The microzooplankton community during winter in NE Atlantic waters and its potential impact on condition and growth of larval Atlantic herring (*Clupea harengus*)

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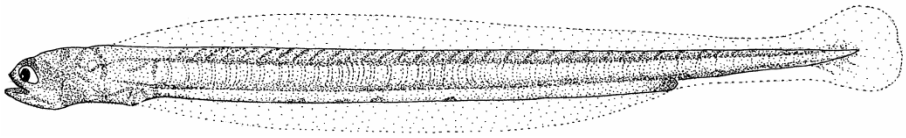

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Contents

List of figures

List of tables

Summary

Microzooplankton is the planktonic size fraction between 20 and 200 µm consisting of unicellular (protozooplankton) and metazoan organisms, such as the early life stages of copepods and other invertebrate larvae. Protozooplankton (PZP) is a key player in the marine carbon cycle as part of the so-called microbial loop. Within the microbial loop, carbon is recycled through a "bacteria - small phytoplankton - PZP – loop" back into the classical food chain. During periods of low productivity, such as the winter in the NE Atlantic, more carbon is cycled via the microbial loop compared to the classical, linear food chain (e.g. from large phytoplankton to copepods to higher trophic levels). While future scenarios predict more beneficial conditions for small-sized cells and, hence, a larger contribution of microbial loop dynamics, it is important to investigate trophodynamics occurring within these planktonic size fractions. For instance, PZP as well as metazoan microzooplankton, serve as a food source for early life stages of fish. Despite the recognition of the importance of microzooplankton the microzooplankton-ichthyoplankton link remains poorly studied. This thesis elucidates the autumn and winter community composition of PZP in the NE Atlantic and uses the larvae of autumn spawning Atlantic herring (*Clupea harengus*) as a case study to investigate the impact of the components of this small plankton size fraction on the distribution, condition and growth of overwintering ichthyoplankton.

Manuscript 1 describes the findings of a broad scale field study on the winter PZP community in the North Sea from the Orkney Islands in the north to the English Channel in the south. The focus was set on the taxonomic composition of the main PZP components, the hetero- and mixotrophic taxa from the phyla Ciliophora (ciliates) and Dinophyta (dinoflagellates). The dominance of small sized cells (<50 µm) in the community suggested that the microbial loop played a pronounced role in fueling the productivity of higher trophic levels. The maximum PZP carbon biomass found was approximately 50 times lower than during bloom conditions. However, highest biomass was observed in southern areas, which are known to be important winter spawning grounds of certain fish species. This work highlights how this type of sampling can be added to a pre- existing survey for fishery

assessment (ICES IBTS) involving marine institutes of several European countries to capture a snap-shot of the PZP community across the North Sea within a relatively short time frame (five weeks). It is hoped that ongoing collaborative monitoring will create a longer-term PZP monitoring, from which ecosystem indicators can be derived.

The potential importance of the microzooplankton-ichthyoplankton link to autumn spawning herring in the Irish Sea was highlighted in **Manuscript 2** using a combination of field sampling, taxonomic plankton identification, biochemical analysis and physiological-based modelling. Synchronous sampling of herring larvae, PZP, MZP and small mesozooplankton (<300 µm) was conducted in two subsequent spawning seasons in November 2012 and 2013. In contrast to plankton distribution patterns in spring and summer the highest PZP biomass, which consisted to a great part of e.g. *Gymnodinium* spp., *Protoperidinium* spp., *Ceratium furca* (Dinophyta) *and Strombidium spp.* (Ciliophora), was found within the main herring spawning sites. Spatial differences in the taxonomic composition of the protozoan community were strongly related to hydrographic characteristics. Statistical analysis indicated a relationship of small copepods as well as dinoflagellates with larval herring abundance and, thus, it can be assumed that grazing dynamics were complex in the lower trophic food web. The small prey size (<200 µm) was implemented for the first time into a foraging and growth model. Comparing the modelled with observed *in-situ* growth rates indicated an important role of PZP for larval growth.

To further resolve the role of microzooplankton for larval fish, the main potential prey sources and their impact on larval nutritional condition (RNA/DNA) and growth was determined using stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) (**Manuscript 3**). A comparison of early feeding larvae (8-14 mm) and their potential prey in two different autumn and winter spawning grounds in 2013 and 2014 in the North Sea (Buchan & Banks and Downs) showed differences in the isotopic composition, depending on season, geographic location or on the sampling year. The trophic level of the autumn as well as the winter spawned larvae suggested a diet based predominantly on prey bigger than 50 µm. The inverse relationship of the C to N ratio (C:N) and larval dry weight found in autumn 2013 may indicate that the larvae were choosing qualitatively higher prey as they grew. A higher total amount of available microzooplankton or feeding on a higher trophic level did not result in a better

nutritional condition of the larvae in autumn 2014 than in 2013. The higher contribution of less preferred prey items, e.g. *Ceratium* spp. and mollusc larvae, compared to early life stages of copepods in the microzooplankton of autumn 2014, may point to mismatch dynamics for larger larvae, which seemed to influence larval growth and potentially overwinter survival.

The results of the present thesis demonstrated the potential importance of microzooplankton, particularly PZP, to the winter carbon cycle. Additionally, the abundance and composition of this plankton size fraction may directly or indirectly impact on the condition and growth of larval herring and, thus, potentially influence survival and recruitment dynamics. The results presented in this thesis demonstrate the need of implementing this kind of data into ecosystem and food web models. Furthermore, to unravel and better understand processes influencing the growth and survival of in early life stages of fish, and potentially recruitment success, and to advance the ecosystem approach to fisheries management, further emphasize should be placed on investigating the microzooplankton-ichthyoplankton link.

Zusammenfassung

Mikrozooplankton, der Bestandteil des Planktons in der Größenordnung von 20 bis 200 µm, besteht sowohl aus einzelligen (Protozooplankton) als auch aus mehrzelligen Organismen, wie z.B. frühe Entwicklungsstadien von Copepoden oder andere Invertebratenlarven. Protozooplankton (PZP) ist Teil der sogenannten mikrobiellen Schleife und nimmt somit eine Schlüsselposition im marinen Kohlenstoffzyklus ein. Durch die mikrobiellen Schleife, bestehend aus Bakterien, kleinem Phytoplankton und PZP, wird Kohlenstoff wieder der klassischen linearen Nahrungskette zugeführt. In Zeiten geringer Produktivität, z.B. im Winter im Nordostatlantik, wird mehr Kohlenstoff durch die mikrobiellen Schleife geleitet als durch die klassische, lineare Nahrungskette (z.B. von großes Phytoplankton zu Copepoden zu höheren trophischen Ebenen). Da Zukunftsszenarien vorteilhafte Bedingungen für kleine Zellen und somit auch einen größeren Beitrag der mikrobiellen Schleife prognostizieren, ist es wichtig Nahrungsdynamiken innerhalb dieser Größenfraktionen des Planktons zu untersuchen. So dient beispielsweise das PZP sowie mehrzelliges Mikrozooplankton frühen Lebensstadien von Fischen als Nahrungsquelle. Der Erkenntnis über die Wichtigkeit von Mikrozooplankton zum Trotz ist der Mikrozooplankton-Ichthyoplankton Link nur in sehr begrenztem Umfang untersucht. Die vorliegende Arbeit beschreibt die Zusammensetzung des PZP im Nordostatlantik in Herbst und Winter und untersucht am Beispiel des herbstlaichenden Atlantischen Herings (*Clupea harengus*) den Einfluss der kleinen Planktongruppen auf die Verteilung, die Kondition und das Wachstum von überwinterndem Ichthyoplankton.

Manuskript 1 beschreibt die Ergebnisse einer umfangreichen Feldstudie über die PZP Zusammensetzung im Winter in der Nordsee, von den Orkneyinseln im Norden bis in den Ärmelkanal im Süden. Der Fokus lag auf der taxonomischen Zusammensetzung der Hauptbestandteile des PZP, den hetero- und mixotrophen Taxa der Phyla Ciliophora (Ciliaten) und Dinophyta (Dinoflagellaten). Kleine Zellen (<50 µm) waren vorherrschend in der PZP Gemeinschaft und deuteten auf eine ausgeprägte Rolle der mikrobiellen Schleife für die Produktivität höherer trophischer Ebenen hin. Die maximale PZP Biomasse war

ca. 50 mal kleiner als zu Zeiten einer Blüte. Allerdings wurde in den südlichen Gebieten, welche als wichtige Winterlaichgebiete bestimmter Fischarten bekannt sind, die höchste Biomasse festgestellt. Diese Arbeit hebt hervor, wie diese Art der Probennahme zu einem schon vorhandenen regelmäßig durchgeführten Fischereimanagementsurveys (ICES IBTS), in welchem wissenschaftliche Institute mehrerer Länder involviert sind, hinzugefügt werden kann, um eine Momentaufnahme der PZP Gemeinschaft quer über die Nordsee in einem relativ kurzen Zeitraum von 5 Wochen zu erhalten. Mithilfe eines weiter andauernden gemeinschaftlichen Monitorings soll künftig ein längerfristiges Planktonmonitoring geschaffen werden.

Die mögliche Bedeutung des Mikrozooplankton-Ichthyoplankton Links für herbstlaichenden Hering in der Irischen See wurde in **Manuskript 2** mit einer Kombination aus Feldarbeit, Planktontaxonomie, biochemischen Analysen und physiologisch basierter Modellierung veranschaulicht. Proben von Heringslarven, PZP, Mikrozooplankton und kleinem Mesozooplankton (<300 µm) wurden in zwei aufeinanderfolgenden Laichzeiten im November 2012 und 2013 genommen. Im Gegensatz zu Verteilungsmustern in Frühjahr und Sommer, war die höchste PZP Biomasse innerhalb der Hauptlaichgründe des Herings zu finden. Sie setzte sich zu einem großen Teil aus *Gymnodinium* spp., *Protoperidinium* spp., *Ceratium furca* (Dinophyta) und *Strombidium spp.* (Ciliophora) zusammen. Räumliche Verteilungsmuster in der taxonomischen Zusammensetzung der Protozoengemeinschaft waren stark von hydrographischen Bedingungen abhängig. Statistische Analysen wiesen auf einen Zusammenhang sowohl zwischen kleinen Copepoden als auch zwischen Dinoflagellaten und der Abundanz von Heringslarven hin und ließen auf komplexe Nahrungsdynamiken in den unteren trophischen Ebenen schließen. Die kleine Beutegrößenklasse (<200 µm) wurde erstmals in ein Nahrungs- und Wachstumsmodell eingefügt. Vergleiche zwischen simulierten und gemessenen *in-situ* Wachstumsraten wiesen auf eine wichtige Rolle des PZP für das larvale Wachstum hin.

Um die Bedeutung des Mikrozooplanktons für Fischlarven genauer zu klären, wurden die hauptsächlichen potentiellen Nahrungsquellen und ihr Einfluss auf den Ernährungszustand (RNA/DNA) und das Wachstum mithilfe stabiler Isotopenanalyse ($\delta^{13}C$ und $\delta^{15}N$) untersucht (**Manuskript 3**). Ein Vergleich von Larven zu Beginn der exogenen Ernährung (8-14 mm) und

ihrer potentiellen Beute in zwei unterschiedlichen Herbst- und Winterlaichgründen in den Jahren 2013 und 2014 in der Nordsee (Buchan & Banks und Downs) zeigte Unterschiede in der Isotopenzusammensetzung in Abhängigkeit von Jahreszeit, geographischer Lage oder des Jahres der Probennahme. Die trophische Ebene, sowohl der im Herbst als auch der im Winter geschlüpften Larven, enthielt Hinweise darauf, dass sich die Nahrung hauptsächlich aus Beute größer als 50 µm zusammensetzte. Die reziproke Beziehung des C zu N Verhältnisses (C:N) zum Trockengewicht der Larven im Herbst 2013 könnte darauf hindeuten, dass die Larven je größer sie wurden Beute von höherer Qualität wählten. Die höhere Gesamtmenge an vorhandenem Mikrozooplankton oder eine Ernährung auf Basis einer höheren trophischen Ebene führte nicht zu einem besseren Ernährungszustand der Larven im Herbst 2014 verglichen mit 2013. Der höhere Anteil von weniger bevorzugten Beuteorganismen im Mikrozooplankton im Herbst 2014, z.B. *Ceratium* spp. oder Molluskenlarven, verglichen mit frühen Entwicklungsstadienstadien von Copepoden, deuteten möglicherweise auf sogenannte „mismatch dynamics“ (d.h. fehlendes Zusammentreffen) hin, die das Wachstum und eventuell das Überleben der Larven im Winter zu beeinflussen schienen.

Diese Ergebnisse zeigten die mögliche Bedeutung von Mikrozooplankton, im Besonderen PZP, im Kohlestoffkreislauf während des Winters. Darüber hinaus kann die Abundanz und Zusammensetzung dieser Planktongruppen möglicherweise direkt oder indirekt Einfluss auf die Kondition und das Wachstum von Heringslarven haben und dadurch eventuell auch auf das Überleben der Larven und die Rekrutierung. Die in dieser Arbeit vorgestellten Ergebnisse zeigen die Notwendigkeit, diese Art von Daten in Ökosystem und Nahrungsnetzmodelle zu integrieren. Zudem sollte der Untersuchung des Mikrozooplankton-Ichthyoplankton Links ein größerer Stellenwert zugewiesen werden, um zum einen Prozesse, die Einfluss auf das Wachstum und Überleben früher Lebensstadien von Fischen und möglicherweise auch auf den Rekrutierungserfolg haben, besser zu begreifen und zum anderen um den Ökosystemansatz im Fischereimanagement weiter voranzubringen.

CHAPTER 1

1. General Introduction

1.1 Microzooplankton in the oceanic realm

Plankton, the passively drifting component of the aquatic biota, consists of autotrophic (phytoplankton) and heterotrophic (zooplankton) organisms forming the base of the food-web in the oceans. Unlike plants in terrestrial ecosystems, phytoplankton is usually very small (0.6-2000 µm, Figure 1.1); nevertheless, its biomass can exceed the biomass of the zooplankton component (Gasol et al. 1997). The majority of planktonic organisms is short lived and has high turnover rates, enabling them to react rapidly to changing environmental conditions. Adequate light and nutrient conditions can lead to immediate exponential growth of phytoplankton (Sommer & Lengfellner 2008), followed by instantaneous growth of the fast-growing zooplankton organisms (e.g. protozoa) grazing on them (Aberle et al. 2007), which are then consumed by higher trophic levels. This is a simplified picture of the energy flow channeled up the food chain in marine systems; in point of fact the variety of planktonic life forms allows energy (i.e. carbon) transfer on various pathways.

The immense diversity in plankton makes exact definitions or distinctions among groups very difficult. Nevertheless, plankton is traditionally separated in functional groups: Virioplankton, bacterioplankton, phytoplankton and zooplankton. Across and within these groups, the organisms are separated into classes distinguished by their size (Sieburth et al. 1978) (Figure 1.1).

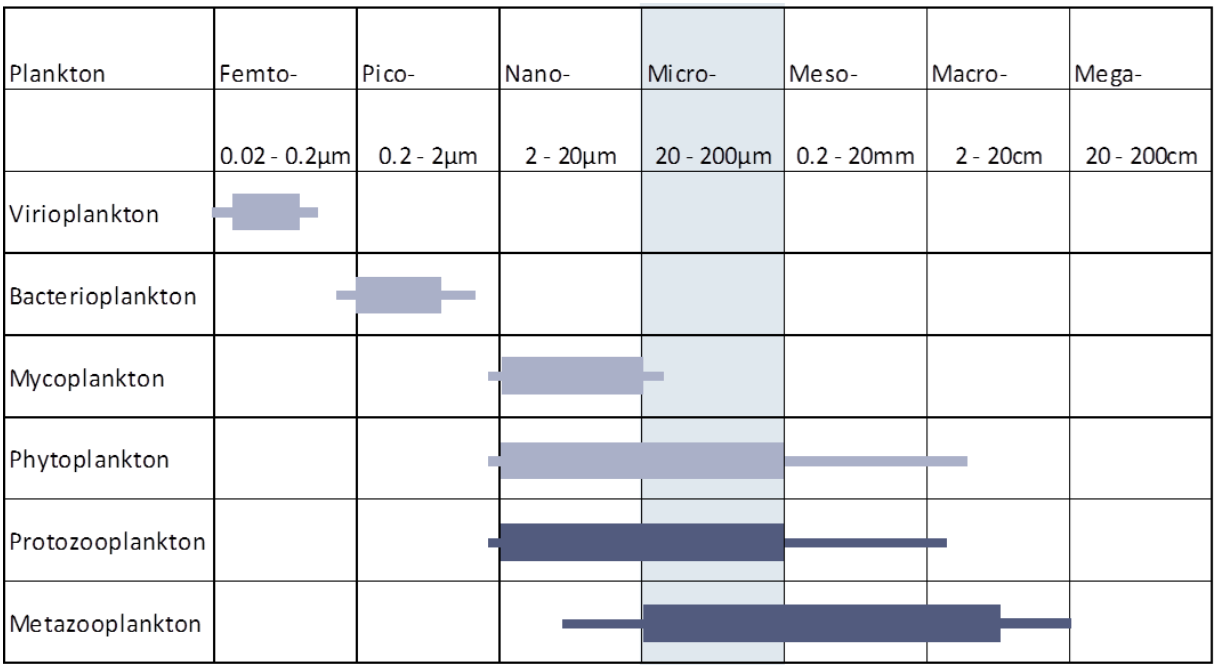

Figure 1.1: Classification of plankton in functional and size groups. In the microzooplankton component (highlighted background) a big part of phytoplankton, protozooplankton and to a smaller extend metazoan organisms are included. Zooplankton components of the microplankton are displayed in dark blue. Modified after Sieburth et al. (1978)

In some extraordinary cases, gelatinous zooplankton, e.g. species from the phylum Cnidaria, can reach a size up to 2 m (megaplankton). The size class from 20 to 200 µm is called microplankton and is divided into microphytoplankton and microzooplankton according to their trophic mode. Organisms defined as microzooplankton share only two characteristics: size (20-200 µm) and trophic mode (not obligate autotrophs). The microzooplankton groups unicellular eukaryotic organisms (the protozooplankton, PZP) as well as metazoan organisms (from here on MZP).

1.1.1 Unicellular microzooplankton (PZP) and the microbial loop

The marine PZP consists of organisms of various phyla and subphyla, among others Dinophyta (dinoflagellates), Ciliophora (ciliates), Foraminifera and Radiolaria (www.marinespecies.org). Numerically, dinoflagellates and ciliates are the most important PZP groups in temperate waters.

Dinoflagellates can either be composed of intracellular cellulose plates (theca), or lacking those plates (athecate). Two flagellae for locomotion enable motility, thus, allowing them them to follow favorable food or nutrient clines in the water column (Klausmeier & Litchman

2001). Traditionally the majority of dinoflagellate species was classified as autotroph, and some as heterotroph or mixotroph. This is widely discussed lately as it is becoming more and more evident that a mixotrophic feeding mode is rather the rule than the exception (Flynn et al. 2013). Heterotrophic and mixotrophic dinoflagellates in the microzooplankton can prey on particulate organic matter (POM), some of them being carnivorous (feeding on other heterotrophic organisms or copepod eggs) (Calbet 2008), but most of them being grazers of phytoplankton cells (herbivory). Their remarkable variety of feeding modes, e.g. complete engulfment of the prey cell (Figure 1.2, panel D) or sucking content of the prey cell via pallium feeding, allows them to feed on cells smaller than 1 µm up to cells much bigger than themselves (e.g. chain forming diatoms) (Sherr & Sherr 1994). Heterotrophic dinoflagellates can achieve growth rates of up to one division per day but seldom more (Jacobson & Anderson 1993).

Ciliates are either surrounded by a lorica, which can contain organic or inorganic material from the surrounding water column (Agatha et al. 2012), or being "naked", i.e. lacking a lorica (aloricate). Most species are known as suspension feeders, drawing in bacteria or small phytoplankton cells with lash-like membranelle structures, the cilia. Unlike dinoflagellates they prefer prey about 1/10 of their own size (Jonsson 1986), but have also been observed to feed on prey of their own size (Smetacek 1981, Aberle et al. 2007). Some species also have been found to be mixotrophic (e.g. *Mesodinium rubrum*). By retaining the chloroplasts of their autotrophic prey, they achieve the ability of energy gain through photosynthesis. The cilia are also used for locomotion, enabling some species to achieve swimming speeds of up to 2 mm sec^{-1} and performing jump like predator escape responses (Jakobsen 2001). Doubling time of ciliate populations can be less than 12 hours (Ohman & Snyder 1991, Dolan 2013).

Foraminifera have a size between 100 µm up to several centimeters with outer or internal shells, which consist either of organic or inorganic compounds like crystalline $CaCO_3$. There are less than 50 planktonic species known contributing to a minor part to zooplankton biomass (Hemleben et al. 1989). Radiolaria can reach sizes of several mm and consist of a silica skeleton with arm-like extensions (axopodia) for prey capture (e.g. bacteria, other PZP).

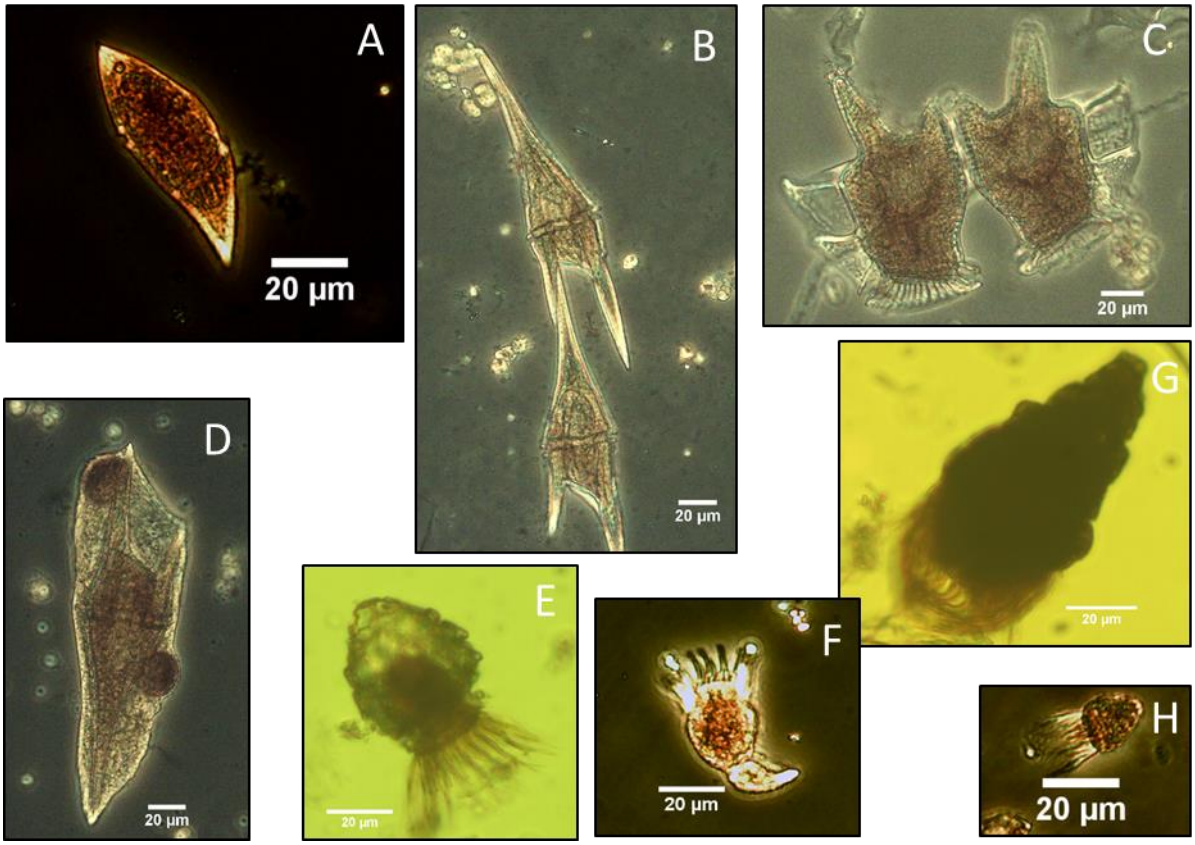

Figure 1.2: **Examples of protozooplankton stained with Lugol's solution. Dinoflagellates: *Gyrodinium* sp. (A), *Ceratium furca* (B), *Dinophysis* sp. (C), *Polykrikos* sp. with ingested *Ceratium* cell (D). Ciliates: *Stenosemella* sp. (E), *Tontonia gracillima* (F), *Laboea strobila* (G) and *Lohmaniella oviformis* (H). Each scale bar represents 20 µm.**

The trophic role of PZP has been generally ignored in the traditional concept of the marine food-chain, which considers copepods as the main grazers of phytoplankton that are then preyed on by bigger zooplankton or planktivorous fish. However, PZP can actually graze more than 60% of the primary production in some areas (Calbet 2008). Investigations on the role of the PZP in food webs and carbon cycling intensified after seminal studies were published on microbial interactions within the traditional food chain, the so-called "microbial loop" (Pomeroy 1974, Azam et al. 1983). Very simplified, the microbial loop is driven by small phytoplankton and heterotrophic bacteria. The latter depend on dissolved organic matter (DOM), which is generated by detritus produced by senescent phytoplankton cells, zooplankton faecal pellets or other detrital material (Lalli & Parsons 2006). Small ciliates and dinoflagellates can prey on bacteria and on bacterivorous and autotrophic nanoflagellates (and on each other) and the energy is channeled back into the classical, linear food chain by grazing of mesozooplankton or higher trophic levels, such as fish larvae (Pomeroy et al. 2007) (Figure 1.3).

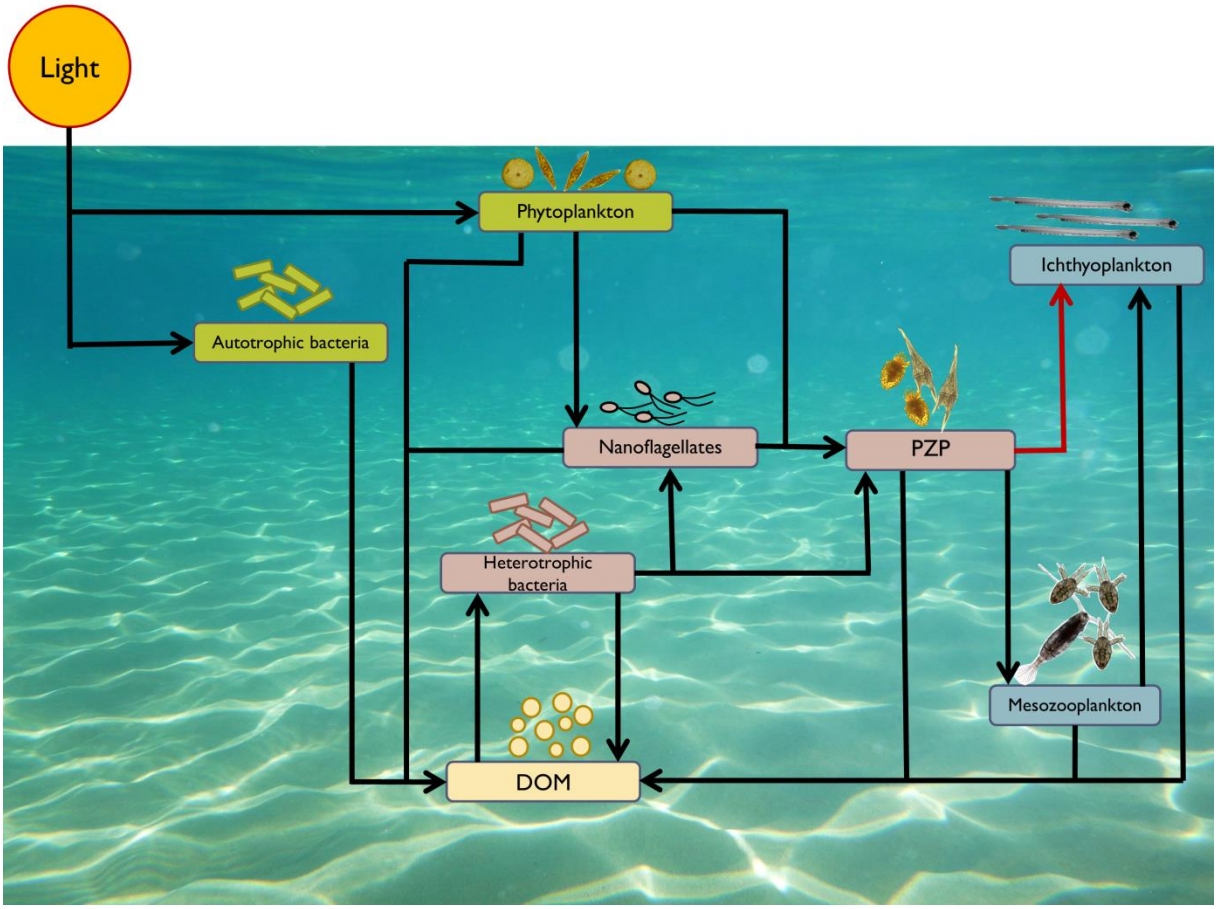

Figure 1.3: **Simplified lower trophic marine food web with microbial loop included. The trophic components are color coded. Yellow: non-living material, green: photosynthetic organisms, red: microbial loop associates, blue: higher trophic levels. Arrows indicate the pathways of the energy flow. The red arrow highlights the protozooplankton-ichthyoplankton link. Modified after Pomeroy (2007). Background photograph by Aylin Klarer.**

The strength of the microbial loop varies with oceanic conditions and between seasons. In general the microbial loop dynamics are considered to play a major role in oligotrophic habitats (e.g. open ocean) compared to nutrient rich waters. The nutrient limitation favors growth of pico- and nanoplankton over large diatoms (Fileman et al. 2011) and due to their small size, they are grazed by PZP which is, in turn, consumed by larger zooplankton (e.g. copepods). This has direct effects on the energy transfer in the food web (trophic transfer efficiency) and the productivity of the oceans. The more trophic levels involved in a food web the more energy is lost from primary production to higher trophic levels (Lalli & Parsons 2006). In nutrient rich temperate waters, large diatoms dominate the phytoplankton community and more biomass (energy) goes directly to large zooplankton. However, in times of low primary production (e.g. winter), the role of PZP and the microbial loop in carbon cycling can be enhanced in temperate waters (Fileman et al. 2011).

This increasing knowledge about MZP and PZP and its role in the oceans is contributing to the ongoing discussion about plankton as ecological indicators, which act as proxies for the environmental state of an ecosystem (Beaugrand 2005). Changes in the species composition or size distribution in lower trophic food webs can help to detect changes in the entire ecosystem due to their high abundance and fast reaction time to environmental changes (Montagnes et al. 2003, Menden-Deuer et al. 2005, Aberle et al. 2012). Until recently the focus was set to mesozooplankton organisms as potential indicators. However, future climate change scenarios predict an increase in stratification (Lozier et al. 2011), which will favor the microbial food web over the classical food chain arising from the increase of small phytoplankton cells (Cushing 1989).

1.1.2 Metazoan microzooplankton (MZP)

The most abundant metazoan MZP are rotifers (Rotatoria) and early life stages of holoplanktonic organisms (e.g. copepods), or meroplanktonic organisms (e.g. trochophora and veliger larvae of gastropods) (Figure 1.4).

Copepods are the dominant components of the mesozooplankton representing a major resource to the higher trophic levels (Johns & Reid 2001) and their early life stages are important prey components of the larvae of several fish species, such as Atlantic herring (*Clupea harengus*) or cod (*Gadus morhua*) (Cohen & Lough 1983, Kristiansen et al. 2014, Denis et al. 2016). In the traditional food web concept copepods are seen as major grazers of phytoplankton and primarily grazing on it (Steele & Frost 1977). This view has shifted towards copepods as omnivors, with microbial loop associated organisms such as PZP contributing to a great part to their diet (Sommer et al. 2005, Saiz & Calbet 2011). Before copepods become sexually mature adults they undergo 6 naupliar and 5 copepodite stages, some of which belong to the microzooplanktonic size category. It can take up to 60 days until a generation cycle is closed in NE Atlantic copepod species (Breteler et al. 1995, Renz et al. 2008). Copepods are the most abundant zooplankton group in the oceans, but due to high fertilization success of some gastropods, their meroplanktonic larvae can dominate plankton coastal community in certain seasons. Until their metamorphosis these larvae can stay up in the water column for several month, e.g. overwinter in the larval stage (Larink & Westheide 2011). Most meroplanktonic larvae in the microzooplankton either rely on yolk

reserves (lecithotrophic) or are herbivorous feeding on nano- and microphytoplankton (Larink & Westheide 2011).

