The noise of the hunt: Effects of noise on predator-prey relationships in a marine ecosystem

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How noise affects prey

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

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Abstract

Anthropogenic noise in the oceans is disturbing marine life. Pelagic fish are a group of species that are also likely to be affected by noise, but have received little attention because of the difficulties of studying them. In wind farms, pelagic fish abundance could be higher than in other areas, due to fisheries restrictions. At the same time, the wind farm construction involves a lot of anthropogenic noise, likely disturbing marine life. Here, we investigate whether bottom-moored echosounders are a suitable tool for studying the effects of anthropogenic sound on pelagic fish in wind farms and explore the types of responses to anthropogenic noise exhibited by pelagic fish. Two bottom-moored echosounders were placed in three different wind farms along the Dutch and Belgian coast, recording presence and behaviour during exposure to the passing by of a full-scale airgun array from an experimental seismic survey and pile driving activity in an adjacent wind farm construction site. The bottom-moored echosounders were successful in detecting variation in the behaviour of pelagic fish residing at the wind farms. Patterns of behaviour and detection of fish schools were significantly different during sound exposure compared to pre-exposure, with fewer, more cohesive schools during the seismic survey and more cohesive schools swimming higher in the water column during pile driving. The types and magnitudes of responses, however, were also observed at the control site. While this stresses the need for thorough replication when investigating responses of pelagic fish to sound exposure, our results indicate that both sound from a seismic survey as well as from pile driving could have the potential to disturb pelagic fish school behaviour.

Introduction

Many aquatic animals change their behaviour in response to increased ambient noise levels. Effects of sound on behaviour range from local changes in water column use (Hawkins et al. 2014b; Neo et al. 2014), to horizontal avoidance of noisy 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; Chapter 6). 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). Specifically, changes in predator-prey interactions have a strong potential to translate to effects on the ecosystem as a whole (Kunc et al. 2016).

Predator-prey interactions are characterized by specific behaviour of the predator – hunting – and responding behaviour of the prey – hiding, escape and defence. 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 cluster to improve protection and reduce risk of attack 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. 2014b; Neo et al. 2014; Rieucau et al. 2014). At the same time, predators aim 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 clusters of animals in deep scattering layers often target their prey at night, when the deep scattering layer migrates closer to the surface (Au et al. 2013; Giorli et al. 2016). Therefore, environmentally induced 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 disadvantageous, for instance if prey become more cohesive and move deeper down the water column, and therefore become more difficult to catch (Voellmy et al. 2014b). In fact, how prey respond to increased noise levels could even determine the response of predators to sound. Cuvier's beaked whales (*Ziphius cavirostris*), for instance, stayed in an area that was frequently disturbed by military sonar exercises, but also contained high prey densities, and did not alter their hunting grounds to an adjacent canyon 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. (2014b) 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). Apart from these studies, that require follow-up and replication, a 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. More and more offshore wind farms are being constructed 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, however, 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 different and more diverse benthic community develops (Lindeboom et al. 2011; Ashley et al. 2014; Raoux et al. 2017), which may also affect the local pelagic community. Therefore, changes in pelagic fish behaviour due to anthropogenic noise can only be discovered with long-term measurements that control for the natural development of the community.

A way to study long-term presence and behaviour of pelagic fauna is through the use of bottom-moored echosounders. An echosounder system operates by transmitting ultrasonic pulses, which are backscattered by objects and detected by a receiver (Lurton 2002). Because of their non-invasiveness and spatial resolution, echosounders are widely used to identify individual species and to observe fish school behaviour in the water column. They are also used to monitor changes in school cohesion and swimming depth (Gerlotto et al. 2004; Weber et al. 2009; Guillard et al. 2010; Hawkins et al. 2014b; Fraser et al. 2018), which are among the expected responses to seismic surveys and pile-driving (Lawson et al. 2001; Colbo et al. 2014; Benoit-Bird et al. 2017).

Here, we explored the effects of two types of anthropogenic sound events – an experimental seismic survey and nearby pile driving – on the spatial behaviour of pelagic fish in the North Sea. We investigated whether bottom-moored echosounders are a suitable tool for studying the effects of anthropogenic sound on pelagic fish, and whether there are longer-term (days) changes in pelagic biomass or behaviour that can be correlated to seismic survey or pile driving sounds. To be able to observe spatial behaviour of the pelagic fish layer, we deployed a pair of bottom-moored echosounders that recorded the entire water column over the course of a month, replicated at three locations in three subsequent periods. By placing an echosounder both inside and outside the wind farms, we aimed to sample the variation in pelagic fish related to these ecologically distinct locations with either rocky or sandy bottom. We expected anthropogenic noise exposure to be correlated with deeper swimming pelagic biomass, as well as fewer, more cohesive, and deeper swimming fish schools.

Materials and methods

Echosounders

Two Acoustic Zooplankton Fish Profilers (AZFPs, ASL Environmental Sciences, Canada) were deployed consecutively at three wind farms. Both AZFP echosounder sets emitted four frequencies, of which three were shared (due to the availability of this equipment at ASL): 125, 200 and 455 kHz. The first and second AZFP also transmitted at 38 and 769 kHz, respectively. The first AZFP was always placed inside the wind farm, whereas the second AZFP was always placed outside the wind farm. Both AZFPs were moored on a frame at the seafloor, at an average depth of 32 m, and had a vertical upward beam (Table 1). The AZFPs recorded 25 consecutive days per location with a ping rate of 1 Hz (sound pulses emitted by the echosounder). Data was extracted after retrieval of the AZFPs.

