



# Identification of marine fish egg predators using molecular probes

Clive J. Fox<sup>1,\*</sup>, Martin I. Taylor<sup>2</sup>, Jeroen van der Kooij<sup>3</sup>, Natasha Taylor<sup>3</sup>,  
Stephen P. Milligan<sup>3</sup>, Aitor Albaina<sup>4</sup>, Sonia Pascoal<sup>2</sup>, Delphine Lallias<sup>2</sup>,  
Marjorie Maillard<sup>2</sup>, Ewan Hunter<sup>3</sup>

<sup>1</sup>Scottish Marine Institute, Scottish Association for Marine Science, Oban PA37 1QA, UK

<sup>2</sup>Molecular Ecology and Fisheries Genetics Laboratory, ECW Building, School of Biological Sciences, Bangor University, Bangor LL57 2UW, UK

<sup>3</sup>Centre for Environment, Fisheries and Aquaculture Science, Pakefield Road, Lowestoft, Suffolk NR33 OHT, UK

<sup>4</sup>Laboratory of Genetics, Department of Genetics, Physical Anthropology & Animal Physiology, Basque Country University, Leioa 48940, Spain

**ABSTRACT:** Mortality during the egg and larval stages is thought to play a major role in determining year-class strength of many marine fish. Predation of eggs and larvae is normally considered to be a major factor but the full suite of predators responsible has rarely been identified. Potential predators on a patch of plaice *Pleuronectes platessa* eggs located in the eastern Irish Sea were mapped using acoustics and sampled by trawl and a plankton multi-net. Gut contents of 3373 fish, crustacea and cephalopods sampled in the area were screened using a plaice-specific TaqMan DNA probe. Herring *Clupea harengus* and sprat *Sprattus sprattus* dominated trawl catches and showed high positive TaqMan responses (77 and 75% of individuals tested respectively). Locations of clupeid schools also broadly corresponded with the distribution of fish eggs in the plankton. Whiting *Merlangius merlangus* were also reasonably abundant in trawl hauls and 86% of their stomachs tested positive for plaice DNA. Species showing lower levels of positive TaqMan response included mackerel *Scomber scombrus*, poor cod *Trisopterus minutus*, squid *Loligo* spp., dogfish *Scyliorhinus canicula* and weever *Trachinus vipera*. Non-reactivity of all negative controls precluded the occurrence of cross-contamination, and positive reactions from demersal species, such as dogfish and weever, may have resulted from secondary predation. No benthic macro-crustaceans tested positive. Samples of planktonic organisms yielded 13% positive TaqMan reactions, mainly from clupeoid or sandeel (Ammodytidae) larvae, but also included some Malacostraca and Amphipoda. Use of the molecular approach allowed rapid screening of a large number of potential predators of plaice eggs, and the results provide a more holistic description of the predator community than has previously been achieved.

**KEY WORDS:** Herring · Sprat · Predation · Fish eggs · Plaice · Recruitment

Resale or republication not permitted without written consent of the publisher

## INTRODUCTION

Despite over 100 years of research, our understanding of the processes involved in controlling year-class strength in marine fish is still limited (Houde 2002, 2008, Platt et al. 2007, Zhang et al. 2010, Fässler et al. 2011). Since Hjort's critical period

hypothesis Hjort (1914), which invoked the onset of larval feeding as the crucial stage, there has been considerable debate about when year-class strength is determined. The current consensus is that it varies with species and stock (Myers & Cadigan 1993, Houde 2008) but there are examples where year-class strength appears to be set at a very early stage.

\*Email: clive.fox@sams.ac.uk

In the southern North Sea, year-class strength of plaice *Pleuronectes platessa* is predictable from the abundance of Stage V eggs (Bannister et al. 1974, Harding et al. 1978, Brander & Houghton 1982), and for herring *Clupea harengus* in the northern North Sea, mortality during the first 30 d of larval life appears critical (Fässler et al. 2011). Further research into the causes of egg and larval mortality should therefore contribute to a better understanding of the conditions which generate periods of good and poor recruitment, at least for some species and stocks (Fässler et al. 2011, Huwer et al. 2011).

Although embryonic malformations (Cameron & von Westernhagen 1997), disease and parasites (Meneses et al. 2003), and exposure to ultra-violet radiation (Browman 2003) and pollutants (Klumpp & von Westernhagen 1995, Cameron & von Westernhagen 1997) can all kill fish eggs, predation is generally thought to be the main cause of egg mortality (Hunter 1981, Sissenwine 1984, McGurk 1986, Bunn et al. 2000). Despite its supposed importance, there are relatively few field studies on predation of fish eggs (Heath 1992, Houde 2008). Because prey are often digested rapidly, identifying items from predator stomach contents can be difficult (Heath 1992). Rapid digestion is a particular problem with regard to larval fish as prey (Folkvord 1993), but the chorions of eggs are generally more resistant to digestion. Egg shells in stomachs may, however, be difficult to assign to species, unless they possess visually distinct external characteristics (Russell 1976, Hunter & Kimbrell 1980, Brewer et al. 1984, Ellis & Nash 1997). Many of the studies that have been conducted have concentrated on the role of fish as predators, but fish eggs (and larvae) are exposed to a wide spectrum of other potential predators including chaetognaths (Feigenbaum & Maris 1984), gelatinous zooplankton (Purcell 1985, Lynam et al. 2005), euphausiids (Theilacker et al. 1993, Krautz et al. 2007) and some of the larger copepods. Obtaining direct visual evidence of consumption from some of these groups, particularly the crustacea, is difficult as they macerate their prey (Theilacker et al. 1993, Taylor & Danila 2005). Finally, visual gut content analysis is a relatively labour-intensive and time-consuming procedure.

Recently, molecular methods have provided additional tools for predation research. Several studies have successfully applied immunological assays to detect predation in the marine environment (Theilacker et al. 1986, 1993, Krautz et al. 2003, Taylor 2004). This approach has certain advantages as the method can sometimes discriminate life-history

stages, e.g. by detecting the presence of yolk proteins (Theilacker et al. 1986, Taylor & Danila 2005). There are also claims that detection times in the presence of digestion may exceed DNA-based methods (Symondson 2002). On the other hand, immunoassays are expensive and difficult to develop and may not be replicable between laboratories. DNA-based methods are therefore becoming increasingly used. In this approach, either a taxon-specific DNA-probe is developed (e.g. Taylor et al. 2002) or cloning or pyro-sequencing are used to evaluate the presence of a broader range of taxa using DNA sequences (e.g. Cytochrome Oxidase I [COI] barcodes). Both probing and sequencing techniques include DNA amplification and can be applied to very small quantities of target DNA. In order to improve the chances of successful detection it is usual to target relatively short nucleotide sequences, since prey DNA becomes fragmented during digestion in the predator gut. Examples of successful applications include Rosel & Kocher (2002), who described a polymerase-chain reaction (PCR) assay to detect cod larvae in mackerel *Scomber scomber* stomachs, and Nejstgaard et al. (2003) and Vestheim et al. (2005) who used similar assays for identifying prey in copepod faecal pellets. Applications using cloning or pyro-sequencing to identify multiple prey taxa include studies on the diets of seals (Deagle et al. 2009) and lobster larvae (Suzuki et al. 2008). Before any molecular method for prey detection is applied to field samples, the method's specificity against a range of potential prey must be tested. In addition, experiments are needed on the extent to which digestion of the target DNA in predator guts reduces detection rates. Given the extreme sensitivity of these methods, stringent efforts are also needed to control cross-contamination during field sampling (Symondson 2002, Passmore et al. 2006).

In previous papers we described the development of a TaqMan probe for plaice DNA, demonstrated its specificity and described the effects of digestion by crustacean and teleost predators on detection efficiency (Albaina et al. 2010, 2012, Hunter et al. 2012). Here we describe the first application of this method to study predation of plaice eggs (and larvae) in the field. Because the TaqMan assay cannot distinguish between the egg and larval stages of plaice, we refer to detection of predation on eggs/larvae throughout. In addition, we assume that consumption of adult plaice by the suite of potential predators that we assayed would not occur, or would be obvious during dissection of predator stomachs. Because plaice spawning occurs

offshore, but nursery grounds are inshore (Fox et al. 2007), we also assume that small, juvenile plaice were not present in the study area. Although remains of plaice eggs can be visually identified in teleost stomachs (Ellis & Nash 1997), we wanted to test the molecular approach as an alternative method for rapid screening of large numbers of potential predators and to identify any non-teleost predators consuming plaice eggs/larvae in the study area.

