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Determination of species-specific spawning distributions of commercial finfish in the Irish Sea using a biochemical protein-based method

O. A. Heffernan[1,*], B. S. Danilowicz[2], S. P. Milligan[1]

[1]The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft Laboratory, Pakefield Road, Lowestoft NR33 0HT, Suffolk, UK

[2]Department of Zoology, University College Dublin, Belfield, Dublin 4, Ireland

ABSTRACT: Knowledge of the spatial and temporal distribution of spawning is essential for an increased understanding of the ecology of the early life-history of a species, and is also a key factor in the management of species that are heavily exploited. Conventionally, the abundance and distribution of spawning are determined for individual species by visual identification of early-stage eggs collected from ichtyoplankton surveys. The accuracy of this method remains contentious for commercial gadoids and pleuronectids, due to potential misidentification of eggs at early developmental stages. In this study, pelagic fish eggs collected on spring ichthyoplankton surveys of the Irish Sea in 2000 were identified using iso-electric focusing (IEF) and histochemical staining of isozymes. Comparison of isozyme patterns obtained from known adult muscle tissue samples or broodstock eggs enabled identification of eggs of ambiguous identity. Application of this technique to 4813 pelagic eggs in the size range 1 to 2 mm collected between late February and May 2000 showed that the following species were present: cod *Gadus morhua*, haddock *Melanogrammus aeglefinus*, plaice *Pleuronectus platessa*, whiting *Merlangius merlangus*, lemon sole *Microstomus kitt* and witch *Glyptocephalus cynoglossus.* The abundance and distribution of newly spawned eggs of cod, haddock, whiting and plaice in the Irish Sea were mapped using kriging as an interpolation method. Times and locations of spawning were considered in relation to the hydrographic characteristics of the region and were compared with distributions estimated based on the morphological identification of eggs. The modification of an established biochemical technique to suit the needs of the present study has enabled the distribution and abundance of the eggs of commercial gadoids in the Irish Sea to be determined on a species-specific basis. The results have a potential application in identifying areas within the Irish Sea that could be targeted for fishery management and in refining spawning-stock biomass estimates used in stock assessments of these species.

KEY WORDS: Spawning distributions · Abundance · Gadoids · Pleuronectids · Iso-electric focusing · Species identification

INTRODUCTION

Most marine demersal teleosts spawn pelagic eggs that drift in the plankton in accordance with the prevailing conditions (Sundby 1996). The distribution of these eggs, determined from ichthyoplankton surveys, has been the primary means of describing and quantifying the spawning of fish species since the beginning of the twentieth century (Harding 1974). Accurate knowledge of species-specific spawning distributions is important from an ecological viewpoint in terms of increased understanding of the complex physical and biological factors influencing the reproductive cycle. From a conservation perspective, knowledge of the spatio–temporal range of spawning enables fisheries management plans to effectively protect species from exploitation at critical points in their life history. Accuracy is of particular importance in the estimation of

*Email: olive@ecoserve.ie

spawning abundance, which is used to quantify egg production from ichthyoplankton surveys as a fishery-independent method of stock assessment (Armstrong et al. 2001).

Spawning has been extensively described and quantified for Irish Sea stocks of cod *Gadus morhua*, haddock *Melanogrammus aeglefinus* and plaice *Pleuronectus platessa* (Simpson 1959, Nichols et al. 1993, Anonymous 1997, Ellis & Nash 1997, Fox et al. 1997, 2000, Nash & Geffen 1999, Armstrong et al. 2001). The accuracy of these estimates remains contentious, however, due to potential misidentification of eggs that are spawned simultaneously in spring in inshore coastal waters of the eastern and western Irish Sea (Anonymous 1997, Fox et al. 2000, Armstrong et al. 2001). Conventionally, marine fish eggs are identified by visual characteristics such as the presence, number and size of oil globules, size of the perivitelline space, yolk homogeneity and embryo pigmentation (Russell 1976). In the early stages of development, eggs of many gadoids and pleuronectids cannot be differentiated on the basis of these morphological characteristics and overlap significantly in their size distributions (Russell 1976). Not until developmental stages IV or V (Lockwood & Nichols 1977), just prior to hatching, do species develop pigmentation of the embryo, which aids in diagnostic identification. Usually, the relative abundance of these late-stage eggs or early-stage larvae is used to estimate the distribution and abundance of newly spawned (Stage IA) eggs (Page et al. 1997, Bradbury et al. 2000). Species that could be mistaken for the target species and are rare as larvae are often assumed to be absent from the plankton when in early developmental stages (Anonymous 1997, Fox et al. 2000, Armstrong et al. 2001). Later developmental stages are likely to have drifted from spawning grounds over time, introducing uncertainty to distribution estimates. A reliable means of identifying early life-stages is clearly crucial to accurately describing and quantifying species-specific spawning distributions.

Mork et al. (1983) showed that iso-electric focusing (IEF) of isozymes and subsequent histochemical staining could be used as a tool to distinguish between species in early life-stages that occur simultaneously in the plankton and that cannot be identified on the basis of morphology alone. This technique has been applied to species identification of gadoids and flatfishes in Norwegian waters (Mork et al. 1983) and to numerous studies of the genetic variability within gadoid species (Mork et al. 1984, 1985, Giaever et al. 1995, Forthun & Mork 1997a,b, Giaever & Forthun 1999, Mork & Giaever 1999, Mattiangeli et al. 2000). In this paper, we describe and evaluate the application of a protein-based biochemical genetic technique, IEF, to identification of newly spawned gadoid and pleuronectid eggs and to estimation of species-specific spawning distributions in the Irish Sea.

MATERIALS AND METHODS

Field work. During 6 consecutive ichthyoplankton surveys of the Irish Sea from 29 February to 26 May 2000, pelagic fish eggs were collected for biochemical identification. Each survey covered a 105-station grid (Fig. 1), pre-stratified into 5 areas, based on expected egg abundance. ICES statistical rectangles are areas measuring 1° of longitude by 0.5° of latitude, that are widely used throughout Europe and Norway for the reporting of commercial fish catches, and are convenient units with which to report plankton catches. These ICES rectangles were split into eighths for the 2000 surveys to attempt to adequately cover the fish spawning distributions in the Irish Sea. This gave a reasonable station density for the whole of the Irish Sea which, given good weather, would allow completion of the survey grid in 5 to 6 d. In areas or strata of expected high egg density (based on 1995 surveys: Armstrong et al. 2001), it was recommended that 2 plankton samples be taken (1 in the north, 1 in the south) of each 1/8 rectangle. In areas or strata of expected low egg density, 1 plankton sample was taken at the mid-point of each 1/8 rectangle.

Plankton at each station was sampled using Gulf VII high-speed plankton samplers (Nash et al. 1998). A PRO-Net electronics system or MAFF/Guildline CTD (Milligan & Riches 1983) relayed real-time data on depth, temperature, conductivity, fluorescence, internal flow (i.e. volume of water filtered) and external flow (for an index of sampler efficiency) to a shipboard

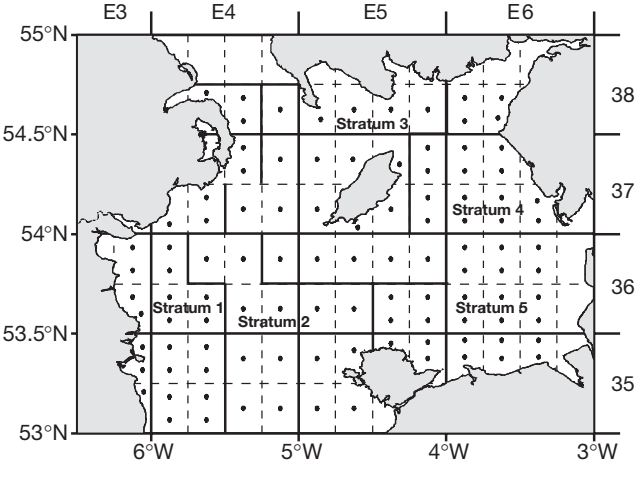

Fig. 1. Sampling stations for the ichthyoplankton surveys in the Irish Sea in 2000

display unit. Gulf VII samplers were fitted with a 280 µm filtering net, for which a 400 µm mesh net was substituted when significant net-clogging was encountered. At each station, the Gulf VII was deployed in an oblique manner from the sea surface to within 2 m of the seabed and towed at a speed of approximately 3.5 to 5.0 knots. The minimum depth of the water column at a station was 12 m, and the maximum bottom depth at a station was 232 m. In shallow water (<30 m), double oblique tows were conducted to enable a sufficient volume of water to be filtered. Sampling was carried out 24 h each day.