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

Location	**Exposure**	**Deployment (2018)**	**Recordings (2018)**	**Location AZFP**	**Depth of deployment (m)**
Wind farm SEISMIC	Seismic survey	10/7 – 20/8	15/7 – 11/8	Inside	37.5
				Outside	38
Wind farm PILE	Pile driving	20/8 – 22/9	22/8 – 22/9	Inside	24
				Outside	25
Wind farm CONTROL	Control	13/11 – 12/12	18/11 – 18/12	Inside	32.7
				Outside	33.1

Study locations

The AZFPs were placed at two wind farms in the Belgian and one wind farm in the Dutch North Sea: 1) Belwind – an offshore wind farm situated on Bligh bank, 40 km from the Belgian coast, 2) C-Power – an offshore wind farm situated at Thornton bank, 27 km off the Belgian coast and 3) Gemini, located 85 km from the Dutch coast, north of Schiermonnikoog (Fig.1). Belwind (wind farm SEISMIC) was exposed to an experimental seismic survey over a period of four days (Fig. 2; PCAD4Cod project, Slabbekoorn et al. 2019). C-Power (wind farm PILE) was exposed to pile driving from a nearby wind farm for 12 separate days during the AZFP deployment period for the construction of the offshore wind farm Norther. Gemini (wind farm CONTROL) was not exposed to any particular anthropogenic activity, other than shipping noise from local maintenance traffic and a nearby shipping lane and functioned as the control.

To investigate differences between the pelagic fish inside and outside the wind farm, at all three locations, 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. AZFPs were always placed at equal distance to the anthropogenic noise disturbance, such that noise levels would not differ between the AZFP inside and outside the wind farm. The AZFPs were placed consecutively in the three wind farms: first at wind farm SEISMIC, next at wind farm PILE and finally at wind farm CONTROL (Table 1).

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 for locations). Water temperatures were quite constant per wind farm: they varied between 16.8 – 18.7 °C at wind farm SEIS-

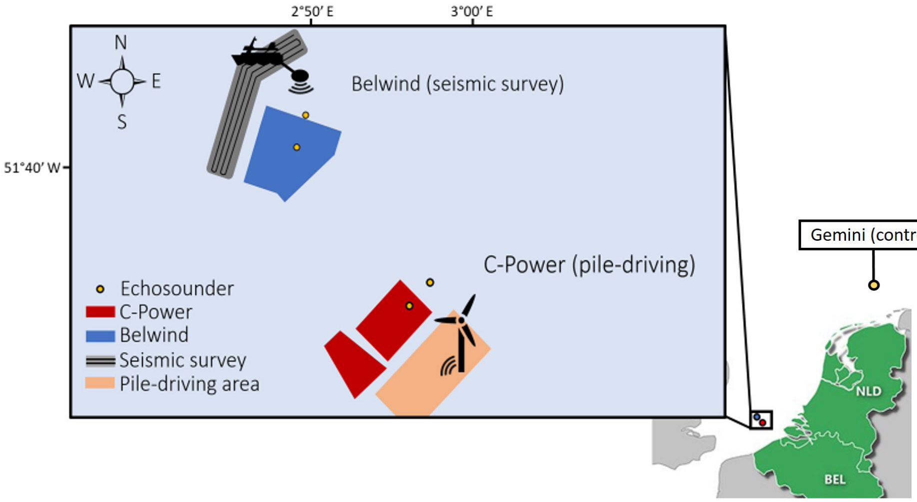

Figure 1: Schematic overview of the placement of the AZFP echosounders (yellow dots in inset) at the two wind farms in the Belgian North Sea. Note the roughly equal distance of the AZFPs to the track of the seismic survey (Belwind) and to the pile driving area (C-Power). At Gemini, there were no periods of impulsive anthropogenic noise during these measurements in the Dutch North Sea.

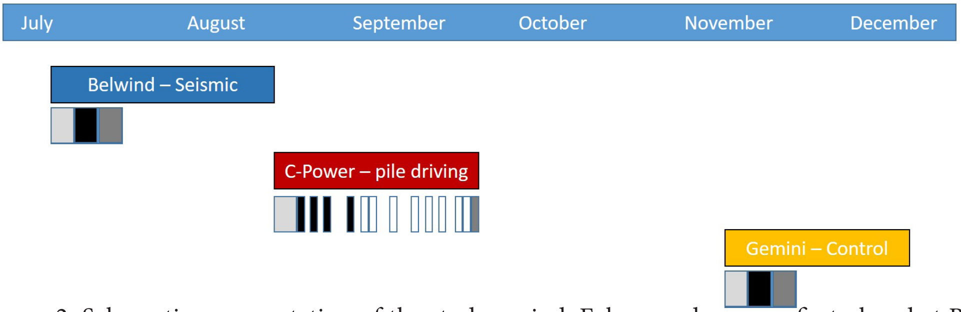

Figure 2: Schematic representation of the study period. Echosounders were first placed at Belwind (wind farm SEISMIC), next at C-Power (wind farm PILE) and finally at Gemini (wind farm CONTROL). Pelagic biomass was measured for the entire recording period (coloured named boxes). Fish schools were measured for four days before the exposure (light grey boxes), four days during the exposure (black boxes), and four days after the exposure (dark grey box). At C-Power pile driving exposure took place on 12 separate days, the first four of which were taken as exposure period (black boxes: measured exposure days; white boxes: not measured exposure days).