## MATERIALS AND METHODS

### Field sampling

Cruises were conducted in 2008 and 2009 on a well-described plaice spawning ground (Fox et al. 2000a, Bunn et al. 2004, Bunn & Fox 2004) in the eastern Irish Sea (Fig. 1). In 2008, the cruise (25 February to 2 March) was affected by poor weather and focused on developing sampling protocols and conducting an initial evaluation of the range of predators taking plaice eggs/larvae. The cruise in 2009 (19 to 28 February) included plankton sampling, to locate the egg patch, trawling and dedicated acoustic grids. In addition, a plankton multi-net (Bedford Institute of Oceanography Net and Environmental Sampling System, BIONESS, Ocean Seas Instrumentation) was deployed. Near-surface temperature and salinity were logged throughout every 5 min using an Aquatracka system (Chelsea Instruments).

### Vertically integrated plankton sampling

On both cruises, a patch of plaice eggs was identified by sampling a grid of approximately 12 plankton stations. The grid was completed at both the start and end of the 2009 cruise but only at the start of the 2008 cruise. Plankton samples were collected using a 76 cm Lowestoft Gulf VII high-speed sampler fitted with a 40 cm diameter nose-cone, 275  $\mu\text{m}$  mesh net and internal and external Valeport flowmeters (Nash et al. 1998). Flow data, salinity and temperature profiles were captured with a Valeport MIDAS-CTD mounted on the body of the sampler. The Gulf VII was deployed in an oblique tow from the sea surface to near the seabed and back at 3 to 4 knots. Upon recovery, the net was washed down and the contents of the cod-end fixed in 4% formalin (4% formaldehyde in distilled water buffered with 2.5% sodium acetate trihydrate, w/v). Samples were sorted on-board for plaice eggs to gain a rapid estimation of the patch location, then subsequently re-sorted in the laboratory for all fish eggs and larvae (Russell 1976). Egg and larval numbers were expressed as numbers per  $\text{m}^2$  of sea surface using the sampled depth and the volume filtered, which was estimated from flume-based calibrations Brander et al. 1993).

### Acoustics

In 2009 the distributions of pelagic fish were mapped using a Simrad EK60 split beam echosounder operating at 38, 120 and 200 kHz. The transducers were mounted on a drop keel; pulse-duration (0.512 ms), ping rate (1 s) and other operational settings followed Korneliussen et al. (2008). At the start of the survey all 3 frequencies were calibrated with the keel lowered at the operating depth (2 m below the hull), using the standard sphere method (Foote et al. 1987). A high resolution acoustic survey was undertaken consisting of a number of equidistant, parallel transects perpendicular to the coast, which covered all but the very eastern- and western-most parts of the plankton sampling area. During acoustic surveying the vessel speed was  $\sim 8$  knots when steaming and 4 knots whilst collecting plankton and trawling.

Acoustic data were analysed using Echoview (version 4.6; Myriax). A filter was created (sum of 3 frequencies above  $-183$  dB) to remove all weaker scatterings using the virtual echogram module, leaving only the acoustic marks associated with fish possessing swim-bladders. A dilation filter was then applied to account for reduced beam overlap between the

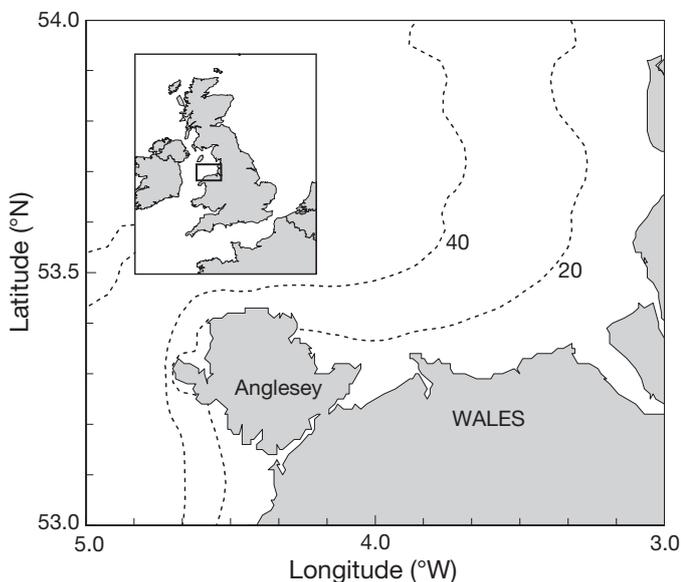


Fig. 1. Study region showing depth contours (m)

transducers at shallow depths and a final erosion filter applied to preserve the original school shape and size. The nautical area backscattering coefficient (NASC,  $s_A$ ,  $m^2$  nautical mile<sup>-2</sup>) was exported and the catch composition from the trawl hauls used to partition the  $s_A$  into species-specific contributions. Length–weight relationships of sprat *Sprattus sprattus* and herring were also obtained from the trawl data and used with length-dependent target strength (TS) measurements from the literature ( $b_{20} = -69.1$  dB for sprat, Robinson 1983 and  $-72.1$  dB for herring, Foote 1987) to convert the  $s_A$  into estimates of abundance and biomass.

### Trawling

For fish sampling in 2008 we used a Portuguese high-headline trawl (PHHT; Cosalt) and in 2009 this was supplemented with a sandeel trawl and a 4 m beam trawl in order to sample a wider range of species. Tow durations with the PHHT varied between 15 and 30 min but were up to 50 min with the sandeel trawl. The PHHT had a wing-end spread of 22.5 m, 140 mm mesh in the wings and throat and 135 mm mesh in the cod-end, with a 20 mm mesh liner. The wing-end spread of the sandeel trawl was 20 m and the mesh varied from 800 mm at the mouth to 120 mm at the cod-end, which had a 10 mm mesh liner. Tows were conducted at approximately 4-hourly intervals over periods of 2 to 3 d. Catches were sorted into species and the total weight of each species and individual total lengths of fish recorded (to the nearest 0.5 or 1 cm interval below following ICES International Bottom Trawl recommendations, IBTS 2010). When large quantities of a species were caught (e.g. sprat), they were randomly sub-sampled by weight, ensuring that at least 30 individual fish length measurements were recorded. Length frequencies were then raised to the total catch, based on the weight sub-sampled. Catch weights were expressed standardised to a 30 min tow. Additional individual weight and length data were collected for sprat and herring in 2009 to allow conversion of acoustic signals to biomass. For beam trawl samples, benthic organisms that might predate on fish eggs and larvae, mainly various species of crab, were removed for stomach content analysis but the entire catch was not identified or weighed. We did not take stomach samples from benthic organisms such as sea urchins and brittlestars as we considered it highly unlikely that they would prey on pelagic fish eggs or larvae.

### Predator stomach sampling from trawl catches

Because of the sensitivity of the TaqMan probe, a key consideration was the avoidance of cross-contamination. Any plaice caught were immediately removed from the catch and transferred to a dedicated measuring station that was not used to process any other species. On each haul a number of negative controls were mixed in with the catch in the hopper. Negative controls consisted of a piece of vinyl disposable glove (representing the stomach) inserted into another glove (representing the fish). Negative controls were processed as for fish samples. After the whole catch had been weighed, sorted and measured, between 20 and 30 individuals of each species (or up to the maximum caught for less abundant species) were selected across the size range. In 2008 both the stomach and hind-guts were dissected out from fish and placed in individual plastic sample bags and immediately frozen to  $-20^\circ\text{C}$ . Based on the TaqMan results from 2008, where all hind-gut samples gave negative results, only the stomachs were preserved in 2009. For organisms such as crabs and squid, the whole animal was frozen. Sampling was discontinued 60 min after the completion of catch sorting to minimise effects of post-mortem degradation. Total time taken for sorting catches ranged from 30 min to 2 h depending on the size of the catch. Each dissection was performed with clean gloves and instruments. Batches of instruments were decontaminated using Microsol detergent (Anachem) between trawl hauls. In addition to the negative controls inserted into the fish hopper, swabs were taken of the non-plaice processing stations and instruments after cleaning, and were then analysed using the TaqMan probe.

### Depth-stratified plankton sampling

In 2009 a single deployment of a multi-net (BIONESS) system was made. The BIONESS comprises a frame with a 1 m square opening containing up to ten 300  $\mu\text{m}$  mesh opening/closing nets. A SeaBird SBE19 CTD is fitted to record environmental parameters. Filtered volumes were estimated using the manufacturer's software reading data from an external General Oceanics flowmeter and frame tilt-sensor. Because of the relatively shallow water depth (33 m) only 4 depth strata were sampled. The BIONESS was deployed around 15:00 on 27 February and sampled strata were: 26.4 to 20.6 m, 20.6 to 13.7 m, 13.7 to 9.2 m and 9.2 m to the surface. On recovery, nets

were washed down and samples pre-sorted on-board. Representative examples of the taxa present were frozen individually in well plates for subsequent TaqMan analysis. The remainder of the samples were fixed in 4% buffered formalin. Samples were subsequently sorted and organisms identified to broad taxonomic categories. Abundance data were converted to numbers  $m^{-3}$  using the estimated volume filtered in each depth-band.

