, 1988) and less variable than fish growth, most probably due to the dual function of the otolith as an organ of balance and hearing (Campana and Casselman, 1993). They are metabolically inert and are therefore unaffected by short-term changes in fish condition (i.e. starvation), which can have irreversible effects on morphometrics. The sagitta is the most commonly analysed otolith and was found to provide slightly better stock discrimination outcomes for cod (*Gadus morhua* L.) than the lapilli or asterisci otoliths (Campana and Casselman, 1993).

Basic methods of otolith shape analysis include manual distance measurements that can be used to calculate size parameters such as area and perimeter. These in turn can be used in a series of mathematical equations to calculate shape indices such as

circularity and roundness which are used to characterise the shape of the otolith (Tuset *et al.*, 2003, Russ, 1990). More complex methods look at the overall shape and use image analysis software to describe the shape of the otolith. Outline methods use trigonometric functions (e.g. polynomials, Fourier series) or other empirical functions (e.g. eigenshape analysis, median axis) to analyse outlines of otolith silhouettes. Many of these methods are incorporated into software packages. Generally, mathematical functions (usually Fourier transforms) are used to generate a set of shape variables from digitised outlines. Each successive shape variable adds increasing detail to the description of the overall shape. These shape variables can be analysed using multivariate analysis such as discriminate function analysis.

In Fourier analysis, the digitised outline of an image is recorded as a set of polar or Cartesian coordinates. Cartesian methods are often preferred as they do not rely on a radiating centroid. This use of a radiating centroid means polar Fourier functions may not work successfully when otoliths are particularly convoluted, or the rostrum has significant curvature that it would be possible for radii to intersect the perimeter at more than one point.

This study uses the elliptic Fourier analysis (EFA) to describe otolith shape in Atlantic herring. EFA uses Cartesian (x,y) coordinates along the outline of an object to characterise its shape. Unlike some other methods (e.g. Fast Fourier Analysis) EFA does not require coordinates to be equally spaced around the outline of the object, therefore it can describe more complex shapes.

Many studies have used otolith shape analysis to separate fish populations and stocks. Bird *et al.* (1986) used Fourier transformations to differentiate between Alaskan (*Clupea pallasii* V.) and Northwest Atlantic herring (*Clupea harengus* L) populations and found that shape showed significant differences between fish of different ages and from different populations. Fourier transformations have also been used in stock identification studies of salmon (*Salmo salar* L.) (Finn *et al.*, 1997, Friedland *et al.*, 1994, Friedland and Reddin, 1994) and orange roughy (*Hoplostethus atlanicus* L.) (Gauldie and Jones, 2000, Robertson and Talman, 2002, Smith *et al.*, 2002) and cod (*Gadus morhua* L.) (Berg *et al.*, 2005, Cardinale *et al.*, 2004), Atlantic mackerel (*Scomber scombrus* L.) (Castonguay *et al.*, 1991) and spotted seatrout (*Cynoscion nebulosus* C.) (Colura and King, 1995). Classification success rates varied from 60-91% for interstock separation depending on the species.

1.8 Summary of objectives

This thesis uses otolith shape measurements to distinguish between winter-spawned Celtic Sea juveniles at Irish Sea nursery grounds (migrant) from those who remained in the Celtic Sea (resident). A new method is developed which uses traces of internal annuli to determine nursery ground origin in adult fish. Otolith shape analysis is also examined as a method for separating juveniles of different hatch types in the Irish Sea (autumn and winter spawned). As nursery ground and spawning area of origin are identified, the current theories on herring stock structure and natal homing behaviour are discussed. Microstructure analysis of archived otolith collections is used to examine temporal trends in the movement of Celtic Sea juveniles into the Irish Sea. The influence of environmental conditions on the growth and distribution of both populations in the Irish Sea is investigated. Each chapter represents a self-contained unit formatted as a journal article. The scope of each chapter is summarized below:

> Chapter 2: *Shape analysis of otolith annuli in Atlantic herring (Clupea harengus); a new method for tracking fish populations.*
> Otolith shape analysis is used to distinguish between resident and migrant winter spawned Celtic Sea juveniles. Based on the successful separation of age-0 fish collected in 2004, the 1st winter ring is traced in age-1 fish collected in 2005 to examine if shape characteristics are preserved to a sufficient level to facilitate identifying nursery ground origin in adult fish.

> Chapter 3: *Otolith shape analysis, its application to discriminating between Irish and Celtic herring (Clupea harengus) stocks in the Irish Sea.*
> In this study otolith shape analysis is presented as an alternative method to otolith microstructure analysis for identifying winter spawned juveniles in the Irish Sea. The two methods are compared with regard to reliability and efficiency, and overall classification success rates.

> Chapter 4: *Otolith shape analysis provides evidence of natal homing in Atlantic herring (Clupea harengus).*

This study completes that examination of a cohort from the larval to the adult phase. Larval otolith microstructure is used to identify winter spawned fish from the Celtic Sea and to determine when the migrant and resident components diverge. Traces of the 1st winter ring in adult (age-2) fish collected in the Celtic Sea in 2006 are used to describe juvenile otolith shape. The relative proportion of each component in adult spawning assemblages in the Celtic Sea was then estimated using mixed stock analysis, and discriminate function analysis and with the age-0 individuals from the same cohort as the reference sample.

Chapter 5: *Temporal trends in the stock composition and abundance of juvenile Atlantic herring (Clupea harengus) in the Irish Sea.*

Otolith microstructure analysis is used in this study to estimate the proportion of winter spawned individuals in samples of age-1 herring from the western Irish Sea over a ten year period (1993-2004). These proportions are used to investigate the potential of splitting juvenile abundance estimates to produce recruitment estimates more appropriate for the Irish Sea herring stock assessment. Temporal trends in abundance and growth rates of winter spawned juveniles in the Irish Sea are also examined in relation to environmental conditions (temperature, wind speed and direction and food availability).
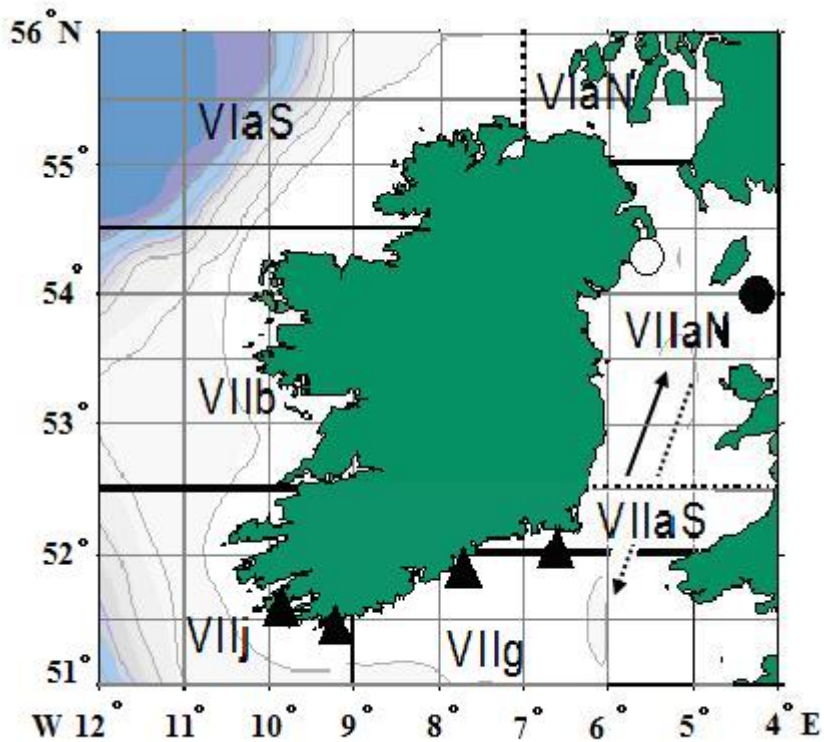
Figure 1.1 Map showing the management units and main spawning locations of herring in the Irish and Celtic Sea. Solid circle; Isle of Man autumn spawners. Open circle; Mourne autumn spawners. Solid triangles; Celtic Sea autumn and winter spawners. The movement of larvae/juveniles from the Celtic Sea into the Irish Sea is indicated by solid arrow. Proposed return route indicated by dashed arrow. The solid and dashed lines mark the boundaries of ICES divisions and subdivisions respectively.

Chapter 2

Shape analysis of otolith annuli in Atlantic herring (*Clupea harengus*); a new method for tracking fish populations[1]

2.1 Abstract

The mixing of Atlantic herring (*Clupea harengus*) stocks during the early life phases has important implications for management of the associated fisheries. There are two components within the winter-spawning Celtic Sea stock with characteristic growth and recruitment patterns; Celtic Sea fish that move into the Irish Sea (migrant component) grow more slowly and hence recruit to the adult population later than those that are retained close to the spawning grounds (resident component). The rate of return of the dispersed component to the Celtic Sea, the relative contribution of each component to the adult stock, and it's inter annual variation are unknown.

A method to discriminate between the migrant and resident components of the winter-spawning Celtic stock, based on shape analysis of the juvenile portion of the otolith, is presented here. Juvenile herring were collected in 2004 and 2005 from nursery grounds in the Irish and Celtic Seas. Autumn- and winter-spawned juveniles were distinguished using larval otolith microstructure measurements. A classification function based on linear otolith measurements and Fourier descriptors was used to differentiate between age-0 winter spawned herring from the two seas. This classification function was applied to age-1 juveniles from the same areas using otolith descriptors of the region bounded by the first annulus. The dispersed and resident components could be distinguished with a high degree of accuracy (>95%). The potential use of otolith characteristics for tracing juvenile origin in adult fish, assessing levels of natal homing and measuring recruitment levels from each nursery area are discussed.

2.2 Introduction

Otolith analysis is an important tool in the study of fish populations. It provides a record of the growth patterns in individuals, cohorts and populations and can accurately estimate age and growth at both the daily and yearly level (Campana and Thorrold, 2001). Increment counts and measurements can provide information on hatching times and growth rates (Fox *et al.*, 2003, Gallego *et al.*, 1999, Munk *et al.*, 1991, Sinclair and Tremblay, 1984) and offer insight into stock structure for numerous species (Ayvazian *et al.*, 2004, Brophy and Danilowicz, 2002, Finn *et al.*, 1997, Mosegaard and Madsen, 1996).Variation in otolith shape is also a useful descriptor for discriminating between fish stocks (Begg and Brown, 2000, Campana and Casselman, 1993, Cardinale *et al.*, 2004, Robertson and Talman, 2002, Stransky, 2005, Turan, 2000)and can also be used in age classification (Bermejo, 2007).

Environmental factors such as sea temperature and food availability have been linked to spatial variation in fish growth rates (Bailey and Alanara, 2006, Campana and Casselman, 1993, Fox *et al.*, 2003, Gallego *et al.*, 1999, Munk *et al.*, 1991). Variation in growth rate produces corresponding variation in otolith microstructure and shape (Gauldie and Nelson, 1990) due to the proportional relationship between otolith growth and somatic growth (Campana and Neilson, 1985). Consequently, groups of fish that maintain distinct distributions for part or all of their lives can be distinguished using microstructure analysis of daily increments, internal measurements of the distances between the nucleus and the annual rings (Begg *et al.*, 2001, Suthers and Sundby, 1993) and shape analysis of the otolith (Berg *et al.*, 2005, Cardinale *et al.*, 2004, Stransky and MacLellan, 2005, Turan, 2000).

As otolith constituents are not altered or reabsorbed during the life of the fish (Campana and Neilson, 1985), annual and daily increments provide a permanent record of previous growth rates. Measurements of these chronological features are specific to particular periods in the life history of the fish. In contrast, shape parameters or truss measurements from the external edge of the otolith reflect the average growing conditions experienced by the fish up to that point in its life because the external outline of the otolith changes as the fish grows. It may be possible to measure the shape of the otolith at a previous point in a fish's life by using the outline of an internal feature such as an annulus. Shape parameters or measurements

generated from an internal annulus would represent growth variation during the period corresponding to the otolith region bounded by the annulus in question. While measurements of growth increments (lengths from nucleus to annual rings) have been used in stock identification studies, shape analysis of internal growth rings has not been investigated. It is likely that, for fish with well-defined annual rings, a trace of the region bounded by the first annulus would correspond well to the shape of the otolith at the time of annulus formation. In this case, shape parameters generated from this internal trace could be used in intraspecific investigations. This study assesses the feasibility of using shape parameters generated from internal traces of the first annulus to identify juvenile origin in herring (*Clupea harengus*).

Atlantic herring are one of the most important pelagic species exploited by the European fishing industry. There are four management units around the Irish coastline, and fish in these areas have their own spawning time and location. Within the Irish Sea, herring spawn in autumn, usually for a 3-4 week period beginning in September (Dickey-Collas *et al.*, 2001) while herring in the Celtic Sea spawn in autumn and winter (Molloy, 1980a). Autumn- and winter-spawned herring display distinct otolith microstructure patterns and can be distinguished using increment width measurements (Brophy and Danilowicz, 2002). Otolith microstructure analysis has shown that herring from the Celtic Sea are transported into the Irish Sea during the first year of life (Brophy and Danilowicz, 2002). Evidence from tagging studies, otolith microstructure and parasite prevalence suggests that Celtic Sea juveniles in the Irish Sea return to join the Celtic Sea spawning stock when they mature (Brophy *et al.*, 2006, Campbell *et al.*, 2007, Molloy *et al.*, 1993). The effect of juvenile mixing on recruitment to the fishery in the Celtic Sea is still unquantified and the importance of the Irish Sea nursery to the Celtic Sea stock has not been assessed or monitored (ICES, 2007).

This study investigates if age-0 winter-spawned herring that drift into the Irish Sea during the first year of life (migrant component) can be distinguished from those that remain in the Celtic Sea (resident component) on the basis of otolith shape. Previous research has found substantial variation in growth rates between these two components (Brophy and Danilowicz, 2002), so it is likely that these growth differences are reflected in otolith shape parameters. The extent to which shape variation is preserved in the internal morphology of the otolith is investigated using

traces of the first winter ring in one year old fish from the same cohort. If shape variation is sufficiently preserved, these internal traces may be used to trace juvenile origin in adult fish. This method could be used to evaluate the extent of movement between nursery, feeding and spawning grounds, thus elucidating the mechanisms implicit in herring stock structure. This would provide critical information where uncertainty in stock assessments has resulted from a lack of reliable information on stock mixing (ICES, 2007). The method may also be useful in separating stocks where distinct growth patterns are only observed during the juvenile phase or where adult stocks are not significantly geographically separated. Measurements of internal annuli were investigated to identify if differences between two different components of the same stock separated by nursery ground origin could be used for discrimination purposes.

2.3 Methods

Fish sampling

Atlantic herring (*Clupea harengus*) were collected in the Irish and Celtic Seas in 2004 and 2005. Sampling was stratified by length in order to target fish from the 2003 cohort as age-0 fish in 2004 (7-19 cm) and age-1 fish in 2005 (15 –23 cm). Fish with no translucent winter ring in the otoliths were classified as age-0 and those with one translucent winter ring were classified as age-1. Irish Sea fish were collected using mid-water trawls during herring acoustic surveys conducted by the Agri-Food and Biosciences Institute (AFBI)[2] in September 2004 and 2005 onboard the RV "Lough Foyle" and RV "Corystes", respectively. Fish from the Celtic Sea were collected during groundfish surveys conducted by the Marine Institute onboard the RV "Celtic Explorer" in November 2004 and 2005. Fish were collected from three sites in the Irish Sea and two sites in the Celtic Sea in 2004, and from one site in the Irish Sea and one site in the Celtic Sea in 2005 (Figure 2.1). Fish were processed onboard or frozen whole at –20˚C. Total standard length and weight were recorded to the nearest 0.1 cm and 0.1 g respectively and both sagittal otoliths were removed and cleaned in water before drying and storing in 5 ml plastic vials.

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[2] Formerly known as The Department of Agriculture and Rural Development , Northern Ireland.

Otolith ageing and microstructure analysis

Otoliths were left to soak in water for up to 24h to improve clarity of the annual rings. Age-0 individuals collected in 2004 and age-1 individuals collected in 2005 were selected for further analysis. The mean lengths and numbers of fish used in the analysis are shown in Table 2.1.

Otolith microstructure analysis was then used to classify individuals as winter- or autumn-spawned based on daily increment widths at the larval core using the method developed by Brophy and Danilowicz (2002). One otolith (typically the right) from each individual was used for microstructure analysis. All otoliths were mounted using TAAB™ resin. Age-0 otoliths were mounted and polished on the sulcus side until the larval core was fully exposed. Age-1 otoliths were initially polished on the anti-sulcus side until light could penetrate the larval core, and were then remounted and polished on the sulcus side until the larval core was fully exposed.

The core was photographed at 1000X using an Olympus™ compound microscope with an Olympus™ Camedia digital camera. Daily increments were measured along the longest axis from the centre to the edge of the image. Otoliths with average increments widths of > 2.3 μm between increments 61-70 were classified as winter-spawned (97% of fish), while fish with average increment widths of < 2.2 μm were classified as autumn-spawned (3% of fish), as determined by Brophy and Danilowicz (2002). Autumn spawned fish were excluded from further analysis. This was done in order to restrict the investigation of otolith shape to fish that had spawned at the same time of year and were most likely of Celtic Sea origin. This removes the potentially confounding influence of hatching time and stock origin on otolith shape and restricts the investigation of otolith shape to the migrant and resident components of the Celtic Sea winter-spawned juvenile herring stock. Autumn-spawned fish were also removed due to the insufficient size of the sample (only 3% of fish sampled).

Otolith shape analysis

Otolith characteristics (size and shape) reflect the growth pattern of the fish as well as being markedly species specific (L'Abee-Lund, 1988). As a result it can be used to differentiate between species (L'Abee-Lund, 1988) as well as between

populations/stocks of the same species (Begg and Brown, 2000, Campana and Casselman, 1993, Cardinale *et al.*, 2004, Robertson and Talman, 2002, Stransky, 2005, Turan, 2000). Otoliths grow throughout the life of the fish and do not appear to be subject to mineral resorption except under extreme circumstances (Mugiya and Uchimura, 1989).

Otolith shape can be described using manual distance measurements to calculate shape indices such as circularity and form factor. More complex methods such as Fourier series shape analysis, use image analysis software to trace otolith outlines and to generate shape coefficients. In this study, a combination of shape indices and elliptical Fourier shape descriptors were used to describe otolith shape variation in juvenile herring from the Irish and Celtic Seas.

Using typically the left otolith, otolith images were digitised using an Olympus™ Camedia digital camera and an Olympus™ SZX7 stereomicroscope at 20X magnification. Where only the right otolith was available, images were horizontally flipped using standard image analysis techniques. Age-0 otoliths were positioned sulcus side down with the rostrum pointing to the left and photographed using transmitted light (Figure 2.2a). Age-1 otoliths were positioned in the same orientation and photographed as bright objects on a dark background to ensure the first winter ring was clearly visible. For each age-1 image the outer edge of the first winter ring was traced manually using Olympus Dp-Soft software (Figure 2.2b). To assess the repeatability of the manual traces, three traces were taken of first winter ring in four otoliths. These traces were then used to obtain a measurement of precision. Coefficients of variation were calculated for feret lengths, feret widths, areas and perimeters of each otolith. All coefficients of variation (CV's) were found to be <1%. This shows that the outline could be traced manually with a high rate of precision. Each trace was filled-in in black using Microsoft Paint and saved for further analysis (Figure 2.2c).

Shape indices

Using the image of each age-0 otolith and the trace from each age-1 otolith, a series of 2-dimensional measurements was taken using Olympus™ Dp-Soft: area, perimeter and feret length and feret width. Feret length and feret width are the length and width

of a box which encloses the otolith. These measurements were used to calculate a number of shape indices (Russ, 1990, Tuset *et al.*, 2003) that are outlined in Table 2.2.

Analysis of otolith outlines

Using images of whole otoliths for age-0 fish and traces of the 1st winter ring filled in black for age-1 fish, digitalised outlines were generated using TpsDig[3] image analysis software. Outlines can be automatically computed around structures that are either dark on a light background or light on a dark background, therefore otolith images were converted to black objects on a white background using TpsDig. Outlines were saved as a series of x, y coordinates as TPS files. The user either accepts the actual number of coordinates generated by TpsDig or can specify a lower number of coordinates. The actual number generated was used in this investigation. Each trace was started at the excisura major, which is the notch located in the anterior rim of the sagittal otolith (see Figure 2.2b). TPS files were then converted into a single data file in the format that is required for the Fourier analysis program EFAwin[4].

Using the EFAwin program, 20 harmonics were generated for each otolith. Each harmonic consisted of four coefficients resulting in 80 coefficients (C) per individual. The program standardises for size and orientation, giving the first three coefficients fixed values of C1 = 1, C2 = C3 = 0. Each individual is therefore represented by 77 unique coefficients (C4-C80). Further details on elliptic Fourier analysis can be found in Bird *et al.* (1986) and Lestrel (1997).

A preliminary trial was conducted to establish the precision associated with the measurement of the shape variables. Nine otoliths were photographed three times. Images were used to generate three separate sets of coordinates. These coordinates were then used to generate three sets of shape variables for each image based on the repeated outline generation. The repeatability of the shape variable measurements was examined across different images of the same otolith and across multiple traces made using the same image using coefficients of variation. Coefficients of variation (CV) were calculated using the formula:

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[3] Public domain program developed by F. J. Rohlf, available freely on the internet at
http://life.bio.sunysb.edu/morph/index.html
[4] Public domain program developed by F. J. Rohlf and S. Ferson, available freely on the internet at
http://life.bio.sunysb.edu/morph/index.html

Coefficient of variation $= \dfrac{Sd}{\bar{x}} * 100$

Where $\bar{x}$ is the mean and Sd is the standard deviation.

High levels of precision were achieved when shape variables were measured using multiple traces of the same image (CV < 10% for 92% of variables). Many of the shape variables could not be measured with satisfactory precision across different images of the same otolith (CV > 20% for 68% of the variables, see Appendix A). This variability may arise due to small variations in the three dimensional positioning of the otolith on the microscope stage. For this reason, further analysis was restricted to variables which could be measured with reasonably high levels of repeatability (CV<18%).

Data Analysis

Growth and condition

Spatial variability in fish size, larval growth rates and juvenile condition was investigated. Total otolith length and the length of the otolith region bounded by the first annulus were used as an index of fish size at the end of year one in 0- and age-1 fish respectively. The mean widths of increments 61-70 were used as a measure of late larval growth rates. Fulton's condition index was calculated using the formula; W/L^3 where W = weight and L= length. Growth and condition variables were first tested for normality and homogeneity of variance to assess their suitability for parametric testing. All tests were carried out with an alpha significance level of 0.05 using MINTAB 14 for windows. Based on the results of these tests, Kruskall-Wallis rank sum tests or univariate ANOVA were used to detect differences in otolith length, larval growth rates and fish condition between components (resident and migrant) for age-0 and age-1 and between sites within components for age-0 fish.