On recovery, the plankton was washed from the net and transferred to a tray on ice, and eggs in the size range 1.0 to 2.0 mm without an oil globule (Russell 1976) were removed from the sample. Eggs of this description were chosen as they included species that could not be visually identified to species level and are of high commercial importance in the region, namely cod, haddock, whiting *Merlangius merlangus* and plaice. A small proportion of eggs <1.00 mm and >2.00 mm were inadvertently included in these samples. A maximum of 50 eggs of the appropriate type and size category was collected at any given station. The remainder of the sample was fixed in 4% buffered formalin (Tucker & Chester 1984). The diameter of each egg collected for IEF identification was measured to an accuracy of 0.01 mm and eggs were categorised as 1 of 6 developmental stages, IA, IB, II, III, IV and V, following the descriptions given for plaice by Simpson (1959) and simplified for cod by Thompson & Riley (1981). Where possible, eggs of late developmental stages were identified visually on the basis of embryonic pigmentation. Eggs were either identified immediately or stored individually within well plates in a freezer prior to identification by IEF. The portion of the catch at each station that was preserved in formalin was subsequently sorted, counted, measured, staged and, where possible, identified.

Laboratory work. ***Preliminary analyses:*** The procedure used for the identification of pelagic fish eggs was a modification of protocols used by Mork et al. (1983) and Anonymous (1997). Survey samples were identified following a period of optimisation, during which the reagents, procedures and conditions for IEF were defined. Species were identified by their expression of the lactate dehydrogenase (LDH) A tetramer that predominates in white skeletal muscle tissue (Mork et al. 1983), and superoxide dismutase (SOD) patterns were also used for identification where visible. The SOD bands showed up as clear zones on a faintly blue background, whereas the LDH patterns show up as dark blue/purple bands, which could easily be distinguished from the light-blue coloration of the gel background. Eggs collected on surveys were identified to species level by comparing the isozyme patterns with those obtained from known broodstock eggs or skeletal muscle tissue homogenate from adult fishes (see Fig. 2). Muscle tissue was homogenised and centrifuged to obtain a clean supernatant for enzyme analysis, but preliminary analyses indicated that raw homogenate was sufficient for analysis of eggs. We used 3 individual adults for determining the banding patterns of each species from muscle tissue samples. Initially, isozyme patterns were identified for cod, haddock, whiting and plaice only. Following identification of target species from the survey samples, however, a number of unidentified banding patterns remained. Isozyme patterns were therefore identified for an additional 4 species; sprat *Sprattus sprattus*, long rough dab *Hippoglossoides hippoglossus*, lemon sole and witch, all of which could potentially be visually misidentified as gadoid or plaice eggs.

IEF Protocol: Groups of eggs were randomly assigned to individual gels for analysis, with a maximum of 30 eggs identified from any individual haul. The IEF was performed using 1 mm thick precast polyacrylamide (PAG) gels, pH 3.5 to 9.5 (T = 5%, C = 3%) containing 2.2% ampholine and <0.2% acrylamide on a Multiphor II horizontal electrophoresis system. Each egg was washed in Milli-Q water and homogenised in 30 µl of Tris buffer, 0.1 M, pH 7.5, using a motorised pellet pestle inserted into a 0.5 ml microtube on ice. The anode solution was 1 mol l^{-1} H_3PO_4 and the cathode solution 1 mol l^{-1} NaOH. Sample application strips with 52 individual wells were placed next to each anode to minimise interference with the resulting banding patterns on the finished gel (Janson & Ryden 1998). The first and last sample wells of each strip were loaded with 20 µl of cod egg homogenate, and 1 well on each side of the gel was loaded with 20 µl of plaice egg homogenate. The power settings were 1500 V, 50 mA, 30 W and 1.5 h for analysis of 100 survey samples and 1500 V, 25 mA, 15 W and 1.5 h for analysis of 50 survey samples. Temperature was maintained at 10°C throughout the loading and IEF period with a Multitemp II cooler. Following IEF, the gel was removed to a glass tray with a 200 ml solution of 0.5 M pure Tris base, 2 ml of DL-lactic acid, 20 mg of b-NAD and 10 mg of both phenazine ethosulfate (PES) and Nitro-Blue Tertazolium (NBT). The gel was left on a lightbox for 15 min to allow SOD bands to develop and was then transferred to an incubator, where staining continued at 30°C until LDH banding patterns were clearly visible. The stain was drained and 200 ml of a reagent (5:4:1 distilled water:ethanol:acetic acid) was added for 30 min to stop the reaction and to wash excess staining reagents from the gel. The gel was positioned face downwards to dry on to a sheet of 3 mm Whatman chromatography paper, labeled with details of the sur-

vey, samples and date. Subsequently, 2 of us (O.A.H and B.S.D.) identified all samples independently by comparison to known standards.

Spawning distributions: The results of the IEF analysis were used to determine the proportion of eggs in each haul accounted for by each of the target species. For each individual haul, the species proportions determined by IEF were assigned to the remainder of the preserved and sorted eggs of 1.00 to 2.00 mm diameter. Egg abundance (individuals m^{-2} sea surface) was determined for each species from the number of eggs caught at a station multiplied by the sampled depth divided by the volume of water filtered (Anonymous 1997). The distribution of Stage IA eggs was taken as an accurate estimate of the spawning distribution of the species, since generally they are at this stage within 3 d of being spawned and are unlikely to have drifted far from the spawning grounds during this period. Kriging was used as an interpolation method (Surfer® Version 7.0, Golden Software) to estimate the abundance of eggs in areas where no samples were taken, based on the parameters fitted to a linear model of a variogram (Barange & Hampton 1997).

RESULTS

Field and laboratory work

Of the 594 stations sampled between February and late May 2000, eggs that fitted that target specification were collected from 328 stations. Although the aim was to sample 105 stations on each survey, this was not always possible due to adverse weather conditions, and 'target' eggs were not present at all sampled stations. On the last survey in late May, so few target eggs were collected that numbers were insufficient to interpolate between stations to determine spawning distributions, so these samples were discounted from subsequent analyses. In total, 4813 pelagic fish eggs collected from the Irish Sea between 29 February and 26 May 2000 were analysed using biochemical genetics. Of these, 2790 (58.0%) were positively identified as one of the target species. Eggs that were deformed or discoloured, probably due to mechanical stress during the sampling procedure (Geldmacher & Wieland 1999), were not analysed, as preliminary results had shown that they rarely produced a reliable LDH or SOD banding pattern. When the remaining target eggs sorted from the preserved samples were added to those identified by IEF, a total of 171 359 eggs were assigned to a given species. These numbers were used to determine the abundance estimates for species on a per station basis prior to interpolating abundance between stations to produce spawning distribution maps.

