Short communication

Otolith shape analysis: its application for discriminating between stocks of Irish Sea and Celtic Sea herring (*Clupea harengus*) in the Irish Sea

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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 living together on 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 as larvae. Measurements of otolith increment width can be used to distinguish between the fast-growing winter-spawned and the slow-growing autumn-spawned stocks, but this method can be time-consuming. Otolith shape analysis is investigated as an alternative method for discriminating between seasonal spawning stocks. Juvenile herring collected from nursery grounds in the Irish Sea in 2006 were classified as autumn- or winter-spawned using increment width measurements. Otolith shape was defined using shape indices and Fourier descriptors. Juveniles were classified successfully 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 juvenile herring in the Irish Sea and its possible use for other mixed-herring stock assessments are discussed.

Keywords: Atlantic herring, *Clupea harengus*, otolith shape analysis, stock discrimination.

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Introduction

Atlantic herring (*Clupea harengus*) is a highly complex species. Many stocks display differences in spawning season and location and life-history parameters (McQuinn, 1997). Because of larval dispersal and adult migrations, stocks that spawn in separate locations often mix at nursery and feeding grounds (Rosenberg and Palmen, 1982; Messieh *et al.*, 1989; Mosegaard and Madsen, 1996), causing uncertainty in their management. This uncertainty has led to extensive research into methods for separating mixed herring stocks. Otolith microstructure (Moksness and Fossum, 1991; Brophy and Danilowicz, 2002; Clausen *et al.*, 2007), 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 (Dahle and Eriksen, 1990; Ruzzante *et al.*, 2006) have all been used to distinguish between stocks or stock components with varying rates of success, depending on the stocks/stock components being investigated.

Around the Irish coast, herring are managed in four 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 3–4-week period from September on (Dickey-Collas *et al.*, 2001), but in the Celtic Sea, spawning takes place in autumn and winter (Molloy, 1980). 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) show that juvenile herring disperse from the eastern Celtic Sea into the Irish Sea during their first year of life, where they mix with the resident Irish Sea stock on 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 populations have been used as a population marker in the Irish and Celtic Seas (Brophy and Danilowicz, 2002), the Norwegian Sea (Moksness, 1992), the North Sea (Mosegaard and Madsen, 1996), and the North Sea and western Baltic (Clausen *et al.*, 2007). In the Irish Sea, Brophy and Danilowicz (2002) found a clear bimodal distribution in width at larval increments 61–70, reflecting the presence of two groups; the slow-growing autumn-spawned fish and the fast-growing winter-spawned fish. These differences in growth pattern could be used routinely to identify winter-spawned Celtic Sea juveniles in the Irish Sea. However, otolith microstructure analysis can be time-consuming. If otolith shape analysis can successfully separate the two components, it would provide a fast and sustainable method to support management. Without the separation of the stocks, accurate estimates of juvenile abundance for the Irish Sea fishery is jeopardized, resulting in a failure to produce a precise recruitment index for the Irish Sea (ICES, 2007).

Otolith shape analysis is 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 because of the dual function of the otolith as an organ of equilibrium and hearing (Campana and Casselman, 1993). Otoliths grow throughout a fish's life, and differential patterns can be caused by environmental conditions such as temperature (Fey, 2001), prey density (Feet *et al.*, 2002), and photoperiod (Dowd and Houde, 1980). Otolith shape has been used in many stock-discrimination studies (DeVries *et al.*, 2002; Cardinale *et al.*, 2004; Stransky *et al.*, 2008), with levels of classification success ranging from 60 to 95% for interstock separation, depending on the species.

Our study assesses the utility of otolith shape analysis as a tool for discriminating between Irish Sea (autumn-spawned) and Celtic Sea (winter-spawned) juvenile herring on Irish Sea nursery grounds, with the aim of using the method in the assessment of the Irish and Celtic Sea herring fisheries, and other mixed herring stocks.

