



Effects of impulsive, low frequency anthropogenic noise on pelagic prey in the North Sea



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1. EXECUTIVE SUMMARY

Wind farms in the Dutch North Sea are rapidly increasing in number, because of the high need for renewable energy. These wind farms change the underwater environment from a sandy bottom with relatively low biodiversity, to richly populated rocky sanctuaries that are free from fishing. At the same time, before and during the construction of a wind farm the area is exposed to a lot of anthropogenic noise from seismic surveys scanning the bottom and pile driving of the wind mill piles. Over the past decades, an increasing number of studies has shown detrimental impact of anthropogenic sound on marine life. These combined positive and negative effects of wind farms are bound to alter the community structure of North Sea life.

Most of the effects of anthropogenic sound have been found in demersal and mesopelagic fish. However, a lot of the fauna residing in wind farms is pelagic, a group of species that has received relatively little attention by science because of the difficulty of studying it. Pelagic fish are generally more difficult to keep in captivity and the turbid waters of the North Sea prevent the use of cameras in field studies. North Sea pelagic fauna includes economically important species, such as commercially-caught fish species like herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*) and might harbour ecologically important zooplankton layers. Therefore, it is important to understand how pelagic fauna will respond to sound from anthropogenic activities.

Bottom-moored, multi-frequency echo sounders might be a solution to this problem. They are placed on the sea floor for longer periods of time and transmit high-frequency sound to record the presence of marine organisms in the water column. Because sound is the medium of detection, low visibility of the water is less likely to influence the recordings. The advantage of bottom-mooring is that the same area can be surveyed over a longer period of time without exposing the study organisms to increased noise levels from shipping activity. Furthermore, by the use of multiple frequencies fish as well as zooplankton can be recorded at the same time.

The purpose of this study was to find out 1) if bottom-moored echo sounders are a suitable tool for studying the fluctuations in time and space of pelagic fauna; 2) how pelagic fauna respond to the sound of a seismic survey; 3) if there is a difference in the response of pelagic fauna inside and outside a wind farm; 4) if pelagic fauna respond differently to sound of pile-driving, and 5) if the pelagic fauna respond to the sound directly, or if a chain-reaction causes the response of some species to influence the behaviour of other species. To answer these questions, two bottom-moored echo sounders were placed for a month sequentially in three different wind farms along the Belgian and Dutch coast. One was exposed to a full-scale seismic survey, the second to pile driving activity, while the third served as a control. The resulting echograms were analysed visually for the presence of zooplankton layers, combined with a more in-depth analysis of fish school presence and behaviour in a period around the sound exposure.

The bottom-moored echo sounders were successful in detecting variation in the behaviour of pelagic fish in a wind farm. However, due to floating particles and turbulent water, it was not possible to detect zooplankton with this method. It is also possible that there were no distinct layers of zooplankton at the sampled locations. Having two echo sounders at the same wind farm showed us that patterns of fish school presence and behaviour were generally similar within the wind farm, while they differed strongly over time and between wind farms. Patterns of behaviour and detection of fish schools were significantly different during sound exposure compared to before, but also varied significantly in the control site. This shows the need for thorough replication when investigating responses of pelagic fish to sound exposure.

2. INTRODUCTION

Many aquatic animals change their behaviour in response to increased ambient noise levels in the ocean. Effects of sound on behaviour range from local changes in water column use (Hawkins et al. 2014; Neo et al. 2014), to horizontal avoidance of acoustically polluted areas (e.g. Carstensen et al. 2006; Kok et al. 2018), and may include changes in mate choice, foraging behaviour, and anti-predator responses (Shafiei Sabet et al. 2015; Simpson et al. 2015; de Jong et al. 2018). Increased noise levels have been found to affect all trophic levels, from invertebrates (Hubert et al. 2018) to top predators, such as marine mammals (Southall et al. 2016). Of all types of behavioural effects of increased noise levels, changes in predator-prey interactions are most likely to have effect on the ecosystem as a whole (Kunc et al. 2016).

Predator-prey interactions characterize themselves by specific behaviour of the predator – hunting – and responding behaviour of the prey – defence and escape. Prey defence tactics in the ocean, where hiding in vegetation or under rocks is no option, often involve aggregation into groups. For example, prey species in deep-scattering layers have been shown to organize themselves by clustering to improve protection of individual animals by being in a group (Benoit-Bird et al. 2017). Furthermore, pelagic animals change their location in the water column in response to predators, often by moving down (Hawkins et al. 2014; Neo et al. 2014; Rieucou et al. 2014). At the same time, predators probably try to maximize their success by targeting high-density prey areas and adapting hunting strategies to prey behaviour (Charnov 1978; Au et al. 2013). For example, marine mammal predators that forage on deep scattering layers, often target their prey at night, when animals of the deep scattering layer migrate and go closer to the surface (Au et al. 2013; Giorli et al. 2016). Therefore, changes in prey defence behaviour are likely to also alter hunting strategies of their predators.

Acoustic disturbance related changes in prey behaviour could have important consequences for predators. It could be beneficial, if prey become less vigilant, and are consequently more easily caught (Simpson et al. 2015). Or it could be detrimental, if prey change their behaviour, for instance by becoming more cohesive and moving deeper down the water column, and therefore become more difficult to catch (Voellmy et al. 2014). In fact, how prey respond to increased noise levels could even determine the response of predators to sound. Cuvier's beaked whales, for instance, stayed in an area that was frequently disturbed by military sonar exercises, but also contained high prey densities, while leaving an area that was less disturbed by sonar but also contained less prey (Southall et al. 2019). So, understanding how prey will respond to sound, might also provide information on the potential effect of sound on their predators.

Many animals from the bottom and middle trophic levels are pelagic, such as zooplankton and schooling fish. However, the effects of sound on pelagic animals have hardly been studied. One benchmark study by Hawkins et al. (2014) indicated changes in cohesion and vertical displacement of pelagic fish and zooplankton when exposed to an artificial, intermittent sound. Additionally, a case study in which zooplankton was experimentally exposed to a seismic survey showed increased mortality compared to the period before the survey (McCauley et al. 2017). A more recent study also revealed increased mortality, but only at very small distances of less than 10m from the source (Fields et al. 2019). Apart from these studies that require follow-up and replication, only very few studies reported changes in fisheries catch rates during and after a noisy human activity, such as a seismic survey (e.g. Skalski et al. 1992; Parry and Gason 2006; Løkkeborg et al. 2012). Consequently, we still lack sufficient insight into changes in spatial behaviour of pelagic species.

Offshore wind farms provide an interesting opportunity to study the pelagic community as well as the potential effects of anthropogenic noise. Offshore wind farms have become increasingly popular and are spreading rapidly to exploit the renewable source of wind energy, with supposedly little or even positive environmental impact (Lindeboom et al. 2011; Ashley et al. 2014; Raoux et al. 2017). In

the pre-construction and construction phase, seismic surveys and pile driving activities typically cause considerable acoustic disturbance in the area. Subsequently, in the exploitation phase, a moderate, low-frequency noise from the wind-driven rotor blades remains, while scour beds and the set of piles and control stations typically introduce a rocky reef at places that were dominated by sandy bottom. Consequently, over time, a more diverse benthic community develops, which may also affect the local pelagic community. Recent studies have indicated increased vertical mixing and elevated zooplankton densities associated with wind farms in the North Sea, but there are no reports on distinct patterns for the pelagic fish community that stand out against already highly variable base line data (Floeter et al. 2017).

The North Sea harbours a variety of fish species, although benthic communities are typically more diverse. In a baseline study on the presence of pelagic fish in 2003 (Grift et al. 2004), nine species were reported from coastal and offshore zones in the Dutch North Sea. From summer to autumn, the dominant species shifted from sandeel (*Ammodytes marinus*) and mackerel (*Scomber scombrus*) to herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). This baseline study (Grift et al. 2004) was conducted with a towed echo sounder, to detect schools and assess biomass, in combination with a trawling net to confirm species presence and identify schooling species. It was labelled a baseline study in the context of potential construction activities such as an artificial island and offshore wind farms. However, the variability with season and across years, with changing average weather conditions, warrants more studies before we can actually speak of sufficient base line insights and to interpret short-term changes correlated to human activities.

Echo sounder applications have a long history and have been used successfully to observe fish school behaviour. They have been used since the late 1960s by the navy, in fisheries, and for mapping distributions of marine fauna and the ocean floor (Lurton 2002; Chu 2011; Colbo et al. 2014). An echo sounder system operates by transmitting ultrasonic pulses, which are backscattered by objects and detected by a receiver (Lurton 2002). This produces an echogram, which shows the variation in intensity of the echoes (i.e. the echo strength) over time (Colbo et al. 2014). In the case of fish, the echo intensity depends on morphological characteristics such as body size, fat percentage, swim bladder shape and orientation to the sound transmitter (Frouzova et al. 2005). Because of their non-invasiveness and spatial resolution, echo sounders are widely used to identify individual species and to observe fish school behaviour (Lawson et al. 2001; Colbo et al. 2014; Benoit-Bird et al. 2017). Various studies have used echo sounders to monitor changes in school cohesion and swimming depth (Gerlotto et al. 2004; Weber et al. 2009; Guillard et al. 2010; Hawkins et al. 2014; Fraser et al. 2018), which are among the expected responses to seismic surveys and pile-driving. Echo sounders therefore seem to be a highly suitable method for examining the impact of seismic survey and pile-driving sounds on fish school behaviour.