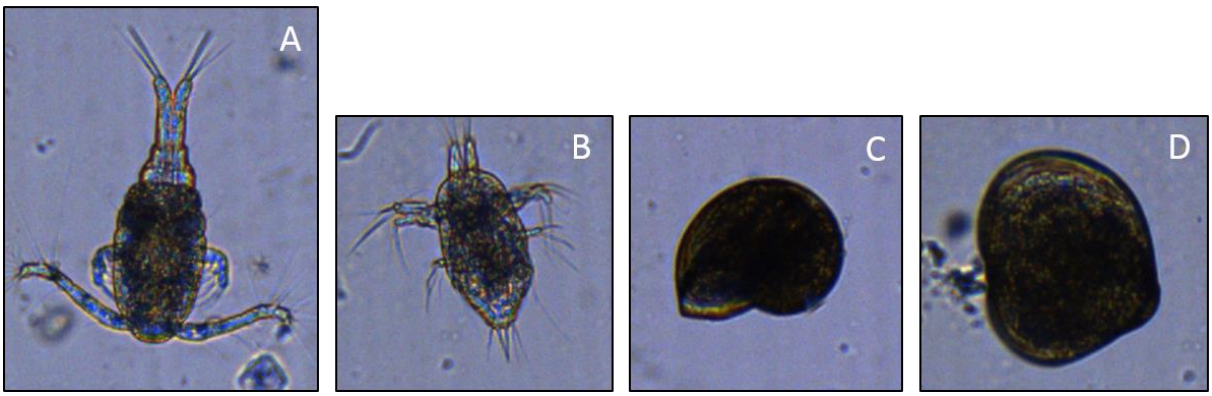

Figure 1.4: **Examples of microzooplankton organisms from plankton assemblage in the North Sea in early autumn. The images were derived from the FlowCam (magnification 4X). Copepodite (A), nauplii (B), veliger larvae (C & D). Images taken by Tim Müller.**

1.1.3 Sampling and analyzing microzooplankton

Plankton is usually sampled either with nets or water sampling devices. Generally ship-based plankton sampling is performed with nets of certain mesh sizes designed for various purposes depending on the target size fraction, e.g. nets sampling vertically through the entire water column (e.g. Apstein net) or multiple plankton nets with closing mechanism with nets of different mesh sizes attached (e.g. MultiNet). Another approach is the frequent use of ships of opportunity additionally to sampling on research cruises to gain high spatial and temporal coverage. Long term datasets of mesozooplankton are derived from e.g. the Continuous Plankton Recorder (CPR). The CPR is a sub-surface plankton sampler, which is towed by commercial ships of opportunity as they traverse specific transects, sampling plankton on a 270µm mesh size gauze (Reid et al. 2003). Due to the delicacy of soft-bodied PZP (i.e. aloricate ciliates and athecate dinoflagellates), sampling is conducted via vertical water sampling instead of using towed nets, which restricts the sampling area and volume of water sampled.

For studying mesozooplankton in situ advanced analyzing techniques have been developed. Using in-situ optical devices, such as the Video Plankton Recorder (VPR) can provide knowledge about distributional and size patterns of mesozooplankton prey (Davis et al. 2005).

Besides manually processing preserved field samples using stereomicroscopy, digital systems using image analysis have been developed (e.g. ZooScan)(Gorsky et al. 2010) allowing identification and measurement of different features at the individual level. For quantifying and identifying PZP the Utermöhl technique, developed in the 1950s (Utermöhl 1958) for phytoplankton is commonly used (Gifford & Caron 2000).

According to the Utermöhl method a defined sample volume depending on the plankton density is settled on the bottom of a cylindrical chamber. The settled PZP cells are counted and identified under an inverted microscope. Size measurements needed for calculation of the biovolume and conversion to carbon content have to be performed manually (Menden-Deuer & Lessard 2000). In recent years, new approaches for analyzing PZP have been developed, such as the FlowCam (Fluid Imaging technologies, Yarmouth, USA), an imaging particle analyzing system, developed for small sized particles and equipped with microscopy objectives from 2x to 20x. This system captures images of the particles within a sample and registers and calculates particle properties, such as size and biovolume.

In contrast to mesozooplankton, there are no advanced sampling devices, such as an in-situ monitoring, for PZP due to technical limits (e.g. optical resolution, inability to capture and preserve organisms). The relatively large mesh size of the CPR does not capture the PZP and MZP size fraction and the preservation method (formalin) causes decomposition of delicate aloricate or athecate PZP. Due to this caveats and difficulties in sampling and processing PZP, longer-term time series programs with a high spatial and/or temporal resolution failed to be developed so far.

1.2 The microzooplankton-ichthyoplankton link

Until the beginning of the 20th century there was consensus among fishermen and fishery scientists that variability in stock size and recruitment was mostly driven by fishing pressure and migratory behavior of the fish stock. In 1914 Johan Hjort suggested for the first time in his “critical period hypothesis” that causes of recruitment variability may be found in the early life stages of fish (Hjort 1914). He proposed that larval survival is determined during the transition from endogenous feeding (yolk sac stage) to exogenous feeding, when the larvae need to find appropriate food supply (“critical period”). Low larval survival due to

failure in first feeding would in consequence lead to low recruitment. Later he extended his hypothesis, stating that low recruitment can as well be caused by unfavorable environmental conditions, winds and ocean currents, drifting larvae away from their feeding grounds ("aberrant drift hypothesis") (Hjort 1926). Based on Hjort's work, new hypotheses were developed during the following decades, focusing on trophodynamic and hydrodynamic factors, controlling recruitment through larval survival. The "match-mismatch" hypothesis developed by David Cushing (1975, 1990) considers a temporal overlap between larvae and availably prey (match) as responsible factor for successful recruitment, which was later shown for cod in the North Sea, where time-series variations in stock size could directly be linked to phytoplankton availability (Beaugrand et al. 2003). Other hypotheses were more specified to certain habitats such as upwelling regions (Lasker 1981, Cury & Roy 1989) and later on also considered top-down processes (e.g. predation) as important bottlenecks for larval survival and recruitment (Bailey & Houde 1989, Leggett & Deblois 1994). However, recruitment processes are generally species-specific and recent research suggests that combined physical and biological processes interact in different ways on different developmental stages of fish to impact recruitment (Houde 2008, Hare 2014).

Nonetheless, finding adequate amounts and qualities of prey items during early life is essential to sustain growth and survival of fish larvae. Copepod nauplii (part of the MZP) have been traditionally considered as the main food source for first-feeding fish larvae. Almost 100 years ago Sir Alister Hardy designed a food web for Atlantic herring (*Clupea harengus*) including protists as prey item for young larvae (Hardy 1924). Since then occasionally the role of protists as food for larval fish has been mentioned in the literature (Lasker et al. 1970, Last 1978), but without affecting the common view of metazoan based nutrition. Recently the importance of PZP for larval diets is gaining attention and their crucial role is becoming more and more evident (Montagnes et al. 2010).

Laboratory approaches provide an opportunity to observe potential effects of PZP on feeding, development and/or survival of larval fish under controlled conditions. Besides the small size of many PZP species, especially the athecate and aloricate PZP components are challenging to detect or even quantify in larval diets. Due to their delicacy, they are rapidly digested and the most often used preservation method for larval fish (formalin) causes PZP

cells to disintegrate. To partially overcome these problems alternative methods have been developed. Prey depletion experiments (Friedenberg et al. 2012), labelling prey organisms with fluorescent microspheres (Fukami et al. 1999), using high resolution microscopy techniques (Denis et al. 2016) or preserving the larvae in alternative fixatives (Figueiredo et al. 2005) give evidence that larval fish do feed on protist prey. Estimates of functional response suggest that feeding on PZP can partially or totally fulfill energetic needs of a fish larva (Figueiredo et al. 2007). Besides few laboratory studies little is known about prey specific selectivity of larval fish, comparing PZP against metazoan prey (Hunt von Herbing & Gallager 2000, Friedenberg et al. 2012).

Investigations of PZP-larval fish dynamics in the field are more challenging as many unknown or uncontrolled conditions occur, such as patchiness of prey (Montagnes et al. 1999), and no direct observations of the behavior and interaction among plankton groups are possible. Additionally, direct linkage of PZP (and MZP) and larval fish abundance as performed for mesozooplankton (Alvarez-Fernandez et al. 2015, Batten et al. 2016) is not yet feasible due to inappropriate spatial and temporal coverage of field sampling.

1.2.1 Tools to investigate the microzooplankton – ichthyoplankton link

Spatial match of potential prey and larvae is difficult to determine, if it is unknown what organisms of the plankton assemblage the larvae prefer and if the available prey field is of sufficient nutritional quality to guarantee or support larval survival. The classical way to determine what a larva has consumed is the analysis of the gut content. It gives a direct insight in the recent consumption, quantity and composition of prey. Nevertheless, this methods neglects differing digestion times of prey classes, specific items lasting longer in the digestive tract and are thus recognized more often than easily digested prey (Figueiredo et al. 2005) and small prey items are often overlooked or not recognized in partially digested stage.

Therefore indirect methods are applied and developed to investigate the link between microzooplankton and larval fish in their natural habitats. Longer term growth rates can be determined via otolith microstructure, giving indications of the conditions the larvae experienced since the day they hatched (Geffen 2009) and the composition of specific stable

isotopes (e.g. $\delta^{18}O$ or Sr:Ca, Ba:Ca) in the otoliths are used to track the origin of the larvae (Morat et al. 2014, Starrs et al. 2016), which can then possibly be linked with the conditions they have experienced so far. Further biochemical proxies that have been applied in the field are for instance the ratio of RNA to DNA (RNA/DNA) and fatty acid composition (Clemmesen 1994, Paulsen et al. 2014), stable isotope analysis (Pepin & Dower 2007), gut fluorescence (Denis et al. 2017) or first approaches on DNA barcoding of larval gut contents (Riemann et al. 2010). Research on the RNA/DNA started in the late 1970s (Buckley 1979). It is based on the fact that the DNA content of an organism is relatively stable, whereas the RNA content changes depending on the amount of protein synthesis. The more protein is synthesized by an organism the better is the status of its metabolism, which is indicative for a good nutritional condition. The method has been constantly improved and standardized in between different analyzing techniques (standardized RNA/DNA, *s*RD) (Caldarone et al. 2006). Now the *s*RD is a widely used proxy to assess recent larval nutritional condition over the past 2-3 days (Peck et al. 2012). In addition, the *s*RD can be used for the calculation of the instantaneous growth rates of a larva (Buckley et al. 2008). In contrast to long-term growth history derived by otolith analysis (e.g. Fox et al. 2003, Geffen 2009), biochemically determined growth rate are instantaneous (Buckley et al. 2008) and can (more or less) directly be linked to recent food availability or environmental conditions (Peck et al. 2015).

Stable isotope analysis (SIA) is a biochemical method to assess trophic predator-prey relationships. It is based on the isotopic fractionation of certain elements. In trophic studies, mainly carbon (C) and nitrogen (N) stable isotopes are used. Unlike gut content analyses, which give a snap shot in time of feeding habits, SIA allows investigation of longer term diet. The heavy isotopes of ^{13}C and ^{15}N are accumulated within the food web by a specific rate and the relative ratio of the heavy isotope (^{13}C and ^{15}N) to the lighter isotope (^{12}C and ^{14}N), which is expressed in parts per million (‰) with the δ notation, gives indications about the role of an organism in the food web. The $\delta^{13}C$ is only slightly enriched in the food web and is therefore used to detect the main source of carbon. The position in the food web is determined by $\delta^{15}N$ as it is enriched in the food web with every trophic step. SIA is widely applied for studying trophic interactions as well as changes in feeding habits during ontogeny or food web dynamics in general (Pepin & Dower 2007, Malzahn & Boersma 2009, Laiz-Carrión et al. 2015).

Finally, a theoretical approach to study the interaction of PZP/MZP with larval fish is the implementation in individual based models (IBM). These models aim to predict how a larva is affected by the surrounding biotic and abiotic conditions, modeling early life history of distinct individuals and taking interaction with other individuals in the population and the biological and hydrographic environment into account (Hermann 2001, Peck et al. 2012). To predict growth rates (and survival) in the field physiological based foraging and growth IBMs have been developed and improved (Werner et al. 2001, Peck & Daewel 2007, Hufnagl & Peck 2011) linking physiological parameters of an individual larvae with the hydrographic and prey conditions during a certain time step. The latest versions are based on a balanced energy budget, which models growth as a function of energy gain, assimilation and metabolic costs. It is depending on various physiological and environmental features, such as larval feeding behavior, assimilation efficiency, prey preferences, prey handling time, temperature and larval size. Until now, models do not account for many MZP and PZP components due to the scarcity of data. Including this prey fraction to the models could show if or to which extend PZP and MZP contribute to larval growth and survival. Hence, it is widely accepted that data on the small sized prey field is urgently needed to make the predictions more realistically (Hufnagl et al. 2015).

1.3 The microzooplankton-ichthyoplankton link in NE Atlantic herring as a case study

The Northeast Atlantic (NE Atlantic) stretches southwards from Arctic waters east of Greenland (~ 80°N) over the Celtic Seas around Ireland and the open Atlantic ocean and is southerly bordered by the Strait of Gibraltar. Eastwards the NE Atlantic includes the adjacent waters of the North and Baltic Seas. Two major current systems influence the NE Atlantic, the cold Greenland current flowing southwards and the North Atlantic current distributing warmer waters north and eastwards, supporting water exchange with the semi-enclosed North- and Baltic Seas. The North Atlantic oscillation (NAO), a major climate forcing, is induced by the strength of westerly winds and has a massive impact on the dynamics in the NE Atlantic by influencing air and sea temperatures, precipitation, water flows and hence ecological processes (Ottersen et al. 2001).

1.3.1 Plankton community in the NE Atlantic

Strong seasonal patterns associated with upper layer stratification processes in the NE Atlantic lead to one (spring) or two (spring and autumn) phytoplankton blooms in the NE Atlantic (Martinez et al. 2011). The cycle of the phytoplankton blooms is acting as a baseline for annual food-web dynamics, by providing food for herbivorous planktonic organisms, such as protozoa and copepods, which are then transferring energy to higher trophic levels, such as planktivorous fish (Platt et al. 2003). To investigate the plankton community and detect long-term changes, several monitoring programs have been installed across the NE Atlantic. Besides the permanent monitoring stations, established by the International council of the exploration of the sea, ICES (www.wgze.org) and dating back to the 1960s, the most ambitious and extensive is the CPR monitoring project (see www.sahfos.ac.uk). As sampling started in 1931 and has expanded since then, the CPR has a large temporal and spatial coverage, especially within the NE Atlantic. There are only scarce data on microzooplankton, especially on PZP in the NE Atlantic covering large spatial or temporal scale. Longer term monitoring (>3 years) on the entire PZP community is so far restricted to one specific site (e.g. station L4 in the English Channel from the ICES working group of zooplankton ecology, wgze). Studies on a bigger spatial scale only cover a few seasonal or annual cycles (Smetacek 1981, Löder et al. 2012) and/or are focused on restricted PZP components (e.g. ciliates) (Montagnes et al. 1999, Yang et al. 2015).

1.3.2 Herring in the NE Atlantic

Atlantic herring (*Clupea harengus*) plays a key ecological and economic role within the NE Atlantic as one of the numerically most important planktivorous fish species (ices.dk). Its adaptability allows to be widely distributed over the NE Atlantic from the Bay of Biscay to Spitzbergen (Figure 1.5). The populations are often mixed except for spawning seasons, when they can be discriminated by their specific spawning times and locations. Atlantic herring is capable of producing offspring year-round (Geffen 2009), but roughly herring can be divided into spring spawning and autumn spawning populations, and within those by their specific spawning sites, which must fulfill certain requirements.

The benthic eggs are sticky and need gravel beds (or vegetation in e.g. the Baltic Sea) as underground. Depending on the season they hatch, the larvae experience different conditions in their early life.

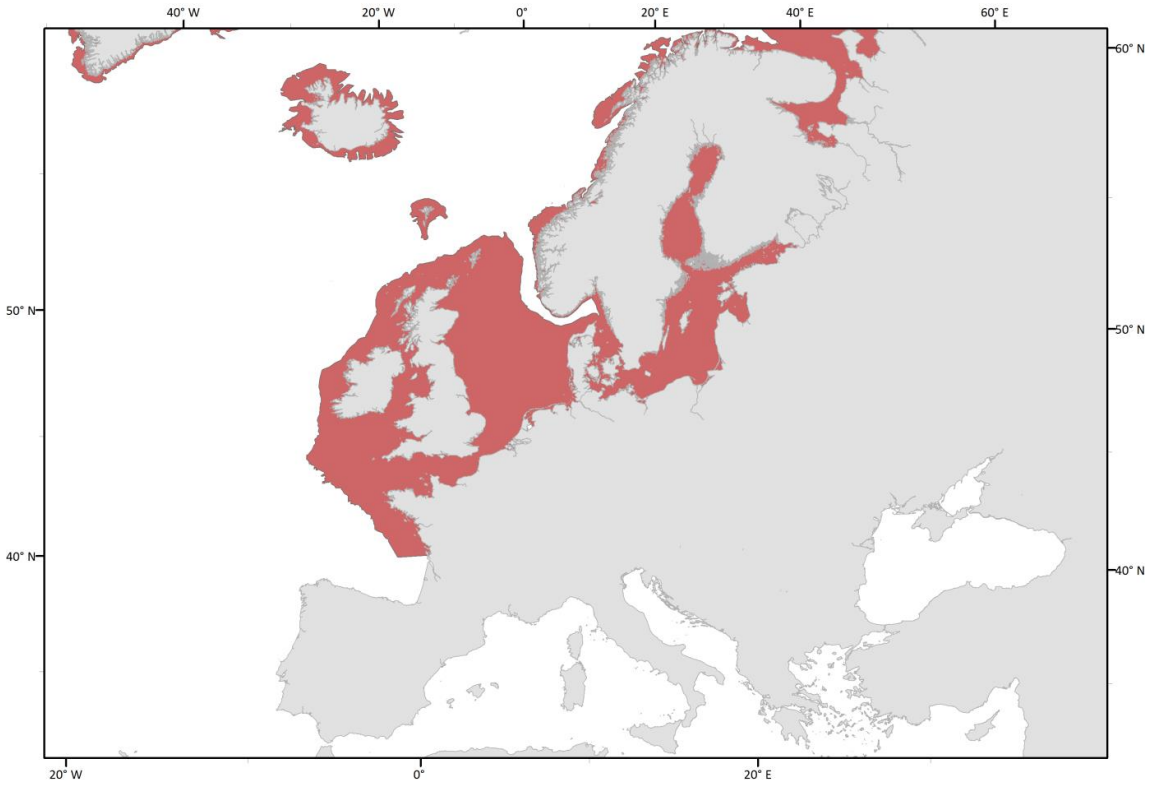

Figure 1.5: Distribution of Atlantic herring (*Clupea harengus*) in the North East Atlantic is displayed by the red shaded areas. The data was kindly provided by FAO. Map drawing by Alexander Schubert.

Larvae hatched during the spring bloom (spring spawners) will experience more favorable conditions in terms of prey, in contrast to the early life stages of autumn spawners which are facing low productivity environments. During their larval stage they drift with the currents on characteristic routes to their nursery grounds and as juveniles join the adult stock (Geffen 2009).

1.3.3 Atlantic herring fisheries and the importance of early life stages

Due to the commercial importance of Atlantic herring and its key position in the NE Atlantic ecosystem, unique time-series have been developed by the ICES to observe and manage the different herring stocks. The outcome of their observations and estimations lead to suggestions for the total allowed catch (TAC) of the EU countries. Big effort by institutions of the ICES member countries are undertaken to investigate different life stages of Atlantic

herring for fisheries management. Adult schools are quantified using hydroacoustic methods, e.g. in the North Sea (HERAS) or on the Malin Shelf west of Scotland (MSHS). Trawling surveys targeting adult and juvenile stages are carried out annualy (e.g. International Bottom Trawl Survey, IBTS, in the North Sea and the Scottish West Coast Ground Fish Surveys). To estimate the spawning stock biomass (SSB) and to calculate recruitment indices herring larvae surveys have been conducted since more than 40 years. The Rügen Herring Larvae Survey (RHLS, carried out by the Thünen Institute of Baltic Sea Fisheries) samples larvae of the Western Baltic spring spawning Herring annually on a weekly basis in the spawning season from March to June in their main spawning ground in the Greifswald Bay. Larvae of autumn spawners are for instance sampled in the spawning grounds in the Irish Sea (Northern Irish Herring Larvae Survey, NINEL) every November since 1993. The International Herring Larvae Survey (IHLS, established 1967) is carried out by IMARES Wageningen (The Netherlands) and Thünen Institute for Sea Fisheries (Germany) and aims to sample newly hatched larvae on the different spawning grounds of North Sea Autumn spawners (NSAS), which is one of the largest herring stocks in the NE Atlantic with estimated SSB of 2.3mio t and annual catches of more than 500 000 t (ICES 2017). Sampling starts in September in the spawning components Buchan and Banks east of Scotland and in the central North Sea. It is then continued in December and January during the spawning season of the Downs component in the English Channel. Later larvae are sampled at the MIK (Midwater Ring Net) survey as part of the IBTS.

Thanks to these time-series it could be recorded that stocks of North Atlantic autumn spawning herring (NAAS) have undergone severe fluctuations in SSB and recruitment during the last century. Massive overfishing leading to stock collapses and thanks to temporary closure of fisheries the stock recovered. While some stocks exhibit a fairly stable recruitment nowadays (e.g. Irish Sea spawning stock (ICES 2016)), the North Sea autumn spawning stock (NSAS) is facing another recruitment depression since the early 2000s. As SSB is stable and fishing pressure on low level (ICES 2014), the reasons for the ongoing depression remain unclear and several hypothesis have arisen since then.

Top-down processes, such as the predation of adult herring on their offspring (Corten 2013), could influence recruitment negatively and also the effect of feeding and parasites on NSAS

recruitment has been discussed recently (Lusseau et al. 2014). Using time series derived data (Fässler et al. 2011) concluded that temperature (directly or indirectly) strongly effects larval survival and adding time-series data from the CPR survey (Alvarez-Fernandez et al. 2015) showed that a mismatch of potential prey and larvae could strongly impact on larval overwinter survival. Hufnagl & Peck (2011, 2015) took advantage of the extensive amount of studies on Atlantic herring and suggest in their IBM that temperature as well as prey abundance and availability has a strong effect on overwinter survival. However, even if suggesting various causes of the recruitment depression, most of these and other hypotheses (e.g. Payne et al. 2013) are united by the assumption, that recruitment of autumn spawning herring is largely established in the overwintering larval stage and that finding the appropriate prey is an obligatory requirement for survival and hence recruitment success.

Additionally to the extending knowledge about stock dynamics and larval distribution patterns a long and successful history of experimental studies on larval herring started already in the late 1800s (Geffen 2009). Thanks to a variety of laboratory studies, covering physiology (Moyano et al. 2016), feeding strategies (Blaxter 1965), prey preferences (Spittler et al. 1990, Munk 1992) or behavior (Illing et al. 2016), a broad knowledge about larvae and their response to environmental drivers exists.

1.3.4 The microzooplankton-larval herring link in the NE Atlantic

Herring larvae need to find appropriate prey as soon as the energy reserves of the yolk sac are absorbed. The prey item a herring larva can capture and ingest strongly depends on prey escape response, larval mouth gape size and capture success (Checkley 1982, Hufnagl & Peck 2011), the latter ones increasing with larval development. Based on the common assumption that herring larvae feed mainly on copepods and their early life stages (nauplii and copepodites) or invertebrate larvae (Checkley 1982, Munk & Kiørboe 1985) most laboratory studies, concerning prey size selectivity (Batty et al. 1990) or foraging behavior (Kiørboe & Munk 1986), are conducted with these prey items. Many field studies on herring larvae feeding presuppose this preference (Alvarez-Fernandez et al. 2015, Paulsen et al. 2017) or, based on gut content analysis, support these findings (Fox et al. 1999, Margoński et al. 2006, Arula et al. 2012). However, it is known for almost 100 years that larval herring also prey on

other, smaller, planktonic organisms, such as phytoplankton and PZP (Hardy 1924). The discrepancy between this knowledge and the majority of the actual findings in gut contents can be explained by three major factors concerning sampling or larval physiology: (i) gut content analysis of herring larvae are challenging in general as they tend to regurgitate their gut content during capture (Hay 1981), (ii) many protists are digested very fast (within minutes) (Ohman et al. 1991) (iii) the larvae are preferably preserved in formalin, which makes delicate plankton, such as PZP, dissolve.

Nevertheless, recent studies are highlighting the importance of PZP as prey for larval herring, supporting older works (Blaxter 1965, Spittler et al. 1990). For example, Figueiredo et al. (2005) suggested that the diet of autumn spawned herring larvae in the Irish Sea is composed up to 70% of PZP prey and Denis et al. (2016) found a big amount of prey smaller than 50 µm in the guts of herring larvae. Research on the feeding ecology of herring larvae is of special importance for early life stages of autumn spawning herring, as they have to withstand periods of low plankton abundance during their first months of life (www.wgze.net). The larvae of the NAAS have to overwinter in the larval stage until they metamorphose the following spring (Heath & Richardson 1989). They are able to survive periods with only little somatic growth (Johannessen et al. 2000), but there is still doubt about feeding strategies of herring larvae during this time of year.

Most of the current studies state that MZP and PZP could play an important role to support larval survival in winter. Nevertheless, for the most part, MZP and PZP are ignored in conceptual models of factors controlling the condition and growth of herring larvae, mostly due to the lack of longer-term data on plankton and the scarce knowledge about PZP/MZP - herring larvae interaction. In general thorough understanding of recruitment dynamics needs a fundamental understanding of the food webs supporting larval fish growth and survival.

1.4 Goals and outline of the thesis

The present study aims to investigate the microzooplankton-ichthyoplankton link during low productivity conditions in temperate waters. Despite the well-known importance of PZP for marine ecosystems, there is still an immense knowledge gap regarding their ecology,

distribution and interaction with other trophic levels. The ecological importance of PZP is thought to be most pronounced during times of limited nutrient or light availability (e.g. winter in temperate waters), when the classical, linear food chain “large phytoplankton–copepods-fish” is replaced by a strengthened microbial loop, i.e. “DOM – bacteria – PZP – mesozooplankton”. North Atlantic autumn spawning herring (NAAS) was chosen to be an optimal case to study the microzooplankton–ichthyoplankton link in the field because of several reasons. As the larvae have to overwinter in the larval stage, their abundance overlaps with the period of a microbial loop dominated system, but nevertheless, the entire feeding dynamics remain unresolved. Due to the multi annual larval sampling as part of fish stock assessment simultaneous sampling of larvae and potential prey items covering different spawning seasons and locations was enabled. Furthermore, a long history of experimental studies on various larval characteristics, such as ecophysiology, prey selection and behavior exist, making Atlantic herring an ideal candidate to transfer this knowledge to the field and merging laboratory based and field derived results in a successfully developed IBM for larval herring. The potential impact of PZP and MZP on Atlantic herring was studied combining field sampling of herring larvae and plankton in the spawning stocks in the North Sea and Irish Sea with laboratory based taxonomic and biochemical analysis. These findings were incorporated in a foraging and growth IBM.

Three major research questions are addressed in this thesis:

1. How is the PZP community composed during wintertime on a large spatial scale? Are there possibilities and needs for time-series?

2. What is the small sized *in-situ* prey field (PZP, MZP) herring larvae experience and how is it related to larval abundance and growth?

3. Can stable isotopes and RNA/DNA help to identify trophic interactions and larval growth and condition in the field?

So far there has been no broad spatial scale investigation of PZP in the North Sea in any season. Manuscript 1 aimed to act as a first overview about the abundance and distribution of PZP in the North Sea during winter. Heterotrophic and mixotrophic dinoflagellates and ciliates were identified to the lowest taxonomic level possible and their distribution patterns

discussed based on hydrographical and species specific features as well as in the light of potential food source for winter spawned fish larvae. This study highlights the importance of further resolve the role of PZP in the ecosystem as, for instance, ecological indicators and to enhance modelling efforts (e.g. NPZD or physiological IBMs). It presents a unique possibility of adding this kind of sampling to routine surveys for fish stock assessment for establishing a PZP time-series on a large spatial scale.

Field studies on the microzooplankton–ichthyoplankton link remain scarce and little is known about the prey fields fish larvae experience during times of low productivity conditions. This is crucial to understand in-situ food-web dynamics and for being able to include these data into IBMs to explore nutritional needs and the role of MZP and PZP for fish larvae nutrition and to further investigate the factors driving recruitment. Manuscript 2 describes a field study, which was conducted in the herring autumn spawning grounds in the Irish Sea. It combined field sampling, statistical modelling and foraging and growth model simulations to examine the autumn PZP assemblage and the potential strength of the microzooplankton-ichthyoplankton link in the Irish Sea herring stock, which does not suffer of low recruitment. This study was undertaken to gain knowledge about the nutritional status and the small prey field conditions (PZP and copepod early life stages) of larvae of a healthy herring stock. The impact of environmental (and prey) factors on plankton and larval fish distribution was tested using a statistical model. For the first time PZP was integrated in an IBM to predict larval growth rates. These predictions were compared to the observed (biochemically-based) in-situ growth rates to explore whether in situ biomass of small plankton were necessary and sufficient to support growth and possibly survival during the larval season of herring in the Irish Sea and if this could be simulated with the model. The results suggest that small planktonic organisms (20–300µm) should be routinely surveyed to better understand factors affecting larval fish feeding, growth and survival.

To further resolve the link between small sized prey and fish larvae it is necessary not only to investigate composition of potential prey in their habitat, but furthermore determine the type of organisms contributing to the larval diet and their impact on larval growth and survival. Manuscript 3 describes a field study undertaken in two different spawning grounds of NSAS (autumn and winter) covering two subsequent spawning seasons. The potential role

of small prey (PZP and MZP) was investigated using taxonomic identification as well as indirect biochemical measurements. The isotopic composition of carbon (δ13C) and nitrogen (δ15N) was used to determine the contribution of PZP and MZP to larval nutrition and to detect changes during larval ontogeny and between spawning grounds. Furthermore nutritional condition indices (RNA/DNA) were measured to reveal potential differences within spawning sites and season, which could be related to available small prey. The results gained in this study give way to hypothesis about the importance of appropriate prey in herring early life stages for growth, survival and potentially recruitment dynamics.

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CHAPTER 2

2. Winter protozooplankton community in the North Sea examined using a routine fisheries survey (Manuscript 1)

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2.1 Abstract

Protozooplankton (PZP) (here size range: 12-200 µm) are rarely sampled in ecosystem monitoring programs despite their trophodynamic importance as grazers in the microbial loop and as prey for larger zooplankton and early life stages of fish. In this study we conducted PZP sampling onboard Dutch, French, German and Norwegian research vessels taking part in the annual ICES coordinated International Bottom Trawl Survey (IBTS) which provides data on fish stock abundances and status for the North Sea. The abundance, biomass, composition and distribution of PZP was examined at 39 stations across the North Sea (from 3.2°W to 7.6°E and 50.5 to 59.8°N) in mid-winter (January-February 2014), a period of the year which is under-investigated so far. Twenty four taxa of dinoflagellates and ciliates were identified. Two groups comprised 89% of the total abundance of PZP: *Gymnodinium* spp. and other athecate dinoflagellates (68%) and *Strombidium* spp. and other naked ciliates (21%). The biomass of PZP at each station ranged between 0.08 and 2.4 µg C L^{-1}, which is much lower than that reported for spring or summer (≥ 100 µg C L^{-1}) in the North Sea. Relatively small-sized (<50 µm) PZP contributed 41% of the total biomass. No significant spatial pattern in the composition of the PZP community was found, although the total abundance of tintinnids was highest in the southern North Sea, an important area for over-wintering marine fish larvae. Using this fish survey (IBTS) as a sampling platform allowed us to obtain a synoptic view of the PZP community and provides an example of how existing monitoring platforms can be augmented in the future to collect relevant data needed to advance the ecosystem-based approach to managing marine systems.

Key words: Microzooplankton, North Sea, time-series, monitoring, International Bottom Trawl Survey, ecosystem-based management

2.2 Introduction

Protozooplankton (PZP), primarily dominated by ciliates and dinoflagellates, are important members of the "microbial loop" (Azam et al. 1983) that can exert considerable grazing pressure on primary production (Calbet 2008). For example, heterotrophic protists can graze up to 60% of the annual phytoplankton biomass in temperate waters (Levinsen & Nielsen 2002, Fileman et al. 2002). By consuming other heterotrophic organisms including bacteria (Aberle et al. 2007, Sherr & Sherr 2007) and by acting as prey for copepods (Calbet & Saiz 2005, Fileman et al. 2007) and even higher trophic levels, such as larval fish (Hardy 1924, Montagnes et al. 2010), PZP play an important role in the carbon cycle of marine systems (Hansen et al. 1999). The PZP community and microbial loop play a particularly important role in food web dynamics when primary productivity is low (either due to low levels of nutrients or intensities of light) (Fileman et al. 2011).