### TaqMan analysis of stomach samples and BIONESS samples

A real-time PCR assay was used to probe potential predators for the presence of plaice DNA. This TaqMan probe, which targets a section of the mitochondrial cytochrome- $\beta$  gene, is highly species-specific and does not cross-react against DNA from a wide range of marine organisms (Albaina et al. 2010). Because the BIONESS samples had been rapidly sorted and frozen at sea (due to concerns about post-mortem degradation of DNA in predator guts), it was necessary to check their identifications before processing them for molecular analysis. Frozen BIONESS samples were therefore defrosted in ethanol and identified to the lowest practical taxonomic level. Because there was significant distortion and damage to the bodies of fish larvae we were unable to identify sprat, herring and sandeel larvae to the species level so they were combined as a Clupeidae/Ammodytidae group. Two different extraction methods were used; a CTAB (hexadecyltrimethylammonium bromide) protocol was used for stomach contents of larger predators and a modified salt extraction protocol was used for stomach contents of smaller predators. Stomach contents from fish (excluding sprat) and crabs were partially homogenized in 1.5 ml autoclaved Eppendorf tubes containing 350  $\mu$ l of 2% CTAB buffer (100mM Tris-HCl pH 8.0; 1.4M NaCl; 20mM EDTA pH 8.0; 2% CTAB; 0.2% 2-mercaptoethanol). Sub-samples of the stomach contents of large predators such as lesser spotted dogfish *Scyliorhinus canicula* were taken, including scraping of the stomach lining. Five microlitres of Proteinase K (Qiagen) was then added and each sample digested overnight at 55°C. DNA was purified using 2 chloroform-isoamyl alcohol washes followed by sodium acetate precipitation (3M, pH 4.8). Due to their relatively small sizes, whole stomachs from sprat, shrimps, prawns, amphipods, and euphausiids and whole organisms from the BIONESS were homogenized in 300  $\mu$ l of extraction buffer (30mM Tris-HCl

pH 8.0, 10mM EDTA pH 8.0 and 1% SDS). Five microlitres of Proteinase K (Qiagen) was then added and each sample digested overnight at 55°C. DNA was purified following a modified salt protocol (Aljanabi & Martinez 1997) using 1.2 ml wells in 96-well blocks (Starstedt MegaBlock). The DNA was resuspended in 100  $\mu$ l ultrapure H<sub>2</sub>O and stored at -20°C. DNA concentrations were quantified using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies).

TaqMan assays were run on a 7900HT Fast Real-Time PCR System (Applied Biosystems) following Albaina et al. (2010). Reactions were run in Optical 96-well reaction plates using Optical Adhesive Covers (Applied Biosystems). Each 20  $\mu$ l reaction contained 0.75  $\mu$ l of plaice probe (10  $\mu$ M; VIC reporter), 1.5  $\mu$ l of the PLA-F and PLA-R primers (10  $\mu$ M; Forward and Reverse primers), 10  $\mu$ l of TaqMan Universal PCR Master Mix (NO UNG + ROX passive reference; Applied Biosystems), 1.25  $\mu$ l tissue culture H<sub>2</sub>O (Sigma), 2.5  $\mu$ l Bovine Serum Albumin (BSA, #B9001S New England Biolabs) and 2.5  $\mu$ l of extracted DNA. Plates were run under real-time conditions on a single dye layer with 2 no template controls (NTCs) and 2 positive controls (DNA extracted from adult plaice muscle tissue) per 96-well plate. The assay was run using the standard protocol including an initial step of 2 min at 50°C and denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 60 s. Because PCR inhibitors are often co-extracted from crustacea stomachs, samples returning negative TaqMan results were rerun following dilution and addition of BSA the following guidelines in Albaina et al. (2010).

TaqMan results were analysed using the Sequence Detection Software version 2.3 (Applied Biosystems). A positive  $\Delta R_n$  threshold of 0.02 was set for the assay and threshold cycle (Ct) values were computed (Albaina et al. 2010); all amplification curves were also checked visually to remove occasional false positive/negative signals.

## RESULTS

### Plankton

#### Oceanography and vertically integrated sampling

In 2008, surface waters were generally cooler inshore (Fig. 2). Vertical profiling showed that the water column was well mixed, with depth integrated

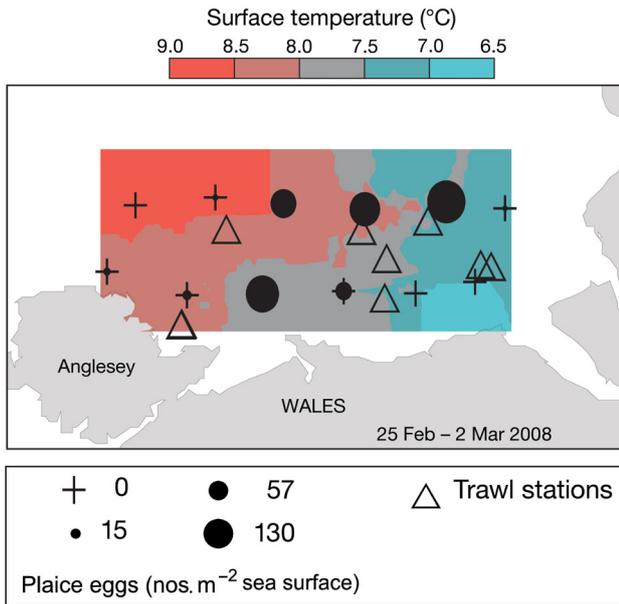


Fig. 2. Sea surface temperature (°C) contoured from Aqua-tracka data, abundance of plai ce eggs (all stages combined, nos. m<sup>-2</sup> sea surface) and location of trawl stations in 2008. Plai ce egg abundance is proportional to the area of the filled circles, with range and interim values shown in the legend

temperatures ranging from 6.74 to 8.39°C. Surface to bottom salinity differences were less than 0.6, except at 1 station, whilst surface salinities ranged from 32.042 to 34.061. Plai ce eggs were concentrated in the north-eastern corner of the sampling grid (Fig. 2) and the spatial distribution of later stage eggs was similar to that of the early stages (data not shown). Plai ce larvae were recorded at only 2 stations, the maximum abundance being 1.6 larvae m<sup>-2</sup>. In 2008, 9 trawl tows were completed using the PHHT. Most trawl stations were located close to the area of high egg abundance but exploratory tows were also conducted to the west of the patch (Fig. 2).

In 2009, sea surface temperatures were up to 1.7°C colder compared with 2008 but the spatial pattern was similar (Fig. 3a,b). The water column was thermally mixed at all stations but surface to bottom salinity differences of up to -1 were noted inshore. Plai ce eggs were more evenly distributed across the study area in 2009 (Fig. 3a) compared with 2008, but by the end of the cruise, they had become slightly more concentrated towards the north-western corner (Fig. 3b). In 2009, plai ce larvae were found at about half of the plankton stations, and the spatial distribution broadly resembled that of the eggs (Fig. 3a,b). At the start of the cruise a maximum abundance of plai ce larvae of 2.3 m<sup>-2</sup> was recorded, although this

increased slightly to a maximum of 7.9 larvae m<sup>-2</sup> in the north-western area by the end of the cruise.

Fish eggs other than plai ce were abundant in both years. In 2008, the maximum number of non-plai ce eggs was 7178 m<sup>-2</sup> and in 2009 it was 1621 m<sup>-2</sup>. The spatial distribution of plai ce and non-plai ce eggs tended to be similar (data not shown). The majority of the non-plai ce eggs were less than 1.1 mm in diameter and, although they could not be identified to species, were probably dab *Limanda limanda* as their larvae are common off the North Wales coast towards the end of March (Bunn & Fox 2004, Bunn et al. 2004). Other identifiable eggs in the Gulf VII samples included late stage cod *Gadus morhua*, dragonets (Callionymidae), rocklings, sole *Solea solea* and sprat, but all of these were present at relatively low abundance. The relative proportion of plai ce versus non-plai ce eggs was also apparent in the BIONESS samples (Fig. 4).

#### Depth-stratified BIONESS sampling

Fish eggs and larvae were the most abundant component of the BIONESS samples and were concentrated towards the surface (Fig. 4). The vast majority of the fish eggs were <1.1 mm in diameter and lacked oil globules or other distinguishing characteristics, but *Callionymus* eggs and a single sandeel egg were caught in the deepest depth strata. In contrast, plai ce and sprat eggs were concentrated in the upper strata. Identifiable larvae included sandeel (Ammodytidae), bull-rout *Myxocephalus scorpius*, dab, flounder *Platichthys flesus*, cod, whiting *Merlangius merlangus*, sea-snail *Liparis montagui* and plai ce. Other larger planktonic organisms caught included various Malacostraca, amphipods and chaetognaths, but these were present at very low densities (<0.3 m<sup>-3</sup>).