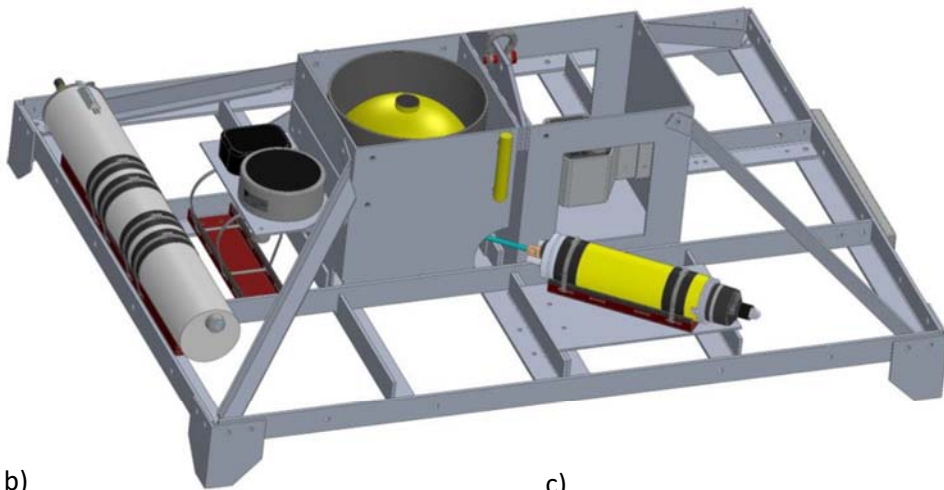
In the current investigation, we studied the effects of two types of anthropogenic sound events – a seismic survey and pile driving – on the spatial behaviour of pelagic prey of the North Sea. Our research questions were: 1) Does the set-up with two bottom-moored echo sounders give insight into fluctuations in time and space of the fish and zooplankton communities that are present in and around a wind farm? 2) How do fish and plankton communities respond to the impulsive sound of a seismic survey? 3) Is there a difference between the response of these communities inside and outside the wind farm? 4) Is there a difference in response of these communities to another source of impulsive sound, pile-driving? 5) Do fish and plankton primarily respond to the sound itself, or do they respond to the behavioural changes in other species of fish or plankton (chain-reaction)? To be able to observe spatial behaviour of the pelagic layer, we deployed a pair of two bottom-moored echo sounders that recorded the entire water column over the course of a month, replicated at three locations in three subsequent periods.

3. MATERIALS AND METHODS

3.1 Echo sounders

Two Acoustic Zooplankton Fish Profilers (AZFPs™) were rented from ASL Environmental Sciences, Canada (Fig. 1a). Both AZFP-echo sounders were able to use multiple frequencies. Each of the two sets emitted four frequencies, of which three were shared (determined by the availability of this equipment at ASL). The first AZFP emitted 38, 125, 200, and 455 kHz. This AZFP was always placed inside the wind farm. The second AZFP emitted 125, 200, 455, and 769 kHz and was always placed outside the wind farm. The AZFPs were calibrated by ASL Environmental Sciences before and after

a)



b)



c)



Fig. 1: a) AZFP mooring device, including two acoustic transducers (38 kHz and 125-200-455 kHz, which was 125-200-455 and 769 kHz for the second one), battery pack and pop-up acoustic release assembly. This mooring was lowered to the seabed using a large A-frame for handling. Image taken from manual AL-77E01F00-R03-PR936, ASL Environmental Sciences Inc. b) The two AZFP-echo sounders on deck of the RV Simon Stevin of the Flemish Government before deployment c) deployment of equipment with the A-frame.

Table 1: Description of deployment locations, exposure type, and period, placement, and depth of deployment.

Location	Exposure	Period (2018)	Location AZFP	Depth of deployment (m)
Belwind	Seismic survey	15/7 – 11/8	Inside	37.5
			Outside	38
C-Power	Pile driving	22/8 – 22/9	Inside	24
			Outside	25
Gemini	Control	18/11 – 18/12	Inside	32.7
			Outside	33.1

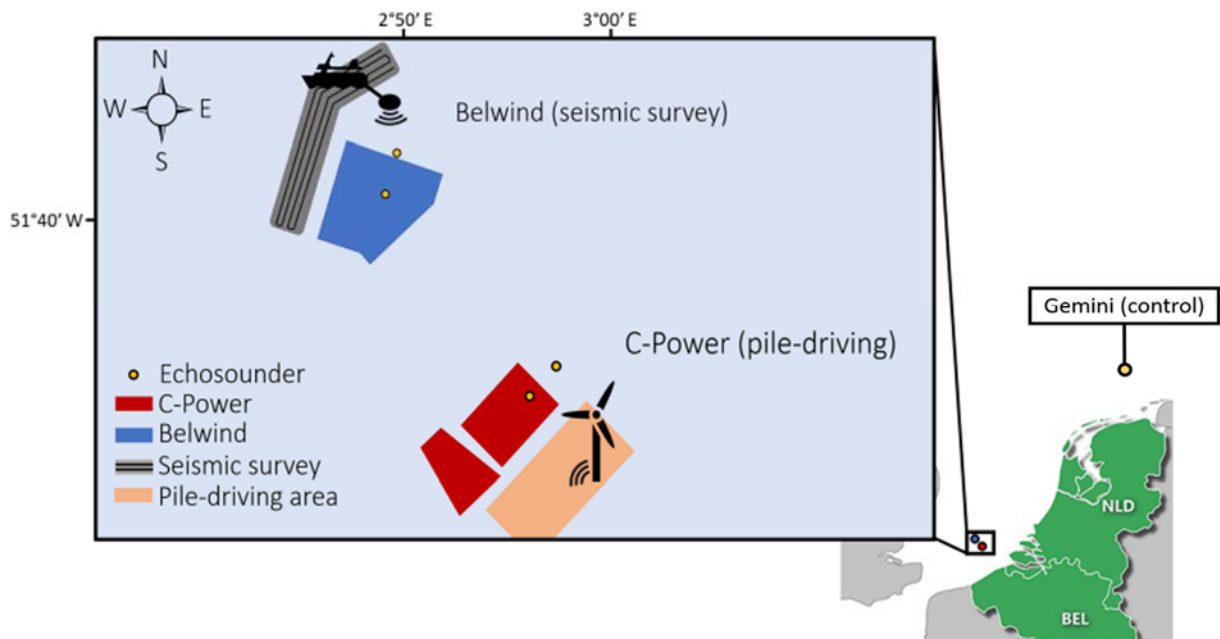


Fig. 2: Schematic overview of the placement of the AZFP echo sounders (yellow dots in inset) at the two wind farms in the Belgian North Sea. Each time, one AZFP was placed inside the wind farm, while the other was placed outside it. Note the roughly equal distance of the AZFPs to the track of the seismic survey (Belwind) and to the pile driving area (C-Power). More detailed maps of AZFP-placement in the exposed wind farms, relative to wind turbine placement, can be found in the supplementary material (Fig. S1 and S2). At Gemini, no specific anthropogenic activity took place and there were no periods of impulsive anthropogenic noise during these measurements in the Dutch North Sea.

deployment and the calibration of one AZFP was verified in a tank at one of the author's research facilities. Both AZFPs were placed on a frame at the seafloor, at an average depth of 32 m, and had a vertical upward beam (Table 1). They were attached to a concrete weight (500 kg) by a ground rope of 75 m. The AZFPs recorded 25 consecutive days per location with a ping rate of 1 Hz. Data were extracted after retrieval of the AZFPs.

3.2 Study locations

The AZFPs were placed at two wind farms in the Belgian and one wind farm in the Dutch North Sea (Fig. 2). The two Belgian wind farms were Belwind – an offshore wind farm situated on Bligh bank, 40 km from the Belgian coast – and C-Power – an offshore wind farm situated at Thornton bank, 27 km

off the coast of Belgium. The Dutch wind farm at which AZFPs were deployed was Gemini, located 85 km from the Dutch coast, north of Schiermonnikoog. At every wind farm, one AZFP was placed inside the farm, 150 m from the centre of the wind farm, while the second AZFP was placed 700 m from the edge of the wind farm, to be able to compare communities inside and outside the wind farm. The AZFPs were placed consecutively in the three wind farms: first at Belwind (deployment July 10 – August 20, 2018; recordings July 15 – August 11, 2018), next at C-Power (deployment August 20 – September 21, 2018; recordings August 22 – September 22, 2018) and finally at Gemini (deployment November 13 – December 12; recordings November 13 – December 11, 2018). Water temperature, wave height and tide records were taken from the Dutch Ministry of Infrastructure and the Environment (waternet.nl, Rijkswaterstaat) from measuring stations close to the wind farms (see Table S1 in the Supplements for locations).

At Belwind, both AZFP frames were deployed at 3.03 km distance from each other on July 10, 2018 with the A-frame of the RV Simon Stevin on dynamic positioning (DP) and were retrieved on August 20, 2018 (Fig. 1b and c). The frames were cleaned, data were downloaded and AZFP and acoustic release was prepared for the next deployment. On the same day as retrieving the AZFP frames, they were deployed at C-Power with the RV Simon Stevin on DP, at 2.35 km distance from each other. Retrieval occurred on September 21, 2018 with the RV Belgica. Finally, at Gemini, the AZFP frames were deployed at 2.44 km distance from each other by the RV Terschelling on November 13, 2018 and were retrieved on December 12, 2018 by the same vessel. The echo sounder inside Belwind and the echo sounder inside C-Power were 15.52 km apart, while the echo sounder inside Gemini was 333 km (as the bird flies) from the echo sounder inside Belwind and 332.6 km from the echo sounder inside C-Power (Table S2).

3.3 Soundscape

The wind farms differed in the soundscape at the time of measurement: Belwind was exposed to an experimental seismic survey over a period of four days (PCAD4Cod project, Slabbekoorn et al. 2019). C-Power was exposed to pile driving from a nearby wind farm for 12 days of the AZFP deployment period (for the construction of the offshore wind farm Northier). Gemini was not exposed to any particular anthropogenic activity, other than shipping noise, from local maintenance traffic and a nearby shipping lane, and was taken as a control. Sound measurements at Belwind were taken with a moored hydrophone (AMAR, M36, rented from JASCO) at 22 m depth inside the wind farm, anchored with a 60 kg rock. Ambient sound levels fluctuated with time but were on average 124 dB re 1 μ Pa at Belwind and 127 dB re 1 μ Pa at C-Power. Ambient sound levels at Gemini were not measured during the study but have previously been reported to range from 80-100 dB re 1 μ Pa²/Hz at 10-500 Hz on average (Lucke 2015).