MIC, 17.3 – 19.2 °C at wind farm PILE, and 9.0 – 10.8 °C at wind farm CONTROL. Distance between the inside and outside frames ranged from 2.3-3.0 km (Table S2). The echosounder locations inside wind farm SEISMIC and wind farm PILE were 15.52 km apart, while the echosounder location inside wind farm CONTROL was 333 km (in a straight line) from both locations inside wind farms SEISMIC and PILE.

Soundscape

The wind farms differed in the soundscape at the time of measurement. Sound measurements at wind farm SEISMIC 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 95-110 dB re 1 μPa^2/Hz at 10-500 Hz at wind farm SEISMIC and 56-87 dB re 1 μPa^2/Hz at 10-500 Hz at wind farm PILE. Ambient sound levels at wind farm CONTROL were not measured during the study but have been reported to range from 80-100 dB re 1 μPa^2/Hz at 10-500 Hz on average in 2013 (Lucke 2015).

Sound exposure

A full-scale airgun array was used for an experimental seismic survey at wind farm SEISMIC from 21-24 July 2018 (Fig. 2). Sound levels at the echosounder ranged from 123 to 195 dB re 1 μPa SPL_p. The survey used 36 airguns (G-Gun II Sercel, 50% operating at a time) with a total volume of 5900 m3 (carried out by CGG, Norway, with the MV Geo Caribbean). 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.

Pile-driving was carried out next to wind farm PILE at a new wind farm site (51° 32’ N, 3° 2’ E) during the construction of additional wind turbines in an adjacent plot. Sound levels for one pile driving period were on average 172 dB re 1 μPa 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 twelve days within this period, all separated by one or more days of no activity. Of the twelve pile-driving days, the first four days were used for the behavioural analysis (Fig. 2). On those four days, all the pile-driving was carried out at daytime. The average pile-driving duration on these days was 148 minutes (range 100-180 minutes).

Observation protocol

To investigate changes in pelagic fish abundance and behaviour between exposure and control conditions, we analysed biomass and school characteristics form the echosounder data. Total biomass was calculated per 1 m depth and 10 min bins for the entire survey period. The exposure period was the entire duration of sound exposure per site, with a control period of sham exposure for wind farm CONTROL (Table S3). All data points that did not fall in the exposure period were considered to be baseline. The presence of fish schools, as well as their distribution and size were measured during 4 day-periods before, during and after the exposure. The 4-day period was selected based on the duration of the seismic survey exposure (Table S3).

For wind farm SEISMIC, 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 wind farm PILE were not 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). Even though pile driving only took place during part of those days, the entire days were sampled as DURING, to maintain similarity in measurement set-up with the other two wind farms. The BEFORE period of wind farm PILE started on the first day of deployment, after an eight-day silent period without pile driving in the area. The AFTER period started on the first day after an exposure period. Piling activity stopped one day before retrieval of the AZFPs, so the AFTER period lasted only one day. There was no period of four days without piling earlier in the deployment. For wind farm CONTROL, the first twelve days of deployment were arbitrarily selected as a control BEFORE, DURING, and AFTER period.

Data analysis

All data analyses were performed using Echoview 9 (Echoview Inc.). The raw data were pre-processed to filter out noise and facilitate school detection (Fig. S1; *sensu* Trygonis et al. 2009). 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 between 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 fish 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 single, but relatively large fish or fish schools of small or large fish. 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 put over 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, echoes that were close to the surface or bottom were regarded as noise (i.e. waves, sediment particles) and were excluded from the data.

Fish school detection

Fish schools were detected automatically using a built-in school detection function of Echoview (detection settings, Table S4). 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 false negatives by the algorithm. Schools were defined in two ways: 1) at least three separate traces of potential fish reflections, present in the same ping (sound pulse from the echosounder), 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. 3). Both school types had to be visible for at least 3 pings before being considered a school.

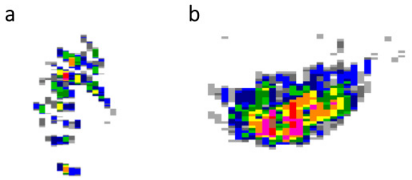

Figure 3: Different forms of a fish school in an echogram. (a) Separate traces with increased echo strength. (b) An area of increased echo strength without any evident separate traces. Colours indicate echo strength intensity, with warmer colours (e.g. orange, pink, and red) being a higher echo strength.

Pelagic biomass measurements

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:

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

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:

$$Centre\ of\ gravity = \frac{\sum momentum}{\sum NASC} \tag{2}$$

With

$$momentum = NASC * D \tag{3}$$

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.