#### Fishing

Trawl catches from both years are summarised in Table 1. Herring (length range 8.5–27 cm), sprat (5.5–14.5 cm), whiting (9–34 cm) and dab (9–28 cm) were recorded in nearly all of the trawl hauls and dominated the catches in terms of weight. Other frequently caught taxa included squid (*Loligo* spp.), grey gurnard *Eutrigla gurnadus* (8–30 cm), plai ce (13–43 cm), poor cod *Trisopterus minutus* (8–17 cm), dragonets (11–25 cm), mackerel (19–30 cm), thornback ray *Raja clavata* (13–76 cm), flounder (21–41 cm) and lesser spotted dogfish (44–69 cm). The

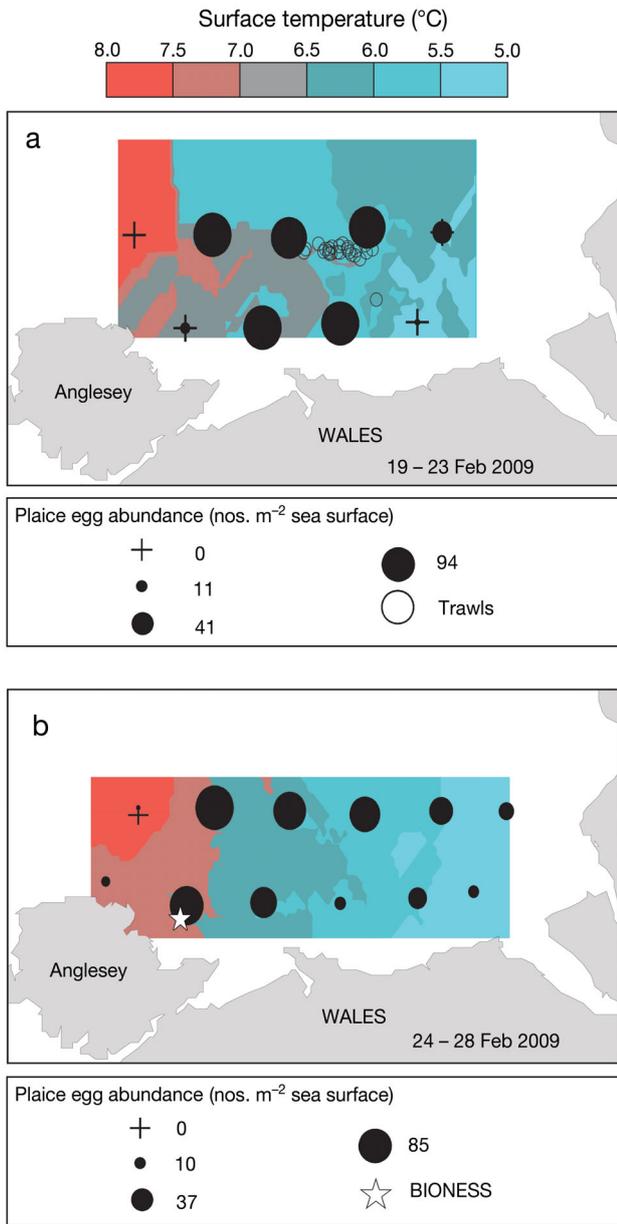


Fig. 3. Sea surface temperature (°C) contoured from Aquatracka data, abundance of plaice eggs (all stages combined, nos. m<sup>-2</sup> sea surface) shown at (a) the start of the 2009 cruise with the trawl locations and (b) the end of the 2009 cruise with the Bedford Institute of Oceanography Net and Environmental Sampling System (BIONESS) station. Plaice egg abundance is proportional to the area of the filled circles, with range and interim values shown in the legend

range of species caught in 2009 was smaller than in 2008, but catch weights of the dominant species, herring and whiting, were higher. In 2009, the range of species caught with the sandeel trawl was larger than with the PHHT but catch weights of herring, sprat and whiting were much lower using this gear.

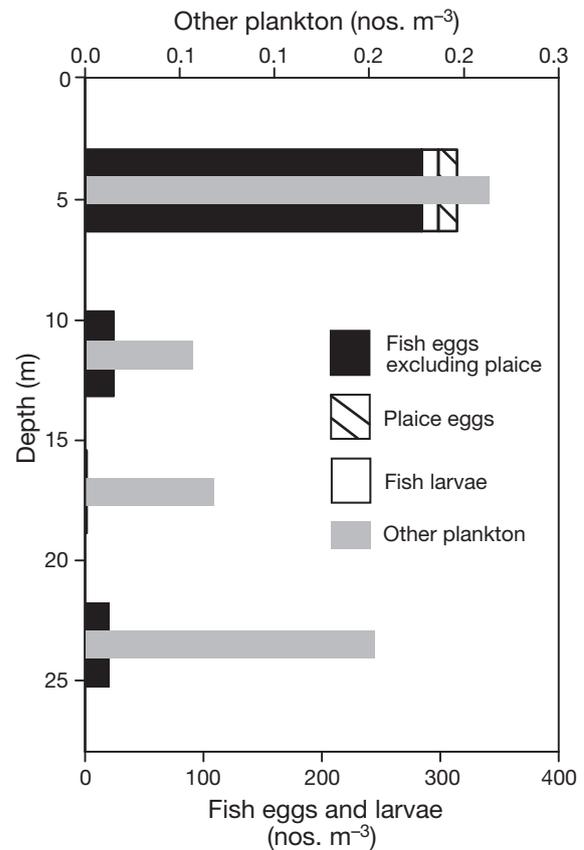


Fig. 4. Vertical distribution of plankton (numbers m<sup>-3</sup>) from the Bedford Institute of Oceanography Net and Environmental Sampling System (BIONESS) deployment

### Acoustics

Trawl catches suggested that the fish community in the water-column consisted of mixed aggregations of herring, sprat and smaller numbers of whiting. There were no apparent diel patterns in species abundance in the trawl data, so results from all trawls were combined and used to partition the acoustic backscatter into relative contributions. Herring and sprat biomass were estimated at 6123 t (93 × 10<sup>6</sup> ind.) and 1117 t (166 × 10<sup>6</sup> ind.) respectively. The distribution of clupeids showed slightly higher densities in the north of the survey area, although schools were found throughout the area (Fig. 5).

### TaqMan analyses

Summaries of the molecular results are shown in Table 2. The results were highly consistent between years. In 2008, sprat and herring showed the highest proportion of positive TaqMan responses (45.5 and

Table 1. Catch summary for the Portuguese high-headline trawl (PHHT) and sandeel trawls, excluding occasional benthic species and the beam trawl tows which were used primarily to provide specimens of benthic organisms. Presence is calculated as percentage of tows in which each species was recorded. Mean catch calculated as mean weights over tow duration

Common name	Scientific name	PHHT				Sandeel	
		2008 (n = 9)		2009 (n = 14)		2009 (n = 10)	
		Presence (%)	Mean catch (kg 30 min <sup>-1</sup> )	Presence (%)	Mean catch (kg 30 min <sup>-1</sup> )	Presence (%)	Mean catch (kg 30 min <sup>-1</sup> )
Herring	<i>Clupea harengus</i>	100	108.38	100	532.54	40	7.16
Sprat	<i>Sprattus sprattus</i>	100	15.58	86	2.93	100	1.58
Whiting	<i>Merlangius merlangus</i>	100	55.00	100	123.80	20	1.06
Dab	<i>Limanda limanda</i>	89	3.76	93	42.10	40	0.25
Squid	<i>Loligo</i> spp.	78	0.19	7	0.08	–	–
Grey gurnard	<i>Eutrigla gurnadus</i>	67	0.18	57	0.08	10	0.01
Plaice	<i>Pleuronectes platessa</i>	67	3.34	93	3.07	–	–
Poor cod	<i>Trisopterus minutus</i>	67	0.22	7	0.09	10	0.03
Dragonets	Callionymidae	44	0.26	14	0.20	10	0.53
Mackerel	<i>Scomber scombrus</i>	44	0.43	29	0.35	20	0.08
Thornback ray	<i>Raja clavata</i>	44	1.28	–	–	10	0.48
Flounder	<i>Platichthys flesus</i>	44	2.98	79	2.00	60	2.82
Crab	<i>Liocarcinus holsatus</i>	44	0.04	–	–	10	<0.01
Lesser spotted dogfish	<i>Scyliorhinus canicula</i>	44	4.40	43	1.63	–	–
Lemon sole	<i>Microstomus kitt</i>	–	–	36	0.38	–	–
Lesser weever	<i>Trachinus vipera</i>	33	1.65	–	–	–	–
Cod	<i>Gadus morhua</i>	22	1.15	–	–	–	–
Scad	<i>Trachurus trachurus</i>	22	0.43	–	–	–	–
Cuttlefish	<i>Sepiolo atlantica</i>	22	0.01	–	–	30	0.01
Pogge	<i>Agonus cataphractus</i>	22	0.02	–	–	10	0.01
Bib	<i>Trisopterus luscus</i>	22	0.41	–	–	–	–
Red gurnard	<i>Aspitrigla cuculus</i>	22	1.05	–	–	–	–
Haddock	<i>Melanogrammus aeglefinus</i>	11	0.44	–	–	–	–
Tub gurnard	<i>Trigla lucerna</i>	11	0.10	–	–	–	–
Cuttlefish	<i>Sepia officinalis</i>	11	0.05	–	–	50	0.01
Octopus	Octopoda	11	0.70	–	–	–	–
Swimming crab	<i>Liocarcinus puber</i>	11	0.03	–	–	–	–
Greater sandeel	<i>Hyperoplus lanceolatus</i>	11	0.04	–	–	20	<0.01
Raitt's sandeel	<i>Ammodytes marinus</i>	11	0.02	–	–	10	<0.01
Brown shrimp	<i>Crangon crangon</i>	11	0.02	–	–	–	–
Pink shrimp	<i>Pandalus</i> sp.	11	0.05	–	–	10	<0.01
Five bearded rockling	<i>Ciliatamustela</i>	11	0.12	–	–	–	–
Nurse hound	<i>Scyliorhinus stellaris</i>	11	0.31	–	–	–	–
Pipefish	<i>Syngnathus rostellatus</i>	11	0.09	–	–	–	–
Crystal goby	<i>Crystallogobius linearis</i>	11	<0.01	–	–	–	–
Tompot blenny	<i>Parablennius gattorugine</i>	–	–	–	–	10	0.47
Gobies	Gobiidae	11	<0.01	–	–	20	<0.01
Three-bearded rockling	<i>Gaidropsarus vulgaris</i>	–	–	–	–	10	0.09
Lumpsucker	<i>Cyclopterus lumpus</i>	–	–	–	–	10	0.04
Thickback sole	<i>Microchirus variegates</i>	–	–	–	–	10	0.03
Scaldfish	<i>Arnoglossus luterna</i>	–	–	–	–	10	0.01
Butterfly blenny	<i>Blennius ocellaris</i>	–	–	7	0.03	–	–