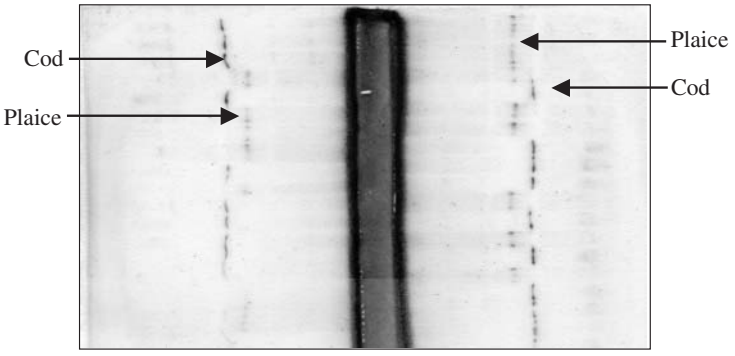

Fig. 2. Iso-electric focusing (IEF) gel showing lactate dehydrogenase (LDH) and superoxide dismutase (SOD) patterns from broodstock eggs of cod *Gadus morhua* and plaice *Pleuronectes platessa*

Species identification

Staining for LDH revealed identifiable activity in the standards for all species. SOD activity, however, was not clearly distinguishable for all species investigated. Comparison of LDH and SOD banding patterns to those of known standards (Fig. 2) demonstrated that the eggs collected were largely of the following species: cod, haddock, plaice, whiting, lemon sole *Microstomus kitt*, and witch *Glyptocephalus cynoglossus*. Most species had unique banding patterns for LDH and SOD (Fig. 3). However, whiting and plaice eggs could not be discriminated from each other biochemi-

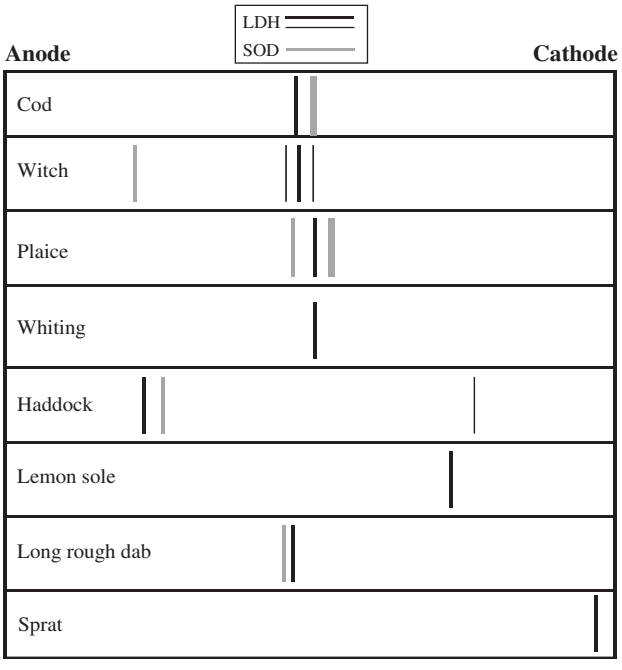

Fig. 3. Observed LDH and SOD banding patterns for species collected during the ichthyoplankton surveys in 2000

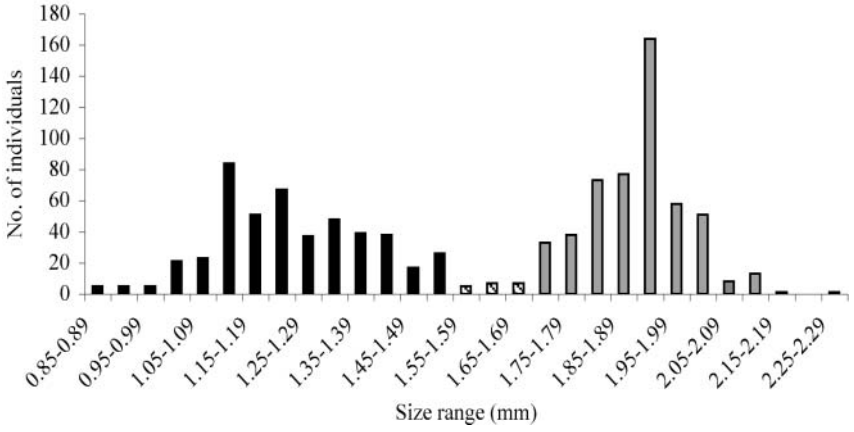

Fig. 4. Size-frequency distribution of eggs with the plaice/whiting banding pattern. Whiting *Merlangius merlangus* (■), unknown (⊠), plaice *Pleuronectes platessa* (■)

cally as their dominant LDH A band was in the same position, and a clear SOD pattern could not always be identified for whiting. Plaice and whiting eggs could, however, be distinguished from other species biochemically and are known not to overlap with each other in size range (Russell 1976, Anonymous 1997). Following their discrimination from other species, they were thus distinguished from each other on the basis of size by analysis of their length-frequency distributions (Fig. 4), which was bimodal, with the lower distribution representing whiting and the upper distribution representing plaice, consistent with their known biology. The upper range of whiting eggs was 1.53 mm and the lower range of plaice eggs was 1.70 mm; 19 eggs with the plaice/whiting banding pattern were in the size range 1.55 to 1.69 mm and were thus labelled as unknowns due to uncertainty regarding their identification. Some of the eggs analysed in the present study expressed additional loci of the LDH isozyme (Fig. 3). These additional bands corresponded to patterns observed in adult tissue samples (as described by Mork et al. 1983) and did not affect the diagnosticity of LDH A. Of the eggs identified using IEF, most were haddock and cod, with whiting and plaice each representing approximately 10% of samples and lemon sole and witch accounting for roughly 1% each (Table 1).

Reliability of IEF technique

Of all samples analysed, 6% expressed a distinct LDH banding pattern that was not identifiable by comparison with the known standards. Approximately 36% of all samples analysed expressed neither LDH nor SOD isozymes. These unidentifiable samples spanned the size range 1.00 to 2.00 mm. There was no significant difference in the proportion of samples without isozyme expression obtained from fresh samples compared to samples stored at −80°C for periods of up to 9 mo after collection ($p > 0.1$). Cod and plaice broodstock eggs transferred from storage at −80°C to storage at −20°C for a period of 6 wk expressed no LDH isozyme patterns, whereas broodstock eggs kept in storage at −80°C over the same period expressed distinguishable LDH banding patterns. The proportion of blank results obtained differed significantly according to the size and developmental stage of the egg. Stage V eggs had a significantly lower proportion of blank results than Stage IB eggs ($p < 0.05$). A higher proportion of blanks was observed in the size category 1.00 to 1.29 mm diameter than in larger eggs ($p < 0.05$).

Species distributions

In general, spawning took place in the eastern and western Irish Sea in coastal waters of less than 50 m depth. The relationship between abundance of spawned eggs and depth was only significant for cod ($p < 0.05$). Overall, 56% of plaice spawning occurred in the eastern Irish Sea (east of 4.5° W), the remainder being concentrated in the coastal waters of the western Irish Sea and to the south west of the Isle of Man. Plaice spawning peaked in early March in both the western and eastern Irish Sea (Fig. 5a) and decreased thereafter through to early May. Approximately 50% of plaice eggs collected from late February to early March were Stage IA (Fig. 6a). Later-stage plaice eggs were recorded from the plankton from as early as late February.

In total, 59% of cod spawning took place in the eastern Irish Sea, the remainder occurring along the east coast of Ireland and, to a lesser extent, west of the Isle of Man and Anglesey (Fig. 7). Cod spawning peaked in

Table 1. Species proportions identified from Irish Sea plankton samples in 2000 using iso-electric focusing

Family	Species	Proportions
Gadidae	Cod *Gadus morhua*	0.17
	Haddock *Melanogrammus aeglefinus*	0.19
	Whiting *Merlangius merlangus*	0.10
Pleuronectidae	Plaice *Pleuronectes platessa*	0.11
	Lemon sole *Microstomus kitt*	0.01
	Witch *Glytocephalus cynoglossus*	0.01