Fish school measurements

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, Roberts, et al. 2014). Reported responses included increased swimming depth and increased 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 (Sv) in dB re 1 m^2/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 biomass of these schools per hour (schooling fish abundance) 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. This way, larger schools would not be overweighed in the data. These values were then summed over each hour.

Statistical analysis

We investigated whether sound exposure influenced biomass depth, as well as school presence, schooling fish abundance, school swimming depth, and school cohesion. All statistical analyses were performed using RStudio (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.

The models concerning school variables also included period (before, during, or after sound exposure) and an interaction between period and location. As temperature correlated strongly with period, we left temperature out of the school data models. 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 (Table S5). 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 lrtest (*lmtest* 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 wind farm SEISMIC and CONTROL but was applied on the exposure days of wind farm PILE 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.

Results

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 wind farm PILE and wind farm CONTROL but not in wind farm SEISMIC (dusk; Fig. 4). Because the fish did not school at night-time, we decided to exclude the data between dusk and dawn from the analyses for both wind farm PILE and wind farm CONTROL (roughly 19:00 – 04:00h for wind farm PILE and 16:30 – 06:20h for wind farm CONTROL). At wind farm SEISMIC, no such pattern was visible, so schools were measured during day and night. 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. 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 wind farm SEISMIC (N = 573, range = 0-53 schools per hour), 10 schools per hour at wind farm PILE (N = 246, range = 0-106 schools per hour) and 7 schools per hour at wind farm CONTROL (N = 251, range = 0-37 schools per hour). The median number of schools inside and outside the wind farm was roughly equal for wind farms SEISMIC (2 schools per hour inside and out-

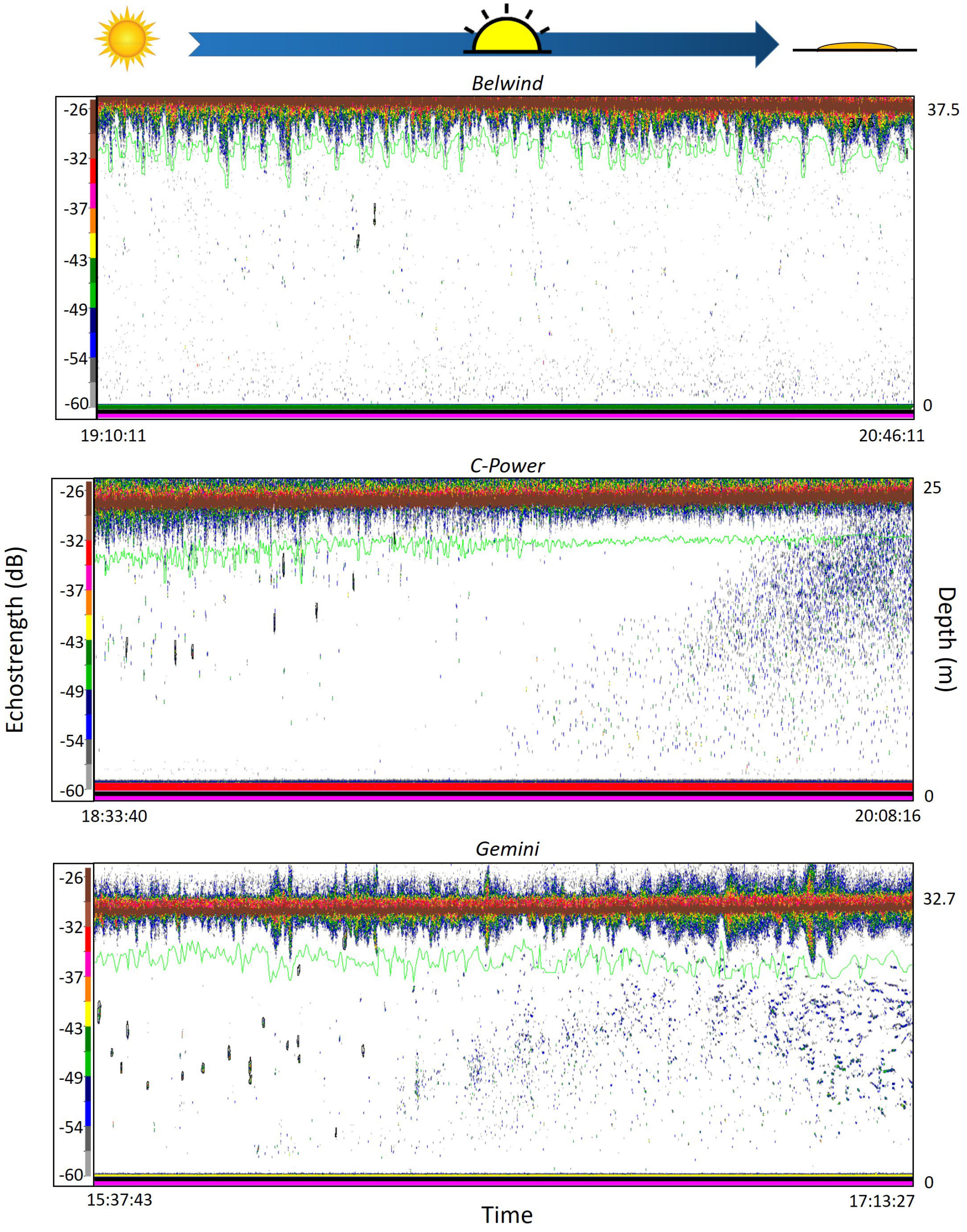

Figure 4: AZFP echosounder view for the period around dusk at the three locations. At wind farms PILE and CONTROL (middle and bottom panel), fish schools dispersed at dusk, with individual fish using the entire water column (PILE) or forming a layer in the middle of the water column (CONTROL). At wind farm SEISMIC, the schooling pattern did not change over the day.