33.1% of samples tested respectively), whilst in 2009 the proportions were even higher (85.3 and 96.1%). When the percentage of individual sprat and herring yielding a positive TaqMan plaice probe result was plotted against the time of day when the fish were caught, there was no obvious trend (data not shown). Likewise, there was no obvious relationship between proportions showing a positive TaqMan response and the total time taken to process the catch. Mackerel also tested positive although this species was much

less numerous than sprat and herring. Whiting were also relatively abundant in both years (Table 1) and around 10% of the individuals tested positive for plaice DNA. Other species showing low levels of positive response included poor cod and squid. Four species expected to be predominantly demersal but which also gave a low incidence of positive results were red gurnard *Aspitrigla cuculus*, grey gurnard, dogfish and lesser weever *Echiichthys vipera*. None of the larger benthic crustacea tested positive (Table 2).

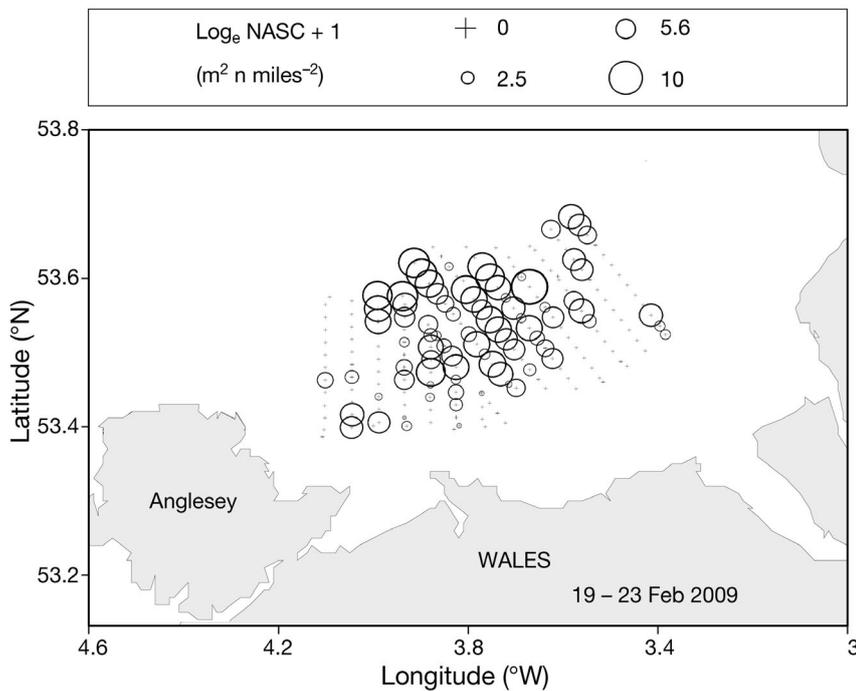


Fig. 5. Acoustically determined clupeid density. Log transformed acoustic signal is proportional to the area of the open circles, with range and interim values shown in the legend. NASC: nautical area backscattering coefficient

From the BIONESS samples, around 13% of animals tested positive with the TaqMan assay. These positive results were dominated by fish larvae (grouped as mixed Clupeidae and Ammodytidae) which yielded 20% TaqMan positives, whilst a few Malacostraca and Amphipoda also tested positive (Table 3).

## DISCUSSION

Our results demonstrate how the application of modern molecular methods can successfully provide a more holistic description of the predator community preying on a patch of fish eggs/larvae compared with visual gut content analysis (Brewer et al. 1984). The dominant predators identified were sprat and herring but the TaqMan probe also revealed that whiting, squid, fish larvae (Clupeidae and/or Ammodytidae) and planktonic malacostraca and amphipods were also consuming plaice eggs/lar-

Table 2. Summary of the TaqMan probe results from trawl samples determined from the percentage of stomachs showing a positive response to the plaice probe. Totals tested include the following species, which were collected in low numbers ( $n < 30$  per species) with no positive TaqMan results: butterfly blenny *Blennius ocellaris*, bib *Trisopterus luscus*, cod *Gadus morhua*, crab *Liocarcinus depurator*, common dragonet *Callionymus lyra*, Dover sole *Solea solea*, edible crab *Cancer pagurus*, gobies *Gobiidae*, greater weever *Trachinus draco*, harbour crab *Liocarcinus depurator*, lemon sole *Microstomus kitt*, lumpsucker *Cyclopterus lumpus*, masked crab *Corystes cassivelaunus*, pogge *Agonus cataphractus*, prawn *Nephrops norvegicus*, rockling *Ciliata mustela*, sandeel *Ammodytes marinus*, scaldfish *Arnoglossus laterna*, scad *Trachurus trachurus*, solenette *Buglossidium luteum*, spotted ray *Raja montagui*, thickback sole *Microchirus variegatus*, thornback ray *Raja clavata*, tub gurnard *Trigla lucerna*, and velvet crab *Necora puber*

Common name	Scientific name	2008		2009		Overall	
		n	% positive	n	% positive	n	% positive
Herring	<i>Clupea harengus</i>	159	33	383	96	542	77
Sprat	<i>Sprattus sprattus</i>	173	45	537	85	710	75
Mackerel	<i>Scomber scombrus</i>	12	33	17	6	29	17
Poor cod	<i>Trisopterus minutus</i>	23	13	10	20	33	15
Red gurnard	<i>Aspitrigla cuculus</i>	14	20	–	–	14	20
Whiting	<i>Melangius merlangus</i>	75	11	293	11	368	11
Squid	<i>Loligo</i> spp.	25	8	1	0	26	8
Lesser weever	<i>Trachinus vipera</i>	13	8	22	0	35	8
Lesser spotted dogfish	<i>Scyliorhinus canicula</i>	23	4	11	0	34	3
Grey gurnard	<i>Eutrigla gurnadus</i>	26	0	37	3	63	2
Dab	<i>Limanda limanda</i>	55	0	181	0	236	0
Crab	<i>Liocarcinus holsatus</i>	15	0	149	0	164	0
Flounder	<i>Platichthys flesus</i>	23	0	63	0	86	0
Brown shrimp	<i>Crangon crangon</i>	–	–	59	0	59	0
Cuttlefish	<i>Sepiolo atlantica</i>	1	0	47	0	48	0
Hermit crabs	<i>Pagurus</i> spp.	–	–	45	0	45	0
Negative controls		25	0	78	0	103	0
Total tested		748		2154		2909	

Table 3. TaqMan results from the Bedford Institute of Oceanography Net and Environmental Sampling System (BIONESS) depth-stratified sampling. n: no. of individuals

Taxa	Total tested (n)	Positive TaqMan (n)	Positive TaqMan (%)
Fish larvae (Clupeidae & Ammodytidae)	348	68	19.5
Malacostraca	138	4	2.8
Amphipoda	31	1	3.2
Chaetognatha	21	0	0
Total	567	73	12.9

vae. These findings extend results from previous studies which reported on a more restricted range of predators (Daan 1976, Ellis & Nash 1997).