3.4 Sound exposure

3.4.1 Seismic survey

A full seismic survey was conducted at Belwind from 21-24 July 2018. Sound levels ranged from 123 to 195 dB re 1 μ Pa SPL_p. The survey was carried out by the Norwegian company CGG with the MV Geo Caribbean, using 36 G-Gun II Sercel airguns (50% operating at a time) with a total volume of 5900 m³. The airgun arrays were towed 204 m behind the vessel, at a depth of 6 m below the surface. The survey involved 19 shooting lines with an average length of 22 km, except for the first line (30 km). Closest approach was 2.1 km from the wind farm. The air guns generated a sound pulse every 10 s, while the vessel maintained an average speed of 2.2 m/s. For the first line a soft-start procedure of 20-40 minutes was used in accordance with mitigation requirements. This procedure

might influence the behavioural response of the fish schools, although similar response levels have been found to a soft-started sound as to a sound without soft start in sea bass (*Dicentrarchus labrax*) (Neo et al. 2016).

3.4.2 Pile-driving

Pile-driving was carried out next to C-Power at a new wind farm site called Norther (51° 32' N, 3° 2' E) during the construction of additional wind turbines. Sound levels were available for one pile driving period and were on average 172 ± 2 dB re 1 μ Pa SPL_p at both AZFPs. A total of 20 turbines were built during the period from 6 August to 25 September 2018, with pile-driving being conducted on twenty days within this period. Some turbines were piled during day time and others during night time. Of the twenty pile-driving days, the first four days were used for the behavioural analysis. On those four days, all the pile-driving was carried out during daylight hours. The average pile-driving duration on these days was 148 minutes, with the shortest and longest durations being 100 minutes and 180 minutes, respectively.

3.5 Analysis procedure

3.5.1 Data periods

Analysis of the collected AZFP data was conducted in two ways: 1) total biomass was calculated per 1 m depth in 10 min bins for the entire survey period. In this case, exposure was taken as the entire duration of sound exposure per site, with a control period of sham exposure for Gemini (Table 2). All data points that did not fall in the exposure period were considered to be baseline. 2) For selected periods around and during the sound exposure, individual fish schools were detected and measured. This period consisted of a period before the exposure (BEFORE), a period during the exposure (DURING), and a period after the exposure (AFTER). The duration of each period was four days, based on the duration of the seismic survey exposure (Table 2).

Table 2: Exposure periods for analysing biomass data and fish school data.

Wind farm	Actual exposure	Exposure period used for biomass data	Exposure period used for fish school data
Belwind	21-07 04:22:42 – 24-07 16:16:08	21-07 04:22:42 – 24-07 16:16:08	21-07 00:00:00 – 24-07 23:59:59
C-Power	26-8 06:00 – 26-08 08:25 28-08 13:40 – 28-08 16:40 30-08 10:30 – 30-08 13:15 03-09 12:10 03-09 13:50 05-09 03:45 – 05-09 05:45 06-09 18:10 – 06-09 20:15 09-09 15:10 – 09-09 16:45 12-09 01:50 – 12-09 04:25 13-09 04:55 – 13-09 07:00 15-09 23:30 – 16-09 02:55 17-09 06:55 – 17-09 09:35 18-09 22:40 – 19-09 02:00	26-8 06:00 – 26-08 08:25 28-08 13:40 – 28-08 16:40 30-08 10:30 – 30-08 13:15 03-09 12:10 03-09 13:50 05-09 03:45 – 05-09 05:45 06-09 18:10 – 06-09 20:15 09-09 15:10 – 09-09 16:45 12-09 01:50 – 12-09 04:25 13-09 04:55 – 13-09 07:00 15-09 23:30 – 16-09 02:55 17-09 06:55 – 17-09 09:35 18-09 22:40 – 19-09 02:00	26-08 00:00 – 26-08 23:59 28-08 00:00 – 28-08 23:59 30-08 00:00 – 30-08 23:59 03-09 00:00 – 03-09 23:59
Gemini	None	20-11 06:30 – 23-11 17:00	20-11 00:00 – 23-11 23:59

For Belwind, the BEFORE, DURING, and AFTER period consisted of four consecutive days before, during, and after the seismic survey. Since the days of pile driving sound exposure at C-Power were not always consecutive, the DURING days were the first four days that contained pile driving after the start of deployment (August 26, 28, 30 and September 3). The BEFORE period of C-Power started at the first day of deployment, but it has to be noted that pile driving had taken place in the area 8 days before the start of deployment. The AFTER period started at the first day after an exposure period and we aimed for four consecutive days of no exposure. However, piling activity stopped one day before retrieval of the AZFPs, so the AFTER period for C-Power lasted only one day. There was no period of four days without piling earlier in the deployment. For Gemini, the first twelve days of deployment were taken as a control BEFORE, DURING, and AFTER period.

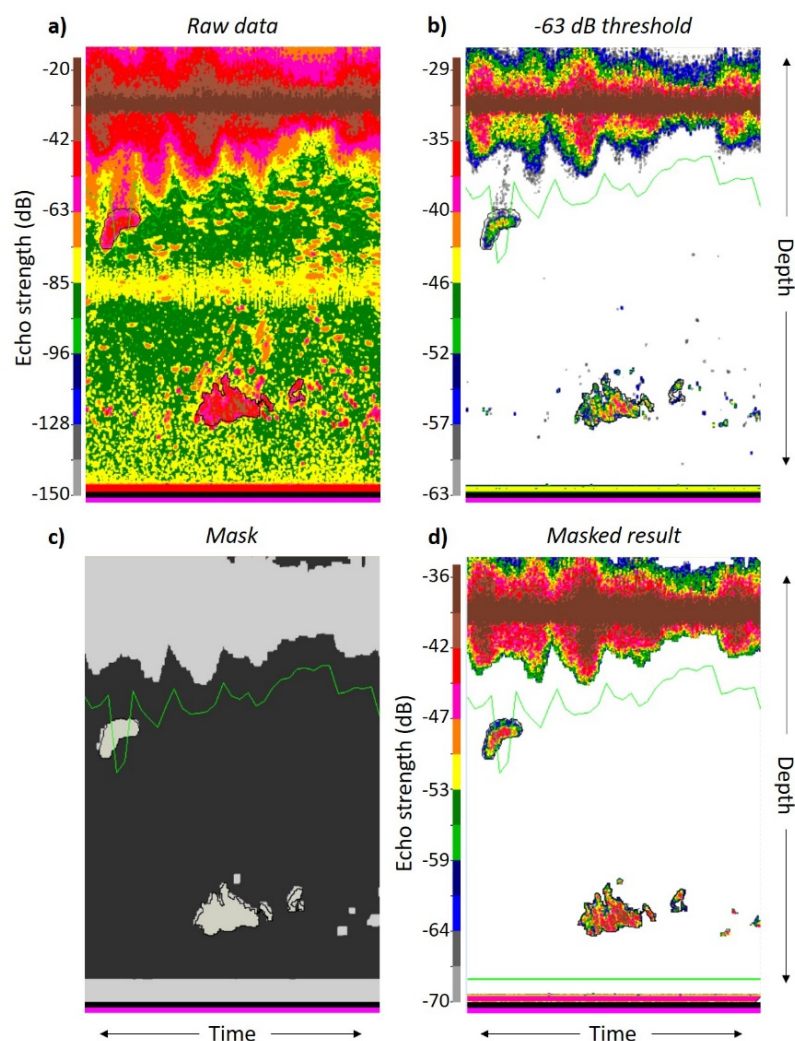


Fig. 3: The raw echo sounder data (a) were pre-processed to reduce noise and enhance the detectability of schools (example data from inside Gemini, November 18). Data of all echo sounder frequencies were filtered with a -63-dB threshold (b) and combined to create a maximum strength echogram. Next, the combined data were eroded and dilated so only clusters of pixels were retained, of which a mask was created (c). The mask was then put over the raw data of 125 kHz, to create the filtered result that could be used for data analyses (d). Noise from the surface and the bottom was excluded from automatic school detection (green lines). Distinct schools that fell (partly) outside of the green lines were manually added to the detected schools.

Table 3: Settings for automatic school detection with Echoview, as used to process data from the two echo sounders for each of the three locations.

Setting	Length (m)	Corresponding number of pings
Min. Total length	1	8.3
Min. Total height	1	n/a
Min. Candidate length	0.5	4.2
Min. Candidate height	0.5	n/a
Max. Vertical linking distance	1.2	n/a
Max. Horizontal linking distance	0.8	10

3.5.2 Data analysis

3.5.2.1 Pre-processing

All data analyses were performed using Echoview 9 (Echoview Inc.). The raw data were pre-processed to filter out noise and facilitate fish school detection (Fig. 3). First, a maximum-strength echogram was calculated from all the measured frequencies, by taking the maximum echo strength from all frequencies per pixel. The optimal frequency for detection differs among species. By taking the maximum echo strength for all frequencies, we made sure that species type did not affect detection probability. Low-signal detections were removed from the maximum-strength echogram by implementing a -63-dB echo strength threshold. This procedure avoids the false detection of pelagic fauna due to reflections that are too minimal to be fish.

To further remove noise in the data from non-biotic particles, we applied an erosion-dilation procedure (Haralick et al. 1987; Reid and Simmonds 1993). This procedure detects clusters of pixels with high echo strength, thereby favouring larger detected objects such as fish, or fish schools. The mask – i.e. a ‘pattern’ of detected and undetected pixels – was created by applying these procedures on the maximum-strength echogram, which was then superimposed onto the raw data of 125 kHz. Data for this frequency were present at both AZFPs, making it possible to compare measurements. Data with the mask were filtered with a threshold of -70 dB. Finally, surface and bottom noise (i.e. waves, sediment particles) were excluded from the data.

3.5.2.2 Fish school detection

Fish schools were detected automatically using a built-in school detection function of Echoview (detection settings, Table 3). Detection settings were based on a comparison of automatically detected schools with manually detected schools in a subsample of the data. After automatic detection, all detected schools were checked manually to correct for false positives and negatives by the algorithm. Schools were defined in two ways: 1) at least three separate traces of fish, present in the same ping, with a maximum vertical distance of 1 m; 2) an area with increased echo strength of at least 1 m high during at least one ping (Fig. 4). Both school types had to be visible for at least 3 pings before being considered a school.