Shape variables

Shape indices and coefficients (henceforth referred to collectively as shape variables) were examined for normality and homogeneity of variance using Kolmorgorov-Smirnov normality tests and Levene's tests respectively. All tests were carried out

with an alpha significance level of 0.05 using MINTAB 14 for windows. Variables that displayed normal distribution and homogeneity of variance were tested using Univariate ANOVA for otolith shape differences between components (resident and migrant) and between sites within each component. Variables that showed no significant difference in shape between sites within each component were deemed representative of that component. Variables that differed significantly between the resident and migrant components were considered potentially useful in the development of a discriminate function analysis (DFA) to classify fish to nursery areas and were selected for further analysis.

Based on the results of these tests on the five shape indices and 77 coefficients calculated, one shape index (rectangularity) and three coefficients (C12, C14 and C25) were selected for further analysis. To ensure that DFA would not be affected by multicollinearity, variables were tested for univariate correlation using Pearson's correlation coefficient using MINITAB 14 for windows and within group correlation using SYSTAT 11 for windows. Multicollinearity can result in the use of redundant predictors and can affect the outcome of the analysis (Graham, 2003). Variables were also tested for homogeneity of covariance matrices by carrying out Box's M test using PAST- version 1.75b (Hammer *et al.*, 2001) to ensure they meet all the assumptions of MANOVA (Gotelli and Ellison, 2004). Using SYSTAT 11 for windows ANCOVA (with otolith length as a covariate) were carried out to determine if there was a significant relationship between otolith length and each variable ($p < 0.05$). No significant interactions were identified. Next, variables were tested for significant correlation with otolith length. Where significant correlation was present the size effect was corrected for using the common within group slope (b) (Begg *et al.*, 2001, DeVries *et al.*, 2002, Galley *et al.*, 2006, Reist, 1985, Tuset *et al.*, 2006). This procedure was used to adjust C14, for which a significant correlation was identified (see Table 2.3). For the age-1 fish, otolith length was taken as the feret length of the trace of the first winter ring. Variables were also tested for correlation with Fulton's condition index and width of increments 61-70 to determine if shape was directly correlated with late larval growth or juvenile condition.

DFA was carried out using rectangularity, C12, C14 (adjusted) and C25 on age-0 fish to determine the proportion of individuals that could be correctly classified to nursery area based on otolith shape. This procedure initially classifies each case into the group

where the value of its classification function is largest. These results may be misleading because the classification rule is evaluated using the same cases that are used to compute it. The jackknifed classification procedure attempts to remedy this problem by removing and replacing each case one at a time and using functions computed from all the data except the case being classified (Engelman, 2004). Both procedures were carried out using the age-0 data. Once the variables that could be used to distinguish between the migrant and resident components had been identified, the next step was to establish if this shape variation was sufficiently preserved in the first winter ring to distinguish between age-1 fish from each component. The discriminate function developed for the age-0 fish was used to classify age-1 fish to nursery area (Celtic Sea-resident / Irish Sea-migratory) by the cross validation procedure. The percentage of individuals correctly classified to each nursery area based on the shape characteristics of the first winter ring was then assessed.

2.4 Results

Analysis of otolith growth and length and fish condition identified distinct differences in growth patterns between the resident component of winter spawned fish collected in the Celtic Sea and the migrant component of winter spawned fish collected in the Irish Sea in both 2004 and 2005. One-way ANOVA showed that for winter-spawned herring there was a significant difference in the mean width of increments 61-70 between the resident component and migrant component in both 2004 ($p < 0.01$) and 2005 ($p = 0.02$) with the migrant component displaying slower growth than its resident counterpart. Fish condition (F) was also shown to be significantly different between the two components ($p < 0.01$), with age-0 juveniles in the Celtic Sea being in better condition than those from the Irish Sea. Otolith length did not meet the assumptions for parametric testing, therefore a Kruskall-Wallis rank sum test was used to test for differences between the components. Significant differences in otolith length at the end of the first year were identified between the resident and migrant components for both the age-0 fish collected in 2004 ($p < 0.01$) and the age-1 fish collected in 2005 ($p < 0.01$). However, otolith length was also found to differ significantly between sites within the Irish Sea ($p < 0.01$) and Celtic Sea ($p < 0.01$) for age-0 fish collected in 2004.

Shape variables were tested for normality and homogeneity of variance and were selected for inclusion in the discriminate function analysis (DFA) based on the spatial and temporal variation revealed by ANOVA. Variables showed significant variability between components and no variability between sites within each component and were therefore representative of that nursery area (see Table 2.4). None of the variables selected showed significant correlations in either univariate Pearson's correlation or within group correlation matrices. Homogeneity of variance was shown using Box's M test (p= 0.67).

Otolith shape variables and fish length and weight were used to investigate the relationship between otolith characteristics and fish growth and condition. These relationships were not clear-cut. Late-larval otolith growth (mean width of increment widths 61-70) was not correlated with any of the selected shape variables. Fulton's condition index was significantly correlated with C25 for the Irish Sea individuals (p = 0.01) but no such correlation was observed in the Celtic Sea fish.

The DFA of age-0 fish gave an overall classification success of 84% and a jack-knifed classification of 83%, with a classification success of over 81% for fish from both the Celtic and Irish Seas (see Table 2.5).

In the cross-validation of the age-1 fish using the DFA developed for the age-0 individuals, 97 % of age-1 individuals were classified correctly. Of the age-1 fish, 93 % of Celtic Sea fish and 100 % of Irish Sea fish were successfully classified to the correct region (see Table 2.5). The canonical scores of age-0 and age-1 fish obtained from the discriminate function analysis and cross validation procedure are shown in Figure 2.3a and 2.3b.

2.5 Discussion

Otolith microstructure analysis was used to distinguish between autumn and winter-spawned juvenile herring from the 2003 cohort, before the analysis of otolith shape variation was carried out. Microstructure analysis has been used to address questions relating to growth rates, age estimates, stock discrimination, dispersal patterns and the effect of temperature and feeding on individuals (Baumann *et al.*, 2006, Clausen *et al.*, 2007, Folkvord *et al.*, 1997, Fox *et al.*, 2003, Morales-Nin *et al.*, 1998, Suthers and Sundby, 1993). In this investigation, microstructure analysis was used to

distinguish between autumn and winter spawned fish. This was achieved using the method developed by Brophy and Danilowicz (2002). The increment widths observed were consistent with the limits set by Brophy and Danilowicz (2002) in their study of juvenile herring in the Irish and Celtic Seas and those observed in adult herring from the same areas (Brophy *et al.*, 2006). The three studies show consistent increment width patterns in Irish and Celtic Sea herring across six year classes indicating temporal stability of this stock marker. Otolith microstructure analysis is therefore an ideal method for identifying the migrant component of Celtic Sea winter-spawned juveniles when mixed with the autumn-spawned Irish Sea stock at Irish Sea nursery grounds. However, increment widths cannot be used to identify the migrant component once it has recruited to the adult stock in the Celtic Sea and mixed with the resident component of winter-spawned fish there, as all fish will display increment widths characteristic of winter growth. This was the rational for using otolith shape to discriminate between the resident and migrant components.

The results of this study show that the shape of otolith annuli can be used to distinguish between the migrant and resident components of the Celtic Sea winter-spawned stock. A high level of classification success (97%) was achieved when fish from each component were classified to nursery area based on the shape of the first winter ring. These findings contrast with the study carried out by Turan (2000) in which herring from the Celtic Sea could not be distinguished from herring from the North Sea based on truss measurements of the otolith outline. The levels of classification success in this study may indicate that internal measurements are a more powerful method for stock discrimination, where juveniles are less migratory / more sedentary and therefore exposed to more constant environmental conditions. This juvenile section of the otolith can be traced at any stage of the fish's life and may facilitate the tracing of nursery origin in adult fish. While this will depend on the thickness of the adult otoliths it is likely for herring given the clarity of increments in older fish (pers. obs.).

As with all morphometric analyses, a number of confounding effects must be considered to ensure that classification is based on shape alone. Sex, age and size may all influence otolith shape (Cardinale *et al.*, 2004), leading to misinterpretation of spatial variation in shape parameters. The fish used in this study were homogenous with respect to age as they were all from the same year class. Sex related differences

are unlikely to influence the results as all fish sampled were immature and of indeterminate sex. In addition, no significant effect of sex on otolith shape has been observed in herring (Bird *et al.*, 1986). In this study, herring from the two components showed considerable differences in otolith length (taken as an indication of size). This is an important effect and can compromise stock discrimination studies if variables are not standardised (Smith, 1992). Only one of the shape variables used in the final analysis was correlated with length. This variable was standardised to ensure the classification of fish to nursery area was independent of length. The observed differences in otolith shape between juvenile herring from the Irish and Celtic Seas are therefore independent of age and size.

In the case of juvenile herring from the Irish and Celtic Seas, the observed length difference was sufficiently great to allow the two groups to be separated purely on the basis of the size of the otolith region bounded by the first annulus. However, discrimination based on size-independent shape variables is preferable for a number of reasons. Firstly, size can be affected by sampling bias and sample design (Dos Reis *et al.*, 1990). Shape on the other hand is not affected by the biases associated with size and represents a more reliable method for classifying individuals. In this study, size showed significant variation between sites within each region as well as between regions. Therefore, unlike the shape variables, the size of fish from one site may not be truly representative of fish from that component and any characterisation based on size will be greatly influenced by choice of site. In addition size differences observed at one point in the life cycle may be less apparent at a later stage due to size-selective mortality. Over-wintering mortality has been shown to be size dependent in herring with smaller fish being more prone to starvation than larger individuals during the first winter (Norcross *et al.*, 2001). Lastly, in cases where there is greater overlap in size between regions, it is likely that a multivariate classification function based on several size-independent shape variables would have greater discriminatory power than size alone. In previous years, the size differences between juvenile herring from the Irish and Celtic Seas were not as marked (Brophy, 2002, Brophy and Danilowicz, 2002) and fish from the two areas could not be separated by size alone. Discrimination based on shape will be more widely applicable across years and regions than separation based on size alone.

The factors influencing otolith shape have not been directly tested in the current study and, in general, are not fully understood. Numerous studies have evaluated the relative importance of genetic / environmental effects on otolith shape in particular fish species in the context of stock discrimination, but a limited number of studies have directly investigated the determinants of otolith shape. Gauldie and Nelson (1990) found that faster growth produced long thin crystals compared with shorter more compacted ones in slower growing fish, which could influence the overall shape of the developing otolith. Gagliano and McCormick (2004) showed that otolith shape was influenced by recent feeding histories in tropical fish species. They established that shape differences could not be attributed to size differences among fish and suggested that shape may reflect fish condition. Other studies have documented a link between shape differences and somatic growth rates (Begg and Brown, 2000, Campana and Casselman, 1993, Cardinale *et al.*, 2004, Simoneau *et al.*, 2000). For some species, the level of classification success obtained using otolith shape analysis increases with the extent of genetic discreteness or geographic separation displayed by study groups (Castonguay *et al.*, 1991, Friedland and Reddin, 1994), implying that otolith shape variation is determined by genetics to at least some degree. Turan (2000) observed a direct relationship between phenotypic divergence and geographic separation of herring in the northeast Atlantic using meristics and truss measurements of otoliths. While both methods were able to separate Icelandic, Baltic and Trondheimsfjord stocks, North Sea and Celtic Sea could not be separated out from one another. Substantial otolith shape differences have also been observed between groups of fish that are close in geographic distribution (Galley *et al.*, 2006, Pothin *et al.*, 2006) and for stocks that could not be separated using genetic techniques (DeVries *et al.*, 2002). Otolith shape differences between reared and wild components of cod (Cardinale *et al.*, 2004) and lake trout (Simoneau *et al.*, 2000) also highlight how environmental factors such as temperature and feeding conditions can produce otolith shape variation in the absence of genetic differences.

It is improbable, that the observed shape differences between the migrant and resident components observed in this study are driven by genetic factors, as the fish collected from both the Celtic and Irish Seas were most likely from the same Celtic Sea winter-spawned stock. Also genetic homogeneity of herring stocks in spawning aggregations around the British Isles has been reported in several investigations (Jorstad *et al.*,

1991, King *et al.*, 1987, Turan, 1997). It is more plausible that environmental factors produce otolith shape variation in the fish sampled. Sea temperatures observed in the Irish and Celtic Seas over the last 50 years have shown a trend, with mean temperatures rarely exceeding 10^oC in the Irish Sea and rarely going below 10^oC in the Celtic Sea (Fox *et al.*, 2000). The link between fish growth rates and temperature is well established (Bailey and Alanara, 2006, Brander, 2000, Young *et al.*, 2005) and may explain the growth and condition differences observed between the migrant and resident components. In the current study, the relationship between otolith shape and fish growth and condition was not clear-cut. While one shape variable was found to be correlated with Fulton's condition index in the Irish Sea, none of the variables were correlated with fish condition in the Celtic Sea. In the absence of any genetic variation between the two components (King *et al.*, 1987), it is likely that the otolith shape variation between the winter-spawned juvenile herring at Irish and Celtic Sea nursery grounds is caused by environmentally driven growth differences.

Studies to date have focused on the shape of the external otolith outline or measurements of internal features. This study highlights the potential for using analysis of internal shape to distinguish between groups of fish that are separated at a particular point in the life history. If, at the time of annuli formation, a stock consists of more than one component distributed at different geographic locations, such as nursery areas, feeding grounds or over-wintering areas, the shape of this internal feature may provide a method for tracing which component an individual belonged to at a later stage in the fish's life history when the fish occurs in a mixed assemblage. The method has potential for intraspecific studies, where internal otolith annuli are well defined (e.g. plaice and cod) and can be reliably traced out. The successful classification of 83% of winter spawned age -0 juveniles is reasonably high compared to other Fourier analysis studies. The success of otolith shape analysis at separating individuals for interstock investigations varies from 60 - >90 % depending on the species (Berg *et al.*, 2005, Bird *et al.*, 1986, Cardinale *et al.*, 2004, Castonguay *et al.*, 1991, Friedland *et al.*, 1994, Gauldie and Jones, 2000, Pothin *et al.*, 2006, Smith *et al.*, 2002). While Pothin *et al.* (2006) achieved >90% classification success, the fish sampled (white goatfish) have been shown to have very high site fidelity. In a tag-release study carried out by Holland *et al.* (1993), 93% of white goatfish recaptures occurred at the site of release. Further knowledge of the movements of species may

help explain why in some studies significant differences are observed between stocks at a local level (Cardinale *et al.*, 2004) while in others the degree of difference increases with geographic separation (Castonguay *et al.*, 1991).

While other studies have investigated the shape of the otolith outline, this is the first study to examine the shape of an internal otolith annuli. The successful classification of 97% of age-1 individuals from the trace of the first winter ring provides promising results for the application of this technique to the separation of stock components that occupy distinct environments during the juvenile phase. The technique could potentially be applied to other calcareous structures that show annual growth rhythms such as scales, bones and spines (Meunier, 2002). Scale morphology has been used to distinguish between fish populations at the local level (Poulet *et al.*, 2005) as well as over wider geographical areas and across numerous year classes (Ibanez *et al.*, 2007, Richards and Esteves, 1997). Classification success rates varied from 57-84% depending on the numbers of samples and year classes used. In a study on measurements of scale annuli, Debarros and Holst (1995) identified significant differences in annuli width between Norwegian spring spawning herring that had spent their juvenile phase at different nursery grounds. The shape of annuli may also be useful for separating these different components.

Regardless of what is causing the observed differences, analysis of internal otolith outlines has great potential for tracing nursery ground origin in adult herring. A method for identifying the migrant component is of value to the management of the associated fishery. The method developed in this study identified no detectable exchange between the resident and migrant components up to the second winter. Evidence from other studies suggests that Celtic Sea winter-spawned juveniles in the Irish Sea return to join the Celtic Sea spawning stock when they mature at age two or three (Brophy and Danilowicz, 2002, Campbell *et al.*, 2007, Molloy, 1980a, Molloy, 1980b). In this study, the migrant component of winter-spawned juveniles in the Irish Sea has been shown to grow at a slower rate than their resident counterparts. Pre-recruitment growth in Celtic Sea herring juveniles has been linked to age at first maturity, with larger fish recruiting to the spawning stock one to two years earlier than fish that had experienced slower growth rates, characteristic of the migrant component (Brophy and Danilowicz, 2003). The delayed recruitment of the migrant component in the Irish Sea to the Celtic Sea stock will have subsequent effects on the

strength of each year class recruiting to the Celtic Sea winter fishery. Regular monitoring of the dispersal of Celtic Sea herring into the Irish Sea and during the first year of life and the return of this component to the adult stock in the Celtic Sea would aid predictions of annual fluctuations in recruitment strength.

If this method is to be used to monitor the return of the migrant component to the Celtic Sea stock, it will need to be ground-truthed on a yearly basis, to account for the effect that inter-annual variation in environmental conditions could have on the otolith shape characteristics of fish from each component. The incorporation of this method into annual herring sampling programmes would increase the level of knowledge obtained from the otoliths of each fish at very little extra cost to the sampler. Other potential nursery areas for the Celtic Sea winter-spawning stock, such as the west and south west coast of Ireland, could be identified using oceanographic dispersal models and juvenile surveys. Such nursery areas, once identified, should then be characterised in terms of otolith shape of the fish residing there to ensure that adults can be reliably assigned to their nursery area of origin.

Other methods that may be useful in this area and show potential for use in conjunction with otolith shape include parasite prevalence's and tagging. A study carried out by Campbell *et al.* (2007), showed differences between the parasites present in juveniles at Irish Sea nursery grounds and those infecting the adult spawning stock. The study also identified a common parasite between spawning adults in the Irish Sea and a spawning sample collected to the south of Ireland. By combining these two methods the rate of classification success achieved with either method on its own could be improved. Results of tagging studies indicate that a considerable amount of juveniles from the Irish Sea migrate southwards to recruit to the Celtic Sea stock (Molloy *et al.*, 1993).

The method presented here could also be applied to other herring stocks that migrate between nursery, feeding and spawning grounds and mix with other stocks in the process. For example, within the North Sea there are two major components of autumn spawning herring: Buchan / Shetland herring, Dogger / Banks herring and a winter spawning component, the Downs herring. Genetic analysis has determined that most aggregations in the North Sea are genetically indistinguishable from each other (Mariani *et al.*, 2005). The three components mix for the majority of the year on feeding grounds and then migrate to specific grounds to spawn. Due to this mixing the

landings are not disaggregated and the three components are managed as a single stock. However all three components have different nursery grounds and have displayed different growth rates, with different mean lengths at the age-1 stage (Daan *et al.*, 1990, Heath *et al.*, 1997). This difference in geographic location during the first year of life and the differences in growth rates may result in shape differences within the first annuli of fish in each component. Shape analysis of this internal feature may provide a method for identifying juvenile origin in adult autumn-spawning herring at the North Sea feeding grounds. Autumn spawned larvae have been shown to drift into the Skagerrak-Kattegat where they remain until they migrate back to the North Sea at age 2/3 (Rosenberg and Palmen, 1982). Shape analysis of the first winter annuli could be investigated to assess its potential for identifying adults in the North Sea who spent their juvenile phase in the Skagerrak-Kattegat.

2.6 Conclusion

The method presented here provides valuable results for otolith science. Shape analysis of internal annuli was used to separate the migrant and resident components of the Celtic Sea winter-spawned juvenile stock, and performed considerably better than size alone. Shape differences can most likely be linked to environmental conditions since all fish were from the same spawning stock. If ground-truthed on a yearly basis this method could be used to monitor and assist in the management of the Celtic Sea herring stocks. The method also has wide application to other stocks and populations that split into separate components at particular stages in their life history or mix at feeding / spawning aggregations. As a method it shows potential for internal shape analysis of not only otoliths but also scales and bones as well. It would be easily incorporated into stock discrimination studies where images of calcified structures are taken and may provide a useful source of information for stock discrimination studies, which play a fundamental role in the management of fish stocks and preserving biodiversity.
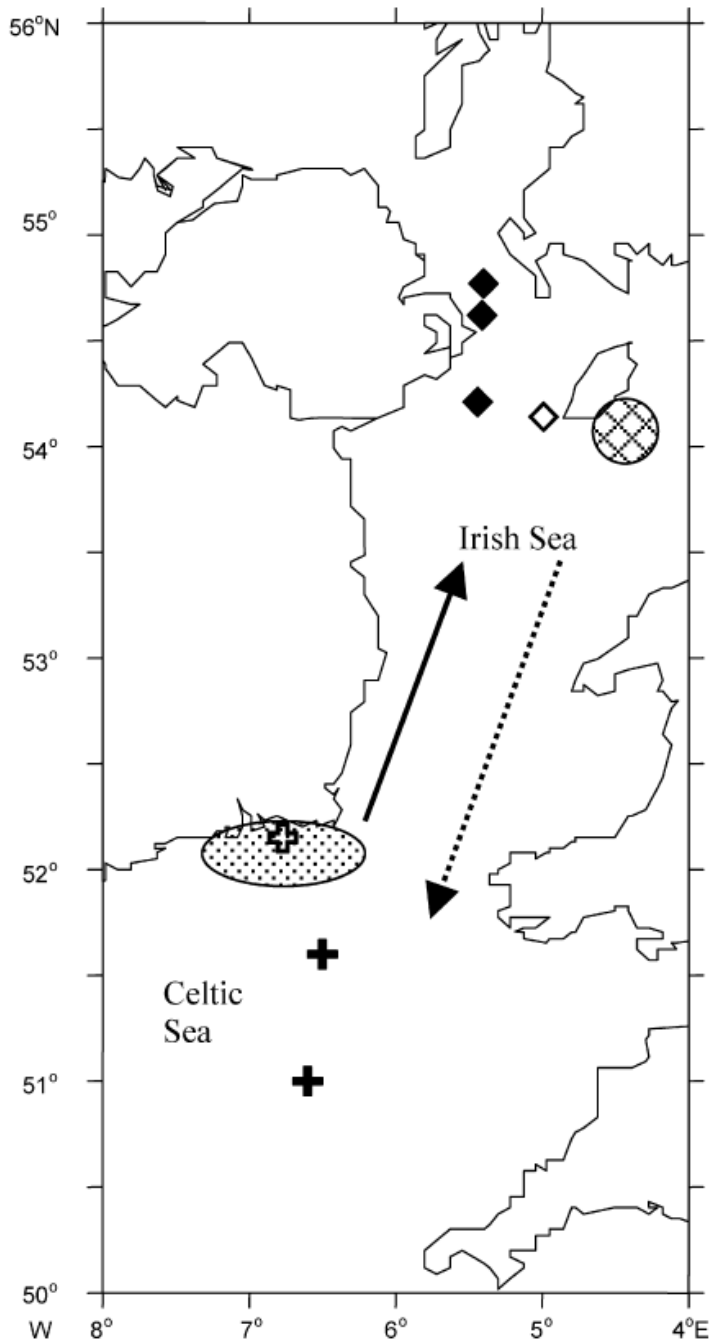
Figure 2.1 Sampling locations in the Celtic Sea (crosses) and Irish Sea (diamonds) of fish from the 2003 cohort as age-0 fish in 2004 (solid) and age-1 fish in 2005 (outlines). Dotted and criss-cross ellipses denote principle spawning areas for winter and autumn spawning fish, respectively. Solid arrow indicates dispersal of Celtic Sea herring to nursery grounds in the Irish Sea. Dashed arrow indicates proposed return route.
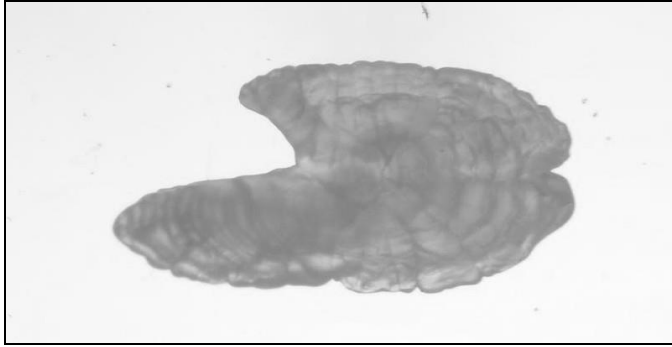
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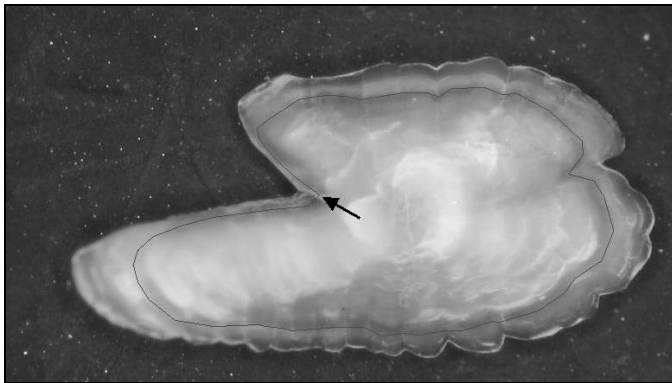
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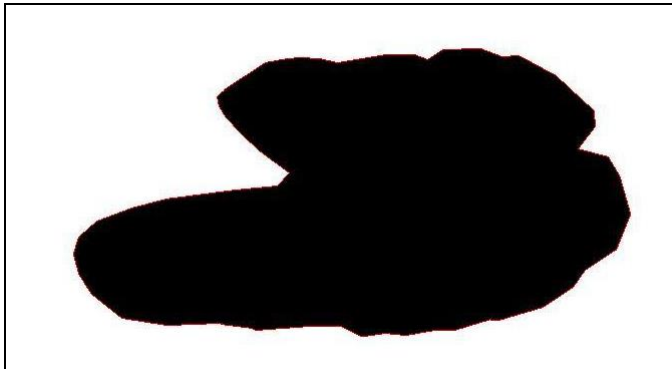
Figure 2.2c