### Clupeids as fish egg predators

Previous studies have implicated sprat and herring as important consumers of fish eggs (Daan 1976, Pommeranz 1981, Ellis & Nash 1997, Köster & Möllmann 2000, Köster et al. 2001, Möllmann et al. 2004, Segers et al. 2007). Ellis & Nash (1997) found that 75 to 97% of sprat stomachs sampled from a small plaice spawning ground off the Isle of Man contained plaice eggs; for herring, the proportions varied from 25 to 48%. TaqMan data from the present study yielded similar values for sprat but higher values for herring (96% of herring stomachs testing positive in 2009). Combined with the potential for individual sprat and herring to consume large numbers of eggs (Ellis & Nash 1997, Plirú et al. 2011), the present results support the idea that clupeids may be the most important predators of plaice eggs/larvae in the Irish Sea. The acoustics surveys also revealed the presence of several schools of sprat and herring in the study area and their spatial distribution broadly corresponded with the abundance of fish eggs. Although this suggests that clupeids might be targeting egg patches, further research is needed to determine whether clupeid shoals remain within egg patches over extended periods and whether they switch feeding modes when eggs are abundant (Batty et al. 1986, Sims et al. 2008).

A number of other species that are known to feed in the water column, including mackerel, whiting, poor cod and squid, were shown to be consuming plaice eggs/larvae in the present study. As far as we are aware, these species have not been previously identified as predators on the early stages of plaice. Harding et al. (1978) did, however, report the presence of plaice eggs in the stomachs of grey gurnard.

Except for whiting, the abundance of these non-clupeid species off the North Wales coast during the present study was low, supporting the idea that sprat and herring were the dominant consumers (Table 1).

The abundance of other potential predators in the plankton was very low (Fig. 4), but the BIONESS samples showed a surprising number of positive responses. In particular, fish larvae appeared to be consuming plaice eggs/larvae. Because of damage to the specimens prior to molecular analysis, these larvae could not be identified to species level and were grouped as mixed Clupeidae/Ammodytidae. Given the low abundance of adult sand-eel in the area (Table 1), it is probable that the majority of these larvae were actually sprat. The relatively high numbers of positive TaqMan reactions from non-plaice fish larvae are unlikely to be due to contamination, since very low numbers of other organisms tested from the BIONESS samples gave positive reactions. Gelatinous predators are also often cited as major consumers of fish eggs and larvae (Purcell & Arai 2001), but in the present study gelatinous predators were not common (Madin et al. 1996). Populations of species such as *Aurelia aurita* and *Cyanea capitella* usually peak in late summer/autumn in the Irish Sea (Houghton et al. 2007) and predation pressure from these organisms should be relatively low early in the year (Purcell & Arai 2001).

### Use of molecular probes in marine predation studies

Using a state-of-the-art molecular approach we were able to rapidly screen nearly 3000 stomach contents and 550 planktonic organisms as potential predators of plaice eggs/larvae. This enabled confirmation of the importance of sprat and herring as consumers of fish eggs but also identified additional predators such as whiting, poor cod, red and grey gurnard and fish larvae. Despite the difficulties of sorting trawl catches, cross-contamination between samples did not appear to be a significant problem, as evidenced by non-reactivity of the negative controls.

Dependent on development stage and temperature, the TaqMan probe has a T90 (the length of time after ingestion that target DNA can be detected in >90% of the predators tested) of between 4 and 5 h (Albaina et al. 2010, Hunter et al. 2012). Field sampling of predators should thus be undertaken regularly every 4 h (for predators showing no diel feeding rhythms), or samples should be collected within 4 h

of peak feeding (for predators showing diel consumption patterns). By fishing every 4 h over several days, detection rates in the present study should have been in the region of 90% or better at some point during each 24 h period. Degradation of prey DNA in predator stomachs post-capture might result from post-mortem pH changes and protease action. Unfortunately, we are not aware of any studies which provide data on how rapidly this can occur so the 2 h limit we used, whilst somewhat arbitrary, seemed a reasonable compromise between allowing full sorting and sampling of the catch and this risk. For sprat and herring, more than 85% of individuals tested positive, regardless of the time of day of sampling, supporting high detection efficiency for the assay and suggesting that degradation of target DNA was not a major problem.

Regurgitation of prey is known to be a particular problem when sampling teleost predators using trawls and can lead to under-estimation of feeding rates (Bowman 1986). It is usually recommended to use tows that are as short as possible to minimise this problem. However, the molecular approach to stomach content analysis may be less sensitive to this problem, since the TaqMan probe has been shown to be capable of detecting residual prey DNA, even when there are no remaining visible signs (Hunter et al. 2012). The opposite problem, that other organisms might consume regurgitated material whilst in the trawl or plankton nets, leading to over-estimation of feeding rates, cannot be dismissed entirely, although for the trawl it is likely that regurgitated material would be rapidly flushed out of the net during towing because of the mesh size of the cod-end. It is feasible that feeding incidence in the BIONESS samples was over-estimated because of this problem (Hunter 1984).

Because of their size and thick wall, plaice eggs and their shells can often be identified in fish stomachs (Ellis & Nash 1997, Plirú et al. 2011). However, eggs of other species cannot be so readily identified; for example cod, haddock *Melanogrammus aeglefinus* and whiting eggs cannot be visually distinguished until the late developmental stages (Fox et al. 2008). For studies targeting predation on species with visually indistinguishable eggs, a visual inspection of predator stomach contents will be unable to assign prey consumed to species level. The application of molecular tools to unambiguously identify fish eggs in the water column (Fox et al. 2008, Goodsir et al. 2008) and in predator guts (present paper) can solve some of these problems. On the other hand, the molecular approach cannot yet provide information

on the number of eggs or larvae consumed, nor on the developmental stages of the prey.

The molecular approach is also a powerful tool for screening predators that macerate their prey (Theilacker et al. 1993). For example, TaqMan results from the BIONESS indicated that some amphipods and malacostraca were consuming plaice eggs/larvae. Euphausiids have also been reported to consume fish eggs and larvae (Bailey et al. 1993) but they were not abundant in the egg patch investigated. Euphausiids are much more common in the deeper parts of the western Irish Sea (Mauchline 1984) where they could be more important as predators of fish eggs, since these areas are also adjacent to fish spawning grounds (Fox et al. 2000a).

Finally, because the TaqMan method is so sensitive, it may detect secondary predation, e.g. the few positive TaqMan results from dogfish stomachs in this study. The diet of dogfish in the Irish Sea is known to include herring (Lyle 1983), which are themselves important consumers of plaice eggs as shown in the present study and by Ellis & Nash (1997).

#### **Relevance to previous studies on predation on fish eggs and larvae**

Our results support and extend data from the southern North Sea (Daan 1976, Segers et al. 2007) and the Irish Sea (Ellis & Nash 1997), where sprat and herring were reported to be important consumers of plaice eggs. Although sprat and herring both feed predominantly on copepods, they will switch to alternate prey when copepods are scarce (Daan 1976, Segers et al. 2007). In UK waters, plaice complete spawning well before the spring increase in zooplankton (Simpson 1959a,b, Fox et al. 2000a, 2007). This was reflected in the present study where eggs of plaice, and other fish, dominated the plankton (Fig. 4). Fish eggs may thus provide a valuable pulse of consumable biomass to pelagic predators early in the year when there is little alternative food available.

Although year-class strength in North Sea plaice appears broadly predictable from the abundance of late-stage eggs (Bannister et al. 1974, Brander & Houghton 1982), further research is required to examine whether this holds true for other plaice stocks and can be linked to levels of egg predation. A further area which requires study is the possible effects of inter-annual changes in water temperature on the activity and feeding rates of egg and larval predators since this could, potentially, explain the observation of a negative correlation between sea

temperatures during late winter and early spring and subsequent recruitment success in several plaice stocks (Fox et al. 2000b). Across a wide range of temperatures, Pepin (1991) found that increased instantaneous mortality was offset by increased development rates so that resulting total mortality across the egg stage was less temperature-sensitive compared with absolute rates. However, there was considerable scatter about this general relationship so that within regions, temperature might still have an impact on overall survival. Results from Daan (1976), Ellis & Nash (1997) and Plirú et al. (2011) suggest that the abundance, distribution and feeding intensity of sprat and herring may be especially important in this respect. Despite several examples where clupeids are thought to play a key role in controlling recruitment of other fish (Cushing 1980, Speirs et al. 2010, Frank et al. 2011), firm links have only been established in the Baltic (Köster et al. 2001, Lindegren et al. 2009) and Barents Sea (Pedersen et al. 2009). In areas such as the North and Irish Seas, the abundance and distribution of herring is relatively well monitored on an annual basis. In contrast, much less is known about changes in sprat abundance and distribution (ICES 2010). Because of their apparent importance as consumers of the early life stages of other species, improvements in monitoring of sprat in particular will probably be required if ecosystem-based approaches to managing marine fish stocks are to succeed (Köster & Möllmann 2000, Kelly et al. 2006, Segers et al. 2007, Lindegren et al. 2009, Speirs et al. 2010, Frank et al. 2011).