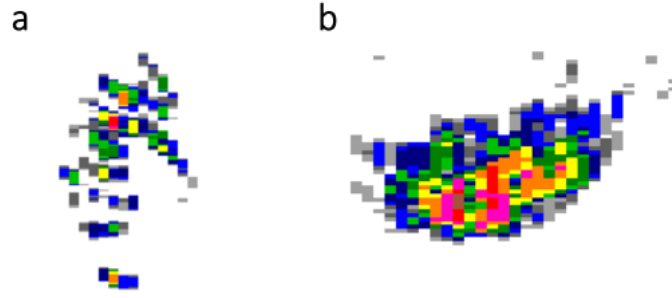


Fig. 4: Different forms of a fish school in an echogram. (a) Separate traces with increased echo strength. (b) An area of increased echo strength without any evident separate traces. Colours indicate echo strength intensity, with warmer colours being a higher echo strength.

3.5.2.3 Day-night patterns

Behaviour of fish schools often differs between day and night. School formation generally takes place between dawn and dusk, while fish scatter more individually at night (Norris and Schilt 1988; Ryer and Olla 1998; Iglesias et al. 2003; Tsagarakis et al. 2012). At Belwind, no such pattern was visible, as schools were apparent throughout the 24 hours of the day during the long-daylight days of summer. We therefore measured schools during all hours of the day for Belwind. However, at C-Power and Gemini, sampled later in the season, there was a distinct diurnal pattern of fish schooling during daylight and a layer of fish in the water column at night (Fig. 5). Because it was not possible to reliably detect schools at night-time, we decided to exclude the data between dusk and dawn from the analyses for these wind farm locations (roughly 19:00 – 04:00h for C-Power and 16:30 – 06:20h for Gemini).

3.5.2.4 Behavioural measurements

3.5.2.4.1 Biomass data

Biomass was calculated as the Nautical Area Scattering Coefficient (the integrated scattering strength of a bin, NASC) for bins of 1 m depth by 10 minutes. NASC is defined as:

$$NASC = 4\pi Nm^2 10^{\frac{Sv}{10}T} \quad (1)$$

Where NASC = Nautical Area Scattering Coefficient in m^2/nm^2 , 4π converts backscattering cross-section to scattering cross-section, Nm = a nautical mile in m (1852 m/nm), Sv = mean volume backscattering strength of the bin being integrated in dB re $1 m^2/m^3$ and T = mean thickness of the bin being integrated.

For the biomass data of the entire survey period, the centre of gravity (i.e. mean depth of the biomass in the water column, henceforth described as biomass depth) was calculated per 10 min bin. The centre of gravity was taken as:

$$Centre\ of\ gravity = \frac{\sum momentum}{\sum NASC} \quad (2)$$

With

$$momentum = NASC * D \quad (3)$$

Where momentum is in m^3/nm^2 and D = distance from the AZFP in m. Since the biomass depth at a certain time point depended on the depth of the previous time point (temporal autocorrelation), the data was resampled to one 10-min bin every 3 hours.

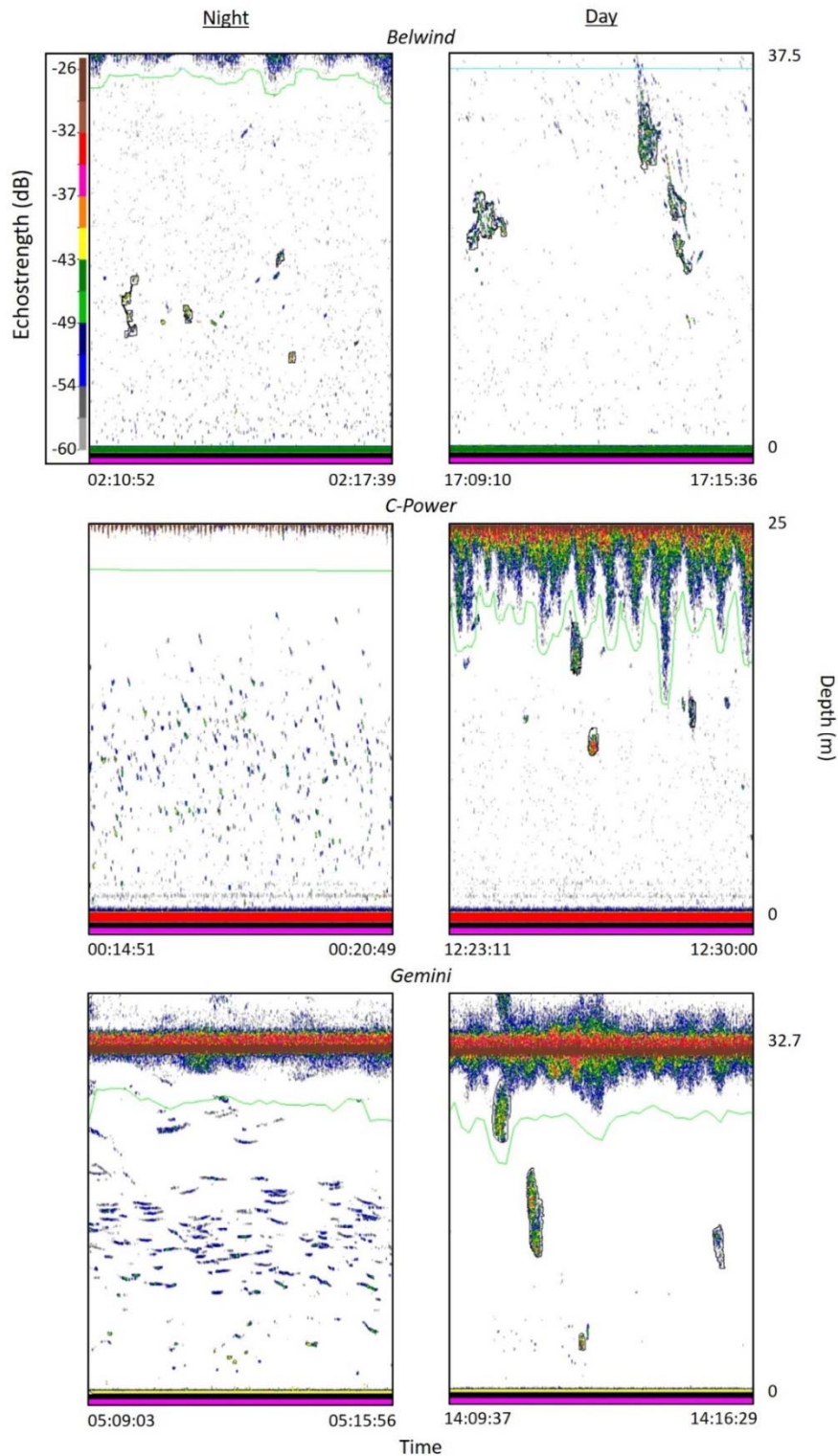


Fig. 5: Fish showed schooling behaviour during both day and night at Belwind. At C-Power and Gemini, however, fish schooled during the day but spread out over the water column at night. Sample echograms were taken from the same day (Belwind: July 18, C-Power: August 26, Gemini: November 24). Note that surface height and surface noise differ between day and night due to tidal influences and variation in wave height.

3.5.2.4.2 School data

We took behavioural measurements from the detected schools based on reported responses of fish to sounds of seismic surveys and pile driving (Fewtrell and McCauley 2012; Hawkins et al. 2014). Reported responses included changes in swimming depth and school cohesion. Swimming depth was measured as the mean distance of the school from the AZFP (which was always at the sea floor) in m. School cohesion was measured by the mean volume backscattering strength of the school (S_v) in dB re $1 \text{ m}^2/\text{m}^3$. An increase in the backscattering strength of the school equals an increase in the school density and thus indicates a smaller distance between individuals, i.e. a higher school cohesion.

Besides taking behavioural measurements per school, we measured fish presence by counting the number of schools present per hour, as well as the total school biomass per hour in NASC calculated per school-region per cell-bin (PRC_NASC, Eq. (1)). The school was divided up in bins of 1 m by 10 min, and only the area of the bin covered by the school was taken. In this way, we prevented that larger schools would get a disproportional impact on the data.

3.6 Statistical analysis

We investigated whether sound exposure influenced biomass depth, and fish school presence, schooling fish abundance, fish school swimming depth, and fish school cohesion. All statistical analyses were performed using R (version 3.5.1). Models were constructed using the biomass depth, school presence, schooling fish abundance, school depth and school cohesion as response variables and location (inside or outside the wind farm), wave height and tide (except in the model for school presence) as common explanatory variables. The model for biomass depth further included treatment (exposure or baseline), total biomass in the water column and temperature as explanatory variables, as well as an interaction between treatment and location. As temperature correlated strongly with period, we left temperature out of the school data models.

The models concerning school variables also included period (before, during, or after sound exposure) and an interaction between period and location. The models for school depth and school cohesion further included the vertical spread of the school as explanatory variable, since that could influence these response variables. Final models were selected using dredging (MuMIn package). After dredging, variable estimates were calculated by bootstrapping (10,000x). If estimates of the explanatory variables did not cross zero in the 95% confidence interval (CI), explanatory variables were considered to be of significant influence on the response variable.

Separate models were created per wind farm to account for the high variability between wind farms in time of year and location. All models used were either linear or generalised linear models. We found the optimal distribution by checking model diagnostics (e.g. the QQ-plot of the model) and by testing for models with higher log-likelihood scores using *lrttest* (*lmttest* package). School presence was evaluated in two ways: first, by applying a rotation test (*tagtools* package; DeRuiter and Solow 2008) that examined whether the pattern of school detection was similar during exposure and before and after periods. This rotation test was applied on the entire 12 days of Belwind and Gemini but was applied on the exposure days of C-Power separately, to account for the discontinuity of the exposure periods. Second, we investigated if the number of schools per hour changed during exposure, using a Hurdle model that consisted of two parts. The first part separately modelled the chance that a school is present by treating all data points larger than zero as 1 (binomial distribution). The second part of the model ignored all data points that are zero and only modelled the number of schools that are present (negative binomial distribution). This part could then tell if the number of schools present was explained by the explanatory variables.

