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Exploring the microzooplankton–ichthyoplankton link: a combined field and modeling study of Atlantic herring (*Clupea harengus*) in the Irish Sea

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The microzooplankton–ichthyoplankton link remains poorly resolved in field studies due to a lack of simultaneous sampling of these predators and potential prey. This study compared the abundance, distribution and growth of larval Atlantic herring (*Clupea harengus*) and the abundance, biomass and composition of micro- and small mesozooplankton throughout the Irish Sea in November 2012 and 2013. In contrast to warmer months, microzooplankton biomass was highest in eastern areas, in the vicinity of the main spawning grounds of herring. Although the protozoan composition differed somewhat between years, dinoflagellates (e.g. *Gymnodinium* spp., *Protoperidinium* spp., *Ceratium furca*) dominated in abundance and/or biomass, similar to other temperate shelf seas in autumn/winter. Spatial differences in the protozoan community were strongly related to hydrographic characteristics (temperature, salinity). Significant relationships between the abundance of larval herring and dinoflagellates (positive) and copepodites (negative) suggested that complex grazing dynamics existed among lower trophic levels. When different, *in situ* size fractions of zooplankton were used as prey in a larval herring individual-based model, simulations that omitted protozooplankton under-predicted observed (biochemically-based) growth of 8–18 mm larvae. This study suggests that small planktonic organisms (20–300 μm) should be routinely surveyed to better understand factors affecting larval fish feeding, growth and survival.

KEYWORDS: autumn-spawning herring; protozooplankton community; microzooplankton–ichthyoplankton link; individual-based model

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INTRODUCTION

Since the introduction of the concept of the "microbial loop" (Azam *et al.*, 1983), the importance of microzooplankton (hetero- or mixotrophic plankton 20–200 μm) to the trophodynamic structure and function of aquatic ecosystems has become more and more evident. Microzooplankton such as protists and early life stages of copepods play an important role as grazers of bacteria and flagellates (Fenchel, 1988; Calbet and Saiz, 2005) and as prey for higher trophic levels (e.g. copepods, larval fish) (Montagnes *et al.*, 2010b; Friedenberg *et al.*, 2012). The role of microzooplankton as prey may be particularly important during time periods characterized by low rates of primary production. For example, during autumn and winter in northern temperate waters the importance of the classical "phytoplankton-copepods-fish" food-chain decreases, and dissolved and particulate organic matter is recycled via the microbial loop. Nevertheless little is known regarding seasonal dynamics, diversity and other basic features of the ecology of these organisms and very few studies have investigated the role of microzooplankton during winter periods, after the termination of the autumn bloom (e.g. Montagnes *et al.*, 2010a; Scherer, 2012; Yang *et al.*, 2015).

In the Irminger Sea (Montagnes *et al.*, 2010a) as well as in the Irish Sea (Scherer, 2012), the relative abundance of the <25 μm microzooplankton size fraction was higher in autumn and winter than during the rest of the year. Montagnes *et al.* (2010a) suggested that the high availability of small (<5 μm) prey during that time of the year explained the high abundance of (and importance of grazing by) small heterotrophic ciliates. Data on microzooplankton are often collected during only one season or on a specific component of that community. Time series data for microzooplankton in the North Atlantic and adjacent waters (e.g. Smetacek, 1981; Montagnes *et al.*, 1988; Edwards and Burkill, 1995; Scherer, 2012; Yang *et al.*, 2015) are often short (<5 years) and collected at only one station. Further challenges to understanding the ecological role of microzooplankton are inconsistencies in the classification of some organisms within this group. For example, dinoflagellates are often classified as phytoplankton despite the fact that most dinoflagellates are considered to be mixotrophic/heterotrophic (Flynn *et al.*, 2013).

Although microzooplankton have long been recognized as part of the diet of the larvae of fish such as Atlantic herring (*Clupea harengus*) (Hardy, 1924), relatively few studies have attempted to understand the role that microzooplankton play in terms of *in situ* feeding and growth of fish larvae (Pepin and Dower, 2007; Montagnes *et al.*, 2010b). This is in stark contrast to many studies examining mesozooplankton–ichthyoplankton interactions (Peck *et al.*, 2012; Llopiz, 2013). Most larvae rapidly digest microplankton and often regurgitate prey when sampled, which makes gut content analyses and the quantitative study of microzooplankton as prey very difficult (Fukami *et al.*, 1999; Nagano *et al.*, 2000; Figueiredo *et al.*, 2005, 2007). For these reasons, the microzooplankton–ichthyoplankton link has been examined most frequently in laboratory experiments using, for example, prey depletion experiments (Friedenberg *et al.*, 2012), prey labelled with fluorescent microspheres (Lessard *et al.*, 1996; Nagano *et al.*, 2000) or by measuring lipid biomarkers (Rossi, 2006) or biochemical condition (Overton *et al.*, 2010; Illing *et al.*, 2015) of the larvae. The results of various laboratory studies suggested that protists can partially or completely fulfil the daily nutritional and energy requirements of the larvae of Atlantic cod (*Gadus morhua)* (Van der Meeren, 1991; Hunt von Herbing and Gallager, 2000), northern anchovy (*Engraulis mordax*) (Ohman *et al.*, 1991), Pacific herring (*Clupea pallasi*) (Fukami *et al.*, 1999; Friedenberg *et al.*, 2012) and Atlantic herring (Illing *et al.*, 2015).

Several recruitment hypotheses for marine fish stem from research performed on clupeids including those focused on early feeding success such as Hjort's critical period hypothesis (Hjort, 1914) or Lasker's stable ocean hypothesis (see Lasker, 1985). It is clear that a variety of physical and biological processes can interact to affect recruitment strength in clupeids and other fishes (Houde, 2008) and that the dominant process(es) may not be stationary (Bakun *et al.*, 2010). Nonetheless, having sufficient prey resources during early larval life will always be a necessary prerequisite for the growth and survival of the larvae of clupeids and other fishes. In autumn and winter spawning herring in the NE Atlantic, feeding conditions are thought to impact on larval survival during the winter (Alvarez-Fernandez *et al.*, 2015; Hufnagl *et al.*, 2015), a period when recruitment is largely established in these stocks (Nash and Dickey-Collas, 2005; Payne *et al.*, 2013). Atlantic herring provides an excellent species to mechanistically explore the link between prey fields and growth in fish larvae since, (i) a wealth of field studies have been conducted on the larvae of different spawning populations (Geffen, 2009), (ii) laboratory experiments have examined the impacts of abiotic and biotic factors on larval feeding and growth, and (iii) this knowledge has been integrated into mechanistic, physiological-based models of foraging activity and growth (Hufnagl and Peck, 2011; Hufnagl *et al.*, 2015).

This study combined field sampling, statistical modelling and individual-based foraging and growth model simulations to examine the autumn protozooplankton assemblage and the potential strength of the microzooplankton–ichthyoplankton link in the Irish Sea. In each of 2 years, physical/hydrographic factors, protists (10–200 μm), nauplii and copepodites (50–300 μm) and herring larvae were sampled on a routine station grid of the ICES-coordinated Northern Irish herring larvae survey (NINEL). This survey has been conducted since 1993 and provided an ideal platform for simultaneous sampling of larvae and their prey. To examine the potential impact of protists and small mesozooplankton on the abundance and distribution of the herring larvae, a generalized linear mixed model (GLMM) was applied. Biochemical-based estimates of nutritional condition and growth of larvae were compared to the microzooplankton community composition and biomass at each station. Finally, these field data were used in larval herring physiological foraging and growth simulations (e.g. see Illing *et al.*, 2016) to explore whether *in situ* biomass of micro- and/or mesozooplankton were necessary and sufficient to support survival and obtain high rates of growth during the larval season of herring in the Irish Sea.

METHOD

Area of study and plankton sampling

Larval fish and zooplankton were collected during two cruises in the northern Irish Sea (north of 53.5° N) in early November 2012 and 2013 on board of the RV Corystes. Field sampling was conducted in the frame of the 62 station-grid of the NINEL (Agri-Food and Bioscience Institute, Belfast, Northern Ireland) (Fig. 1). Herring larvae were sampled using a Gulf VII high speed net (280 μm mesh size, 0.4 m nose cone opening), towed in double oblique hauls (3–5 knots) from the surface to 2–3 m above the seafloor. This net was equipped with a flowmeter (Valeport Midas) and a CTD (Valeport) with a fluorometer (Seapoint chlorophyll). Sampling with this high-speed gear was conducted day and night and according to McGurk (1992), the day/night catch ratio for larvae <10 mm is close to 1, increases with increasing larval size and never exceeds 2.5 in larvae <25 mm. The majority of the larvae caught in this study were <12 mm, hence, day/night differences were neglected.

At each station, all larvae were removed, counted and the standard length (SL) of each individual (or a subsample when stations contained >250 larvae) was measured (Olympus SZ40, ± 0.1 mm). Some larvae were immediately transferred to individual Eppendorf vials containing RNAlater©, a storage reagent preserving RNA and DNA in unfrozen tissue up to 4 months at 5°C (see below), and all the rest of the sample was preserved in 70% ethanol. Depth-integrated larval abundance (ind*m^{-2}) was calculated as

$$\text{Abundance (no.*m}^{-2}\text{)} = \text{Density(no.*m}^{-3}\text{)} * \text{bottom depth (m)}$$

Besides the routine NINEL larval herring sampling, nine stations in 2012 and 12 stations in 2013 were sampled for protozooplankton and microzooplankton (52–300 μm) (Fig. 1). Protozooplankton was sampled with Niskin bottles in a CTD rosette (Seabird 19plus V2). Water samples were collected from near the surface (max. 4 m depth) and a 500-mL subsample was immediately stored in a brown glass bottle and preserved with neutral Lugol's solution (2% final concentration). Larger microzooplankton (52–300 μm) was sampled with a small plankton net (PUP-net, 52 μm mesh size) mounted on the Gulf sampler to simultaneously sample larval fish and their potential prey. These samples were preserved in 4% buffered formalin and, once in the laboratory, divided in two size fractions (52–100 and 100–300 μm).