*Acknowledgements.* We thank the officers and crew of the RV 'Endeavour' and the staff of the Centre for Environment, Fisheries & Aquaculture Science (Cefas) plankton sorting team whose hard work made this research possible. Probe development as well as the research discussed in this paper was funded by Department for Environment, Food and Rural Affairs (Defra) contracts M0432 PREDATE and M1102 MEMFISH. Whilst writing the paper, C.J.F. was supported by the Natural Environment Research Council (NERC) Oceans2025 program WP4, Predators and prey.

#### LITERATURE CITED

- Albaina A, Fox CJ, Taylor N, Hunter E, Maillard M, Taylor MI (2010) A TaqMan real-time PCR based assay targeting plaice (*Pleuronectes platessa* L.) DNA to detect predation by the brown shrimp (*Crangon crangon* L.) and the shore crab (*Carcinus maenas* L.)—assay development and validation. *J Exp Mar Biol Ecol* 391:178–189
- Albaina A, Taylor MI, Fox CJ (2012) Molecular detection of plaice remains in the stomachs of potential predators on a flatfish nursery ground. *Mar Ecol Prog Ser* 444:223–238
- Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res* 25:4692–4693
- Bailey KM, Brodeur RD, Merati N, Yoklavich MM (1993) Predation on walleye pollack (*Theragra chalcogramma*) eggs and yolk-sac larvae by pelagic crustacean invertebrates in the western Gulf of Alaska. *Fish Oceanogr* 2: 30–39
- Bannister RCA, Harding D, Lockwood SJ (1974) Larval mortality and subsequent year-class strength in the plaice (*Pleuronectes platessa* L.). In: Blaxter JHS (ed) *The early life history of fish*, Vol. 1. Proc Int Symp Dunstaffnage Mar Res Lab, Oban, Scotland. Springer-Verlag, Berlin, p 21–37
- Batty RS, Blaxter JHS, Libby DA (1986) Herring (*Clupea harengus*) filter-feeding in the dark. *Mar Biol* 91:371–375
- Bowman R (1986) Effect of regurgitation on stomach content data of marine fishes. *Environ Biol Fishes* 16:171–181
- Brander K, Houghton RG (1982) Predicting the recruitment of North Sea plaice from egg surveys. *ICES CM* 1982/G: 5, p 1–3
- Brander KM, Milligan SP, Nichols JH (1993) Flume tank experiments to estimate the volume filtered by high-speed plankton samplers and to assess the effect of net clogging. *J Plankton Res* 15:385–401
- Brewer GD, Kleppel GS, Dempsey M (1984) Apparent predation on ichthyoplankton by zooplankton and fishes in nearshore waters of southern California. *Mar Biol* 80: 17–28
- Browman HI (2003) Assessing the impacts of solar ultraviolet radiation on the early life stages of crustacean zooplankton and ichthyoplankton in marine coastal systems. *Estuaries* 26:30–39
- Bunn N, Fox CJ (2004) Spring plankton surveys of the Irish Sea in 2000: hydrography and the distribution of fish eggs and larvae. *Sci Ser Data Rep No. 41*. Centre for Environment, Fisheries and Aquaculture Science, Lowestoft
- Bunn NA, Fox CJ, Webb T (2000) A literature review of studies on fish egg mortality: implications for the estimation of spawning stock biomass by the annual egg production method. *Sci Ser Tech Rep* 111. Centre for Environment, Fisheries and Aquaculture Science, Lowestoft
- Bunn N, Fox CJ, Nash RDM (2004) Spring plankton surveys of the eastern Irish Sea in 2001, 2002 and 2003: hydrography and the distribution of fish eggs and larvae. *Sci Ser Data Report No. 42*. Centre for Environment, Fisheries and Aquaculture Science, Lowestoft
- Cameron P, von Westernhagen H (1997) Malformation rates in embryos of North Sea fishes in 1991 and 1992. *Mar Pollut Bull* 34:129–134
- Cushing DH (1980) The decline of the herring stocks and the gadoid outburst. *J Cons Int Explor Mer* 39:70–81
- Daan N (1976) Some preliminary investigations into predation on fish eggs and larvae in the southern North Sea. *ICES CM* 1976(L):15, p 1–5
- Deagle BE, Kirkwood R, Jarman SN (2009) Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Mol Ecol* 18:2022–2038
- Ellis T, Nash RDM (1997) Predation by sprat and herring on pelagic fish eggs in a plaice spawning area in the Irish Sea. *J Fish Biol* 50:1195–1202
- Fässler SMM, Payne MR, Brunel T, Dickey-Collas M (2011) Does larval mortality influence population dynamics? An analysis of North Sea herring (*Clupea harengus*) time series. *Fish Oceanogr* 20:530–543

- Feigenbaum DL, Maris RC (1984) Feeding in the chaetognatha. *Oceanogr Mar Biol Annu Rev* 22:343–392
- Folkvord A (1993) Prey recognition in stomachs of cannibalistic juvenile cod (*Gadus morhua* L.). *Sarsia* 78:97–100
- Foote KG (1987) Fish target strengths for use in echo integrator surveys. *J Acoust Soc Am* 82:981–987
- Foote KG, Knudsen HP, Vestnes G, MacLennan DN, Simmonds EJ (1987) Calibration of acoustic instruments for fish density estimation: a practical guide. Report No. 144. ICES, Copenhagen
- Fox CJ, O'Brien CM, Dickey-Collas M, Nash RDM (2000a) 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 generalized additive modelling. *Fish Oceanogr* 9:33–49
- Fox CJ, Planque BP, Darby CD (2000b) Synchrony in the recruitment time-series of plaice (*Pleuronectes platessa* L.) around the United Kingdom and the influence of sea temperature. *J Sea Res* 44:159–168
- Fox CJ, Geffen AJ, Taylor N, Davison P, Rossetti H, Nash RDM (2007) Birth-date selection in early life stages of plaice *Pleuronectes platessa* in the eastern Irish Sea (British Isles). *Mar Ecol Prog Ser* 345:255–269
- Fox CJ, Taylor M, Dickey-Collas M, Fossum P and others (2008) Mapping the spawning grounds of North Sea cod (*Gadus morhua*) by direct and indirect means. *Proc R Soc B Biol Sci* 275:1543–1548
- Frank KT, Petrie B, Fisher JAD, Leggett WC (2011) Transient dynamics of an altered large marine ecosystem. *Nature*.
- Goodsir F, Armstrong MJ, Witthames PR, Maxwell DL, Fox CJ (2008) The use of species-specific TaqMan probes for identifying early stage gadoid eggs following formaldehyde fixation. *ICES J Mar Sci* 65:1573–1577
- Harding D, Nichols JH, Tungate DS (1978) The spawning of plaice (*Pleuronectes platessa* L.) in the southern North Sea and English Channel. *Rapp P-V Réun Cons Int Explor Mer* 172:102–113
- Heath MR (1992) Field investigations of the early life stages of marine fish. *Adv Mar Biol* 28:1–174
- Hjort J (1914) Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. *Rapp P-V Réun Cons Int Explor Mer* 20:1–228
- Houde ED (2002) Mortality. In: Fuiman LA, Werner RG (eds) *Fishery science, the unique contributions of early life stages*. Blackwell Science, Oxford, p 64–87
- Houde ED (2008) Emerging from Hjort's shadow. *J Northwest Atl Fish Sci* 41:53–70
- Houghton JDR, Doyle TK, Davenport J, Lilley MKS, Wilson RP, Hays GC (2007) Stranding events provide indirect insights into the seasonality and persistence of jellyfish medusae (Cnidaria: Scyphozoa). *Hydrobiologia* 589: 1–13
- Hunter JR (1981) Feeding ecology and predation of marine fish larvae. In: Lasker R (ed) *Marine fish larvae: morphology, ecology and relation to fisheries*. Washington Sea Grant, Seattle, WA, p 33–77
- Hunter JR (1984) Inferences regarding predation on the early life stages of cod and other fishes. In: Dahl E, Danielssen DS, Moksness E, Solemdal P (eds) *The propagation of cod *Gadus morhua* L.* Flødevigen rapportser 1: 533–562
- Hunter JR, Kimbrell CA (1980) Egg cannibalism in the northern anchovy, *Engraulis mordax*. *Fish Bull* 78: 811–816
- Hunter E, Taylor N, Fox CJ, Maillard M, Taylor MI (2012) Effectiveness of TaqMan probes for detection of fish eggs and larvae in the stomach contents of a teleost predator. *J Fish Biol* 81:320–328
- Huwer B, Clemmesen C, Grønkjær P, Köster FW (2011) Vertical distribution and growth performance of Baltic cod larvae—field evidence for starvation-induced recruitment regulation during the larval stage? *Prog Oceanogr* 91:382–396
- IBTS (International Bottom Trawl Survey) (2010) Manual for the International Bottom Trawl Surveys revision VIII. ICES, Copenhagen
- ICES (International Council for the Exploration of the Sea) (2010) Report of the Herring Assessment Working Group (HAWG) for the area south of 62°N. ICES CM 2010: ACOM:06, ICES, Copenhagen
- Kelly CJ, Codling EA, Rogan E (2006) The Irish Sea cod recovery plan: some lessons learned. *ICES J Mar Sci* 63: 600–610
- Klump DW, von Westernhagen H (1995) Biological effects of pollutants in Australian tropical coastal waters: embryonic malformations and chromosomal aberrations in developing fish eggs. *Mar Pollut Bull* 30: 158–165
- Korneliusson RJ, Diner N, Ona E, Berger L, Fernandes PG (2008) Proposals for the collection of multifrequency acoustic data. *ICES J Mar Sci* 65:982–994
- Köster FW, Möllmann C (2000) Trophodynamic control by clupeid predators on recruitment success in Baltic cod? *ICES J Mar Sci* 57:310–323
- Köster FW, Hinrichsen HH, St. John MA, Schnack D, MacKenzie BR, Tomkiewicz J, Plikshs M (2001) Developing Baltic cod recruitment models. II. Incorporation of environmental variability and species interaction. *Can J Fish Aquat Sci* 58:1534–1556
- Krautz MC, González M, Castro LR (2003) Detection of anchoveta (*Engraulis ringens* Jenyns 1842) eggs in euphausiid diets using immunoassays (ELISA). *J Exp Mar Biol Ecol* 294:27–39
- Krautz MC, Castro LR, González M (2007) Interaction of two key pelagic species in the Humboldt Current: euphausiid predation on anchoveta eggs estimated by immunoassays. *Mar Ecol Prog Ser* 335:175–185
- Lindgren M, Möllmann C, Nielsen A, Stenseth NC (2009) Preventing the collapse of the Baltic cod stock through an ecosystem-based management approach. *Proc Natl Acad Sci USA* 106:14722–14727
- Lyle JM (1983) Food and feeding habits of the lesser spotted dogfish, *Scyliorhinus canicula* (L.), in Isle of Man waters. *J Fish Biol* 23:725–737
- Lynam CP, Heath MR, Hay SJ, Brierley AS (2005) Evidence for impacts by jellyfish on North Sea herring recruitment. *Mar Ecol Prog Ser* 298:157–167
- Madin LP, Bollens SM, Horgan E, Butler M and others (1996) Voracious planktonic hydroids: unexpected predatory impact on a coastal marine ecosystem. *Deep-Sea Res II* 43:1823–13298
- Mauchline J (1984) Euphausiid, Stomatopod and Leptostracan crustaceans: keys and notes for the identification of the species, Vol 30. Linnean Society of London, Estuarine and Brackish-water Sciences Association, E. J. Brill, London
- McGurk MD (1986) Natural mortality of marine pelagic fish eggs and larvae: role of spatial patchiness. *Mar Ecol Prog Ser* 34:227–242