Despite the importance of PZP in planktonic food-webs, these organisms are largely ignored in monitoring programs (Stern et al. 2015). Generally, the PZP community has been sampled only at specific stations and/or in specific seasons in temperate shelf seas (Dolan & Coats 1990, Edwards & Burkill 1995, Tillmann & Hesse 1998, Löder et al. 2012) or open ocean waters (e.g. Pacific (Stoecker et al. 1996), Antarctic (Dolan et al. 2013) and Arctic (Monti & Minocci 2013)). There are a few exceptions such as the plankton monitoring program in Chesapeake Bay from 1984 – 2001 (Coats & Revelante 1999). The availability of such long-term as well as broad-scale data on PZP would be particularly valuable to help validate models designed to depict seasonal plankton dynamics such as NPZD (Nutrient Phytoplankton Zooplankton Detritus) or broader end-to-end models (D'Alelio et al. 2016).

The North Sea is a relatively large shelf sea (750,000 km^2) situated on the northeast Atlantic with a shallow southern half (≤ 50 m) and a deeper (max. 700 m) northern half with dominant inflows of Atlantic water from the north and a cyclonic current regime which is strongest in the southern region; a thorough review of the hydrography and physical forcing is been provided by Sündermann und Pohlmann (2016). The plankton dynamics of this shelf ecosystem have been well-studied using long-term monitoring stations such as Helgoland Roads (Wiltshire et al. 2010), Plymouth L4 (Harris 2010) and several other locations (www.wgze.net). Phyto- and mesozooplankton are also monitored using ships of

opportunity as part of the Continuous Plankton Recorder (CPR) program (O'Brien et al. 2013, ICES 2016). Unfortunately, plankton monitoring programs in the North Sea have largely ignored PZP, except at Plymouth Station L4 where microplankton is routinely collected and various taxa quantified. Moreover, much less monitoring of plankton (from phyto- to zooplankton) has been conducted in the North Sea during winter, when plankton blooms are absent and, consequently, the microbial loop is considered to play a major role in energy transfer to higher trophic levels (Fileman et al. 2011).

The present study aims to reveal if potential differences in the PZP community composition, abundance or biomass are related to the oceanographic conditions in the North Sea in mid-winter. This large scale sampling campaign was conducted within the framework of the ICES coordinated International Bottom Trawl Survey (IBTS) in January/February 2014. The IBTS is a fish survey conducted twice a year in the North Sea by several European countries which, during winter (IBTS quarter 1, Q1), also samples the overwintering larvae of autumn spawning herring (*Clupea harengus*) to calculate an index of recruitment strength (ICES 2016). Sampling PZP during the IBTS was considered to be highly relevant given the potential importance of PZP as prey for herring larvae (Figueiredo et al. 2005, Friedenberg et al. 2012, Illing et al. 2015, Denis et al. 2016, Bils et al. 2017) and the suggestion that recruitment success of herring may be linked to feeding conditions experienced by overwintering larvae (Payne et al. 2013, Hufnagl et al. 2015).

2.3 Material & Methods

2.3.1 Sampling

Water samples were collected on the IBTS Q1, between January 15th and February 19th 2014, by marine institutes of Norway, the Netherlands, France and Germany, respectively. A total of 157 stations were sampled covering the area bounded by 49.4°N to 61.2°N and 2.5°W to 7.8°E (Figure 2.1, Table 3.1). PZP sampling did not require any special permit besides the standard work permit issued by each coastal state in which territorial waters and exclusive economic zones (EEZ) the IBTS is operating (for details see 2.8 Supporting information). Sampling did not involve any disturbances of the seabed or of endangered or protected species.

Water samples were taken at 10-m depth using a Niskin bottle attached to a CTD rosette. This depth was chosen to guarantee sampling in the photic surface zone, practicable even under adverse sea conditions. The samples were immediately transferred into brown, 500-mL glass bottles and preserved with neutral Lugol's iodine solution (2% final concentration). Samples were stored in darkness. Environmental factors were recorded by a CTD (RV Tridens, RV G.O. Sars & RV Walther Herwig: Seabird SBE 911, RV Thalassa: Seabird SBE 19+) on 254 stations including the stations sampled for PZP. Fluorescence was recorded only during the French and the German coordinated cruises hence the factor fluorescence was excluded from statistical analyses.

Table 2.1: Survey dates and research vessels used during the International Bottom Trawl Survey Q1 in the present study. Time period of sampling and number of samples from each survey analysed in this study are provided.

Country	Ship	Survey Period 2014	Period analysed	Number of samples
Norway	G.O. Sars	14.1. - 19.2.	7.2. - 14.2.	9
Germany	Walther Herwig	23.1. – 24.2.	30.1. - 19.2.	14
France	Thalassa	13.1. - 14.2.	19.1. - 8.2.	8
Netherlands	Tridens	26.1. - 28.2.	27.1. - 6.2.	8

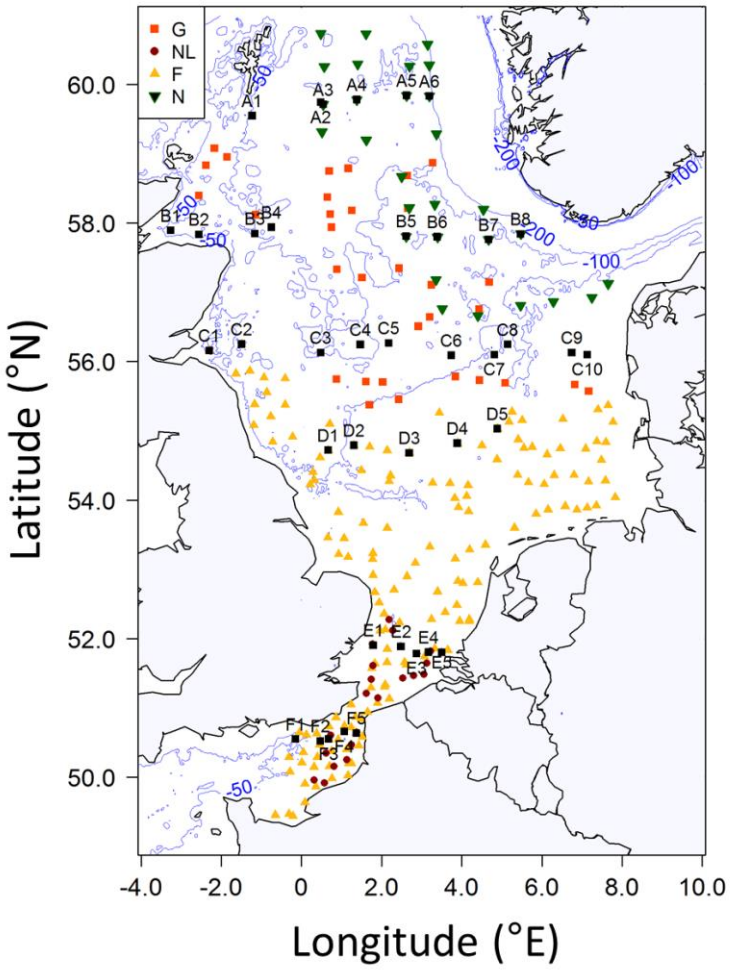

Figure 2.1: **Protozooplankton sampling stations in the North Sea during the International Bottom Trawl Survey Q1 in 2014. Stations are color- and shape-coded according to the country in charge of the IBTS sampling. Samples analysed in the present study are highlighted in black, labelled from North to South alphabetically and from West to East numerically. Abbreviations: N = Norway, G = Germany, F = France, NL = The Netherlands, see text for further details.**

2.3.2 PZP biomass, abundance and community composition

A total of 39 stations in 6 latitudinal transects (A – F, North to South) were selected for further analyses in order to maximize the coverage of the sampling area (Figure 2.1). On each transect, stations were labelled numerically from West to East. Due to very poor weather conditions and sea state which limited PZP sampling, no longitudinal transect was possible between 52 and 54°N.

Water samples were settled in a 100-mL sedimentation chamber (HydroBios, Germany) for 48 h, and PZP was counted and identified under an inverted microscope (Leica DMI 3000,

x200) using the method described by Utermöhl (1958). Due to the low density of cells, the whole plate was counted to avoid underrepresentation of less abundant groups. Heterotrophic and mixotrophic ciliates and dinoflagellates >12 µm were identified to the lowest taxonomic level possible, following a combination of references and identification keys (Dodge & 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) was used to determine trophic status. The ciliate species *Mesodinium rubrum* and *Laboea strobila* were included as obligate mixotrophs due to their photosynthetic capabilities coupled with optional phagotrophy (Stoecker et al. 1988, Crawford 1989). To ensure more precise biomass calculations, the most abundant taxa were separated into 2 to 4 size classes. Images were taken with a camera system (Moticam) attached to the microscope. Biovolumes (μm^3) were estimated for each taxon (n >10) using image analysis (Image J, 1.6.0, freeware, Wayne Rasband J), applying specific geometric shapes (Hillebrand et al. 1999, Olenina et al. 2006, Strüder-Kypke et al. 2006). The biovolume of less abundant taxa was estimated using literature values (Olenina et al. 2006, Strüder-Kypke et al. 2006, Löder et al. 2012). Biovolume was converted to carbon biomass (µg C L^{-1}) using the Carbon to Volume (C:Vol) relationship for protists (Menden-Deuer & Lessard 2000).

2.3.3 Data analysis

The data analysis was performed using PRIMER 6 (Clarke & Warwick 2005) and R software (R core team 2014). Hierarchical clustering and Principal Component Analysis (PCA) was conducted with the Euclidean distances to explore regional differences of the sampling area based merely on temperature and salinity. Bottom depth was not included in the analysis. Similarities in PZP abundance among stations were examined with Correspondence Analysis (CA), using the χ^2 distances calculated with the R-package 'vegan' (Oksanen et al. 2016).

As sampling was conducted 24 h per day and the sampling lasted for 5 weeks, an analysis of similarity (ANOSIM) was performed to test for the influence of light and/or sampling week on the PZP composition. 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.

Linear dependence of PZP abundance and environmental/geospatial factors (temperature, salinity, longitude, latitude) was tested using Pearson correlation coefficient ($p < 0.05$). In

order to account for potential (dis)similarities in the diversity of the PZP community among stations or areas, the Shannon diversity index (H) was calculated for each station (Shannon & Weaver 1949)

$$H = \sum_{i=1}^{n} pi \cdot \ln pi$$

Where p_i is the proportional abundance of species *i* and n is the number of taxa.

Care should be taken when attempting to compare diversity values calculated in this and other studies, as each taxon was not always identified to the same taxonomic level.

2.4 Results

2.4.1 Area of study and plankton sampling

Weather conditions were generally stormy and the water column was well mixed (data not shown). Temperatures ranged from 4.0°C in the northern North Sea (55.12°N, 7.77°E) to 10.9°C in the English Channel (50.08°N, 0.29°W) and were generally higher in the shallower southern areas compared to central and northern areas (Figure 2.2). Salinity ranged from 31.4 in coastal areas (53.86°N, 6.87°E) to 35.4 in offshore areas and in the English Channel (50.53°N, 0.47°E). Fluorescence was generally low (<0.4 relative fluorescence units) and only a few stations had values above 0.6.

Temperature explained 93.5% of the variability in the PCA among stations (Eigenvector PC 1: 0.98). Two groups of stations could be identified by hierarchical clustering: 'warm' (English Channel) and 'cold' (central and northern North Sea). Four stations (B7, C6, C8 and C9), located in the Northwest, were not classified in any of these two groups (Figure 2.3).

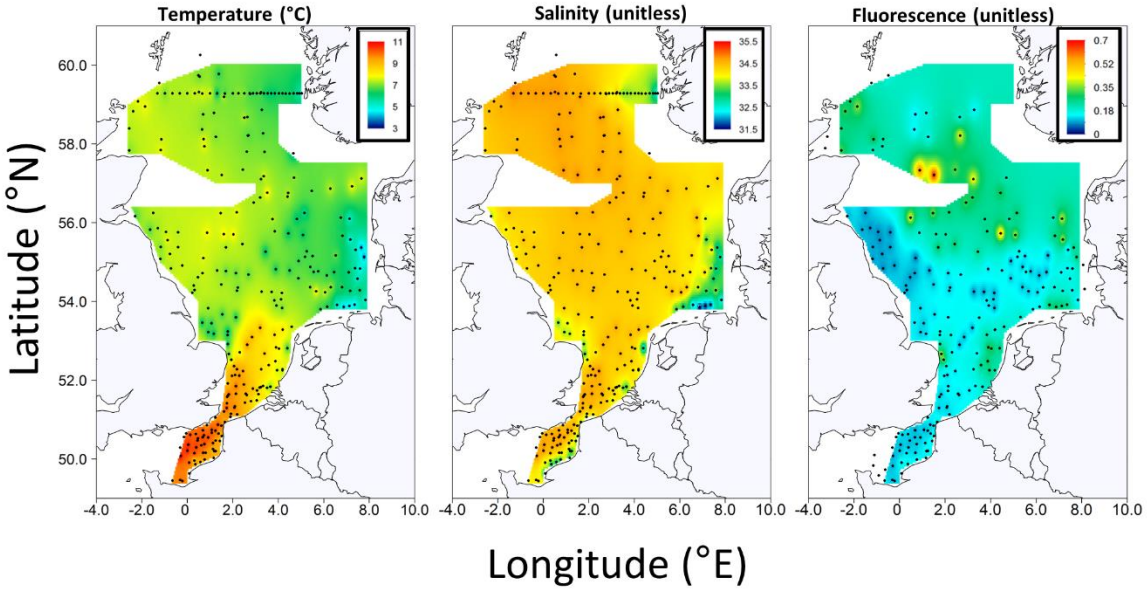

Figure 2.2: **Temperature (°C), salinity and relative fluorescence (unitless) at 10 m depth during winter in the North Sea. Values are interpolated over the time frame of the survey (5 weeks), and sampling stations are displayed as black dots.**

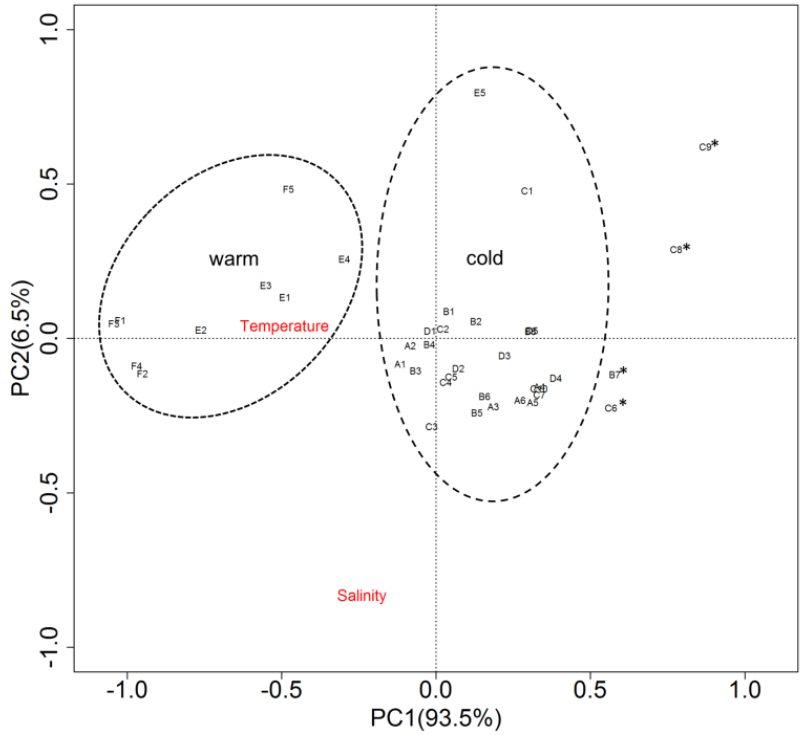

Figure 2.3: **Principal component analysis (PCA) of the environmental data (temperature, salinity) of the 39 sampling stations in the North Sea during mid-winter 2014. Note two clear groups were observed mainly due to temperature (PC1): cold and warm stations. Outliers are marked (*).**

2.4.2 PZP abundance and carbon biomass

In total 10 ciliate and 14 dinoflagellate taxa were found, of which eight dinoflagellate and two ciliate taxa were considered to be mixotrophs (Table 2.2). Athecate dinoflagellates and naked ciliates represented 68% (40%) and 21% (32%) of the total abundance (biomass) of PZP, respectively. *Gymnodinium* spp. was the only taxon occurring at every station, *Strombidium* spp. was the most abundant ciliate taxon (present at 95% of the stations) and about 50% of the cells were <20 µm. Overall, small cells (<50 µm) accounted for 77% (58% dinoflagellates and 19% ciliates) of the total mean abundance.

Total biomass ranged from 0.08 (Station A4) to 2.4 µg C L^{-1} (Station D3, Figure 2.4).

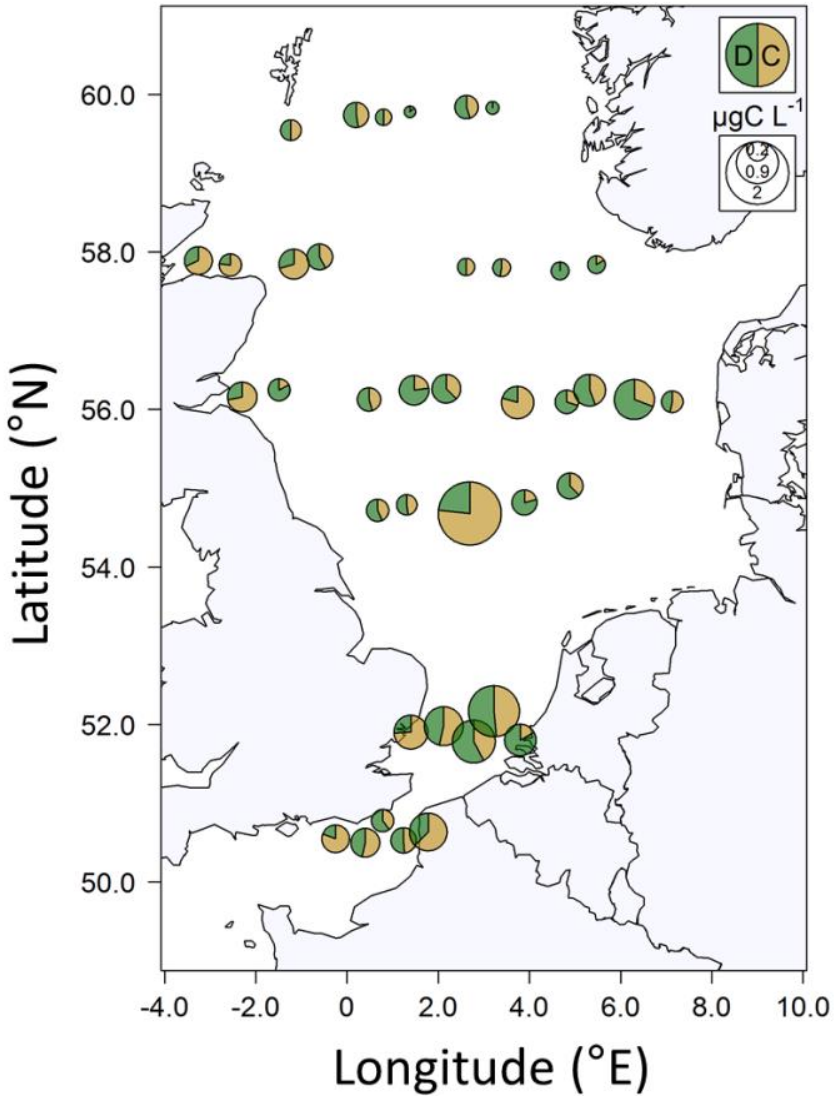

Figure 2.4: **Biomass (µg C L^{-1}) of ciliates C (yellow) and dinoflagellates D (green) during mid-winter 2014 in the North Sea. The size of the pie represents the total biomass and the color the relative contribution of ciliates/dinoflagellates.**

Ciliates accounted for 52% and dinoflagellates for 48% of the total biomass (Table 2.2). In 66% of the stations, dinoflagellates formed >50% of the total carbon biomass (Figure 2.5). In two of the northerly stations (A6, B7), dinoflagellates exceeded 99% of the total biomass, whereas they contributed <25% at four stations (B2, C6, D3, F1) distributed across the sampling area (2.8 Supporting information Table 2.4). *Strombidium* spp. and *Gymnodinium* spp. accounted for 22% and 16% of the total PZP biomass. Other taxa with a significant contribution to the total mean biomass were *Torodinium robustum* (11%) and *Protoperidinium* spp. (10%). In general, the small PZP size fraction (<50 µm) accounted for 47% of the biomass.

The total PZP biomass was negatively correlated with latitude (Pearson, -0.55, $p < 0.005$) and depth (-0.422, $p < 0.05$). Furthermore, a negative correlation was found for dinoflagellate biomass and salinity ($R = -0.523$, $p < 0.0005$). The time (week) of sampling had a weak, albeit significant, effect on the community composition (1-way ANOSIM, $R = 0.161$, $p < 0.005$) while time of day (absence/presence of daylight) had no significant effect ($R = -0.066$, $p > 0.5$). Thus, these two factors were not included in the analysis.

In terms of spatial patterns, the first two components of the CA only explained 26.7% and 14.3%, respectively, of the variation in the PZP community composition among stations (Figure 2.6). Northeastern stations (B7, B8, C7, C8, C9 and C10) were characterized by a high abundance of the athecate dinoflagellates *Torodinium robustum* and *Katodinium* sp., and the absence of the ciliates *Mesodinium rubrum* and *Spathidium* sp. which, in turn, were most abundant in two central stations (C1, D3). Stations in the English Channel (E1, E2, E3, E5 and F5) as well as one northern station (B5) could be grouped due to the high abundance of *Prorocentrum micans* and the abundance of *Tintinnida* was associated with southern stations. The total abundance of loricated ciliates correlated negatively with latitude ($R = -0.43$, $p < 0.05$). The Shannon diversity index of the PZP community ranged from 1.3 to 2.3 and was not related to temperature, salinity, latitude, longitude or bottom depth (Pearson correlation, $p > 0.5$).

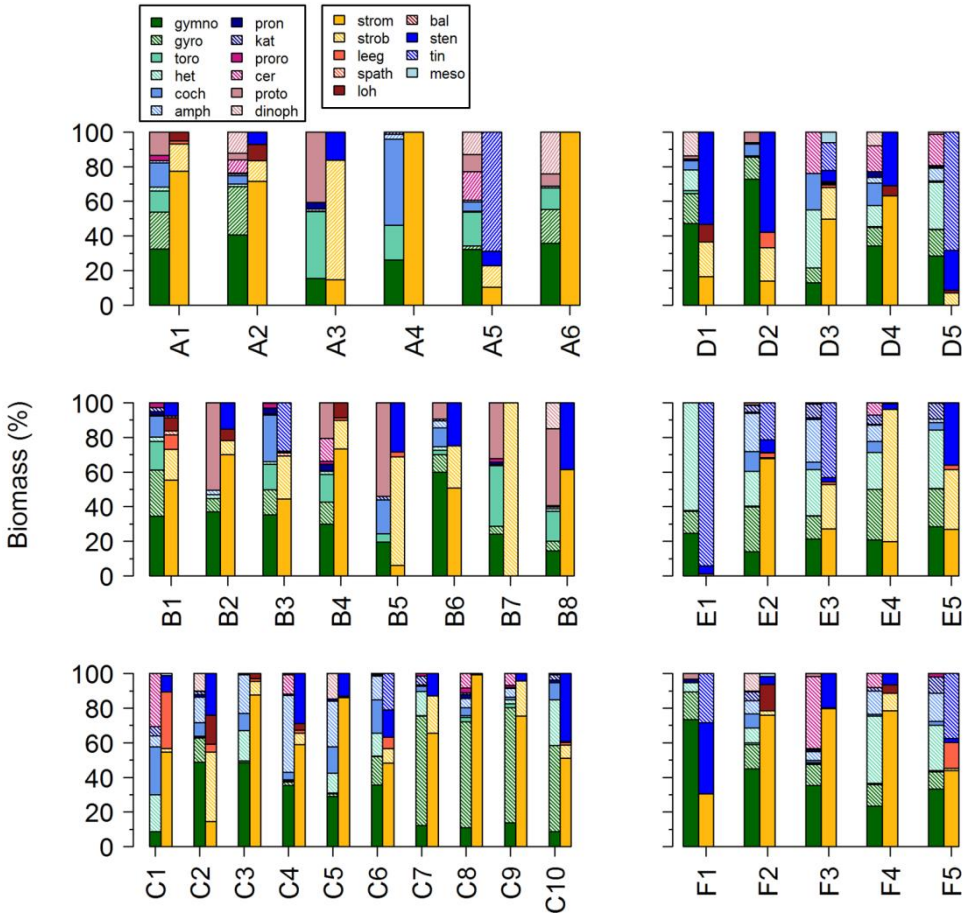

Figure 2.5: **Relative biomass contribution of the different PZP taxa in the North Sea during mid-winter 2014. See Figure 2.1 for the station coding. Left bars on each panel represent dinoflagellate biomass, right bars represent ciliate biomass. Note that taxa occurring at less than 20 ind L^{-1} at every station were excluded. Abbreviations: gymno = *Gymnodinium* spp., toro = *Torodinium robustum*, het = *Heterocapsa* cf. *rotundata*, proto = *Protoperidinium* spp., gyro = *Gyrodinium* spp., coch = *Cochlodinium* sp., amph = *Amphidinium* sp., pror = *Prorocentrum* cf. *micans*, kat = *Katodinium* sp., cer = *Ceratium* spp., pron = *Pronoctiluca* cf. *pelagica*, dinoph = *Dinophysis* spp. strom = *Strombidium* spp., strob = *Strobilidium* spp., leeg = *Leegardiella* cf. *ovalis*, spath = *Spathidium* spp., loh = *Lohmaniella oviformis*, bal = *Balanion* cf. *comatum*, sten = *Stenosemella* spp., tin = Tintinnida, meso = *Mesodinium rubrum*.**

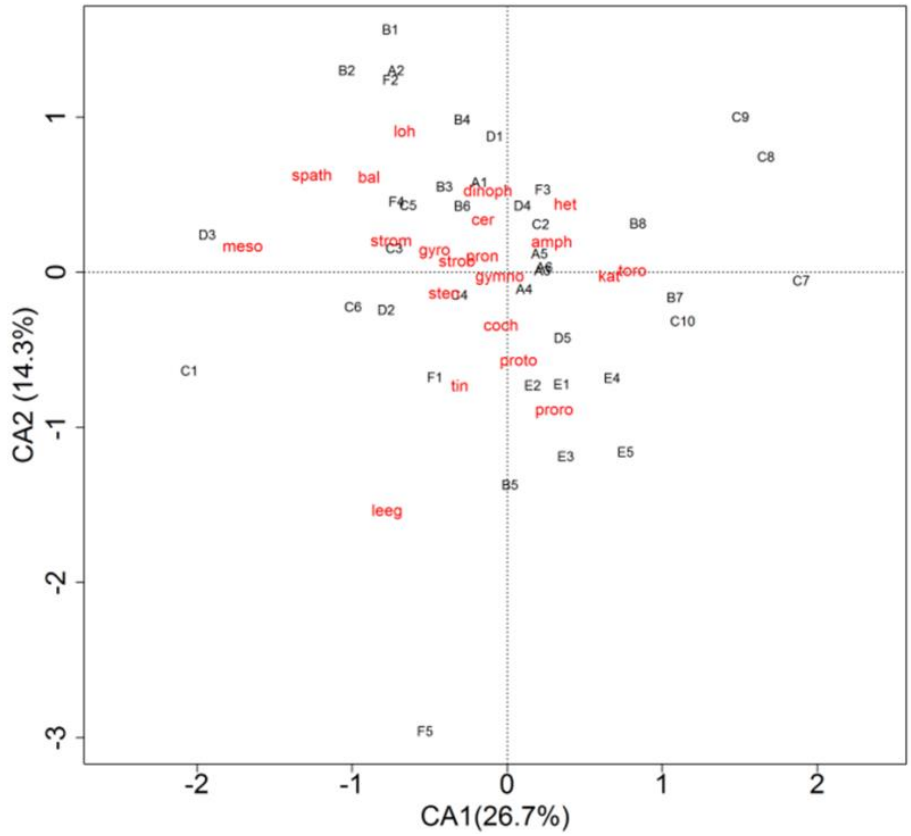

Figure 2.6: **Correspondence analysis (CA) performed on the winter PZP community composition of the North Sea, including the sampling stations and the PZP taxa, whereas no higher resolution than genus level was applied. No significant spatial distribution pattern was observed. For abbreviations see Figure 2.5.**

Table 2.2: **Dinoflagellate and ciliate taxa identified during mid-winter in the North Sea. Maximum and mean biomass (µg C L^{-1}) and abundance (ind L^{-1}) are listed. Taxa occurring with maximum abundances of <20 ind L^{-1} are not included. Size and biomass estimates used from previous studies are marked with a *(Olenina et al. 2006) or**(Strüder-Kypke et al. 2006). For ***(Löder et al. 2012), only biomass, no size data available. Abbreviations: H = heterotroph, M = mixotroph**

			Biomass		Abundance		
	trophic position	mean size (µm)	max (µg C L^{-1})	mean (µg C L^{-1})	max (ind L^{-1})	mean (ind L^{-1})	station with max biomass
DINOFLAGELLATES							
Gymnodiniales							
Gymnodium spp. <20 µm	H	14.9	0.024	0.01	320	125.64	F5
Gymnodium spp. <30 µm	H	23.3	0.077	0.028	220	77.18	C4
Gymnodium spp. >30 µm	H	33	0.079	0.028	100	34.36	E4
Gyrodinium spp.	H	30.1	0.025	0.004	150	22.56	D3
Gyrodinium spirale	H	51.8	0.094	0.016	160	25.88	D3
Torodinium robustum <40µm	H	27.7	0.167	0.023	960	121.54	C8
Torodinium robustum >40 µm	H	47.7	0.412	0.03	700	46.41	C8

			Biomass		Abundance		
	trophic position	mean size (µm)	max (µg C L^{-1})	mean (µg C L^{-1})	max (ind L^{-1})	mean (ind L^{-1})	station with max biomass
Katodinium sp.	H	22.1	0.01	0.001	110	14.36	C8/C9
Amphidinium sp.	H	19.2	0.01	0.002	120	21.03	D4
Cochlodinium sp.	H	39.3	0.181	0.026	230	31.28	G 134
Peridiniales							
Protoperidinium spp. <20 µm	H	15.0*	0.004	0.001	80	11.28	E4
Protoperidinium spp. <30 µm	H	25.0*	0.047	0.007	150	19.74	E4
Protoperidinium spp. <50 µm	H	40.0*	0.145	0.033	100	21.28	E5
Heterocapsa cf. *rotundata*	M	12.8	0.014	0.002	700	88.46	C9
Gonyaulacales							
Ceratium tripos	M	35.0*	0.087	0.009	20	2.05	D3
Ceratium lineatum	M	20.0*	0.03	0.002	20	1.28	F3
Ceratium fusus	M	20.0*	0.028	0.002	20	1.28	C8
Prorocentrales							
Prorocentrum cf. *micans*	M	43.7	0.046	0.006	130	17.18	E5
Dinophysiales							
Dinophysis spp.	M	NA***	0.046	0.006	20	2.56	C5
Noctilucales							
Pronoctiluca cf. *pelagica*	H	35.0*	0.008	0.001	30	4.87	B4/D2
CILIATES							
Strombidiida							
Strombidium spp. <20 µm	H	13	0.045	0.005	460	48.97	D3
Strombidium spp. <30 µm	H	24.7	0.112	0.014	260	28.97	D3
Strombidium spp. <50 µm	H	38.3	0.343	0.049	220	29.23	D3
Strombidium spp. <100 µm	H	61	0.231	0.035	100	14.1	F5
Strombidium spp. >100 µm	H	NA***	0.317	0.019	30	1.8	D3
Laboea strobila	M	85.0**	0.127	0.003	20	0.51	C8
Choreotrichida							
Strobilidium spp. <20 µm	H	13	0.006	0.001	50	6.67	B3
Strobilidium spp. <30 µm	H	25.5	0.042	0.006	70	9.74	D3
Strobilidium spp. <50 µm	H	35	0.05	0.008	50	7.18	D3
Strobilidium spp. <100 µm	H	57	0.551	0.03	120	6.15	E4
Leegaardiella cf. *ovalis* <30 µm	H	19.7	0.081	0.004	420	21.02	F5
Leegaardiella cf. *ovalis*	H	NA***	0.119	0.004	50	7	C1

			Biomass		Abundance		
	trophic position	mean size (µm)	max (µg C L^{-1})	mean (µg C L^{-1})	max (ind L^{-1})	mean (ind L^{-1})	station with max biomass
Lohmanniella oviformis	H	16	0.04	0.005	200	22.31	F2
Cyclotrichiida							
Mesodinium rubrum	M	35.0*	0.04	0.001	100	3.33	D3
Mesodinium rubrum	M	17.5*	0.074	0.002	30	0.77	D3
Haptorida							
Spathidium sp.	H	NA***	0.014	0.001	20	1.03	D3
Prorodontida							
Balanion cf. *comatum*	H	16	0.003	0	50	4.36	B1/D3
Tintinnida							
Stenosemella spp. <70 µm	H	41	0.151	0.028	140	23.87	F1
Tintinnid	H	150	0.477	0.046	50	4.62	E1

2.5 Discussion

Ecosystem-based approaches to manage marine habitats require a broad range of physical and biological data collected using efficient methods. The present study provides an example of how measurements of PZP can be added to a fish stock assessment survey (seven nations performing cruises on the IBTS). This type of opportunistic sampling can take advantage of pre-existing surveys to provide a cost-effective way of collecting information on factors relevant for ecosystem monitoring and a more thorough understanding of processes contributing to the trophodynamic structure and function of marine habitats, including Good Environmental Status (GES) for the pelagic system (Dickey-Collas et al. 2017). The fisheries survey was conducted across the North Sea which allowed large-scale (49°N – 61°N) patterns in the abundance, biomass and community composition of PZP to be examined within a relatively short time window (5 weeks) during winter.