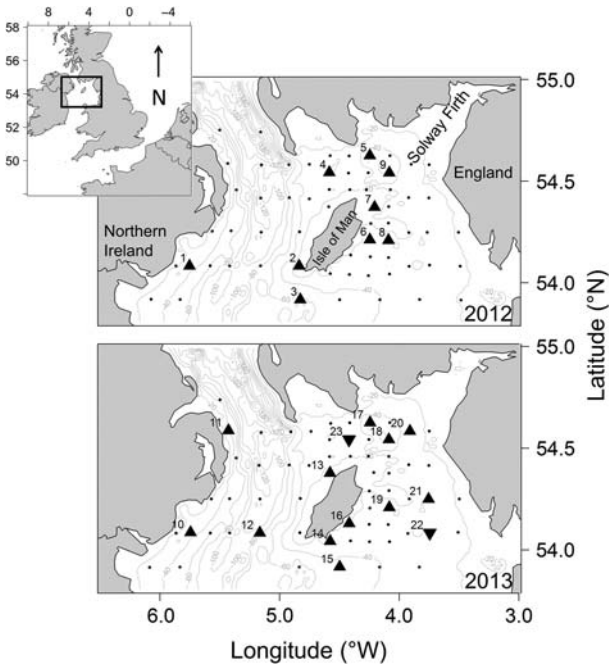

Fig. 1. Sampling stations of the Irish Sea herring larvae survey in the autumns of 2012 and 2013 (·). Stations with additional microzooplankton sampling (▲) are labeled from 1 to 9 for 2012 and 10–21 for 2013, labelling is from West to East. Stations 22 and 23 (▼) were not sampled for microzooplankton, only herring larvae were sampled.

All nauplii and copepodites were counted using a stereomicroscope (Leica MZ16 magnification 2.5×). For each sample and size fraction, the prosome length of a minimum of 30 nauplii and copepodites was measured using image analysis (ImagePro, Media Cybernetics). No correction for shrinkage was applied. In order to calculate the biomass of both size classes, a mean value of carbon contents from abundant copepod species in the North and Baltic Seas was used based on length–weight relationships derived from different studies (see Kühn *et al.*, 2008).

Hydrographic conditions

Among station differences in hydrographic characteristics, i.e. temperature, salinity, fluorescence at 10-m depth and bottom depth, of stations were analyzed with Principal Component Analysis (PCA) using the euclidean distance with PRIMER 6 (Clarke and Warwick, 2005).

Protozooplankton identification and community composition analysis

Once in the laboratory, water samples were settled in a 100-mL sedimentation chamber (HydroBios) for 48 h (HELCOM, 2014) and counted under an inverted microscope (Leica DMI 3000, 200× with Moticam camera attached) using methods described by Utermöhl (1958). The whole chamber was counted to avoid under-representation of less abundant groups. Ciliates and dinoflagellates were identified to the lowest taxonomic level possible, following Dodge and Hart-Jones (1982); Montagnes (1996); Olenina *et al.* (2006); Strüder-Kypke *et al.* (2006); Hoppenrath *et al.* (2009); Kraberg *et al.* (2010). The classification of Löder *et al.* (2012) for the trophic status of dinoflagellates was used. Some taxonomic groups were separated by size class to ensure more precise calculations of biomass. The ciliate *Mesodinium rubrum*, commonly classified as mixotroph, was included in the analysis to cover the whole ciliate community. Other groups were not identified because they were >200 µm and/or underrepresented in the samples (e.g. copepod nauplii, invertebrate larvae). Additionally, *Heterocapsa* sp. and small Gymnodiniales were included in the analysis because of their high abundance at stations although their size was <20 µm.

For the most abundant groups, the size of the organisms ($n > 10$ per taxon) was measured using image analysis (Image J, 1.6.0, freeware, Wayne Rasband) without correction for shrinkage, and then their biovolume (μm^3) was estimated assuming specific geometric shapes (Hillebrand *et al.*, 1999; Olenina *et al.*, 2006; Strüder-Kypke *et al.*, 2006). The biovolume of less abundant groups was estimated from literature values (Olenina *et al.*, 2006; Strüder-Kypke *et al.*, 2006; Löder *et al.*, 2012). Biovolume (μm^3) was converted to *in situ* carbon biomass ($\mu g\ C*L^{-1}$) using the C:Vol relationship for protists reported by Menden-Deuer and Lessard (2000).

The structure of the protozooplankton community was studied using multivariate analysis using PRIMER 6 (Clarke and Warwick, 2005). Then, hierarchical agglomerative cluster analysis in conjuction with nonmetric multidimensional scaling were applied to identify protozooplankton assemblages. To avoid the noise-derived effects of rare taxa, only taxa present at ≥2 stations were included in the analysis. Biomass data were $\log_{10}(x+1)$ transformed and a Bray–Curtis similarity matrix was built. Hierarchical agglomerative clustering was conducted on this matrix to find natural groupings of samples. The adequacy of the groups was tested with a similarity profile (SIMPROF). To elucidate which specific taxa defined the groups, all taxa contributing 90% to the (dis)similarity were identified performing a similarity percentages routine (SIMPER). Finally, the BioEnv routine was performed to find the best match between multivariate among sample patterns of protozooplankton assemblages and environmental variables (depth, salinity, temperature and fluorescence) associated to those samples. The correlation is expressed as weighted Spearman rank correlation ρ.

An analysis of similarity (ANOSIM) was performed to reveal statistically significant similarities within the years. The ANOSIM results are based on the test statistic R (between 0 and 1), which gives the strength of the factor on the samples and the significance level p ($\alpha = 0.05$).

Larval herring nutritional condition

In the laboratory, RNAlater was removed and the samples were stored at −80°C until further analysis. Larvae were freeze dried (Christ Alpha 1, 4 LSC) and dry weight (DW) was determined using a microbalance (Sartorius Genus SE2, ± 0.1 µg). In some cases, crystals of RNAlater biased the DW measurements, and DW was estimated from SL according to: $DW = 0.057* SL^{3.36}$ (MAP, unpublished data). The RNA–DNA ratio was measured according to a modified protocol of Caldarone *et al.* (2001) with ethidium bromide as fluorescent dye. Measurements were done on individual larvae except for those <140 µg which were pooled. The RNA–DNA ratio was standardized (sRD) based on the slopes of the RNA and DNA standards (a factor of 2.4 was used) (Caldarone *et al.*, 2006). The sRD was used to calculate the instantaneous growth rate ($*d^{-1}$) (Buckley *et al.*, 2008).

Microzooplankton–ichthyoplankton link

Generalized linear mixed model

Because of the low spatial frequency of plankton sampling, a weighted average of the plankton abundance was calculated for each herring station using the inverse distance method. A spatial distance between stations of 5, 10, 15 and 20 km, allowed 21, 39, 60 and 85 stations, respectively, to be included in the model. This reduced the spatial resolution of the larval sampling, but allowed comparison of larval and plankton distribution using GLMMs (Zuur, 2009). GLMMs were applied to each dataset using abundances of nauplii, copepods, ciliates and dinoflagellates as explanatory variables. Station depth was included in the model as a random intercept, to avoid the hydrographical differences between stations biasing the analyses. The Gamma family with a log transformation showed best results when modelling the data.

After an overall model was fit to the data, the non-significant explanatory variables at the >5% level were excluded one by one until only significant variables remained in the model. All models with only significant explanatory variables were compared by using the Akaike Information Coefficient (AIC).

Foraging and growth model

The *in situ* temperature and the concentration of microozooplankton in each of three size classes, <100 μm, 100–200 μm, 200–300 μm, representing protozooplankton, nauplii and copepodites, were used in individual-based model (IBM) simulations of larval herring survival and growth at each station. The general assumptions and extensive validations of the larval herring IBM were previously reported (Hufnagl and Peck, 2011; Hufnagl *et al.*, 2015; Illing *et al.*, 2016). In brief, the model is based on a balanced energy budget where energy available for growth (G) in each time step (1 h) was determined from the energy consumed (C), which was based on foraging success minus different loss terms. The latter are assimilation efficiency (β) and metabolic costs associated with routine activity (R) as well as the digestion of a meal (specific dynamic action, SDA):

$$G = C^{*}\beta^{*}(1 - \mathrm{SDA}) - R.$$

Both larval and prey size influence C via changes in handling time, encounter rate and capture success, and C is regulated by temperature via gut evacuation rate. Furthermore, size and temperature determine R. Thus, to predict G, information on prey size and concentration, larval length and temperature are required. From each sampling station, these data were extracted and growth (over a period of 5 days) was predicted. This relatively short time period was chosen to better compare the model results to the biochemically based growth rates. Prey in the model was classified by size using 100 μm bins and weight of individual prey particles was represented by the weight of the middle of the bin. The potential importance of microzooplankton to herring growth was examined by estimating G using three different prey scenarios including (i) all prey (protozooplankton, nauplii and copepodites) represented as 50, 150 and 250 μm prey, (ii) only microozooplankton (50 μm prey), and (iii) only nauplii and copepodites (150 and 250 μm prey). When G was zero, the larvae died in the simulation. Modelled and *in situ* (sRD-based) growth rates were compared for small-, medium- and large-sized (7–10, 11–14 and 15–18 mm length) larvae.

The model is also capable of determining the DW condition factor (CF = dry weight-at-length) of larvae as length and weight growth are partially uncoupled. Well fed and poorly fed larvae increase and decrease their CF, respectively, in the model. Since *in situ CF* of the larvae was not available, simulations were started with a set of larvae having the same length but different CFs (0.8, 0.9, 1.0, 1.1 and 1.2 times the average *DW* of a larva at a specific length; see Hufnagl and Peck, 2011) so that different feeding histories at the beginning of the simulation could be taken into account.

RESULTS

Hydrographic conditions

In both years, the water column at all stations was well mixed and the shallower waters in the eastern Irish Sea were colder, had lower salinity and higher fluorescence (relative values) compared to the deeper waters of the western Irish Sea (Fig. 2). In general, water temperature, salinity and fluorescence were higher in 2013 than in 2012. The mean (depth integrated) water temperature ranged from 9.9 to 12.9°C and from 11.1 to 12.9°C in 2012 and 2013, respectively (Fig. 2A and B). The range in salinity (~31.4 to 34.4) was similar in both years but 75% of the stations had a higher salinity in 2013 (Fig. 2C and D).