3.6.2 School data

3.6.2.1 School presence

School presence was evaluated in two ways: first, by applying a rotation test on the data of Belwind and C-Power (*tagtools* package; DeRuiter and Solow 2008) that examined whether the pattern of school detection was similar during exposure and baseline periods. This rotation test was applied on the entire 12 days of Belwind but was applied on the exposure days of C-Power separately, to account for the discontinuity of the exposure periods.

Second, the number of schools per hour was used to investigate if the number of schools detected changed during exposure. The data followed a zero-inflated negative binomial distribution. Therefore, we used a Hurdle model that consisted of two parts. The first part separately modeled the chance that a school is present by treating all data points larger than zero as 1 (binomial distribution). The second part of the model ignored all data points that are zero and only modeled the number of schools that are present (negative binomial distribution). This part can then tell if the number of schools present is explained by the explanatory variables. The explanatory variables included in the full models were: Period (BEFORE, DURING, AFTER exposure), Location (Inside or Outside the wind farm), and Wave Height. As Temperature correlated strongly with Period, we left Temperature out of the school data models.

3.6.2.2 School biomass

The total school biomass per hour was calculated only for the hours in which schools were present. The data followed a normal distribution after $\log_{10}$ transformation, so a linear model was applied for all three wind farms. As explanatory variables, we included Period, Location, mean Water Depth per hour (calculated by adding tidal levels at the time point of school measurements to the average water depth per location), and mean Wave Height per hour.

3.6.2.3 School depth

The depth of each school was taken as the mean distance of the school from the transducer. Data followed a normal distribution for Belwind, had to be square-transformed for C-Power, and had to be 10-log-transformed for Gemini. After transformation, a linear model could be applied for data of all three wind farms. Explanatory variables of the full models included: Period, Location, Water Depth, Wave Height, and mean Height of the school (in m). Height of the school was included because it might influence how high a school can be in the water column.

3.6.2.4 School cohesion

Mean Sv (volume backscattering strength) generally followed a Gamma distribution, so we used a GLM with Gamma distribution for all three wind farms. Because a Gamma distribution requires positive data, we used the absolute Sv_mean values, instead of the measured negative values. As explanatory variables, we included Period, Location, Water Depth, Wave Height, and mean Height of the school.

4. RESULTS

4.1 General patterns

There was a distinct diurnal pattern of fish schooling during daylight and a layer of scattered individual fish in the water column at night with a clear transition between the two states at dawn and dusk in both C-Power and Gemini but not in Belwind (dusk; Fig. 6). Because the fish did not

school at night-time, we decided to exclude the data between dusk and dawn from the analyses for both C-Power and Gemini (roughly 19:00 – 04:00h for C-Power and 16:30 – 06:20h for Gemini). At Belwind, no such pattern was visible, so schools were measured during day and night. Distinct zooplankton layers could not be distinguished and resuspended sediment particles are a likely cause for interference, which we inferred from fluctuating conditions related to the tide (Fig. 7). Weather conditions varied considerably over the deployment periods, and calm to rough sea surface conditions were found for all wind farms, with decreased detection possibility of fish schools during rough sea states (Fig. 8). Wave height could reach up to ~2.5 m at all wind farms.

Fish schools were found both inside and outside the wind farm at all three wind farms. There were a median of 2 schools per hour at Belwind (N = 573, range = 0-53 schools per hour), 10 schools per hour at C-Power (N = 246, range = 0-106 schools per hour) and 7 schools per hour at Gemini (N = 251, range = 0-37 schools per hour). The median number of schools inside and outside the wind farm was roughly equal for Belwind (2 school per hour inside and outside) and Gemini (6 inside, 7 outside). For C-Power, there were considerably more schools outside the wind farm (17 schools) than inside (7 schools).

Abiotic characteristics influenced almost all biotic variables that were measured. Temperature negatively influenced the biomass depth, with the mean biomass being closer to the bottom when temperatures were higher (Table S3 in Supplements). Wave height led to deeper swimming schools, as well as influencing biomass depth, the number of schools per hour and school cohesion, although patterns for these other variables were not always consistent between wind farms (Tables S4-7). The third abiotic characteristic, tide, influenced all variables. Tidal influences were consistent for the abundance of schooling fish, which was higher at low tide than at high tide for all wind farms and for school cohesion, which was lower at low tide for two out of three wind farms.

4.2 Belwind

During the seismic exposure, the biomass depth outside the wind farm was significantly shallower than during the baseline (Fig. 9a & b; Table S4). Fish school presence also changed during the exposure by the seismic survey (Fig 9c & d; Table S5): fewer fish schools were present than before or after the exposure, although the abundance of schooling fish did not change. This indicates that fish were present in fewer, but larger schools during the seismic survey. The probability that a school was present did not change between periods, i.e. there was no change in the number of hours with schools present. This was matched by the non-significant result of the rotation test ($p > 0.1$). The fish schools that were present during exposure tended to swim shallower (non-significant trend; Fig. 9e; Table S6) and schools inside the wind farm were more cohesive (Fig. 9f; Table S7). After the seismic exposure, schools inside the wind farm were also more cohesive than before the exposure and schools both inside and outside the wind farm swam higher in the water column.

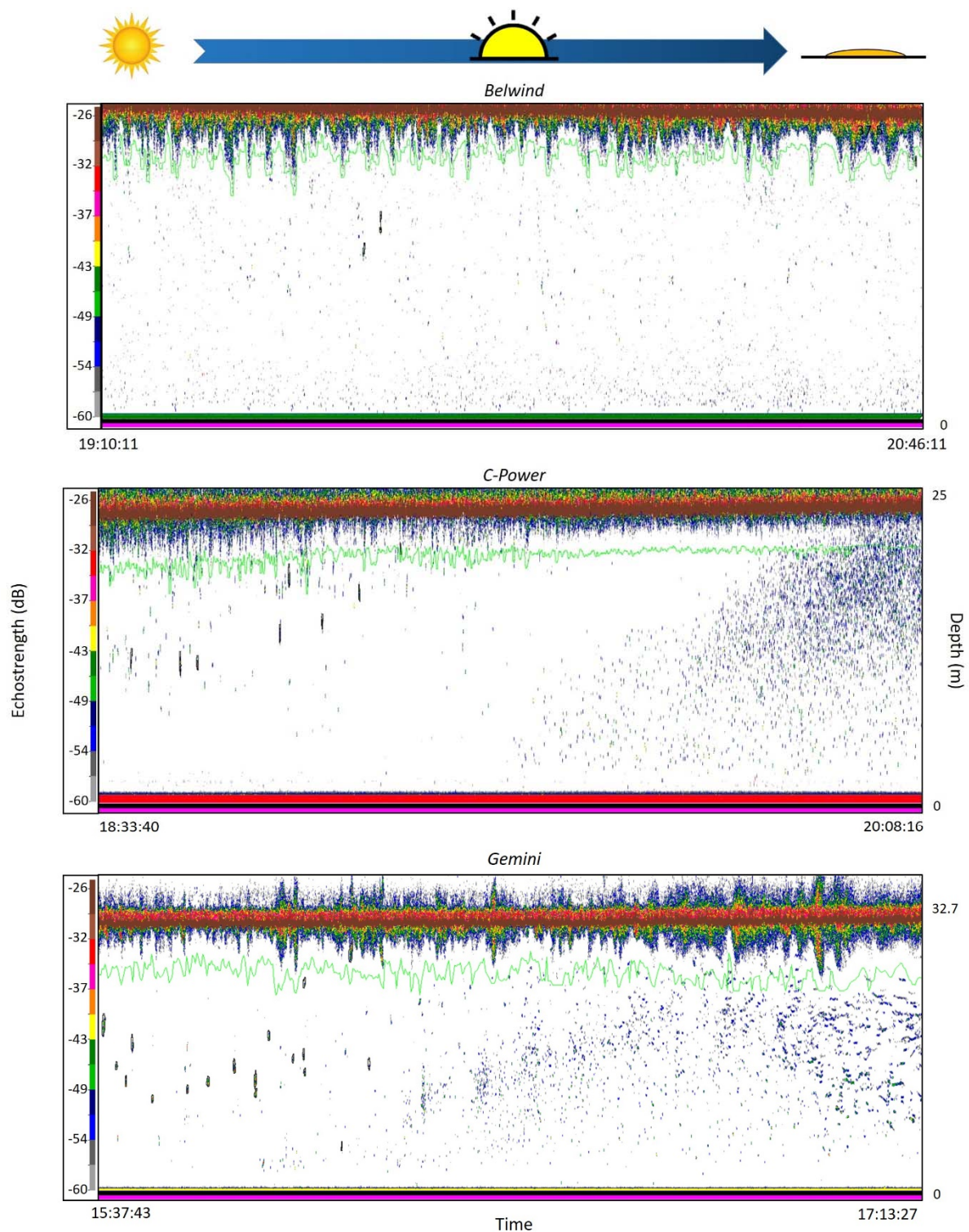


Fig. 6: At C-Power and Gemini, fish schools dispersed at dusk, with individual fish using the entire water column at C-Power and forming a layer in the middle of the water column at Gemini. At Belwind, such a pattern was not evident.