Our study suggests that the PZP community is homogenously distributed in the North Sea during wintertime. This PZP community consisted mainly of naked ciliates and athecate dinoflagellates including *Gymnodinium* spp. and *Strombidium* spp., which are characteristic for winter communities in the North Sea (Löder et al. 2012) as well as other temperate

regions during seasons with low primary productivity (Levinsen & Nielsen 2002, Figueiredo et al. 2009, Scherer 2012). The PZP biomass was low (mean 0.51 µg C L^{-1}) and similar values have been reported in other studies conducted during autumn/winter (Levinsen & Nielsen 2002, Scherer 2012, Löder et al. 2012). Furthermore, the community was dominated by small-sized (<50 µm) cells, a pattern which is typical of places and/or times with low productivity when the phytoplankton community is dominated by pico- and nanophytoplankton (Legendre & Rassoulzadegan 1995, Levinsen & Nielsen 2002). As small-sized PZP can be important grazers of bacteria, pico- and nanophytoplankton (Sherr & Sherr 2002), considerable proportions of carbon may be channeled via PZP to higher trophic levels, such as copepods and ichthyoplankton, especially in winter. Thus, PZP can be considered as a key component of the winter food web and a component which requires further monitoring.

Total PZP biomass showed a clear north-south gradient with higher total biomass in the English Channel. Although southern stations were, on average about 2°C warmer than central or northern stations, the observed patterns in PZP were unrelated to differences in temperature. The biomass of dinoflagellates was negatively correlated with salinity, but this might be of minor relevance since only marginal differences in salinity were observed across sampling stations (34.79 ± 0.42). As observed in this study for PZP, Fransz & Gonzalez, 2001 (Fransz & Gonzalez 2001) also reported no distinct spatial differences in the diversity of mesozooplankton in the North Sea during winter. Thus, our results support the assumption, that at no location within the study area certain taxa would dominate the PZP composition or that specific taxa were rigorously underrepresented in the community. The higher abundance of loricate ciliates (i.e. *Tintinnid* spp. and *Stenosemella* sp.) in the southern area contradicts the results of a previous broad-scale tintinnid study conducted in the North Sea in spring 1986 (Cordeiro et al. 1997), where the lowest tintinnid abundance was found in the southern areas. Data from the CPR survey also suggest that the long-term mean abundance of tintinnids is lower in the English Channel compared to the central and northern North Sea (Barnard et al. 2004). One reason for the discrepancy may be that members of the genus *Stenosemella,* which accounted for the majority of the loricated taxa in the present study, are not efficiently captured by the CPR (Hinder et al. 2012). In their 2.5 - years time-series at Helgoland Roads Löder et al. (2012) also observed that *Stenosemella* spp. can dominate the

tintinnid community. This genus is listed as neritic (Pierce & Turner 1993), which makes it plausible to find it mainly in the English Channel. As this study provides only a snap shot in time, one can only speculate on the processes (e.g. growth, mortality, transport) that may have recently occurred to create such distributional patterns. For example, any increase in PZP growth rate due to increasing water temperature (Montagnes et al. 2003, Aberle et al. 2015) may be counterbalanced by increased mortality due to predation, e.g. by fish larvae (Montagnes et al. 2010, Illing et al. 2015).

Laboratory experiments have demonstrated that the larvae of many fish species feed on and can nutritionally benefit from PZP (Hunt von Herbing & Gallager 2000, Friedenberg et al. 2012, Illing et al. 2015) and field studies have documented PZP in the diets of herring larvae and other larval fish (Figueiredo et al. 2005). For example, tintinnids have been found in the larval guts of Pacific (*Clupea pallasii*) and Atlantic herring (Purcell 1990, Bollens & Sanders 2004), Pleuronectiforms (Spittler et al. 1990), blue whiting (*Micromesistius poutassou*) (Hillgruber & Kloppmann 1999) and winter flounder (*Pseudopleuronectes americanus*) (Shaheen et al. 2004). The relatively high winter biomass of PZP (including a high contribution of tintinnids of up to 78% of the total PZP biomass) in the southern North Sea is highly relevant because this area is a winter nursery ground for the larvae of several marine fish species including Atlantic herring and European plaice (*Pleuronectes platessa*). The tintinnid taxa found in this study have a comparable size and carbon content to small copepod nauplii (see e.g. Kühn et al, 2008 (2008)). Moreover, population growth rates of tintinnids can exceed 50% d^{-1} at temperatures <10°C (Verity 1985), which would allow them not only to exert (as predators) but also to support (as prey) considerable grazing pressure. One has to keep in mind, however, that the sum of tintinnids biomass in transects E and F was lower than 2 µg C L^{-1} and the PZP biomass we observed in the southern North Sea (maximum of 1.5 µg C L^{-1}) is somewhat lower than the carbon concentration reported to be required for the survival and growth of young larvae of species such as herring (~2 µg C L^{-1}, (see Munk & Kiørboe 1985, Figueiredo et al. 2005, Peck et al. 2012)). Nevertheless PZP can make an important contribution to larval feeding requirements during autumn/winter when the abundance of other potential prey (e.g. copepod nauplii) is low (Wesche et al. 2007, Bils et al. 2017). Furthermore, the presence of PZP can increase the magnitude, intensity and duration of first-feeding by larval fish on metazoan prey (copepod nauplii) (Overton et al.

2010, Illing et al. 2015). Studies have hypothesized that reductions observed in the growth rates of overwintering herring larvae were due to shifts in available prey rather than changes in temperature (Payne et al. 2013, Alvarez-Fernandez et al. 2015), but the lack of data on potential prey fields during winter seriously hampers our understanding of "bottom-up" recruitment mechanisms in North Sea herring (Hufnagl et al. 2015). Therefore, dedicated field process-studies undertaken in parallel with the type of broad-scale survey conducted in the present study would ultimately be needed to reveal the role of PZP as prey in the North Sea food web during winter and the factors influencing the spatial patterns observed in PZP abundance, distribution and community composition.

The PZP community can serve as a plankton indicator for ecosystem changes (Beaugrand 2005), as they are known to respond rapidly to changes in abiotic and biotic conditions such as changes in temperature (Montagnes et al. 2003, Aberle et al. 2012) or food concentrations (Menden-Deuer et al. 2005). For example, at monitoring station Plymouth L4, ciliates and heterotrophic dinoflagellates have recently occurred at relatively low (ciliates) and high (dinoflagellates) abundance, respectively, compared to long-term trends (O'Brien et al. 2013). The reason for these recent changes is unknown but differences in the abundance of ciliates versus dinoflagellates are expected to affect food web dynamics as these groups play different roles in carbon cycling. Dinoflagellates are able to prey on diatoms larger than themselves (Sherr & Sherr 2007, Calbet 2008) and, thus, may act as strong competitors for food with copepods. On the other hand, ciliates prey on small phytoplankton and can channel that energy to higher trophic levels (Calbet & Saiz 2005) as copepods often prefer ciliates over dinoflagellates as prey (Vincent & Hartmann 2001). Commonly, long-term data of phytoplankton and mesozooplankton have been used as indicators for fish recruitment dynamics (Beaugrand et al. 2003, Platt et al. 2003). As PZP fulfils an important role in the food-web it can be assumed that changes in the abundance or community composition will affect lower or higher trophic levels. Nevertheless, in contrast to phytoplankton and fish, relatively few (laboratory) studies have explored the ecophysiology of zooplankton (including PZP) to reveal mechanisms that can be combined with field survey data (on abundance and composition) to improve the realism and prediction capacity of end-to-end models (Mitra et al. 2014).

As future climate scenarios predict a shift in the phytoplankton community towards small sized cells (Rodríguez et al. 2001, Caron & Hutchins 2013) energy transfer through the microbial loop may be enhanced which reduces trophic transfer efficiency due to the increased number of trophic steps required before energy reaches upper trophic levels.

In contrast to many mesozooplankton organisms the ecology and distributional patterns of PZP are not only poorly studied in the North Sea, but also worldwide and this lack of knowledge may be due to a number of potential factors. First, using feeding mode to classify organisms as either phytoplankton or zooplankton has led to confusion since mixotrophy is often the rule rather than the exception in the PZP community (Flynn et al. 2013) with important consequences for estimating carbon cycling, nutrient remineralization and energy transfer within food-webs (Mitra et al. 2016). Second, naked PZP dissolve in formalin, which is the main fixative in routine plankton surveys (e.g. CPR). Lugol's iodine solution (commonly used for phytoplankton and PZP) avoids this issue but does not allow long-term storage of samples (Gifford & Caron 2000). Finally, manual identification requires considerable expertise and is time-consuming which also limits the number of samples or stations that can be examined.

The present study is the first to document the broad-scale patterns in the abundance, biomass and community composition of PZP in the North Sea during winter. Although the water column is generally well mixed during wintertime, patchiness in the distribution of PZP may exist which would not be captured by the sampling protocol used here. For example, the extraordinarily high biomass in station D3 might result from sampling within a patch. Moreover, considerable time was required to manually resolve the taxonomic composition of the PZP community and this protocol may or may not be necessary depending on what information is deemed most important for understanding the ecology of the North Sea (in general) and changes in prey fields of fish larvae (more specifically). Automated water collection and DNA sequencing for microbial diversity (Stern et al. 2015) and/or automated image recognition systems (e.g. FlowCAM) identifying nano- and microplankton communities (Alvarez et al. 2013) are more rapid and may be more cost effective than manual identification. Furthermore, the routine use of already augmented gear on this survey (e.g. a 335 µm MIKey M net) for sampling mesozooplankton and by adding nutrient and chl *a* measurements would allow spatial patterns in potential bottom-up and top-down

processes to be resolved. This sampling campaign reported here was repeated in the ICES IBTS surveys in 2015, 2016 and 2017 with the intention of establishing a time series on the North Sea winter PZP community and offering an example of the type of holistic sampling platforms that will be necessary to collect the wide range of data required to advance integrated ecosystem assessments and management advice.

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2.8 Supporting information

Table 2.3: Political bodies of each country that issued the work permits in the waters the IBTS did enter for sampling in 2014. Only the permissions required for the four nations considered in this study are given.

Country	IBTS work permits issued by
The Netherlands	Ministerie van Economische Zaken; Rijksdienst voor Ondernemend Nederland, Vergunningen & Handhaving, Visserijregelingen
United Kingdom	The Marine Policy Unit of the Foreign and Commonwealth Office, and the Crown Estate
Norway	Norwegian Armed Forces, Norwegian Joint Headquarters
Denmark	Ministry of Foreign Affairs
Germany	Bundesministerium für Ernährung und Landwirtschaft (BMEL), Referat 614 – Seefischereimanagement und –kontrolle, IWC
France	Ministère des Affaires Étrangères et du Développement International. Direction générale de la mondialisation, de la culture, de l'enseignement et du développement international
Belgium	Service public fédéral Affaires étrangères, Commerce extérieur et Coopération au Développement

Table 2.4: Details for each of the 39 North Sea stations sampled for PZP: Research vessel, sampling date, sampling position, ciliate and dinoflagellate abundance (ind L^{-1}) and biomass (µg C L^{-1}).

Ship	Country	Station ID	Lat (°N)	Long (°E)	Date	Abundance (ind L^{-1})		Biomass (µg C L^{-1})	
						D	C	D	C
Thalassa	France	C1	56.16	-2.30	27.01.2014	180	460	0.15	0.38
Thalassa	France	C2	56.25	-1.49	27.01.2014	730	110	0.24	0.05
Thalassa	France	D1	54.72	0.67	25.01.2014	610	220	0.17	0.13
Thalassa	France	D2	54.79	1.31	25.01.2014	380	260	0.13	0.12
Thalassa	France	D3	54.68	2.69	24.01.2014	790	1740	0.56	1.84
Thalassa	France	D4	54.82	3.89	08.02.2014	780	120	0.3	0.08
Thalassa	France	D5	55.03	4.89	07.02.2014	510	80	0.25	0.15
Thalassa	France	F1	50.55	-0.15	19.01.2014	260	160	0.09	0.37
Thalassa	France	F3	50.55	0.68	20.01.2014	480	70	0.18	0.12
Walther Herwig III	Germany	A1	58.95	-1.85	14.02.2014	720	190	0.13	0.13
Walther Herwig III	Germany	A2	59.08	-2.17	14.02.2014	620	280	0.2	0.18
Walther Herwig III	Germany	B1	58.39	-2.56	11.02.2014	640	480	0.15	0.32
Walther Herwig III	Germany	B2	58.19	-3.17	11.02.2014	280	270	0.07	0.23
Walther Herwig III	Germany	B3	56.24	1.47	07.02.2014	580	280	0.16	0.38
Walther Herwig III	Germany	B4	58.12	-1.14	10.02.2014	700	280	0.23	0.17
Walther Herwig III	Germany	C3	57.21	1.51	19.02.2014	640	290	0.18	0.15
Walther Herwig III	Germany	C4	56.51	2.92	07.02.2014	930	170	0.41	0.12
Walther Herwig III	Germany	C5	56.64	3.20	02.07.2014	770	230	0.31	0.19
Walther Herwig III	Germany	C6	56.25	5.15	03.02.2014	290	410	0.13	0.5
Walther Herwig III	Germany	C7	56.13	6.74	31.01.2014	1000	70	0.23	0.1
Walther Herwig III	Germany	C8	56.10	7.13	30.01.2014	1920	160	0.34	0.27
Walther Herwig III	Germany	C10	55.67	6.82	30.01.2014	400	110	0.13	0.15
Walther Herwig III	Germany	C9	55.57	7.17	30.01.2014	2100	150	0.67	0.3
Tridens II	Netherlands	E1	50.55	0.60	29.01.2014	220	80	0.18	0.51
Tridens II	Netherlands	E2	52.26	1.88	28.01.2014	1190	310	0.42	0.49
Tridens II	Netherlands	E3	51.90	3.74	27.01.2014	1450	200	0.64	0.47
Tridens II	Netherlands	E4	56.13	3.30	04.02.2014	2200	340	0.81	0.76
Tridens II	Netherlands	E5	56.11	-2.29	06.02.2014	1320	100	0.5	0.1
Tridens II	Netherlands	F2	50.96	1.62	30.01.2014	820	550	0.23	0.27
Tridens II	Netherlands	F4	50.32	1.01	30.01.2014	460	320	0.2	0.19
Tridens II	Netherlands	F5	49.96	0.26	29.01.2014	1230	630	0.32	0.54
GO Sars	Norway	A3	59.71	0.54	07.02.2014	510	10	0.19	0
GO Sars	Norway	A4	59.78	1.38	11.02.2014	170	50	0.08	0.08
GO Sars	Norway	A5	59.84	2.62	14.02.2014	440	90	0.18	0.15
GO Sars	Norway	A6	59.83	3.19	14.02.2014	260	20	0.1	0
GO Sars	Norway	B5	57.81	2.61	11.02.2014	220	40	0.07	0.01
GO Sars	Norway	B6	57.80	3.39	11.02.2014	180	50	0.09	0.09
GO Sars	Norway	B7	57.76	4.67	11.02.2014	410	130	0.09	0.1
GO Sars	Norway	B8	57.84	5.47	12.02.2014	450	30	0.16	0.03

CHAPTER 3

3. Exploring the microzooplankton-ichthyoplankton link: A combined field and modeling study of Atlantic herring (*Clupea harengus*) in the Irish Sea (Manuscript 2)

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3.1 Abstract

The protozooplankton-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

3.2 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 & Saiz 2005) and as prey for higher trophic levels (e.g. copepods, larval fish) (Montagnes, Dower, et al. 2010, 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 (Montagnes, Allen, et al. 2010, Scherer 2012, Yang et al. 2015).

In the Irminger Sea (Montagnes, Allen, et al. 2010) 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.(2010) 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 and time series data for microzooplankton in the North Atlantic and adjacent waters (Smetacek 1981, Montagnes et al. 1988, Leakey et al. 1993, Edwards & 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 & Dower 2007, Montagnes, Dower, et al. 2010). This is in stark contrast to the multitude of 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) 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 & 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 impact on 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 & 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 & 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 (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.

3.3 Method

3.3.1 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) (Figure 3.1). 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

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

Besides the routine NINEL larval herring sampling, 9 station in 2012 and 12 stations in 2013 were sampled for protozooplankton and microzooplankton (52-300 µm) (Figure 3.1).

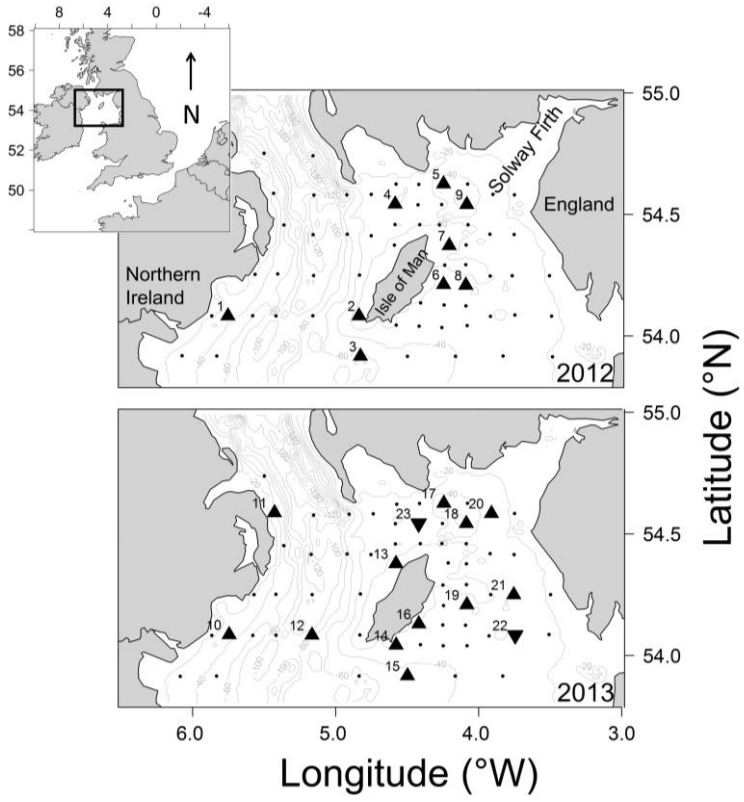

Figure 3.1: **Sampling stations of the Irish Sea herring larvae survey in the autumns of 2012 and 2013 (·). Stations with additional microzooplankton sampling (▲) are labelled from 1 to 9 for 2012 and 10-21 for 2013, labelling is from West to East. Stations 22 and 23 (▼) were not sampled for microzooplankton, only herring larvae were sampled.**

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). All nauplii and copepodites were counted using a stereomicroscope (Leica MZ16 magnification 2.5x). For each sample and size fraction, the prosome length of a minimum of 30 nauplii and copepodites was measured using image analysis (ImagePro, Media Cybernatics). 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).

3.3.2 Hydrographic conditions

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

3.3.3 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, 200x 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 following Dodge and Hart-Jones (1982), Montagnes (1996),Olenina et al. (2006), Strüder-Kypke et al. (2006), Hoppenrath et al. (2009) and 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 & Lessard (2000).

The structure of the protozooplankton community was studied using multivariate analysis using PRIMER 6 (Clarke & Warwick 2005). Then, hierarchical agglomerative cluster analysis in conjuction with non-metric multidimensional scaling (MDS) was 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 to 90% to the (dis)similarity were identified performing a similarity percentages routine (SIMPER). Finally, the Bio-Env 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 expresses as weighted Spearman rank correlation ρ.

An analysis of similarity (ANOSIM) was performed to reveal statistical 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 ($\alpha = 0.05$).

3.3.4 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).

3.3.5 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 & 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*):

$$G=C*\beta*(1-SDA)-R.$$

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* (*s*RD-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 & Peck 2011)) so that different feeding histories at the beginning of the simulation could be taken into account.

3.4 Results

3.4.1 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 (Figure 3.2). 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 (Figure 3.2 A,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 (Figure 3.2 C,D).

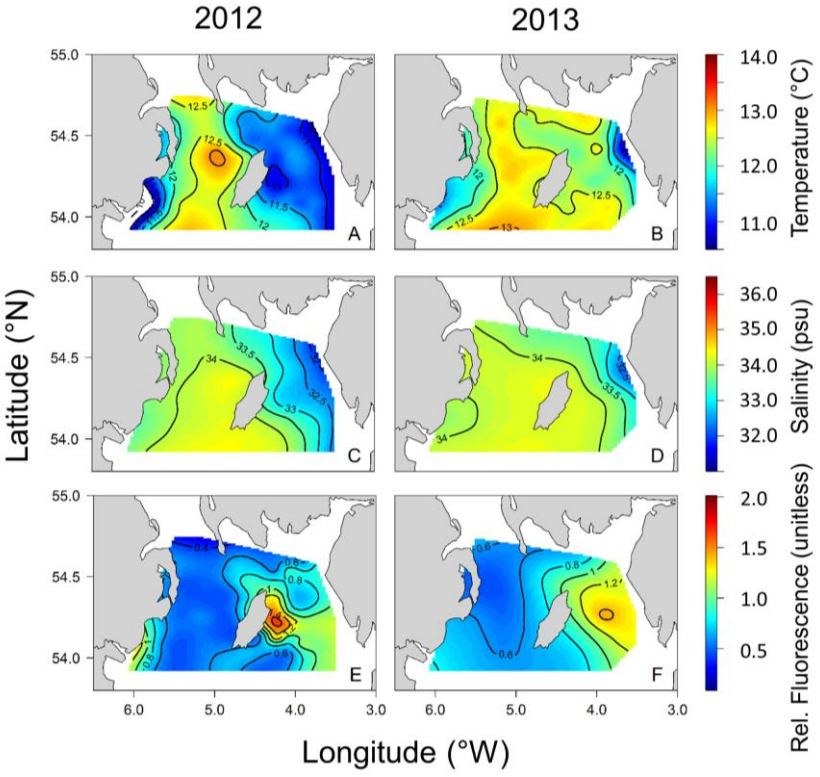

Figure 3.2: **Surface temperature (Panel A,B), salinity (Panel C,D) and fluorescence (Panel E,F) in the Irish Sea during the autumns of 2012 and 2013.**

Stations were grouped into three classes according to differences in their hydrographic characteristics revealed by the PCA (Figure 3.3): 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 3.1). A total of 21.9% of the variation was explained by PC2, primarily driven by bottom depth.

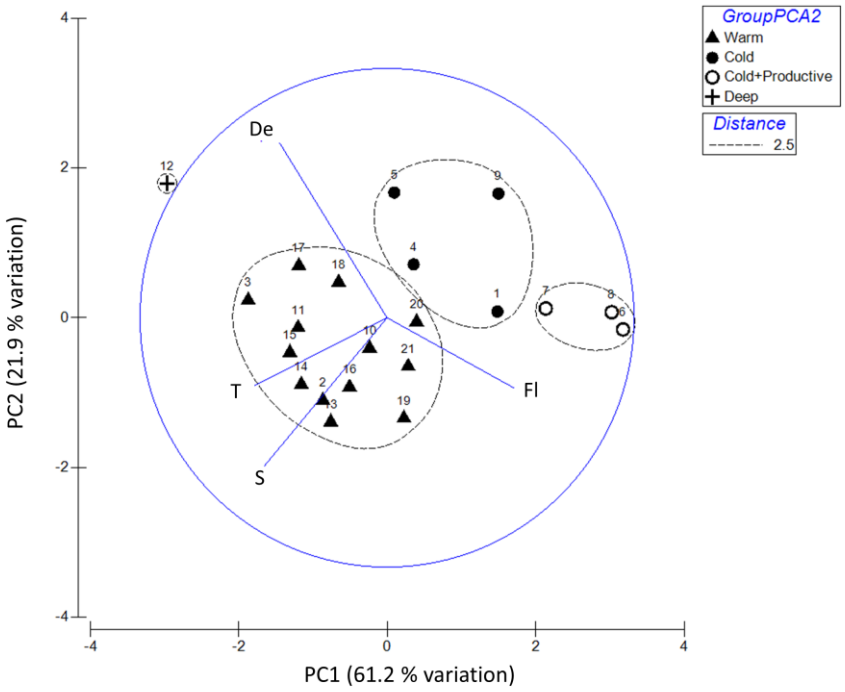

Figure 3.3: **PCA for the plankton sampling stations 2012 and 2013. Using the available environmental data (De = depth, *T* = temperature, *S* = salinity and *Fl* = fluorescence at 10 m depth) 3 groups could be distinguished: Warm, Cold and Cold+Productive.**

Table 3.1: **Eigenvectors of the two principal components (PC) gained from the PCA (Principal Component Analysis) including all available hydrographic factors from 2012 and 2013 combined.**

Variable	PC1	PC2
Depth	-0.434	0.7
Temperature	-0.549	-0.279
Salinity	-0.496	-0.594
Fluorescence	0.513	-0.281

3.4.2 Micro- and small mesozooplankton abundance and biomass

A total of 29 different taxonomic groups of dinoflagellates and ciliates were identified (Table 3.2). 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 taxa 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 3.2). For ciliates, *Strombidium* spp. was the most abundant taxon.

Table 3.2: 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.	37760	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

			Abundance (Ind*L^{-1})			Biomass (µg C*L^{-1})			
Family	**Species**	**2012**	**rel. A%**	**2013**	**rel. A%**	**2012**	**rel. C%**	**2013**	**rel. C%**
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

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 (Figure 3.4). *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 (Figure 3.5 A, 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 (Figure 3.5 C-F). Note, the flowmeter malfunctioned at Station 6 in 2012 and the nauplii and copepodites from that station could not be used in analyses.

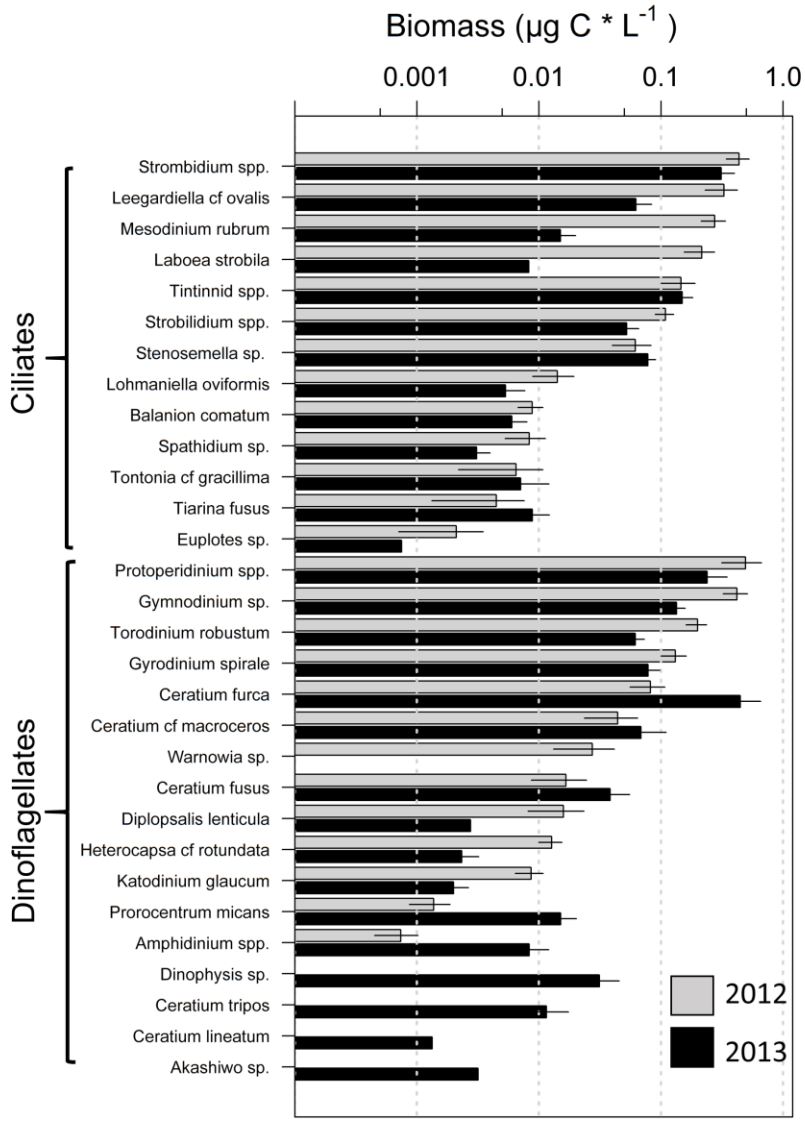

Figure 3.4: **Mean biomass ± SE (µg C*L^{-1}) of all ciliate and dinoflagellate taxa identified in 2012 and 2013 during the Irish Sea herring larvae survey. Taxa are ranked first by group and then by the biomass of 2012. Missing bars indicate the absence of the taxon.**

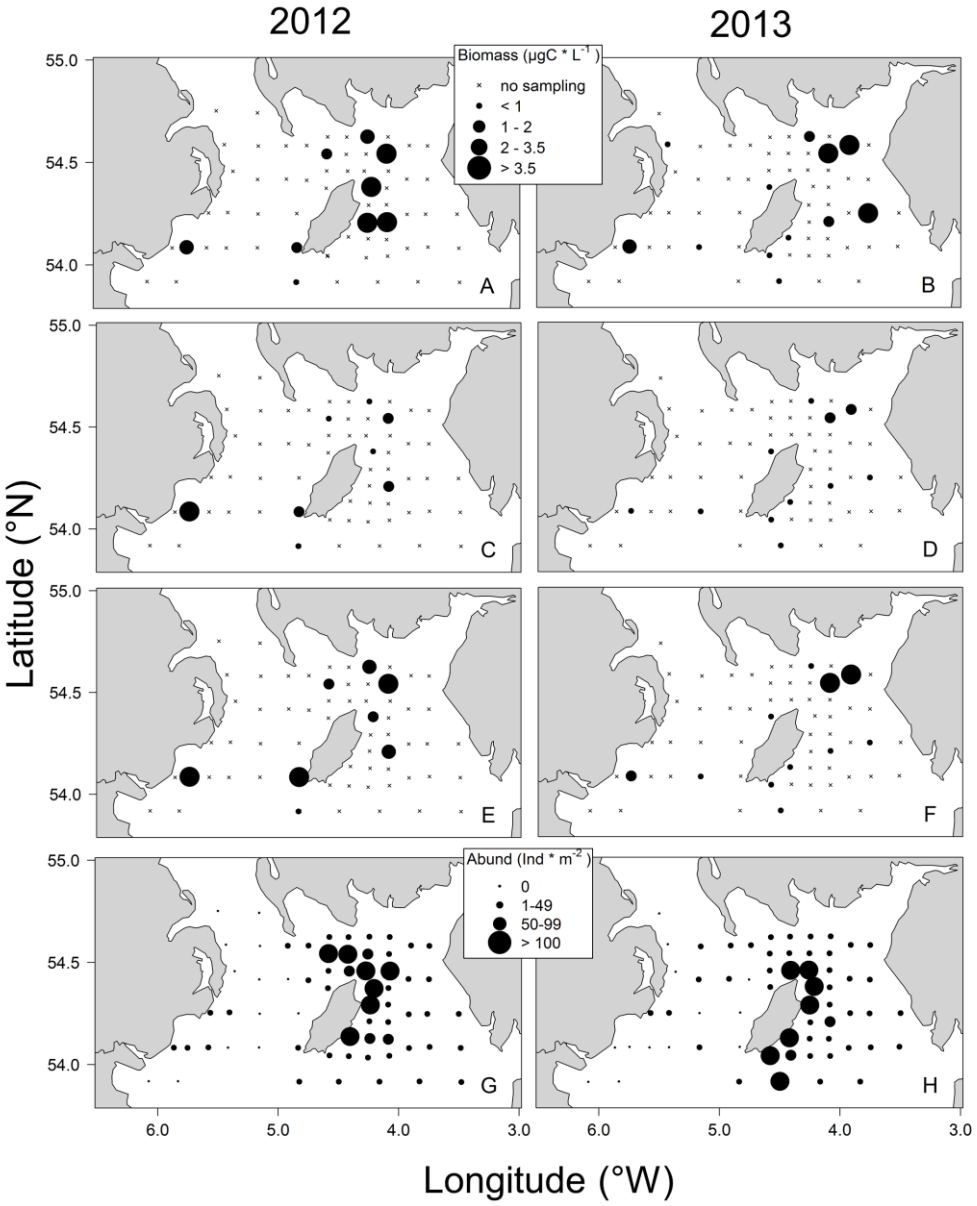

Figure 3.5: **Biomass (µg C*m^{-3}) of ciliates and dinoflagellates (Panel A,B), nauplii (Panel C,D) and copepodites (Panel E,F) of the 21 stations sampled and abundance (ind*m^{-2}) of herring larvae <12mm (Panel G,H) during the Irish Sea herring larvae survey in 2012 and 2013.**

3.4.3 Protozooplankton community structure

Hierarchical clustering (together with the SIMPROF) identified three main protozooplankton groups (Figure 3.6): "East 2013","East 2012" and "Mixed". Only a maximum of 10 taxa explained 90% of the similarity in all three groups (Table 3.3). 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. and *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 3.4).