Stations were grouped into three classes according to differences in their hydrographic characteristics revealed by the PCA (Fig. 3): Warm (T > 11.9°C), Cold (T < 11.5°C) and Cold + Productive (T < 11.5°C, Fluorescence >1.5). The PC1 explained 61.2% of the variation between the stations, mainly due to the effect of temperature and relative fluorescence (Table I). A total of 21.9% of the variation was explained by PC2, primarily driven by bottom depth.

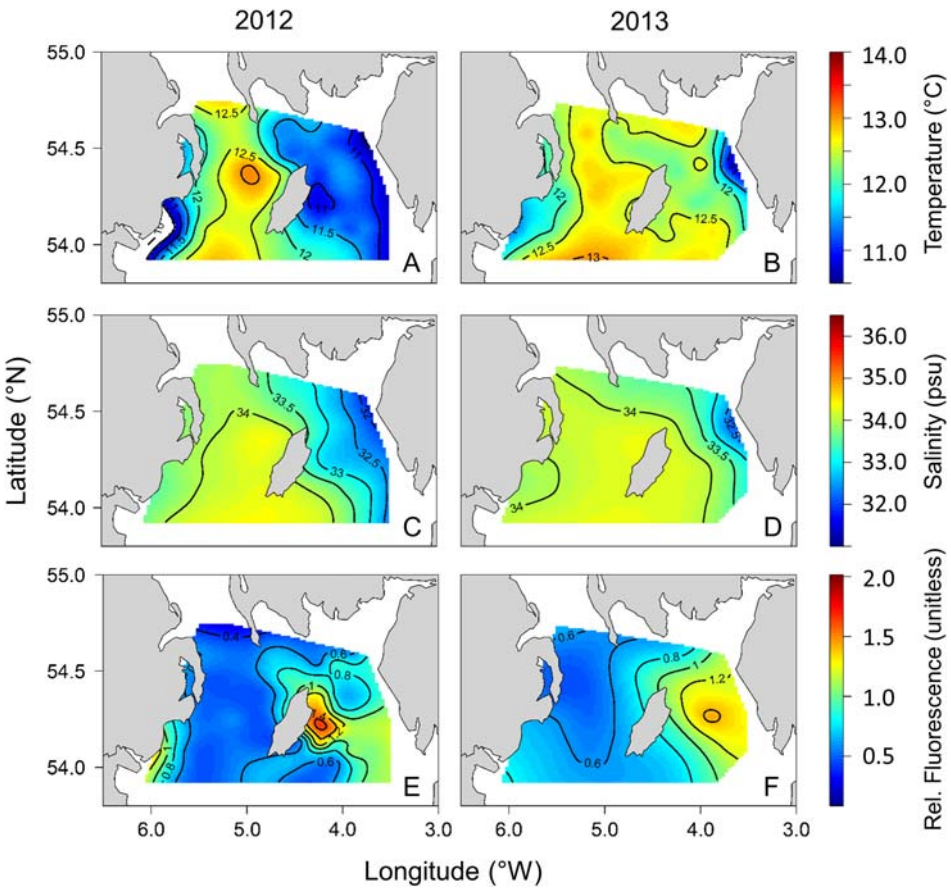

Fig. 2. Surface temperature (Panel **A**,**B**), salinity (Panel **C**,**D**) and fluorescence (Panel **E**,**F**) in the Irish Sea during the autumns of 2012 and 2013.

Micro- and small mesozooplankton abundance and biomass

A total of 29 different taxonomic groups of dinoflagellates and ciliates was identified (Table II). In both years, dinoflagellates were more abundant than ciliates and accounted for 75% of the total abundance in 2012 and 61% in 2013. The dinoflagellate *Gymnodinium* spp. was the most abundant taxon and occurred at all sampling stations. This taxon dominated the protozooplankton community in 2012 (>50.0%), but not in 2013 (24.1%) when other taxa such as *Strombidium* spp. or *Gyrodinium spirale* were also relatively abundant (>18.0%) (Table II). For ciliates, *Strombidium* spp. was the most abundant taxon.

The mean total biomass of dinoflagellates and ciliates was higher in 2012 (3.06 μg C*L^{-1}) compared to 2013 (1.85 μg C*L^{-1}). The contribution of dinoflagellates to total biomass was <50% in 2012 and >60% in 2013. The dinoflagellate contributing most to biomass was *Protoperidinium* spp. (16.6%) in 2012 and *Ceratium furca* (24.4%) in 2013 (Fig. 4). *Strombidium* spp was the ciliate taxon contributing most to the mean biomass in both years.

The highest biomass of ciliates and dinoflagellates was found in the shallow area east of the Isle of Man (Fig. 5A and B). The maximum biomass (5.32 μg C*L^{-1}) was recorded at Station 6 (2012), different ciliate taxa, such as *Strombidium* spp. and *M. rubrum* accounted for 65% of the biomass. In 2013, a maximum biomass of 4.37 was found at Station 20, where *C. furca* made up 43% of the biomass. The average biomass of copepod nauplii (2.83 μg C*L^{-1} in 2012 and 1.15 μg C*L^{-1} in 2013) and copepodites (1.35 μg C*L^{-1} in 2012 and 0.51 μg C*L^{-1} in 2013) was higher in 2012 than in 2013. In contrast to the protists, the highest biomass of nauplii and copepodites was found in shallow waters on the coast of Northern Ireland in 2012 (Fig. 5C–F). Note, the flowmeter malfunctioned at Station 6 in 2012 and the nauplii and copepodites from that station could not be used in further analyses.

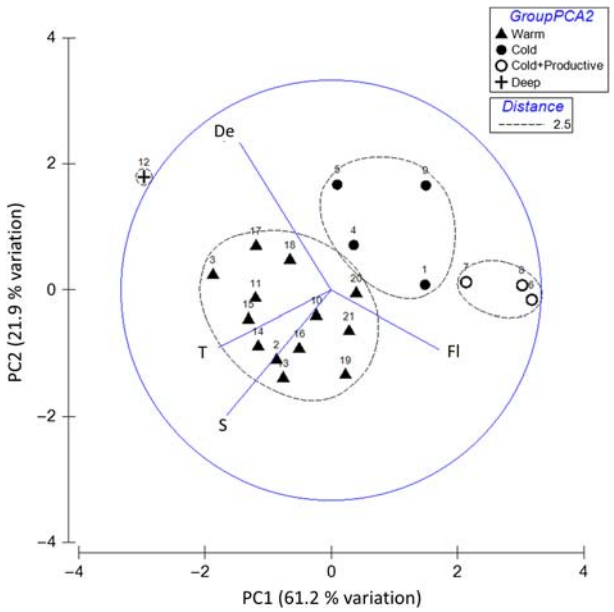

Fig. 3. PCA for the plankton sampling stations 2012 and 2013. Using the available environmental data (De = depth, *T* = temperature, *S* = salinity and Fl = fluorescence at 10 m depth) three groups could be distinguished: Warm, Cold and Cold+Productive.

Table I: Eigenvectors of the two principal components (PC) gained from the PCA including all available hydrographic factors from 2012 and 2013 combined

Variable	PC1	PC2
Depth	−0.434	0.700
Temperature	−0.549	−0.279
Salinity	−0.496	−0.594
Fluorescence	0.513	−0.281

Protozooplankton community structure

Hierarchical clustering (together with the SIMPROF) identified three main protozooplankton groups (Fig. 6): "East 2013," "East 2012" and "Mixed." Only a maximum of 10 taxa explained 90% of the similarity in all three groups (Table III). The "Mixed" group was mainly composed by the ciliate taxon *Strombidum* spp. (~31%), and in lower numbers *Gymnodinium* spp., *Stenosemella* sp. and *Tintinnid* spp. On the other hand the "East 2012" group had high numbers of *Protoperidinium* spp. (17%) and *Gymnodinium* spp. (16%), and the "East 2013" group of *C. furca* (30%). The main taxa separating each group (Dissimilarity/standard deviation, Diss/SD > 1.3) were *Protoperidinium* spp., *Leegardiella* cf *ovalis* and *Gymnodinium* sp ("East 2012" vs "Mixed"), *C. furca* and *Protoperidinium* spp. ("East 2013" vs "Mixed") and *C. furca* ("East 2012" vs "East 2013") (Table IV).

Salinity best explained the variability in protozooplankton community (BIO-ENV, $\rho = 0.54$) followed by the combination of temperature and salinity ($\rho = 0.51$). The effect of light (day, night) was positive ($R = 0.29$) but was not significant ($P > 0.05$); therefore, this factor was not included in the analysis.

Herring larvae abundance and nutritional condition

In both years, yolk-sac and first-feeding larvae (<12 mm SL) were most abundant to the east and north of the Isle of Man (Fig. 5 G–H) where they reached peak abundances of 430 and 365 ind*m^{-2} in 2012 and 2013, respectively. In 2012, larvae were between 5.2 and 31.3 mm SL, and most of the largest larvae (>22 mm SL) were found southeast of the Isle of Man. In 2013, all larvae were <22 mm SL and only a few larvae were found south east of the Isle of Man. The mean (±SE) sRD of 8- to 14-mm SL larvae was 3.35 (±0.89) and 2.86 (±0.97) in 2012 and 2013, respectively.

Microzooplankton–ichthyoplankton link

Generalized linear mixed model

The model that best described patterns of abundance of herring larvae was:

$$\log(\mathcal{N}\_\text{Larvae})_{ij} = (\beta_0 + b_{0i}) + (\beta_1 \times \mathcal{N}\_\text{Dinoflagellates}) + (\beta_2 \times \mathcal{N}\_\text{Copepodites}) + \varepsilon_{ijk},\ \varepsilon_{ij} \sim \mathcal{N}(0, \sigma_{2k})$$

where $(\beta_0 + b_{0i})$ represents the intercept (and its random variation), and β_1 and β_2 represent the slopes of regressions using dinoflagellates and copepodites, respectively. Parameter β_1 was consistently positive and β_2 consistently negative, representing a positive and negative effect of dinoflagellates and copepodites on herring abundance (ind*m^{-2}), respectively (Table V). This model did not depend on the type of spatial averaging (coupling distance) used to create the data set. The only variable that was also significant and slightly improved the model was fluorescence, but this was only true for a coupling distance of 20 km. The improvement was not enough to be considered according to the Akaike Information Coefficient (ΔAIC < 2).