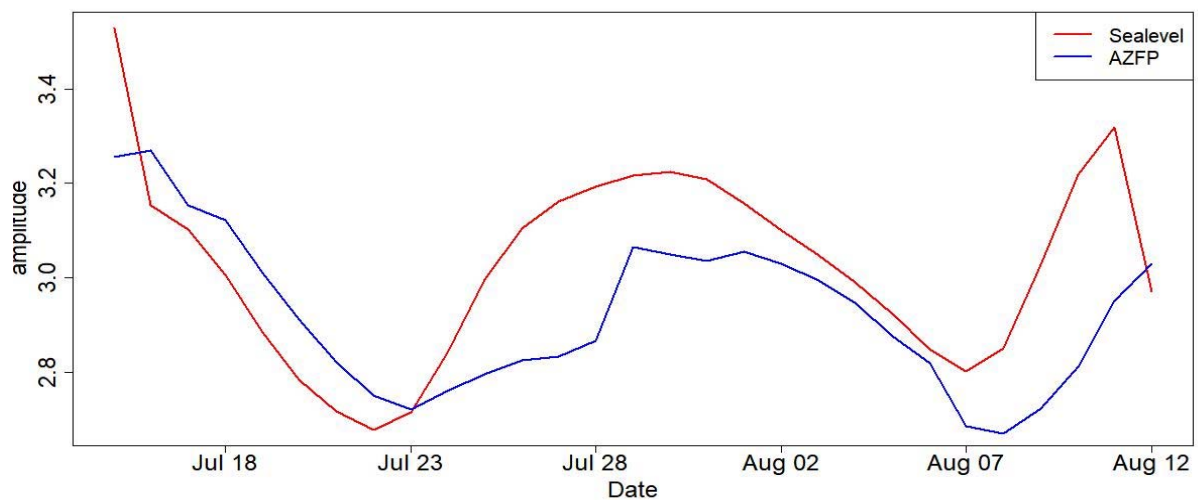


Fig. 7: Daily average fluctuations of the sea level (red) and the backscatter at 769 kHz (blue) from the echo sounder outside Belwind. The congruency between the patterns indicates that the backscatter is dominated by resuspended sediment particles, rather than zooplankton.

4.3 C-Power

The biomass depth was not significantly different from baseline, inside or outside, during the exposure to pile driving (Fig. 10a & b; Table S4). On each day of exposure, the probability of school detection in the actual hours of exposure also did not differ from the probability in the other hours of that day (rotation tests >0.1). However, the number of fish schools per hour as well as the probability that a school was present decreased in the AFTER period, without a change in abundance of schooling fish (Fig. 10 c & d; Table S5). In the DURING period, schools were present higher in the water column, but recovered after the exposure (Fig. 10e; Table S6). Inside the wind farm, school cohesion was significantly higher in the DURING period compared to BEFORE, while it decreased again AFTER exposure and was lower than BEFORE the exposure period (Fig. 10f; Table S7). Outside C-Power, however, there were no significant differences in cohesion between the periods.

4.4 Gemini

At the control wind farm Gemini, the biomass depth was not significantly different from baseline, inside or outside the wind farm (Fig. 11a & b; Table S4). However, the number of fish schools significantly increased in the DURING period (Fig. 11c & d; Table S5). No other significant factors were detected by the model for fish school numbers. The rotation test did not indicate a significant change in school presence pattern ($p>0.1$). The abundance of schooling fish tended to be lower in the DURING, but not the AFTER period. Combined with the results on the number of fish schools per hour, this means that there were more, similar-sized schools in the DURING period. Also, DURING schools swam higher in the water column compared to BEFORE as well as AFTER, and tended to be less cohesive (Fig. 11e & f; Table S6 & S7). In the AFTER period, school cohesion was the same as in the BEFORE period.

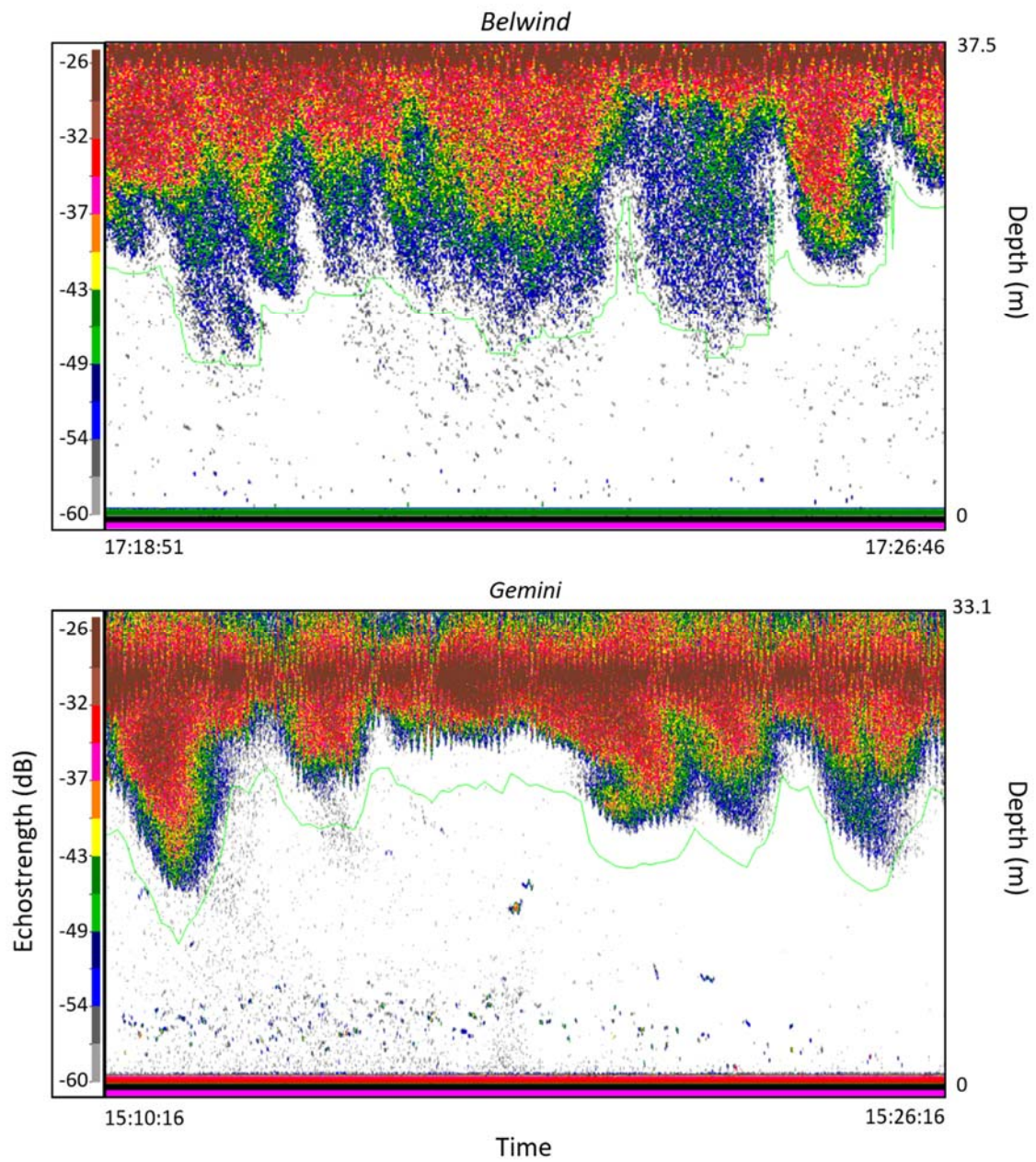


Fig. 8: Examples of rough weather conditions decreasing the detection area for fish schools at Belwind and Gemini. The warm-coloured area at the top (everything above the green line) is surface noise, while small, warm-coloured areas below the green line are fish(schools).

Belwind - Seismic

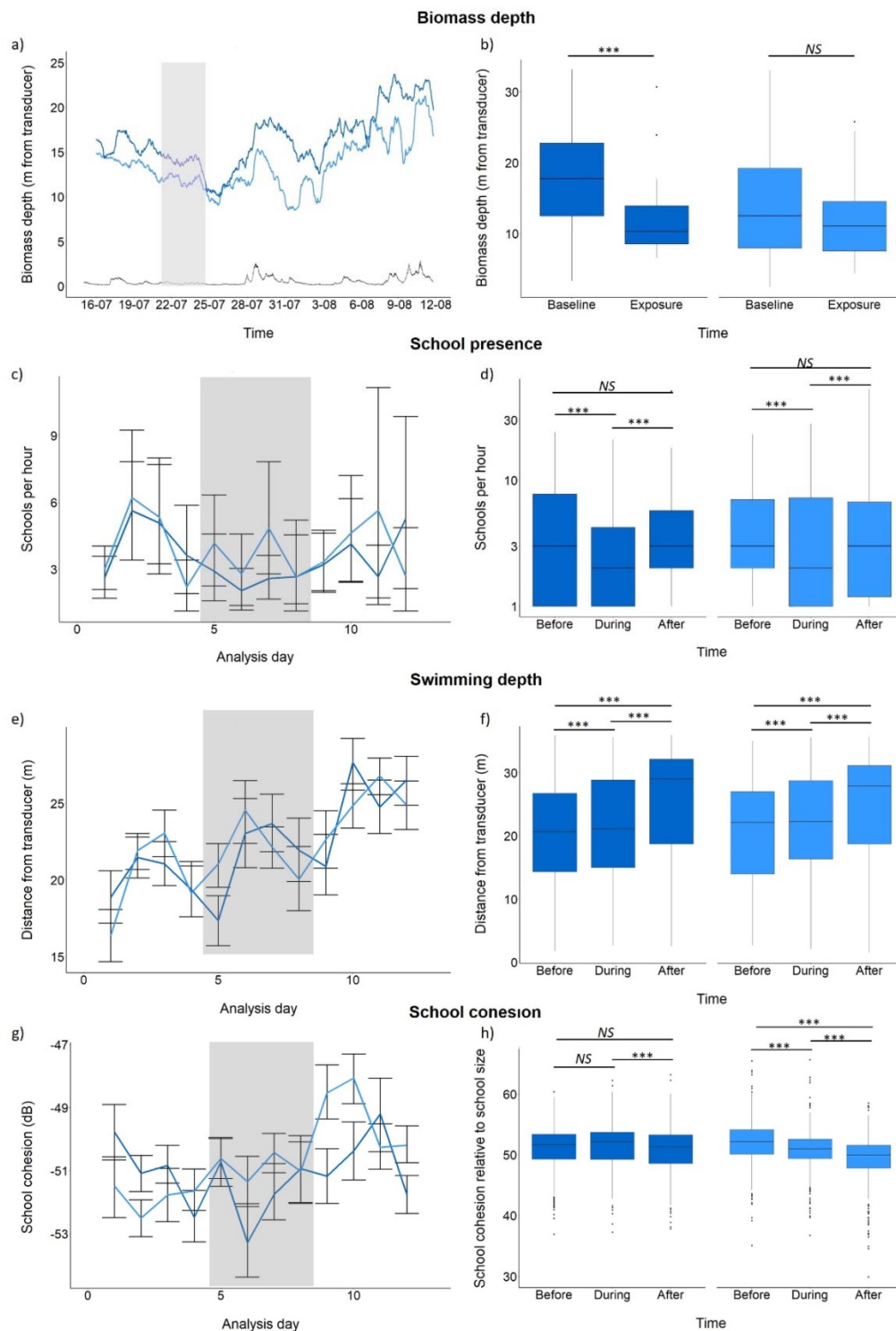


Fig. 9: The seismic survey correlated with changes in a & b) biomass depth, c & d) number of fish schools per hour, e&f) school swimming depth and g&h) school cohesion. Dark coloured lines and boxes represent the AZFP outside the wind farm, light coloured lines and boxes represent the AZFP inside the wind farm. Shaded areas depict exposure periods. Error bars (c, e, h) depict bootstrapped 95% CI. Boxplots (b, d, f, h) show median (black line), first and third quartile (box) and 1.5 inter-quartile range (whiskers). Dots represent any data point outside of this range. Biomass depth is depicted as a) rolling mean (window length: 24 h), with wave height (black line) and b) taken every 3 hours during baseline and exposure.