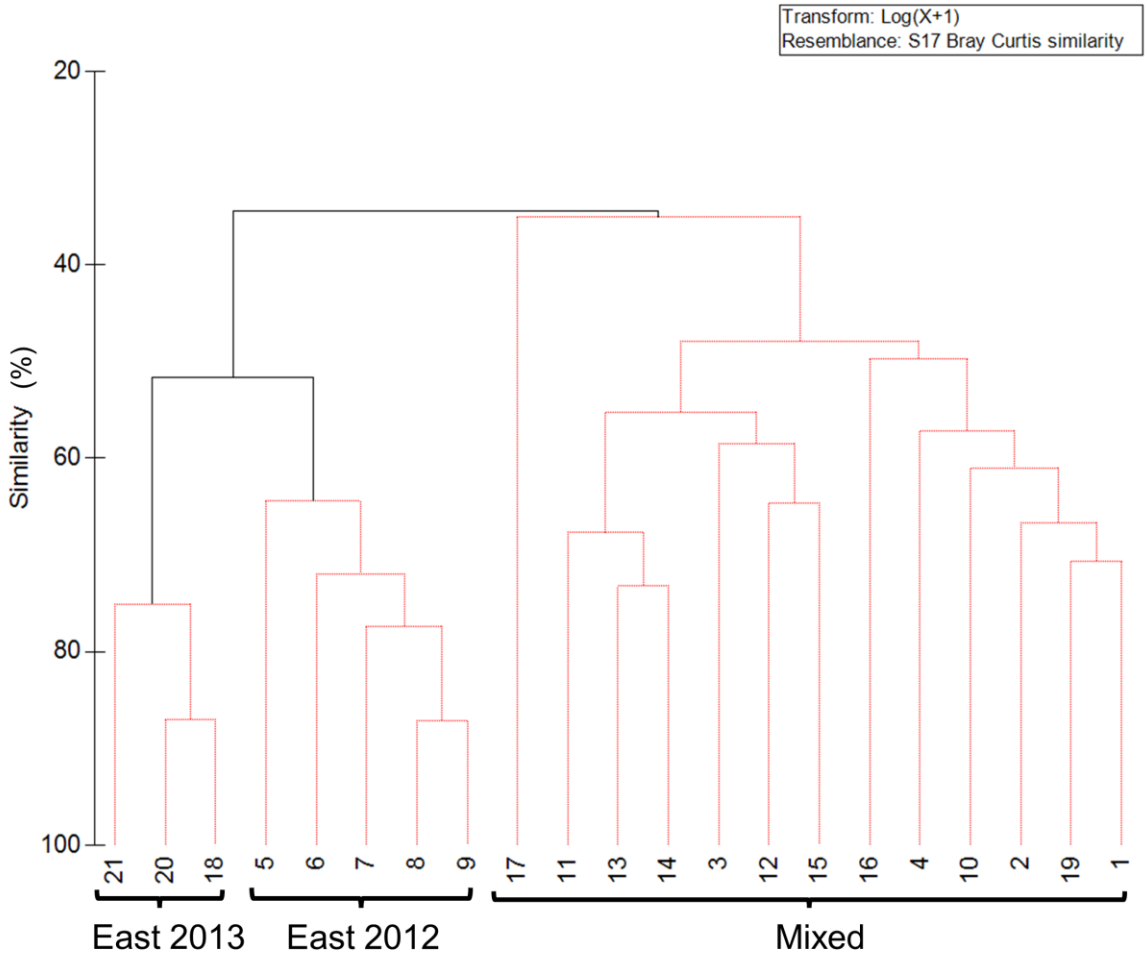

Figure 3.6: **Hierarchical clustering of the microzooplankton community (Ciliates, Dinoflagellates) during Irish Sea herring larvae survey in autumn 2012 and 2013. Three groups of stations were identified using the SIMPROF analysis (see text for further details).**

Table 3.3: SIMPER of the biomass of protists in the Irish Sea among stations for the groups distinguished by the SIMPROF analyses. 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).

	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
Legardiella 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

Salinity best explained the variability in protozooplankton community (BIO-ENV, ρ = 0.54) followed by the combination of temperature and salinity (ρ = 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.

3.4.4 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 (Figure 3.5 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) *s*RD of 8- to 14-mm SL larvae was 3.35 (± 0.89) and 2.86 (± 0.97) in 2012 and 2013, respectively.

Table 3.4: SIMPER results for the three protist groups identified in Figure 3.6. The most important taxa contributing to the average similarity 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.

Species	**Mixed**			**East 2012**			**East 2013**		
	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

3.4.5 Microzooplankton-ichthyoplankton link

Generalized linear mixed model

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

$$\log(N\_Larvae)_{ij} = (\beta_0 + b_{0i}) + (\beta_1 \times N\_Dinoflagellates) + (\beta_2 \times N\_Copepodites) + \varepsilon_{ijk},\ \varepsilon_{ij} \sim N(0, \sigma_{2k})$$

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 3.5). 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 (Δ AIC <2).

Table 3.5: 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

Foraging and growth model (IBM)

The *in situ* (*s*RD-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 (Figure 3.7). 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 (*s*RD- derived growth).

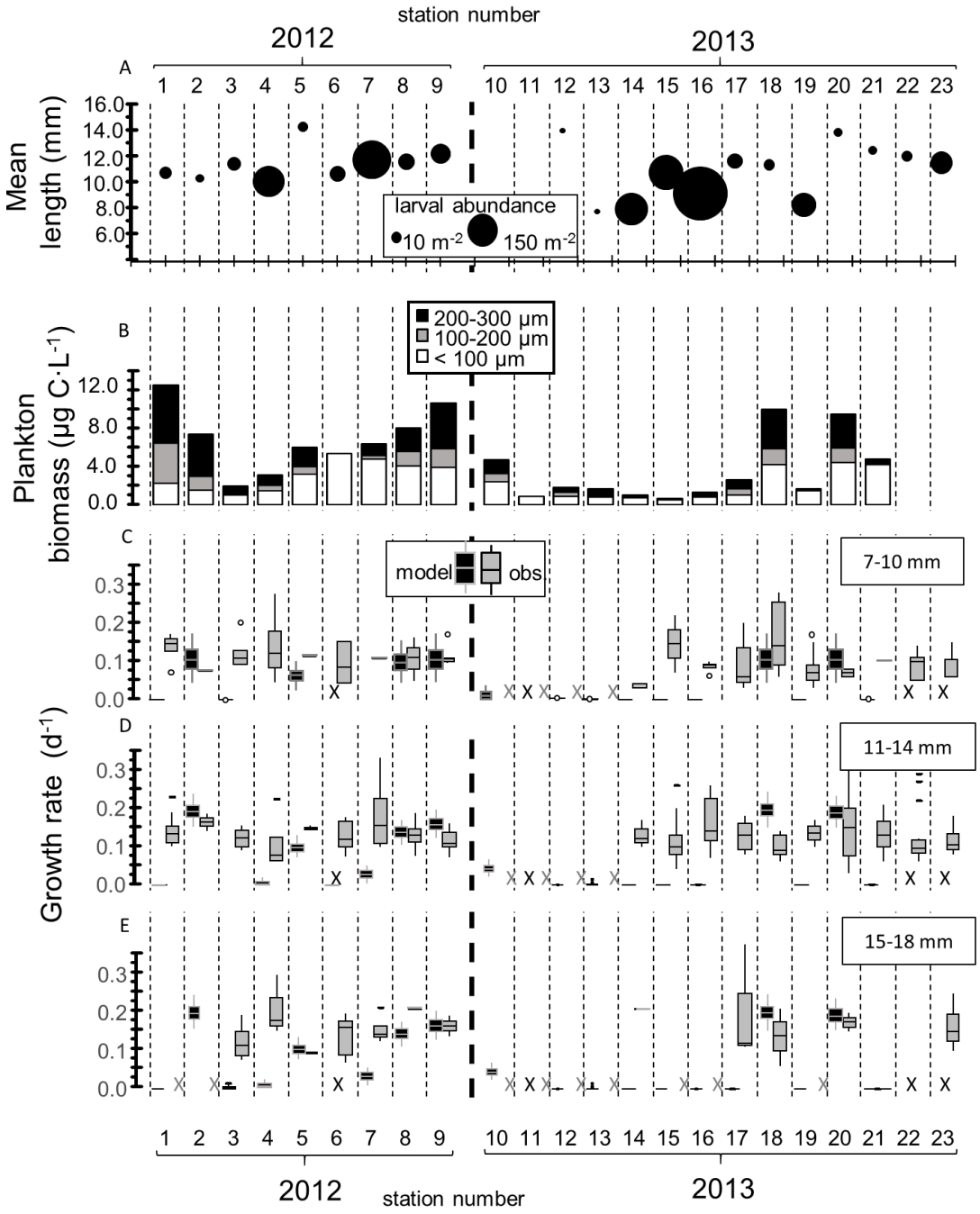

Figure 3.7: **Larval herring abundance (Ind * m^{-2}) and mean SL (mm) (Panel A), and plankton biomass of different size classes (µg C * L^{-1}) for the stations sampled during the Irish Sea herring larvae survey in 2012 and 2013. Observed (biochemically estimated) and modelled growth rates (*d^{-1}) for each of these stations are displayed for small (Panel C) and large herring larvae (Panel D). Note, X indicates that no larvae were measured for sRD and X indicates that no plankton was sampled.**

3.5 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. This study 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.

3.5.1 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 (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 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 correspondence to 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.

3.5.2 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 and 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 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 & 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 & Warwick 1994). Stations classified by hierarchical clustering as “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 & Morello 1998) depending on food availability and thermal conditions of the system (Johansson et al. 2004, Aberle et al. 2007).

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%).

3.5.3 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 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 & Kiørboe 1985, Figueiredo et al. 2005, Peck et al. 2012, Huebert & 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 a *s*RD >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 15-18 mm larvae at each station and in both years. In smaller (7-14 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 zooplanktonic prey field during spring and summer in Newfoundland (NE Canada).

Based on the mean protozoan biomass across the Irish Sea, our IBM results suggested 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.

3.6 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 to 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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CHAPTER 4

4. The impact of microzooplankton on the nutritional condition and growth of marine fish larvae using stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) (Manuscript 3)

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4.1 Abstract

In this study the potential role of small prey (<200 µm) for larval fish nutrition and condition was exemplified on autumn spawning Atlantic herring (*Clupea harengus*) in the North Sea. Sampling of protozooplankton (PZP), metazoan microzooplankton and larvae was conducted in two different spawning sites, Buchan and Banks in autumn 2013 and 2014 and Downs in winter 2014. Using a combination of taxonomic analysis of PZP and MZP and biochemical analysis (Stable isotope analysis (SIA), RNA/DNA) we aimed to reveal, if the main larval prey differs between spawning sites/seasons and if the abundance of appropriate prey can be related to larval growth and survival. We also show the limits of bulk SIA on this kind of comparative field studies. In autumn and winter the potential small prey was dominated by cells <50 µm (e.g. Gymnodiniales and *Strombidium* spp.), *Pronoctiluca pelagica* (autumn 2013) and *Ceratium* spp. (autumn 2014). In contrast to the nauplii, small copepodites were more abundant in the microzooplankton in winter than in autumn 2014. Trophic enrichment ($\Delta^{15}N$) suggested that the larvae fed predominantly on prey <50 µm rather than on the small PZP size fraction. A higher trophic level or higher total abundance of potential prey was not necessarily beneficial for larval nutritional condition, but the differences in taxonomic plankton composition may have impacted on larval growth and potentially overwinter survival and hence recruitment dynamics. The present study demonstrates the potential importance of small zooplankton for larval fish and highlights the need to further investigate this understudied plankton component.

Key words: microzooplankton-ichthyoplankton link, Atlantic herring, protozooplankton, North Sea, stable isotopes, RNA/DNA, trophic relationship

4.2 Introduction

Although it has generally been accepted for more than a century that the survival and growth of marine fish early life stages form a bottleneck for marine fish recruitment (Cushing 1975, 1990, Iles & Sinclair 1982, Houde 2008), understanding the mechanisms driving fluctuations in year class success remains a major challenge for fisheries scientists. In Atlantic herring (*Clupea harengus*), year-class strength of North and Baltic Sea stocks has been correlated with survival during the first month of life (Nash & Dickey-Collas 2005, Polte et al. 2014). For autumn-spawners in the North Sea (NSAS), recruitment appears to be determined at some point as larvae overwinter (Nash & Dickey-Collas 2005, Payne et al. 2009) and negative correlations with winter temperature (Fässler et al. 2011) and other studies on prey field dynamics (Hufnagl & Peck 2011, Alvarez-Fernandez et al. 2015) suggest that starvation may play an important role. However, the strength of any particularly recruitment driver may vary among NSAS spawning grounds (Fässler et al. 2011) because each subcomponent spawn at a unique time and location (e.g. early/mid-September, early October and December/January within the Orkney-Shetland (northernmost), Buchan and Banks (central) and English Channel (southernmost), respectively (Hufnagl et al. 2015)). Metazoan zooplankton such as early life stages of copepods have been considered to be the main prey of the larvae of herring and other marine fishes, (Munk & Kiørboe 1985, Pepin & Penney 1997). Munk & Kiørboe 1985)

To achieve a better understanding of larval feeding dynamics in the field Llopiz (2013) highlighted the importance of a thorough taxonomic identification of ingested metazoan prey. However, the importance of other potential sources of food such as protozooplankton (PZP) and phytoplankton has been largely ignored (Montagnes, Dower, et al. 2010, Denis et al. 2016, Bils et al. 2017) and the contribution of these specific groups to larval diets is still largely unknown.

In the laboratory, larvae of species such as Atlantic cod (*Gadus morhua*) (Hunt von Herbing & Gallager 2000), surgeonfish (*Paracanthurus hepatus*) (Nagano et al. 2000) and northern anchovy (*Engraulis mordax*) (Ohman et al. 1991) have been reported to actively forage on PZP. Recent studies on larvae of Pacific herring (*Clupea pallasii*) (Friedenberg et al. 2012) and Atlantic herring (Illing et al. 2015) have emphasized the importance of PZP for larval nutrition

and survival. There is little knowledge, however, on the relative roles that PZP and metazoan organisms in the microzooplankton (20-200 µm) play for larval diets in the field (Fukami et al. 1999, Figueiredo et al. 2005, Pepin & Dower 2007). The few studies available on field-caught larvae suggest that small prey (<200 µm) can form a large portion of the prey field of larval fish (Figueiredo et al. 2005, Denis et al. 2016). During larval ontogeny, the sizes and types of prey ingested by larval fish change with increasing mouth gape size (Llopiz 2013 and references therein), larval length (Hufnagl & Peck 2011) and prey capture success (Munk 1992). Using a physiological-based foraging model, Bils et al (2017) suggested that small planktonic organisms (10-300 µm) play a key role in larval growth of herring in the Irish Sea. When the small prey classes were excluded from foraging simulations, modelled growth rates were under-estimated compared to rates observed in the field (Bils et al. 2017).

The majority of studies on the diet of marine fish have relied on gut content analysis conducted on fish stored in formalin, a preservative unsuitable for PZP. Unfortunately, it is challenging to directly assess the importance of PZP to larval fish (e.g. the potential preference of these prey) since these prey items are rapidly digested and the larvae of some fish species, e.g. herring, are known to regurgitate prey during capture (Hay 1981). For this purpose indirect techniques are applied, such as stable isotope analysis (SIA), to detect trophic relationships of fish larvae. The relative amount of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes is commonly used to study food-web dynamics, to detect the trophic position of an organism and to achieve information about long-term integrated dietary composition (Peterson & Fry 1987, Vander Zanden & Rasmussen 1999, Post 2002). As prey items can be characterized by their specific stable isotope composition, differences in feeding ecology of fish larvae inhabiting different habitats (Laiz-Carrión et al. 2015, Costalago et al. 2016), changes in the dietary composition during ontogeny (Wells & Rooker 2009) or changing environmental conditions (Malzahn & Boersma 2009) can be revealed by SIA. The general nutritional condition and recent growth rates can be derived biochemically via the ratio of RNA to DNA (RNA/DNA) (Buckley et al. 2008). The fatty acid composition provides information about quality of the ingested prey (Malzahn et al. 2007). The use of molecular methods for studying diets of fish is a growing scientific field (Riemann et al. 2010), but to date it has not been used to study PZP in larval fish diet.

The present study explored the potential contribution of PZP and other microzooplankton to the nutritional condition of young herring larvae across different years (2013 & 2014) and at different spawning grounds in the North Sea (Buchan and Banks, and Downs subcomponents). We compared measurements of trophic position (SIA) and nutritional condition (RNA/DNA) (Ferron & Leggett 1994, Clemmesen 1994) to the taxonomic composition of PZP and MZP items simultaneously collected with larval herring at field stations.

We tested three hypotheses: (i) larvae spawned later in the year (winter – Downs) rely more on PZP than on metazoan microzooplankton to cover their energetic demands, (ii) larval herring target different prey items depending on larval size and spawning ground, and (iii) the survival of herring larvae is correlated to the availability of suitable prey. A better understanding of in situ prey availability, preferred prey categories and potential impacts of prey on the condition of herring larvae is needed to improve our knowledge on environmental conditions favoring overwinter survival and thus, how bottom-up drivers may influence recruitment variability.

4.3 Methods

4.3.1 Plankton and herring larvae sampling

Sampling of plankton (protozoo- to ichthyoplankton) was conducted during the International Herring Larvae Survey (IHLS) in 2013 and 2014 undertaken by the IMARES Wageningen on board of the RV Tridens. Samples were collected in September of 2013 and 2014 at the Buchan and Banks spawning grounds (BB13 and BB14, respectively, Figure 3.1) and in January 2014 at the Downs spawning ground in the English Channel (DO14). Water samples were collected with Niskin bottles mounted on a CTD rosette (Seabird SBE 911). Samples for Chlorophyll *a* (Chl *a*) were taken at 15 m depth and samples for SIA of particulate organic matter (POM) near the surface (max. 4 m depth) at 6 (BB13), 10 (BB14) and 8 (DO14) stations (Figure 3.1). Up to 1,000 mL of water was filtered through pre-combusted filters (Whatman GFC, 1.2-µm pore size). Duplicate filter samples were collected at each station for Chl *a* and POM and frozen at -80°C until further analysis. For PZP taxonomic analysis a subsample from the Niskin bottles sampled near the surface was immediately stored in a

brown 500-mL glass bottle and preserved with neutral Lugol's solution (2% final concentration).

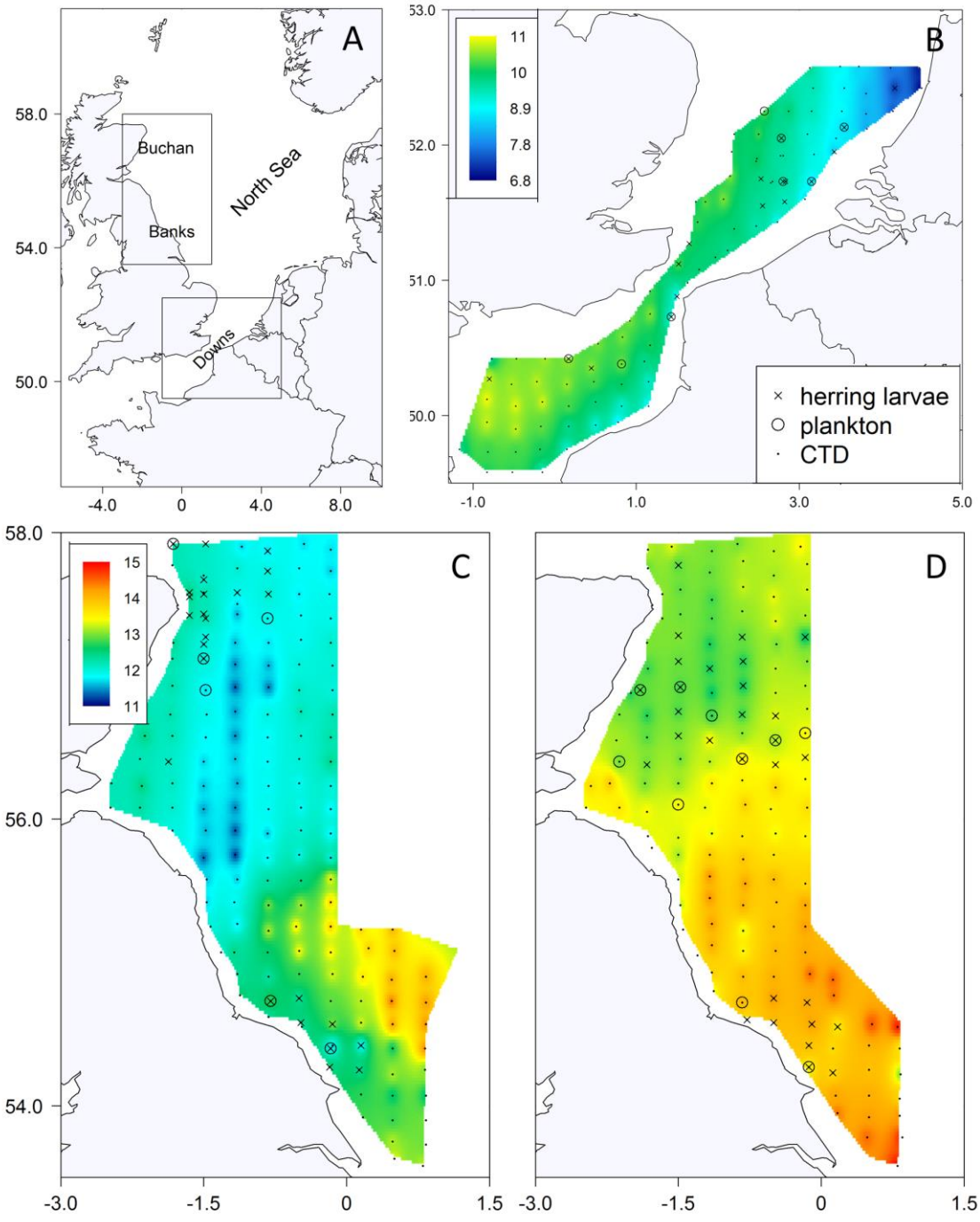

Figure 4.1: **Overview of the herring spawning grounds in the North Sea considered in the previous study (panel A). Sea surface temperature during IHLS in the Downs spawning area, DO14 (panel B) and the Buchan-Banks spawning grounds in 2013, BB13 (panel C) and 2014, BB14 (panel D). Stations, where particulate organic matter (POM), protozooplankton (PZP) and microzooplankton (MZP) (crosses) or larval herring (circles) were sampled for taxonomic analysis and/or biochemical analysis are highlighted. Note the temperature interpolation was conducted with all CTD stations in the grid. Note the different scaling for BB and DO14.**

A Gulf VII high-speed sampler (280 µm mesh size, 0.4 m nose cone opening) and a PUP-net (52 µm mesh size) mounted thereon were used to capture herring larvae and microzooplankton (52-200 µm, MZP), respectively, and towed in double oblique hauls at a speed of 5 knots from the surface to 5 m above the seafloor. A sample of herring larvae was taken approximately every hour. The Gulf VII net was equipped with a flowmeter (Valeport) and a CTD (Seabird 911plus). Temperature and salinity was measured at every IHLS station (n = 165, 145 and 96 stations in BB13, BB14, and DO14, respectively). At the stations with PZP sampling, the MZP sample was split (Motodo plankton splitter) and half of the sample was fractionated into two size classes (52-100 µm and 100-200 µm) and filtered on pre-combusted GFC filter for SIA. The other half of the sample was transferred to buffered 4% formalin for subsequent taxonomic analysis. Herring larvae caught with the Gulf sampler were preserved in 4% buffered formalin. Larval length measurements and abundance calculations were done by IMARES Wageningen after termination of the survey. No correction for shrinkage due to preservation and handling time was applied. The day/night catch ratio for larvae <10 mm is close to 1 (McGurk 1992). It increases with larval size but never exceeds 2.5 in larvae <25 mm. As the majority of larvae caught during the IHLS was <12 mm (>80% of total catches), day/night differences can be neglected. For biochemical analysis (SIA and RNA/DNA), a minimum of 20 larvae per station were frozen at -80 °C onboard at 25 (BB13), 31 (BB14) and 20 (DO14) random stations where the total amount of larvae exceeded 100 individuals (based on onboard estimations) (Figure 3.1).

4.3.2 Plankton abundance

Chlorophyll was extracted with acetone (90%), incubated overnight and Chl *a* concentration was calculated after Jeffrey and Humphrey (1975).

PZP water samples were analysed according to Utermöhl (1958). Due to the low PZP cell abundance, the samples were settled in a 100-mL sedimentation chamber (HydroBios) for 48 h (HELCOM 2014). PZP cells were counted under an inverted microscope (Leica DMI 3000, 200x with Moticam camera attached). The whole chamber plate was counted to avoid under-representation of less abundant PZP groups. PZP, comprising heterotrophic and mixotrophic ciliates and dinoflagellates, were identified to the lowest taxonomic level possible (Dodge & Hart-Jones 1982, Montagnes 1996, Olenina et al. 2006, Strüder-Kypke et

al. 2006, Hoppenrath et al. 2009, Löder et al. 2012). Other protists and small-sized metazoans occurring sporadically in the samples (e.g. Foraminifera and copepod nauplii) were not counted.

The PUP net samples (52-200 µm) were processed for MZP abundance with a FlowCam VS2 (Fluid Imaging technologies, Yarmouth, USA), an imaging particle analyzer, using a 4x magnification. A subsample of each sample was analyzed for BB13 and BB14 and the abundance (ind L^{-1}) of the six most abundant planktonic groups calculated: *Ceratium* spp. (Dinophyceae), *Dinophysis* spp. (Dinophyceae), copepod nauplii, copepodite stages (further referred as copepods), mollusc larvae (Bivalvia and Gastropoda) and tintinnids (Ciliophora). In DO14 the whole sample was processed due to the low overall MZP abundance in the samples. In DO14 only two groups (nauplii and small copepods/copepodites) were quantified, as other zooplankton organisms were very rare (Raudenkolb 2016). No copepod staging was applied. Coefficient of variation (CV, %) was calculated for PZP and MZP abundances to visualize the differences in the abundances.

4.3.3 Biochemical analysis

The GFC filters with POM and MZP were dried at 60°C for 24 h prior to SIA and duplicate GFC filter samples were used for SIA of POM samples. SIA of MZP (52-100 µm and 100-200 µm) was performed on 1/4 of each filter to obtain the correct range of C and N. Each GFC filter was cut into quarters and two of those pieces were analyzed separately and mean values calculated for these (pseudo)replicates.

For biochemical analysis, larvae between the lengths of 8 and 14 mm were chosen. This size range covers the life phase immediately after yolk absorption through the end of successful first feeding. The ^{13}C and ^{15}N was analysed on 117, 95 and 171 larvae, from 9 (BB13), 10 (BB14), and 10 (DO14) stations, respectively. Due to changes in C and N content with larval size, SIA was measured in small (S = 8 to 9 mm), medium (M =1 0 to 12 mm) and large (L = 13 to 14 mm) length classes representing early, first and advanced feeding larvae, respectively. At each station, larvae were pooled within these length classes to achieve the amount of C and N required for SIA. The number of larvae required for measurements needed to be pooled from two (BB13 and BB14, respectively) and four (DO14) (neighboring) stations.

Whole larvae were analysed without removing the gut since gut contents account for a maximum of 2% of total body weight (Pepin & Penney 2000). Larvae and filters were packed in tin capsules prior to SIA. All measurements of SIA (^{13}C and ^{15}N) were conducted at the Stable Isotope Facility at UC Davis, California, USA. The GFC filters (POM and MZP) were analyzed using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples containing <100 µg C or <20 µg N were excluded from further analysis.

The ^{13}C and ^{15}N isotopes of the herring larvae were analysed using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

δ^{13}C values of the larvae were corrected for lipid content as suggested by Post et al. (2007) for a C-N ratio >4:

$$\delta^{13}\mathrm{C}_{normalized=}\, \delta^{13}C_{uncorrected} - 3.32 + 0.99 \cdot C{:}N.$$

The stable isotope ratios were expressed as conventional δ notation

$$\delta\mathrm{X}\ (‰) = \left[\left(R_{Sample}{:}\, R_{Standard}\right) - 1\right] \cdot 1000$$

where δX =^{15}N or ^{13}C, R =^{15}N:^{14}N or ^{13}C:^{12}C and the relative international standards of Vienna PeeDee Belemnite for nitrogen (±0.3‰ SD) and Air for carbon (±0.2‰ SD).

A total of 391 (BB13), 190 (BB14), and 729 (DO14) herring larvae was classified as intact and larval length was measured to the nearest mm with a stereomicroscope (Leica MZ16). Larvae were freeze dried at -50°C (Christ Alpha 1, 4 LSC) and dry weight (DW, µg) was determined using a microbalance (Sartorius Genus SE2, ±0.1 µg). The RNA/DNA was determined on single larvae following a modified protocol of Caldarone et al. (2001) that uses ethidium bromide as binding fluorescent dye and restriction enzymes for RNA and DNA. The standardized RNA/DNA (s*RD*, (Caldarone et al. 2006)) was estimated using a factor of 2.4 based on the slopes of the RNA and DNA standards.

The instantaneous growth rate (Gi, d^{-1}) was calculated from s*RD* and in situ water temperature (T, °C) using an interspecific equations generated by Buckley et al. (2008):

$$G\mathrm{i} = 0.0145 \cdot sRD + 0.0044 \cdot (sRD \cdot T) - 0.078.$$

4.3.4 Data analysis

All statistical and graphical analysis were performed using the R software (V 3.1.0 R core team 2014). Temperature was mapped using inverse distance interpolation in the RGeostats package (Renard et al. 2016).

The $\delta^{15}N$ of the POM samples ($\delta^{15}N_{POM}$) was used as a baseline to estimate the trophic position (TP) in a one-source food web model (Hobson & Welch 1992). A trophic fractionation factor of 3.4‰ between trophic levels for $\delta^{15}N$ was assumed (Minagawa & Wada 1984, Peterson & Fry 1987, Post 2002):

$$TP = \frac{1 + \delta^{15}N_{Consumer} - \delta^{15}N_{POM}}{3.4}.$$

To test for significant differences in $\delta^{15}N$ and $\delta^{13}C$ of the larvae and the plankton, respectively, one-way ANOVA's were performed within as well as among larval length classes, spawning grounds and spawning seasons followed by a Bonferroni correction of the p-value.

For each spawning ground, a one-way ANOVA was performed on the square root transformed data and a regression model fitted to reveal potential effects of larval length and/or environmental variables on s*RD*. Larvae were grouped into the three length classes for SIA (S, M and L) (see previous section). Samples collected at BB13 and BB14 were from two different spawning grounds, Buchan in the north (BB13N and BB14N) and Banks in the south (BB13S and BB14S) (Figure 4.2). Therefore, the areas are treated as separated variables for statistical analysis of nutritional condition, growth rate and stable isotope composition.

4.4 Results

4.4.1 Environmental conditions and larval prey abundances

In BB13 sea surface temperature (SST) ranged from 11.6°C to 14.7°C (Figure 3.1) with colder waters in the north (BB13N) compared to the south (BB13S). The same pattern was observed between Buchan and Banks in 2014, but SST was higher and ranged from 13.0 to 15.2°C. In January 2014 (DO14) the SST was between 6.9 and 10.9°C. A salinity gradient existed in the English Channel with higher salinities towards the Atlantic in the West and lower salinities in the eastern part of the sampling grid (29.2 - 35.2). In BB13 and BB14, the surface salinity ranged from 34.0 to 35.1 and 34.0 to 34.9, respectively, with the highest salinities in the northern part of the sampling area. In Banks and offshore Buchan area the water column was thermally stratified in BB13 and BB14 (at 59 and 81 stations, respectively) in contrast to DO14, where all stations were well mixed. The mean (±SD) Chl *a* concentration was slightly higher in BB13 (3.19 (±2.86) µg L^{-1}) compared to BB14 (2.70 (±1.19) µg L^{-1}) (Table 3.1). During the winter survey, (DO14), the mean (±SD) Chl *a* concentration was 1.1 (±0.59) µg L^{-1}.