Table II: Protists identified in water samples collected from the Irish Sea during the autumns of 2012 and 2013. The total abundance and biomass, and the relative abundance and biomass are listed

			Abundance (Ind*L^{-1})				Biomass (µg C*L^{-1})			
	Family	Species	2012	rel. A%	2013	rel. A%	2012	rel. C%	2013	rel. C%
Ciliates	Strombidiidae	*Strombidium* spp	7030	9.6	6190	20.1	3.92	14.8	3.73	16.9
	Strobiliidae	*Strobilidium* spp	1210	1.6	920	3.0	0.98	3.7	0.63	2.8
	Lohmanniellidae	*Lohmaniella oviformis*	760	1	110	0.4	0.13	0.5	0.06	0.3
	Mesodiniidae	*Mesodinium rubrum*	3070	4.2	500	1.6	2.48	9.3	0.18	0.8
	Tintinnidiidae	*Tintinnid* spp.	220	0.3	300	1.0	1.31	4.9	1.79	8.1
	Codonellopsidae	*Stenosemella* sp.	280	0.4	1300	4.2	0.55	2.1	0.93	4.2
	Balanionidae	*Balanion comatum*	1150	1.6	740	2.4	0.08	0.3	0.07	0.3
	Euplotidae	*Euplotes* sp.	20	0	10	0.0	0.02	0.1	0.01	0
	Leegaardiellidae	*Leegardiella* cf *ovalis*	4000	5.5	1510	4.9	2.94	11.1	0.75	3.4
	Tontoniidae	*Laboea strobila*	190	0.3	10	0.0	1.94	7.3	0.1	0.4
	Tontoniidae	*Tontonia* cf *gracillima*	30	0	30	0.1	0.06	0.2	0.09	0.4
	Colepidae	*Tiarina fusus*	30	0	60	0.2	0.04	0.2	0.11	0.5
	Spathidiidae	*Spathidium* sp	220	0.3	240	0.8	0.07	0.3	0.03	0.2
Dinoflagellates	Gymnodiniaceae	*Gymnodinium* spp	37 760	51.5	7410	24.1	3.76	14.1	1.61	7.3
	Gymnodiniaceae	*Gyrodinium spirale*	870	1.2	4240	13.8	1.18	4.4	0.94	4.2
	Gymnodiniaceae	*Akashiwo* sp.	0	0	10	0.0	0	0	0.04	0.2
	Gymnodiniaceae	*Amphidinium* spp	120	0.2	380	1.2	0.01	0	0.1	0.4
	Gymnodiniaceae	*Torodinium robustum*	4560	6.2	2370	7.7	1.79	6.7	0.74	3.3
	Gymnodiniaceae	*Katodinium glaucum*	510	0.7	120	0.4	0.08	0.3	0.02	0.1
	Dinophysiaceae	*Dinophysis* sp.	0	0	170	0.6	0.00	0	0.31	1.4
	Protoperidiniaceae	*Diplopsalis lenticula*	140	0.2	20	0.1	0.14	0.5	0.1	0.5
	Protoperidiniaceae	*Protoperidinium* spp	1850	2.5	990	3.2	4.42	16.6	2.68	12.1
	Warnowiaceae	*Warnowia* sp.	210	0.3	0	0.0	0.25	0.9	0.18	0.8
	Ceratiaceae	*Ceratium tripos*	0	0	30	0.1	0	0	0.14	0.6
	Ceratiaceae	*Ceratium* cf *macroceros*	90	0.1	200	0.7	0.40	1.5	0.82	3.7
	Ceratiaceae	*Ceratium furca*	140	0.2	1180	3.8	0.74	2.8	5.36	24.2
	Ceratiaceae	*Ceratium fusus*	110	0.1	360	1.2	0.15	0.6	0.46	2.1
	Ceratiaceae	*Ceratium lineatum*	0	0	10	0.0	0	0	0.02	0.1
	Prorocentraceae	*Prorocentrum micans*	90	0.1	540	1.8	0.01	0	0.18	0.8
	Peridiniaceae	*Heterocapsa* cf *rotundata*	8730	11.90	790	2.6	0.11	0.40	0.03	0.10

Foraging and growth model (IBM)

The *in situ* (sRD-based) instantaneous growth rate of larvae ranged from 0.05 to 0.37*d^{-1}, the highest mean growth was observed for larvae >11 mm SL at Stations 2, 8 and 9 in 2012, and 18 and 20 in 2013 (Fig. 7). At those stations, the IBM predicted a median growth rate of ~0.2*d^{-1} and maximum growth rates of 0.24, 0.17, 0.20 (2012) and in 2013 of 0.25 and 0.24 (*d^{-1}), respectively. In the IBM, the maximum growth of smaller (<11 mm) larvae ranged from 0 (*d^{-1}) (3, 4, 6, 11–17, 19) to 0.01 (5) and 0.02 (7) to 0.17 (*d^{-1}) (2, 8, 9, 18, 20), respectively. These results were obtained when all prey categories (<100,100–200 and 200–300 µm) were used in the simulation. If only microzooplankton (<200 µm, no copepodites) was used, modelled growth rate was zero at all stations. If only large microzooplankton and mesozooplankton were used (100–300 µm), larvae survived but G was 49% of that simulated when all prey classes, including protozooplankton, were included. In both years, the IBM predicted zero growth (no survival) at several stations where larvae exhibited positive *in situ* growth (sRD-derived growth).

DISCUSSION

This study combined *in situ* sampling, laboratory taxonomic analyses, statistical analyses as well as IBM simulations to explore the potential link between the abundance and condition of herring larvae and the biomass and composition of micro- and small mesozooplankton. It included two consecutive years of station grid sampling in the Irish Sea. Both years, 2012 and 2013, appear to be "typical" in terms of the spatial distribution and abundance of herring larvae in this survey (Dickey-Collas *et al.*, 2001; ICES, 2014). Larvae were generally more abundant in shallow waters near adult spawning grounds, and less abundant in deeper areas such as the Northern channel.

Microzooplankton community composition

In both years, the microzooplankton assemblage was dominated by protozooplankton <50 µm which agrees well with the results of previous studies conducted during autumn and winter in the adjacent North Sea

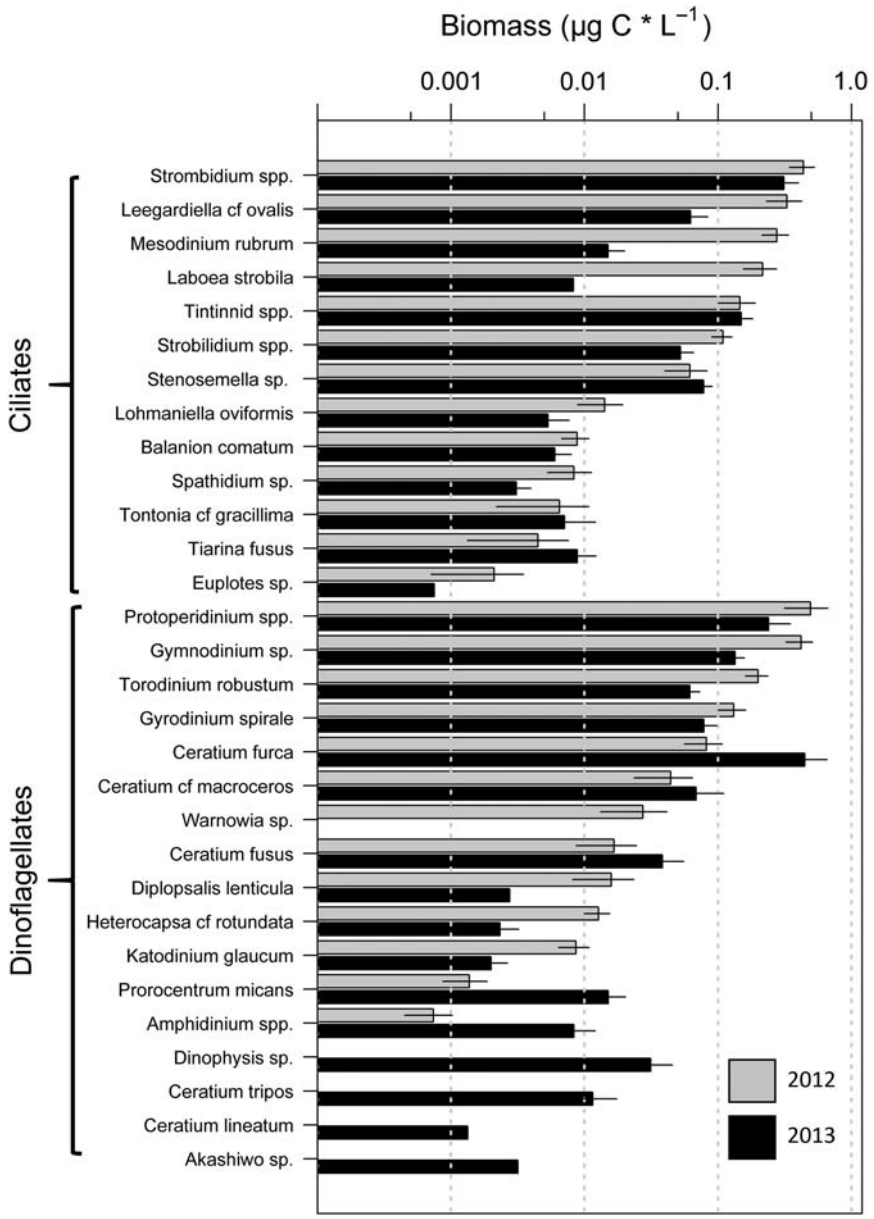

Fig. 4. Mean biomass ± SE (µg C*L^{-1}) of all ciliate and dinoflagellate taxa identified in 2012 and 2013 during the Irish Sea herring larvae survey. Taxa are ranked first by group and then by the biomass of 2012. Missing bars indicate the absence of the taxon.

(Löder *et al.*, 2012; Yang *et al.*, 2015). The most abundant components were dinoflagellates of the order Gymnodiniales and specimens of *Protoperidinium* spp. along with various ciliates such as members of the genus *Strombidium*. These results correspond well with previous studies conducted in the Irish Sea in autumn/winter (Figueiredo *et al.*, 2009; Scherer, 2012) and also in other temperate shelf seas such as the North Sea where Gymnodiniales and Strombidiids dominated the microzooplankton community (Löder *et al.*, 2012; Yang *et al.*, 2015). Similar to the results of the present study, those studies reported that the relative abundance of loricated ciliates was low compared to aloricate species. The dinoflagellate *C. furca*, which occured at high biomass at a few stations in the east in 2013, is commonly classified as an autotroph (and therefore not considered in most protozoa studies). However, *Ceratium* spp. are common in the Irish Sea (Montagnes *et al.*, 1999; Scherer, 2012) and, can potentially dominate the dinoflagellate biomass (Figueiredo *et al.*, 2009).