Figure 2.2. Otolith Images: a) image of age-0 herring otolith taken using transmitted light; b) age-1 herring otolith with trace of the first winter ring marked by black line. Outline traces started at excisura major marked by arrow; and c) age-1 herring otolith trace filled in black in preparation for elliptic Fourier analysis.
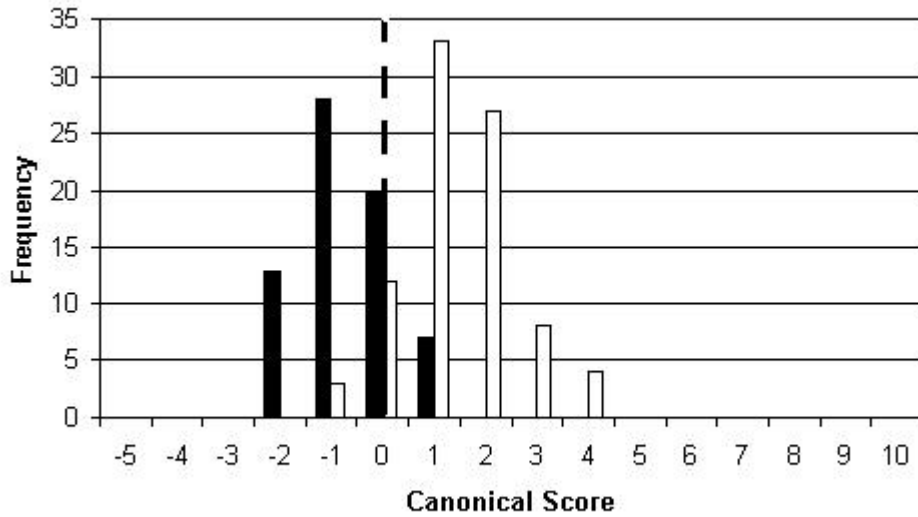
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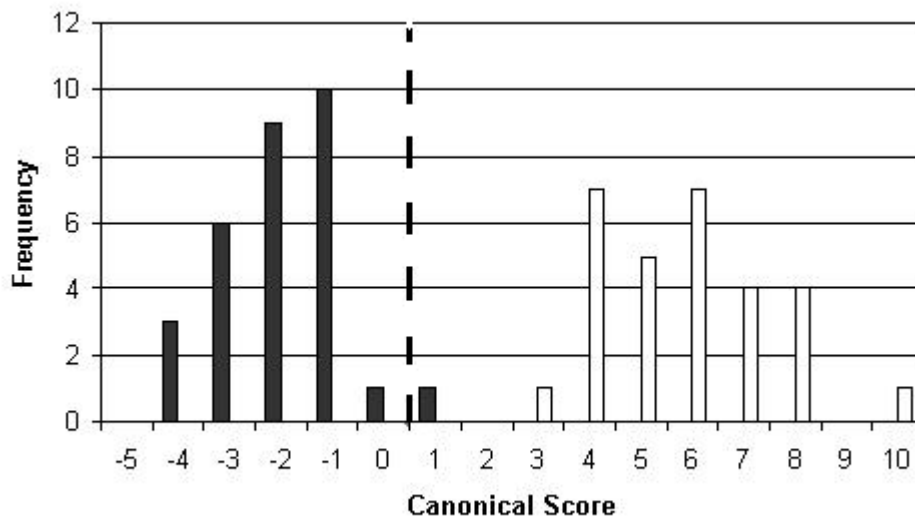
Figure 2.3b

Figure 2.3. Frequency distribution of canonical scores of (a) age-0 fish obtained from discriminant function analysis, and b) age-1 fish that were classified using the same classification function. All fish are from the 2003 cohort. Black bars indicate Celtic Sea fish; open bars indicate Irish Sea fish. Dashed lines indicate approximated separation of components.

Table 2.1 Mean length ± standard deviation and mean increment width from increment 61-70 ± standard deviation of winter-spawned herring captured in 2004 and 2005.

| Region | n | 2004 age-0 | | n | 2005 age-1 | |
		Fish length (cm)	Increment width (μm)		Fish length (cm)	Increment width (μm)
Irish Sea	90	9.7 ± 1.1	3.1 ± 0.5	29	18.2 ± 0.6	3.2 ± 0.4
Celtic Sea	68	15.3 ± 1.0	3.4 ± 0.5	28	20.5 ± 0.6	3.4 ± 0.3

Table 2.2 Size parameters and resulting shape indices calculated for analysis of each otolith in age-0 and age-1 fish from the 2003 cohort.

Size Parameters	Shape Indices
Area (A)	Circularity = P/A^2
Perimeter (P)	Rectangularity = $A / (FL*FW)$
Feret Weight (FW)	Form-Factor = $(4\pi A)/P^2$
Feret Length (FL)	Roundness = $(4A) / (\pi FL^2)$
	Ellipticity = $(FL-FW) / (FL+FW)$

Table 2.3 Pearson's correlation coefficients for variables selected for discriminate function analysis for age-0 fish from the 2003 cohort from the Irish and Celtic Seas.

| | Celtic Sea age-0 | Irish Sea age-0 | |
	Length	Length	B
Rectangularity	-0.20 (0.10)	-0.11 (0.32)	
C12	0.01 (0.99)	-0.17 (0.12)	
C14	0.12 (0.34)	0.27 (0.01)	$7 * 10^{-5}$
C14 adjusted	0.02 (0.86)	0.17 (0.12)	
C25	-0.08 (0.55)	-0.18 (0.09)	

p values are indicated in parenthesis.

Table 2.4 Results of ANOVAs investigating otolith shape differences between sites within each area and between areas (Celtic and Irish Seas) for age-0 juveniles from the 2003 cohort.

	Between sites within Irish Sea '04			Between sites within Celtic Sea '04			Celtic Sea vs. Irish Sea '04		
	F	df	p	F	df	p	F	df	P
Rectangularity	0.99	2	0.37	3.05	1	0.09	11.73	1	<0.01
C12	1.88	2	0.16	2.42	1	0.13	36.32	1	<0.01
C14	0.61	2	0.55	0.12	1	0.73	40.53	1	<0.01
C25	1.09	2	0.34	0.04	1	0.84	34.81	1	<0.01

Table 2.5 Jackknifed classification matrix for discriminate function analysis of the age-0 juveniles and cross validation of age-1 fish from the Irish and Celtic Seas. All fish sampled from the 2003 cohort.

Actual origin		Classified to		
	Age	Celtic Sea	Irish Sea	% Correct
Celtic Sea	0	55	13	81
	1	28	2	93
Irish Sea	0	14	73	84
	1	0	29	100
Total	0	69	83	83
	1	28	31	97

Chapter 3

Otolith shape analysis, its application for discriminating between Irish and Celtic Sea herring (*Clupea harengus*) stocks in the Irish Sea[5].

3.1 Abstract

The extensive movement of Celtic Sea juvenile Atlantic herring (*Clupea harengus*) during the first year of life into the Irish Sea results in two stocks of herring occurring together at Irish Sea nursery grounds: the resident autumn spawned juveniles that originate in the Irish Sea and the winter spawned juveniles that hatch in the Celtic Sea and drift into the Irish Sea during their larval stage.

Measurements of otolith increment widths can be used to distinguish between the fast growing winter-spawned and slow growing autumn-spawned stocks; however this method can be time consuming. This study investigates otolith shape analysis as an alternative method for discriminating between seasonal spawning stocks. Juvenile herring were collected from nursery grounds in the Irish Sea in 2006 and were classified as autumn or winter spawned using increment width measurements. Otolith shape was defined using shape indices and Fourier descriptors. Juveniles were successfully classified to hatch type with a high degree of accuracy (86-87%) using shape variables. The potential use of otolith shape analysis for identifying Celtic Sea juveniles in the Irish Sea and its possible use for other mixed herring stock assessments is discussed.

3.2 Introduction

Atlantic herring (*Clupea harengus*) is a highly complex species. Numerous stocks display differences in spawning season and location and life history parameters (McQuinn, 1997). Due to larval dispersal and adult migrations stocks, that spawn in separate locations often mix at nursery and feeding grounds (Messieh *et al.*, 1989, Mosegaard and Madsen, 1996, Rosenberg and Palmen, 1982) causing much uncertainty for management. This uncertainty has led to extensive research into methods for separating mixed herring stocks. Otolith microstructure (Brophy and

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[5] Chapter submitted to ICES Journal of Marine Science. Authors: Noirin Burke, Deirdre Brophy and Pauline A. King.

Danilowicz, 2002, Clausen *et al.*, 2007, Moksness and Fossum, 1991), otolith morphometric analysis (Messieh *et al.*, 1989, Turan, 2000, Burke *et al.*, 2008), vertebral counts (Mosegaard and Madsen, 1996), parasite prevalence (Campbell *et al.*, 2007) and genetics (Ruzzante *et al.*, 2006, Dahle and Eriksen, 1990) have all been used to distinguish between stocks or stock components with varying success rates depending on the stocks / stock components being investigated.

Around the Irish coastline, herring are managed in four separate units, of which the Irish Sea (ICES division VIIaN) and the Celtic Sea (ICES division VIIaS, VIIg-k) are two. Within the Irish Sea, herring spawn in autumn, usually during a three to four week period from September onwards (Dickey-Collas *et al.*, 2001), while in the Celtic Sea spawning occurs in autumn and winter (Molloy, 1980a). Evidence from larval drift studies (Özcan, 1974), length and vertebral count distributions (Bowers, 1964), tagging studies (Molloy *et al.*, 1993) and otolith increment widths (Brophy, 2002) show that juvenile herring disperse from the east of the Celtic Sea into the Irish Sea during their first year of life and mix with the resident Irish Sea stock at nursery grounds in the Irish Sea. Evidence of the winter-spawned Celtic Sea herring returning to join the Celtic Sea winter spawning stock when they mature has been provided by tagging experiments (Molloy *et al.*, 1993), otolith microstructure (Brophy *et al.*, 2006) and parasite prevalence (Campbell *et al.*, 2007).

Otolith microstructure analysis has been used extensively in herring research since the discovery of daily increments within otoliths (Panella, 1971). Differences in microstructure between seasonal herring stocks have been used as a stock marker in the Irish and Celtic Sea (Brophy, 2002) , the Norwegian Sea (Moksness, 1992), the North Sea (Mosegaard and Madsen, 1996) and the North Sea and western Baltic (Clausen *et al.*, 2007). Within the Irish Sea, Brophy and Danilowicz (2002) found a clear bi-modal distribution in width at larval increments 61-70 reflecting the presence of two groups: the slower growing autumn spawned individuals and faster growing winter spawned fish. These growth pattern differences could potentially be used to identify winter spawned Celtic Sea juveniles in the Irish Sea on a routine basis. However otolith microstructure analysis can be time consuming, if otolith shape analysis could successfully separate the two components it could provide a fast and sustainable method for management. Without the separation of this stock, accurate juvenile abundance estimates for the Irish Sea fishery is hampered and results in the failure to produce a precise recruitment index for the Irish Sea (ICES, 2007).

Otolith shape analysis is also widely used for fish species identification and stock classification. Otolith shape is markedly species specific (L'Abee-Lund, 1988) and less variable than fish growth, presumably due to the dual function of the otolith as an organ of equilibrium and hearing (Campana and Casselman, 1993). Otolith shape has been used in numerous stock discrimination studies (Cardinale *et al.*, 2004, DeVries *et al.*, 2002, Stransky *et al.*, 2008) with levels of classification success ranging from 60-95 % for interstock separation depending on the species.

This study assesses the usefulness of otolith shape analysis as a tool for discriminating between Irish Sea (autumn spawned) and Celtic Sea (winter spawned) juveniles at Irish Sea nursery grounds are considers its value in the assessment of the Irish and Celtic Sea herring fisheries and other mixed herring stocks.

3.2 Methods

Fish sampling

Atlantic herring (*Clupea harengus*) were collected in the Irish Sea in September 2006 using mid water trawls during the herring acoustic survey onboard the RV "Corystes" with the Agri-Food Biosciences Institute (AFBI)[6], Northern Ireland. Individuals ranging from 7-19cm were collected in order to target fish from the 2005 year class as age-0 fish (i.e. fish with no translucent winter ring in the otoliths). Sampling was spatially stratified with four stations selected from the east of the Isle of Man and four from the west (see Figure 3.1). Fish were processed onboard or frozen whole at -20°C. Total standard length and weight were recorded to the nearest 0.1cm and 0.1g respectively and both sagittal otoliths were removed and cleaned in water before being stored dry in 5ml plastic vials.

Otolith microstructure analysis

Examination of otolith annuli was used to verify that all fish used in the analysis were at the age-0 stage. The mean lengths and numbers of fish used in analysis are shown in Table 3.1.

Otolith microstructure analysis was then used to classify individuals as winter or autumn spawned based on daily increment widths at the larval core using the method

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[6] Formerly known as The Department of Agriculture and Rural Development, DARD.

developed by Brophy and Danilowicz (2002) . Otoliths were processed following the method described in Burke *et al.* (2008).

Otoliths with an average increment width of ≥ 2.3 μm between increment 61-70 were classified as winter spawned, while fish with mean increment widths of ≤ 2.2 μm were classified as autumn spawned. Approximately 50% of individuals were classified using manual increment measurements. A blind test of 10 randomly selected otoliths was carried out to assess the success of classification based on visual inspection. Classification based on visual inspection was feasible due to the distinct growth patterns displayed by autumn and winter spawned fish (Brophy and Danilowicz, 2002) (see Figure 3.2a). The achieved classification success to hatch type was 100%, and remaining individuals were classified based on visual inspection alone.

Otolith shape analysis

Otolith shape can be described in a number of ways, one of the simplest being manual distance measurements. These measurements can be used in a series of mathematical equations that calculate shape indices which in this study included circularity, rectangularity, roundness, form-factor and ellipticity. More complex methods use image analysis software to generate coefficients that describe the shape of the otolith, such as Fourier series shape analysis. In this study, elliptic Fourier analysis was used to generate 77 shape coefficients (C4-C80) to describe the shape of each otolith outline. A combination of shape indices (form-factor) and coefficients (C12, C19 and C21) were used to describe otolith shape variation in juvenile autumn and winter spawned herring collected at nursery grounds in the Irish Sea. Methods used for obtaining shape indices and elliptic Fourier shape coefficients are described in Burke *et al.* (2008). Both otolith microstructure analysis and otolith shape analysis procedures were timed to estimate the processing time required for each method.

Data analysis

For the purpose of data analysis, winter and autumn spawned fish were treated as two separate stocks. Variability in growth was examined using fish and otolith lengths (Table 3.1). Lengths were first screened for normality and homogeneity of variance using Kolmorgorov-Smirnov normality tests and Levene's tests respectively. All tests were carried out with an alpha significance of 0.05 using MINITAB 14 for windows. Attempts to transform the data were unsuccessful so Kruskall- Wallis rank sum tests

were used to test for differences in fish and otolith lengths between the stocks and between sites within each stock.

Shape indices and elliptic Fourier shape coefficients (henceforth referred to collectively as shape variables) were screened using the same procedure. Variables which did not meet the assumptions for parametric tests were tested using the non-parametric equivalent.

Univariate ANOVAs or Kruskall- Wallis rank sum tests were used to test for shape differences between the stocks and between sites within each stock using MINITAB 14 for windows. Variables that showed no difference in shape between sites within stocks were deemed representative of that stock. Variables that differed significantly between stocks were considered potentially useful for classifying fish to spawning season and were selected for further analysis. Next, variables were tested for significant correlations with each other. Where two variables were found to have a high correlation coefficient (>0.5) only one was selected for use in the final analysis. Based on the results of these tests form-factor and three coefficients (C12, C19 and C21) were selected for further analysis.

Next, variables were tested for significant correlation with fish length to identify any size effects using Pearson's correlation. ANCOVAs (with fish length as a covariate) were carried out using SYSTAT 11 for windows to determine if there was a significant relationship between fish length and each variable ($p<0.05$) within each stock. If no significant interaction is identified size effects can be corrected for using the common within group slope (Begg *et al.*, 2001, DeVries *et al.*, 2002, Galley *et al.*, 2006, Reist, 1985). Form-factor and C21 were identified as significantly correlated with fish length and were adjusted accordingly as no significant interaction was identified. This adjustment successfully removed the significant correlation with fish length.

Variables were also tested for within group correlation using SYSTAT 11 for windows to ensure multicollinearity would not result in use of redundant predictors in the final analysis. Box's M test was carried out using PAST-version 1.75b (Hammer *et al.*, 2001) to test for heterogeneity of covariance matrices and was identified as significant ($p = 0.02$)

Where the assumptions of equal covariance matrices are violated the optimal classification is achieved using a quadratic function rather than a linear (Seber, 2004).

Quadratic discriminate function analysis (QDFA) was carried using form-factor (adjusted), C12, C19 and C21 (adjusted) to determine the proportion of individuals that could be correctly classified as belonging to the autumn or winter spawned stock based on otolith shape. This procedure initially classifies each case into the group where the value of its classification functions is highest. These results may be misleading because the classification rules are evaluated using the same cases that are used to compute them. The jackknifed classification procedure attempts to remedy this problem by removing and replacing each case one at a time and using functions for all the data except the one being classified (Engelman, 2004). Both procedures were carried out in SYSTAT 11 for windows for age-0 autumn-spawned and winter-spawned stocks.

3.4 Results

Of 244 fish analysed, 115 were classified as autumn-spawned while 129 were classified as winter-spawned based on otolith increment widths. Ninety seven percent of the individuals classified as autumn-spawned were from stations in the east Irish Sea, while 92% of individuals classified as winter were from stations in the west Irish Sea (see Figure 3.1). Of the individuals classified by manual measurement of the increment widths (54% of total) the same split in mean increment widths at increment 61-70 was identified as Brophy and Danilowicz (2002). Processing times took approximately five minutes per individual for otolith shape analysis and 20 minutes for microstructure analysis where manual increment measurements were taken. This was reduced to approximately 12 minutes when classification was carried out by visual inspection alone.

Kruskall-Wallis rank sum tests identified differences in both otolith and fish lengths between the two stocks (p<0.05). However both otolith and fish length was also found to differ significantly between sites within the autumn-spawned (p<0.05) and winter-spawned stock (p<0.05). Fish length was also observed to overlap between the autumn and winter components and not display the clear bi-modal distribution that otolith microstructure did (see Figure 3.2b).

Individuals were classified to spawning type using Quadratic discriminate function analysis. Classification rates varied from 87% for overall classification and 86% for

jackknifed classification. The mahal distances were plotted to show visually how the two stocks separated from one another (see Figure 3.3).

3.5 Discussion

The distribution of Celtic Sea winter spawned juveniles to the West of the Isle of Man (92% of fish sampled) and Irish Sea autumn spawned juveniles to the East of the Isle of Man (97% of fish sampled) in 2006 suggests that both stocks inhabit distinct locations within the Irish Sea nursery areas. However this segregation was not observed at all sites or in previous work on juvenile herring in the Irish Sea (Brophy and Danilowicz, 2002, Burke *et al.*, 2008). Brophy and Danilowicz (2002) found inter-annual variation in the distribution patterns of winter and autumn spawned juveniles in the Irish Sea with winter-spawned fish occurring on both sides of the Irish Sea. With the distribution of juveniles across the Irish Sea varying from year to year their separation based on geographical location alone would not be temporally stable. Similarly, fish size can not be used to separate autumn and winter spawned juveniles. While fish and otolith length did differ significantly between autumn and winter spawned fish, they also differed between sites within each spawning group. Unlike otolith microstructure measurements, fish length did not display a bimodal distribution and overlapped between autumn and winter spawned fish. This overlap in size between autumn and winter spawned juveniles was also observed by Brophy and Danilowicz (2002) in the Irish Sea in 1999 and 2000. This, together with the differences observed between sites within each component, suggests that size is more influenced by environmental conditions during the juvenile phase than by hatching date or stock origin. This would indicate that size is not a suitable parameter for separating the two components.

Shape variables that displayed significant correlation with fish length were adjusted for the size effect using the common within group slope. This correction was crucial as size effects can compromise stock discrimination studies if variables are not standardised (Smith, 1992). The adjustment of variables did not remove differences between spawning groups making discrimination based on shape more widely applicable across years and regions than separation based on size alone.