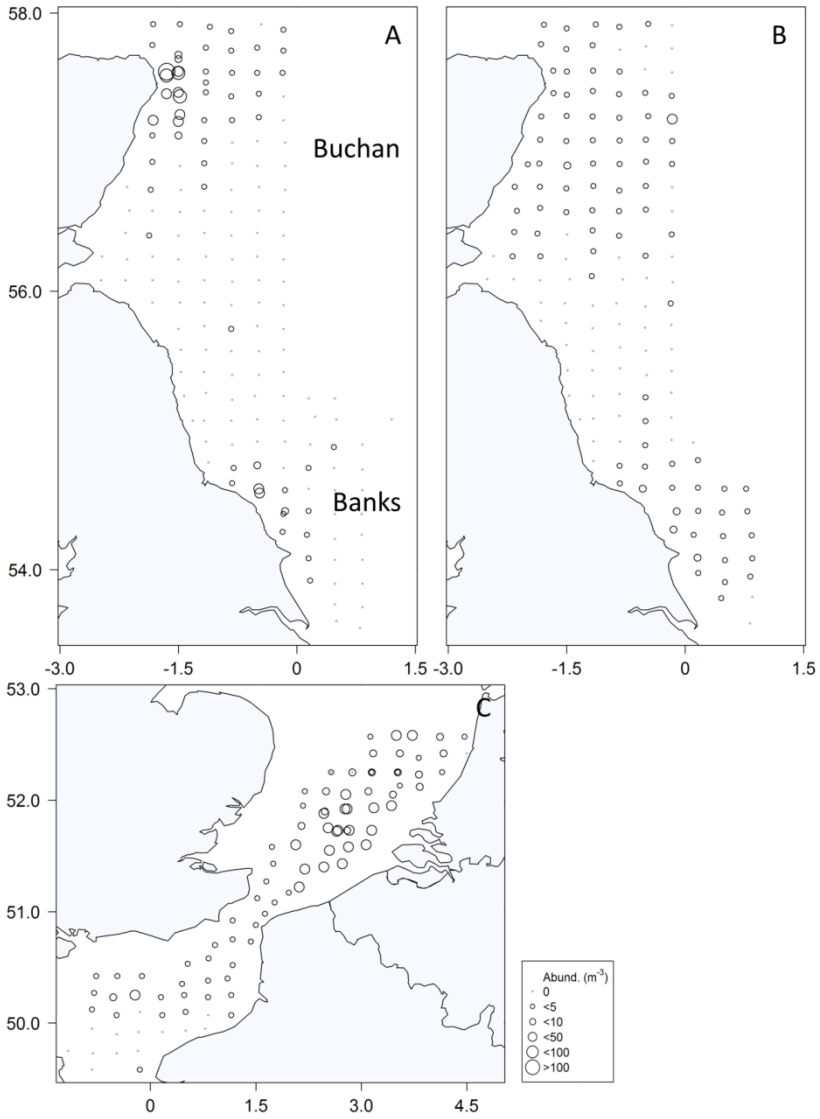

Figure 4.2: **Abundance (no. m^{-3}) of 8 to 14 mm length Atlantic herring (*Clupea harengus*) larvae sampled on the routine station grid of the IHLS. Abundances in September 2013, BB13 (panel A) and 2014, BB14 in panel B and January 2014, DO14 (panel C) are displayed. Size of the circles represents the larval abundance.**

The PZP abundance varied between and within sampling areas (Table 3.1 and Table 4.2). The mean abundance was 29,120 (CV: 80%) ind L^{-1} in BB13N and 33,780 (CV: 90%) ind L^{-1} in BB13S and consisted mostly of cells <50 µm (73% and 72% of total abundance, respectively). Dinoflagellates (mostly unspecified Gymnodiniales) comprised almost 90% of the total abundance in BB13N and BB13S (97 and 89%, respectively). The areas differed in the mean abundance of the thecate dinoflagellates *Ceratium* spp. (e.g. 3 ind L^{-1} (CV: 237%) in BB13N and 1,067 ind L^{-1} (CV: 337%) in BB13S) and the athecate *Pronoctiluca pelagica* (9,588 ind L^{-1} (CV: 125%) in BB13N, 5 ind L^{-1} (CV: 141%) in BB13S). The ciliate community was dominated

by aloricate specimens of the genus *Strombidium* spp. (2,318 ind L^{-1} (CV: 159%) in BB13N and 2,995 ind L^{-1} (CV: 109%) in BB13S). In BB13N *Mesodinium rubrum* was also an abundant ciliate taxon (mean 1,183 ind L^{-1} (CV: 172%)). In BB14 the total PZP abundance was five times higher in BB14N compared to BB14S (mean abundance of 27,575 ind L^{-1} (CV: 51%) in BB14N and 5,125 ind L^{-1} (CV: 33%) in BB14S) and was dominated by cells <50 µm (82% and 75%, respectively). Similar to BB13, the majority of the PZP community consisted of dinoflagellates, 78% in BB14N and 83% in BB14S, mostly composed of unspecified Gymnodiniales (mean 9,044 ind L^{-1} (CV: 77%) in BB14N and 13,950 ind L^{-1} (CV: 18%) in BB14S). Thecate dinoflagellates of the genus *Ceratium* were very abundant in BB14N (mean 484 ind L^{-1} (CV: 310%)). The ciliate community consisted mainly of *Strombidium* spp. in BB14N as well as in BB14S (mean 5,404 ind L^{-1} (CV: 86%) and 715 ind L^{-1} (CV: 82%), respectively). In contrast to BB13, *M. rubrum* was rare in BB14N (mean 173 ind L^{-1} (CV: 163%)) and absent in BB14S. In January (DO14), the PZP abundance was one order of magnitude lower (mean 1,045 ind L^{-1} (CV: 41%)) than in September. PZP assemblage in DO14 consisted mostly of (small celled (<50 µm)) dinoflagellates (81%), Gymnodiniales being most abundant (mean 360 ind L^{-1} (CV: 48%)). Other important taxa for the dinoflagellate composition were *Torodinium robustum* (mean 189 ind L^{-1} (CV: 74%)) and *Protoperidinium* spp. (mean 108 ind L^{-1} (CV: 36%)). The aloricate ciliates *Strombidium* (mean 80 ind L^{-1} (CV: 86%)) and *Strobilidium* (mean 78 ind L^{-1} (CV: 77%)) dominated the ciliate community. Contrasting to BB13 and BB14 tintinnida were the third most abundant ciliates contributing 12% to total ciliate abundance. The mean MZP abundance (±SD) was 18 (CV: 59%) ind L^{-1} in BB13N and 17 ind L^{-1} (CV: 20%) in BB13S. In BB13N the community was dominated by nauplii (mean abundance 12 ind L^{-1} (CV: 102%)), in BB13S by nauplii (mean 7 ind L^{-1} (CV: 32%) and the thecate dinoflagellate *Ceratium* spp. (mean 9 ind L^{-1} (CV: 90%)).

Table 4.1: Overview over sampling position, environmental factors, abundance (ind L^{-1}) of main groups of PZP and MZP and contribution (%) of PZP size fractions to total abundance at the stations sampled in September 2013 (BB13), September 2014 (BB14) and January 2014 (DO14). Positions of the sampling stations are ordered from North to South within each area.

							PZP abundance (Ind L^{-1})		contribution (%) to total PZP abundance			MZP abundance (Ind L^{-1})					
area	sampling date	°lat	°lon	SST (°C)	sal	chl a (mg L^{-1})	dinoflagellates	ciliates	cells <30µm	cells 30-50µm	cells >50µm	nauplii	copepods	*Ceratium* spp.	*Dinophys* is spp.	mollusc larvae	tintinnida
BB13N	21.09.2013	57.92	-1.82	12.61	34.75	1.83	1360	4850	70.37	12.96	16.67	30.25	1.15	0.43	0.97	0.72	0.04
BB13N	19.09.2013	57.4	-0.83	12.28	34.75	1.92	4310	7860	15.23	2.58	82.19	5.64	1.04	0.96	0.79	0.63	0.00
BB13N	20.09.2013	57.12	-1.5	12.36	34.68	2.86	43270	2100	84.52	8.70	6.79	5.50	0.92	2.77	2.04	0.38	1.15
BB13N	20.09.2013	56.9	-1.48	12.11	34.64	8.95	52490	240	48.47	50.79	0.74	6.33	0.75	5.36	3.04	0.50	2.36
BB13S	24.09.2013	54.73	-0.8	13.08	34.38	2.16	53920	1270	31.85	43.71	24.44	5.22	0.14	9.15	4.20	0.75	0.08
BB13S	24.09.2013	54.4	-0.17	12.33	34.45	1.39	6490	5880	58.81	9.44	31.75	8.26	0.18	2.08	3.07	0.60	0.49
BB14N	18.09.2014	56.92	-1.48	13.21	34.43	2.32	23020	16400	78.59	19.16	2.26	4.23	0.00	8.83	72.57	11.09	1.03
BB14N	19.09.2014	56.9	-1.9	13.26	34.26	2.27	14880	1490	63.62	19.94	16.44	4.14	0.05	11.37	96.00	27.82	0.79
BB14N	18.09.2014	56.72	-1.15	13.16	34.46	2.10	7530	4230	75.40	22.17	2.43	2.25	0.05	1.94	14.79	4.37	0.14
BB14N	16.09.2014	56.6	-0.17	13.75	34.73	2.07	2020	5420	75.24	20.26	4.50	5.44	0.19	11.36	48.28	15.86	0.55
BB14N	17.09.2014	56.55	-0.48	13.52	34.49	2.60	21210	8970	91.25	7.46	1.29	1.31	0.00	0.43	12.99	1.73	0.45
BB14N	17.09.2014	56.42	-0.83	13.91	34.43	3.05	37940	5920	65.48	32.64	1.88	3.46	0.06	7.83	26.49	9.71	0.59
BB14N	19.09.2014	56.4	-2.12	13.37	34.28	2.82	38340	3380	34.90	16.79	48.32	11.16	0.14	486.97	433.05	535.29	1.24
BB14N	18.09.2014	56.1	-1.5	13.76	34.38	4.86	26270	3580	18.93	16.49	64.58	12.69	0.04	279.87	325.42	344.45	1.26
BB14S	23.09.2014	54.72	-0.83	14.34	34.15	2.22	4990	1320	45.98	29.57	24.46	23.22	0.15	10.20	484.89	34.66	1.74
BB14S	24.09.2014	54.27	-0.13	13.87	34.22	NA	3540	400	54.59	19.06	26.35	6.46	0.19	4.01	148.11	30.36	0.25
DO14	20.01.2014	52.25	2.57	10.23	35.10	0.56	540	40	44.78	34.33	20.90	0.31	0.00	NA	NA	NA	NA
DO14	23.01.2014	52.13	3.55	8.63	34.35	2.06	1520	230	44.00	42.86	13.14	6.30	0.40	NA	NA	NA	NA
DO14	23.01.2014	52.05	2.78	9.70	34.97	0.77	720	60	38.80	50.27	10.93	3.20	0.28	NA	NA	NA	NA
DO14	23.01.2014	51.73	3.15	8.73	34.26	1.37	1040	210	41.60	41.60	16.80	5.28	0.00	NA	NA	NA	NA
DO14	23.01.2014	51.73	2.8	9.47	34.65	1.84	510	80	44.97	46.15	8.88	7.42	0.25	NA	NA	NA	NA
DO14	22.01.2014	50.73	1.43	9.19	34.20	0.81	1000	500	54.67	34.00	11.33	1.43	0.15	NA	NA	NA	NA
DO14	21.01.2014	50.42	0.17	10.57	35.24	0.65	750	230	68.38	20.51	11.11	0.53	0.00	NA	NA	NA	NA
DO14	22.01.2014	50.38	0.82	10.39	35.23	0.69	720	210	59.18	29.59	11.22	1.01	0.19	NA	NA	NA	NA

Table 4.2: **PZP taxa identified from the IHLS in September 2013 (BB13N, BB13S), September 2014 (BB14N, BB14S) and January 2014 (DO14). Abundances are given in ind L^{-1}. Positions of sampling stations are ordered from North to South within each spawning ground and season.**

			PZP abudance (Ind L^{-1})																						
			BB13N				BB13S		BB14N								BB14S		Downs14						
° lat			57.92	57.4	57.12	56.9	54.73	54.4	56.92	56.9	56.72	56.6	56.55	56.42	56.4	56.1	54.72	54.27	52.25	52.13	52.05	51.73	51.73	50.73	50.42	50.38
° long			-1.82	-0.83	-1.5	-1.48	-0.8	-0.17	-1.48	-1.9	-1.15	-0.17	-0.48	-0.83	-2.12	-1.5	-0.83	-0.13	2.57	3.55	2.78	3.15	2.8	1.43	0.17	0.82
	order	taxon																								
Dinoflagellates	Gymnodiniales	spp.	680	380	15720	38690	36430	3250	9500	4180	4510	760	18180	20240	9920	5060	1220	1570	170	720	240	480	280	300	350	340
	Gymnodiniales	*Gyrodinium* spp.	170	3480	1750	260	460	150	320	720	120	90	130	400	200	320	170	20	10	50	30	30	10	30	10	50
	Gymnodiniales	*Torodinium robustum*	10	0	210	80	50	80	960	510	1020	340	620	1600	1400	1240	220	20	50	420	220	290	60	290	140	40
	Gymnodiniales	*Katodinium* sp.	80	90	70	20	40	30	4320	1110	870	280	460	5140	3760	360	1330	430	10	40	20	10	10	20	10	40
	Gymnodiniales	*Amphidinium* spp.	0	0	20	40	70	400	160	220	140	20	110	140	80	50	50	60	0	20	0	20	10	10	40	20
	Gymnodiniales	*Akashiwo* spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	10	0	0
	Gymnodiniales	*Polykrikos kofoidii*	0	0	0	0	0	0	0	20	0	0	0	20	0	210	0	0	0	0	0	0	0	0	0	0
	Noctilucales	*Pronoctiluca* cf. *pelagica*	310	0	25160	12880	10	0	80	60	0	0	20	0	60	130	0	10	0	0	0	0	0	0	0	0
	Dinophysiales	*Dinophysis* sp.	10	30	30	30	170	40	20	290	0	0	0	60	3300	6720	900	790	0	0	0	0	0	10	0	0
	Gonyaulacales	*Alexandrium* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	20	0	0	0	0	0
	Gonyaulacales	*Ceratium tripos*	10	0	10	0	60	0	0	40	0	10	0	40	300	910	80	0	0	0	0	0	0	0	0	0
	Gonyaulacales	*Ceratium macroceros*	0	0	0	0	40	0	0	30	0	10	0	0	40	310	10	0	0	0	0	0	0	0	0	0
	Gonyaulacales	*Ceratium furca*	0	0	0	10	90	0	20	100	0	0	0	20	6900	2050	70	20	0	0	0	0	0	0	0	10
	Gonyaulacales	*Ceratium lineatum*	0	0	0	30	12480	120	40	410	0	0	10	100	5540	5770	110	110	0	0	0	0	0	0	0	0
	Gonyaulacales	*Ceratium fusus*	0	0	0	10	10	0	0	150	0	0	0	0	120	250	0	10	0	0	0	0	0	0	0	0
	Gonyaulacales	*Ceratium* spp.	0	0	0	0	0	0	0	0	0	0	0	0	40	0	10	10	0	0	0	0	0	0	0	0
	Peridiniales	*Heterocapsa* sp.	90	290	140	300	140	1550	7280	5520	820	510	1630	10000	3320	780	580	350	0	60	10	20	40	120	100	60
	Peridiniales	*Protoperidinium* spp.	0	30	90	70	3800	870	300	620	40	0	50	100	760	760	190	110	100	140	50	120	70	170	90	120
	Prorocentrales	*Prorocentrum* spp.	0	10	70	70	70	0	20	900	10	0	0	80	2600	1350	50	30	140	70	130	70	30	40	10	40
Ciliates	Strombidiida	*Strombidium* spp.	190	7770	1290	20	680	5310	15960	1200	3310	3680	7720	5220	3080	3060	1130	300	10	30	30	70	50	220	130	100
	Strombidiida	*Tontonia gracillima*	80	0	0	0	40	120	0	10	70	40	100	140	0	0	10	0	0	0	0	0	0	10	0	0
	Strombidiida	*Laboea strobila*	0	0	50	100	0	180	0	0	10	80	10	120	0	0	0	0	0	0	0	0	0	0	0	0
	Prorodontida	*Balanion* sp.	280	0	150	0	110	0	0	0	30	500	270	0	0	20	10	10	0	0	0	10	0	10	0	0
	Prorodontida	*Tiarina fusus*	0	0	0	0	0	50	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
	Haptorida	*Spathidium* sp.	0	0	0	0	0	0	0	60	0	0	70	0	10	0	0	10	0	0	0	10	0	0	0	0
	Cyclotrichiida	*Mesodinium rubrum*	4220	0	430	80	170	10	0	0	170	820	280	100	0	10	0	0	0	0	0	0	0	0	0	0
	Choreotrichida	*Strobilidium* spp.	40	50	70	0	150	130	380	80	630	300	490	100	220	130	60	50	20	170	10	90	20	150	80	80
	Choreotrichida	*Lohmanniella* sp.	0	0	0	20	100	0	10	60	0	0	0	0	40	330	0	0	0	0	20	0	0	0	20	0
	Choreotrichida	*Leegaardiella* spp.	20	0	100	10	10	0	0	10	0	0	20	20	0	10	40	0	0	0	0	0	0	40	0	0
	Tintinnida	spp.	20	20	0	0	10	60	0	10	10	0	10	200	0	10	10	0	0	0	0	0	0	10	0	0
	Tintinnida	*Tintinnopsis* sp.	0	20	10	10	0	20	50	60	0	0	0	20	20	10	60	30	10	30	0	30	10	60	0	30

Mean copepod abundance was 0.97 ind L^{-1} (CV: 18%) and 0.16 ind L^{-1} (CV: 15%) in BB13N and BB13S, respectively). In BB14 mean abundance was about 20 times higher than in BB13 with 355 ind L^{-1} (CV: 155%) in BB14N and 372 ind L^{-1} (CV: 69%) in BB14S. The majority of the abundance was composed of thecate dinoflagellates in BB14N with mean abundance of 115 ind L^{-1} (CV: 145%) and mean abundance of 162 ind L^{-1} (CV: 139%). Nauplii had mean abundances of 6 (CV: 74%) and 15 ind L^{-1} (CV: 80%) (BB14N and BB14S, respectively) and copepods did not exceed 0.19 ind L^{-1} in both areas. In DO14, the abundance of nauplii was lower than in September with mean abundance of 3 ind L^{-1} (CV: 88%). Mean copepod abundance (0.25 ind L^{-1}, CV: 59%) was lower than in BB13N, albeit higher than in BB13S and BB14.

4.4.2 Herring larvae abundance and distribution

In BB13 and BB14, larval herring were mostly found in the northern (Buchan) and southern (Banks) portions of the survey grid (Figure 4.2). In BB13, peak abundance was 270 ind m^{-3}, mostly composed of larvae 6-8 mm in length (Fig. 3). The following year (BB14), larvae reached a maximum abundance of 13 ind m^{-3}.

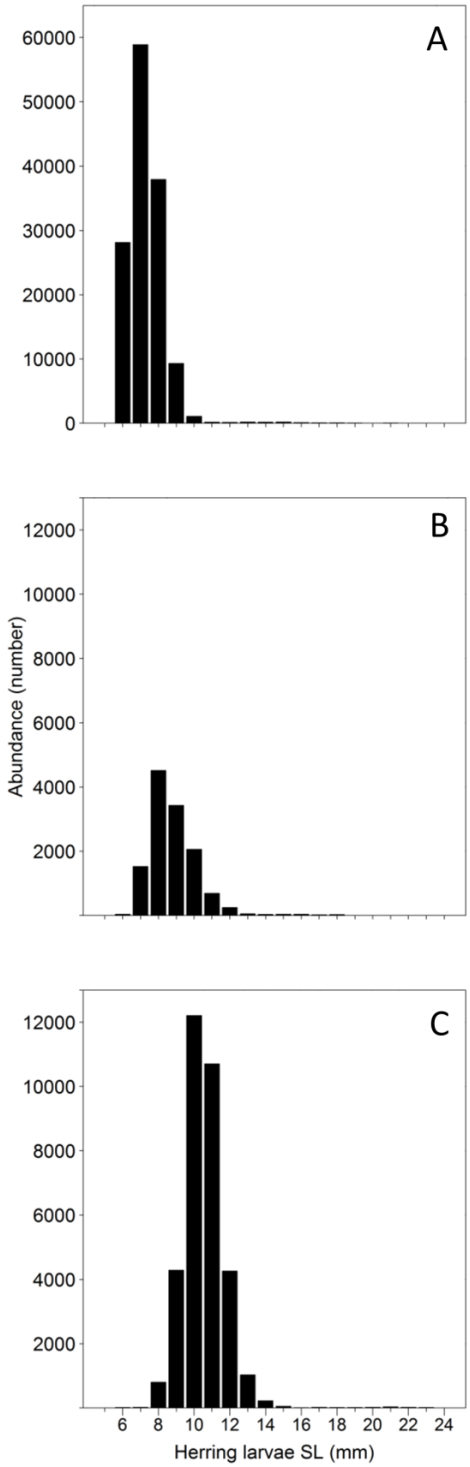

Figure 4.3: **Length-frequency distribution of herring larvae (Standard length (SL) vs. total number of larvae caught during the IHLS survey). The three surveys considered in this study are displayed: September 2013, BB13 (panel A), September 2014, BB14 (panel B) and January 2014, DO14 (panel C). Please note the different scaling of y-axis.**

The majority of the sampled larvae had a SL between 8 and 10 mm. In DO14, the maximum abundance of larvae was 50 ind m^{-3} and consisted mostly of 10- to 11-mm individuals.

4.4.3 Herring larvae trophic position

POM samples had an average $\delta^{15}N$ of <7‰ in every cruise (Table 4.3). A significant difference in $\delta^{13}C$ between the plankton size classes (50-100 µm and 100-200 µm) was found only for BB14N. Thus, the size classes were pooled for the analysis and are further referred to as MZP. MZP was significantly more enriched in $\delta^{13}C$ ($p < 0.001$) in BB13N compared to BB13S.

Table 4.3: Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of microzooplankton (MZP) and particulate organic matter (POM), derived from bulk filter samples, in September 2013 (BB13), September 2014 (BB14) and January 2014 (DO14). MZP in BB13 is displayed separately for BB13N and BB13S due to statistical differences in stable isotope composition between the areas.

Cruise	sample type	n	$\delta^{15}N$ (±SD)	$\delta^{13}C$ (±SD)
BB13	POM	6	6.79 ±0.42	-21.66 ±1.46
BB13N	MZP	5	7.81 ±0.29	-12.30 ±2.12
BB13S	MZP	3	7.71 ±0.59	-20.05 ±0.69
BB14	POM	10	6.60 ±0.62	-22.61 ±1.86
BB14	MZP	10	7.24 ±0.69	-20.49 ±4.19
DO14	POM	8	6.97 ±1.57	-18.36 ±1.57
DO14	MZP	8	7.56 ±1.42	-6.44 ±1.42

The $\delta^{15}N$ signature of the larvae showed no statistical differences in between the spawning grounds BB13N and BB13S for all length classes ($p > 0.05$). However, in BB14N, the $\delta^{15}N$ was significantly higher for length class M compared to BB14S ($p = 0.049$) (Table 4.4 and Figure 4.4).

In BB13N, larvae of length class S were more depleted in $\delta^{13}C$ than larvae of length class L ($p < 0.001$). Differences in $\delta^{13}C$ were significant for length class S and L (not enough data points to test for length class M) between BB13N and BB13S ($p < 0.001$), with larvae in BB13N having higher $\delta^{13}C$ signatures, which coincides with the differences we found for the MZP. In DO14, $\delta^{15}N$ of all length classes were higher and differed significantly from the values in BB13 and BB14 ($p < 0.001$). For $\delta^{13}C$, significant differences in DO14 compared to BB13 and

BB14 were found between larvae of length class M ($p < 0.001$, $p = 0.02$, respectively). MZP was less than one trophic level above baseline (Figure 4.4) and similar in all cruises with TP 1.27 (BB13), 1.19 (BB14) and 1.17 (DO14). Larvae of all length classes in BB13 showed a TP between 2.08 and 2.28, independent from the spawning ground.

Table 4.4: Stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) and biochemically derived nutritional condition (*sRD*) and growth rate (G_i) of the herring larvae sampled during the IHLS in September 2013 (BB13), September 2014 (BB14) and January 2014 (DO14). Data is displayed separately for the areas and the larval length classes.

				stable isotopes (‰)			condition	
area	sample type	length class	n	$\delta^{15}N$ (±SD)	$\delta^{13}C$ (±SD)	n	*sRD* (±SD)	G_i (±SD)
BB13N	herring larvae	S	9	10.88 ±0.33	-18.51 ±0.43	82	3.08 ±1.26	0.21 ±0.09
BB13N	herring larvae	M	3	10.91 ±0.58	-18.09 ±0.18	9	3.91 ±0.80	0.27 ±0.05
BB13N	herring larvae	L	7	11.15 ±0.22	-17.70 ±0.32	6	4.08 ±0.90	0.27 ±0.06
BB13S	herring larvae	S	5	11.1 ±0.40	-19.66 ±0.45	12	2.75 ±1.30	0.19 ±0.09
BB13S	herring larvae	M	0	NA	NA	6	3.50 ±0.77	0.24 ±0.05
BB13S	herring larvae	L	4	10.45 ±0.76	-19.80 ±0.34	2	3.31 ±1.83	0.23 ±0.14
BB14N	herring larvae	S	3	11.07 ±0.44	-19.45 ±0.26	12	2.34 ±0.91	0.16 ±0.06
BB14N	herring larvae	M	7	10.61 ±0.49	-19.50 ±0.41	63	3.49 ±1.22	0.24 ±0.08
BB14N	herring larvae	L	0	NA	NA	3	2.32 ±1.00	0.16 ±0.07
BB14S	herring larvae	S	5	11.63 ±0.34	-19.40 ±0.22	11	2.69 ±1.59	0.19 ±0.11
BB14S	herring larvae	M	3	11.54 ±0.79	-19.28 ±0.29	6	1.94 ±0.78	0.14 ±0.05
BB14S	herring larvae	L	0	NA	NA	0	NA	NA
DO14	herring larvae	S	7	13.29 ±0.40	-19.22 ±0.32	9	2.24 ±0.84	0.13 ±0.05
DO14	herring larvae	M	12	12.96 ±0.59	-19.01 ±0.24	139	2.13 ±0.71	0.12 ±0.04
DO14	herring larvae	L	8	13.47 ±0.47	-18.86 ±0.29	23	2.5 ±0.71	0.13 ±0.04

The trophic enrichment ($\Delta^{15}N$) relative to the SI of MZP was in the range of the estimated enrichment (3.4‰) between two trophic levels (3.07-3.39‰) except for the largest larvae in BB13S (2.74‰). In BB14, $\Delta^{15}N$ relative to MZP was higher and reached values exceeding one trophic level (up to 4.39‰ in BB14S). The larvae in DO14 showed an enrichment of >5‰ relative to MZP.

The relation of the total amount of carbon to nitrogen in the larvae decreased significantly with larval dry weight in BB13 (Figure 4.5). Larvae of BB14 showed no significant relationship of C:N ratio with larval weight, DO14 even showed a slight, albeit not significant, increase ($R^2 = 0.006$, $p = 0.004$). However, larvae of length class S (max. 165 µg dry weight) did not show any differences in C:N ratio between cruises ($p > 0.5$).

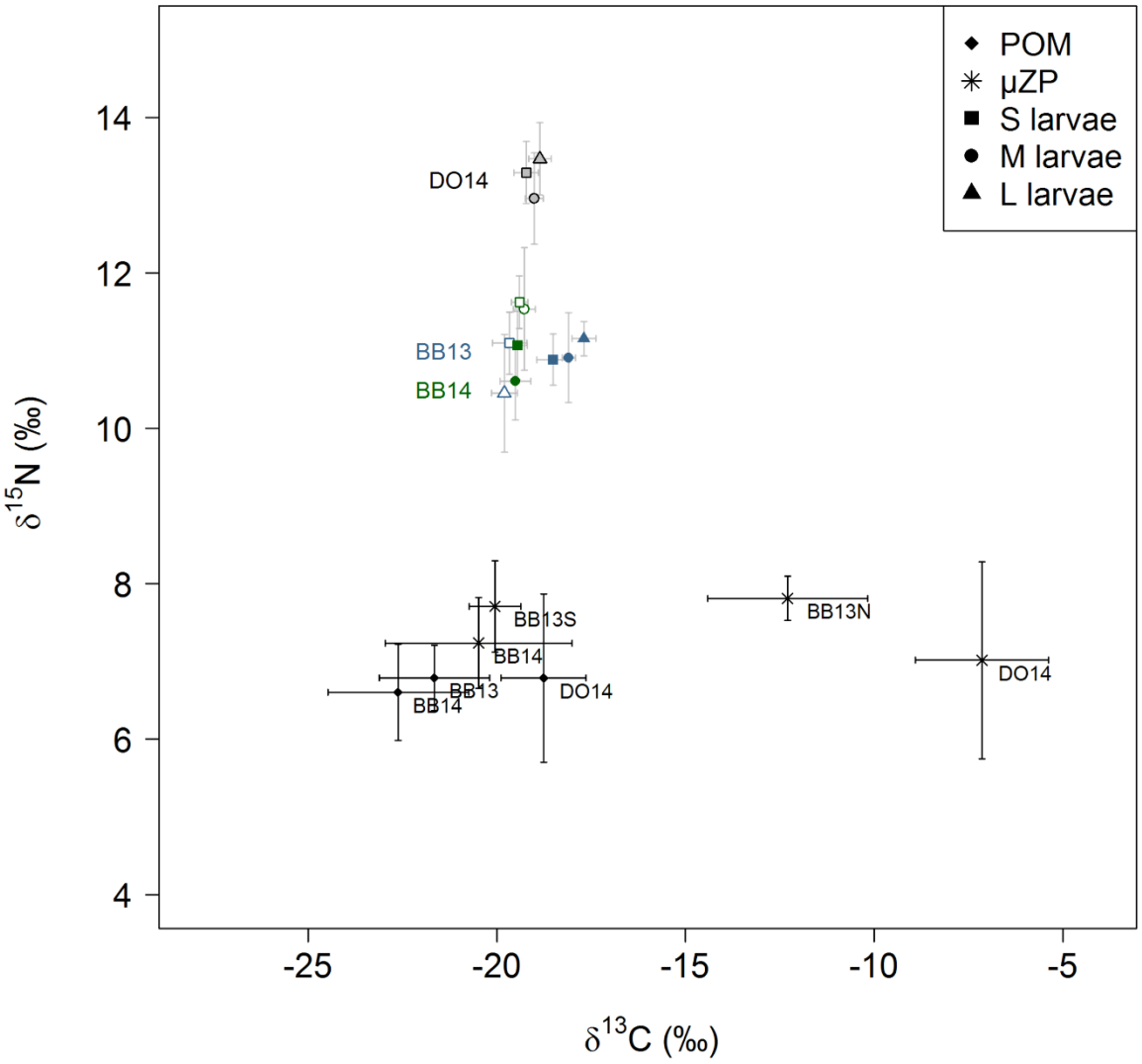

Figure 4.4: **Biplot of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures for bulk POM and MZP, and herring larvae samples of the three surveys (±SD) in September 2013 (BB13), September 2014 (BB14) and January 2014 (DO14), size classes indicated by the symbols (diamond =POM, asterisk =MZP). MZP samples in BB13 are displayed separately for Buchan (BB13N) and Banks (BB13S) due to statistical significant differences. Herring spawning grounds are color coded, blue filled =BB13N, blue open =BB13S, green filled =BB14N, green open =BB14S and grey =DO14. Larval length classes are displayed by symbols (S =rectangle, M =circle, L =triangle).**

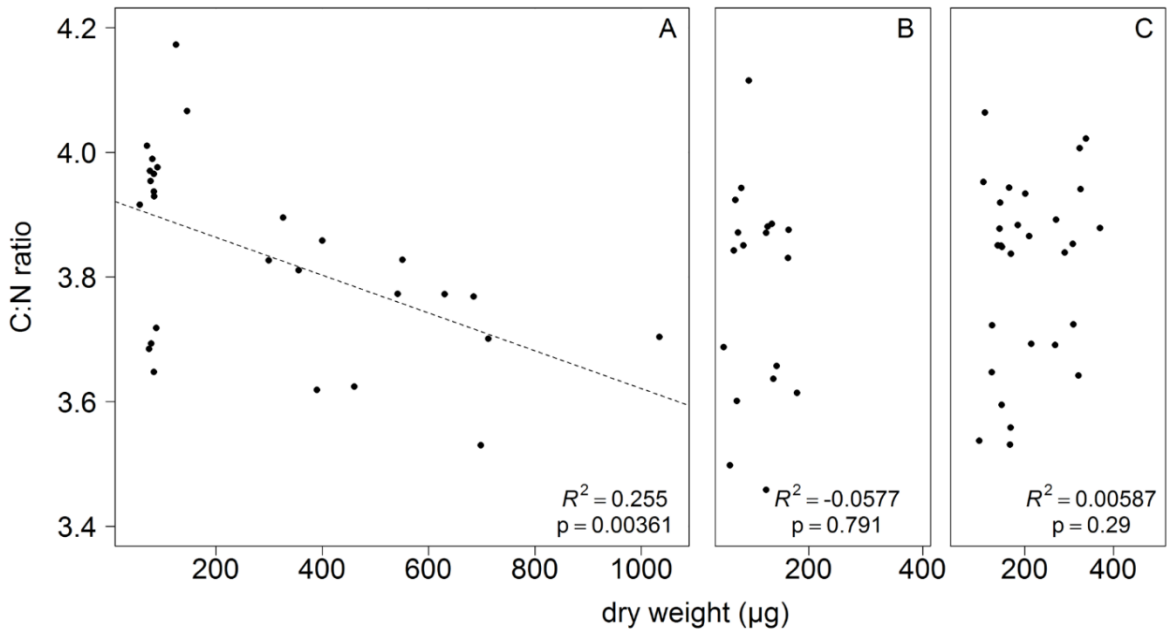

Figure 4.5: **C:N ratio versus dry weight (µg) of the herring larvae used for stable isotope analysis (SIA) in September 2013, BB13 (panel A), September 2014, BB14 (panel B) and January 2014, DO14 (panel C). As larvae were pooled for the analysis, the mean dry weight per sample per larva was used for the C:N analysis. Note that only for BB13 larvae with a mean weight >400 µg were used.**

4.4.4 Herring larvae nutritional condition and growth rate

Larval nutritional condition (s*RD*) did not significantly differ between the length classes in each area (Table 4.4 and Figure 4.6) ($p > 0.05$), except for larvae of length class M in BB14S, which showed a significant lower s*RD* than the other larvae in BB14 ($p = 0.03$).