Table 2: the biomass depth was influenced by several factors, as shown by general linear models of wind farm SEISMIC, wind farm PILE and wind farm CONTROL data. Significant factors were found by bootstrapping estimate values. If the 95% CI did not cross zero, factors were considered of significant influence (indicated with an asterisk). Note that since the models used a Gamma distribution, estimate values have to be converted before comparing to the data.

Wind farm	**Variable**	**2.5% limit estimate**	**97.5% limit estimate**	**5% limit estimate**	**95% limit estimate**
SEISMIC	Intercept*	0.086	0.21	0.095	0.20
	Treatment				
	Exposure*	0.0065	0.035	0.0087	0.033
	Location				
	Inside*	0.0099	0.021	0.011	0.020
	Wave height*	-0.017	-0.0081	-0.016	-0.0088
	Temperature*	-0.0078	-0.0010	-0.0071	-0.0015
	Total biomass	$-2.87*10^{-5}$	$9.5*10^{-6}$	$-2.72*10^{-5}$	$4.38*10^{-6}$
	Treatment:Location	-0.037	0.0056	-0.033	0.002
PILE	Intercept*	0.13	0.28	0.14	0.27
	Treatment				
	Exposure	-0.013	0.0066	-0.012	0.0043
	Location				
	Inside*	0.0046	0.013	0.0053	0.012
	Wave height*	0.012	0.021	0.013	0.020
	Temperature*	-0.012	-0.0039	-0.011	-0.0043
	Tide*	-0.012	-0.0054	-0.012	-0.0060
CONTROL	Intercept*	0.11	0.20	0.12	0.19
	Treatment				
	Exposure	-0.0073	0.019	-0.0049	0.017
	Temperature*	-0.011	-0.0018	-0.010	-0.0025
	Tide*	0.00057	0.022	0.0022	0.020

side) and CONTROL (6 inside, 7 outside). For wind farm PILE, 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 2). 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 2-5). The third abiotic characteristic, tide, influenced all variables (Tables 2-6). 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.

Table 3: model results of number of schools per hour. Note that covariate estimates were produced using a negative binomial (Count) and binomial (zero) Hurdle model, so the estimate values have to be converted before comparing to the data.

Wind farm	**Model part**	**Variable**	**2.5% limit estimate**	**97.5% limit estimate**	**5% limit estimate**	**95% limit estimate**
SEISMIC	Count	Intercept*	0.74	1.76	0.85	1.69
		Period				
		During*	-0.74	-0.018	-0.68	-0.075
		After	-0.35	0.39	-0.28	0.33
		Location				
		Outside+	-0.60	0.0048	-0.56	-0.044
		Wave height	-0.64	0.24	-0.57	0.17
		Tide	-0.19	0.35	-0.15	0.31
	Zero	Intercept*	0.36	1.38	0.44	1.28
		Period				
		During	-0.72	0.27	-0.63	0.20
		After	-0.086	0.11	-0.78	0.033
		Location				
		Outside	-0.088	0.68	-0.024	0.63
		Wave height*	0.072	1.36	0.15	1.22
		Tide+	-0.64	0.011	-0.58	-0.043
PILE	Count	Intercept*	2.80	3.90	2.89	3.81
		Period				
		During	-0.57	0.12	-0.51	0.069
		After*	-4.68	-2.55	-4.35	-2.69
		Location				
		Outside*	0.16	0.72	0.20	0.68
		Wave height*	-0.95	-0.16	-0.88	-0.22
		Tide*	0.095	0.61	0.14	0.56
	Zero	Intercept*	2.24	20.34	2.52	10.75
		Period				
		During*	-17.99	-0.058	-4.93	-0.34
		After*	-20.95	-2.85	-8.96	-3.08
		Location				
		Outside*	0.12	2.95	0.32	2.58
		Wave height	-2.55	1.30	-2.16	0.97

Table 3 continued.

Wind farm	**Model part**	**Variable**	**2.5% limit estimate**	**97.5% limit estimate**	**5% limit estimate**	**95% limit estimate**
CONTROL	Count	Intercept*	1.36	1.90	1.40	1.86
		Period				
		During*	0.32	0.93	0.37	0.88
		After*	0.19	0.79	0.24	0.74
		Location				
		Outside	-0.086	0.34	-0.048	0.31
		Tide	-0.39	0.11	-0.35	0.066
	Zero	Intercept*	1.82	4.69	1.95	4.34
		Period				
		During+	-0.10	2.40	0.090	2.08
		After*	0.22	4.34	0.42	3.73
		Location				
		Outside*	-3.21	-0.38	-2.89	-0.54
		Tide	-4.71	-0.99	-4.20	-1.20

Table 4: Covariate estimates influencing school depth at wind farm SEISMIC, wind farm PILE and wind farm CONTROL. Covariate estimates were converted back, so can be used without modification.