The factors that influence shape are not fully understood and are not investigated directly in this study. Numerous studies on stock discrimination have evaluated the

relative importance of genetics / environmental conditions on otolith shape but few studies have directly examined the subject. Gauldie and Nelson (1990) found growth rates had a direct link to otolith shape with faster growth producing longer thinner crystals while Gagliano and McCormack (2004) found that recent feeding regimes influenced otolith shape in tropical fish species. Other studies have linked shape differences to rates of somatic growth (Begg and Brown, 2000, Cardinale *et al.*, 2004, Simoneau *et al.*, 2000). Some studies have found that classification success from otolith shape increased as genetic discreteness or geographic separation increased (Cardinale *et al.*, 2004, Friedland and Reddin, 1994) implying the genetic differences were responsible for shape variation. Other studies have shown substantial differences between groups with little or no geographic separation (Galley *et al.*, 2006, Pothin *et al.*, 2006), or which could not be separating using genetic techniques (DeVries *et al.*, 2002). Reared and wild components of the same genetically distinct stock have also been shown to display differences in otolith shape in response to difference in environmental conditions (Cardinale *et al.*, 2004, Simoneau *et al.*, 2000). It is uncertain if the shape differences between the autumn and winter stocks observed in this study are driven by genetic factors or by differences in environmental conditions experienced by the components during the first year of life. Little genetic difference have been observed between stocks around the British Isles to date (King *et al.*, 1987, Jorstad *et al.*, 1991, Turan, 1997) and both spawning types experience different environmental conditions due to the difference in their spawning times.

Regardless of what is causing the observed differences, shape analysis has great potential for providing a fast, reliable and sustainable method of identifying components of fish within a mixed fishery. It is less time consuming than otolith microstructure analysis and has lower running costs with software being freely available for carrying out analysis once images are taken. The procedure is also far less destructive to the otoliths as only images of whole otoliths are used; microstructure analysis relies on the polishing or sectioning of the otolith to expose the larval core. Otoliths can easily be damaged or destroyed during the microstructure process rendering the otolith worthless.

Within the Irish Sea otolith shape analysis may provide an alternative or collaborative method to otolith microstructure analysis for separating trawl catches of juveniles into autumn and winter spawned fish. This would benefit the management of herring in both the Irish and Celtic Seas (ICES, 2007). Regular monitoring of the proportion of

Celtic Sea juveniles in the Irish Sea would improve the juvenile abundance estimates for the Irish Sea and increase the accuracy of recruitment indices for the Irish Sea spawning stock. The incorporation of this method into annual herring sampling programmes would supply information to the scientist at very little extra cost or effort.

Otolith shape analysis may have applications in the management of other mixed herring stocks. In the North Sea autumn spawned juveniles drift into the western Baltic during their first year of life and mix with western Baltic spring spawned herring. At present they are monitored in the western Baltic where the catch is split into autumn and winter spawned using otolith microstructure analysis (ICES, 2007). Western Baltic spring spawned juveniles that drift into the North Sea during the first year of life are identified using vertebral counts and otolith microstructure analysis (ICES, 2007, Clausen *et al.*, 2007). If the seasonal spawning stocks in these areas show variability in otolith shape the method could facilitate their rapid separation and be incorporated into routine assessment.

3.6 Conclusion

The method presented here shows potential for separating components of fish in mixed stock fisheries. The results correspond well with other studies that have assessed otolith shape analysis as a method for separating stocks. The advantages of this method over current methods such as otolith microstructure analysis include speed of analysis, availability of software, and preservation of otolith for further / alternative analysis. The potential to adjust shape variables for size effects where significant correlations exist make them more useful than characteristics based on size / growth alone. As a method it could be easily incorporated in stock discrimination studies where otolith analysis is already carried out.
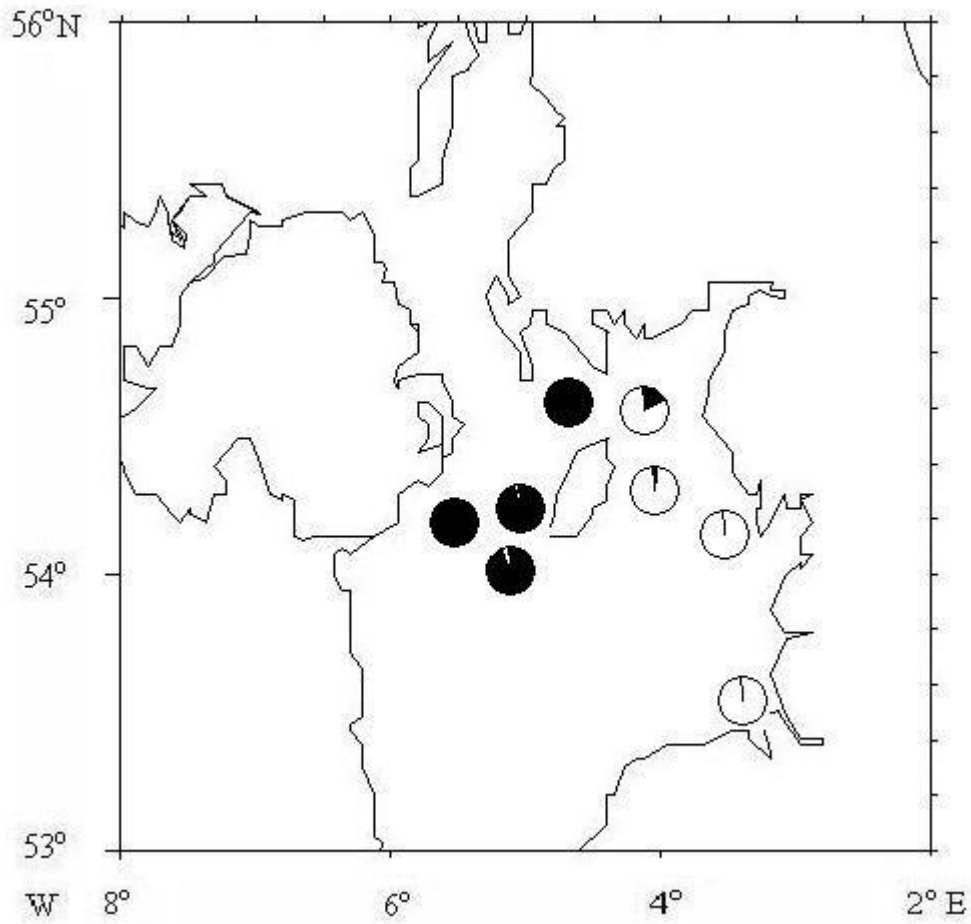
Figure 3.1 Map showing relative proportion of autumn- (white slice) and winter- (black slice) spawned age-0 herring collected in the Irish Sea in 2006.
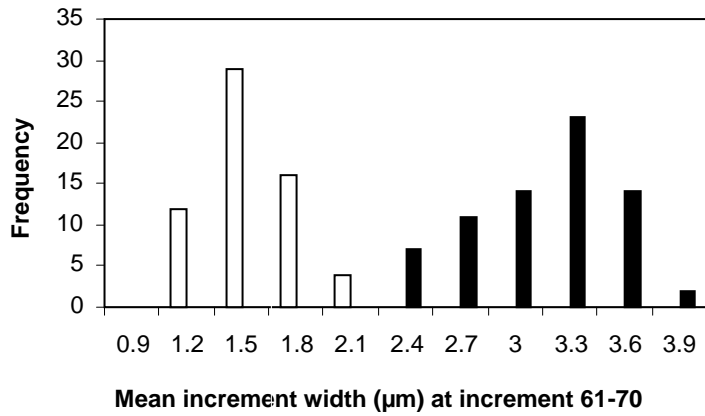
a)
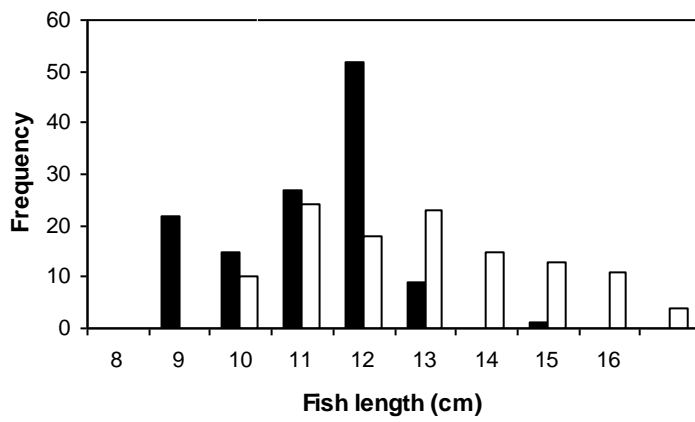
b)

Figure 3.2 Frequency distribution of a) mean increment width at increment 61-70 and b) mean fish lengths; of age-0 fish captured in the Irish Sea in 2005. Black bars indicate winter spawned Celtic Sea fish; open bars indicate autumn spawned Irish Sea fish.
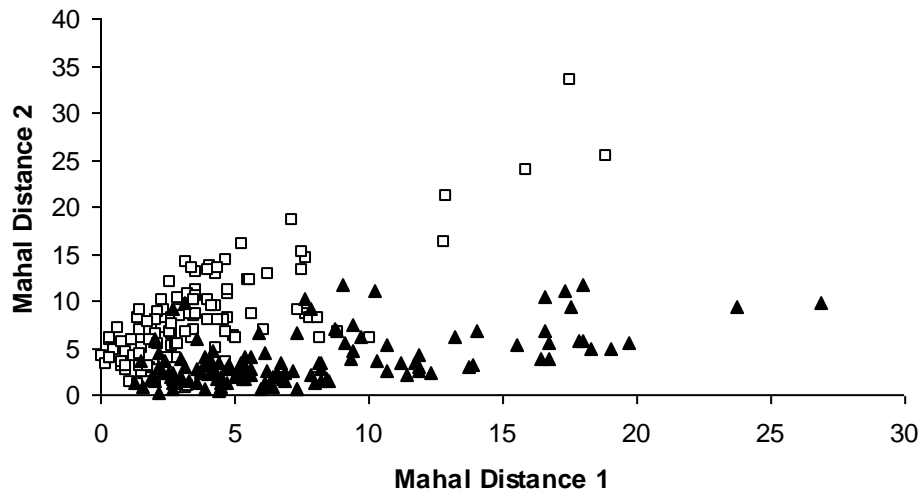
Figure 3.3 Frequency distribution of mahal distances obtained from quadratic discriminate function analysis of age-0 fish collected in 2005. Black triangles indicate winter spawned Celtic Sea fish; open squares indicate autumn spawned Irish Sea fish.

Table 3.1 Mean fish length and otolith length ± standard deviation for autumn- and winter-spawned age-0 fish captured in the Irish Sea in 2006.

Spawning group	N	Fish length (cm)	Otolith length (mm)
Autumn	118	11.5 ± 1.9	2.2 ± 0.2
Winter	126	10.7 ± 1.3	2.0 ± 0.1

Chapter 4

Otolith shape analysis provides evidence of natal homing in Atlantic herring (*Clupea harengus*)

4.1 Abstract

Herring stock structure continues to be the focus of extensive research. Studies which focus on stock discrimination attempt to clarify the mechanisms that maintain complexities such as separate spawning times and locations in a migratory species, where stocks often mix throughout their adult lives. Within the Celtic Sea there are two components of winter-spawned Atlantic herring (*Clupea harengus*); those that spend their juvenile phase at Celtic Sea nursery grounds (resident), and those that drift into Irish Sea nursery grounds during their first year of life (migrant). Previous work has established that the two groups show variation in otolith shape and outline analysis of the first winter ring can be used to determine the nursery ground origin of 1 year old fish. This study applies this separation technique to spawning adults collected from the Celtic Sea in winter. The results indicate that approximately 42% of these fish spent their juvenile phase in the Irish Sea, confirming that despite extensive dispersal during early life, Celtic Sea migrants return to their natal stock to spawn. This is the first study to show strong evidence of natal homing in a cohort of Atlantic herring and the studies findings offer valuable insights into the mechanisms maintaining population structure.

4.2 Introduction

The ambition to isolate the factors that regulate fish populations provides many challenges for fisheries science and assessments and is often a driving factor behind fisheries research, especially for commercial species. Even the definitions of such terms as the "unit stock" has provided much debate in scientific literature (Waldman, 2005). The mechanisms that maintain fish population structure and integrity are often hard to identify in nature. In the marine environment, understanding of stock structure is complicated by the lack of obvious physical barriers to groups of fish mixing throughout their life history. With migratory species the issue is further confused as

fish move between nursery, feeding, wintering and spawning grounds as part of their reproductive strategy. While physical barriers such as ocean currents (Wilson and Boehlert, 2004, Wroblewski *et al.*, 2000) may provide the limiting factor for some populations, behavioural cues may be more influential in other groups such as salmonidae (Quinn *et al.*, 2006, Satou *et al.*, 1998) and reef fish (Almany *et al.*, 2007, Gerlach *et al.*, 2007).

Atlantic herring (*Clupea harengus*) is a highly complex migratory species of substantial economic importance within the northern hemisphere. In the north east Atlantic there are numerous herring management and assessment units each with their own spawning time and location (Parrish and Saville, 1965). There are several examples of herring stocks mixing at juvenile nursery areas and adult feeding grounds (Husebo *et al.*, 2005, Rosenberg and Palmen, 1982, Ruzzante *et al.*, 2006, Brophy and Danilowicz, 2002). A number of theories have been proposed to explain how herring population structure is maintained. Iles and Sinclair (1982) hypothesized that herring population structure is maintained through the retention of larvae near natal spawning grounds by ocean currents. Adults home to specific natal spawning grounds at precise spawning times, keeping local populations reproductively isolated. In contrast, Smith and Jamieson (1986) proposed that there was no stable population structure in herring, that larval and juvenile mixed randomly: with some fish homing to their natal spawning grounds while others stray, making gene flow between populations significant. They suggested that herring populations would expand and contract their range in relation to environmental factors and fishing pressures. According to McQuinn's "adopted migrant" hypothesis (McQuinn, 1997) homing is learnt through social transmission and adult herring display repeat homing instead of natal homing. Larvae and juveniles that stray from the natal population learn their homing and migration patterns from the adults with which they associate and recruit to this adoptive population. Evidence from herring stocks does not provide definitive support for any one theory. Larval and juvenile herring have been shown to drift away from their natal spawning area and mix with juveniles from other herring stocks (Brophy and Danilowicz, 2002, Rosenberg and Palmen, 1982) which is inconsistent with the closed life cycle model of Iles and Sinclair (1982). There is indirect evidence to indicate that these juveniles return to spawn with their natal spawning stock providing support for the occurrence of natal rather than repeat homing (Brophy and Danilowicz, 2002, Rosenberg and Palmen, 1982, Molloy *et al.*, 1993, Campbell *et al.*, 2007,

Brophy *et al.*, 2006). The complexity of herring populations in the North East Atlantic, each with their own spawning time and location would suggest that populations are not in dynamic balance (Parrish and Saville, 1965).

Around the Irish coastline, there are four management units, of which the Irish and Celtic Seas are two (ICES Divisions VIIa and VIIj respectively). Within the Irish Sea, herring spawn in autumn, usually for a 3-4 week period beginning in September (Dickey-Collas *et al.*, 2001). In the Celtic Sea, spawning takes place in both autumn and winter (Molloy, 1980b) and it is now widely accepted that a considerable proportion of Celtic Sea winter-spawned juveniles drift into the Irish Sea nursery grounds during the first year of life (ICES, 2007). Otolith increment width measurements have been used to identify Celtic Sea winter-spawned juveniles that drift into Irish Sea nursery grounds (Brophy and Danilowicz, 2002, Burke *et al.*, 2008). Evidence from tagging studies (Molloy *et al.*, 1993); microstructure analysis (Brophy *et al.*, 2006) and parasite prevalence (Campbell *et al.*, 2007) suggests that these migrant Celtic Sea juveniles return from the Irish Sea to join the Celtic spawning stock when they mature; however no previous study has traced a cohort from the larval to the adult phase to provide direct evidence of natal homing.

Burke *et al.* (2008) used otolith shape analysis to distinguish between winter spawned juveniles collected in the Irish Sea (migrant) from those sampled in the Celtic Sea (resident). This shape difference was found to be preserved to an adequate level in the trace of the first winter annuli of the otolith to differentiate between the resident and migrant components in older fish. This method is used in the current study to determine the juvenile origin of two year old adult fish collected from spawning assemblages in the Celtic Sea in winter (the same cohort that was examined in the previous study). If members of the migrant component are shown to return to the Celtic Sea stock this would provide direct evidence of natal homing in Celtic Sea herring. This would be a very significant finding, providing insight into herring population structure and natal homing in marine fish populations. The importance of the Irish Sea nursery grounds to the Celtic Sea spawning stock is also evaluated.

4.3 Methods

Fish sampling

Juveniles

Atlantic herring were collected in the Irish Sea and Celtic Sea in 2004. Fish sampling was stratified by size (9-17cm) in order to target herring from the 2003 cohort as juveniles. Irish Sea samples were collected using mid water trawls during the herring acoustic survey conducted by the Agri-Food and Bio-sciences Institute in Northern Ireland[7] onboard the RV "Lough Foyle". Celtic Sea fish were collected during the ground fish survey conducted by the Marine Institute onboard the RV "Celtic Explorer". Fish were collected in 2004 at three sites in the Irish Sea and two sites in the Celtic Sea (see Figure 4.1). Fish were processed onboard or frozen at -20°C. Total length and weight were recorded to the nearest 0.1cm and 0.1g respectively. Sagittal otoliths were removed and cleaned in water before being dried and stored in 5ml plastic vials. Full details of juvenile sampling is given in Burke *et al*. (2008).

Adults

Adult herring were collected in the Celtic Sea in 2006. Adults (fish classified as having two translucent winter rings in their otoliths) were targeted in 2006 by collecting fish between 18-26 cm. Samples were collected during the herring acoustic survey conducted by the Marine Institute onboard the RV "Celtic Explorer" at four sites in the Celtic Sea (Figure 4.1). Fish were processed onboard or frozen at -20°C. Total length and weight were recorded to the nearest 0.1cm and 0.1g respectively. Sex and maturity stages of adult herring were determined by visual inspection of the gonads according to the eight point maturity scale as used by the Irish Marine Institute (Landry and McQuinn, 1988). Sagittal otoliths were removed and cleaned in water before being dried and stored in 5ml plastic vials.

Otolith aging and microstructure analysis

Otoliths were placed in water and age was determined based on visual inspection of the translucent and opaque bands. Fish from the 2003 cohort were selected for the analysis (ie. age-0 fish from the 2004 collections and age-2 fish from the 2006

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[7] Formally known as the Department of Agriculture and Rural Development.

collections). The mean length and numbers of fish used in analysis are shown in Table 4.1.

Otolith microstructure analysis was used to classify individuals as autumn or winter spawned based on the method developed by Brophy and Danilowicz (2002). One otolith (typically the right) was used for microstructure analysis for each fish. Juvenile otoliths were mounted in TAAB resin on the sulcus side and polished until the larval core was exposed. Adult otoliths were mounted on the anti-sulcus side first and polished until light could penetrate the larval core. Each otolith was then remounted sulcus side down and polished on the opposite side until the larval core was fully exposed. Fish with a mean increment width of >2.3 μm between increments 61-70 from the core were classified as winter spawned, while fish with a mean increment width <2.2 μm in the same region were classified as autumn spawned. For some adult otoliths classification was based on visual inspection alone without measurement of increment widths. This was feasible due to the distinct otolith growth patterns displayed by autumn and winter spawned fish (Brophy and Danilowicz, 2002). To determine the reliability of the method a blind test was carried out using a random selection of 10 otoliths containing both autumn and winter spawned fish. Here 100% classification success to hatch type was achieved.

Autumn spawned fish were excluded from further analysis (2.5% of total fish sampled) in order to restrict the study to fish that had all spawned at the same time of year and were most likely of Celtic Sea origin. This limited the examination to resident (juveniles that remained at Celtic Sea nursery grounds) and migrant (juveniles that were present in Irish Sea nursery grounds) components of the Celtic Sea winter spawned herring stock.

Otolith shape analysis

Juveniles

Otolith shape can be described in a number of ways. Otolith size measurements can be used to calculate shape indices such as rectangularity / roundness which describe specific elements of the shape of the otolith. More complex methods include image analysis techniques such as Fourier analysis that characterise the outline of the otolith. In this study, a combination of shape indices and Fourier analysis were used to describe the shape of each otolith. A classification function was developed to differentiate between age-0 winter spawned herring from the Irish Sea (migrant

component) and the Celtic Sea (resident component). Full details of this method are given in Burke *et al.* (2008) and summarised below.

Using typically the left otolith (where only right otoliths were available, images were flipped using standard image analysis techniques) images were digitalised using an Olympus™ Camedia digital camera and an Olympus™ stereomicroscope at 20X magnification. Otoliths were positioned sulcus side down with the rostrum pointing to the left and photographed using transmitted light. Digital otolith images were obtained using an Olympus™ Camedia digital camera and an Olympus™ stereomicroscope at 20X magnification. Using these images a series of two dimensional measurements were taken using the image analysis package Olympus™ DP-Soft. These measurements were then used to calculate a series of shape indices (Tuset *et al.*, 2003, Russ, 1990) that are outlined in Table 4.2. Images were also used to obtain a digitalised outline of each otolith using TpsDig[8] software and to generate elliptic fourier harmonics using EFAwin[9], to describe the otolith shape for each individual. Each harmonic consisted of four coefficients resulting in 80 coefficients per individual (C1-C80).

Shape indices and coefficients (henceforth referred to collectively as shape variables) were examined to identify variables suitable for describing shape differences between the migrant and resident component in the juvenile sample. Variables selected for further analysis met the assumptions of normality and homogeneity of variance and displayed significant differences between components. The selected variables also showed no significant variation in fish from different sampling sites within each area. One shape index (rectangularity) and three coefficients (C12, C14 and C25) were selected based on these criteria. Any correlation with otolith length was removed using the common within group slope (b) (Turan, 2000, Tuset *et al.*, 2006, Reist, 1985). This was carried out to ensure size effects would not influence the classification of individuals, as site differences within components were observed in otolith length ($p < 0.05$). Discriminate function analysis was carried out in SYSTAT 11 for windows to determine the proportion of individuals that could be correctly classified to nursery area based on otolith shape.

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[8] Public domain program developed by F. J. Rohlf, available freely on the internet at http://life.bio.sunysb.edu/morph/index.html
[9] Public domain program developed by F. J. Rohlf and S. Ferson, available freely on the internet at http://life.bio.sunysb.edu/morph/index.html

Otolith microstructure was used to examine larval growth patterns in both migrant and resident components. By analysing larval growth patterns, the stage in the life cycle at which the two components separated was assessed. Larval drift studies (Özcan, 1974) have indicated that this separation occurs quite early on in the larval stage. As growing conditions differ between the Celtic and Irish Seas, different growth patterns will be observed in the two components once they separated. Average increments widths between days 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, and 61-70 were examined in both components to investigate when separation takes place.

Adults

Using typically the left otolith (where only right otoliths were available, images were flipped using standard image analysis techniques) images were digitalised using an Olympus™ Camedia digital camera and an Olympus™ stereomicroscope at 20X magnification. Adult otoliths were positioned sulcus side down with the rostrum pointing to the left, as a bright object on a dark background to ensure the first winter ring was clearly visible. For each adult fish the outer edge of the first winter ring was traced manually using Olympus™ DP-soft. The feasibility of using the trace of the first winter ring to determine juvenile origin was previously established by Burke at al. (2008) using age-1 juveniles. Each trace was filled-in in black using Microsoft paint and saved for further analysis.

Using the trace of the first winter ring shape indices were calculated as described for the juvenile otoliths.