The lowest *sRD* was found in DO14 with 0.42 in length class M, the highest in BB14N with 6.39 in length class M. Overall temperature had a strong positive effect on larval s*RD* ($p < 0.001$), but no correlation was found within each single cruise.

All larvae in the three surveys showed positive G_i (>0.02 d^{-1}). For length class S, G_i was lowest in DO14 ($p = 0.008$) and highest in BB13N ($p = 0.006$) with maximum G_i of 0.4 d^{-1}. Larvae of length class M exhibited a higher G_i in BB13N and BB14N than in BB14S and DO14 ($p < 0.001$). As for larvae of length class S larvae of length class L showed significantly higher G_i in BB13N than in the other areas ($p < 0.001$) with max. G_i of 0.43 in BB13N.

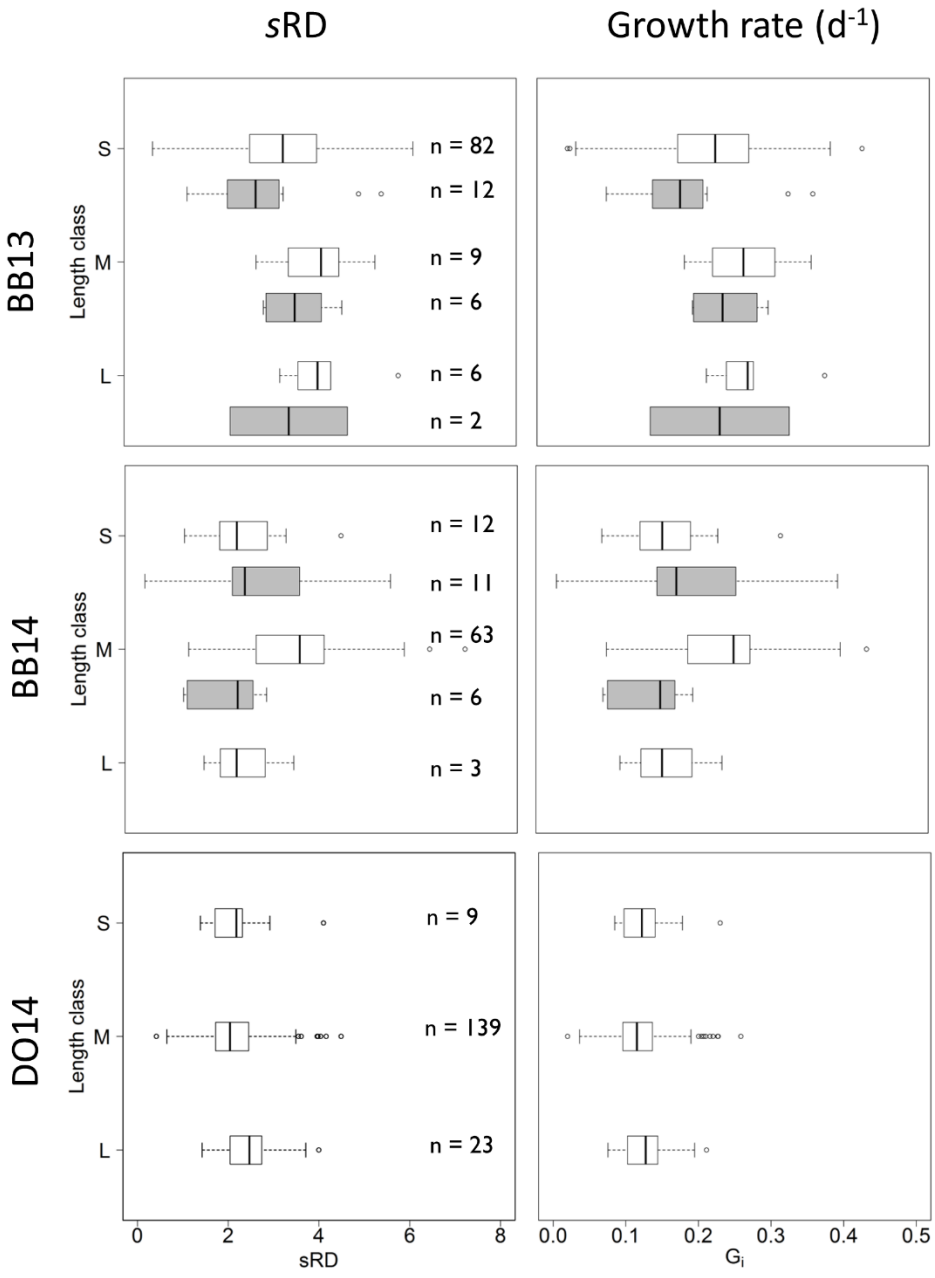

Figure 4.6: **Standardized RNA/DNA (*sRD*) and corresponding instantaneous growth rate (G_i) of Atlantic herring (*Clupea harengus*) larvae from September 2013 (BB13), September 2014 (BB14) and January 2014 (DO14) from three different length classes: S (8-9 mm), M (10-12 mm) and L (13-14 mm). White boxplots in BB13 and BB14 display Buchan area and grey boxes Banks area. Number of larvae used for the analysis is given for every length class.**

4.5 Discussion

Data on PZP and MZP are generally scarce, especially for autumn and winter periods (Bils et al., submitted, Widdicombe et al. 2010, Löder et al. 2012) and, thus, very little knowledge exists about the role of these organisms as prey items for larval fish in the field (Montagnes, Dower, et al. 2010). When the classical food chain, consisting of large phytoplankton cells, copepods and higher trophic levels, decreases after the autumn bloom in the NE Atlantic, the carbon (re)cycling is mainly driven by microbial loop associated organisms, such as small ciliates and heterotrophic dinoflagellates. As a consequence of the lowered availability of mesozooplankton prey, PZP and MZP are thought to gain importance as prey items for larval fish during that time of year. We chose Atlantic herring NSAS as a case study to further explore the relation of small prey fields and larval fish. Due to its commercial importance, there have been many thorough laboratory studies conducted covering various aspects of herring early life history in the past century, from ecophysiology (Hufnagl & Peck 2011 and references therein) to behaviour (Munk 1992, Illing et al. 2016) and prey preferences (Checkley 1982). The present study provides a unique sampling and analysing approach. It combines simultaneous field sampling of herring larvae and plankton, taxonomic analysis of the potential small prey fields and biochemical analysis (stable isotopes, condition and growth) in three spawning grounds covering two seasons (autumn & winter). This way we hope to gain further insights in the trophic ecology of larvae of NSAS and the possibilities to study the relationship between the lower trophic food web and larval fish in general.

In the following we will discuss the three hypothesis presented in the introduction.

4.5.1 Larvae spawned later in the year (winter – Downs) rely more on PZP than on MZP to cover their energetic demands

The contribution of the different spawning components to recruitment varies between years, but the Downs component has gained importance since 1990s with contributions up to 60% (Schmidt et al. 2009). The reason and ultimate mechanisms behind these recruitment dynamics remain unknown.

Low productivity conditions with Chl *a* concentrations <1.5 µg Chl *a* L^{-1} (www.wgze.net, this study) measured in the English Channel during herring spawning season from mid-December

until end of January, affect higher trophic levels and thus prey availability for herring larvae. Therefore it has been suggested, that first feeding larvae, usually associated with the classical food-chain (phytoplankton-copepods-larval fish), might rely on prey organisms from the microbial food web such as heterotrophic ciliates and dinoflagellates (Figueiredo et al. 2005, Peck, Huebert, et al. 2012).

PZP abundance was lower in the winter than in autumn at DO14, but both periods displayed a similar composition with athecate dinoflagellates dominating the community relative to ciliates, also observed in North Sea plankton studies in autumn and winter (Bils et al., submitted, Widdicombe et al. 2010, Löder et al. 2012). The total PZP and nauplii winter abundance was in the range of long term trends observed in the English Channel (Widdicombe et al. 2010) and at one monitoring site in the eastern North Sea (Wesche et al. 2007). Thus, the small prey field was considered as typical for Downs larvae.

The elevated $\delta^{15}N$ of Downs larvae compared to autumn spawned larvae indicate difference in dietary composition. A diet based predominantly on heterotrophic (carnivorous) unicellular organisms can result in a higher trophic level of the larvae as strictly herbivorous copepods as main food source (Pepin & Dower 2007). Additionally, during times of lower primary production (i.e. winter), the size distribution of plankton may shift from bigger autotroph and herbivorous organisms to smaller (bacterivorous) cells leading to a stronger influence of the microbial loop (Levinsen & Nielsen 2002), which influences stable isotope signals. However, the community composition of PZP did not show any drastic differences to autumn. Most of the taxa found in both seasons are known to be herbivorous, feeding on autotrophic or heterotrophic nanoflagellates, or bacterivorous (e.g. *Heterocapsa* sp., Gymnodiniales, *Strombidium* spp.) (Jeong et al. 2010, Montagnes, Allen, et al. 2010) and a higher proportion of potential predatory PZP, feeding on other dinoflagellates or ciliates, such as species of the genus *Protoperidinium* (Jeong et al. 2010) could not be found in the winter samples. Additionally, a higher enrichment in $\delta^{15}N$ would have been expected for POM and MZP samples if carni- and omnivorous microbial loop cells were more abundant compared to herbivorous organisms. The signals of the bulk samples of POM and MZP do not indicate any increase in $\delta^{15}N$ in the entire system. However, there is a presumed lack of detectable trophic fractionation within the protozoan food-web (Gutiérrez-Rodríguez et al.

2014), which contradicts the hypothesis of higher trophic consumer level due to increased feeding on protozoans. An additional indication, that the bulk samples do not reflect the potential prey items are the $\delta^{13}C$ values of the MZP above average measured for pelagic plankton organisms (Kürten et al. 2013). This pattern might be partially explained by higher lipid content of the MZP causing an elevation of $\delta^{13}C$ signal (Waite et al. 2007).

Potentially, the shallowness of the entire sampling area (mean depth 38 m) and the rough weather conditions during the sampling period (van Damme & Bakker 2014) could have led to a high contribution of benthic detrital material and sediments in the MZP net, which is towed down to 5 m above ground. Benthic systems rely on the use of HCO_3^- rather than dissolved CO_2, resulting in a less negative $\delta^{13}C$ (Marconi et al. 2011). Some benthic cells (van Beusekom and Calbet, pers. comm) were visually observed in the samples, supporting this hypothesis. This might also have influenced the $\delta^{15}N$ signal of pelagic consumers.

Furthermore, $\delta^{15}N$ can be negatively related to temperature in zooplankton species (Power et al. 2003) and fish, observed for European seabass *Dicentrarchus labrax* (Barnes et al. 2007), most likely due to reduced nitrogen assimilation with increasing metabolic activity.

Assuming that even very young herring larvae prefer prey >29 µm (Spittler et al. 1990), it is important to note that, in the present study, average contribution of cells <30 µm was consistently above 40% in winter (Table 4.1). Contrary to Spittler et al. (1990), Denis et al. (2016) found different protist prey items ≥3 µm (diatoms and dinoflagellates) in the guts of Downs larvae <12mm. It can be assumed that aloricate ciliates and dinoflagellates were also part of their diet, but could not be detected due to the preservation method. Whether the contribution of PZP prey was still important when metazoan plankton was readily available to larvae (BB13 and BB14) remains unknown. To further investigate the contribution of PZP organisms in larval diet and the potential importance of (specific) taxonomic groups for larval survival, DNA barcoding could be an alternative to overcome the problem of prey in the digestive tract (Roslin et al. 2016), as successfully reported for larvae of European eel (*Anguilla anguilla)* (Riemann et al. 2010). Nevertheless, sampling the available prey field to account for prey preferences and how changes in prey composition impact larval survival will remain a crucial step. The sampling and effective monitoring of PZP and MZP has been widely neglected so far but is put more and more into focus as there is growing evidence for

the importance of PZP and MZP for larval fish nutrition in general (Figueiredo et al. 2005, 2007, Montagnes, Dower, et al. 2010, Friedenberg et al. 2012, Denis et al. 2016, Bils et al. 2017).

4.5.2 Larval herring target different prey items depending on larval size and spawning ground

Larval length at hatch increases at colder incubation temperatures (Peck, Kanstinger, et al. 2012) and the Downs component usually hatch at a length of 9 mm (Heath et al. 1997), while larvae in Buchan and Banks hatch at 7 and 8 mm, respectively (Blaxter & Hempel 1963). In addition, growth and development of larval fish is usually decelerated in winter due to lower temperatures (Pepin 1991). Furthermore, the larvae shrink when captured and can shrink further after being placed in formalin (Hay 1982). Despite these uncertainties, SL was chosen for comparisons on the trophic ecology of larvae from different feeding grounds as feeding success and preferred prey sizes are related to larval size (Hufnagl & Peck 2011).

The lack of differences in $\delta^{15}N$ among larvae sampled in September 2013 (BB13) suggests that larvae of all size classes were feeding on organisms of the same trophic level. Differences in $\delta^{13}C$ between small larvae (8-9 mm) and larger larvae (13-14 mm) within and between the spawning grounds Buchan and Banks make it plausible that the larvae use different primary carbon sources (difference in $\delta^{13}C$) from the same trophic level (no differences in $\delta^{15}N$). Another possible explanation, matching the elevated $\delta^{13}C$ of the MZP in BB13N, is that the organisms of the MZP fraction in this area possessed higher lipid storages (and thus higher nutritional value) compared to those in BB13S. Such high lipid storage would lead to a higher $\delta^{13}C$ signal, which can be then reflected in the $\delta^{13}C$ larval signal (Waite et al. 2007). In the following spawning season (BB14), lower $\delta^{15}N$ (and lower $\Delta^{15}N$) were observed for length class M in BB14N compared to BB14S, which could indicate feeding on lower trophic organisms considering that neither the POM nor MZP signals differed between the areas. Gutiérrez-Rodríguez et al. (2014) showed in an experimental study that there is no or only little trophic fractionation between heterotrophic protists and their autotrophic prey, in contrast to metazoan organisms. According to these findings larval diets rich in PZP (rather than MZP) would lead to a lower $\delta^{15}N$. Such speculation would also be

supported by the lower PZP abundances found in the Banks area compared to Buchan. Moreover, the lower trophic position in BB13 was favourable for the larval s*RD* and G_i.

The $\delta^{15}N$ signals measured for BB13 and BB14 match signals derived from lab experiments on herring larvae (Aberle & Malzahn 2007) but are lower than mean signals known for adult herring in the North Sea (e.g. Jansen et al. 2012). This indicates that $\delta^{15}N$ of herring larvae from Banks clearly provided a first/advanced feeding signal when compared to yolk-sac larvae that usually reflect the maternal signal. It has to be noted that some individuals of the smallest larval size class (S) might have partially reflected the maternal isotopic signal (Aberle & Malzahn 2007), a possible explanation of the higher mean $\delta^{15}N$ signals of small-sized larvae compared to size class M.

It is thought that the variability in $\delta^{15}N$ decreases with size as the niche width of captured prey becomes narrower (Malzahn & Boersma 2009). This was not observed in the present study, indicating that the larvae investigated are not specifically becoming more selective as they grow or selectively prey on organisms consuming a wide range of prey (Wells & Rooker 2009). Trophic enrichment ($\Delta^{15}N$) in BB13 did not show an increase with larval size, thus suggesting that the larvae of all length classes preferred prey from the MZP size fraction rather than from the POM size fraction. Larvae from BB14S achieved higher enrichment than estimated for one trophic level. However, the signals we derived from the plankton samples might not directly reflect the prey spectrum these larvae feed on. This can be associated with the caveat of stable isotope signatures derived from bulk samples, where phytoplankton and non-living detrital material also affect isotope signals (Kürten et al. 2013). The size classes in DO14 did not differ from each other in terms of stable isotope composition, but $\delta^{15}N$ was >1‰ higher than observed for BB13 and BB14. Adult herring tend to have higher $\delta^{15}N$ in coastal and southern areas of the North Sea (Jansen et al. 2012) and this may be one factor affecting the elevated signal observed in larval in the present study. However, zooplankton organisms also displayed a higher trophic position during wintertime (Kürten et al. 2013), which contradicts the MZP $\delta^{15}N$, which is in the same range in winter (DO14) than in autumn (BB13 and BB14). The reason for that might also be found in the analysis of bulk samples described above.

Shifts in larval diet during ontogeny are known and revealed by SIA, e.g. for the larvae of various marine fish species in the Gulf of Mexico (Wells & Rooker 2009) or during the settlement process in red drum (*Sciaenops ocellatus*) (Herzka et al. 2002). However, changes in stable isotope composition do not necessary need to account for a severe dietary shift. If prey feeding habits and thus $\delta^{15}N$ and $\delta^{13}C$ signals change (Irigoien et al. 2003) it can possibly influence the stable isotope composition of the predators consuming them (e.g. larval fish). For NSAS in the English Channel Denis et al. (2016) showed a shift in diet at length of around 12 mm using Scanning Electron Microscopy (detecting microplanktonic organisms in larval guts, but without accounting for naked PZP). In the present study, these results could not be confirmed by SIA, which might be related to pooling several larvae (i.e. up to 7) for the analysis. However, C:N ratio was inversely related to dry weight for larvae in BB13, suggesting that the larvae were feeding on qualitatively higher food sources as they grow. Similar to our results, Ehrlich (1974) measured that C:N ratio in feeding herring larvae decreased with development from 4.05 at the end of the yolk-sac stage to 3.63 at 21 mm larvae.

The present study gives indications about different feeding habits between larval sizes and spawning grounds, but shows the limitations of SIA approaches as well. Bulk samples of plankton size classes of potential prey do not account for the high variability of trophic modes and thus stable isotope signals. Samples of single MZP and PZP species/groups and controlled laboratory experiments based on recent findings of gut content analysis (Figueiredo et al. 2005, Denis et al. 2016) are needed to investigate feeding changes during ontogeny and potential consequences for larval development and survival.

4.5.3 The survival of herring larvae is correlated to the availability of suitable prey

Environmental conditions the larvae experienced in the spawning seasons and grounds considered, were comparable to long-term time series data from permanent monitoring sites and CPR data (continuous plankton recorder) in terms of Chl *a* concentrations (www.wgze.net, Bresnan et al. 2015). SST in DO14 and BB14 was about one degree higher than in previous years (van Damme & Bakker 2014) which is in line with positive anomalies observed since mid 1990s (O'Brien et al. 2013).

In the present study we are covering two spawning seasons associated with high and low recruitment of Atlantic autumn spawning herring. In 2014 strong recruitment (larvae from BB13 and DO14 being part of that year-class) was observed comparable to levels seen before the depression in the early 2000s. Recruitment in 2015 (including larvae from BB14) dropped below average, being among the lowest since the beginning of the time-series (ICES 2016).

In 2014, peak larval abundances were found to be at larger larval sizes than in the previous year, indicating that spawning had occurred earlier that year, maybe induced by higher temperatures (Blaxter & Hempel 1963). Differences in larval condition between the two subsequent autumn surveys were striking for larvae between 10 and 14 mm, especially for larvae from Banks component. Larvae in BB13 exhibited higher mean G_i pointing at favorable conditions in this specific year. The higher availability of PZP in BB13 could have been indirectly beneficial for the larvae by providing sufficient food supply for metazoan prey organisms (e.g. copepods).

In both, BB13 and BB14, athecate dinoflagellates (e.g. order Gymnodiniales) and aloricate ciliates (e.g. *Strombidium* spp.) were abundant throughout the area. These cells are within the 10-120 µm size range and, therefore, could be appropriate prey for smaller larvae (Peck, Huebert, et al. 2012). But as larvae >12 mm prefer prey >350 µm (Munk 1992), the scarce abundance of larvae >12 mm in BB14S could also be a consequence of sub-optimal conditions for growth and feeding in that area. In fact the total MZP abundance was higher in BB14 than BB13, but mostly due to high abundances of thecate dinoflagellates (*Ceratium* spp., *Dinophysis* spp.) and mollusc larvae. The latter was shown to be positively selected by younger larvae but rejected by larvae >13mm (Checkley 1982). Thecate dinoflagellates have not been specifically mentioned as larval fish prey, contrasting to athecate dinoflagellates and ciliates, which were found to be abundant in larval fish guts (Fukami et al. 1999, Figueiredo et al. 2005). The abundance of small copepods and copepodite stages was lower in BB14 compared to BB13 and DO14 and less than half as suggested as minimum prey threshold (Munk & Kiørboe 1985, Figueiredo et al. 2005, Hufnagl & Peck 2011) to sustain growth. In contrast to the present study, where larvae of the Downs component exhibited minimum instantaneous growth rates of 0.02 d^{-1}, Denis et al. (2017) reported growth rates of <0 d^{-1} for Downs larvae in 2015 (part of recruitment in 2015), pointing at poorer feeding

conditions. According to several previous studies (Fässler et al. 2011, Payne et al. 2013) larval mortality can have a strong impact on fish stock dynamics. The lower availability of small potential prey in BB14 and coinciding differences in nutritional condition and stable isotope signatures compared to BB13 (and to a lesser part DO14), might be an indicator of a match-mismatch situation causing increased larval mortality in BB14 resulting in lower recruitment.

4.6 Conclusion

The biochemical analysis and prey abundance data from two different herring spawning grounds (autumn and winter) pointed out the relevance of temperature and availability of appropriate prey sizes in the early life history of larval fish. SIA ($\delta^{15}N$ and $\delta^{13}C$) of herring larvae and bulk plankton samples revealed no differences in trophic level between larvae from 8 to 14 mm within each spawning ground. Nevertheless, differences in larval growth and condition were observed in the autumn-spawned larvae suggesting that feeding on a higher trophic level is not directly linked to higher growth rates or better nutritional condition. The $\Delta^{15}N$ calculations led to the assumption that the larvae hatched in autumn are covering their nutritional needs mostly by prey of the MZP. Winter spawned larvae in the English Channel exhibited $\delta^{15}N$ values >1‰ higher than autumn-spawned larvae; higher $\delta^{15}N$ values being associated with southern North Sea and winter in general. This signal was, however, not reflected in the plankton samples, which might be explained by either higher lipid content or a stronger benthos-derived ^{13}C contribution. Higher temperatures, total Chl *a* and plankton abundance in autumn 2014 compared to 2013 did not promote a positive effect on growth and condition nor did it alter $\delta^{15}N$ or $\delta^{13}C$ signals. In the MZP in BB14 large thecate dinoflagellates, such as *Ceratium* spp., were the most abundant organisms. This composition might have been an inadequate food source to obtain optimal feeding in herring larvae, emphasizing the importance of thorough plankton sampling, monitoring and analysis. There were indications but no clear patterns in prey availability, trophic level or nutritional condition, which point to the resulting high (year class from BB13 and DO14) or low (year class from BB14) recruitment. Future studies should consider a thorough size-sorting of plankton samples instead of using bulk or only rough size-fractioned samples for SIA to study the impact of different plankton size classes/types on trophic level and growth

of the larvae with a higher resolution. In addition, combined approaches using SIA and gut content analysis based on molecular approaches are likely to have a high potential to provide valuable estimates on the contribution of small size classes to the diets of consumers e.g. larval fish.

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CHAPTER 5

5. General Discussion

In times of severe overfishing of many fish stocks and climate change associated factors altering ocean dynamics, understanding the processes driving and interacting in marine ecosystems, becomes a major task to unravel the factors influencing the recruitment of fish. There are strong indications that, in general, PZP and MZP contribute to a substantial part to ecosystem functioning and, more specific, can be crucial for larval fish survival. But the ecology and distribution patterns of these small zooplankton components remain widely unexplained and there is still a long way to go to fully uncover their links to higher trophic levels in the food-web. The NE Atlantic suits perfectly as a case study to explore microzooplankton and its interactions with larval fish. The strong seasonality in the NE Atlantic characterized by productive seasons during the spring, the less pronounced early autumn phytoplankton bloom and an autumn and winter period with low productivity, where the microbial loop is thought to be the main driver of the carbon cycle within lower trophic levels. The autumn and winter period coincides with the spawning and larval period of autumn spawning Atlantic herring (*Clupea harengus*), making this an ideal candidate to study the microzooplankton-ichthyoplankton link.

In order to shed some light in the microzooplankton community in the NE Atlantic during autumn and winter, for the first time, a broad-scale assessment of the winter PZP community in temperate waters was conducted as a first step towards a longer-term time series (Manuscript 1). In order to investigate the link between PZP/MZP and early life stages of Atlantic herring, a key player in the NE Atlantic ecosystem, field sampling in different spawning components was combined with laboratory and modeling approaches. In spawning grounds in the Irish Sea the abundance/biomass of PZP/MZP was directly linked to the abundance of larval herring and the impact of the small plankton components on larval growth and survival was investigated by implementing field and laboratory derived data into

an IBM (Manuscript 2). By comparing two spawning grounds and seasons in the North Sea this thesis aimed to reveal if larval condition and growth is impacted by the availability of suitable prey and if the actual contribution of PZP and MZP to larval nutrition could be assessed via stable isotope analysis (Manuscript 3). This thesis contributes to a deeper understanding of microzooplankton-larval fish interactions in general and, in specific, as potential factor influencing overwinter survival of larval herring and, hence, recruitment dynamics.

5.1 PZP and MZP community under low productivity conditions

After termination of the autumn bloom and during the winter months, life in the temperate NE Atlantic waters is thought to function on low level due to reduced primary production. Overall Chl *a*, phyto- and zooplankton abundance is relatively low (www.wgze.net), but there are still ongoing trophic processes in the water column. During this low productivity periods, the base of the food web and carbon cycling is driven by bacteria, pico-, nano- and microplanktonic organisms (Azam et al. 1983).

5.1.1 PZP and MZP community composition

The PZP and MZP community under non-bloom conditions is structured differently than in other, more productive seasons. The PZP community in late autumn and winter in temperate waters is mostly dominated by heterotrophic dinoflagellates and ciliates, whereas abundance/biomass of PZP is higher in autumn compared to winter (Levinsen & Nielsen 2002, Scherer 2012, Löder et al. 2012, Manuscript 1, 2 & 3), when there is no possibility to profit from structures provided by the terminated autumn bloom. There are contradictory reports on the dominant taxa in the PZP in the North Atlantic with some studies suggesting dinoflagellates while others suggest ciliates (Burkill et al. 1993, Montagnes et al. 2010, Fileman et al. 2011), making it challenging to relate a specific season to increased occurrence of one or the other. Most likely, the contribution of ciliates and dinoflagellates depends on specific local conditions and processes. The number of species found is generally higher during more productive seasons in the NE Atlantic, (see e.g. Seuthe et al. 2011, Löder et al. 2012). During autumn and winter the taxa contributing to the PZP community are the same as those occurring during other seasons (Edwards & Burkill 1995, Löder et al. 2012).

Athecate and aloricate organisms, such as *Strombidium* spp. or dinoflagellates from the order Gymnodiniales, were found to be most abundant (e.g. Figueiredo et al. 2005, Fileman et al. 2011, Scherer 2012, Manuscript 1, 2 & 3). Besides *Strombidium* spp. *Leegardiella* spp., *Lohmaniella oviformis* and *Balanion* spp. are abundant components of the ciliate community (Edwards & Burkill 1995) and were observed to occur in autumn and winter in the NE Atlantic (Seuthe et al. 2011, Löder et al. 2012, Yang et al. 2015, Manuscript 1, 2 & 3). Especially during productive seasons the mixotrophic *Mesodinium rubrum* is very abundant in the PZP and can form intense blooms due to its strong phototrophic capability (Montagnes et al. 2008, Fileman et al. 2011), but reaching minimum abundances or being completely absent in autumn and winter (Leakey et al. 1993, Löder et al. 2012, Yang et al. 2015, Manuscript 1, 2 & 3). In contrast to other studies conducted in the NE Atlantic during more productive seasons (e.g. Burkill et al. 1993, Edwards & Burkill 1995, Yang et al. 2015), the diversity of frequently occurring Tintinnida in autumn and winter is low (Graziano 1989, Seuthe et al. 2011, Manuscript 1, 2 & 3) and often dominated by *Stenosemella* spp. or *Tintinnopsis* spp. (Edwards & Burkill 1995, Yang et al. 2014, Manuscript 1 & 2). Long-term annual data derived from the CPR survey suggest that tintinnids were rare in the English channel and Strait of Dover and more pronounced in the central North Sea and the NE Atlantic (Hinder et al. 2012). During the winter, the opposite situation was observed for the North Sea assemblage (Manuscript 1). It should be noted that, when making these comparisons, the dominant taxa found in the winter assemblage, *Stenosemella* spp., is too fragile to be sampled with the CPR (see 5.2.2) (Hinder et al. 2012) and thus, the occurrence of *Stenosemella* spp. in the English Channel might not be a specific winter phenomenon. Nevertheless, this genus seems to be of minor importance during late spring in the North Sea (Cordeiro et al. 1997) or strongly related to coastal areas (Fileman et al. 2011). Even though the abundance may be low, tintinnids can contribute to a significant amount to winter PZP biomass thanks to their large size compared to the dominating small celled PZP fraction (Manuscript 1 & 2).

Examples of common dinoflagellate taxa in the PZP community during low productivity conditions, apart from the dominating Gymnodiniales, are *Torodinium robustum*, *Protoperidinium* spp., *Cochlodinium* spp. and *Pronoctiluca pelagica* (Edwards & Burkill 1995, Löder et al. 2012, Manuscript 1, 2 & 3). The phagotrophic dinoflagellate *Polykrikos* spp.

reached maximum abundance in autumn in the North Sea, whereas it was absent in winter (Löder et al. 2012, Manuscript 1 & 3). Other taxa, such as *Pronoctiluca pelagica* could occur in high densities in certain areas in autumn (Manuscript 3), while being completely absent in surrounding waters or in other NE Atlantic waters (Scherer 2012, Löder et al. 2012, Manuscript 2 & 3). From CPR data it was derived that in the North Sea *Ceratium* species are the dominant (phyto)plankton component on yearly average, which could be observed at some stations in terms of biomass/abundance in this thesis in autumn (Manuscript 2 & 3). One has to keep in mind that the CPR neglects organisms under a certain size and does not account for athecate and aloricate species. Looking only at conditions with low primary production, *Ceratium* spp. tend to be at low levels (Bresnan et al. 2009, Fileman et al. 2011) and seems to play a subsidiary role.

In general there is a homogenous PZP community composition observed in mixed waters (Edwards & Burkill 1995, Montagnes et al. 2010, Manuscript 1). Differences in (ciliate) community composition can mostly be observed on smaller (meter) than on broader (km) spatial scales (Montagnes et al. 1999) and in between hydrographic discontinuities, such as mixed versus stratified waters (Edwards & Burkill 1995).

Regarding MZP, increased abundance of early stages of invertebrate larvae was mostly associated with the early autumn season (www.wgze.net Station L4; Manuscript 3) in comparison to the late autumn (Manuscript 2) or winter (Denis et al. 2016, Manuscript 1 & 2). The abundance of copepod nauplii was observed to be highly variable in NE Atlantic waters in autumn (Manuscript 2 & 3), decreasing in winter (Hay et al. 1991, Denis et al. 2016) and increasing in late January (Hay et al. 1991). In contrast to PZP, a higher biomass of copepods and their early life stages was observed in the northern North Sea during a broad-scale study covering late autumn and winter seasons in the North Sea, making grazing pressure of mesozooplankton on PZP a possible explanation for the patterns of change observed in the winter PZP community (Manuscript 1). According to Hay et al. (1991) overall biomass of nauplii reaches a minimum in January and increases again towards March with varying species contributions across the area. The copepod overwintering strategies are generally not completely resolved in the NE Atlantic and seem to be species- and area-specific (Wesche et al. 2007).

