Broad-scale distribution of the winter protozooplankton community in the North Sea

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Abstract :

Protozooplankton (PZP) (here size range: 12-200 mu m) are rarely sampled over a broad scale, especially 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 sampled PZP from Dutch, French, German and Norwegian research vessels taking part in the annual ICES coordinated International Bottom Trawl Survey (IBIS) which provides data on fish stock abundances and status for the entire North Sea. The abundance, biomass, composition and distribution of PZP were examined at 39 stations across the North Sea (from 3.2 degrees W to 7.6 degrees E and 50.5 to 59.8 degrees 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 dinofiagellates (68%) and Strornbidium spp. and other naked ciliates (21%). The biomass of PZP at each station ranged between 0.08 and 2.4 mu g C L-1, which is much lower than that reported for spring or summer (>= 100 mu g C L-1) in the North Sea. Relatively small-sized (< 40 mu m) PZP contributed 46% 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 over-wintering area for marine fish larvae. Using this fish survey (IBTS) as a sampling platform allowed us to obtain a synoptic view of the PZP community over a large area. The present collaborative effort provides an example of how existing monitoring platforms can be augmented in the future to collect relevant data and potential ecological indicators needed to advance the ecosystem-based approach to managing marine systems.

Highlights

► Winter protozooplankton community examined using a routine fisheries survey. ► Homogenous spatial distribution of the protozooplankton community composition ► Small sized cells (<40 µm) dominated the system. ► PZP community mainly composed of athecate dinoflagellates and aloricate ciliates. ► Highest protozooplankton biomass found towards southern North Sea areas.

Keywords : Microzooplankton, Time-series, Monitoring, International bottom trawl survey, Ecological indicators, Ecosystem-based management

1. 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). Heterotrophic protists can graze up to 60% of the annual phytoplankton biomass in temperate waters (Fileman et al., 2002; Levinsen and Nielsen, 2002). By consuming other heterotrophic organisms including bacteria (Aberle et al., 2007) and acting as prey for copepods (Calbet and Saiz, 2005) and even higher trophic levels, such as larval fish (Montagnes et al., 2010), PZP fulfils an important function in the carbon cycle of marine systems (Hansen et al., 1999). The strength and contribution of the "microbial loop" to the carbon cycle varies depending on oceanographic conditions and season. In oligotrophic systems, the phytoplankton community is dominated by small cells (pico- and nanophytoplankton); size classes which are poor prey for mesozooplankton, but favour the growth of PZP (Fileman et al., 2011). In nutrient-rich waters, the role of the microbial loop can be enhanced during times of low primary productivity, when conditions are unfavourable for large phytoplankton cells, such as diatoms (either due to low levels of nutrients or light intensity).

PZP may be useful as an ecological indicator as these organisms i) respond quickly to physical and biogeochemical changes and ii) contribute to the EU's "Good Environmental Status" by enabling life cycle closure of marine organisms and foster increased rates of biogeochemical cycling (Dickey-Collas et al., 2017). In that sense the availability of such long-term as well as broad-scale data on PZP would be valuable to help validate models designed to depict seasonal plankton dynamics such as NPZD (Nutrient Phytoplankton Zooplankton Detritus) models or broader end-to-end models (D'Alelio et al., 2016). Despite the importance of PZP in planktonic food-webs, these organisms are largely ignored in monitoring programs (Stern et al., 2015). Except for a few plankton monitoring programs, such as in Chesapeake Bay, USA, from 1984 – 2001 (Coats and Revelante, 1999), the PZP community has been sampled with limited coverage either spatially (Löder et al., 2012; Yang et al., 2014), or temporally (Tillmann and Hesse, 1998, Löder et al., 2012). Monitoring is also generally limited to productive seasons (Dolan and Coats, 1990, Edwards and Burkill, 1995) in temperate shelf seas or open ocean waters, such as the Pacific (Stoecker et al., 1996) and the Antarctic (Dolan et al., 2013). These studies are very valuable for understanding the PZP community, but more large-scale temporal and spatial coverage is needed to gain a deeper knowledge on processes impacting on the PZP community.