Traces of first winter rings filled in black were also used to generate elliptic Fourier coefficients to describe each otolith outline using TpsDig[10] and EFAwin[11] software. Data on the relevant shape variables (Rectangularity, C12, C14 C25) were selected for determination of juvenile origin. C14 was adjusted using the common within group slope of the relationship with otolith length to remove size effects. The slope was calculated using data from both juvenile and adult otoliths.

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[10] Public domain program developed by F. J. Rohlf, available freely on the internet at
http://life.bio.sunysb.edu/morph/index.html
[11] Public domain program developed by F. J. Rohlf and S. Ferson, available freely on the internet at
http://life.bio.sunysb.edu/morph/index.html

Mixed stock analysis

Integrated stock mixture analysis (ISMA) was used to determine the proportion of migrant and resident individuals present in the sample of spawning herring from the Celtic Sea using the juvenile otoliths as the reference sample.

The ISMA method used here is a modified version of the maximum-likelihood-based method based on the EM algorithm by Millar (1987) refined by S.E. Campana and S. J. Smith (Campana *et al.*, 1999). To evaluate the accuracy of this method for classifying individuals based on shape, 10 simulation tests were carried out. A random selection of juveniles were removed from the juvenile reference sample and tested as the unknown sample against the remaining individuals. Classification success was compared to a perfect classification success of 100%, where all individual fish were assigned to the correct component.

Discriminate function analysis (DFA) was also used to determine the juvenile origin of the adult fish. The cross validation procedure was used to ascertain which individuals fell into the migrant and resident categories. Discriminant analysis is less suitable than ISMA for separating group mixtures when there is no prior knowledge of the composition of the mixed assemblage (Campana *et al.*, 2000). However, unlike ISMA, it can be used to assign each individual to a reference group. It facilitated the comparison of growth and maturity in the fish classified to either component. DFA was carried out with the prior probabilities set at the levels determined by ISMA.

Based on the results of this analysis, the migrant and resident components of the adult sample were examined for differences in maturity and condition to determine if nursery ground of origin had subsequent effects on the condition or age at maturity of adult fish. Fish condition was quantified using Fulton's condition index. Fulton's condition indices are calculated using the formula, W/L^3 where W is the total fish weight and L is total fish length. Condition indices were tested for normality and homogeneity of variance using Kolmorgorov-Smirnov normality tests and Levene's tests respectively. Tests were carried out in MINITAB 14 for windows to an alpha significance of 0.05. A Univariate ANOVA was then used to compare fish condition between components. Variation in maturity stages between the groups was assessed using an interactive chi-squared test (Preacher, 2001).

Otolith length was examined to identify growth differences between the migrant and resident components at the juvenile phase. While growth differences were identified between migrant and resident components, otolith length was also identified as

significantly different between sample sites within resident (p = 0.006) and migrant (p < 0.001) components. This within component variation indicates otolith size is not a reliable indicator of component type. It may vary from one sample site to the next and may be altered due to sample site selection and may also be influenced by size selective mortality.

4.4 Results

Analysis of larval growth identified distinct differences between migrant and resident components in otolith increment widths from as early as increments 1-10 after the hatch check (see Table 4.3 and Figure 4.3).

Using shape variables selected for DFA of the juvenile sample, an overall classification success of 84% and a jackknifed classification of 83% was obtained. A more detailed analysis of juvenile results is present in Burke *et al.* (2008).

An average of 86% classification success was achieved when ten ISMA simulation tests were conducted using juvenile otoliths of known nursery ground origin (Table 4.4). These classification success rates indicate that the margin of error associated with the determination of nursery ground origin in the adult samples is in the region of ± 7%.

Using ISMA analysis the estimated proportions of the resident and migrant component in the samples of spawning adults were 58% and 42% respectively. Considering the margin of error obtained in simulation tests, the contribution from the resident component could range from 51% -65% and the migrant from 35%-49%. ISMA was also carried out for samples from each site in the Celtic Sea separately. Adult fish which had spent the juvenile period in the Irish Sea occurred at all four stations. The proportion of these migrant individuals ranged from 34% - 52% depending on the station sampled. The station with the highest number of migrant individuals was the inshore station with the most easterly orientation closest to the Irish Sea (Figure 4.2, Table 4.5).

The DFA was used to assign each adult individual to the migrant or resident component based on the canonical scores. Prior probabilities were set to 0.58 and 0.42 based on the results of ISMA (if priors are not set manually they with automatically be set to 0.5 and 0.5). An important element of mixed stock analysis is that all groups contributing to the mix are properly represented in the reference sample. If fish from

other areas are contributing this may undermine the accuracy of the classification of the adult sample. Canonical scores were used to identify the potential that other groups were contributing to the adult sample, such as herring from the West coast of Ireland, however the shape of otoliths from other stocks around the Irish coastline were not included in the classification process. Distribution of canonical scores shows that the otolith shape of the adult sample corresponds well with the shape of the two reference groups of juvenile fish. This indicates that members of the adult group have not spent their juvenile phase outside of the reference areas (Celtic Sea and Irish Sea). Canonical scores of juvenile and adult fish are shown in Figure 4.4a and b. The results of both methods are summarised in Table 4.5.

Chi-squared analysis revealed no significant difference in the maturity stage of migrant and resident adults ($X^2 = 0.78$, $p > 0.05$). Fish condition was also found not to differ significantly between components using univariate ANOVA ($p > 0.05$) (Table 4.5).

4.5 Discussion

ISMA based shape analysis of the juvenile portion of the otolith (region bounded by the first annulus) indicates that approximately 42% percent of the adult fish collected from the spawning assemblages in the Celtic Sea in winter had spent their juvenile phase in the Irish Sea. Results from simulation tests indicated an error rate of $\pm 7\%$ in ISMA, however by observing canonical score results we identified a number of fish who spent their juvenile phase in the Irish Sea with a high degree of certainty.

This is the first study to track a stock throughout the life cycle and provide direct evidence of natal homing in Atlantic herring. Integration across the entire life history and consideration of the dispersive larval stages are crucial elements of natal homing investigations (Bradbury and Laurel, 2007). Winter spawned juveniles identified in the Irish Sea are most likely of Celtic Sea origin (Brophy and Danilowicz, 2002, Brophy *et al.*, 2006, Özcan, 1974). Otolith microstructure allowed the reconstruction of growth patterns and was used to identify juveniles of Celtic Sea origin that dispersed into the Irish Sea early in life (Brophy and Danilowicz, 2002). Otolith shape differences between the two components at the age-0 stage were identified and were found to be preserved to an adequate level in the trace of the first winter ring in age-1 fish (Burke *et al.*, 2008). Using both otolith microstructure and shape analysis, a

cohort of Celtic Sea herring was traced from the larval to adult phase. This study shows adult spawning assemblages in the Celtic Sea contain fish that spent their juvenile phase in the Irish Sea and demonstrates natal homing in Celtic Sea herring.

 The concept of fish returning to their natal site for reproduction has inspired many studies which attempt to identify the means by which fish find their way home. There are two schools of thought on how fish find their way home: learned behaviour and larval imprinting. In this study, learned behaviour can be ruled out as migrant juveniles who associated with autumn spawned Irish Sea adults did not recruit to the Irish Sea spawning stock (Brophy *et al.*, 2006). If larval imprinting is the mechanism enabling homing behaviour it would take place before migrant individuals left their natal spawning ground. The examination of otolith microstructure measurements found significant differences between resident and migrant components at increments 1-10 indicating that migrant larvae drift out of the natal area quite quickly, meaning larval imprinting must occur quite early in the larval phase. Herring hatch checks are thought to appear 10 days after hatching, when the yolk sac is absorbed (Geffen, 1982). Based on these timelines, migrant herring larvae move out of their natal spawning area 10-20 days after hatching. If they leave their natal spawning area at such a young age, how do they remember where home is? Are larval herring's sensory abilities sufficiently developed to facilitate detection and storage of characteristics of their spawning area?

The best examples of studies on homing behaviour in fish have been carried out on salmon (Quinn *et al.*, 2006, Satou *et al.*, 1998) and reef fish (Almany *et al.*, 2007, Gerlach *et al.*, 2007). Olfactory sensors, visual cues and detection of the earth's magnetic field have been shown to play an important role in homing behaviour (Almany *et al.*, 2007, Gerlach *et al.*, 2007, Mitamura *et al.*, 2005, Quinn *et al.*, 2006, Satou *et al.*, 1998). Pre-settlement reef fish larvae show a preference for the odour of their natal reef (Gerlach *et al.*, 2007) and are capable of returning to a very small target reef (Almany *et al.*, 2007). When tested in relation to visual cues Mitamura *et al.* (2005) found that black rockfish relied more on their olfactory system than their sight to find their way back to their natal site. Salmon have been shown to prefer home water over non-home water and it has been demonstrated that laceration of the olfactory sense organ seriously damages an individuals ability to detect home water and their upstream-running behaviour (Satou *et al.*, 1998). The exact stimulus for natal homing in Atlantic herring is unknown, however studies on herring sensory systems

have shown their importance at the larval and juvenile phase (Dempsey, 1978, Skajaa *et al.*, 2004). Skajaa *et al.* (2004) found that herring showed increased responsiveness to predators with age and condition. Dempsey (1978) found that herring juveniles show a preference for water which has already contained herring and that severing of the olfactory nerves resulted in the failure to detect food. Atlantic herring have been shown to produce fast repetitive tick sounds in association with bubble expulsion from the anal duct region indicating their sense of hearing is also used for communication (Wilson *et al.*, 2004). Perhaps a combination of olfactory, visual and hearing systems is used to guide individuals, with vision and smell being used to help individuals navigate to the right general location while sound is used to find specific shoals. Individuals may also use their olfactory systems to smell out spawning aggregations. Female goldfish have been shown to release a urinary pheromone into the water to advertise to males their condition and location (Appelt and Sorensen, 2007). Individuals may be predisposed to spawn at a particular time of year, determined by time of hatching. This may regulate the timing of their reproductive system, and cause them to orientate themselves with the closest spawning aggregation, which they locate by smell. The exact stimulus used by herring in homing behaviour is a fascinating subject and warrants further investigation.

This study has not investigated the spatial resolution of the homing behaviour. Otolith shape and microstructure analyses are probably not sufficiently sensitive to distinguish between fish from individual spawning beds. Other methods such as mass marking could be used to investigate the spatial scale of homing in Celtic Sea herring. Mass marking methods such as maternal transmission of stable isotopes have been used to investigate the fate of juveniles after they are spawned (Almany *et al.*, 2007, Thorrold *et al.*, 2006, Thorrold *et al.*, 2001). Thorrold *et al.* (2006) showed that isotope markers (barium 137) injected into pre-spawning females were transferred to the otoliths of embryonic juveniles. The method was particularly effective in benthic spawners. This application holds promise for future investigations of the spatial resolution of natal homing in herring which are also benthic spawners.

Mass marking may also provide a method for identifying the level of straying between the two stocks. Genetic analysis has found no significant difference between herring in the Irish and Celtic Seas (Jorstad *et al.*, 1991) indicating that there is some degree of gene flow between the two stocks. Straying between the two stocks was proposed by Brophy *et al.* (2006), when a small proportion of individuals (2%), identified in

autumn spawning aggregations, were classified as winter spawned, and shown switching spawning times. By monitoring the levels of natal homing and environmental conditions on a yearly basis, the conditions that trigger the homing response, such as sea temperature and water currents could be investigated. If chemical cues released in the water trigger fish to smell their way home, wind direction will have a significant effect on homing. Wind driven currents carrying chemical cues from the Celtic Sea into the Irish Sea will simulate migrant fish to return to their natal spawning sites. On the other hand, years when wind conditions are not favourable may cause higher numbers of migrant fish to stray and switch spawning times or die and ultimately be lost from the Celtic Sea spawning stock. It has been suggested that migrant Celtic Sea juveniles identified in the Irish Sea could be added to Celtic Sea assessment values and used to improve the accuracy of Celtic Sea recruitment indices (ICES, 2007, Burke *et al.*, 2008). However if homing behaviour is linked to environmental conditions, than weather patterns in each sample year will have to be factored in. If water temperatures simulate homing behaviour, wind conditions may also be important, as water temperatures in the Irish Sea have been shown to be influenced by wind direction (see chapter 5).

4.6 Conclusion

The results presented in this study provide exciting findings for the natal homing hypothesis. They also have important implications for herring stocks in the Irish and Celtic Seas and have wider applications to other herring stocks and herring stock structure theories.

Larval imprinting is suggested as the mechanism for homing behaviour in this study as learnt homing behaviour has not been demonstrated by the component of herring which moved into the Irish Sea during the early larval stage.

Studies of sensory responses in other fish species indicate that the olfactory system plays a role in homing, and smell has been shown to be important for food detection in herring. The level of development in sensory abilities in larval herring and the cues that trigger the homing response in adult herring are largely unknown. Further investigation into the links between environmental conditions and homing behaviour may indicate the factors that influence migrant Celtic Sea herring to home from the Irish Sea.

This is first study to trace a cohort of herring from larval to adult life and provide direct evidence of natal homing in Celtic Sea herring. Further research using methods such as mass marking may indicate the spatial scale of this homing ability and the levels of straying between the two stocks.
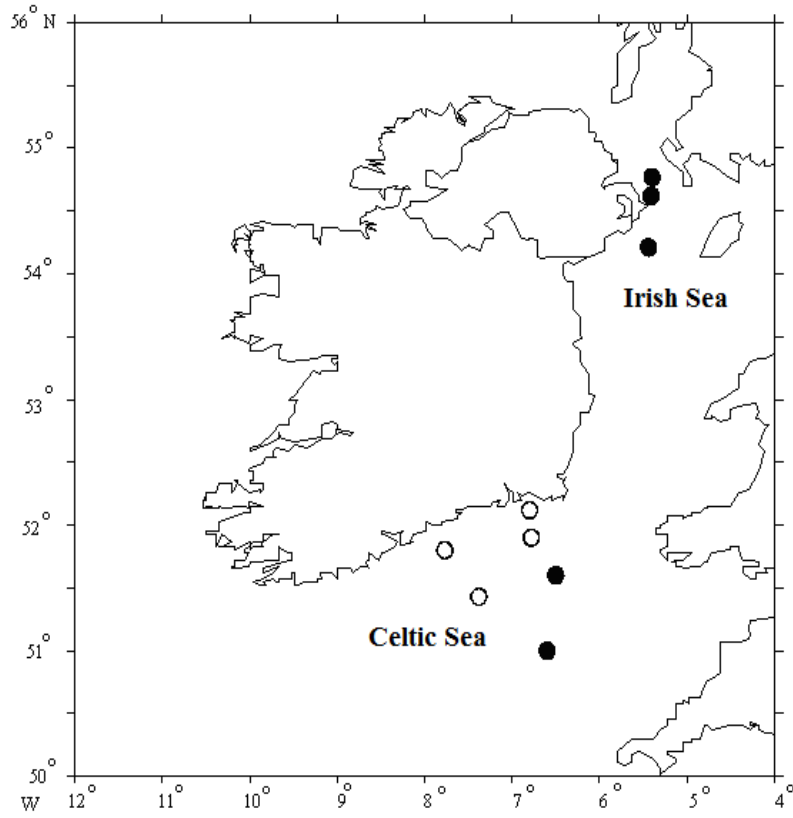
Figure 4.1 Sampling locations in the Irish Sea and Celtic Sea for fish from the 2003 cohort collected as juvenile fish in 2004 (solid circles) and from the Celtic Sea as adult fish in 2006 (open circles).
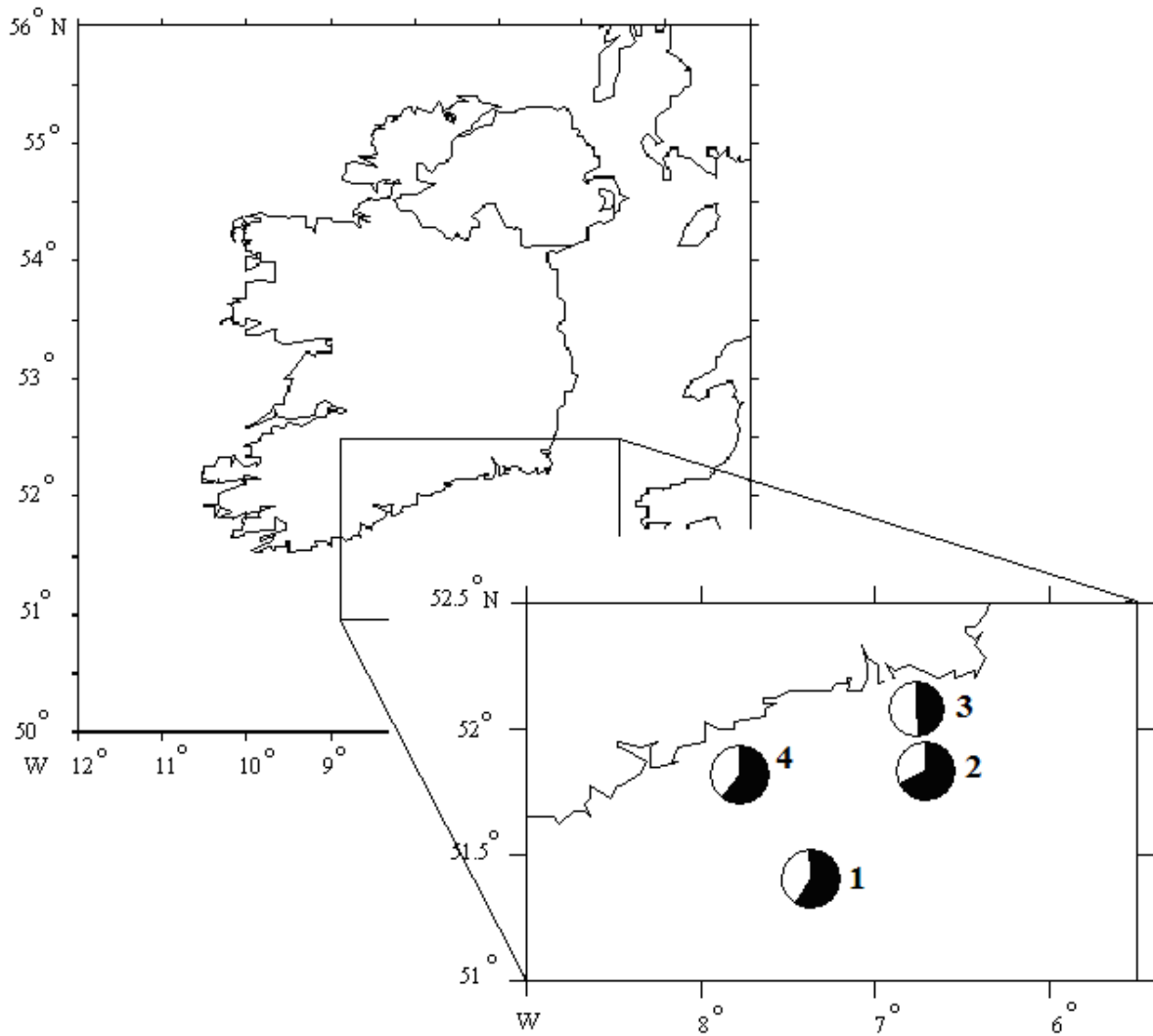
Figure 4.2 Map showing the relative proportion of resident (black slice) and migrant (white slice) adult fish collected in the Celtic Sea stations in 2006. Station code shown to right of pie chart.
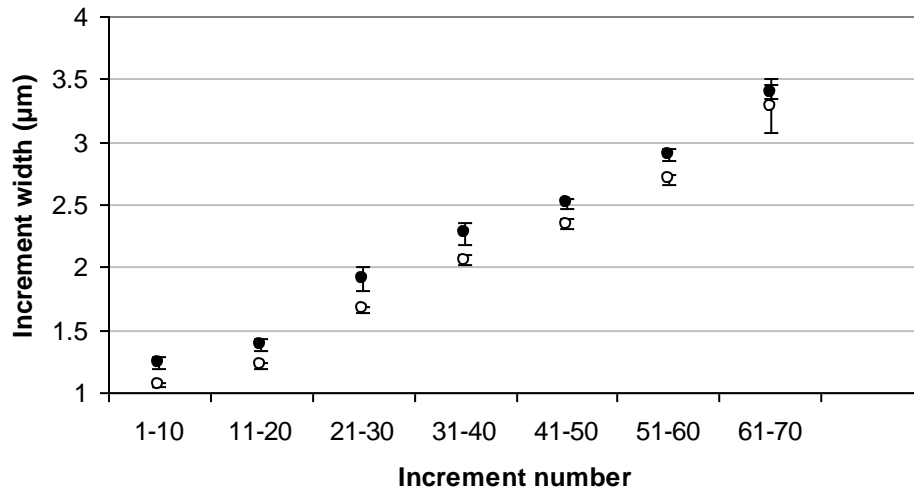
Figure 4.3 Mean increment widths, plotted against increment number for resident (black) and migrant (white) juvenile herring collected in 2004. Error bars show the standard error of the mean values.
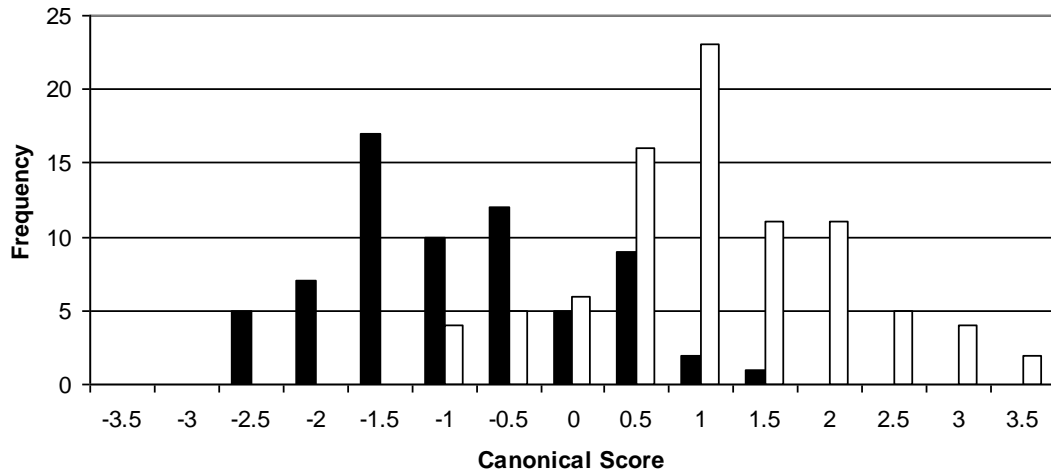
(a)
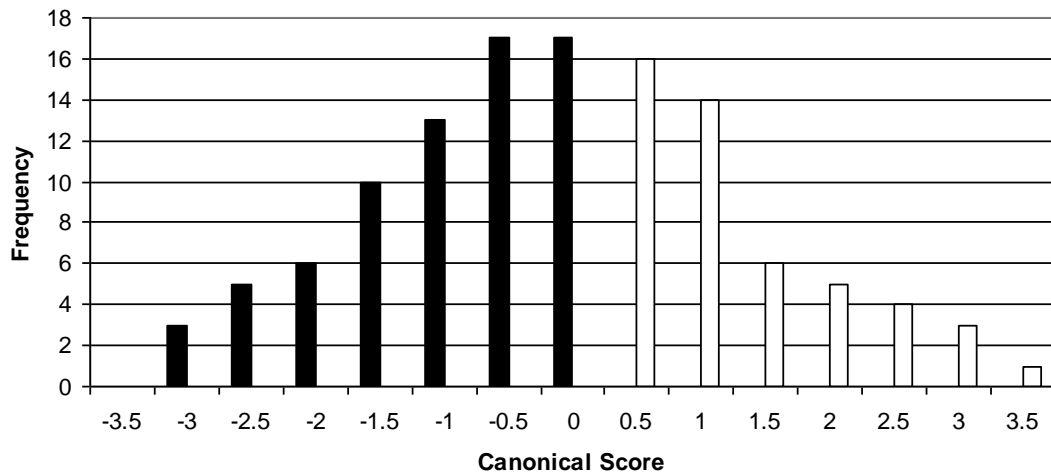
(b)

Figure 4.4 Frequency distribution of canonical scores obtained from discriminant function analysis of otolith shape variables for (a) juvenile fish, and (b) adult fish that were classified using the same classification function. All fish are from the 2003 cohort. Black bars indicate Celtic Sea fish; open bars indicate Irish Sea fish.