Figueiredo *et al.* (2005) examined the protozoan biomass at three stations located on the east coast of the

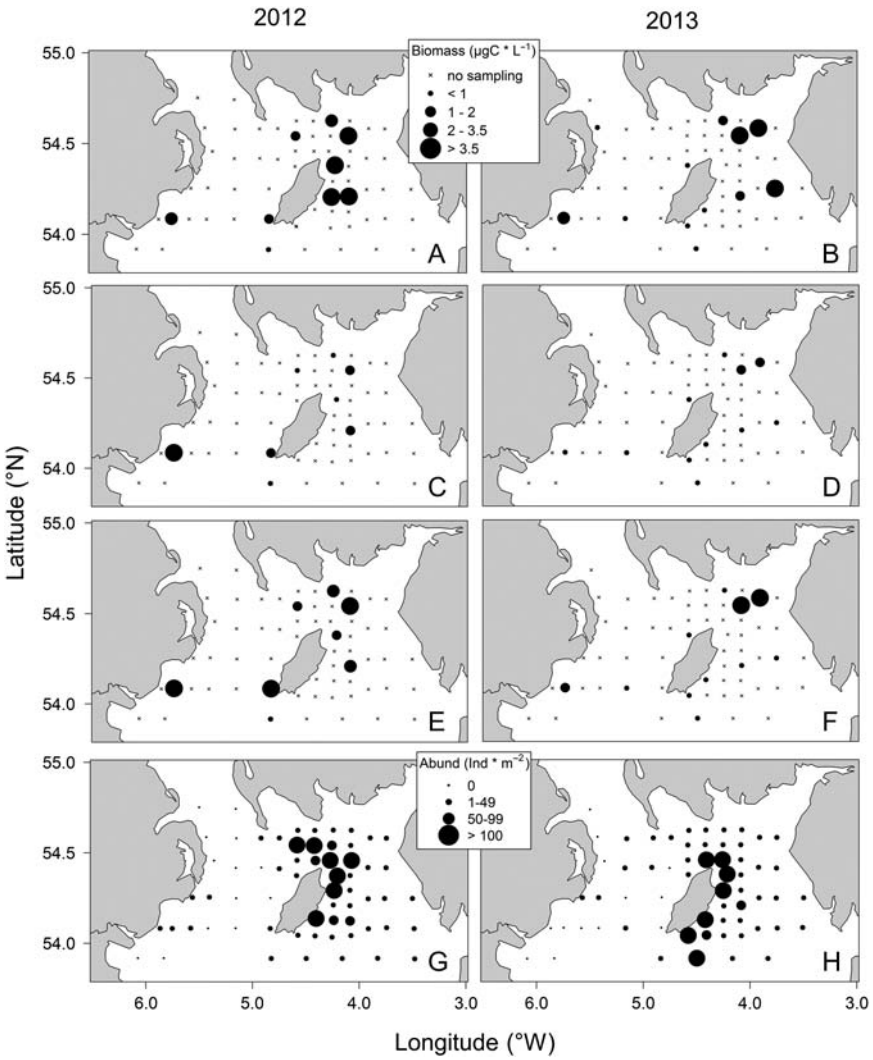

Fig. 5. Biomass (µg C*L^{-1}) of ciliates and dinoflagellates (Panel **A**,**B**), nauplii (Panel **C**,**D**) and copepodites (Panel **E**,**F**) of the 21 stations sampled and abundance (Ind*m^{-2}) of herring larvae <12 mm (Panel **G**,**H**) during the Irish Sea herring larvae survey in 2012 and 2013.

Isle of Man in autumn and found a maximum biomass of ~6 µg C*L^{-1}, which is comparable to that (5.37 µg C*L^{-1}) estimated in the present study. The average total biomass in the present study agrees well with previously reported biomass during autumn/winter in the Irish Sea (Figueiredo *et al.*, 2005, Scherer, 2012). Therefore, the composition and biomass of the microzooplankton community for the eastern and western Irish Sea found in 2012 and 2013 appears to be representative for this area and season.

In good agreement with our study, no other relevant mesozooplankton groups besides nauplii, copepodites and copepods were reported to occur in autumn by Figueiredo *et al.* (2005). In that study conducted in late October, copepods accounted for a substantial portion (>90%) of the total biomass (11.5 µg C*L^{-1}).

Our study observed a 4-fold lower copepod biomass (2.7 µg C*L^{-1}), but it is important to keep in mind that only size classes <300 were included in the calculation.

Spatial distribution of microzooplankton and small mesozooplankton

Throughout spring and summer, the western Irish Sea is characterized by the development of a cyclonic gyre which persists until October, leads to stratification and increases the retention times of plankton (Department of Energy and Climate Change, 2009). With the onset of autumn storms, the gyre subsides and Irish Sea waters become well mixed during winter. The coastal waters in the eastern Irish Sea are exposed to high riverine inflow, leading to lower salinities and higher nutrient

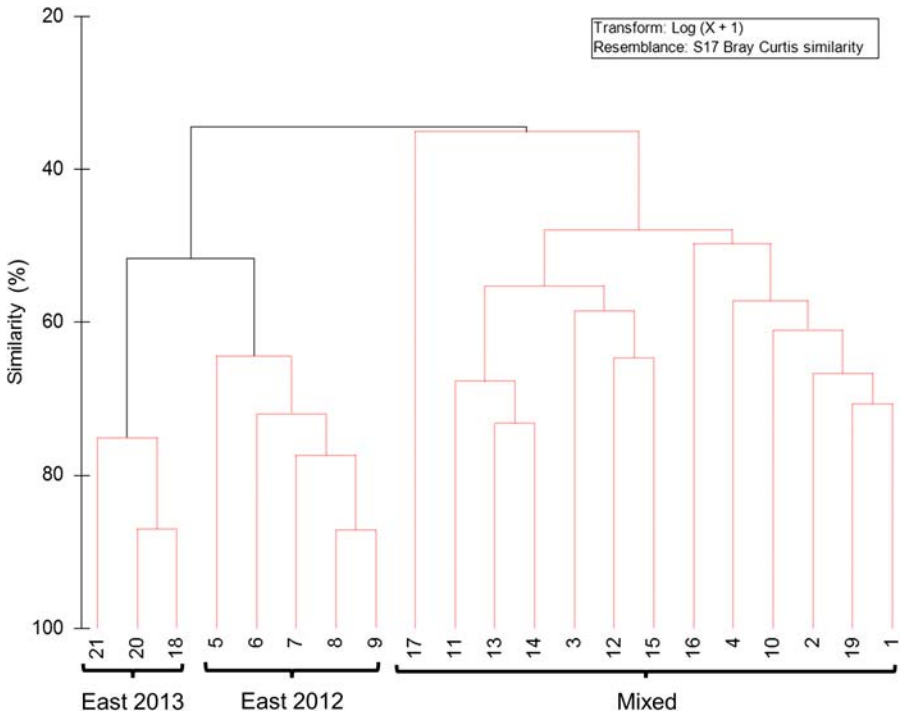

Fig. 6. Hierarchical clustering of the microzooplankton community (Ciliates, Dinoflagellates) during Irish Sea herring larvae survey in autumn 2012 and 2013. Three groups of stations were identified using the SIMPROF analysis (see text for further details).

Table III: Similarity percentage analysis (SIMPER) of the biomass of protists in the Irish Sea among stations for the groups distinguished by the SIMPROF analyses

	Mixed			East 2012			East 2013		
Species	Av.Sim	Sim/SD	Contrib%	Av.Sim	Sim/SD	Contrib%	Av.Sim	Sim/SD	Contrib%
Strombidium spp.	15.5	1.7	**30.97**	8.48	7.05	11.84	5.09	23.26	6.41
Gymnodinium spp.	6.51	1.61	13.02	11.49	3.93	16.05	5.62	11	7.07
Stenosemella sp.	5.57	1.78	11.12						
Tintinnid spp.	5.42	0.92	10.83				3.46	1.36	4.35
Torodinium robustum	3.41	1.91	6.81	5.93	6.37	8.27	3.15	8.64	3.97
Strobilidium spp.	2.72	1.09	5.44	3.25	4.79	4.54			
Gyrodinium spirale	2.63	1.84	5.26	4.3	4.21	6	4.94	25.88	6.22
Leegardiella cf *ovalis*	2.29	1.63	4.57	9.26	2.21	12.93	2.86	6.58	3.6
Mesodinium rubrum	1.63	0.75	3.26	6.28	4.72	8.76			
Protoperidinium spp.				11.84	2.33	**16.53**	18.75	17.71	23.62
Ceratium furca				2.8	2.44	3.91	23.91	2.67	**30.11**
Laboea strobila				2.78	0.98	3.88			
Ceratium cf *macroceros*							4.37	1.54	5.51

The taxa contributing most to the similarity are listed, the most contributing is printed in bold. The list contains taxa contributing to 90% of the similarity (smaller contributions are not shown).

concentrations (Howarth, 2005). Previous studies on the microzooplankton of the Irish Sea focused on time periods characterized by stratification and the presence of the gyre (Edwards and Burkill, 1995; Montagnes *et al.*, 1999). In contrast to those studies, the present study was conducted in autumn when the gyre was absent, likely explaining why the highest microzooplankton biomass was found in the eastern Irish Sea. This is important because herring spawning grounds are located in the eastern Irish Sea.