The North Sea is a relatively large continental shelf sea (750 000 km^2) situated on the northeast Atlantic with a shallow southern half ($\leq$ 50 m) and a deeper northern half (max. 700 m) with dominant inflows of Atlantic water from the north and a cyclonic current regime which is strongest in the southern region (for a thorough review of the hydrography see Sündermann and Pohlmann (2016)). The plankton dynamics of this shelf ecosystem have been well-studied using long-term monitoring stations such as Helgoland Roads (Wiltshire et

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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). Unfortunately, plankton monitoring programs in the North Sea, and adjacent areas, have largely ignored PZP, except at Plymouth Station L4 where microplankton is routinely collected and various taxa are 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 investigates the abundance, biomass and community composition of PZP in the North Sea in mid-winter (January / February) 2014. We explored whether spatial patterns in PZP existed and how those might be related to key abiotic characteristics such as water temperature or salinity. Furthermore, this study acts as a proof-of-concept for the feasibility of adding PZP sampling to an existing, large-scale fish survey (the ICES coordinated International Bottom Trawl Survey (IBTS)). The semi-annual IBTS also samples the overwintering larvae of autumn-spawning herring (*Clupea harengus*) to calculate an index of recruitment strength (ICES, 2017). Sampling PZP during this phase of the IBTS was considered to be highly relevant given the potential importance of PZP as prey for herring larvae (Bils et al., 2017; Denis et al., 2016; Figueiredo et al., 2005; Friedenberg et al., 2012; Illing et al., 2015). It has been suggested that recruitment success of herring may be linked to feeding conditions experienced by overwintering larvae (Hufnagl et al., 2015; Payne et al., 2013).

2. Materials & Methods

2.1 Sampling

Water samples were collected on the IBTS Q1, between January 15th and February 19th 2014, by marine institutes in the Netherlands, Norway, Germany, and France 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 (Fig 1, Table 1), of which 39 were chosen for PZP analysis.

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 nine stations of the French and the 14 stations of the German coordinated cruises and no calibration of the sensors was performed. Hence, the factor fluorescence was

excluded from most statistical analyses. Density σ_t was calculated using the temperature and salinity recorded at 10m depth.

Table 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

2.2. PZP biomass, abundance and community composition

The 39 stations chosen were located on 6 latitudinal transects (A – F, North to South) (Fig 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.
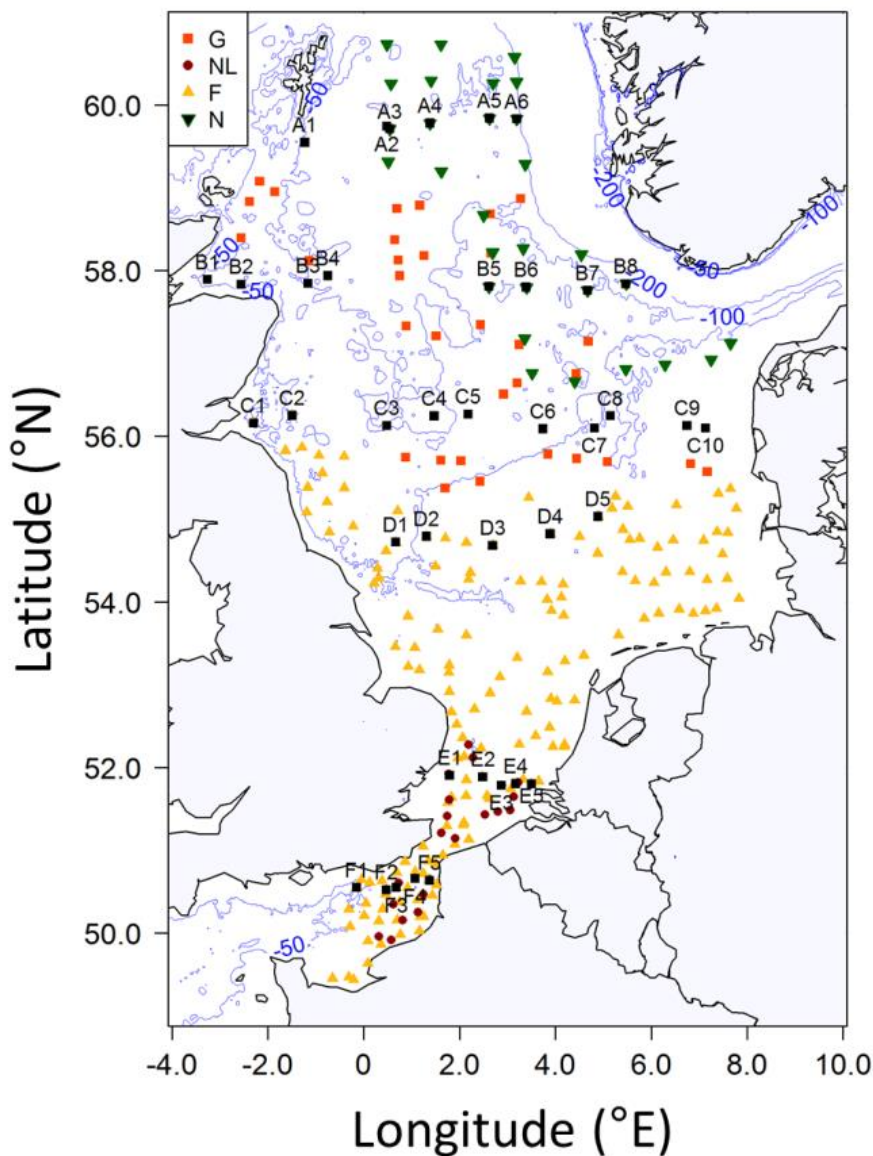
Figure 1: Protozooplankton sampling stations in the North Sea during the International Bottom Trawl Survey Q1 in 2014. Stations are colour- and shape-coded according to the country in charge of the IBTS sampling. Samples analysed in the present study are highlighted in black, labelled from North to South alphabetically and from West to East numerically. Abbreviations: N = Norway, G = Germany, F = France, NL = The Netherlands.