- Meneses I, Vendrell C, Stratoudakis Y (2003) Mackerel (*Scomber scombrus*) eggs parasitized by *Ichthyodinium chabelardi* in the north-east Atlantic: an overlooked source of mortality. *J Plankton Res* 25:1177–1181
- Möllmann C, Kornilovs G, Fetter M, Köster FW (2004) Feeding ecology of central Baltic Sea herring and sprat. *J Fish Biol* 65:1563–1581
- Myers RA, Cadigan NG (1993) Density-dependent juvenile mortality in marine demersal fish. *Can J Fish Aquat Sci* 50:1576–1590
- 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
- Nejstgaard JC, Frischer ME, Raule CL, Gruebel R, Kohlberg KE, Verity PG (2003) Molecular detection of algal prey in copepod guts and fecal pellets. *Limnol Oceanogr Methods* 1:29–38
- Passmore AJ, Jarman SN, Swadling KM, Kawaguchi S, McMin A, Nicol S (2006) DNA as a dietary biomarker in Antarctic krill, *Euphausia superba*. *Mar Biotechnol* 8:686–696
- Pedersen OP, Pedersen T, Tande KS, Slagstad D (2009) Integrating spatial and temporal mortality from herring on capelin larvae: a study in the Barents Sea. *ICES J Mar Sci* 66:2183–2194
- Pepin P (1991) Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Can J Fish Aquat Sci* 48:503–518
- Platt T, Sathyendranath S, Fuentes-Yaco C (2007) Biological oceanography and fisheries management: perspective after 10 years. *ICES J Mar Sci* 64:863–869
- Plirú A, van der Kooij J, Engelhard GH, Fox CJ, Milligan SP, Hunter E (2011) Is recruitment of plaice in the Irish Sea constrained by predation of eggs by sprat? Report No. ICES CM 2011/H:33
- Pommeranz T (1981) Observations on the predation of herring (*Clupea harengus* L.) and sprat (*Sprattus sprattus* L.) on fish eggs and larvae in the southern North Sea. *Rapp P-V Réun Cons Int Explor Mer* 178:402–404
- Purcell JE (1985) Predation on fish eggs and larvae by pelagic cnidarians and ctenophores. *Bull Mar Sci* 37:739–755
- Purcell JE, Arai MN (2001) Interactions of pelagic cnidarians and ctenophores with fish: a review. *Hydrobiologia* 451:27–44
- Robinson BJ (1983) In situ measurements of the target strength of pelagic fishes. *FAO Fisheries Report No. 300. Food and Agriculture Organisation of the United Nations, Rome*, p 99–103
- Rosel PE, Kocher TD (2002) DNA-based identification of larval cod in stomach contents of predatory fishes. *J Exp Mar Biol Ecol* 267:75–88
- Russell FS (1976) The eggs and planktonic stages of British marine fishes. Academic Press, London
- Segers FHID, Dickey-Collas M, Rijnsdorp AD (2007) Prey selection by North Sea herring (*Clupea harengus*), with special reference to fish eggs. *ICES J Mar Sci* 64:60–68
- Simpson AC (1959a) The spawning of the plaice (*Pleuronectes platessa*) in the Irish Sea. Fisheries Investigations Series II, Vol. XXII, No. 8. Ministry of Agriculture, Fisheries and Food, Lowestoft
- Simpson AC (1959b) The spawning of the plaice (*Pleuronectes platessa*) in the North Sea. Fisheries Investigations Series II, Vol. XXII, No. 7. Ministry of Agriculture, Fisheries and Food, Lowestoft
- Sims DW, Southall EJ, Humphries NE, Hays GC and others (2008) Scaling laws of marine predator search behaviour. *Nature* 451:1098–1102
- Sissenwine MP (1984) Why do fish populations vary? In: May RM (ed) Exploitation of marine communities. Springer-Verlag, Berlin, p 59–94
- Speirs DC, Guirey EJ, Gurney WSC, Heath MR (2010) A length-structured partial ecosystem model for cod in the North Sea. *Fish Res* 106:474–494
- Suzuki N, Hoshino K, Murakami K, Takeyama H, Chow S (2008) Molecular diet analysis of phyllosoma larvae of the Japanese spiny lobster *Panulirus japonicus* (Decapoda: Crustacea). *Mar Biotechnol* 10:49–55
- Symondson WOC (2002) Molecular identification of prey in predator diets. *Mol Ecol* 11:627–641
- Taylor DL (2004) Immunological detection of winter flounder (*Pseudopleuronectes americanus*) eggs and juveniles in the stomach contents of crustacean predators. *J Exp Mar Biol Ecol* 301:55–73
- Taylor DL, Danila DJ (2005) Predation on winter flounder (*Pseudopleuronectes americanus*) eggs by the sand shrimp (*Crangon septemspinosa*). *Can J Fish Aquat Sci* 62:1611–1625
- 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
- Theilacker GH, Kimball AS, Trimmer JS (1986) Use of an ELISPOT immunoassay to detect euphausiid predation on larval anchovy. *Mar Ecol Prog Ser* 30:127–131
- Theilacker GH, Lo NCH, Townsend AW (1993) An immunological approach to quantifying predation by euphausiids on the early stages of anchovy. *Mar Ecol Prog Ser* 92:35–50
- Vestheim H, Edvardsen B, Kaartvedt S (2005) Assessing feeding of a carnivorous copepod using species-specific PCR. *Mar Biol* 147:381–385
- Zhang T, Bailey KM, Chan KS (2010) Recruitment forecast models for walleye pollock *Theragra chalcogramma* fine-tuned from juvenile survey data, predator abundance and environmental phase shifts. *Mar Ecol Prog Ser* 417:237–248

Editorial responsibility: Edward Durbin,  
Narragansett, Rhode Island, USA

Submitted: November 1, 2011; Accepted: April 5, 2012  
Proofs received from author(s): July 6, 2012