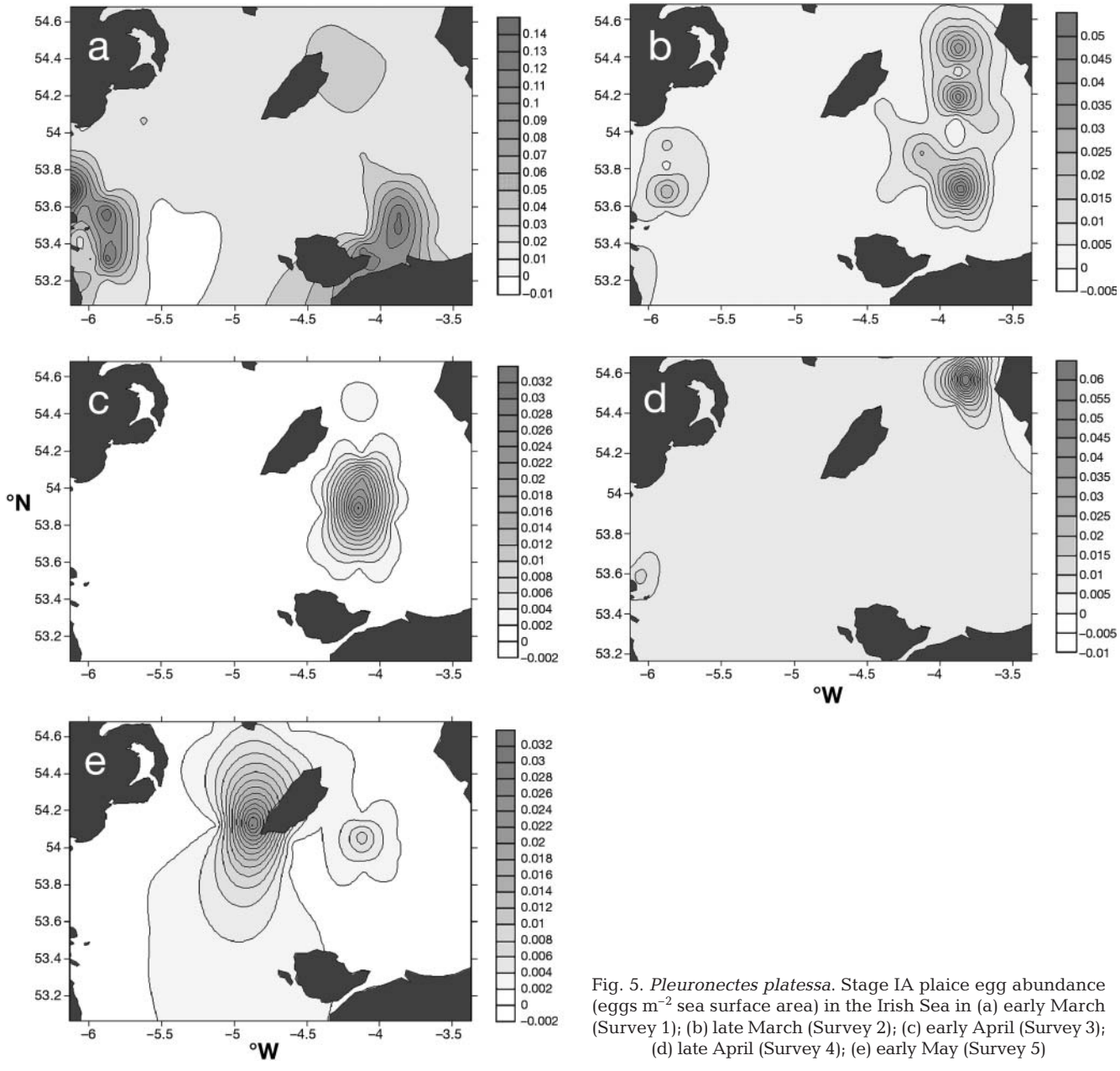

Fig. 5. *Pleuronectes platessa*. Stage IA plaice egg abundance (eggs m^{-2} sea surface area) in the Irish Sea in (a) early March (Survey 1); (b) late March (Survey 2); (c) early April (Survey 3); (d) late April (Survey 4); (e) early May (Survey 5)

March, occurring mostly along the east coast of Ireland in early March. In late March, spawning was more spatially diffuse and occurred mostly in the eastern Irish Sea along the coast from Cumbria to Wales. The abundance of Stage IA cod eggs decreased in April and cod eggs of all stages were virtually absent from the plankton by the surveys in May (Fig. 7e). The vast majority of cod eggs identified during the first survey were Stage I (Fig. 6b), with late-stage eggs in low abundance.

Overall, 79% of haddock spawning occurred in the eastern Irish Sea in 2000, in coastal waters from Wales to Cumbria. The remaining Stage IA haddock eggs were collected in coastal waters of the western Irish Sea (Fig. 8). In early March, approx. 60% of haddock eggs were Stage IA, decreasing to approx. 30% by late March (Fig. 6c). Some Stage III, and a very small proportion of Stage IV and V, haddock eggs were identified in early March. Haddock spawning peaked in the eastern Irish Sea in late April; spawning occurred here to a lesser extent in March (Fig. 8a–d). In the western Irish Sea, haddock spawning mainly occurred in early April off Dublin Bay, but the intensity was almost 20 times lower than that recorded later in April in the eastern Irish Sea. Haddock spawning had virtually ceased by early May (Fig. 8e).

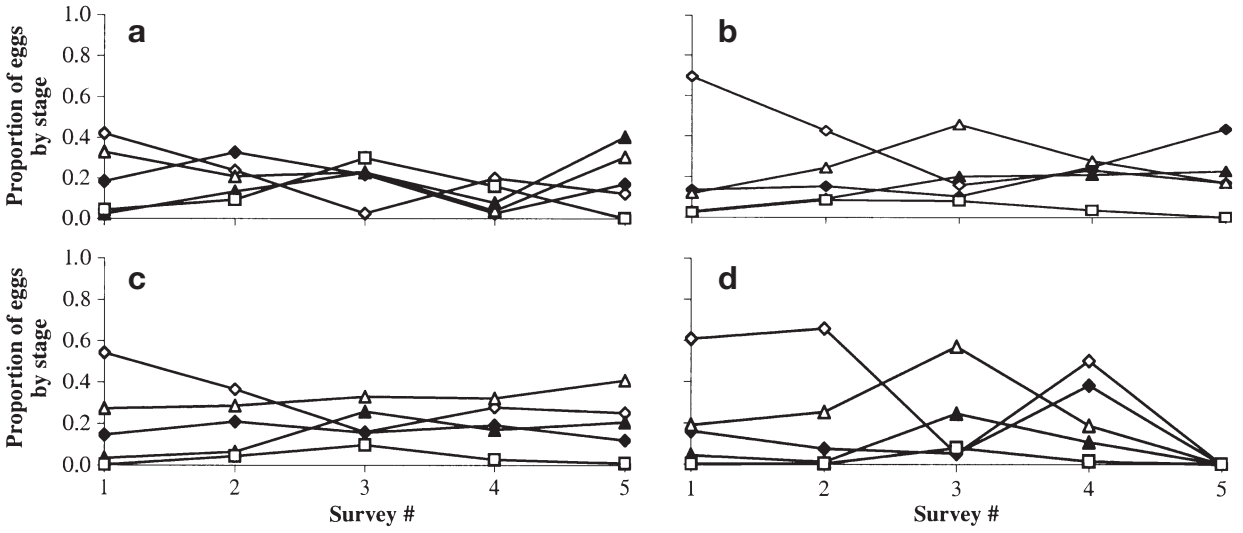

Fig. 6. Proportion of eggs of (a) plaice *Pleuronectes platessa*, (b) cod *Gadus morhua*, (c) haddock *Melanogrammus aeglefinus* and (d) whiting *Merlangius merlangus* of each developmental stage identified from the ichthyoplankton surveys in 2000. (◇) Stage IA; (◆) Stages IB/II; (△) Stage III; (▲) Stage IV; (□) Stage V. Stages IB and II are grouped as there are known difficulties in differentiating between the 2 stages

In total, 88% of whiting spawning occurred in the eastern Irish Sea in coastal waters between Wales and Cumbria. Some whiting spawned around Dublin Bay in the western Irish Sea in late March. Spawning peaked throughout March, and was concentrated in the eastern Irish Sea (Fig. 9a,b). Relatively low concentrations of whiting eggs were recorded in early April in the eastern Irish Sea, but there appeared to be a second peak of spawning activity in late April (Fig. 9c,d). Relatively few late-stage whiting eggs were identified from the first survey in late February to early March, and whiting of all stages were absent from the plankton in May (Fig. 6d).

In total, 26 eggs were identified as lemon sole. These were all collected from the western Irish Sea, mostly in March to early April in the coastal waters off Dublin Bay. A total of 25 witch eggs was collected throughout March and April, mostly from the western Irish Sea, but some from the eastern Irish Sea. There were insufficient numbers to derive meaningful abundance or distribution estimates for either species.

DISCUSSION

The ability to accurately define the spatio-temporal spawning distributions of species increases the understanding of basic ecology and has large-scale implications for species conservation and fisheries management. Knowledge of the spawning distributions of designated stocks is critical to understanding the extent of stock and sub-stock mixing, and thus has implications for identifying population-based parameters. Accurate baseline distribution and abundance data are crucial for assessing changes in stocks over time and for determining the initial need for conservation measures. Unambiguous identification of spawning areas and seasons provides a means for determining the efficacy of management measures designed to protect designated stocks and for increasing the accuracy of abundance estimation, a powerful tool in stock management and assessment of commercially exploited species.