C-Power - Pile driving

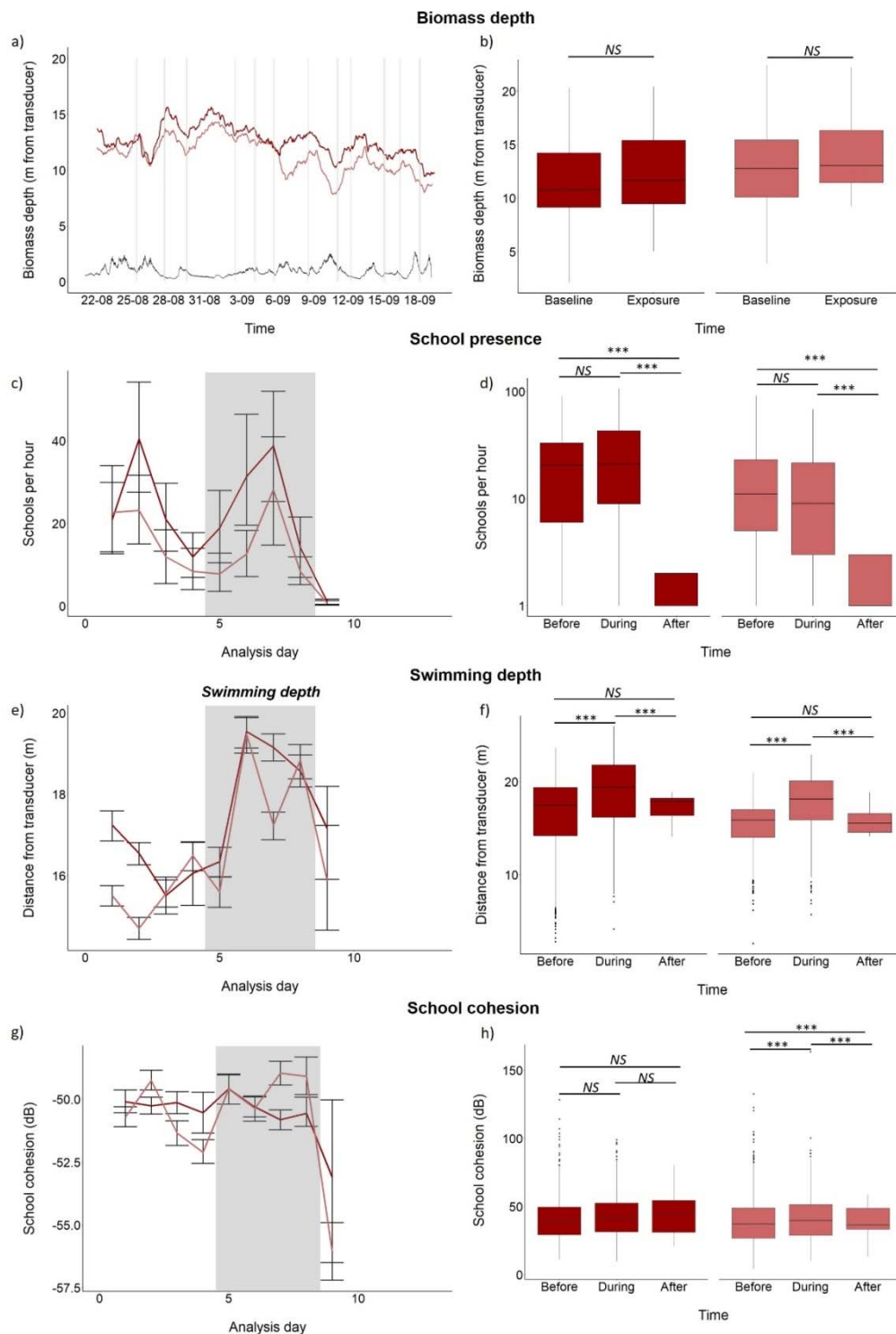


Fig.10: When fish were exposed to pile driving, there were no changes in a & b) biomass depth and c & d) number of fish schools per hour. The number of fish schools per hour did change after exposure. During exposure, school behaviour changed with schools e & f) swimming shallower and g & h) being more cohesive (only inside the wind farm). Dark coloured lines and boxes represent the AZFP outside the wind farm, light coloured lines and boxes represent the AZFP inside the wind farm. Shaded areas depict exposure periods (note the 12 brief exposure periods for the biomass depth in thin lines). Error bars (c, e, g) depict bootstrapped 95% CI. Boxplots (b, d, f, h) show median (black line), first and third quartile (box) and 1.5 inter-quartile range (whiskers). Dots represent any data point outside of this range. Biomass depth is depicted as a) rolling mean (window length: 24 h), with wave height (black line) and b) taken every 3 hours during baseline and exposure.

Gemini - Control

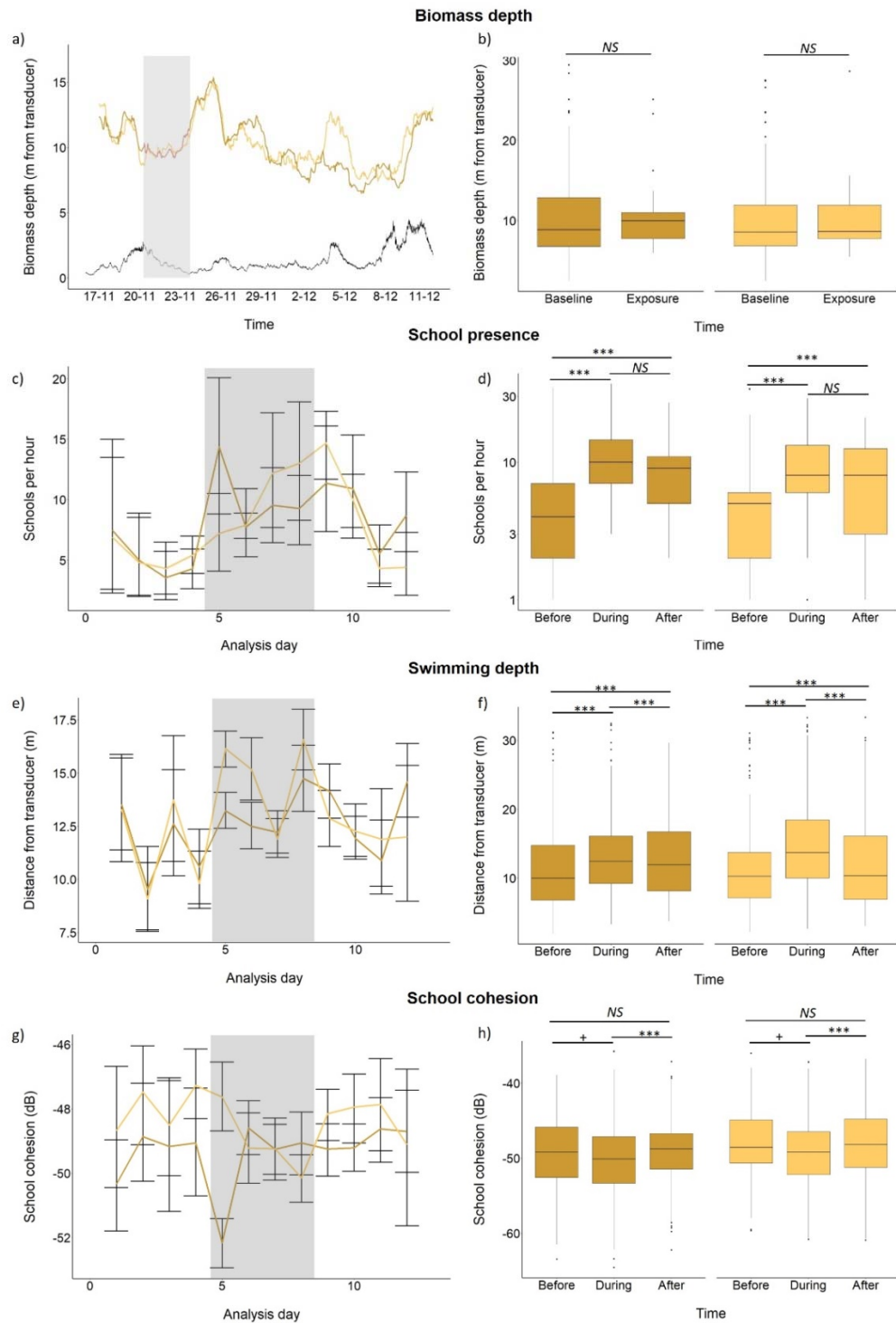


Fig. 11: At wind farm CONTROL, there were no changes in a & b) biomass depth during the no-exposure period. The number of fish schools per hour (c & d) did change in the DURING period. Also, in the DURING period fish schools e & f) swam shallower inside the wind farm and g & h) were less cohesive. Dark coloured lines and boxes represent the AZFP outside the wind farm, light coloured lines and boxes represent the AZFP inside the wind farm. Shaded areas depict exposure periods (note the 12 brief exposure periods for the biomass depth in thin lines). Error bars (c, e, g) depict bootstrapped 95% CI. Boxplots (b, d, f, h) show median (black line), first and third quartile (box) and 1.5 inter-quartile range (whiskers). Dots represent any data point outside of this range. Biomass depth is depicted as a) rolling mean (window length: 24 h), with wave height (black line) and b) taken every 3 hours during baseline and exposure.