Wind farm	**Variable**	**2.5% limit estimate**	**97.5% limit estimate**	**5% limit estimate**	**95% limit estimate**
SEISMIC	Intercept*	-148.87	-101.46	-145.01	-105.49
	Period				
	During^{+}	-0.015	1.64	0.11	1.50
	After*	4.36	5.93	4.48	5.81
	Location				
	Outside*	-1.51	-1.29	-1.23	-1.25
	School Height*	0.25	0.86	0.29	0.82
	Wave height*	-4.68	-2.54	-4.50	-2.72
	Tide*	3.26	4.52	3.37	4.42
PILE	Intercept*	17.69	25.63	18.44	25.07
	Period				
	During*	6.90	7.83	6.97	7.76
	After	-2.47	7.06	-1.12	6.73
	Location				
	Outside*	6.64	7.54	6.68	7.36
	School Height	-1.17	2.62	-0.89	2.49
	Wave height*	-9.23	-8.07	-9.14	-8.18
	Tide^{+}	-3.72	0.65	-3.56	0.85
CONTROL	Intercept*	5.25	6.92	6.03	6.76
	Period				
	During*	1.48	1.86	1.51	1.82
	After*	1.15	1.45	1.17	1.41
	Location				
	Outside*	1.12	1.06	-1.05	-1.05
	School Height*	1.16	1.21	1.16	1.21
	Wave height	-1.01	1.072	-1.0042	1.07
	Interaction				
	During:Outside*	-1.40	-1.04	-1.37	-1.07
	After:Outside	-1.40	1.09	-1.38	1.06

Table 5: Covariate estimates influencing school cohesion at wind farm SEISMIC, wind farm PILE and wind farm CONTROL. Note that covariate estimates were calculated using a Gamma-distributed GLM and have to be converted before comparing to the data.

Wind farm	Variable	2.5% limit estimate	97.5% limit estimate	5% limit estimate	95% limit estimate
SEISMIC	Intercept*	0.033	0.042	0.034	0.041
	Period				
	During*	0.00021	0.00059	0.00023	0.00057
	After*	0.00082	0.00012	0.00086	0.0012
	Location				
	Outside*	0.00048	0.00052	0.00062	0.00056
	School Height*	0.00023	0.00036	0.00024	0.00035
	Wave height^{+}	-0.00048	8.2 *10^{-7}	-0.00044	-2.4 *10^{-5}
	Tide*	-0.00060	-0.00039	-0.00059	-0.00040
	Interaction				
	During:Outside	-0.00087	-0.00028	-0.00082	-0.00032
	After:Outside	-0.0012	-0.00066	-0.0012	-0.00070
PILE	Intercept*	0.0010	0.0060	0.0014	0.0056
	Period				
	During*	0.00051	0.00052	0.00024	0.00049
	After*	-0.0031	-0.0013	-0.0029	-0.0014
	Location				
	Outside*	-0.00043	-0.00060	-0.00046	-0.00051
	School Height*	0.00036	0.00051	0.00037	0.00050
	Tide*	0.00054	0.00075	0.00056	0.00073
	Interaction				
	During:Outside*	-0.00062	-.000023	-0.00059	-0.00026
	After:Outside*	0.0016	0.0030	0.0020	0.0011
CONTROL	Intercept*	0.0026	0.019	0.0041	0.018
	Period				
	During^{+}	-0.00055	4.31	-0.0050	-2.86 *10^{-6}
	After	-0.00015	0.00043	-0.00010	0.00039
	Location				
	Outside*	-0.00069	-0.00049	-0.00059	-0.00056
	School Height*	5.43 *10^{-5}	0.00020	6.54 *10^{-5}	0.00019
	Tide*	3.35 *10^{-5}	0.00054	7.42 *10^{-5}	0.00050

Table 6: Covariate estimates influencing school biomass per hour at wind farm SEISMIC, wind farm PILE and wind farm CONTROL. Note that covariate estimates were calculated using a 10-log-distributed GLM and have to be converted before comparing to the data.

Wind farm	**Variable**	**2.5% limit estimate**	**97.5% limit estimate**	**5% limit estimate**	**95% limit estimate**
SEISMIC	Intercept*	5.15	14.78	5.91	13.99
	Period				
	During	-0.25	0.14	-0.21	0.11
	After	-0.50	0.18	-0.17	0.14
	Tide*	-0.39	-0.13	-0.37	-0.15
	Wave Height	-0.41	0.066	-0.37	0.030
PILE	Intercept*	2.62	11.09	3.30	10.35
	Period				
	During	-0.19	0.32	-0.15	0.28
	After	-0.57	0.19	-0.51	0.12
	Wave Height	-0.15	0.36	-0.11	0.32
	Tide*	-0.46	-0.10	-0.42	-0.13
CONTROL	Intercept*	0.91	20.74	2.55	19.10
	Period				
	During^{+}	-0.56	0.034	-0.51	-0.0091
	After	-0.51	0.12	-0.45	0.067
	Tide*	-0.62	-0.016	-0.57	-0.067

Wind farm SEISMIC

During the seismic exposure, the biomass depth outside the wind farm was significantly shallower than during the baseline (Fig. 5a & b; Table 2). Fish school presence also changed during the exposure by the seismic survey (Fig. 5c & d; Table 3): fewer fish schools were present than before or after the exposure, although the abundance of schooling fish did not change (Table 6). 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. 5e; Table 4) and schools inside the wind farm were more cohesive (Fig. 5f; Table 5). 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.