5.1.2 PZP and MZP as indicators

Observations on the size structure of PZP are very valuable as they can quickly respond to changing conditions due to their high growth rates compared to e.g. mesozooplankton organisms (Calbet 2008). This way they can provide insight into ongoing ecosystem trophic dynamics. While in freshwater or sewage water treatment, the ciliate community is commonly used as ecological indicator (Berger & Foissner 2004, Serrano 2008), in marine environments this is still a widely unacquainted field. There is ongoing research how to use community composition, size structure of ciliates or abundance of specific PZP species as indicator for environmental pollution (Jiang et al. 2012, Wu et al. 2016) or climate change effects (e.g. McLeod et al. 2012, Jiang et al. 2013). In North Atlantic waters a northward shift of some tintinnid species since the mid 1990s was observed (Hinder et al. 2012), which might be associated with overall warming SST. Hinder et al. (2012) provide the only long-term data on a broad scale on a PZP component (Tintinnida), admitting that the sampling method (CPR) does not capture all tintinnid species efficiently. In general only snapshots of the PZP community in the NE Atlantic exist so far, covering the ciliate as well as the dinoflagellate component. They are restricted either in time (single surveys on a broader scale) (Edwards & Burkill 1995, Montagnes et al. 2010) or in space (longer term monitoring on a fixed station), Plymouth L4 (Harris 2010) and Helgoland Roads (Löder et al. 2012). Monitoring programs or studies in the NE Atlantic focus on phyto- and mesozooplankton (www.wgze.net, CPR program), ignoring not only the PZP but the MZP in the NE Atlantic as well (Hay et al. 1991). The structure of the PZP community provides some valuable aspects which have the potential to serve as ecological indicators.

During non-bloom conditions small cells (<50 µm) dominate the phyto- and protozooplankton (Montagnes et al. 2010, Fileman et al. 2011, Scherer 2012, Manuscript 1, 2 & 3) while larger cells dominate during bloom conditions, such as, increased abundance of large diatoms (Aberle et al. 2007, Strom et al. 2007, Fileman et al. 2011). Since small PZP (especially ciliates) are known to prey on bacteria, nanoflagellates and nanophytoplankton (Sherr & Sherr 2002), their dominance is a strong indication of ongoing microbial loop dynamics as main drivers of the carbon cycling and energy transfer to higher trophic levels (Legendre & Rassoulzadegan 1995, Levinsen & Nielsen 2002). Information about the drivers in carbon cycling in a given system can be gained by investigating the feeding strategies of

the PZP and MZP community. For example, the presence of some small copepod species (and their early life stages) could suggest a microbial-loop driven system, as these copepods feed predominantly on heterotrophic protists, rather than on phytoplankton (Turner 2004 and references therein). Also, the absence of specific microzooplankton grazers can directly be linked to disappearance of nanophytoplankton cells (Löder et al. 2011), indicating a shift in the sources of primary production.

Ciliates and dinoflagellates differ in some key functions concerning feeding habits and growth patterns. Pallium- or peduncle-feeding dinoflagellates are not limited by prey size and can prey on a wide variety of organisms (Jeong et al. 2010) in contrast to ciliates, which tend to be constrained to consuming cells no larger than themselves (Hansen 1992) and are, therefore, more restricted to small sized prey. On one hand ciliates can achieve higher growth rates than dinoflagellates (Strom & Morello 1998) and thus react more rapidly to changes in environmental conditions. On the other hand, dinoflagellates prey on a higher diversity of prey and are capable of withstanding longer periods of starvation (Hansen 1992, Sherr & Sherr 2007). This might be a reason for the (mostly) dinoflagellate dominated community composition observed in all field surveys presented in this thesis as well as in other areas under conditions of low prey abundance (Fileman et al. 2011, Löder et al. 2012).

5.2 Challenges in studying PZP and MZP

Knowing about the importance of these small zooplankton size fractions as grazers of bacteria and primary producers and prey for higher trophic levels (Calbet 2008), there is still a lack of long-term monitoring programs, which is urgently needed as abundance and composition can vary from year to year (Manuscript 3). The reasons behind this scarce research on the PZP and MZP component in the field is probably due to problems related to sampling, preservation and categorizing the trophic mode of the organisms.

5.2.1 Sampling

As PZP is often not evenly distributed, but rather occurs in patches, field sampling and abundance estimations are difficult to determine (see Montagnes et al. 1999, Quevedo 2003). Additionally, the sampling methods (i.e. plankton nets) used for mesozooplankton are

not adequate for MZP and fragile PZP organisms. First, net induced turbulence and pressure can cause destruction of the PZP cells (Sherr & Sherr 1993, Gifford & Caron 2000). Second, the mesh size of plankton nets usually used for plankton investigations is too coarse to retain MZP organisms (<200 µm). For example, the CPR, providing data for many time series analysis (e.g. Edwards & Richardson 2004, Beaugrand 2005, Hinder et al. 2012, Beaugrand et al. 2015) uses a 280 µm mesh, missing the entire MZP fraction.

There is a growing perception that, to effectively manage marine areas, marine ecosystems have to be understood and monitored as a whole, from primary producers to top-predators, and not merely with monitoring of specific, "managed" species/components. This is the so-called ecosystem approach to management. An ecosystem-based approach offers new opportunities for studying PZP and MZP. In this thesis, I provide an example of how routine annual international surveys for fish stock assessment can be augmented to add PZP and MZP sampling (Manuscript 1). Such an approach opens the chance to (i) study and monitor PZP and MZP on a broad spatial and temporal scale and with this data (ii) reveal, if changes in PZP and MZP abundance or composition are indicators for changes in the upper trophic levels (plankti- and herbivorous fish) and (iii) use this data to implement them in different kind of models (e.g. food-web or biogeochemical models).

5.2.2 Preservation

Zooplankton samples are generally preserved in buffered formalin (4%) or ethanol due to their long-term storage capacity (Postel et al. 2000). Such preservation allows epifluorescence measurements (as primary production estimate) on the formalin-fixed sample (Stoecker et al. 1994), or genetic/molecular analysis on the ethanol-preserved sample (Bucklin 2000). However, such preservation causes lysis of many of the athecate and aloricate PZP cells. Stoecker et al. (1994) calculated that more than 50% of the cells were lost compared to preservation in Lugol's solution. In contrast to formalin, Lugol's solution is iodine based and not hazardous and provides the traditional fixative for PZP, because it causes little cell loss (Strüder-Kypke et al. 2006). But the Lugol's solution also has several downsides: (i) storage capacity is limited to maximum one year (Karlson et al. 2010), (ii) it causes cell shrinkage (Menden-Deuer & Lessard 2000), and (iii) the strong coloration of the sample does not allow identification to species level in many cases nor the detection of

trophic mode as it conceales fluorescence (Gifford & Caron 2000). Alternative fixatives, such as Glutaraldeyde or Bouin's solution also provide some advantages and disadvantages. Both of them are very toxic and also cause cell shrinkage. Glutaraldeyde is able to preserve autofluorescence of the cells, but has a short shelf life (Gifford & Caron 2000). Considering all the above, there is unfortunately no universal method that can be used for PZP/MZP that allows high resolution taxonomical identification, carbon content measurements and preservation of cell fluorescence.

5.2.3 Mixotrophy of PZP

The impact of the used fixation method on the organisms is of importance as it provides a possibility to distinguish cells performing photosynthesis from phagotrophic ones. In the PZP this is a major challenge since most of the species in the PZP should be identified as mixotrophs, contributing to both primary and secondary production, making the classical distinction in "phytoplankton" and "zooplankton" difficult or even obsolet (Flynn et al. 2013). Among dinoflagellates mixotrophy is a widespread feeding mode, but the degree they depend on either photosynthesis or heterotrophic feeding mode varies among species and seasons. In any case, it is common that these organisms ingest a variety of different phytoplankton species (Jeong et al. 2010) and can graze down a substantial proportion (up to 20%) of specific phytoplankton cells (Du Yoo et al. 2009). Among ciliates mixotrophy is often being enabled by kleptoplasty, a phenomenon by which a ciliate predator retains the plastids from its autotrophic prey and is then capable of photosynthesis. As many PZP species are fragile and problematic in culturing, laboratory experiments on feeding mode are mostly restricted to culturable species (Löder et al. 2011), which neglects the wide variety in feeding habits or adaptations to changes in environmental conditions.

Mixotrophic protists are especially gaining importance in the seasonal cycle of food-web dynamics during non-bloom conditions, when there are no favorable conditions whether for strictly autotrophs (high nutrients) or strictly heterotrophs (high phytoplankton biomass) (Mitra et al. 2014). In this thesis, many identified taxa are potentially mixotrophs, such as some Gymnodiniales, *Dinophysis* sp., species of *Ceratium* spp., *Mesodinium rubrum* and *Laboea strobila*. Unfortunately, the fixation method used (Lugol's solution) does not allow to identify their current feeding mode (see 5.2.2). The uncertainties in trophic position make it

difficult to assess the bulk PZP and MZP role in carbon cycling. This can result in wrong assumptions of the productivity of a system and misleading outcomes of e.g. nutrient-phytoplankton-zooplankton-bacteria (NPZB) food web models (Mitra et al. 2014).

In situ tools for fluorescence measurements, such as the FluoroProbe, cannot yet distinguish between fluorescence derived from phytoplankton or PZP. In experimental studies in general, prey labelling can detect and quantify potential mixotrophy (Smalley et al. 2003) and in mesocosm studies in specific (Aberle et al. 2007, Calbet et al. 2014) processes in the plankton can be investigated and the conditions favoring for instance mixotrophy can be studied under semi-controlled conditions. In field and laboratory studies, another possible way of accounting for the rate of mixotrophy could be the analysis of life samples of PZP with an image particle analyzer, such as the FlowCam, which is able to count and identify particles and additionally use laser detection of fluorescence.

5.3 Impact of PZP and MZP on larvae of autumn spawning herring

If the plankton community does not provide the appropriate prey types or if the nutritional value of the available prey is low, it can severely impact the larvae. It has been shown that nutrient limitations of primary producers can be transferred to higher trophic levels and result in reduced nutritional condition of the larvae (Malzahn et al. 2007). During bloom conditions the nutrient supply for the phytoplankton is high and thus its nutritional value, which is lowered towards the end of the bloom at nutrient depleted conditions (Boersma et al. 2008). This is most likely the case during the entire phase of low primary production from the termination of the autumn bloom until onset of the spring bloom. Comparing biochemically derived nutrition factors from larvae of two subsequent spawning seasons and spawning sites (Denis et al. 2017, Manuscript 3) there are indications that potential mismatch dynamics and elevated temperatures during the larval phase have influenced larval survival.

5.3.1 Impact of PZP and MZP abundance on larval herring

Autumn-spawned herring larvae face challenging conditions compared to larvae spawned in spring: (i) prey availability is lower (www.wgze.net), (ii) larval stage duration is longer due to

the reduced growth rates associated to low temperatures (Gamble et al. 1985, Peck et al. 2012), and (iii) vulnerability to predation is higher due to the longer larval periods, although predator activity is reduced during wintertime (Pepin 1991). These larvae will then overwinter in the larval stage and take advantage of the spring bloom to complete metamorphosis (Bainbridge et al. 1974). Albeit metabolic activity is reduced due to colder winter temperatures, the larvae are hinged on sufficient food supply to sustain overwinter survival until the onset of the spring bloom. An increasing number of laboratory (Figueiredo et al. 2007, Friedenberg et al. 2012) and field studies (Figueiredo et al. 2005, Denis et al. 2016) indicate that fish larvae may rely on MZP or PZP prey in times of scarce abundance of larger prey.

Payne et al. (2009) suggested that the recruitment depression of NSAS herring might be attributed to the change in plankton community (regime shifts) observed in the North Sea in the late 1980s and 2000s. In recent years the highest larval abundance is provided by the Downs spawning component in the southern North Sea (Schmidt et al. 2009), the reasons for this remain unknown. Lower mean annual ciliate abundance and higher dinoflagellate abundance compared to long term trends on station L4 (Plymouth, www.wgze.net) in the English Channel might have effected conditions in a favorable direction for survival of larval herring. Since these are the only long-term data on PZP in the NE Atlantic, derived from a single coastal station there are various assumption in either direction possible. Comparing winter PZP across the North Sea, high PZP biomass was observed on the major winter spawning grounds of herring (Manuscript 1), compared to other, more northerly located sampling stations. However, the PZP biomass reported on those autumn stations represented only part of the estimated carbon requirements of larval herring (Munk & Kiørboe 1985, Figueiredo et al. 2005, Peck et al. 2012), suggesting that PZP might not be sufficient to act as main prey source, but that it is available in amounts that can support larval survival. One needs to keep in mind that direct correlations of potential available prey and larval herring do not fully consider distinct ocean dynamics. Plankton is not homogenously distributed and thus snapshot PZP sampling might misinterpret prey abundance by over- or underestimating it. Furthermore, PZP can react very fast to changing condition so that local patches are more likely to occur than mesozooplankton patches (Montagnes et al. 2010). As the larvae are also known to migrate vertically in the water

column (Haslob et al. 2009), plankton sampling on a distinct depth does not directly reflect the prey field actual experienced by the larvae.

Considering the low field data availability of MZP and PZP the results gained so far can serve as first steps to disentangle potential predator-prey interactions and we have to keep in mind that not only prey abundance can impact on larval abundance, but also vice versa by top-down processes. Predation pressure of larval fish on the zooplankton community is thought to have a minor impact during highly productive seasons in temperate waters (Nielsen & Munk 1998, Pepin & Penney 2000), but for autumn or winter, when zooplankton production is at low level, this is not studied so far. The negative impact of the abundance of small copepods on larval herring abundance revealed by a generalized linear mixed model (GLMM) approach (Manuscript 2) could either point to copepod depletion by the herring or a competition of copepods and small larvae for the same prey items. But the modeled relationships are not necessarily directly impacting the larvae. The positive relationship between dinoflagellate and larval herring abundance (Manuscript 2) could be possibly explained by the larvae having depleted the dinoflagellate grazer and thus providing improved growth conditions for them. These and other possible small scale interactions in the plankton will not be captured by short term investigations and model efforts on abundances in the field.

Recent modeling approaches are attempting to reveal potential interactions between different mesozooplankton groups, also including abiotic factors and/or various individual larval characteristics. Using longer-term CPR data in the Gulf of Alaska, Batten et al. (2016) showed a strong correlation of the abundance of small mesozooplankton on growth patterns of larval Pacific herring (*Clupea pallasii*). Alvarez-Fernandez et al. (2015) used a generalized additive mixed model (GAMM) to support the hypothesis that the survival of Atlantic herring larvae in the English Channel is rather depending on the availability of specific mesozooplankton prey types than on temperature. In this same line, an Individual based model (IBM) developed for larval herring (Hufnagl & Peck 2011, Hufnagl et al. 2015) suggests that prey availability seems to be an important prerequisite for larval overwinter survival (Hufnagl et al. 2015). When adding field data of PZP and MZP to that model, which was restricted to prey >200 µm hitherto, larvae could not survive on the available PZP biomass

alone. But excluding this prey size fraction and only leaving metazoan prey <300 µm as potential prey, the growth rate of all pre-metamorphose larvae in the model decreased dramatically (Manuscript 2). These model approaches take into account the total abundance or biomass of potential prey types, but do neglect prey preferences or nutritional value.

5.3.2 Indirect methods of studying PZP/MZP – herring larvae link

Despite being one of the best studied species worldwide, we still have limited knowledge on the feeding preferences of herring larvae. The role of copepods in larval diets is well known (Munk & Kiørboe 1985), but the importance of other organisms has rarely been quantified. An experimental study showed that herring larvae prefer prey >29 µm (Spittler et al. 1990), which would exclude a major proportion of the PZP community during autumn and winter (Manuscript 1, 2 & 3). Controversially, a field study on Downs herring larvae revealed that the larvae positively selected prey items from the small protist community, while refusing other abundant planktonic prey (Denis et al. 2016). This thesis explored the dominance of PZP, MZP or mesozooplankton component in larval diets using SIA (Manuscript 3), but the results were not very definite. Biases related to sampling season or sampling site distort the comparability of samples and the variety of trophic modes from autotrophy up to higher consumer level present in bulk plankton samples do not allow to precisely define the nutritional source of the larval diet. Nevertheless, the overall trophic fractionation of $\delta^{15}N$ found in autumn spawned larvae in the North Sea indicates that MZP might predominantly fulfill their nutritional needs during that season.

The RNA/DNA and the growth rate (G_i) derived from it has been shown to be a good proxy for the nutritional condition of a larva (e.g. Clemmesen 1994, Buckley et al. 2008, Meyer et al. 2012). The comparison of G_i of larvae from different spawning grounds and seasons indicated more favorable conditions for larvae spawned in autumn 2013 compared to 2014 or winter conditions (Manuscript 3). Whether the higher PZP abundance during that time was directly, indirectly or not at all contributing to the larval condition still needs to be investigated.

Denis et al. (2017) found a strict shift in diet composition at a size of ca. 13-14 mm in the English Channel. With bulk SIA analysis this ontogenetic shift could not be observed

(Manuscript 3), but decreasing C:N with larval size relate to an increased selection of high quality prey with larval growth (Ehrlich 1974, Manuscript 3). Paulsen et al. (2014) observed that the nutritional condition and G_i of herring larvae in the Baltic Sea depended on the planktonic composition, thus only taking mesozooplankton into account. But it would be misleading to conclude that a high abundance of potential prey is associated with favorable conditions for the larvae, resulting in high growth rates (Manuscript 3). The actual prey choice a larva is making and the longer-term diet can not be revealed by a single method approach. For example, gut content analyses only provide snapshots and are restricted by larval anatomy and digestive capacity. On the other hand, SIA of bulk plankton samples give only indications about the larval diets as they are facing various caveats in marine systems: (i) $\delta^{15}N$ in animals for instance can vary naturally depending on season or geographic location (Jansen et al. 2012, Kürten et al. 2013), (ii) trophic steps in lower trophic food webs do function different than in higher trophic levels and are to a great part invisible using SIA (Aberle & Malzahn 2007, Gutiérrez-Rodríguez et al. 2014).

Table 5.1: List of advantages and disadvantages of direct and indirect measurement methods in trophic ecology studies of larval fish

Method	Advantage	Disadvantage	References
Gut content	direct, cheap, qualitatively	Short-term dietary composition, time-consuming	Figueiredo et al. (2005), Denis et al. (2016)
Stable Isotope Analysis (SIA)	longer term dietary composition, indication of trophic position, possibly qualitatively	bulk tissue samples, variable baseline, unprecise microbial detections, not quantitatively	Boecklen et al. (2011), Gutiérrez-Rodríguez et al. (2014)
Amino Acid Compound Specific Isotope Analysis (AA-CSIA)	strong, more precise fractionation than SIA	lower reproducability than SIA, vulnerable to measurement errors, semi-quantitatively	Boecklen et al. (2011)
Fatty Acid Analysis (FA)	good indicator for food quality	limited knowledge about lipid processing in different organisms, not quantitatively	Dalsgaard et al. (2003)
DNA barcoding, Next Generation Sequencing (NGS)	detection of rare prey species in digestive tract, potentially quantitatively	poor species resolution of PZP, bias of secondary prey items	Riemann et al. (2010)

The use of other or additional trophic markers has been shown to be useful in food-web studies (Table 5.1). First, amino acid compound specific stable isotope analysis (AA-CSIA) provide a relatively novel approach to measure the stable isotopes within single amino acids, some of which show a much higher trophic fractionation in $\delta^{15}N$ and $\delta^{13}C$ than bulk SIA. They are related to the consistent fractionation of the stable isotope in specific amino acids with each trophic step rather than being related to a (often variable) baseline. AA-CSIA provides a wider scope and more precise estimates for unrevealing trophic interactions (McClelland & Montoya 2002, Hannides et al. 2009), but requires a higher amount of tissue per sample hitherto and also bears some measurement caveats (Boecklen et al. 2011). Second, the nutritional quality of the planktonic prey can be detected by measuring the content of certain essential fatty acids (Dalsgaard et al. 2003). Fatty acid trophic markers have been shown to give a proxy of the food quality of the potential food source and consequently of the nutritional condition of the predator (larval fish) (Rossi 2006, Paulsen et al. 2014). The

fatty acid profile of the prey is reflected in the larva's profile and can potentially trace down the main prey source (Fraser et al. 1989, Dalsgaard et al. 2003), which was shown to influence nutritional condition in larval herring in the Baltic Sea (Paulsen et al. 2014). And finally, molecular methods can provide valuable and more detailed information about the recent food intake of an animal. DNA metabarcoding methods, such as next generation sequencing (NGS), enables to reveal the taxonomic composition in a given sample. As a prerequisite the approximately expectable species in the sample and the adequate barcodes for the respective species have to be known (Pompanon et al. 2012). Barcoding of the protist community in the marine realm is starting (Caron et al. 2016), some studies focusing on the community composition (Stern et al. 2010), some on the similarities in distribution patterns (Gimmler et al. 2016). In the guts of eel larvae (*Anguilla anguilla*) the main ingested prey types could be identified using existing DNA barcodes of plankton (Riemann et al. 2010). Considering the advantages and limits a combination of these techniques in laboratory experiments as well as in field studies can contribute to further and more precisely investigate the role of MZP and PZP (and/or specific components within these groups) for larvae of Atlantic herring and other fish species.

5.4 Future scenarios for PZP/MZP – ichthyoplankton link

There is no doubt anymore that e.g. rising temperatures, ocean acidification, or other human induced factors are influencing marine ecosystems (e.g. Guinotte & Fabry 2008, Pörtner et al. 2014). With ongoing changing climate it is important to know how organisms itself react and what impact it has on ecosystem level. Changes in the base of the food web, such as altered community structure in phytoplankton (Dutkiewicz et al. 2015) or size structure and biomass of copepod communities (Taucher et al. 2017) under increasing ocean acidification will consequently affect ecosystem structures. Recently regime shifts during the 20th century in different parts of the world's oceans have been found to be associated with temperature (Beaugrand et al. 2015). The hypothesis that changes in phytoplankton dynamics can effect recruitment of some fish species has been shown true using long-term data (Beaugrand et al. 2003, Platt et al. 2003). Taking the formally discussed potential importance of MZP and PZP for larval fish into consideration, it can be assumed that changes in the PZP and MZP community will possibly affect early life stages of fish as it was for

instance investigated for specific copepod species and the abundance of larval herring in the English Channel (Alvarez-Fernandez et al. 2015).

Rising temperatures and ocean acidification, as examples for consequences of global change, impact on the physiology and development of fish larvae. Elevated temperatures are thought to be more critical for fish with a narrow thermal window (Llopiz et al. 2014). However, even Sardine larvae (*Sardina pilchardus)* exhibited an increased mortality rate and reduced feeding success when exposed to elevated temperature (Faleiro et al. 2016) and the fact that rising temperatures are associated with higher metabolic costs could become critical in seasons with low general prey abundance, such as for autumn spawned herring larvae. Additionally, larval development is enhanced with increasing temperatures (Pepin 1991), which may result in a match-mismatch situation for Atlantic herring due to early metamorphosis prior to the onset of the spring bloom.

Another widely discussed man made problem in marine habitats is ocean acidification. While some studies reveal drastic negative impacts on larval fish with an increase in pCO_2 in the oceans, concerning behaviour, physiology, growth or survival (Munday et al. 2009, Frommel et al. 2014, 2016, DePasquale et al. 2015) others conclude no distinct direct effects of ocean acidification on the larvae of the investigated species (Munday et al. 2011, Frommel et al. 2013), making species specific responses to this environmental stressor very likely.

Besides direct effects of climate change associated factors on the larvae itself, indirect effects via impacts on prey fields (such as the availability and suitability of PZP and MZP) may also be important. Studies on impacts of climate change associated factors on microbial loop organisms remain scarce. Temperature increases predicted for end of this century showed reduced nauplii development in a laboratory study (Vehmaa et al. 2013). The ciliate community exhibited faster species turnover rates with higher temperatures in a simulated spring bloom, which can result in temporal mismatch between specific ciliate species and their consumers (Aberle et al. 2012). The species-specific response to increasing temperatures among different plankton components may lead to important changes in trophodynamic structure and function at upper trophic levels by, for example, leading to strong changes in match-mismatch dynamics, e.g. of nauplii and phytoplankton bloom dynamics (Sommer et al. 2006). In the consequence this will also affect larval fish, which

depend on a temporal as well as spatial match of sufficient prey for survival. Reduced pH is known to cause shell lysis of calcifying organisms, such as some foraminifer species (Guinotte & Fabry 2008). For the other PZP components the picture is not that clear. While some studies (Rose et al. 2009, Aberle et al. 2013) did report a high tolerance against elevated pCO_2 other studies did describe negative impacts on ciliates (Calbet et al. 2014) or species specific responses to changing pH (Pedersen & Hansen 2003). Larval stages of bivalves and molluscs can suffer from shell malformations and increased mortality rates (Kurihara 2008). Copepods and their early life stages are thought to be impacted more of rising temperatures than of ocean acidification itself (Garzke et al. 2016).

The predicted increase in stratification can also alter food-web dynamics. In their review Caron and Hutchins (2013) propose the hypothesis that the growth of heterotrophic bacteria could be favored due to the increase in DOM released by the phytoplankton, resulting in higher abundance of small bacterivorous PZP. Also the phytoplankton community is thought to be shifted to smaller sized cells under future climate scenarios (Rodríguez et al. 2001, Caron & Hutchins 2013), which could enhance microbial loop dynamics and reduce trophic transfer efficiency due to multiplied trophic steps in the food chain. This lowered energy transfer is consequently affecting nutrition of larval fish.

To make more precise predictions of impacts of climate change scenarios on the community level and on potential effects of higher trophic levels, e.g. larval fish, there is still too little information on organism level. We still have for instance very limited knowledge about prey preferences of PZP and how a potential lowered food quality or starvation could affect them (John & Davidson 2001, D'souza et al. 2017). However, as a shift towards more microbial loop dominated systems is predicted (Caron & Hutchins 2013), it is crucial to further resolve the link between microbial loop associated organisms and higher trophic levels.

5.5 Outlook

Besides natural human curiosity, the ongoing and future changes in the marine habitats due to anthropogenic threats like overfishing, environmental pollution and climate change, provide good reason to gain further and deeper knowledge on the functioning of ecosystems. We have a certain understanding about the processes involved in the carbon

cycling and energy transfer in the food web. But the conditions during periods of low primary production remain poorly studied, making it difficult to resolve trophic interactions or understand processes in the ecosystem, such as larval overwinter survival and potential impacts on recruitment dynamics.

It is evident, that these tasks cannot be solved with a single scientific approach. In future studies it is important to collaborate between various scientific fields, involving microbiologist, larval fish biologists, modelers and fishery scientists, performing experimental, field and theoretical studies.

Experimental studies under controlled laboratory conditions are needed on the single PZP and MZP species to discover behavior and metabolic reactions to different environmental stressors, which can possibly be transferred to other species and make implications for consequences of other trophic levels. At the same time multi species experiments from microbial loop to higher trophic levels have to be conducted, such as mesocosms as simulated miniature ecosystems, to see how community dynamics and species interactions (e.g. PZP - larval fish) develop in semi-natural and also manipulated environmental conditions. The emphasis needs to be on the so far neglected low productivity conditions as well to see how e.g. nutritional quality of potential prey organisms develops.

Results, experiences and open questions from laboratory studies can be applied in field conducted studies to gain more information about PZP and MZP community and its role in the ecosystem. Including PZP and MZP in routine monitoring programs is essential as they are important players in carbon cycling and should not be ignored in the ecosystem approach.

In this thesis the successful implementation of PZP sampling on a well established survey has led to an ongoing, multi-national sampling collaboration, which, at the writing of this thesis has continued for 3 years beyond the data presented here. The goal of the collaboration is to establish a longer-term winter time series for PZP in the North Sea. Monitoring changes in PZP abundance and composition can act as one additional component in investigating winter conditions with respect to general microbial loop dynamics as well as PZP as potential food source for higher trophic levels, e.g. larval fish.

To further track down the role of PZP and MZP in larval fish nutrition, combined analysis on prey types, selectivity and ingestion rates have to be performed keeping in mind that each single method suffers from differing caveats.

This thesis revealed several problems of studying the PZP/MZP-ichthyoplankton link in the field. Raising solid conclusions about trophic links is certainly difficult as scientific field cruises generally just provide a snapshot of the current situation. In this sense, the combined use of measurements of biochemical condition (RNA/DNA) and SIA helped to identify some potential relationships of larval herring, PZP and MZP. For example, independent of spawning ground and season herring larvae tended to prefer microzooplankton prey of a size class >50 µm over smaller items. Rather the composition of PZP and MZP than the overall abundance seemed to be a pivotal factor for nutritional condition, growth and presumable survival. But for future studies it seems that the combination of direct methods, such as gut content analysis, and indirect biochemical and molecular techniques (RNA/DNA, SIA, CSIA, FA and NGS) will be a more fruitful path to investigate larval fish prey preferences as well as long- and short term diets under controlled laboratory and in field studies.

Implementing field data on PZP and MZP into statistical and individual-based models are a valuable addition to the techniques described above. This thesis shows that using field-derived larval data (abundances and growth rates) and prey abundance/biomass in model approaches resulted in new knowledge on the factors determining larval distribution patterns. This highlights once more, the need for additional measurements of the PZP and MZP community in order to advance the predictive capacity of mechanistic models of larval survival. The implementation of these data into an IBM can help one represent natural conditions experienced by larvae and indicated the importance of PZP and MZP for larval growth and survival. This work also highlights that almost all models of larval feeding and growth are potentially missing key elements of the prey field.

These results underline the urgent need of generating more and thorough data on long- and short-term patterns in PZP and MZP abundance and the role of these potential prey items in general and of specific groups. Dynamics of PZP as a key component of the carbon cycle are needed to model how energy is transferred and recycled in marine systems. The number of steps involved in transferring energy to higher trophic levels informs about the amount of

carbon/energy available for e.g. fish and their offspring. Data on the small plankton components is not only urgently needed to improve ecosystem models and IBMs of larval fish, but also to advance the ecosystem-based management in fisheries. If plankton sampling becomes part of routine surveys for fisheries management, the data will be valuable as an indicator of ecosystem status and, if changes in the PZP can be related to early life stage survival, these data may be used as an indicator of recruitment success. For the latter, building a longer time series of the abundance, distribution and community composition of small plankton components, PZP and MZP, during winter is a pre-requisite.

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Outline of publications

The chapters of this thesis are written as manuscripts and are either published, submitted or prepared for submission in a peer-reviewed scientific journal. In the following the scientific contributions of each author are described in detail.

Manuscript 1

Winter protozooplankton community in the North Sea examined using a routine fisheries survey

Franziska Bils, Marta Moyano, Nicole Aberle, Cindy van Damme, Richard D.M. Nash, Matthias Kloppmann, Christophe Loots & Myron A. Peck

The study was designed by Franziska Bils (FB), Marta Moyano (MM) and Myron A. Peck (MAP). Sampling was conducted by Cindy van Damme, Richard D.M. Nash, Matthias Kloppmann and Christophe Loots. Sample and data analysis was performed by FB under close cooperation with Nicole Aberle (NA), MM and MAP. The manuscript was written by FB under supervision of MM, NA and MP. All authors contributed to reviewing and editing the manuscript.

The manuscript has been re-submitted to *PLOS ONE,* a peer-reviewed journal.

Manuscript 2

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

Franziska Bils, Marta Moyano, Nicole Aberle, Marc Hufnagl, Santiago Alvarez-Fernandez & Myron A. Peck

The study was designed by FB, MM and MAP. Sample and data analysis was conducted by FB under supervision of NA, MM and MAP. Statistical modelling was performed by Santiago Alvarez-Fernandez and Marc Hufnagl (MH). All figures, except Figure 3.7 (produced by MH), displaying the results of the IBM, were produced by FB. The manuscript was written by FB under supervision of MM and MAP. MH wrote the section about the IBM. All authors contributed to reviewing and editing the final version of the manuscript.

The manuscript is published in *Journal of Plankton Research* (2017), 39:1, 147–163, https://doi.org/10.1093/plankt/fbw074

Manuscript 3

The impact of microzooplankton on the nutritional condition and growth of marine fish larvae using stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$)

Franziska Bils, Marta Moyano, Nicole Aberle & Myron A. Peck

The study was designed by FB and MM. Sample and data analysis was performed by FB under close cooperation with MM and NA. The first draft of the manuscript was written by FB under close cooperation with MM, NA and MAP. All authors contributed to editing and reviewing the manuscript.

The manuscript is being prepared for submission to a peer-reviewed scientific journal.

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Declaration on oath

I hereby declare, on oath, that I have written the present dissertation

„The microzooplankton community during winter in NE Atlantic waters and its potential impact on condition and growth of larval Atlantic herring (*Clupea harengus*)“

by my own and have not used other than the acknowledged resources and aids.

Hamburg, 01.11.2017

Franziska Bils

Certification of written English quality

I hereby confirm that this dissertation by Franziska Bils entitled „The microzooplankton community during winter in NE Atlantic waters and its potential impact on condition and growth of larval Atlantic herring (*Clupea harengus*)“ has been prepared according to excellent written English language standards.

Hamburg, 01.11.2017

Prof. PhD Myron A. Peck