Material and methods

Atlantic herring (*C. harengus*) were collected in the Irish Sea in September 2006 using midwater trawls during the herring acoustic survey aboard the RV "Corystes" with the Agri-Food Biosciences Institute (AFBI; formerly known as the Department of Agriculture and Rural Development, DARD), Northern Ireland. Herring ranging from 7 to 19 cm were collected to target fish from the 2005 year class as age-0 (i.e. with no translucent winter ring in the otoliths). Sampling was spatially stratified, four stations being occupied east of the Isle of Man, and four west of the island (Figure 1). Fish were processed on board 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 being stored dry in 5-ml plastic vials.

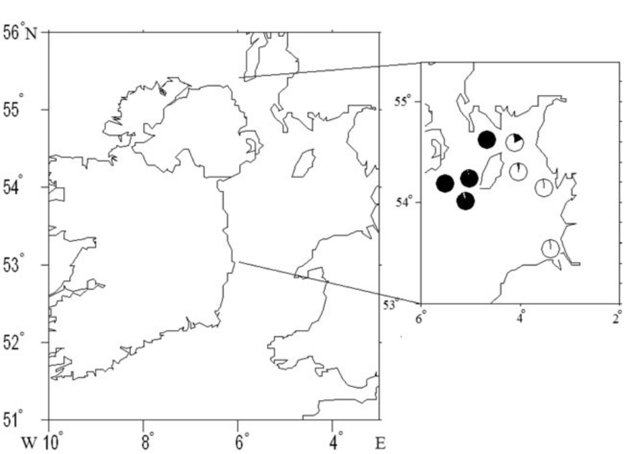

Figure 1. Map showing the relative proportions of autumn- (white slice) and winter-spawned (black slice) age-0 herring collected in the Irish Sea in 2006.

Table 1. Mean fish length and otolith length ± standard deviation for autumn- and winter-spawned 0-group 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

Otolith microstructure analysis

Examination of otolith annuli was used to verify that all fish used in the analysis were age-0. The mean lengths and numbers of fish used in the analysis are shown in Table 1. Otolith microstructure analysis was then used to classify the herring as winter- or autumn-spawned, based on daily increment widths at the larval core, using the method 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 increments 61 and 70 were classified as winter-spawned, and fish with mean increment width of ≤ 2.2 μm as autumn-spawned. Approximately 50% of the fish were classified using manual increment measurement. At this stage, a blind test of ten randomly selected otoliths was carried out to assess the success of the classification based on visual inspection. Classification based on visual inspection was feasible because of the presence of distinct growth patterns in autumn- and winter-spawned fish (Brophy and Danilowicz, 2002). The classification success to hatch type achieved was 100%, so the remaining fish were classified based on visual inspection alone.

Otolith shape analysis

Otolith shape can be described in many ways, one of the simplest being manual distance measurement. Such 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 (Russ, 1990). More complex methods use image-analysis software to generate coefficients that describe the shape of the otolith, such as Fourier series shape analysis. Here, elliptic Fourier analysis was used to generate 77 shape coefficients (C4–C80), to describe the outline shape of each otolith. A combination of shape indices (form-factor) and coefficients (C12, C19, and C21) was used to describe otolith shape variation in juvenile autumn- and winter-spawned herring collected on the nursery grounds in the Irish Sea. The methods used to obtain shape indices and elliptic Fourier shape coefficients are described in Burke *et al.* (2008). Both otolith microstructure analysis and otolith shape analysis were timed, to estimate the processing time required for each method.

Data analysis

For the purposes of data analysis, winter- and autumn-spawned fish were treated as separate stocks. Variability in growth was examined using fish and otolith length (Table 1). Lengths were first screened for normality and homogeneity of variance using Kolmogorov–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 Kruskal–Wallis rank sum tests were used to test for differences in fish and otolith lengths between stocks and between sites within each stock.