Table 4.1 Mean fish length and otolith length ± standard deviation of winter spawned herring from the 2003 cohort captured as juvenile fish in the Irish and Celtic Seas in 2004 and adult fish in the Celtic Sea in 2006. Otolith length of adult fish refers to feret length of first winter ring.

Region	Age	n	Fish length (cm)	Otolith length (mm)
Irish	0	90	9.7 ± 1.1	1.9 ± 0.2
Celtic	0	68	15.3 ± 1.0	2.8 ± 0.1
Celtic	2	120	23.3 ± 0.7	2.5 ± 0.2

Table 4.2 Size parameters and resulting shape indices calculated for analysis of each otolith in 0- and adult fish from the 2003 cohort.

Size Parameters	Shape Indices
Area (A)	Circularity = P/A^2
Perimeter (P)	Rectangularity = $A / (FL*FW)$
Feret Weight (FW)	Form-Factor = $(4\pi A)/P^2$
Feret Length (FL)	Roundness = $(4A) / (\pi FL^2)$
	Ellipticity = $(FL-FW) / (FL+FW)$

Table 4.3 Comparison of larval otolith increment widths between winter spawned age-0 fish sampled at nursery grounds in the Irish and Celtic Sea in 2004. (Kruskall-Wallis tests with Bonferroni adjusted probably level for multiple comparisons.) Significant differences marked with asterisk.

	Mean Increment Width (µm)			
	Celtic Sea	Irish Sea		
Increments	(n=70)	(n=90)	H	P
0-10	1.24	1.06	9.80	0.002 *
11-20	1.32	1.22	8.93	0.003 *
21-30	1.76	1.66	6.20	0.013
31-40	2.20	2.06	7.86	0.005 *
41-50	2.51	2.35	9.02	0.003 *
51-60	2.90	2.70	8.43	0.004 *
61-70	3.40	3.20	10.98	0.001 *

Bonferroni adjusted significance level: $0.05/7 = 0.007$.

Table 4.4 Summary of Simulation Tests carried out using ISMA (Integrated Stock Mixture Analysis).

Test Number	% Celtic Sea Origin	% Classified as Celtic Sea	% Irish Sea Origin	% Classified as Irish Sea	% Classified Correctly
1	50	45	50	55	90
2	50	42	50	58	85
3	50	57	50	43	86
4	40	28	60	72	70
5	35	43	75	57	88
6	60	63	40	37	93
7	45	54	55	46	84
8	25	25	75	75	100
9	55	40	45	60	73
10	60	61	40	39	97
Average % of individuals Classified Correctly					86

Table 4.5 Mixed stock analysis of adult herring collected in the Celtic Sea. Discriminate function analysis of adult herring tested as new group using classification function developed for juvenile sample. Resident = individuals who spent their juvenile phase at Celtic Sea nursery grounds. Migrant = individuals who spent their juvenile phase in Irish Sea nursery grounds. DFA= discriminate function analysis. ISMA = integrated stock mixture analysis.

Group		DFA ± Error	ISMA ± Error	Numbers at Maturity		Fulton's Condition Index
				Stage 3	Stage 4	
All Stations Pooled	Resident	59.0 ± 7	58.0 ± 7	43	25	$8.62 * 10^{-3}$
	Migrant	41.0 ± 7	42.0 ± 7	33	19	$8.57 * 10^{-3}$
Station 1	Resident	57.0 ± 7	58.0 ± 7			
	Migrant	43.0 ± 7	42.0 ± 7			
Station 2	Resident	67.0 ± 7	66.0 ± 7			
	Migrant	33.0 ± 7	34.0 ± 7			
Station 3	Resident	50.0 ± 7	48.0 ± 7			
	Migrant	50.0 ± 7	52.0 ± 7			
Station 4	Resident	53.0 ± 7	60.0 ± 7			
	Migrant	47.0 ± 7	40.0 ± 7			

Chapter 5

Temporal trends in the stock composition and abundance of juvenile Atlantic Herring (*Clupea harengus*) in the Irish Sea

5.1 Abstract

Celtic Sea herring (*Clupea harengus*) show extensive movement into the Irish Sea during their first year of life where they mix with resident fish. This influences the assessment of Irish Sea herring as juvenile abundance at Irish Sea nursery grounds does not provide a reliable recruitment index. Otolith microstructure can be used to separate Irish Sea autumn spawned and Celtic Sea winter spawned juveniles. The incorporation of this technique into routine monitoring of the fishery could facilitate the development of a recruitment index for the Irish Sea stock.

Otolith microstructure was used in this study to estimate the proportion of winter-spawned individuals in samples of age-1 herring (fish with one translucent winter ring in the otolith) from the western Irish Sea over a ten year period (1993-2003). These proportions were combined with data from the assessment of the Irish Sea stock to produce separate abundance estimates for each component. After adjustment, the abundance of age-1 autumn spawned Irish Sea juveniles was significantly correlated with the abundance of age 3 fish (fish with three translucent winter rings in the otolith) from the same year class estimated from commercial catch and acoustic survey data ($r > 0.8$, $p < 0.05$). Temporal trends in the abundance, growth and condition of the components were analysed in relation to environmental variables (temperature, wind and food availability). There was a significant negative correlation between the abundance of winter spawned fish in the Irish Sea and the incidence of strong prevailing winds from a south westerly direction during the larval phase ($r = -0.96$, $p < 0.05$).

These findings have important implications for the monitoring and assessment of herring in the Celtic and Irish Sea and the prediction of recruitment to the fishery.

5.2 Introduction

The movement of fish stocks between management areas and the mixing of stocks at feeding and nursery areas greatly complicates fisheries management. When stocks are

managed separately despite mixing throughout their lifespan, estimates become uncertain and an accurate assessment of the state of the stock is hard to ascertain (Daan *et al.*, 1990). These uncertainties pose particular problems for fisheries management where fish stocks straddle national boundaries and are targeted by fisheries from multiple jurisdictions.

The uncertainty in many mixed stock assessments has lead to the development of numerous methods for identifying stocks. Natural population markers include body morphometrics and meristics, otolith microstructure, shape and chemistry, parasite prevalence, gene frequencies and fatty acid profiles. Artificial tags and otolith marking techniques can also be used to trace populations (Friedland and Waldman, 2005).

Otolith microstructure analysis has been used extensively in fisheries research since the discovery of daily growth markings in otoliths (Panella, 1971). Otoliths grow throughout the life of the fish and once deposited are unlikely to be reabsorbed or altered by negative growing conditions (Campana and Neilson, 1985). Their growth rates can be influenced by a number of environmental factors such as temperature (Fey, 2001), prey density (Feet *et al.*, 2002), and photoperiod (Dowd and Houde, 1980). These properties make otoliths the ideal structure for tracing stocks that experience different environmental conditions during their life history. Otolith microstructure has proven particularly useful for distinguishing between seasonal spawning groups of herring (Brophy and Danilowicz, 2002, Clausen *et al.*, 2007, Moksness and Fossum, 1991, Mosegaard and Madsen, 1996, Munk *et al.*, 1991).

Within the northeast Atlantic, there are approximately fourteen separate herring stocks each with its own spawning time and location and there are numerous examples of stocks mixing throughout their life histories (Parrish and Saville, 1965). Around the Irish coastline there are four management units of which the Irish Sea and Celtic Sea stocks are two (ICES Divisions VIIa and VIIJ respectively). These stocks are managed separately; however, it is now widely accepted that there is extensive mixing of the two stocks during the juvenile phase (Bowers, 1964, Brophy and Danilowicz, 2002, Molloy *et al.*, 1993, Özcan, 1974). Within the Irish Sea there are two autumn spawning components, the Manx and the Mourne stock, with the majority of spawning occurring in a four-week period between September and October (Dickey-Collas *et al.*, 2001). In the Celtic Sea spawning takes place in both autumn and winter between October and January (Molloy, 1980a). Evidence from tagging studies

(Molloy *et al.*, 1993), length and vertebral count distributions (Bowers, 1964), larval drift studies (Özcan, 1974) and otolith microstructure analysis (Brophy and Danilowicz, 2002) has shown that juveniles from the Celtic Sea mix with juveniles from the Irish Sea at Irish Sea nursery grounds. These juveniles are not present in the adult spawning stock in the Irish Sea (Brophy *et al.*, 2006) but return to the Celtic Sea as adults to spawn (see chapter 4). The contribution of the migrant component to the total abundance of juveniles in the Irish Sea varies from year to year (Brophy and Danilowicz, 2002, Burke *et al.*, 2008) and the cause of this variation is unknown. It has been suggested that large wind events could increase the dispersal of Celtic Sea herring larvae into the Irish Sea (O'Brien & Little, 2006) and in this study the relationship between the prevailing winds and the abundance of winter spawned juveniles in the Irish Sea is examined.

As a result of this movement of Celtic Sea fish, juvenile abundance estimates from nursery grounds in the Irish Sea do not provide a reliable index of recruitment to the Irish Sea stock and it has been recommended that separation of Irish Sea juveniles into autumn and winter spawned fish based on otolith microstructure and/or length compositions be used to produce a recruitment index more appropriate to the Irish Sea stock (ICES, 2007). However the success of the splitting technique may be hampered by the limitations of otolith microstructure analysis. Microstructure analysis can only classify individuals from different spawning seasons. It cannot be used to differentiate between autumn spawned juveniles of Celtic Sea origin in the Irish Sea, and the resident autumn spawned population. Similarly winter spawning individuals in the Irish Sea cannot be broken down into those from the Celtic Sea spawning stock, and any resident winter spawned individuals who were spawned there, or individuals from the Clyde west of Scotland stock.

Recruitment indices produce early information for fisheries managers on year class strength and are used in stock assessments and virtual population analysis. They are important for monitoring fish stocks and give an early warning of poor year classes that may affect the overall biomass levels. Accurate and reliable recruitment indices are especially important in the management of species with highly variable recruitment such as herring (Parrish and Saville, 1965).

Otolith microstructure is used in this study to assess the relative proportions of winter and autumn spawned fish present in the Irish Sea over a ten year time span. These proportions are combined with data from the assessment of the Irish Sea stock to

produce separate abundance estimates for each component. Correlations with adult abundance estimates are investigated and the potential for using these split estimates of juvenile abundance to develop recruitment indices for the Irish and Celtic Sea herring stocks is evaluated. The influence of environmental conditions such as wind speed and direction, sea surface temperature and food availability on abundance and growth of autumn and winter spawned juveniles is also examined.

5.3 Methods

Fish sampling and otolith collection

Atlantic herring (*Clupea harengus*) otoliths were selected from the Agri-food and Biosciences Institute Northern Ireland (AFBI)[12] historical archive. These otoliths had been collected during herring acoustic surveys in the Irish Sea from 1993 – 2003. During these surveys fish were collected using midwater trawls. Samples were stratified by length, with a minimum of two individuals taken from each length class where possible. Total length and weight were recorded to the nearest 0.1cm and 0.1g respectively and sex and maturity stage recorded. Sagittal otoliths were removed and cleaned in water before being dried and stored in plastic blocks. Otoliths were used for ageing and set in resin in plastic storage blocks.

For the current study, otoliths classified as age-1 (one translucent winter ring) were selected from the historical collection. The maximum available number of individuals was selected for each year. (It was necessary to leave one set of otoliths for each length class unprocessed in order to preserve the AFBI archive). Years where a minimum sample size of 20 individuals was not achieved were excluded from further analysis. Selection of individuals indicated that the majority of samples were from the west Irish Sea. It was decided to restrict the study to this geographical area. Selected otoliths were photographed within their resin/plastic blocks and then removed from the plastic blocks using a coping saw. Each individual piece was then heated to soften the resin and facilitate the removal of the otoliths from the plastic blocks. Once removed the otoliths were stored in plastic eppendorf tubes. Additional age-1 samples were obtained from samples collected during other studies conducted by Brophy and

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[12] Formally known as the Department of Agriculture and Rural Development Northern Ireland

Danilowicz (2002). The total number of otoliths that were examined for each year is shown in Table 5.1 with sample locations displayed in Figure 5.1.

Otolith analysis

Age estimates were confirmed based on visual inspection of the translucent and opaque bands at 20X using an Olympus™ stereoscopic microscope. Otolith microstructure analysis was used to classify individuals as autumn / winter spawned based on the method developed by Brophy and Danilowicz (2002). This method classifies individuals based on the growth patterns displayed in the larval core of the otolith. All otoliths were initially mounted sulcus side up in 1.5 ml eppendorf lids in TAAB™ epoxy resin and polished until light could penetrate the larval core. The blocks of resin were then inverted and remounted to facilitate polishing on the sulcus side. Otoliths were then polished until the larval core was fully exposed.

The core was photographed at 1000X using an Olympus™ compound microscope with an Olympus™ Camedia digital camera attached. Daily increments were measured along the longest visible axis from the nucleus to the image edge. Otoliths with an average increment width ≥ 2.3 µm between increments 61-70 were classified as winter spawned, while fish with an average of ≤ 2.2 µm between increment 61-70 were classified as autumn. Total length to the edge of the first winter ring in age-1 fish were measured to obtain a growth measurement for the first year.

Fisheries data

Age structured abundance estimates from commercial catches and annual acoustic surveys in the Irish Sea were obtained from the ICES herring working group report (ICES, 2007). Information from assessments on the mean length and weight -at-age was also used in analysis. Annual estimates of abundance for one group fish from the Integrated Catch at Age analysis (Patterson, 1998) were split according to the proportions of autumn and winter spawned fish in the otolith samples to produce separate estimates of abundance for each stock. Estimates of juvenile abundance (before and after splitting) were investigated for significant correlations with catch at age three and the acoustic estimate of herring age three in the Irish Sea for the same year class to determine if adjustment would improve the relationship. The correlation between the abundance of autumn spawned juveniles in the Irish Sea and the spawning stock biomass of the Irish Sea stock two years previously was examined.

The correlation between the abundance of winter spawned juveniles in the Irish Sea and the acoustic estimates of abundance of three year old adults from the same year class in the Celtic Sea stock two years later was investigated. The relationship between abundance of winter spawned juveniles and spawning stock biomass of the Celtic Sea stock two years previously was also examined.

The larval growth (increments widths 1-70) and juvenile growth (length to the outer edge of the first winter ring) of autumn spawned fish were tested for a significant correlation with the mean length-at-age two, three and four; and the mean weight-at-age two, three and four taken from catch data from the Irish Sea for the same year class. This was carried out to determine if larval or juvenile growth has a subsequent effect on the size of adult fish in the Irish Sea. Larval growth and juvenile growth of both components were also examined in relation to abundance of winter spawned juveniles in the Irish Sea to establish if growth was density dependent.

Environmental data

Temperature data was obtained from the coastal temperature network and ferry route programme carried out by CEFAS (Joyce, 2004). Using this dataset, the mean monthly sea surface temperature was obtained for Port Erin (54.083 N, 4.766 W) in the Irish Sea and the Angle station (51.683 N, 5.083 W) at the south west tip of Wales (Figure 5.1). Annual sea surface temperatures were examined for significant correlations with the abundance of winter spawned juveniles in the Irish Sea and with larval and juvenile growth rates in both components.

Comparisons of larval growth rates between the migrant and resident components of Celtic Sea juveniles indicate that the migrant component disperses quite early in the larval phase (see chapter 4). Back calculation of spawning dates using otolith microstructure analysis showed the migrant component were spawned between January and February (Brophy and Danilowicz, 2002) which is consistent with the observed spawning behaviour of the adult winter spawning stock . Therefore wind conditions between January and March may influence the transportation of larva into the Irish Sea. Hourly wind speed and direction data was obtained from MET Eireann from the Rosslare weather station (52.255 N, 6.334 W) which is located on the south-east corner of Ireland (Figure 5.1).

Daily wind speed and direction were calculated and a single wind variable (wind factor) was derived by combining data from the months when movement of herring

larva into the Irish Sea was most probable (January – March). Wind factor was calculated by summing daily wind speed values for each month. Prevailing winds (SSW to SW; 191-236^0) were given a positive value. Winds from the opposite direction were given a negative value (SE to SEE; 123-169^0). The number of days when the wind direction was from the prevailing direction (191-236^0) was also calculated for January, February and March separately and all three months combined. The number of days when wind speed was > 10 knots, >15 knots and >20 knots was calculated both in regards to prevailing winds (191-236^0) and general turbulence (all directions) for January, February and March separately and for all three months combined. Wind variables and abundance of winter spawned one group fish in the Irish Sea were investigated for significant correlations. Wind conditions were assessed for all year-classes except for the 1996/97 year class, as no wind speed data could be obtained for 1997. The relationship between wind speed and direction and the sea surface temperature at Port Erin and Angle was also investigated to determine if wind conditions improved growing conditions for juveniles in the Irish Sea. Wind roses were produced using WRPLOT View version 5.3[13] to visual display wind conditions between January and March in each sample year. The average daily wind speed were used to construct the wind rose for January to March 2000 as hourly wind speeds were not available for this year. It is important to note that during the winter period (January-March), larvae dispersing from the Celtic Sea into the Irish Sea are not subject to the cyclonic gyre which forms in the western Irish Sea in spring/summer each year. This gyre forms a dome of cold dense water that lies beneath a strong thermocline and affects the movement of water in the Irish Sea (Horsburgh and Hill, 2003).

Levels of *Calanus finmarchicus* in the Irish Sea were taken as an indicator of food availability. Data on the abundance of *C. finmarchicus* was obtained from the continuous plankton recorder survey carried out by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS). Samples were taken monthly in the Irish Sea and the numbers of samples ranged from 0-37, with a mean of 16 samples been taken in each month. In this study we used mean monthly counts for the Irish Sea from 1993 – 2003. The average mean monthly count of *Calanus finmarchicus* in the Irish Sea was 0.26 per 3m^{-3}. Average counts were calculated for each sample year. Correlations

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[13] Freely available at http://www.weblakes.com/lakewrpl.html

between annual levels of *Calanus finmarchicus* and the abundance and growth of winter and autumn spawned juveniles in the Irish Sea were investigated. However it should be noted that *Calanus finmarchicus* is not always the dominant species of Calanus in the Irish Sea (Nash and Geffen, 2004). Nash and Geffen (2004) found *Calanus helgolandicus* to be more abundance in six out of the seven years they carried out sampling in the western Irish Sea.

5.4 Results

The separation of individuals into autumn and winter spawned revealed significant fluctuations in the proportions of winter spawned juveniles present in the Irish Sea (24-89%) over the period of the study (Figure 5.1 and Table 5.1). This shows that adjusting the abundance of one group fish using a standard scaling factor would not improve the accuracy of herring assessments. If adjustment of the abundance estimates of one group fish is to be carried out routinely as part of annual herring assessments the proportions of autumn and winter spawned fish will need to be assessed on a yearly basis.

Abundance of one group fish in the Irish Sea taken from herring acoustic surveys was reduced to account for the proportion of winter spawned juveniles present. This adjustment produced a marked improvement in the relationship between the abundance of one group fish and the number of fish age three for the same year class taken in commercial catches (unadjusted juvenile abundance: $r = 0.44$, $p = 0.33$; adjusted juvenile abundance: $r = 0.82$, $p = 0.03$, Figure 5.2). The adjustment also improved the relationship between abundance of one group fish and the acoustic estimate of age three fish from herring acoustic estimates for the same year class (unadjusted juvenile abundance; $r = 0.68$, $p = 0.09$; adjusted juvenile abundance: $r = 0.88$, $p < 0.01$, Figure 5.3).

No significant correlation was identified between the abundance of autumn spawned fish in the Irish Sea and the spawning stock biomass two years previously ($p > 0.05$), however the temporal coverage of the spawning stock biomass estimates for the Irish Sea stock was not complete, so the relationship could only be investigated over five years, which may have resulted in the exclusion of key years.

The relationship between the abundance of winter spawned juveniles in the Irish and the spawning stock biomass in the Celtic Sea two years previously was just outside

significance (r = 0.73, p = 0.06, see Figure 5.4). There was no correlation between the abundance of winter spawned juveniles in the Irish Sea and the acoustic estimate of three year old fish from the same year class in the Celtic Sea, however this may also reflect the exclusion of a number of years as the the temporal coverage of the acoustic index of abundance for the Celtic Sea stock was not complete, so the relationship could only be investigated over five years.

There were no significant correlations between larval or juvenile growth rates of autumn spawned juveniles and the subsequent length or weight at age two/ three or four in Irish Sea fish (p > 0.03, see Bonferroni corrections Table 5.2).

There were no significant relationships between temperature, and the abundance of Celtic Sea winter spawned juveniles in the Irish Sea, or larval and juvenile growth rates in either autumn or winter spawned components (p > 0.05).

The prevailing winds in the south of Ireland are from the southwest. In the current study, wind was predominantly from the southwest between January to March in all but one of the years examined, 1996 (Figure 5.5). There was a significant negative correlation between the abundance of winter spawned juveniles in the Irish Sea and the number of days during the January-March period when wind speed was >15 knots and wind direction was from 191-236° (SSW-SW) (r =-0.96, p < 0.01, Figure 5.6). The abundance of winter spawned juveniles in the Irish Sea was not correlated with the other wind variables (wind factor or wind strength for all directions).

Wind variables were also tested for significant correlations with temperature. Wind factor and the mean monthly temperature at Port Erin for February were significantly correlated (r = 0.93, p = 0.02) possibly because warmer water is carried from the Celtic Sea into the Irish Sea with south westerly winds (Figure 5.7).

There were no significant correlations between mean counts of *Calanus finmarchicus* and the abundance or growth of winter and autumn spawned juveniles in the Irish Sea (p > 0.05). A summary of variables examined for significant correlations is shown in table 5.2.