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 and Hart-Jones, 1982; Hoppenrath et al., 2009; Kraberg et al., 2010; Montagnes, 1996; Olenina et al., 2006; Strüder-Kypke et al., 2006). The classification of Löder et al. (2012) was used to determine trophic status. The ciliate species *Mesodinium rubrum* and *Laboea strobila* were included as mixotrophs due to their photosynthetic capabilities

coupled with optional phagotrophy (Crawford, 1989; Stoecker et al., 1988). To ensure more precise biomass calculations, the most abundant taxa were separated into up to four 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 (Löder et al., 2012; Olenina et al., 2006; Strüder-Kypke et al., 2006). Biovolume was converted to carbon biomass ($\mu g \ C \ L^{-1}$) using the carbon to Volume (C:Vol) relationship for protists (Menden-Deuer and Lessard, 2000).

2.3. Data analysis

Maps and figures were produced with the R software. Interpolated environmental variables were calculated and graphically displayed using RGeostats (Renard et al., 2016) in the R software (R core team, 2014).

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.

BIO-ENV routine was performed to identify the key impacts of environmental variables on the PZP community composition. The Euclidean distances of the environmental variables (temperature and salinity) was calculated and a Bray-Curtis similarity matrix was built on the log10(x+1) transformed biomass data ($\mu g \ C \ L^{-1}$) of all taxa. The best subset explaining the dissimilarities in the PZP community are then expressed as Spearman correlation ρ. To account for potential bias of rare taxa, the BIO-ENV routine was repeated with the biomass of the seven most dominant taxa (*Gymonodinium* spp., *Gyrodinium* spp., *Protoperidinium* spp, *Torodinium robustum*, *Stenosemella* spp., *Strombidium* spp. and *Strobilidium* spp.). Dominance was defined as contributing not less than 3% to the total PZP biomass at each station in at least 75% of the stations (29 stations). ANOSIM and BIO-ENV routines were performed using PRIMER 6 (Clarke and Warwick, 2005)

Similarities in abundance (Ind L^{-1}) of the single PZP taxa identified among stations were examined with Correspondence Analysis (CA), using the χ^2 distances calculated with the package 'vegan' (Oksanen et al., 2016) using R software. This allows for grouping stations according to the occurrence of specific taxa and, thus, to identify potential regional clusters.

Potential (dis)similarities in the diversity of the PZP community among stations or areas were examined with the Shannon diversity index (H). It was calculated for each station (Shannon and 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.

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3. Results

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. In order to explore potential factors impacting on the total biomass of PZP (µg C L-1), linear dependence of PZP biomass and environmental/geospatial factors (temperature, salinity, fluorescence, longitude, latitude) was tested using Pearson correlation coefficient (p <0.05).