The station groups identified by hydrography (PCA) and plankton composition (hierarchical clustering) were similar. In terms of the latter, the high ratio of similarity to SD (>1.3) suggested that plankton was a good discriminator of station groups (Clarke and Warwick, 1994). Stations classified by hierarchical clustering as

Table IV: Similarity percentage analysis (SIMPER) results for the three protist groups identified in Fig. 6

	Mixed/East 2012			Mixed/East 2013			East2012/East 2013		
Species	Av.Diss	Diss/SD	Contrib%	Av.Diss	Diss/SD	Contrib%	Av.Diss	Diss/SD	Contrib%
Protoperidinium spp	11.74	1.96	**18.09**	13.64	4.7	**20.53**	3.03	1.44	6.28
Leegardiella cf *ovalis*	8.22	2.58	**12.68**	2.48	1.44	3.74	4.2	2.03	8.72
Gymnodinium spp.	8.06	2.33	**12.42**	2.27	1.59	3.42	4.22	2.28	8.76
Mesodinium rubrum	5.43	2.14	8.36				4.31	2.78	8.94
Strombidium spp	4.91	1.44	7.56	3.17	0.98	4.77	2.99	1.27	6.21
Torodinium robustum	4.18	2.56	6.45				1.99	2.19	4.13
Laboea strobila	4.14	1.31	6.37	1.46	0.82	2.2	2.76	1.28	5.72
Gyrodinium spirale	3.02	2.64	4.66	3.02	3.17	4.54			
Tintinnid spp.	2.83	1.05	4.36	2.61	1.27	3.93	1.95	1.36	4.05
Ceratium furca	2.72	1.96	4.2	20.63	3.06	**31.06**	12.06	3.08	**25.03**
Strobilidium spp	1.9	1.63	2.94				1.31	1.97	2.72
Ceratium cf *macroceros*	1.73	1.23	2.67	5.44	2.1	8.19	2.53	1.49	5.26
Ceratium fusus				2.37	2.49	3.57	1.15	1.74	2.38
Dinophysis sp.				1.88	1.68	2.83	1.32	1.85	2.75
Torodinium robustum				1.4	1.79	2.12			

The most important taxa contributing to the average dissimilarity among two groups are listed (percentage of contribution, Contrib%). Taxa with a high contribution (>10%) to the dissimilarity between groups are printed in bold. The ratio of the average dissimilarity between two groups to the SD (Diss/SD) is shown for each species.

*Table V: Fixed effects estimates ± SE of the GLMM relating the abundance of herring larvae (N_Larvae, ind*m^{-2}), dinoflagellates (N_Dino, ind*m^{-3}) and copepodites (N_Cop, ind*m^{-3}) in the Irish Sea in autumn using a coupling distance of 5 km*

	Estimate	SE	*t* value	*P*-value
Intercept	2.410 e^{+00}	3.507 e^{-01}	6.871	6.36 e^{-12}
Dinoflagellate abundance	2.337 e^{-04}	7.992 e^{-05}	2.925	0.00345
Copepod abundance	−1.724 e^{-01}	4.528 e^{-02}	−3.808	0.00014

"East 2012" and "East 2013" were characterized by colder and warmer temperatures, respectively. In general, stations were warmer in 2013. The remaining stations classified as "Mixed" were located close to the coast and/or in the western part of the Irish Sea. The similarity of "East 2012" was caused primarily by athecate dinoflagellates (*Protoperidinium* spp. and *Gymnodinium* spp.), whereas the thecate and mixotrophic dinoflagellate *C. furca* contributed most to the similarity of "East 2013" and the ciliate *Strombidium* spp. contributed most to "Mixed" group.

The more coastal and warmer "Mixed" stations may have exhibited favourable conditions for the dominance of ciliates. Ciliates can achieve higher growth rates than dinoflagellates (Hansen, 1992; Strom and Morello, 1998) depending on food availability and thermal conditions of the system (Johansson *et al.*, 2004; Aberle *et al.*, 2006). The lower water temperatures seemed to favour dinoflagellate growth but, due to a lack of nutrient data, we can only assume that the high abundance of the mixotrophic dinoflagellate *C. furca* at northeastern "East 2013" stations was caused by a high nutrient input from the Solway Firth as suggested from elevated fluorescence values (and thus higher primary production). Facultative autotrophs, which include many dinoflagellate taxa (Flynn *et al.*, 2013), would benefit from elevated nutrient levels associated with less saline waters in the vicinity to the Solway Firth. Hence, it may not be surprising that variability in the composition of this community was best explained by salinity (54%).

Microzooplankton-herring larvae link

Previous work has stressed the importance of protozoans such as dinoflagellates and nonloricate ciliates in the feeding, growth and survival of the larvae of clupeoid fish (Lasker, 1978; Ohman *et al.*, 1991). The present study employed indirect methods to examine the potential connection between the abundance and composition of prey and the abundance and nutritional condition of fish larvae. Direct methods examining this connection pose challenges including problems associated with gut content analyses in young larvae potentially feeding on extremely small, easily digested prey. Moreover, the usual fixation method for larval fish gut content analysis uses buffered formalin which decomposes naked protozoans, a major component of the microzooplankton community found in the present study. Figueiredo *et al.* (2005) speculated that up to 70% of the diet of larval herring could be composed of protozoan prey which suggests that a major component of the diet has been overlooked in the vast majority of field studies on larval

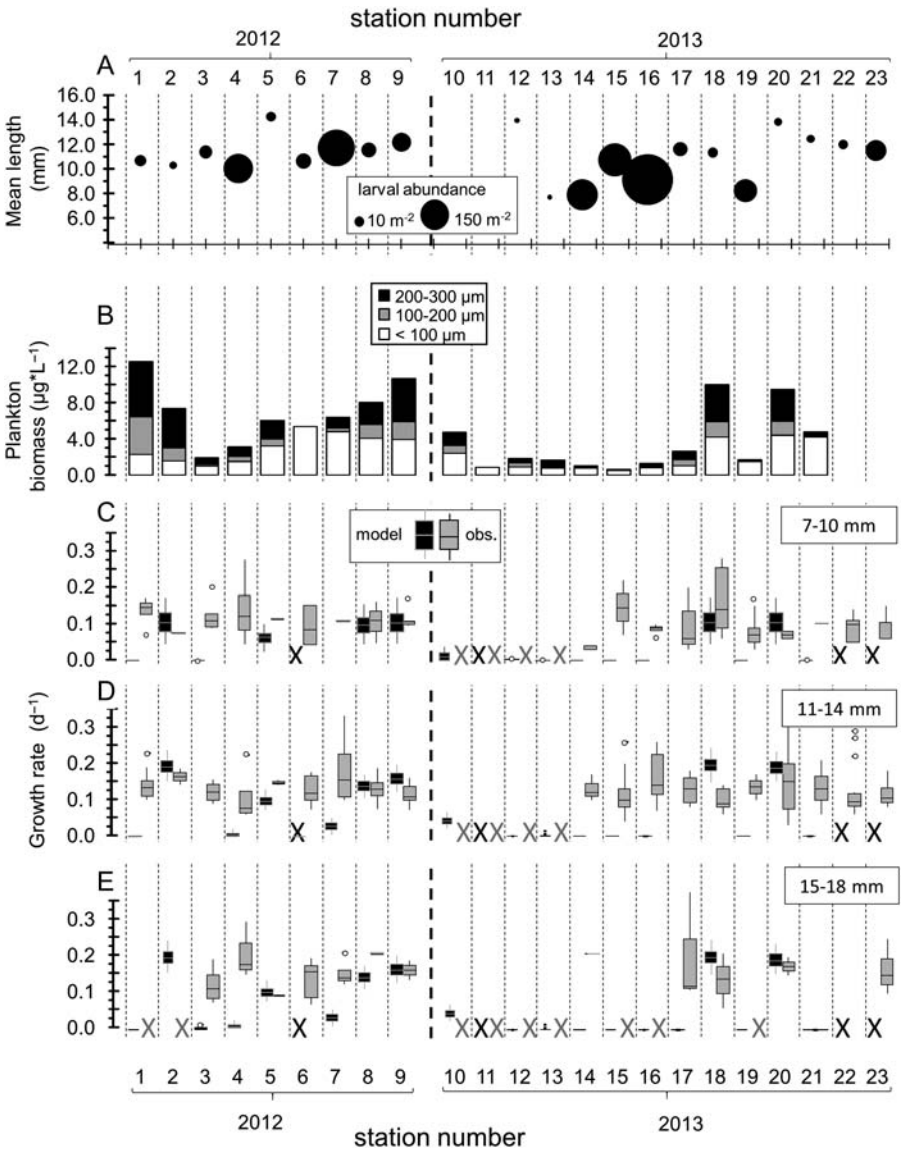

Fig. 7. Larval herring abundance (Ind*m^{-2}) and mean SL (mm) (Panel **A**), and plankton biomass of different size classes (µg C*L^{-1}) for the stations sampled during the Irish Sea herring larvae survey in 2012 and 2013. Observed (biochemically estimated) and modelled growth rates (*d^{-1}) for each of these stations are displayed for small (Panel **C**) and large herring larvae (Panel **D**). Note, X (grey) indicates that no larvae were measured for sRD and X indicates that not all plankton size fractions were sampled.

herring (and potentially other species) in this and other regions.

Based on our statistical analysis we can assume that the abundance of both dinoflagellates and copepodites was closely related to the abundance of herring larvae. Since neither size nor length was taken into consideration (for plankton or larvae) contradicting hypotheses can be drawn regarding the link between plankton and herring abundance. For example, copepodites could be considered either competitors of small herring larvae (i.e. both group consume microzooplankton), or as preferred prey consumed by larvae. Competition seems likely for larvae <8 mm SL, a size class accounting for ~25% of the total larval abundance. These young, small larvae have a maximum ingestible prey size of ~300 µm as estimated by Hufnagl and Peck (2011) and, therefore, copepodites are not expected to form a large portion of their diet. Similarly, a positive correlation between small larvae and dinoflagellate abundance may be expected because the former can consume the latter. On the other hand, dinoflagellate abundance is expected to increase as the predators of dinoflagellates (such as nauplii and large ciliates), are removed via predation by larvae. A larger dataset for the abundance of the three

groups (microzooplankton, mesozooplankton and larval herring) would be needed in order to make a strong conclusion. In the present study, spatial resolution was coarse and the model explained <20% of the overall variation in herring abundance. Although the GLMM did not identify strong causal relationships, these statistical model results provide a useful baseline for future efforts to conceptualize and understand the relationship between microzooplankton and larval fish.