The application of a simple biochemical technique in the present study has resolved a long-term problem of misidentification of early-stage marine pelagic fish eggs collected during ichthyoplankton studies in the Irish Sea (Nichols et al. 1993, Anonymous 1997, Fox et al. 1997, 2000, Armstrong et al. 2001). DNA-based gene probes have recently been shown to be a useful means of distinguishing between cod, haddock and whiting tissue samples (Taylor et al. 2002). However, for distinguishing between pelagic eggs on ichthyoplankton surveys of the Irish Sea, this method has thus far been limited to those species for which a primer has been developed (cod, haddock and whiting). Furthermore, application of the technique has been limited to a small sample size (352 individuals). In the present study, analysis of samples from surveys with extensive spatial and temporal coverage of the Irish Sea during spring enabled identification of the likely spawning times and locations of commercially important species in the area.

The benefits of protein-based methods for identifying components of the plankton include the ability to

process a large quantity of samples rapidly, the comparative simplicity and relatively low cost of the procedures, the ability to analyse several loci simultaneously and the reliability of the results (Park & Moran 1994, Mustafa 1999). As a method of protein analysis, IEF is one of the most highly resolving of all separation techniques (Moss 1982). In the past decade, there has been an upsurge in the use and development of DNA-based molecular procedures in fisheries science. The evolution of such techniques has been necessary, given the limitations of protein-based methods. Electrophoresis is limited to detecting genetic variation affecting genes that express proteins detectable with a histochemical stain, which account for only a small proportion of the entire genome (Park & Moran 1994). Furthermore, enzymatically active proteins are required for protein electrophoresis, which can pose a serious challenge in terms of tissue collection and storage. However, DNA-based methods of species identification are extremely time-consuming and expensive compared with protein-based methods. The identification of standards for comparison with eggs of unknown identity takes a few days for IEF-based procedures, but requires the development and validation of primers for DNA-based methods, a costly and lengthy procedure. In cases where large quantities of data need to be generated

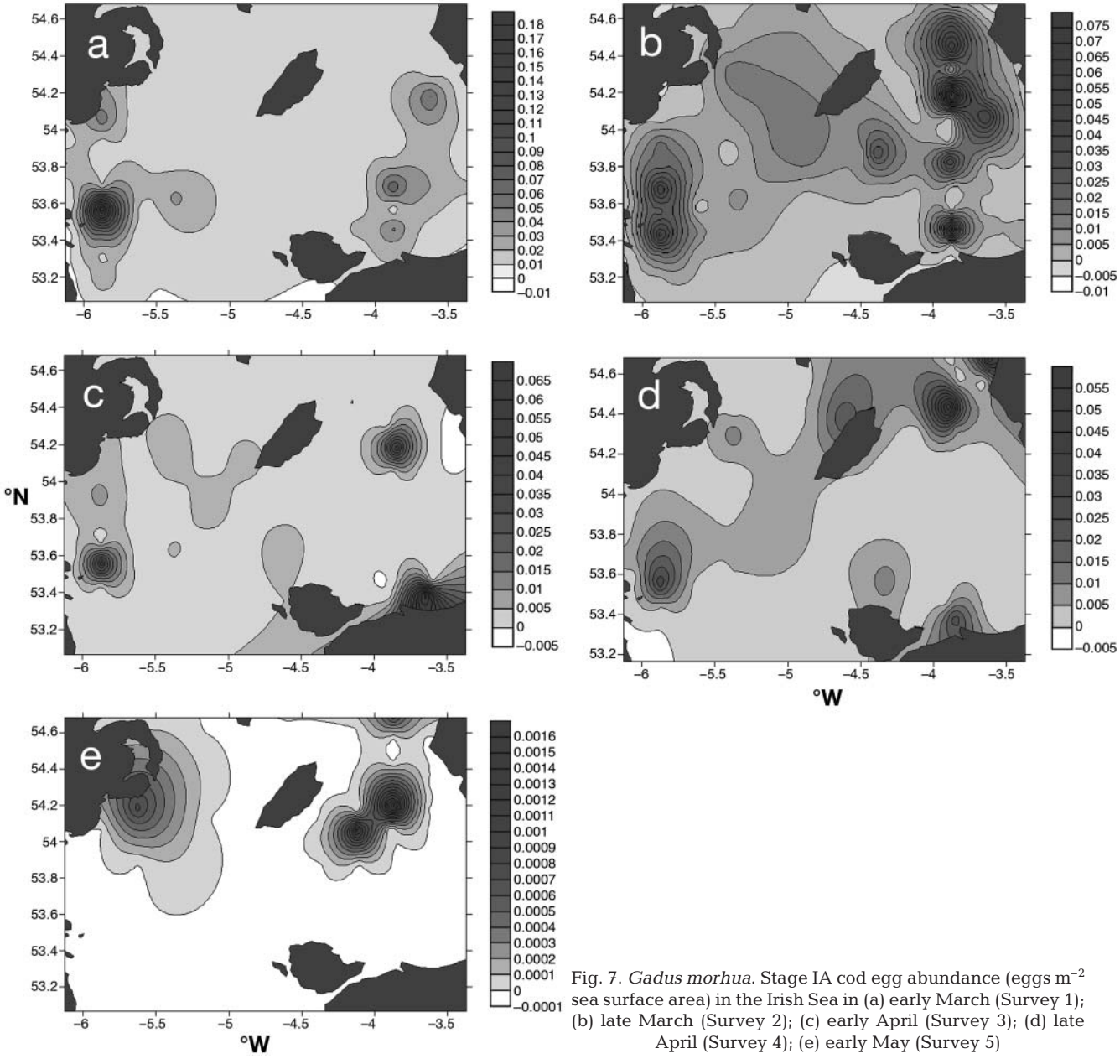

Fig. 7. *Gadus morhua*. Stage IA cod egg abundance (eggs m^{-2} sea surface area) in the Irish Sea in (a) early March (Survey 1); (b) late March (Survey 2); (c) early April (Survey 3); (d) late April (Survey 4); (e) early May (Survey 5)

reliably over a relatively short time period, protein electrophoresis is recommended as the most viable option (Park & Moran 1994). IEF could be potentially used to identify samples on board research vessels during the course of a survey and to thus identify 'hot spots' of spawning, which could be targeted for adaptive sampling.

In general, spawning in 2000 occurred in coastal waters less than 50 m deep in both the western and eastern Irish Sea. The most concentrated aggregations of early-stage cod eggs were found along the Irish coast in the western Irish Sea, and between the Isle of Man and Cumbria and the Isle of Man and Wales in the eastern Irish Sea. Previous studies have reported cod spawning in these areas (Nichols et al. 1993, Anonymous 1997, Fox et al. 1997, 2000, Armstrong et al. 2001). Plaice spawning was concentrated primarily off Great Orme Head and between the Isle of Man and Cumbria in the eastern Irish Sea and in Dublin Bay in the western Irish Sea. Spawning of plaice was generally confined to shallow sandy regions (Armstrong et al. 2001), which are suitable grounds for demersal adult plaice (Ellis et al. 2000). These locations are entirely consistent with historical records of plaice spawning (Simpson 1959, Anonymous 1997, Fox et al. 2000). In early May, spawning was recorded off the