Shape indices and elliptic Fourier shape coefficients (hereafter referred to collectively as shape variables) were screened using the same procedure. Variables that did not meet the assumptions for parametric tests were tested using the non-parametric equivalent. 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 in classifying fish to spawning season and were selected for further analysis. The selected variables were tested for significant correlations with each other. Where two variables had a high correlation coefficient (>0.5), only one was selected for use in the final analysis. Based on the results of these tests on five shape indices and 77 coefficients, one shape index, form-factor, and three coefficients (C12, C19, and C21) were selected for further analysis.

The 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 whether 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 (Reist, 1985; Begg *et al.*, 2001; DeVries *et al.*, 2002; Galley *et al.*, 2006). Form-factor and C21 were identified as significantly correlated with fish length and were adjusted because 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 that multicollinearity would not result in the 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, an optimal classification is achieved using a quadratic function rather than a linear one (Seber, 2004).

Quadratic discriminate function analysis (QDFA) was carried out using form-factor (adjusted), C12, C19, and C21 (adjusted) to determine the proportion of fish that could be classified correctly as autumn- or winter-spawned 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, however, 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, using functions for all the data except that being classified (Engelman, 2004). Both procedures were carried out in SYSTAT 11 for Windows for age-0 autumn- and winter-spawned stocks.

Results

Of 244 fish analysed, 118 were classified as autumn-spawned and 126 as winter-spawned, based on otolith increment widths. Some 97% of the fish classified as autumn-spawned were from stations in the eastern Irish Sea, and 92% of herring classified as winter-spawned were from stations in the western Irish Sea (Figure 1). Of the fish classified by manual measurement of increment width (54% of the total), the split in mean increment width at increments 61–70 was the same as identified by Brophy and Danilowicz (2002). Processing times took ~5 min per fish for otolith shape analysis, and 20 min per fish for microstructure analysis, where manual increment measurements were made, but just ~12 min when classification was carried out by visual inspection alone.

Kruskal–Wallis rank sum tests identified differences in otolith and fish lengths between the two stocks ($p < 0.05$). However, both these lengths also differed significantly between sites within autumn- ($p < 0.05$) and winter-spawned stocks ($p < 0.05$). Fish length also overlapped between autumn- and winter-spawned components and did not display the clear bimodal distribution shown by otolith microstructure (Figure 2).

Fish otoliths were classified into spawning type using QDFA, classification rates being 87% for overall classification and 86% for jackknifed classification. Mahal distances were plotted to show visually how the two stocks separated from each other (Figure 3).

Discussion

The distribution of Celtic Sea winter-spawned juvenile herring 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.*,

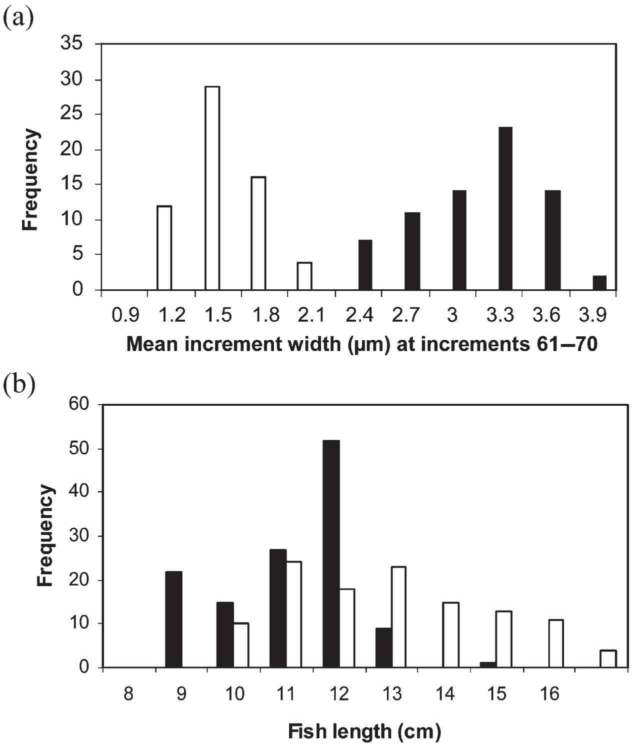

Figure 2. (a) Frequency distribution of mean increment width at increments 61–70. (b) Frequency distribution of fish lengths of age-0 fish captured in the Irish Sea in 2005. Black histograms indicate winter-spawned Celtic Sea fish and white histograms autumn-spawned Irish Sea fish.