Various studies employing different methods have estimated that herring larvae need ~2 μg C*L^{-1} of prey biomass to survive at temperatures close to those observed in the present study (Munk and Kiørboe, 1985; Figueiredo *et al.*, 2005; Peck *et al.*, 2012; Huebert and Peck 2014). The biomass of ciliates and dinoflagellates at some stations in our study would surpass this threshold but, at most stations and in both years, additional prey biomass from nauplii and copepodites would also be required to support observed (*in situ*) growth rates. There are a number of caveats related to this simple estimate. First, the zooplankton sampling conducted here represents only a snapshot in time. Second, average abundances calculated from net tows likely do not reflect what larvae actually encounter / experience in nature where prey is often distributed in patches (see Montagnes *et al.*, 1999; Young *et al.*, 2009). Working in well-mixed waters in the Irish Sea, Edwards and Burkill (1995) did not report any depth-related differences in the abundance of microzooplankton during summer, whereas Figueiredo *et al.* (2005) reported higher abundance at 20 m compared to the surface during autumn. Finally, biomass estimates of protozooplankton prey included all organisms between 10 and 200 μm and potential differences in food quality and prey preference were ignored. At Irish Sea stations, more than half of the total protozoan biomass consisted of cells <50 μm and yolk sac larvae of herring prefer prey sizes >29 μm (Spittler et al., 1990). This suggests that a narrow window of preferred prey sizes may have existed for the smallest larvae sampled in this study. Regardless, in both years, herring larvae were in relatively good nutritional condition with all individuals having an sRD > 1.6, which is above the negative growth threshold (sRD < 1.3) (Buckley *et al.*, 2008). This indicates that the larvae must have had appropriate feeding conditions throughout the sampling area.

The RD is an index of recent growth and responds relatively rapidly (days) to changes in feeding conditions (Peck *et al.*, 2012). Using *in situ* plankton abundance, IBM-based (simulated) growth rates of larvae tended to be lower than most (but not all) of the observed growth rates of 15- to 18-mm larvae at each station and in both years. In smaller (7- to 14-mm SL) larvae, where microzooplankton is thought to play a major role in the diet (Friedenberg *et al.*, 2012), the IBM drastically underpredicted *in situ* growth rates. The model predicted that larvae would not grow at some stations (Station 1 in 2012 and Stations 14, 15, 16 and 19 in 2013) where *in situ* (biochemical-based) growth rates were positive. The discrepancy could be due to temporal changes in prey fields occurring at each station a few days prior to sampling. Spatiotemporal change has ramifications for our ability to compare simulated and *in situ* growth rates of herring larvae. For example, at stations with good herring growth but low prey biomass, one could speculate that the micro- and small mesozooplankton community had been grazed down by herring larvae and other predators shortly before sampling. Pepin and Penney (2000) suggested the predation pressure exerted by the larval fish community (including *C. harengus*) could deplete the zooplankton prey field during spring and summer in Newfoundland.

Based on the mean protozoan biomass across the Irish Sea, our IBM results suggest that a diet based predominately on microzooplankton would not be sufficient to support larval growth and survival. This is especially true if the larvae are unable to filter feed or passively drink but must actively "snatch" microzooplankton. To pay for the costs of foraging and growth, larvae would need to feed on larger zooplankton (such as nauplii or copepodites). It is important to note, however, that excluding the microzooplankton fraction from the IBM prey field (i.e. feeding the larvae only with >100 μm zooplankton) resulted in lower predicted growth rates in all size classes of larvae because larger prey are associated with decreased capture success and longer handling times. Moreover, smaller larvae are expected to rely more heavily on smaller size classes of prey compared to larger larvae, suggesting that using prey fields in the IBM derived from higher resolution sampling of microzooplankton would provide more realism in the depiction of the foraging and growth of young, small larvae. Unfortunately, field data on the abundance, composition and biomass of prey <200 μm remain scarce in the Irish and North Seas, especially during autumn and winter months. Our results suggest that small microzooplankton may be important prey for larval fish, especially during time periods (such as the winter period) when larger microzooplankton may not be available.

CONCLUSIONS

The study combined field sampling of plankton (protozooplankton to fish larvae) and statistical and physiological-based modelling to examine the potential

relationship between micro- and small mesozooplankton and larvae of autumn-spawning herring in the Irish Sea. In the autumn of both 2012 and 2013, the protozoan community was dominated by Gymnodiniales and Strombidiids. The variability in the protozooplankton community was largely explained by salinity (54%), and thus it may be due to higher nutrient loads at coastal stations favouring the growth of mixotrophic protozoans. In contrast to other seasons, the highest biomass was found in the eastern Irish Sea in the vicinity of the main spawning ground for herring. Despite this spatial overlap, the total abundance of dinoflagellates and copepodites explained only 20% of the overall variation in larval abundance. In both years, the plankton was dominated by organisms smaller than the sizes preferred by herring larvae and physiological-based IBM simulations suggested that protozoans could not act as the sole or a major food source for larval herring. However, that model underestimated *in situ* growth of herring and larval growth declined when microzooplankton <100 μm were excluded from the simulations. This study highlights the importance of collecting field data not only on the spatial but also on the temporal changes in the abundance, composition and biomass of microplankton (20–200 μm) organisms. These data are scarce in most regions and need to be collected on standard ichthyoplankton surveys to adequately understand how the environment may limit the growth and survival of temperate marine fish.

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REFERENCES

Aberle, N., Lengfellner, K. and Sommer, U. (2006) Spring bloom succession, grazing impact and herbivore selectivity of ciliate communities in response to winter warming. *Oecologia*, **150**, 668–681.

Alvarez-Fernandez, S., Licandro, P., van Damme, C. J. G. and Hufnagl, M. (2015) Effect of zooplankton on fish larval abundance and distribution: a long-term study on North Sea herring (*Clupea harengus*). *ICES J. Mar. Sci.*, **72**, 2569–2577.

Azam, F., Fenche, T., Field, J. G., Gray, J. S., Meyer-Rei, L. A. and Thingstad, F. (1983) The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.*, **10**, 257–263.

Bakun, A., Babcock, E. A., Lluch-Cota, S. E., Santora, C. and Salvadeo, C. J. (2010) Issues of ecosystem-based management of forage fisheries in "open" non-stationary ecosystems: the example of the sardine fishery in the Gulf of California. *Rev. Fish Biol. Fish. Sci.*, **20**, 9–29.

Buckley, L. J., Caldarone, E. M. and Clemmesen, C. (2008) Multi-species larval fish growth model based on temperature and fluorometrically derived RNA/DNA ratios: results from a meta-analysis. *Mar. Ecol. Prog. Ser.*, **371**, 221–232.

Calbet, A. and Saiz, E. (2005) The ciliate-copepod link in marine ecosystems. *Aquat. Microb. Ecol.*, **38**, 157–167.

Caldarone, E. M., Clemmesen, C. M., Berdalet, E., Miller, T. J., Folkvord, A., Holt, G. J., Olivar, M. P. and Suthers, I. M. (2006) Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish: Intercalibration of RNA/DNA protocols. *Limnol. Oceanogr. Methods*, **4**, 153–163.

Caldarone, E. M., Wagner, M., St. Onge-Burns, J. and Buckley, L. J. (2001) Protocol and Guide for Estimating Nucleic Acids in Larval Fish Using a Fluorescence Microplate Reader. *NEFSC.* Ref. Doc. 01–11, 1 –22.

Clarke, K. R. and Warwick, R. (2005) *Primer-6 Computer Program.* Natural Environment Research Council, Plymouth.

Clarke, K. R. and Warwick, R. M. (1994) *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation.*. Natural Environment Research Council, Plymouth, pp. 172.

Department of Energy and Climate Change (2009) Offshore Energy Strategic Environmental Assessment Appendix 3-Environmental baseline, 357–360.

Dickey-Collas, M., Nash, R. D. and Brown, J. (2001) The location of spawning of Irish Sea herring (*Clupea harengus*). *J. Mar. Biol. Assoc. UK.*, **81**, 713–714.

Dodge, J. D. and Hart-Jones, B. (1982) *Marine Dinoflagellates of the British Isles.* Her Majesty's Stationary Office, London.

Edwards, E. S. and Burkill, P. H. (1995) Abundance, biomass and distribution of microzooplankton in the Irish Sea. *J. Plankton Res.*, **17**, 771–782.

Fenchel, T. (1988) Marine plankton food chains. *Annu. Rev. Ecol. Syst.*, **19**, 19–38.

Figueiredo, G. M., Nash, R. D. M. and Montagnes, D. J. S (2005) The role of the generally unrecognised microprey source as food for larval fish in the Irish Sea. *Mar. Biol.*, **148**, 395–404.

Figueiredo, G. M., Nash, R. D. M. and Montagnes, D. J. S. (2007) Do protozoa contribute significantly to the diet of larval fish in the Irish Sea? *J. Mar. Biol. Assoc. UK*, **87**, 843.

Figueiredo, G. M., Montagnes, D. J. S. and Nash, R. D. M. (2009) The importance of protozooplankton as prey for copepods in the coastal areas of the central Irish Sea. *Hydrobiologia*, **628**, 227–239.

Flynn, K. J., Stoecker, D. K., Mitra, A., Raven, J. A., Glibert, P. M., Hansen, P. J., Graneli, E. and Burkholder, J. M. (2013) Misuse of the phytoplanktonzooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *J. Plankton Res.*, **35**, 3–11.

Friedenberg, L. E., Bollens, S. M. and Rollwagen-Bollens, G. (2012) Feeding dynamics of larval Pacific herring (*Clupea pallasi*) on natural prey assemblages: the importance of protists. *Fish. Oceanogr.*, **21**, 95–108.

Fukami, K., Watanabe, A., Fujita, S., Yamaoka, K. and Nishijima, T. (1999) Predation on naked protozoan microzooplankton by fish larvae. *Mar. Ecol. Prog. Ser.*, **185**, 285–291.

Geffen, A. J. (2009) Advances in herring biology: from simple to complex, coping with plasticity and adaptability. *ICES J. Mar. Sci.*, **66**, 1688–1695.

Hansen, P. J. (1992) Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. *Mar. Biol.*, **114**, 327–334.

Hardy, A. C. (1924) The herring in relation to its animate environment. Part 1. The food and feeding habits of the herring with special reference to the east coast of England. *Fish. Invest. Ser. II*, **7**, 1–53.

HELCOM (2014) Manual for Marine Monitoring in the COMBINE Programme of HELCOM_PartC_AnnexC6.pdf. http://www.helcom.fi/Documents/ [Verified 19 August 2015]

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U. and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.