5.5 Discussion

This study was primarily based on historical otoliths from AFBI herring acoustic survey carried out between 1993 and 2004. While the best possible use was made of the material available, the study was limited by sample sizes. All otoliths used to

estimate the proportion of winter spawned fish in the Irish Sea came from the west Irish Sea as samples from the east Irish Sea were not present in the archive in sufficient numbers. However, previous research has shown that the abundance of winter spawned fish increases from east to west (see chapter 4). Therefore, the abundance of winter spawned fish may be overestimated by excluding samples from the east Irish Sea. If carried out as part of annual sampling programmes larger samples from a wider range of sites would be recommended to estimate the relative proportions of autumn and winter spawned fish at Irish Sea nursery grounds. Ideally a sample of juveniles would be taken at each sampling station during the herring acoustic surveys. Samples would then be split into autumn and winter spawned components using otolith microstructure analysis. The relative proportion of each component would be calculated for the Irish Sea and the autumn spawned component could be used for producing recruitment indices for the Irish Sea herring stock. Despite the crude methods used during the current study to estimate the abundance of winter spawned juveniles in the Irish Sea, the splitting of abundance estimates based on the proportions of autumn and winter spawned juveniles leads to a significant improvement in the relationship between juvenile and adult abundance estimates. This indicates that, if in future years this splitting technique was carried out more rigorously, as part of the routine monitoring of the stock, a very reliable recruitment index for the Irish Sea could be produced.

One limitation of the otolith microstructure method is its inability to identify autumn spawned juveniles who have not originated in the Irish Sea. Herring spawn in autumn and winter in the Celtic Sea and it has been recognised that a considerable proportion of autumn spawned juveniles are present in the Celtic Sea during certain years (ICES, 2007). This limitation also applies to winter spawned herring. While the majority of spawning in the Irish Sea occurs in autumn, a small amount of spawning has been observed up to January (Dickey-Collas *et al.*, 2001). These fish may contribute to the abundance of Celtic Sea winter spawned juveniles in the Irish Sea and undermine the accuracy of this abundance estimate. However the relationship between Celtic Sea SSB and the abundance of winter spawned juveniles in the Irish Sea two years on ($p=0.06$) would indicate that resident Irish Sea winter spawned fish do not contribute significantly to abundance of winter spawned fish in the Irish Sea. The results of this study indicate that, while the presence of autumn spawned juveniles from the Celtic Sea, or resident winter spawned juveniles at Irish Sea nursery grounds cannot be ruled

out at present, it does not significantly confound the adjustment of recruitment indices using the separation of autumn and winter spawned fish. It must also be considered that herring from other areas may contribute to the juvenile assemblages in the Irish Sea, such as Scottish, Clyde stocks. Estimated of hatch date using microstructure analysis indicated that juveniles in the Irish Sea were either autumn or winter spawned (Brophy and Danilowicz, 2002), indicating that spring spawned juveniles do not contribute significantly to juvenile assemblages in the Irish Sea.

It has been suggested that if Celtic Sea juveniles could be identified during Irish Sea herring surveys it could contribute to the calculation of recruitment indices for Celtic Sea herring (ICES, 2007). Previous studies have shown that this migrant component are not present in the Irish Sea spawning stock (Brophy *et al.*, 2006) and that they display natal homing behaviour as adult fish (see chapter 4). In this study, the abundance of Celtic Sea winter spawned juveniles in the Irish Sea was not significantly correlated with acoustic estimates of abundance for three year old fish from the same year class. This may reflect the fact that this component is only a proportion of the juvenile stock. Annual fluctuations in survival at other nursery grounds (e.g. in the Celtic Sea) may be independent of variation at Irish Sea nursery grounds. The Celtic Sea fishery includes a mixture of autumn and winter spawned individuals and both components are assessed a single stock. The recruitment index considered here for the Celtic Sea stock excludes the contribution of the autumn spawned component. This introduces another possible source of error in the juvenile-adult recruitment relationship. If splitting procedures are instigated on a routine basis in the Irish Sea, the link between the abundance of winter spawned juveniles in the Irish Sea and year class strength in the Celtic Sea adult stock could be examined in greater detail. The recruitment index for the Celtic Sea stock may be improved if juvenile abundance data from the Irish Sea is combined with juvenile data from Celtic Sea nursery grounds.

Juvenile abundance estimates are used to predict year-class strengths and predict the numbers of fish that will recruit to the spawning stock in the future. A number of studies have successfully used juvenile abundance estimates of redfish, herring and cod to predict year class strength for the associated adult fishery (Axenrot and Hansson, 2003, Helle *et al.*, 2000, Magnusson and Johannesson, 1997). They provide an early warning signal of weak year classes, and give fisheries scientists the opportunity to put precautionary measures in place to protect the stock. Several

herring stocks have collapsed in the past and where management action was taken the stocks have been shown to recover; where no management action was taken no signs of recovery have been observed (Zheng, 1996). In the North Sea, information from herring stock assessments have identified poor recruitment over the last six years and has lead management to recommend quota restrictions in response to this pattern (ICES, 2007).

Splitting techniques are already used in the assessment of North Sea autumn spawners (NSAS) and Downs herring and Western Baltic spring spawners (WBSS). NSAS and Downs herring drift into ICES area IIIa as larvae and mix with adult WBSS that also move into the area as adult. WBSS also migrate as far as the North Sea. The components are identified within samples from commercial catches using otolith microstructure and vertebral counts (Clausen *et al.*, 2007, ICES, 2007). North Sea catch estimates are calculated by taking the catches from the North Sea and subtracting the WBSS individuals and adding in NSAS and Downs herring sampled in area IIIa (ICES, 2007).

The proportion of winter spawned juveniles in the Irish Sea was found to vary considerable from year to year (24-89%). This variability may arise due to a combination of factors. Larval abundance will be affected by variations in spawning activity in the Celtic Sea. High numbers of winter spawned juveniles in the Irish Sea may reflect a high spawning stock biomass in the Celtic Sea in the year of hatching. In this study the abundance of winter spawned juveniles in the Celtic Sea did appear to increase with spawning stock biomass in the Celtic Sea two years previous. This relationship might improve if the time series were extended or if the contribution of the autumn spawning component could be removed from the estimate of spawning stock biomass.

Abundance of winter spawned juveniles at Irish Sea nursery grounds will also be influenced by mortality at the egg, larval and juvenile stages. Compensatory patterns in herring stocks have been linked to density dependence and possible environmental processes (Fox, 2001, Nash and Dickey-Collas, 2005, Zheng, 1996). At the egg stage, hatching success may be lowered due to overcrowding of eggs on spawning beds leading to anoxia and developmental retardation, which has been demonstrated for Pacific herring (*Clupea pallasii* V.) (Stratoudakis *et al.*, 1998), however this will only effect stocks where spawning beds are limited in size (Johannessen, 1986). Density dependence may also become important when herring group together and begin to

shoal. At this stage there may be more competition for food between individuals (Purcell and Sturdevant, 2001) and higher levels of predation from other species (Walter *et al.*, 1986). Variability in mortality during the egg, larval and juvenile stages will dampen the effect of high spawning stock biomass and reduce its correlation with juvenile abundance.

An inverse relationship was identified between the prevailing wind conditions (southwest) and the abundance of Celtic Sea winter spawned juveniles in the Irish Sea. There are two scenarios that may lead to this negative relationship. The first is that stronger than average south westerly winds increase the flow of water through the Irish Sea and out through the North Channel, thus flushing more larvae out of the Irish Sea. Xing and Davies (2001) demonstrated that strong northerly flow through St. Georges Channel affects water in the western Irish Sea and that these strong northerly flows continue on into the North Channel where water exits the Irish Sea.

The transportation of Celtic Sea juveniles into the Irish Sea will be influenced by wind speed and direction, the effect of wind conditions on water currents and the position of the herring larvae within the water. The prevailing winds in this area are generally southwest between January to March and circulation models of surface currents for the Irish Sea and George's channel indicate that these would influence the transportation of Celtic Sea juveniles into the Irish Sea (Davies, 1994, Davies and Jones, 1992, Davies and Xing, 2003, Xing and Davies, 2001). Cooper (1960) demonstrated that both south and southwest winds along the southern coast of Ireland will promote the movement of surface water from the Celtic Sea into the Irish Sea, even when Ekman's spiral is taken into account. Ekman's theory predicts that wind stress taken together with the Coriolis effect (the deflection of water relative to the earth's rotation), will cause water to move at a right angle to the wind (Price *et al.*, 1987). However this movement refers to surface water and the position of the herring larvae within the water column may have a significant effect on their direction of transportation. Heath *et al.* (1988) investigated the vertical distribution of herring larvae (6-18mm) and found that in general they are confined to the upper layers in the open-ocean. They found a general layering of larvae in response to high surface irradiance and a downward displacement and dispersal of the population in relation to high wind velocities. Otolith increments indicate that Celtic Sea winter spawned larvae that are transported into the Irish Sea begin their move between days 1-10 after hatching. Based on the size of herring larvae from the Downs and Dogger stocks at

hatching (presented in Parrish and Saville 1965), larvae would be >7mm when they began their dispersal from the Celtic Sea into the Irish Sea. Larval herring have been shown to move along transport routes caused by topography and coastal currents (Sætre *et al.*, 2002, Townsend, 1992), and wind patterns have been shown to play an important role in the distribution of larval fish for numerous species including cod and plaice (Hinrichsen *et al.*, 2003, Catalán *et al.*, 2006, Nielsen *et al.*, 1998). Strong wind events have been shown to negatively affect settlement patterns in wrasse *Symphodus ocellatus* L. with calmer weather during the planktonic period increasing settlement success (Raventos and Macpherson, 2005). Further research is needed to assess the effect of wind conditions on the movement of sub-surface waters and larval studies would provide more information on the position of the larvae within the water column during the early larval phase.

The second scenario is that winter spawned juveniles experience higher mortality rates while at nursery grounds in the Irish Sea in years of strong south westerly winds due to associated changes in other environmental conditions (e.g. food availability).

The abundance of winter spawned juveniles may also be influenced by the resident autumn spawned stock. Autumn spawned fish will be between 4 and 6 months old when winter spawned larvae drift into the Irish Sea at an inferior developmental stage. Autumn and winter spawned fish may compete for food and may be subject to the same predation pressures. Their larger size may give autumn spawned herring an advantage over winter spawned herring in both cases. Growth-selective mortality has been identified in fish with smaller individuals being more vulnerable to predation (Takasuka *et al.*, 2003) and age has been demonstrated to be more important than size to herring larvae's responsiveness to predators (Skajaa *et al.*, 2004). Monitoring the variability in abundance and growth patterns of the winter spawned juveniles in the Irish Sea and explaining the factors that produce this variability may contribute to management predictions for the Celtic Sea stock in particular and to understanding of herring recruitment dynamics in general.

Temperature and prey availability are important factors in fish growth (Bailey and Alanara, 2006, Baumann *et al.*, 2006, Fey, 2001, Gallego *et al.*, 1999, Michalsen *et al.*, 1998, Husebø *et al.*, 2007, Hakala *et al.*, 2003). However, in this study, larval or juvenile growth rates did not appear to be linked to temperature or abundance of *Calanus finmarchicus*. This may be because the food availability and temperature conditions to which herring in the Irish Sea are exposed is not accurately reflected by

the environmental measurements used (sea surface temperature and continuous plankton recorder measurements of *Calanus finmarchicus*).

5.6 Conclusion

Despite the limitations of the splitting procedure, the adjustment of age-1 abundance estimates lead to significant improvements in correlations with abundance estimates of age three fish from the same year class from commercial catches and acoustic surveys in the Irish Sea. These improvements highlight the value of this technique in annual herring sampling programmes and indicate that the splitting technique will generate recruitment indices more appropriate for Irish Sea herring stock assessments. The abundance of winter spawned juveniles in the Irish was inversely related to the incidence of strong south westerly winds between January and March. The underlying cause of this relationship is not clear, however, it may reflect dispersal of larvae out of the Irish Sea or increased juvenile mortality during years of frequent strong south westerly winds. Modelling studies and larval surveys may help to establish the effect of wind on dispersal and survival.
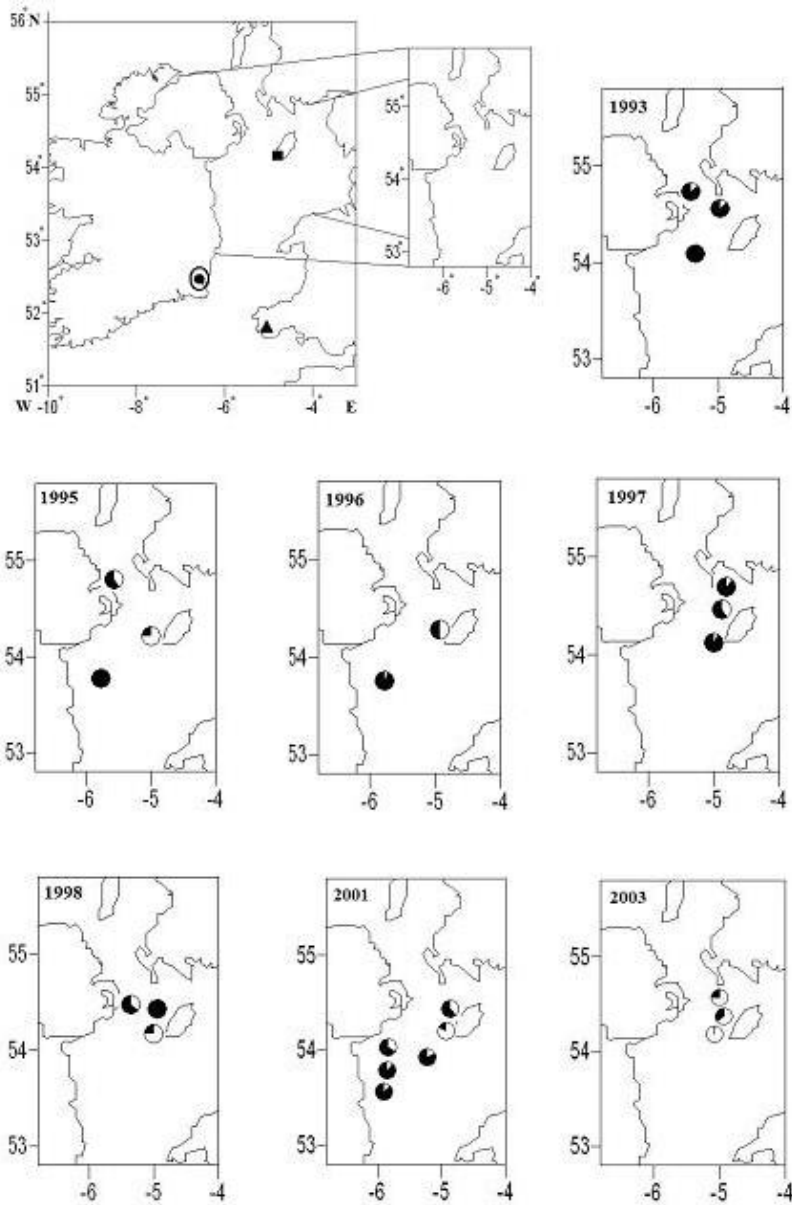
Figure 5.1 Maps showing the locations of wind station at Rosslare (bulls-eye in left top panel), and sea surface temperature stations at Port Erin (solid square in top left panel) and Angle station (solid triangle in top left panel); and the relative proportion of autumn (open slice) and winter (closed slice) spawned individuals at each sample site in 1993, 1995, 1996, 1997, 1998, 2001, and 2003.
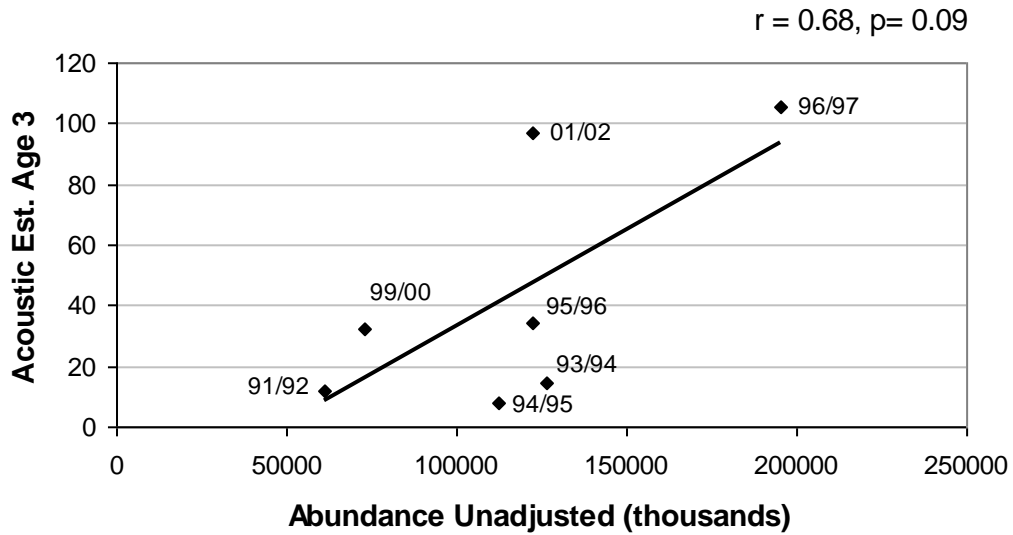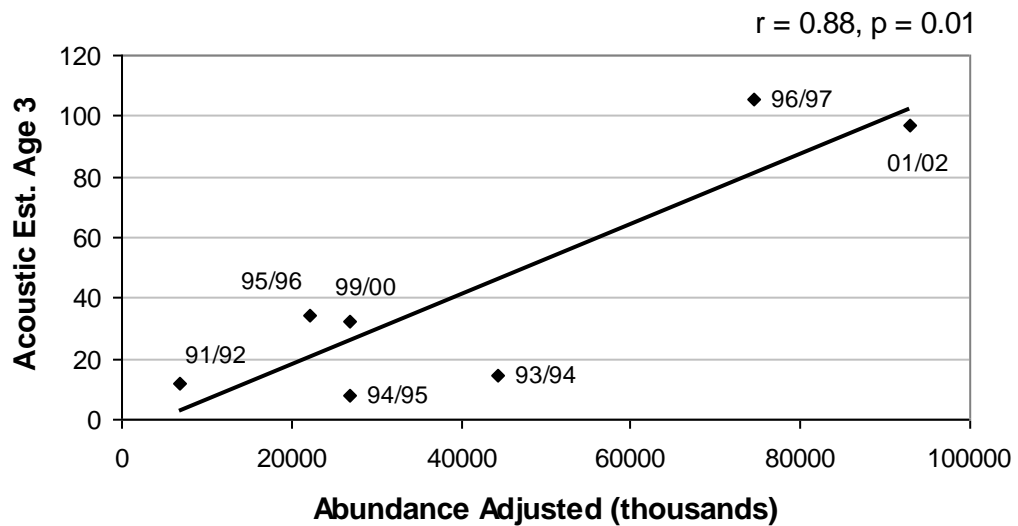
r = 0.45, p = 0.33

a)

r = 0.82, p = 0.03
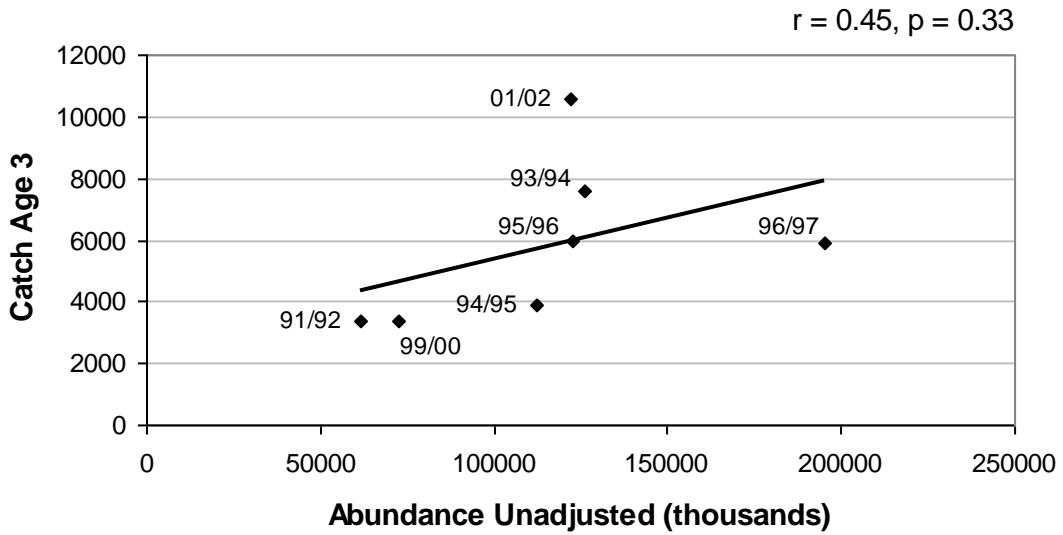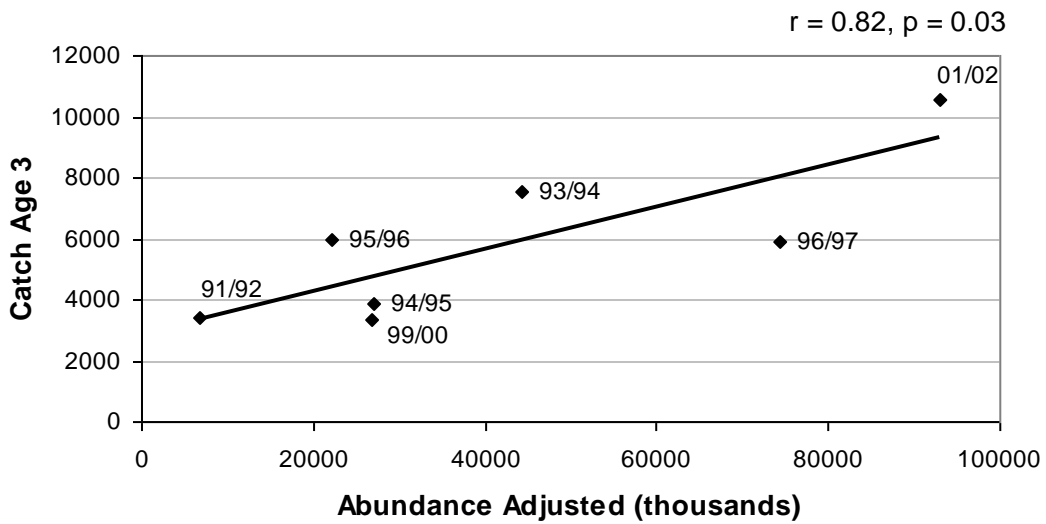
b)

Figure 5.2 Relationship between the abundance of one-group fish from Irish Sea herring stock summary and catch age 3 for the same year class for a) unadjusted abundance and b) abundance adjusted to remove proportion of winter spawned juveniles present. Catch in numbers (thousands) for herring division VIIa (N). Labels refer to hatch year.

r = 0.68, p= 0.09

a)

r = 0.88, p = 0.01

b)