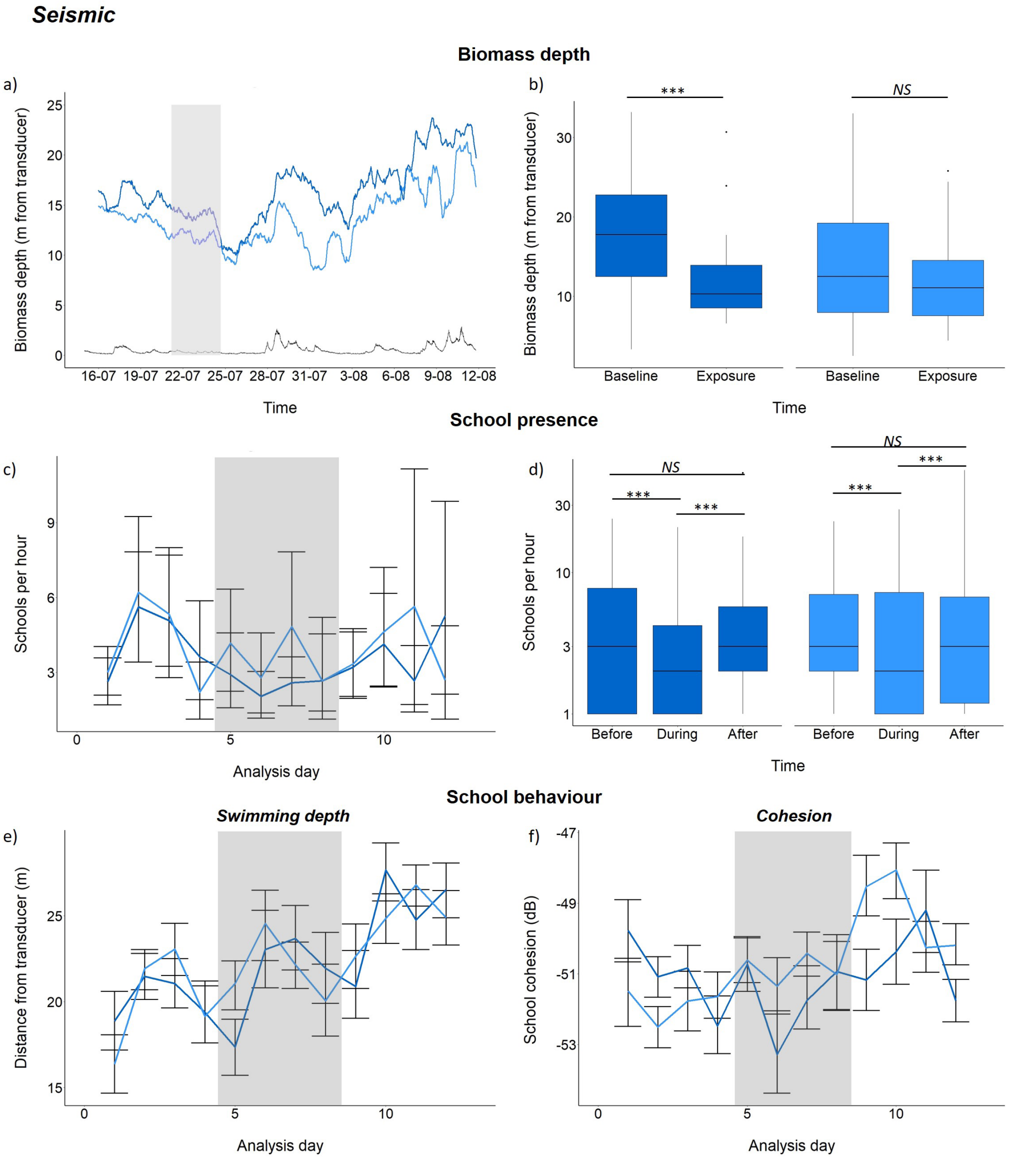

Figure 5: The seismic survey correlated with changes in a & b) biomass depth, c & d) number of fish schools per hour, e) school swimming depth and f) school cohesion. Dark coloured lines and boxes represent the AZFP outside the wind farm, light coloured lines and boxes represent the AZFP inside the wind farm. Shaded areas depict exposure periods. Error bars (c, e, f) depict bootstrapped 95% CI. Boxplots (b, d) 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.

Wind farm PILE

The biomass depth was not significantly different from baseline, inside or outside, during the exposure to pile driving (Fig. 6a & b; Table 2). 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. 6c & d; Table 6). In the DURING period, schools were present higher in the water column, but recovered after the exposure (Fig. 6e; Table 4). 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. 6f; Table 5). Outside wind farm PILE, however, there were no significant differences in cohesion between the periods.