3.1. Environmental conditions

Weather conditions were generally harsh (ICES, 2014), wind speeds often in excess of 18 ms^{-1} and significant wave heights in excess of 3m (R. Nash & M. Kloppmann pers. Obs.) 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 (Fig 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). Where fluorescence was measured, it was generally low (<0.4 relative fluorescence units) and only a few stations had values above 0.6. Density σ_t ranged from 24.9 in the German Bight (53.86°N, 6.87°E) to 27.6 in the northern North Sea (59.28°N, 1.32°W).
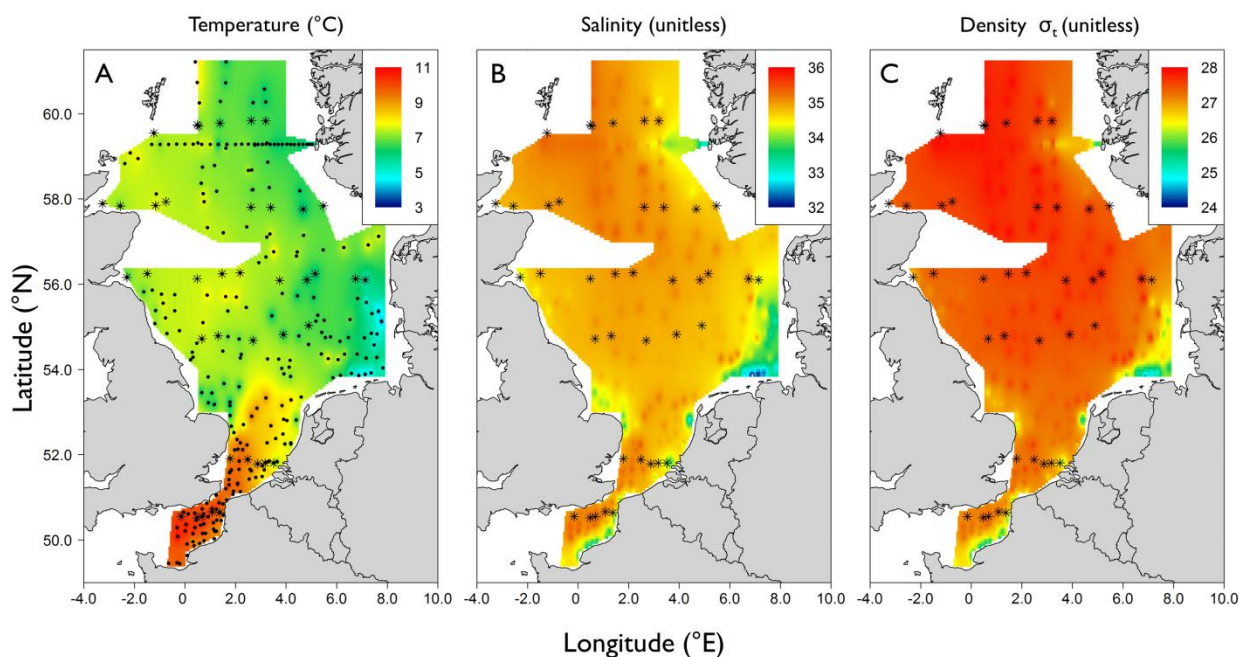

Figure 2: Temperature (°C, panel A), salinity (unitless, panel B), and density σ_t (unitless, panel C) at 10-m depth during mid-winter 2014 in the North Sea. Values are interpolated over the time frame of the IBTS survey (5 weeks), sampling stations are displayed as black dots in panel A and stations analyzed for PZP are asterisked.

3.2. PZP abundance and carbon biomass

In total 10 ciliate and 14 dinoflagellate taxa were found, of which eight dinoflagellate and four ciliate taxa were considered to be mixotrophs (or containing mixotrophic species within this taxonomic group) (Table 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 (<40 μm) accounted for 80% (60% dinoflagellates and 20% ciliates) of the total abundance (Fig 3).
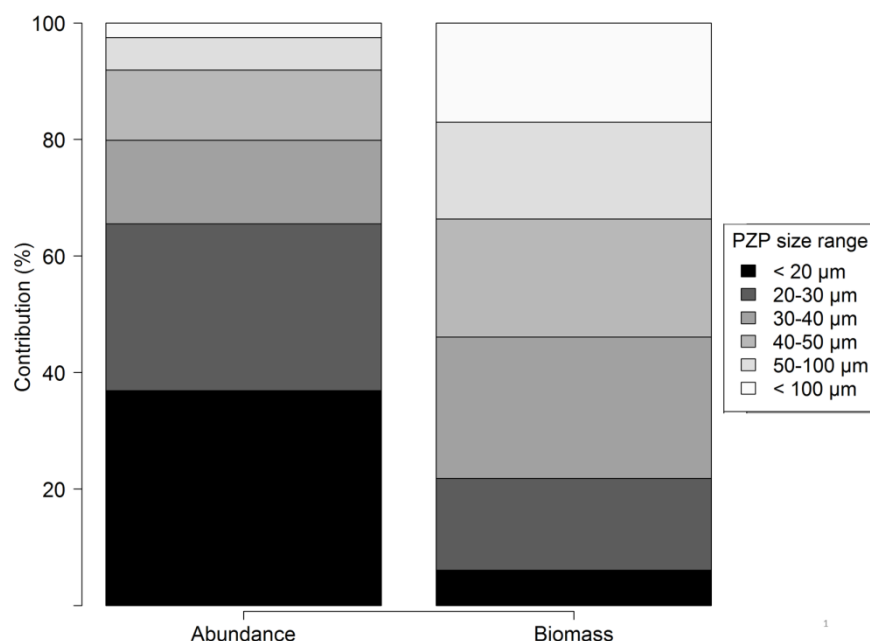