Figure 5.3 Relationship between the abundance of one-group fish from Irish Sea herring stock summary and acoustic estimate of herring abundance age three for the same year class from ABFI surveys for a) unadjusted abundance and b) abundance adjusted to remove proportion of winter spawned juveniles present. Labels refer to hatch year.
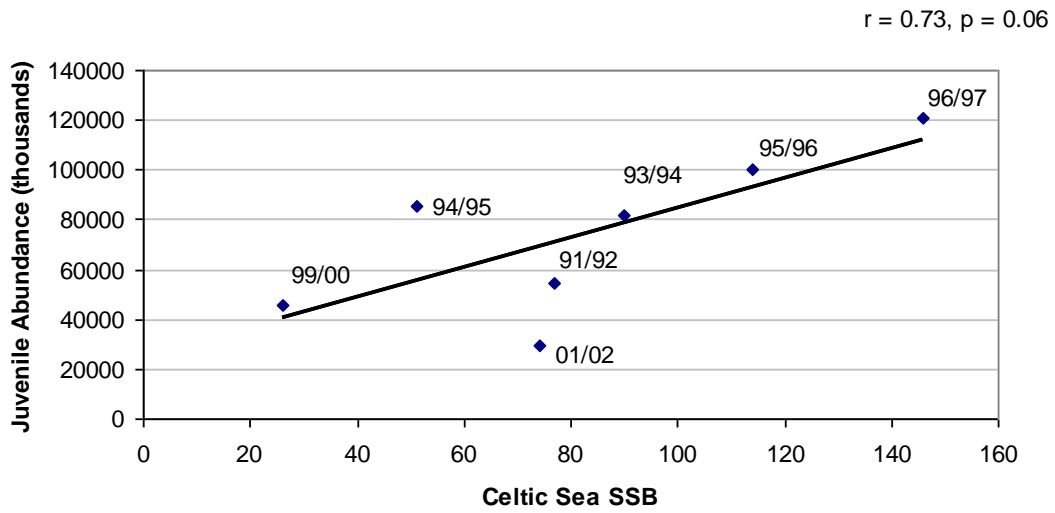
Figure 5.4 Relationship between juvenile abundance of winter spawned fish and Celtic Sea spawning stock biomass. Labels refer to hatch year.
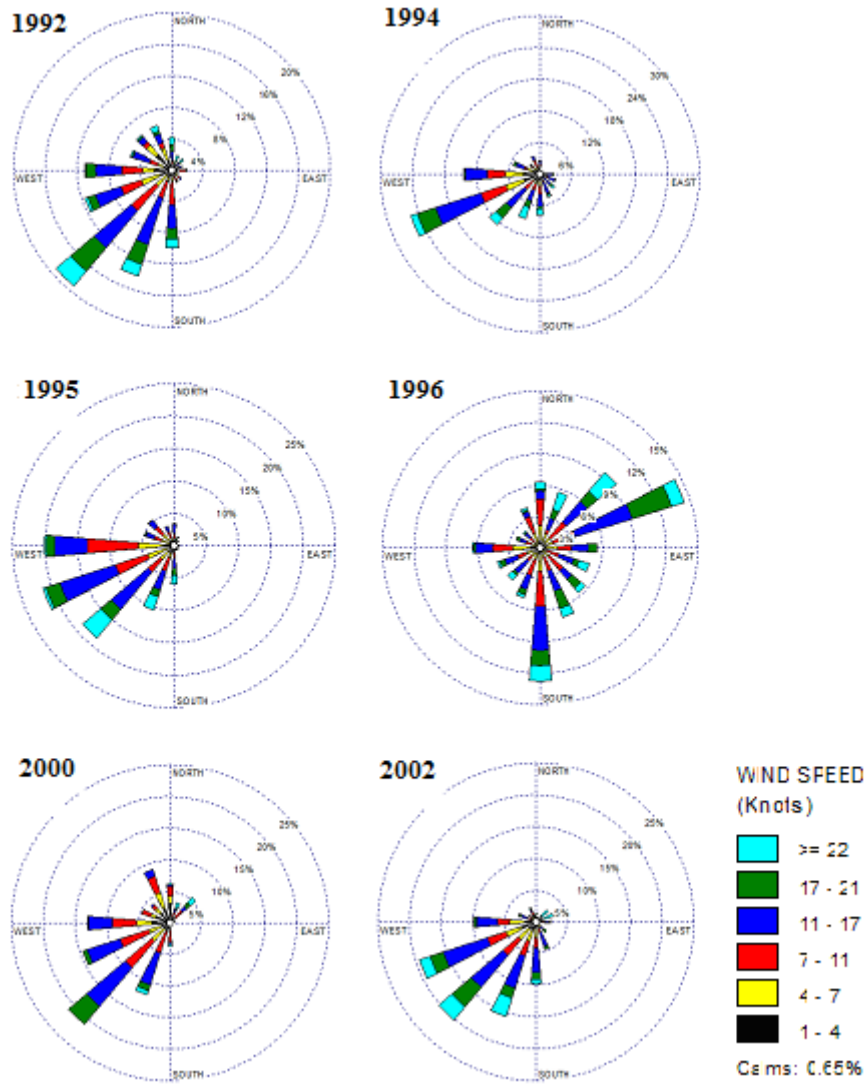
Figure 5.5 Wind roses summarising the wind conditions for January to March in 1992, 1994, 1995, 1996, 2000, and 2002. Wind speed is shown in knots.

Figure 5.6 Relationship between juvenile abundance of winter spawned fish and number of days in January to March when wind speed > 15 knots and wind direction between 191-236°. Labels refer to hatch year.
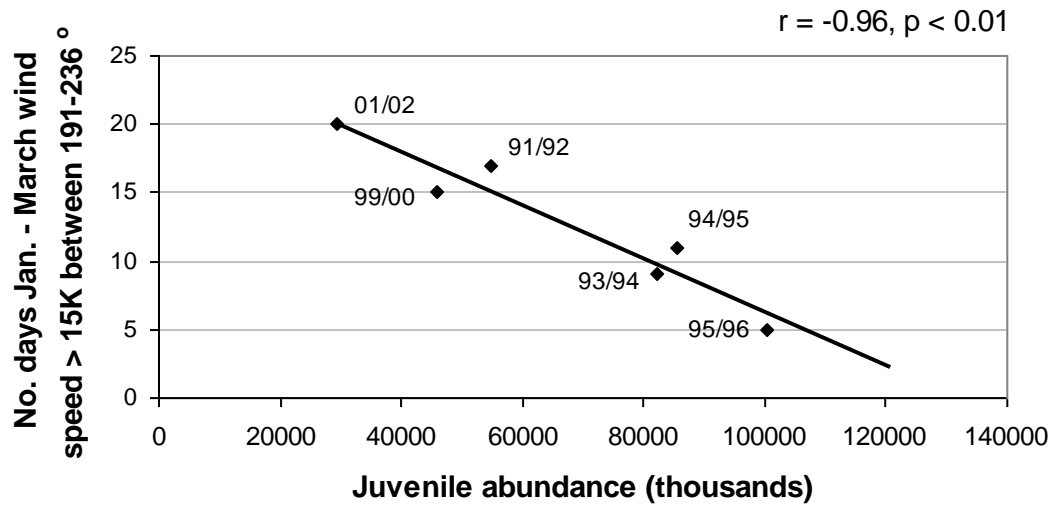
r = 0.93, p = 0.02
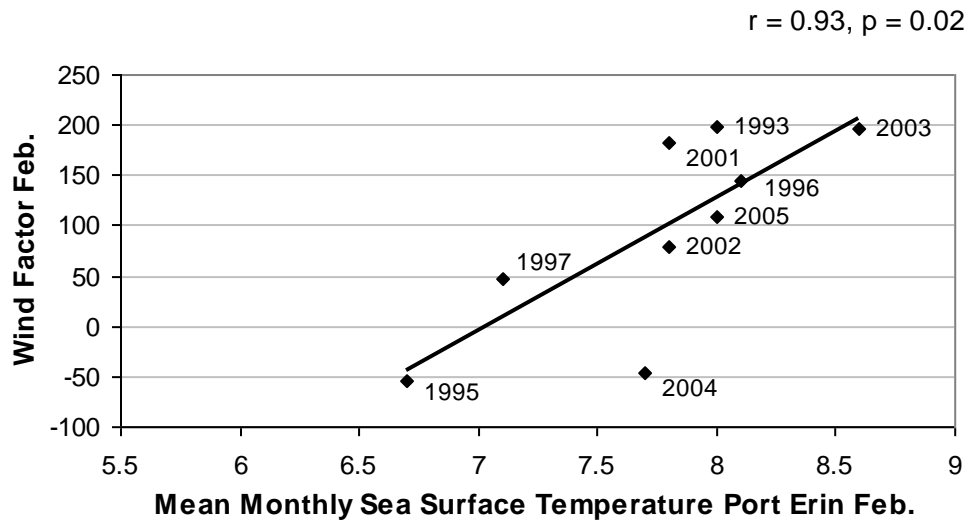
Figure 5.7 Relationship between wind factor and the mean monthly sea surface temperature for Port Erin in the Irish Sea for the month of February.

Table 5.1 Summary data for otoliths from age-1 juveniles collected from 1993-2003. The table shows the number of sites from which otoliths were available, total individuals sampled (n) and the number of individuals classified as autumn-spawned (n_a) and winter-spawned (n_w).

Year	Year class	Number of sites	N	n_a	n_w
1993	91/92	3	21	16	5
1995	93/94	3	20	7	13
1996	94/95	2	21	5	16
1997	95/96	3	22	4	18
1998	96/97	3	21	8	13
2001	99/00	12	70	26	44
2003	01/02	3	21	16	5

Table 5.2 Summary of variables examined for significant correlations where a) fisheries data b) larval and juvenile growth c) environmental data and larval and juvenile growth and d) environmental data. JA = juvenile abundance; JA AS = juvenile abundance autumn spawned; JA WS = juvenile abundance winter spawned; LG AS = larval growth autumn spawned; JG AS = juvenile growth autumn spawned; Catch IS = catch age 3 Irish Sea; AC IS = acoustic estimate age 3 Irish Sea; SSB IS = spawning stock biomass Irish Sea; AC CS = acoustic estimate age 3 Celtic Sea; SSB CS = spawning stock biomass Celtic Sea. P-values shown in italics. Significant relationships marked with an asterisk.

a)

	JA	JA AS	JA WS	LG AS	JG AS
JA WS		-0.05 *0.91*		0.16 *0.73*	-0.18 *0.70*
Catch IS	0.44- *0.33*	0.82 *0.03*			
AC IS	0.68 *0.09*	0.88 *0.01**			
SSB IS	0.00 *0.99*	-0.70 *0.19*			
AC CS			0.04 *0.95*		
SSB CS			0.73 *0.06*		
Calanus finmarchicus		-0.27 *0.61*			

b)

		LG AS	JG AS
Fish Length (cm)	Age 2	-0.09 *0.86*	-0.67 *0.10*
	Age 3	0.38 *0.40*	-0.18 *0.70*
	Age 4	-0.49 *0.26*	-0.77 *0.04*
Fish Weight (g)	Age 2	0.01 *0.98*	-0.65 *0.12*
	Age 3	0.15 *0.74*	-0.33 *0.47*
	Age 4	-0.46 *0.30*	-0.79 *0.03*

c)

		JA WS	LG AS	JG AS	LG WS	JG WS
Temperature Port Erin	Jan.	-0.73	-0.40	-0.11	0.53	0.00
		0.06	*0.38*	*0.82*	*0.23*	*1.00*
	Feb.	-0.59	0.41	0.23	0.10	0.47
		0.16	*0.36*	*0.62*	*0.83*	*0.29*
	March	-0.65	0.62	0.60	-0.28	0.89
		0.12	*0.14*	*0.15*	*0.55*	*0.01**
	Annual	-0.39	0.61	0.60	0.03	0.56
		0.38	*0.14*	*0.15*	*0.95*	*0.19*
Temperature Angle Station	Jan.	-0.43	-0.44	-0.54	0.52	-0.21
		0.34	*0.32*	*0.21*	*0.23*	*0.65*
	Feb.	-0.54	0.44	0.17	0.14	0.48
		0.22	*0.32*	*0.71*	*0.77*	*0.28*
	March	-0.49	0.73	0.76	-0.45	0.84
		0.27	*0.06*	*0.05*	*0.31*	*0.02*
	Annual	0.40	0.63	0.23	-0.28	0.13
		0.38	*0.13*	*0.62*	*0.55*	*0.79*
Abundance *Calanus finmarchicus*		0.40	0.63	0.23	-0.28	0.13
		0.38	*0.13*	*0.62*	*0.55*	*0.79*

d)

		Temperature Port Erin					Temperature Angle Station			
		JA. WS	Jan	Feb	March	Annual	Jan	Feb	March	Annual
Wind Factor	Jan-Mar.	-0.72			0.85	0.71			0.90	0.44
		0.11			*0.03*	*0.11*			*0.02*	*0.38*
	Jan.	-0.48	0.04	0.44	0.50	0.57	0.15	0.55	0.77	0.46
		0.33	*0.94*	*0.39*	*0.31*	*0.24*	*0.77*	*0.26*	*0.07*	*0.37*
	Feb.	-7.84		0.95	0.87	0.90		0.91	0.72	0.24
		0.07		*<0.01**	*0.02*	*0.02*		*<0.01**	*0.11*	*0.65*
	Mar.	-0.11			0.19	-0.09			0.58	0.44
		0.84			*0.72*	*0.87*			*0.22*	*0.38*
Days January - March wind 191-236°	All Speeds	-0.49			0.93	0.78			0.91	0.30
		0.27			*<0.01**	*0.04*			*<0.01**	*0.51*
	>10k	-0.79			0.90	0.79			0.85	0.27
		0.06			*0.01**	*0.07*			*0.03*	*0.60*
	>15k	-0.96			0.98	0.76			0.90	-0.06
		<0.01*			*<0.01**	*0.08*			*0.02*	*0.91*
	>20k	-0.05			0.15	-0.27			0.33	-0.01
		0.92			*0.77*	*0.61*			*0.52*	*0.99*
Days January - March wind 360°	>10k	0.23			-0.21	0.04			-0.35	-0.23
		0.66			*0.69*	*0.95*			*0.49*	*0.66*
	>15k	-0.17			0.02	-0.01			-0.27	-0.90
		0.75			*0.96*	*0.99*			*0.60*	*0.02*
	>20K	0.15			-0.16	-0.42			-0.43	-0.71
		0.78			*0.77*	*0.41*			*0.11*	*0.11*

Bonferroni adjusted significance level: $0.05/3 = 0.016$, $0.05/4 = 0.012$

Chapter 6

General Discussion

6.1 Otolith shape analysis and stock discrimination

Classification success rates from stock discrimination studies based on otolith shape vary from 60-90% depending on the species (Bird *et al.*, 1986; Castonguay *et al.*, 1991; Friedland *et al.*, 1994; Gauldie and Jones, 2000; Smith *et al.*, 2002; Cardinale *et al.*, 2004; Berg *et al.*, 2005; Pothin *et al.*, 2006). The levels of classification success achieved in this study (81- 100%) show that otolith shape is useful for discriminating between juvenile herring from different nursery areas and spawning components and in particular for tracing nursery ground of origin in winter spawned Celtic Sea fish using the shape of the first winter ring. The method could be applicable to other herring that show movement of juveniles outside their natal area. In particular, the North Sea autumn spawners and Downs herring display a similar pattern of mixing. Juveniles have been shown to migrate into the Skagerrak where they mix with Western Baltic spring spawners until they migrate back at age 2/3 (Rosenberg and Palmen, 1982). While the juveniles are currently identified in the western Baltic using otolith microstructure and vertebral counts (Clausen *et al.*, 2007, ICES, 2007), the proportion of adults in the North Sea who spent their nursery period in the western Baltic is not known. Shape analysis of otolith annuli could be investigated as a method of tracing this migrant component, and to identify if a similar stock structure is evident. Shape analysis of internal annuli may also prove useful for tracing nursery origin in mixed feeding stocks. There is much potential for the method to be applied to questions of herring stock structure and migration patterns in the northeast Atlantic, as many stocks cannot be classified using genetic analysis (Bekkevold *et al.*, 2007, Hatfield *et al.* 2007, Mariani *et al.*, 2005). Shape analysis of internal structures in other hard parts that show annual growth rhythms such as scales, bones and spines (Meunier, 2002) could also be useful in stock discrimination. Scale morphology has been used to distinguish between fish population, resulting in classification success rates of between 57-84% (Poulet *et al.*, 2005, Richards and Esteves, 1997, Debarros and Holst, 1995, Ibanez *et al.*, 2007)

6.2 Structure of herring stocks around Ireland

The mixing that occurs between herring stocks from the Irish and Celtics Seas is verified by studies of larval drift (Özcan, 1974), parasite prevalence (Campbell *et al.*, 2007), tagging studies (Molloy *et al.*, 1993) and otolith microstructure (Brophy, 2002, Brophy and Danilowicz, 2002). The study of juvenile and adult Celtic Sea winter spawned fish provides strong evidence that Celtic Sea herring that disperse into the Irish Sea return to their natal area to spawn. While previous studies provided indirect evidence of natal homing (Brophy *et al.*, 2006, Molloy *et al.*, 1993), this is the first study to trace the movement of a cohort from juvenile to adult stage and show direct evidence of natal homing. Despite extensive mixing of the early life stages Celtic and Irish Sea herring form two distinct stocks with little mixing between them. This contrasts with the lack genetic differences observed in other herring stocks to the west of Britain and Ireland. A multidisciplinary study (WESTHER) used body morphometrics, otolith shape, meristic characters, internal parasites, otolith microchemistry, otolith microstructure and genetics in an attempt to separate the stocks (Hatfield *et al*., 2007). No apparent differences were observed across the "Malin shelf unit" which includes various temporal and spatially distinct spawning assemblages, and encompasses seven ICES management areas (VIaN, VIaS and VIIb, Clyde and the Irish Sea). It is suggested that the fish in this area form a metapopulation with large amounts of exchange between subgroups. The study successfully discriminated Celtic Sea fish from the other subgroups. The recommendations arising from the WESTHER study are that herring to the west of Britain and Ireland are managed as two units: the Malin shelf unit (containing the current ICES stocks VIaN, VIaS, VIIb, Clyde and Irish Sea) and the Celtic Sea unit (containing the current Celtic Sea and VIIj stock).

6.3 Herring stock structure theories

Herring populations are distributed throughout the Northern hemisphere with approximately 20 stocks in the North Atlantic. Six are located in the northwest Atlantic, while the remaining 14 are found in the northeast. Of these, the Atlanto-Scandian complex of herring (Icelandic summer spawners and Norwegian Spring spawners) are currently that largest group in the world (FAO, 2004). Herring

populations can be highly variable and many stocks have displayed population collapses and recoveries over the last 50 years (Zheng, 1996). As they migrate between spawning, nursery and feeding grounds herring from different management areas and stocks mix. However the distinct spawning times and locations, and morphometric characteristics displayed by many herring stocks suggests that although genetic differences are often not found, stocks are not identical and therefore should not be managed as just one unit. This issue presents a particular challenge for fisheries scientists.

Many efforts have been made to construct a unifying theory to explain herring population structure. Theories range from complete isolation to complete transience (Iles and Sinclair, 1982, Smith and Jamieson, 1986), with the metapopulation theory proposed by McQuinn (1997), offering a compromise between the two.

In some areas herring stocks show distinct structure while in others stock boundaries are more fluid. North Sea and Downs herring spawn in different geographic locations and at different times of the year. They mix as juveniles where some drift into the Skagerrak where they remain until age 2/3 (Rosenberg and Palmen, 1982). While both stocks display distinct spawning times and locations, little or no genetic differences have been identified between them (Mariani *et al.*, 2005). This is similar to the situation displayed in the Malin shelf area as described above.

In contrast, genetic differences have been observed between herring populations in the Baltic Sea (Jorgensen *et al.*, 2005) . Bekkevold *et al.* (2005) identified genetic differences between herring populations from the North Sea and the Baltic. In both cases, salinity gradients are proposed as a mechanism for maintaining isolation between spawning populations (Bekkevold *et al.*, 2005). The distinct spawning units observed in the Irish and Celtic Seas (Burke *et al.*, 2008, Hatfield *et al.*, 2007) may be maintained by environmental differences between the two areas.

As new techniques are applied to stock identification and more information becomes available on stock structure it emerges that perhaps no one theory can explain structure in all herring populations. It also becomes evident that environmental conditions can have a significant effect on structure. As climate conditions change adaptive responses cause stock boundaries to change, leading to increased or decreased levels of exchange between groups.

6.4 Archival otolith samples

The results presented in chapter 6 highlight the value of historical otolith collections for studies of fish population dynamics and stock structure. The AFBI otolith collections provided information over a long time series and facilitated the development of a recruitment index for the Irish Sea stock. The archive was also used to examine temporal trends in abundance and growth in relation to environmental conditions.

Otolith archives provide an enormous data resource. Fisheries institutes around the world hold collections of historical otoliths, which can be utilised at very little cost to the researcher. Most research studies are conducted over a three year time span. The use of archival otoliths facilitates extending the temporal range of the study. In an age of increasing stock depletions and climate change (>25% of the worlds fish and shellfish stocks are currently overexploited or significantly depleted (UNEP, 2006), it is increasingly important to understand long term trends in fish populations. Also, as new techniques are developed, the potential for unlocking information from historical otoliths increases. Archived otoliths have been used to examine genetic stability in populations over time in cod (Nielsen *et al.*, 1998, Poulsen *et al.*, 2006) and have also been used to link trends in growth, recruitment and population abundances to environmental conditions and climate change in cod (Pilling *et al.*, 2007), sole (Millner and Whiting, 1996), and striped mullet (McDonough and Wenner, 2002). It is critical that otolith collections are properly preserved and achieved to be used to their full potential.

Appendix A:

Average coefficients of variation for each shape variable (SV) generated in Image and outline trials using elliptic Fourier analysis (EFA)

Method	EFA Image Trail	EFA Outline Trial
SV	Average (%)	Average (%)
4	0.66	0.01
5	16.9	0.13
6	4.68	0.27
7	94.66	2.65
8	2.47	0.08
9	5.68	0.08
10	3.86	0.18
11	8.29	0.21
12	13.59	0.1
13	5.31	0.13
14	9.18	0.02
15	2.79	0.27
16	209.91	6.83
17	8.44	0.27
18	140.65	0.82
19	4.4	0.35
20	8.93	0.36
21	17.91	0.54
22	12.11	0.79
23	26.57	1.56
24	9.58	0.38
25	6.94	0.41
26	186.47	1.14
27	51.64	1.01
28	14.4	1.05
29	65.54	16.06
30	101.88	0.51
31	54.45	8.81
32	40.53	2.97
33	87.69	1.2
34	5.7	0.15
35	28.17	0.24
36	19.43	9.43
37	12.12	0.28
38	85.45	1.22
39	15.92	0.48
40	30.26	0.47

SV	Average	Average
41	23.48	0.38
42	60.59	1.72
43	49.47	4.62
44	263.9	0.83
45	30.2	0.98
46	11.26	0.56
47	37.56	13.98
48	482.19	10.68
49	38.55	0.23
50	46.77	0.26
51	51.38	26.34
52	1783.48	0.36
53	36.95	0.97
54	98.2	2.75
55	41.39	0.06
56	520.96	9.5
57	30.98	1.01
58	16.94	0.95
59	21.23	1.61
60	114.08	1.01
61	51.32	0.46
62	79.65	3.95
63	160.12	1.71
64	88.58	5.53
65	53.77	0.31
66	21.05	1.64
67	74.11	2.96
68	68.84	67.59
69	55.01	4.37
70	146.65	1.36
71	262.12	4.38
72	98.98	2.47
73	271.44	2.41
74	45.75	7.34
75	46.02	10.6
76	204.87	2.85
77	168.14	1.48
78	45.08	3.71
79	182.47	4.12
80	45.98	6.25

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Acknowledgements