Hjort, J. (1914) Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer*, **20**, 1–228.

Hoppenrath, M., Elbrächter, M. and Drebes, G. (2009) *Marine Phytoplankton: Selected microphytoplankton species from the North Sea around Helgoland and Sylt*, 1st edn. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart. Kleine Senckenberg-Reihe 49.

Houde, E. D. (2008) Emerging from Hjort's Shadow. *J. Northwest Atl. Fish. Sci.*, **41**, 53–70.

Howarth, M. J. (2005) Hydrography of the Irish Sea. *SEA6 Technical Report*, Department of Trade and Industry offshore energy Strategic Assessment programme.

Huebert, K. B. and Peck, M. A. (2014) A day in the life of fish larvae: modeling foraging and growth using quirks. *PloS One*, **9**, e98205.

Hufnagl, M. and Peck, M. A. (2011) Physiological individual-based modelling of larval Atlantic herring (*Clupea harengus*) foraging and growth: insights on climate-driven life-history scheduling. *ICES J. Mar. Sci.*, **68**, 1170–1188.

Hufnagl, M., Peck, M. A., Nash, R. D. M. and Dickey-Collas, M. (2015) Unravelling the Gordian knot! Key processes impacting overwintering larval survival and growth: a North Sea herring case study. *Prog. Oceanogr.*, **138**, 486–503.

Hunt von Herbing, I. and Gallager, S. M. (2000)) Foraging behavior in early Atlantic cod larvae (*Gadus morhua*) feeding on a protozoan (*Balanion* sp.) and a copepod nauplius (*Pseudodiaptomus* sp.). *Mar. Biol.*, **136**, 591–602.

ICES (2014) *Report of the Working Group of International Pelagic Surveys (WGIPS)*, Copenhagen, Denmark.

Illing, B., Moyano, M., Berg, J., Hufnagl, M. and Peck, M. A. (2016) Behavioral and physiological responses to prey match-mismatch in larval herring. *Estuarine, Coastal Shelf Sci.*, doi:10.1016/j.ecss.2016.01.003 (in press).

Illing, B., Moyano, M., Niemax, J. and Peck, M. A. (2015) Direct effects of microalgae and protists on herring (*Clupea harengus*) yolk sac larvae. *PLoS One*, **10**, e0129344.

Johansson, M., Gorokhova, E. and Larsson, U. (2004) Annual variability in ciliate community structure, potential prey and predators in the open northern Baltic Sea proper. *J. Plankton Res.*, **26**, 67–80.

Kraberg, A., Baumann, M. and Dürselen, C. D. (2010) *Coastal Phytoplankton: Photo Guide for Northern European Seas*, 1st edn. Verlag Dr. Friedrich Pfeil, München.

Kühn, W., Peck, M. A., Hinrichsen, H.-H., Daewel, U., Moll, A., Pohlmann, T., Stegert, C. and Tamm, S. (2008) Defining habitats suitable for larval fish in the German Bight (southern North Sea): an IBM approach using spatially- and temporally-resolved, size-structured prey fields. *J. Mar. Syst.*, **74**, 329–342.

Lasker, R. (1985) What limits clupeoid production. *Can. J. Fish. Aquat. Sci.*, **42**, 31–38.

Lasker, R. (1978) The relation between oceanographic conditions, and larval anchovy food in the California Current: identification of factors contributing to recruitment failure. Rapp. *P.-V. Reun.* - Cons. Int. Explor. Mer, **173**, 212–230.

Lessard, E. J., Martin, M. P. and Montagnes, D. J. S. (1996) A new method for live-staining protists with DAPI and its application as a tracer of ingestion by walleye pollock (*Theragra chalcogramma* (Pallas)) larvae. *J. Exp. Mar. Biol. Ecol.*, **204**, 43–57.

Llopiz, J. K. (2013) Latitudinal and taxonomic patterns in the feeding ecologies of fish larvae: a literature synthesis. *J. Mar. Syst.*, **109–110**, 69–77.

Löder, M. G. J., Kraberg, A. C., Aberle, N., Peters, S. and Wiltshire, K. H. (2012) Dinoflagellates and ciliates at Helgoland Roads, North Sea. *Helgol. Mar. Res.*, **66**, 11–23.

McGurk, M. D. (1992) Avoidance of towed plankton nets by herring larvae: a model of night-day catch ratios based on larval length, net speed and mesh width. *J. Plankton Res.*, **14**, 173–181.

Menden-Deuer, S. and Lessard, E. J. (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol. Oceanogr.*, **45**, 569–579.

Montagnes, D. (1996) Growth responses of planktonic ciliates in the genera Strobilidium and Strombidium. *Mar. Ecol. Prog. Ser.*, **130**, 241–254.

Montagnes, D., Allen, J., Brown, L., Bulit, C., Davidson, R., Fielding, S., Heath, M., Holliday, N. *et al* (2010a) Role of ciliates and other microzooplankton in the Irminger Sea (NW Atlantic Ocean). *Mar. Ecol. Prog. Ser.*, **411**, 101–115.

Montagnes, D. J. S., Dower, J. F. and Figueiredo, G. M. (2010b) The protozooplankton-ichthyoplankton trophic link: an overlooked aspect of aquatic food webs. *J. Eukaryot. Microbiol.*, **57**, 223–228.

Montagnes, D. J. S., Lynn, D. H., Roff, J. C. and Taylor, W. D. (1988) The annual cycle of heterotrophic planktonic ciliates in the waters surrounding the Isles of Shoals, Gulf of Maine: an assessment of their trophic role. *Mar. Biol.*, **99**, 21–30.

Montagnes, D. J. S., Poulton, A. J. and Shammon, T. M. (1999) Mesoscale, finescale and microscale distribution of micro-and nanoplankton in the Irish Sea, with emphasis on ciliates and their prey. *Mar. Biol.*, **134**, 167–179.

Munk, P. and Kiørboe, T. (1985) Feeding behaviour and swimming activity of larval herring (*Clupea harengus*) in relation to density of copepod nauplii. *Mar. Ecol. Prog. Ser.*, **24**, 15–21.

Nagano, N., Iwatsuki, Y., Kamiyama, T. and Nakata, H. (2000) Effects of marine ciliates on survivability of the first-feeding larval surgeonfish, *Paracanthurus hepatus*: laboratory rearing experiments. *Hydrobiologia*, **432**, 149–157.

Nash, R. D. M. and Dickey-Collas, M. (2005) The influence of life history dynamics and environment on the determination of year class

strength in North Sea herring (*Clupea harengus* L.). *Fish. Oceanogr.*, **14**, 279–291.

Ohman, M. D., Theilacker, G. H. and Kaupp, S. E. (1991) Immunochemical detection of predation on ciliate protists by larvae of the northern anchovy (*Engraulis mordax*). *Biol. Bull.*, **181**, 500 –504.

Olenina, I., Hajdu, S., Edler, L., Andersson, A., Wasmund, N., Busch, S., Göbel, J., Gromisz*et al* (2006) Biovolumes and Size-classes of Phytoplankton in the Baltic Sea. *HELCOM Balt. Sea Environ. Proc.*, pp. 144

Overton, J., Meyer, S., Støttrup, J. and Peck, M. (2010) Role of heterotrophic protists in first feeding by cod (*Gadus morhua*) larvae. *Mar. Ecol. Prog. Ser.*, **410**, 197–204.

Payne, M., Stine, D., Worsøe Clausen, L., Munk, P., Mosegaard, H. and Nash, R. (2013) Recruitment decline in North Sea herring is accompanied by reduced larval growth rates. *Mar. Ecol. Prog. Ser.*, **489**, 197–211.

Peck, M. A., Huebert, K. B. and Llopiz, J. K. (2012) Intrinsic and extrinsic factors driving match–mismatch dynamics during the early life history of marine fishes. *Adv. Ecol. Res*, **47**, 177–302.

Pepin, P. and Dower, J. F. (2007) Variability in the trophic position of larval fish in a coastal pelagic ecosystem based on stable isotope analysis. *J. Plankton Res.*, **29**, 727–737.

Pepin, P. and Penney, R. (2000) Feeding by a larval fish community: impact on zooplankton. *Mar. Ecol. Prog. Ser.*, **204**, 199–212.

Rossi, S. (2006) Lipid biomarkers and trophic linkages between phytoplankton, zooplankton and anchovy (*Engraulis encrasicolus*) larvae in the NW Mediterranean. *J. Plankton Res.*, **28**, 551–562.

Scherer, C. (2012) Developing and testing an index of change in microplankton community structure in temperate shelf seas. PhD thesis, Edinburgh Napier University, 336 p.

Smetacek, V. (1981) The annual cycle of protozooplankton in the Kiel Bight. *Mar. Biol.*, **63**, 1–11.

Spittler, P., Brenning, U. and Arlt, G. (1990) Protozoans—the first food of larval herring (*Clupea harengus* L.)? *Int. Rev. Gesamten Hydrobiol. Hydrogr.*, **75**, 597–603.

Strom, S. L. and Morello, T. A. (1998) Comparative growth rates and yields of ciliates and heterotrophic dinoflagellates. *J. Plankton Res.*, **20**, 571–584.

Strüder-Kypke, M., Kypke, E., Agatha, S., Warwick, J. and Montagnes, D. (2006) The Planktonic Ciliate Project Online - Introduction. The user-friendly guide to coastal planktonic ciliates. http://www.zooplankton.cn/ciliate/intro.htm [Verified 19 August 2015]

Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Plankton-Methodik. *Mitt. Int. Ver. theor. angew. Limnol.*, **9**, 1–38.

Van der Meeren, T. (1991) Algae as first food for cod larvae, *Gadus morhua* L.: filter feeding or ingestion by accident? *J. Fish Biol.*, **39**, 225–237.

Yang, J., Löder, M. G. J., Gerdts, G. and Wiltshire, K. H. (2015) Structural composition and temporal variation of the ciliate community in relation to environmental factors at Helgoland Roads, North Sea. *J. Sea Res.*, **101**, 19–30.

Young, K. V., Dower, J. F. and Pepin, P. (2009) A hierarchical analysis of the spatial distribution of larval fish prey. *J. Plankton Res.*, **31**, 687–700.

Zuur, A. F. (2009) *Mixed Effects Models and Extensions in Ecology with R.* Springer, New York.