5. DISCUSSION

The two bottom-moored echo sounders provided detailed insight into the presence, schooling behaviour, and swimming depth of pelagic fish. Floating particles in the turbulent, shallow water of the North Sea prevented detection of zooplankton. However, we were able to assess distinct patterns of variation for the pelagic fish community: among the three wind farms, within and outside the wind farm, and among the before, during, and after periods of four days, for the seismic survey and pile driving sound exposure site, and the control site. Exposure to the seismic survey was related to a deeper biomass centre of gravity (outside windfarm) and lower school numbers (both inside and outside windfarm). Pile driving was related to higher swimming and less cohesive schools. However, also the sham-exposure period at the control site revealed changes in the measured variables: more schools were present, which swam higher in the water column, and which were less cohesive.

Generally, we found very similar patterns inside and outside the windfarm, but abiotic conditions affected almost all of the parameters observed, indicating that these have to be considered when studying the responsiveness of fish schools to noisy human activities. The tidal conditions, for example, fluctuate in time, may also vary spatially, but will affect data from bottom-mounted echo-sounders anywhere (not only at wind farms). The current data set concerns a proof of concept with unreplicated treatments. This means that it shows that bottom-mounted AZFP-echo sounders are suitable to assess patterns of variation in sufficient detail to detect sound event related changes in presence, group cohesion, and swimming depth in the pelagic fish community, as shown for the data from Belwind and C-power. However, causal explanations for the correlations between exposure conditions and associated changes in fish schooling behaviour requires replication, as was shown by the significant variation in the control data from Gemini.

5.1 Changes in fish school presence and behaviour related to sound exposure

The number of fish schools per hour decreased during exposure to the seismic survey at Belwind. Earlier studies on reduction in catch rates and changes in fish abundance found varying results. Effects of seismic surveys on catch rates was difficult to disentangle from the inherent variability in catch rates due to natural fluctuations (Parry and Gason 2006; Thomson et al. 2014; Bruce et al. 2018). In another study, direct observations of reef fish abundance before and during a seismic survey nearby showed a marked decrease in the number of fish present, but mostly in the evenings (Paxton et al. 2017). The results of our study are in line with these earlier findings: the sound event-related decrease in fish schools is suggestive for fish leaving the area, or altering their behaviour such that they become invisible for the echo sounder, but large variability in fish school abundance calls for caution in this interpretation. Proper replication is required before drawing conclusions about sound event related deterrence.

Sound events were related to changes in schooling behaviour with fish schools going up in the water column in response to both the seismic survey and the pile driving, although they also went up at the control site. School cohesion became less during both sound exposures, while it increased at the control site. Interestingly, our results do not follow the general pattern found in literature of fish diving to deeper water upon acoustic disturbance (Slotte et al. 2004; Doksæter et al. 2012; Fewtrell and McCauley 2012; Hawkins et al. 2014; Neo et al. 2014). However, there are also some studies that report fish swimming higher in the water column, either during or immediately after exposure (Chapman and Hawkins 1969; Sarà et al. 2007; Neo et al. 2015). The variation in response tendency and nature reported may depend on species type (bento-pelagic going down; pelagic going up or down), but also on exposure type (nearby vessels or seismic surveys coming from the surface; pile driving, dredging, or mining having a more diffuse directionality from whole water column), and other environmental (see below 5.2) or previous exposure conditions. We find the discrepancy

between the patterns found for fish schools and the total pelagic biomass in our data is difficult to explain. It could be caused by behavioural differences in schooling fish compared to other species that make up the pelagic biomass, but this would have to be verified in future research.

School cohesion became higher during both sound exposures, while it decreased at the control site. Typically, fish schools initially decrease cohesion with a sudden exposure, followed by increased school cohesion (Doksæter et al. 2012; Fewtrell and McCauley 2012; Hawkins et al. 2014; Neo et al. 2014; Neo et al. 2015). Since the reports in the literature are typically observations over brief time periods (minutes to hours), while we report a response pattern analysed at a resolution of days, the increased school cohesion found matches with what would be expected for long-term responses of fish schools to sound. The consistency of this pattern between both exposure sites suggests that school cohesion is a variable that should be measured in any future investigations into effects of sound exposure that lasts for longer periods of time.

5.2 Effect of abiotic conditions

Fish behaviour was affected by wave height and water temperature, as well as tide in some cases. We found that fluctuations in wave height affected swimming depth and school cohesion, with fish shifting down and (at Belwind) becoming more coherent schools in rough weather. Such weather dependent patterns have been reported before (e.g. Kaartvedt et al. 2017) and have been attributed to be a response to a decreased visibility (Tsuda et al. 2006) and a destratification of the water column (Secor et al. 2019). These patterns have further been correlated to increased wind speed (Lagardère et al. 1994) and a drop in barometric pressure (Heupel et al. 2003). Sensitivity to weather conditions may be species-specific and future integration with species (group) identification may yield more insights in this respect (e.g. distinguishing between species with swim bladder, such as herring and sprat, and without swim bladder, such as sandeel and mackerel).

Another interesting pattern was distinct variation in schooling behaviour between day and night. Typical nocturnal behaviour with a release of clustering in schools to spread out individually across the water column was found for C-Power and Gemini, but not for Belwind. There could be several explanations for this. The dominant fish species may have been different for the different wind farms in the sampling periods and species may vary in their tendency to break up schools nocturnally. Sandeel, for example, is known to school during day time and hide for predators during night time, burrowed in the sand (Freeman et al. 2000). However, maybe an even more logical explanation is that Belwind was sampled first in the summer with long daylight periods, while C-Power and Gemini were sampled later in the autumn to winter, with already much shorter days and more distinct nocturnal parts of the day.

5.3 Using echo sounders for monitoring long-term changes in fish behaviour

This study has shown the potential of echo sounders for studying long-term changes in the behaviour of pelagic fish. The advantage of using bottom-moored echo sounders over a longer period of time, is that the behaviour can be studied at various time scales. In this study, we focussed on long-term changes in the order of days, but shorter-term changes, such as the transition from schooling to individual swimming at dusk, can be studied as well (c.f. Freeman et al. 2004). Depending on the duration a school is in view of the echo sounder, it might even be possible to study behaviour at the scale of seconds to minutes, as has been done previously with echo sounders that were not bottom-moored but towed from a little boat (Hawkins et al. 2014). Additional analyses planned for the current data set concerns a finer time scale to test for changes in water column use and schooling behaviour of pelagic fishes in response to pile driving at C-power (not days, but hours).

Fish behaviour, especially schooling behaviour, can vary considerably with daylight, tide, wave height and temperature. This was evident from all the behavioural variables measured. Inherent variability in the data may lead to false positive results, for example if a natural peak in the data coincides with the exposure event. Therefore, it is important to use considerable replication for exposure and control sites, as well as allowing for enough baseline data per site. At the same time, data from the two echo sounders placed in each wind farm were strikingly similar. This suggests that data from one echo sounder may be representative of an area that is far larger than the beam width of the echo sounder itself. Our results concur with a previous study that combined data from moored echo sounders with those of ship-based surveys and found a homogeneity of fish density distribution of 50 nmi² (De Robertis et al. 2018).

A better understanding of fish behaviour may be achieved when echo sounder deployment is combined with bio-sampling to verify the species composition on site. One goal of this project was to investigate the effect of sound on zooplankton behaviour. However, no zooplankton layers were visible in the echograms. Bio-sampling could reveal if zooplankton was indeed absent during the deployment, or whether the zooplankton was simply not picked up by the echo sounder. Another consideration is the use of multi-beam echo sounders or even an autonomous vehicle with a mounted echo sounder (Chu 2011; Benoit-Bird et al. 2017). Multi-beam echo sounders make it possible to study horizontal movement, while an autonomous vehicle can get close to fish schools and follow them over a longer period of time, thereby getting detailed information of the behaviour of a particular school. Regardless, bottom-moored single-beam echo sounders, as used here, are very well capable of monitoring short-term vertical movements of fish (an often observed change in response to sound) and long-term changes in fish school presence and dynamics.

5.4 Conclusions

We have shown that bottom mounted AZFP-echo sounders are a very suitable method to monitor fluctuations in time and space for pelagic fish – but not for zooplankton – in a relatively shallow part of the Belgian and Dutch North Sea in the vicinity of offshore wind farms. We found considerable variation among wind farms, which could be related to site, period of sampling, and weather conditions. We also found some variation, but largely similar fluctuations in time, for the within and outside wind farm sampling locations, which were just a few kilometres apart and sampled synchronously. The data were also suitable to detect density and behavioural changes related to the sound exposure events of an experimental seismic survey and pile driving activity for another nearby wind farm. Fish at Belwind and C-power were less abundant and swam shallower in less coherent schools in the days during the exposure than in the days before the exposure. However, we refrain from drawing strong conclusions about a causal relationship here as we stress that these data concern case studies and just serve as a proof of concept. The sound event related changes in fish density and schools are unreplicated samples of patterns that fluctuate in time naturally. This was shown clearly by significant changes in fish school presence and fish behaviour at our control site in the Gemini wind farm. We therefore recommend continuing the use of bottom-mounted echo sounders for the future, like performed in the current study, and then replicated at the same and more wind farms in the North Sea region (Belgium, Netherlands, UK, Denmark, Germany). We also believe that placement within, just outside, or maybe further away if possible, from wind farms in a well-replicated design (at least 6x2, but aiming for 10x2) should yield a better understanding of the pelagic fish community and potential effects of wind farm ecology and associated anthropogenic noise on biomass presence, species diversity, and schooling behaviour.

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