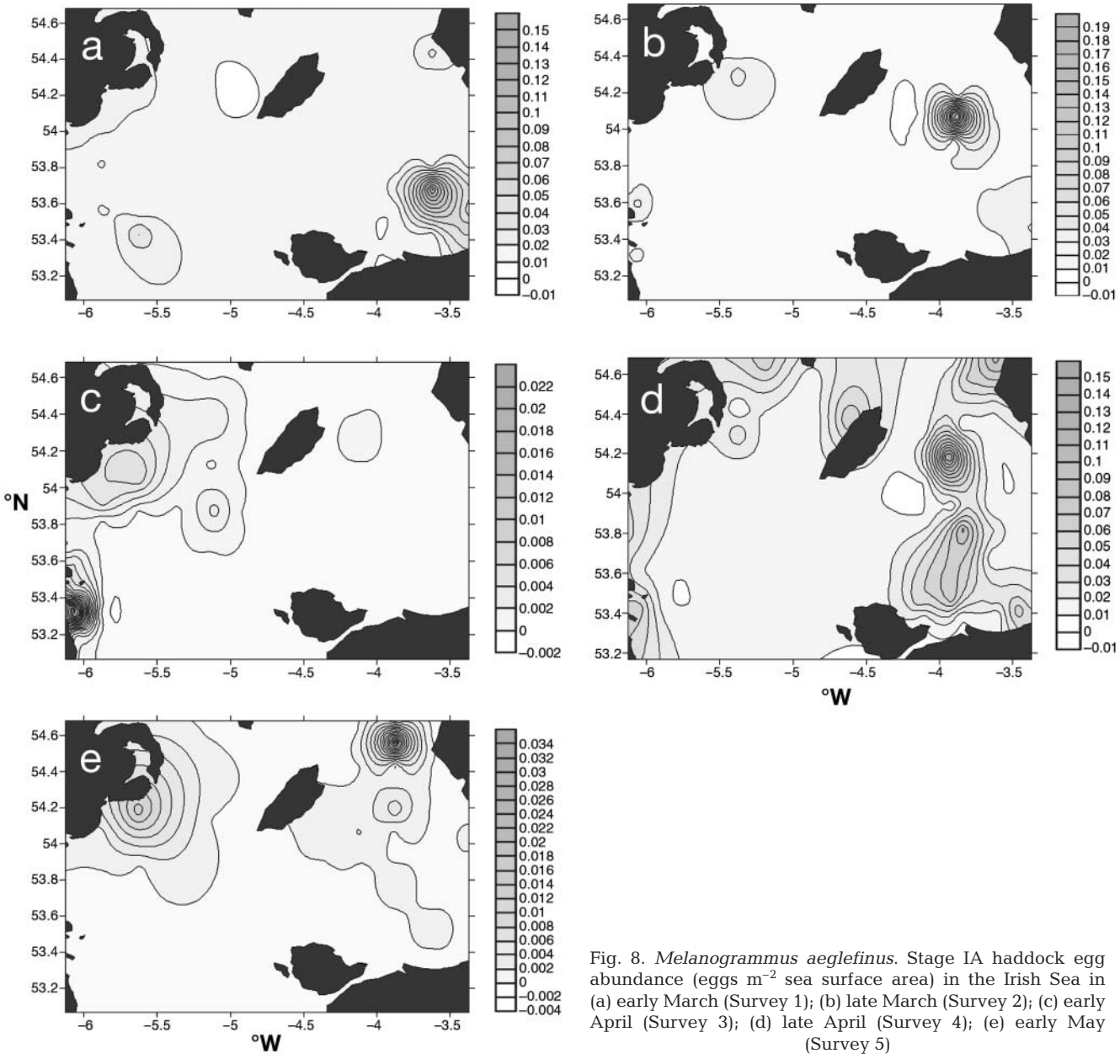

Fig. 8. *Melanogrammus aeglefinus*. Stage IA haddock egg abundance (eggs m^{-2} sea surface area) in the Irish Sea in (a) early March (Survey 1); (b) late March (Survey 2); (c) early April (Survey 3); (d) late April (Survey 4); (e) early May (Survey 5)

southwest tip of the Isle of Man. This spawning ground was recorded in 1991 (Ellis & Nash 1997), and in 1996 and 1997 (Nash & Geffen 1999). Spawning of plaice in this area may have been more extensive during 2000, but these stations were generally omitted during adverse weather conditions. The presence of late-stage plaice eggs in the plankton in late February to early March suggests that plaice spawning began, and may have peaked, prior to the survey period. Stage IA whiting eggs were most abundant between the Isle of Man and St. Bee's Head and off Great Orme Head in Liverpool Bay. Whiting spawned to a lesser extent off Dublin Bay in the western Irish Sea. This location has previously been inferred to be a whiting spawning ground on the basis of larval and juvenile fish distributions (Dickey-Collas et al. 1996). Haddock spawned primarily in the eastern Irish Sea in 2000, with some haddock spawning also taking place in coastal waters of the western Irish Sea. The presence of late-stage haddock eggs, larvae and juveniles has previously been used to infer haddock spawning in the western Irish Sea, with relatively small densities recorded from the eastern Irish Sea (Dickey-Collas et al. 2003). In 2000, haddock spawning was restricted to the eastern Irish Sea in March and the western Irish Sea in April, and occurred in both areas during May. These results suggest that haddock may spawn over a wider spatial area than previously assumed from egg and larvae surveys. Alternatively, haddock spawning in the eastern Irish Sea may be a relatively recent phenomenon, coincident with the expansion of the stock to the west of the British Isles, including the Irish Sea, since the mid-1990s (Dickey-Collas et al. 2003).

The concurrence between spawning of whiting, plaice and cod in the Irish Sea in 2000 and in previous years suggests that these species may exhibit site-fidelity to spawning grounds within the Irish Sea. Spawning at restricted times and locations has been widely documented in North Atlantic cod stocks (Bergstad et al. 1987, Rose 1993, Lawson & Rose 2000). Dunn & Pawson (2002) provide evidence from tagging studies of spawning ground fidelity in plaice, particularly in older individuals, which may learn orientation. A whole suite of factors such as age, size, condition, behaviour and external factors such as temperature can influence timing of spawning (Kjesbu 1994).

Pelagic fish eggs are often spawned at times and in locations with hydrographical conditions that retain

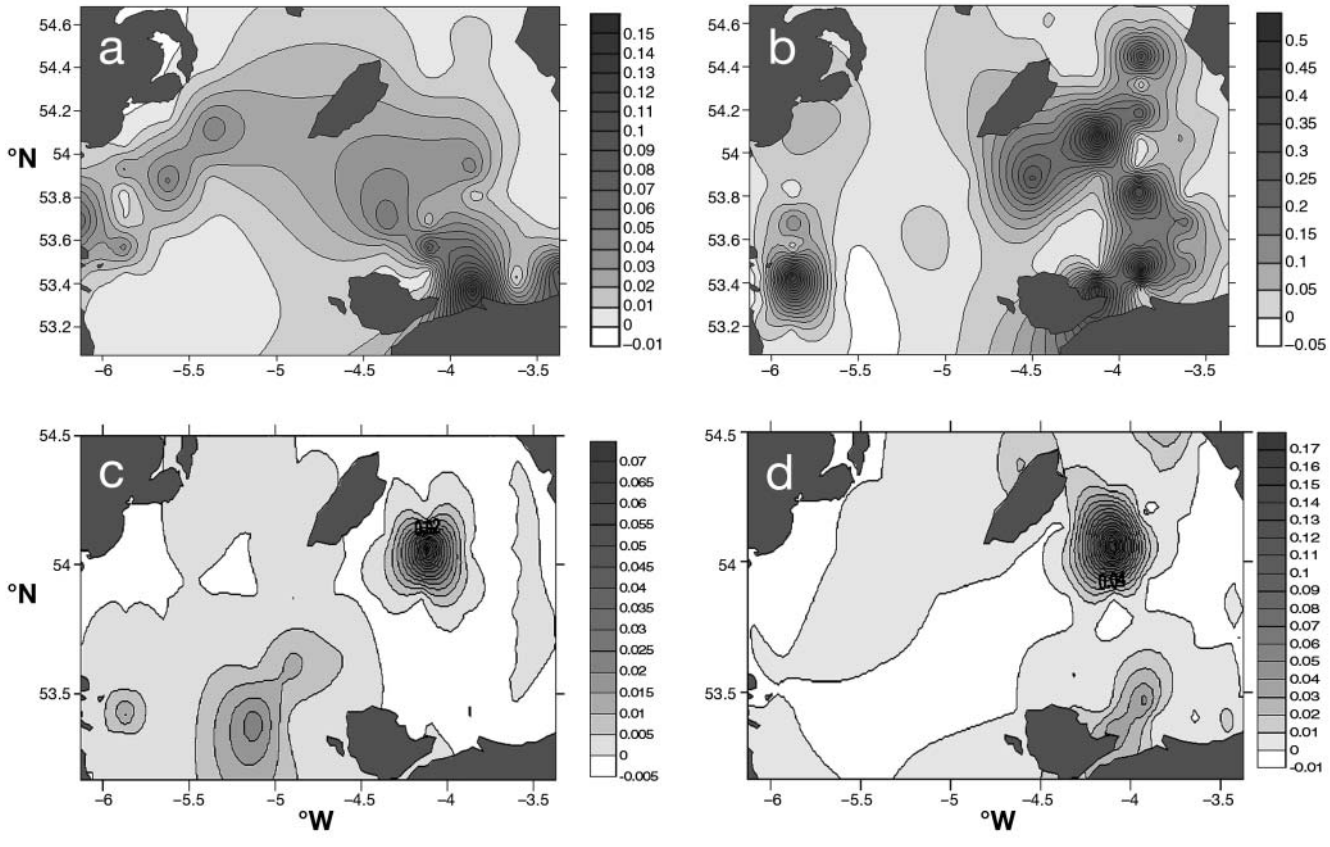

Fig. 9. *Merlangius merlangus*. Stage IA whiting egg abundance (eggs m^{-2} sea surface area) in the Irish Sea in (a) early March (Survey 1); (b) late March (Survey 2); (c) early April (Survey 3); (d) late April (Survey 4)