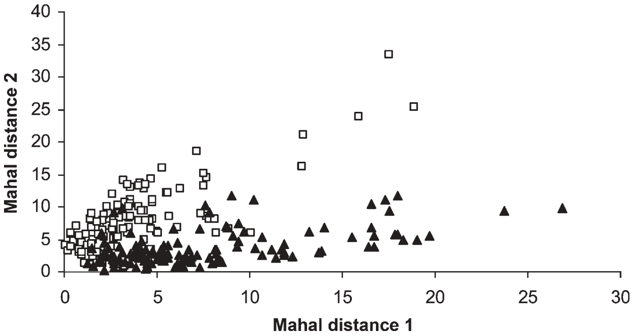

Figure 3. Frequency distribution of Mahal distances obtained from QDFA of age-0 fish collected in 2005. Black triangles indicate winter-spawned Celtic Sea fish and open squares autumn-spawned Irish Sea fish.

2008). Brophy and Danilowicz (2002) found interannual variation in the distribution patterns of winter- and autumn-spawned juvenile herring in the Irish Sea, winter-spawned fish being found on both sides of the Irish Sea. With the distribution of juvenile herring across the Irish Sea varying from year to year, their separation based on geographic location alone would not be stable temporally.

Similarly, fish size cannot be used to separate autumn- and winter-spawned juveniles. Although 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 measurement, 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 juvenile herring 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. Therefore, size is unlikely to be 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, because size effects can compromise stock discrimination studies if variables are not standardized (Smith, 1992). Adjustment of the 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 here. Many studies on stock discrimination have evaluated the relative importance of genetics/environmental conditions on otolith shape, but few have examined the subject directly. Gauldie and Nelson (1990) found that growth rates had a direct link to otolith shape, with faster growth producing longer thinner crystals, and Gagliano and McCormack (2004) stated 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; Simoneau *et al.*, 2000; Cardinale *et al.*, 2004). Some studies have found that classification success from otolith shape increased as genetic discreteness or geographic separation increased (Castonguay *et al.*, 1991; Friedland and Reddin, 1994), implying that genetic differences were responsible for the 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 have failed to be distinguished genetically (DeVries *et al.*, 2002). Reared and wild components of the same genetically distinct stock can display differences in otolith shape in response to differences in environmental conditions (Simoneau *et al.*, 2000; Cardinale *et al.*, 2004). It is uncertain whether the shape differences between the autumn- and winter-spawned stocks observed here are driven by genetic factors or by differences in environmental conditions experienced during the first year of life. Little genetic difference has been observed between herring 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 attributable to differences in their spawning seasons.

Regardless of what is causing the 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 the software for carrying out the analysis once images are taken being freely available. The procedure is also far less destructive to otoliths, because 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 for age determination or structural analysis.

For the Irish Sea stock, 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 juvenile herring in the Irish Sea would improve the estimates of juvenile abundance for the Irish Sea and increase the accuracy of recruitment indices for the Irish Sea spawning stock. Incorporation of this method into annual herring sampling programmes would supply valuable information 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 juvenile herring drift into the western Baltic during their first year of life, where they 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 juvenile herring that drift into the North Sea during their first year of life are identified from vertebral counts (ICES, 2007). If the seasonal spawning stocks in those areas show variability in otolith shape, the method could facilitate their rapid separation and be incorporated into routine assessment.

The method presented here shows potential for separating components of fish in mixed stock fisheries. The results correspond well with those from other studies that have evaluated 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 maintenance of the otolith in a condition suitable 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, therefore, it could be incorporated easily into stock discrimination analysis where otolith analysis is already carried out.

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