Figure 3: Relative contribution (%) of the different size fractions to the abundance and biomass of the PZP community in mid-winter in the North Sea.

Total biomass ranged from 0.08 (Station A4) to 2.4 μg C L^{-1} (Station D3) (Fig 4). Ciliates accounted for 52% and dinoflagellates for 48% of the total biomass. In 66% of the stations, dinoflagellates formed >50% of the total carbon biomass (Fig 4). 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 (Table S1). *Strombidium* spp. and *Gymnodinium* spp. dominated the PZP community (Fig 5) and 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 (<40 μm) accounted for 46% of the biomass (Fig 3).
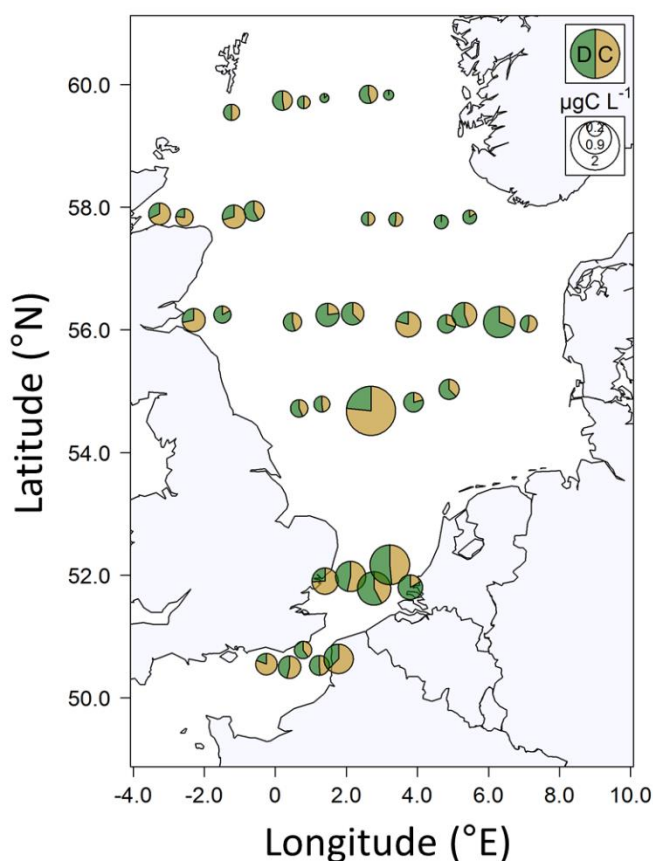
Figure 4: Biomass (µg C L^{-1}) of ciliates C (yellow) and dinoflagellates D (green) during mid-winter 2014 in the North Sea. The size of the pie represents the total biomass and the colour the relative contribution of ciliates/dinoflagellates.

Table 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 *(Olenina et al., 2006) or **(Strüder-Kypke et al., 2006). For ***(Löder et al., 2012), only biomass, no size data were available. Abbreviations: H = heterotroph, M = mixotroph, H/M = Trophic position depending on species

			Biomass		Abundance		
DINOFLAGELLATES	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
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 < 40 µm	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
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

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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/M	13	0.045	0.005	460	48.97	D3
Strombidium spp. <30 μm	H/M	24.7	0.112	0.014	260	28.97	D3
Strombidium spp. <50 μm	H/M	38.3	0.343	0.049	220	29.23	D3
Strombidium spp. <100 μm	H/M	61	0.231	0.035	100	14.1	F5
Strombidium spp. >100 μm	H/M	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/M	13	0.006	0.001	50	6.67	B3
Strobilidium spp. <30 μm	H/M	25.5	0.042	0.006	70	9.74	D3
Strobilidium spp. <50 μm	H/M	35	0.05	0.008	50	7.18	D3
Strobilidium spp. <100 μm	H/M	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
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

3.3. PZP distribution patterns

The total PZP biomass was negatively correlated with latitude (Pearson correlation = -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). Due to the missing calibration of the fluorescence probes, the correlation measurements were performed separately for the PZP biomasses from stations sampled on the German and on the French coordinated cruise (see table S1 for details). At the French stations a correlation of total PZP biomass and fluorescence was found (R = 0.713, p <0.05).