eggs and larvae in favourable conditions (Norcross & Shaw 1984, Lough et al. 1996, Page et al. 1997, Sanvicente-Anorve et al. 2000). As a result of progressive heating of surface waters, the deep waters of the western Irish Sea become thermally stratified during late spring to early summer (Dickey-Collas et al. 1997). This stratification isolates a dome of cold, deep, bottom water in the region, resulting in a cyclonic gyre that dominates the circulation regime (Gowen et al. 1995, Dickey-Collas et al. 1997, Hill et al. 1997, Mills et al. 1997). In this region, spawning takes place in inshore coastal waters and later stages subsequently move offshore, where they become entrained within the western Irish Sea gyre as pelagic juveniles (Dickey-Collas et al. 1996, 1997). Within the coastal region where spawning occurs, there is an early spring bloom of phytoplankton (Dickey-Collas et al. 1996, Mills et al. 1997), a prolonged production period (Gowen et al. 1995) and a high biomass of zooplankton early in the year (Dickey-Collas et al. 1996). Both the timing and location of spawning indicate that it coincided both temporally and spatially with the commencement of biological production in the western Irish Sea in 2000, with all of the target species spawning to some extent in the inshore waters of the western Irish Sea in spring.

In the present study, unidentified patterns accounted for 6% of the patterns identified. These random patterns may have been target eggs that were not identified correctly due to distortion of the gel, or may have been a result of error such as inclusion of more than 1 egg in the micro-tube, leading to an unrecognisable isozyme pattern thus labelled as 'unknown'. Alternatively the occurrence of 'unknowns' may due to the possibility that pelagic fish eggs not corresponding to the description of the target species were erroneously selected for IEF identification, or species with eggs that met the description of the target species were not included as standards. The latter rationalisation only really applies to eggs at the very lower end of the target size range. Pollack *Pollachius* sp., flounder *Platyichthys flesus*, dab *Limanda limanda*, and *Trisopterus* species have morphological characteristics similar to those of the target species, but have very small eggs, ranging from 0.9 to 1.1 mm in median diameter. Furthermore, they mostly spawn in deep stratified waters outside the spatial distribution of spawning of the target species, and so are unlikely to have been mistaken for target species. As only 5% of the samples collected were less than 1.1 mm in diameter, it was decided not to analyse banding patterns for these species, as they would have accounted for an extremely small fraction of the samples. Prior to the surveys in 2000, the size range of cod, haddock and plaice eggs was believed to be outside the range of these species, but IEF results show that a small proportion of cod and haddock eggs could potentially overlap in size with larger individuals of these species.

The unidentified samples that were 'blank' are believed to be primarily the result of isozyme degradation, and this view was supported by laboratory experiments on the effects of storage of samples at various temperatures. The importance of storage temperature was demonstrated by the loss of isozyme activity from samples transferred from storage at –80°C to storage at – 20°C for a period of 6 wk, compared with clearly identifiable isozyme expression from samples maintained at –80°C for the same period. The susceptibility of LDH isozymes, particularly A-subunits, to temperature changes has been widely documented in the literature (Odense et al. 1969, Pasteur et al. 1988, Janson & Ryden 1998). An unsuitable temperature regime can adversely affect enzyme functionality and isozyme expression. Although every precaution was taken to keep samples at a suitable temperature during the selection process, transportation, storage and subsequent identification, lack of enzyme activity may have been responsible for the 'blank results' obtained for some of the samples analysed in the current study. The higher proportion of unidentified eggs among smaller size categories has implications for abundance estimation. Specifically, haddock eggs are most likely to be underrepresented, as they have the highest frequency of occurrence of the target species in this size category.

The size ranges of the eggs of the target species identified by biochemical analyses were wider than those previously documented by Russell (1976). Cod eggs were in the size range 1.01 to 1.89 mm, compared with 1.16 to 1.89 mm documented for cod eggs around the British Isles (Russell 1976). The size range of haddock eggs was 0.97 to 1.87 mm (1.20 to 1.70 mm; Russell 1976), plaice eggs were in the size range 1.7 to 2.26 mm (1.66 to 2.17 mm; Russell 1976), and eggs identified as whiting were 0.87 to 1.53 mm (0.97 to 1.32 mm; Russell 1976). Mork et al. (1984) noted that biochemical identification of species often results in revised size-frequency distributions, as those determined from visual identification are often based on a small number of measurements, or on measurements of eggs from a limited number of parents, and thus cannot be representative of all populations.

The abundance of Stage IA eggs from ichthyoplankton surveys is used as basis from which to estimate egg production, which is extrapolated to spawning stock biomass (SSB) estimates using the annual egg production (AEP) method for species with determinate annual fecundity in the Irish Sea. Accurate estimate of the SSB is an important tool in the management of exploited species (Armstrong et al. 2001). The urgent need for effective management of cod stocks has been highlighted by the collapse of the cod fishery in the NW

Atlantic and by the implementation of recovery plans in the Irish Sea since 2000. The identification of species-specific spawning grounds for gadoids described herein has important implications for production and SSB estimates given that species-specific abundance estimates are clearly lower than generic abundance estimates for gadoid eggs. Furthermore, the accurate identification of spawning distributions is important in terms of highlighting appropriate locations and periods for implementing recovery measures such as restricted or closed areas.

Future work in this area should focus on comparing protein-based and DNA-based methods of pelagic egg identification in terms of cost-effectiveness, reliability and, importantly, in terms of the feasibility of applying such techniques to surveys with extensive spatial and temporal coverage.

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LITERATURE CITED

Anonymous (1997) Estimation of fish biomass in the Irish Sea by means of the annual egg production method. EU Report. Queen's University, Belfast

Armstrong MJ, Connolly P, Nash RDM, Pawson MG and 7 others (2001) An application of the annual egg production method to estimate the spawning biomass of cod (*Gadus morhua* L.), plaice (*Pleuronectes platessa* L.) and sole (*Solea solea* L.) in the Irish Sea. ICES J Mar Sci 58:183–203

Barange M, Hampton I (1997) Spatial structure of co-occuring anchovy and sardine populations from acoustic data: implications for survey design. Fish Oceanogr 6:94–108

Bergstad OA, Jorgensen T, Dragesund O (1987) Life history and ecology of the gadoid resources of the Barents Sea. Fish Res 5:119–161

Bradbury IR, Snelgrove PVR, Fraser S (2000) Transport and development of eggs and larvae of Atlantic cod, *Gadus morhua*, in relation to spawning time and location in coastal Newfoundland. Can J Fish Aquat Sci 57: 1761–1772

Dickey-Collas M, Gowen RJ, Fox CJ (1996) Distribution of larval and juvenile fish in the western Irish Sea: relationship to phytoplankton, zooplankton biomass and recurrent physical features. Mar Freshw Res 47:169–181

Dickey-Collas M, Brown J, Fernand L, Hill AE, Horsburgh KJ, Garvine RW (1997) Does the western Irish Sea gyre influence the distribution of pelagic juvenile fish? J Fish Biol 51:206–229

Dickey-Collas M, Armstrong MJ, Officer RA (2003) Growth and expansion of haddock (*Melanogrammus aeglefinus* L.) stocks to the west of the British Isles in the 1990s. ICES J Mar Sci 219:271–282

Dunn MR, Pawson MG (2002) The stock structure and migrations of plaice populations on the west coast of England and Wales. J Fish Biol 61:360–393