The time (week) of sampling had a weak, albeit significant, effect on the community composition (1-way ANOSIM, R =0.197, p <0.005) while time of day (absence/presence of daylight) had no significant effect (R = -0.004, p >0.5). Thus, these two factors were not included in the analysis. Temperature explained not more than 4% of the variability in the entire PZP community (BIO-ENV, ρ =0.035) neither a combination of temperature and salinity in the dominant taxa (BIO-ENV, ρ =0.025).
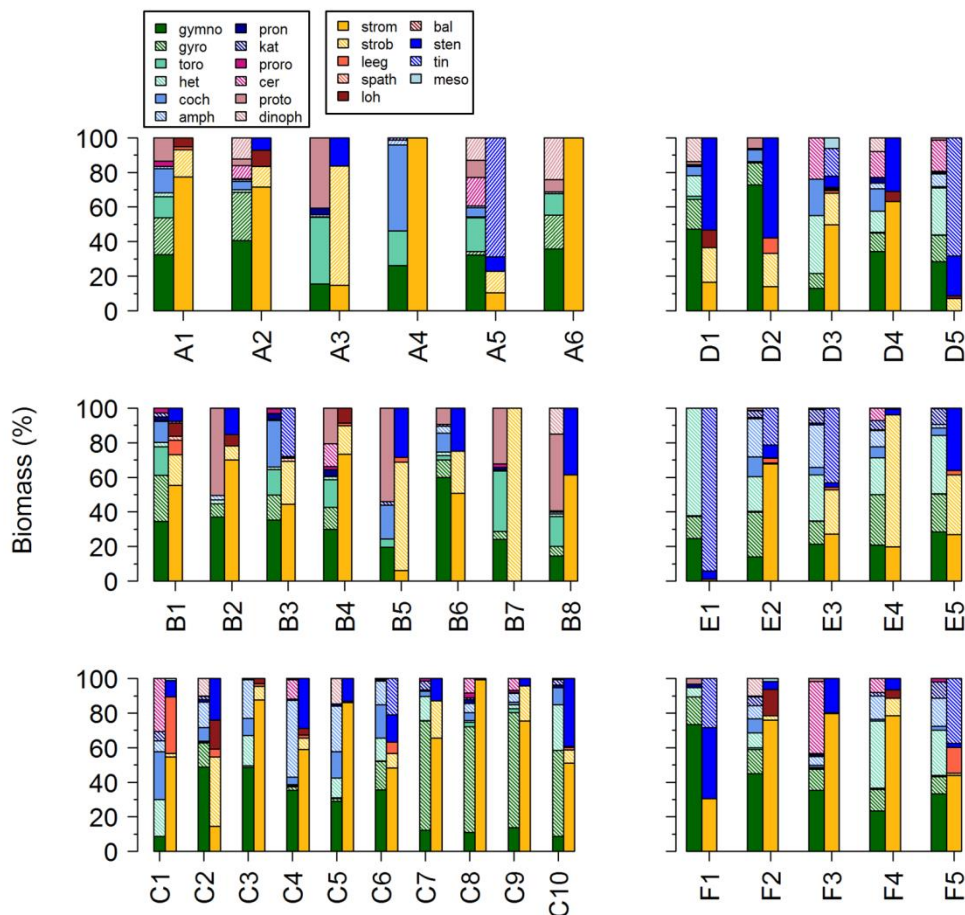
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Figure 5: Relative biomass contribution of the different PZP taxa in the North Sea during mid-winter 2014. See Figure 1 for the station coding. Left bars on each panel represent dinoflagellate biomass, right bars represent ciliate biomass. Note that 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*.

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 (Fig 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

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was not related to temperature, salinity, bottom depth or geographic location (Pearson correlation, p >0.5).
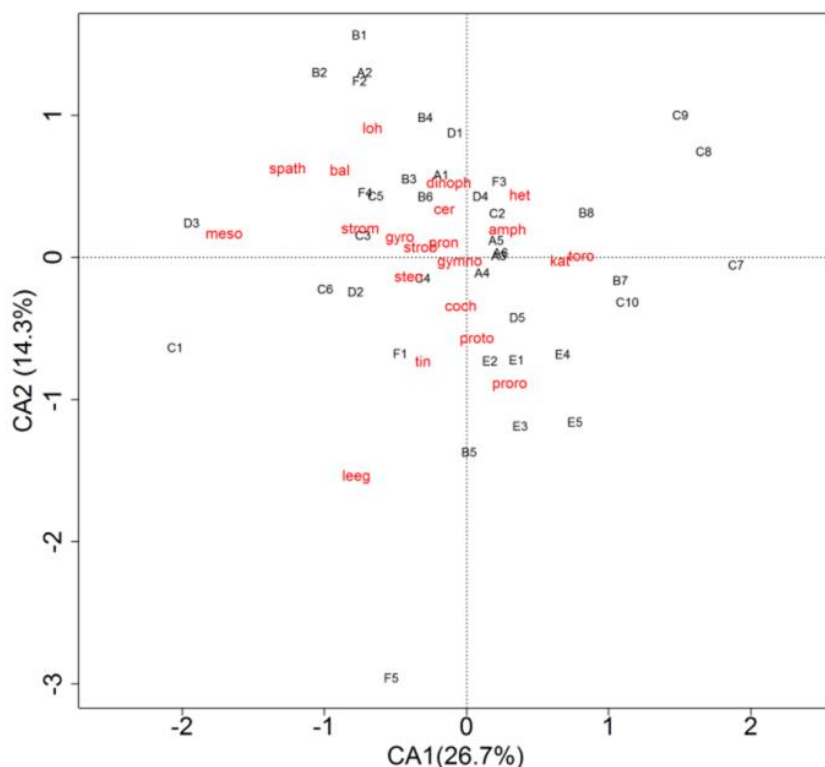

Figure 6: Correspondence analysis (CA) performed on the PZP community composition of the North Sea in mid-winter 2014, including the sampling stations and the PZP taxa, whereas no higher resolution than genus level was applied. No significant spatial distribution pattern was observed. For abbreviations see Fig 5.

4. Discussion

Ecosystem-based approaches to manage marine habitats require a broad range of physico-chemical 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.

4.1. Environmental conditions and PZP abundance, biomass and community composition

In terms of environmental conditions, 2014 was an atypical year. In contrast to this study, where the temperature was below 8°C except for the English Channel and surrounding

areas, temperatures in the northern North Sea were characterized in previous (2006 – 2013) and following years (2015 - 2017) by higher temperatures (>8 °C) similar or even above the temperatures observed in the English Channel.

Our study suggests that the PZP community was homogenously distributed in the North Sea during wintertime 2014. 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 (Figueiredo et al., 2009; Levinsen and Nielsen, 2002; 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 and Nielsen, 2002; Löder et al., 2012; Scherer, 2012). Furthermore, the community mainly consisted of small-sized cells (<40 µm), a pattern which is typical of places and/or times with low productivity when the phytoplankton community is dominated by pico- and nanophytoplankton (Legendre and Rassoulzadegan, 1995; Levinsen and Nielsen, 2002). It has been observed that the average size of *Strombidium* spp. can be four times larger during spring bloom compared to non-bloom conditions (Fileman et al., 2011). As small-sized PZP can be important grazers of bacteria, pico- and nanophytoplankton (Sherr and Sherr, 2002), in winter considerable proportions of carbon may be channeled via PZP to higher trophic levels, such as copepods and ichthyoplankton. Thus, PZP can be considered as a key component of the winter food web and a component which requires further monitoring.

4.2. PZP distribution patterns

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, salinity and density. The correlation of fluorescence and total PZP biomass at the French coordinated stations could indicate a positive impact of phytoplankton on PZP growth. Previous studies have shown that high fluorescence values (or chl *a*) are often correlated with high abundances of oligotrich ciliates (Dolan and Perez, 2000 and references therein), observed in the present study at station D3, which exhibited the highest fluorescence values and the highest PZP biomass and abundance, mostly consisting of *Strombidium* spp., an oligotrich ciliate. But since the PZP, neither ciliates nor dinoflagellates, showed any correlation with fluorescence on the German coordinated stations, nor are there fluorescence data available for the remaining 16 stations, the trend seen at the French stations cannot be generalized for the entire sampling grid.

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) 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.