Ellis JR, Rogers SI, Freeman SM (2000) Demersal assemblages in the Irish Sea, St George's Channel and Bristol Channel. Estuar Coast Shelf Sci 51:299–315

Ellis T, Nash RDM (1997) Spawning of plaice *Pleuronectes platessa* L. around the Isle of Man, Irish Sea. ICES J Mar Sci 54:84–92

Forthun J, Mork J (1997a) Genetic variability at isozyme loci in two gadoid species; whiting (*Merlangius merlangus* L.) and saithe (*Pollachius virens* L.) from the Norwegian coast. Int Counc Explor Sea Comm Meet AA:06

Forthun J, Mork J (1997b) Description of polymorphisms at tissue enzyme loci in East Atlantic haddock (*Melanogrammus aeglefinus* L.): tissue manifestation, electrophoretic conditions and estimates of basic population genetic parameters. Int Counc Explor Sea Comm Meet AA:07

Fox CJ, Dickey-Collas M, Winpenny AJ (1997) Spring plankton surveys of the Irish Sea in 1995: the distribution of fish eggs and larvae. Minist Agric Fish Food Lowestoft Sci Ser Tech Rep No. 104

Fox CJ, O'Brien CM, Dickey-Collas M, Nash RDM (2000) Patterns in the spawning of cod (*Gadus morhua* L.), sole (*Solea solea* L.) and plaice (*Pleuronectes platessa* L.) in the Irish Sea as determined by generalised additive modelling. Fish Oceanogr 9:33–49

Geldmacher A, Wieland K (1999) Implications of mechanical deformation and formaldehyde preservation for the identification of stage-specific characteristics of Baltic cod eggs. J Appl Ichthyol 15:75–79

Giaever M, Forthun J (1999) A population genetic study of haddock (*Melanogrammus aeglefinus*) in Northeast Atlantic waters based on isozyme data. Sarsia 84:89–98

Giaever M, Forthun J, Mork J (1995) Genetic variability at isozyme loci in haddock (*Melangrammus aeglefinus*) from Norwegian fjord and coastal waters. Int Counc Explor Sea Comm Meet G:16

Gowen RJ, Stewart BM, Mills DK, Elliott, P (1995) Regional differences in stratification and its effect on phytoplankton production and biomass in the northwestern Irish Sea. J Plankton Res 17:753–769

Harding D (1974) The biology of fish larvae with particular reference to the plaice (*Pleuronectes platessa* Linn.). In: Harden Jones FR (ed) Sea fisheries research. Elek Science, London

Hill AE, Brown J, Fernand L (1997) The summer gyre in the western Irish Sea: shelf sea paradigms and management implications. Estuar Coast Shelf Sci 44(Suppl A):83–95

Janson JC, Ryden L (1998) Protein purification: principles, high resolution methods and applications. J Wiley & Sons, New York

Kjesbu OS (1994) Time of start of spawning in Atlantic cod (*Gadus morhua*) females in relation to vittellogenic oocyte diameter, temperature, fish length and condition. J Fish Biol 45:719–735

Lawson G, Rose G (2000) Seasonal distribution and movements of coastal cod (*Gadus morhua* L.) in Placentia Bay, Newfoundland. Fish Res 49:61–75

Lockwood SJ, Nichols JH (1977) The development rates of mackerel (*Scomber scombrus* L) eggs over a range of temperatures. Int Counc Explor Sea Comm Meet 1977/J:13

Lough RG, Caldarone EM, Rotunne TK, Broughton EA, Burns BR, Buckley LJ (1996) Vertical distribution of cod and haddock eggs, feeding and condition in stratified and mixed waters on southern Georges Bank, May 1992. Deep-Sea Res II 43:1875–1904

Mattiangeli V, Bourke EA, Ryan AW, Mork J, Cross,TF (2000)

Allozyme analyses of the genus *Trisopterus*: taxonomic status and population structure of the poor cod. J Fish Biol 56:474–494

Milligan SP, Riches BF (1983) The new MAFF/Guildline high speed plankton samplers. Int Counc Explor Sea Comm Meet 1983/L:7

Mills DK, Gowen RJ, Woods EA (1997) Observation and simulation of the spring bloom in the north-western Irish Sea. J Plankton Res 19:63–77

Mork J, Giaever M (1999) Genetic structure of cod along the coast of Norway: results from isozyme studies. Sarsia 84: 157–168

Mork J, Solemdal P, Sundnes G (1983) Identification of marine fish eggs: a biochemical genetics approach. Can J Fish Aquat Sci 40:361–369

Mork J, Solemdal P, Sundes G (1984) Biochemical genetic identification and population genetic studies of marine fish eggs. In: Dahl E, Danielssen DS, Moksness E, Solemdal P (eds) The propagation of cod *Gadus morhua* L. Flodevigen Rapp Ser 1:712–719

Mork J, Ryman N, Stahl G, Utter F, Sundnes G (1985) Genetic variation in Atlantic Cod (*Gadus morhua*) throughout its range. Can J Fish Aquat Sci 42:1580–1587

Moss DW (1982) Isoenzymes. Chapman & Hall, London

Mustafa S (1999) Genetics in sustainable fisheries management. Fishing New Books, London

Nash RDM, Geffen AJ (1999) Variability in Stage I egg production and settlement of plaice *Pleuronectes platessa* on the west side of the Isle of Man, Irish Sea. Mar Ecol Prog Ser 189:241–250

Nash RDM, Dickey-Collas M, Milligan SP (1998) Descriptions of the Gulf VII/PRO-NET and MAFF/Guildline unencased high-speed plankton samplers. J Plankton Res 20:1915–1926

Nichols JH, Haynes GM, Fox CJ, Milligan SP, Brander KM, Chapman RJ (1993) Spring plankton surveys of the Irish Sea in 1982, 1985, 1987, 1988 and 1989: hydrography and the distribution of fish eggs and larvae. Fish Res Tech Rep Minist Agric Fish Food Lowestoft No. 111

Norcross BL, Shaw RF (1984) Oceanic and estuarine transport of fish eggs and larvae: a review. Trans Am Fish Soc 113: 153–165

Odense PH, Leung, TC, Allen, TM, Parker E (1969) Multiple forms of lactate dehydrogenase in the cod, *Gadhus morhua* L. Biochem Genet 3:317–334

Page FH, Sinclair M, Naimie CE, Loder JW, Losier RJ, Berrien PL, Lough RG (1997) Cod and haddock spawning on Georges Bank in relation to water residence times. Fish Oceanogr 8:212–226.

Park LK, Moran P (1994) Developments in molecular genetics in fisheries. Rev Fish Biol Fish Spec Issue 4: 272–299

Pasteur N, Catalan J, Pasteur G, Bonhomme F, Britton-Davidian J (1988) Practical isozyme genetics. Prentice-Hall, Englewood Cliffs, NJ

Rose GA (1993) Cod spawning on a migration highway in the north-west Atlantic. Nature 366:458–461

Russell FS (1976) The eggs and planktonic stages of British marine fishes. Academic Press, London

Sanvicente-Anorve L, Flores-Coto C, Chiappa-Carrara X (2000) Temporal and spatial scales of ichthyoplankton distribution in the southern Gulf of Mexico. Estuar Coast Shelf Sci 51:463–475

Simpson AC (1959) The spawning of plaice (*Pleuronectes platessa* L.) in the British Isles. Fish Invest II Mar Fish GB Minist Agric Fish Food 22:30

Sundby S (1996) Turbulence and ichtyoplankton: influence on vertical distributions and encounter rates. Sci Mar 61: 159–176

Taylor MI, Fox CJ, Rico I, Rico C (2002) Species-specific TaqMan probes for simultaneous identification of cod (*Gadus morhua* L.), haddock (*Melanogrammus aeglefinus* L.) and whiting (*Merlangius merlangus* L.). Mol Ecol Notes 2: 599–601

Thompson BM, Riley JD (1981) Egg and larval development studies in the North Sea cod (*Gadus morhua* L.). J Cons Int Explor Mer 178:553–559

Tucker JWJ, Chester J (1984) Effects of salinity, formalin concentration and buffer on quality of preservation of southern flounder (*Paralichthys lethostigma*) larvae. Copeia 1984:981–988

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