One group within the ciliate community, the tintinnids, has been focused on in several studies in the North Atlantic (Barnard et al., 2004; Cordeiro et al., 1997; Hinder et al., 2012). Due to the tolerance against formalin of many tintinnid species they can be captured by e.g. the CPR, enabling for example, longer-term or broad scale studies. Previous studies on tintinnid abundance in the North Sea suggest that the mean abundance of tintinnids is lower in the English Channel compared to the Central and Northern North Sea (Barnard et al., 2004; Cordeiro et al., 1997). This differs from studies using Lugol as preservative (this study; Fileman et al., 2011), where highest tintinnid abundances were observed in coastal stations compared to open waters and members of the genus *Stenosemella* accounted for a great part of the tintinnid community. One reason for the discrepancy might be that this taxon is not efficiently captured by the CPR (Hinder et al., 2012), which gives one more reason for establishing a PZP time series to avoid missing a potential important component of the plankton community. 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 (Aberle et al., 2015; Montagnes et al., 2003) may be counterbalanced by increased mortality due to predation, e.g. by fish larvae (Illing et al., 2015; Montagnes et al., 2010).

4.3. Role of PZP in the winter food web

Laboratory experiments have demonstrated that the larvae of many fish species feed on and can nutritionally benefit from PZP (Friedenberg et al., 2012; Hunt von Herbing and Gallager, 2000; Illing et al., 2015) and field and laboratory studies have documented PZP in the diets of herring larvae and other larval fish (e.g. Bollens and Sanders, 2004; Denis et al., 2016; Figueiredo et al., 2005; Hillgruber and Kloppmann, 1999). Thus, the comparably high winter biomass of PZP in the southern North Sea in contrast to the Northern North Sea is highly relevant as this area acts as a winter nursery ground for the larvae of several marine fish species including Atlantic herring and European plaice (*Pleuronectes platessa*). For instance, 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), an important prey item of many fish larvae. One has to keep in mind, however, that 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 Figueiredo et al., 2005; Munk and Kiørboe, 1985; Peck et al., 2012)). Nevertheless PZP can make an important contribution to larval feeding during autumn/winter when the abundance of other potential prey (e.g. copepod nauplii) is low (Bils et al., 2017; Wesche et al., 2007). 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 (Alvarez-Fernandez et al., 2015; Payne et al., 2013), 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.

4.4. PZP as ecological indicator

Changes in the community composition or size distribution of lower trophic components in the plankton can serve as indicators for ecosystem changes (Beaugrand, 2005) due to their high abundance and fast response time to environmental changes, such as temperature or food concentrations (Aberle et al., 2012; Menden-Deuer et al., 2005; Montagnes et al., 2003). 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 have different roles in carbon cycling. Dinoflagellates are able to prey e.g. on diatoms larger than themselves (e.g. 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 and Saiz, 2005) as copepods often prefer ciliates over dinoflagellates as prey (Vincent and 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) and Mitra et al. (2014) urged the integration of e.g. protozooplankton to a greater extent into fisheries models. 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. Racault et al. (2014) suggest three different attributes for phytoplankton to observe the state of an ecosystem: Composition, structure and functioning. Within these attributes several indicators are presented, which can be achieved by different monitoring methods. Transferring this approach to PZP our type of sampling was able to cover indicator measurements as composition attribute (by observing the most dominant groups) and as structure attribute (by size measurements). Further indicators, such as Chl *a* are planned to be added to the survey. On board measurements (e.g. fluorescence on live samples with a FlowCam) could provide estimations of mixotrophy (as an indicator for functioning of the ecosystem) and community composition.

As future climate scenarios predict a shift in the phytoplankton community towards small sized cells (Caron and Hutchins, 2013; Rodríguez et al., 2001)

energy transfer through the microbial loop may be enhanced which might in turn reduce trophic transfer efficiency due to the increased number of trophic steps required before energy reaches upper trophic levels. On a North-South transect across the Atlantic on a time scale from 2003–2010, microphytoplankon abundance declined while nano- and picophytoplankton abundance increased throughout the area (Racault et al., 2014). This will probably affect all trophic levels as it is known that for instance copepods shift from a phytoplankton based diet to PZP if small phytoplankton cells dominate the community (Lewandowska et al., 2014).

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 and Caron, 2000).

4.5. Future perspectives for PZP monitoring

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. For example, the extraordinarily high biomass of PZP at station D3 might have resulted from sampling within a patch. Moreover, as considerable time was required to manually resolve the taxonomic composition of the PZP community the protocol could be simplified, for example, by focusing on functional types of the North Sea ecosystems (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 the gear on this survey (e.g. a 335 µm MIKey M net; see ICES 2017) 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. Whether the unusual temperature patterns observed in this study did influence PZP community, abundance or biomass, will be analyzed in future work thanks to the availability of subsequent PZP data from the IBTS Q1. 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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7. References

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8. Supporting information

Table S1: 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 ($\mu g\ C\ L^{-1}$).