Wind farm CONTROL

At wind farm CONTROL, the biomass depth was not significantly different from baseline, inside or outside the wind farm (Fig. 7a & b; Table 2). However, the number of fish schools significantly increased in the DURING period (Fig. 7c & d; Table 3). 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 (Table 6). 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. 7e & f; Table 4 & 5). In the AFTER period, school cohesion was the same as in the BEFORE period.

Discussion

Our results provide detailed insight into the presence, schooling behaviour, and swimming depth of pelagic fish in relation to seismic and pile driving exposure. Exposure to the seismic survey was related to a deeper biomass centre of gravity (outside wind farm), higher school cohesion (inside the wind farm) and lower school numbers (both inside and outside wind farm). Pile driving was related to shallower and more cohesive schools (inside the wind farm). In contrast, during the no-exposure period at the control site more schools were present, which swam higher in the water column and which were less cohesive.

Generally, we found very similar patterns within and outside the wind farm. This suggests that the environmental conditions of the wind farm itself did not change baseline behaviour of the pelagic fish community. Other abiotic conditions, in particular wave height and tidal differences, affected almost all of the parameters observed. Hence, these have to be considered when studying the responsiveness of fish schools to noisy human activities. However, the current data set shows that bottom-mounted AZFP-echosound-

Pile driving

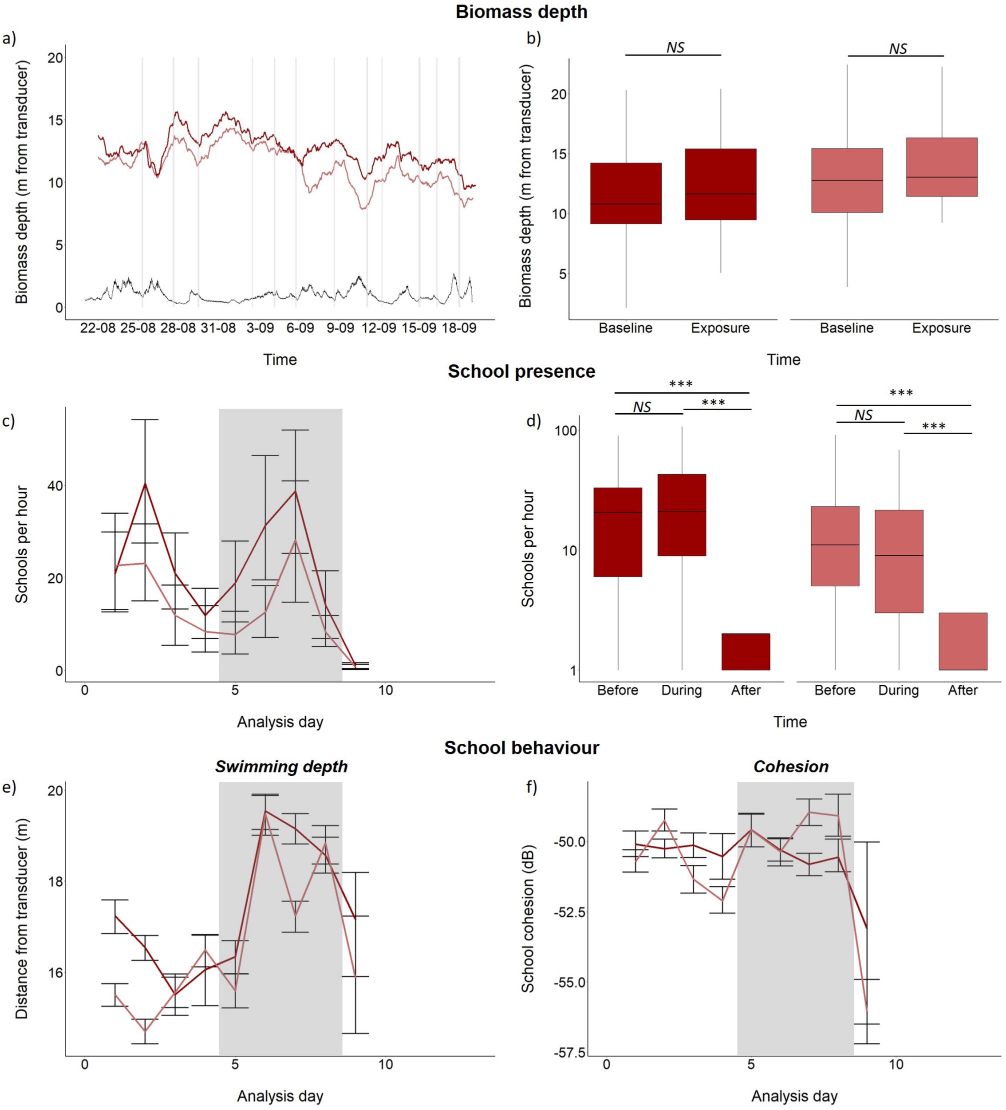

Figure 6: When fish were exposed to pile driving, there were no changes in a & b) biomass depth and c & d) number of fish schools per hour. The number of fish schools per hour did change after exposure. During exposure, school behaviour changed with schools e) swimming shallower and f) being more cohesive (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, f) depict bootstrapped 95% CI. Boxplots (b, d) 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.

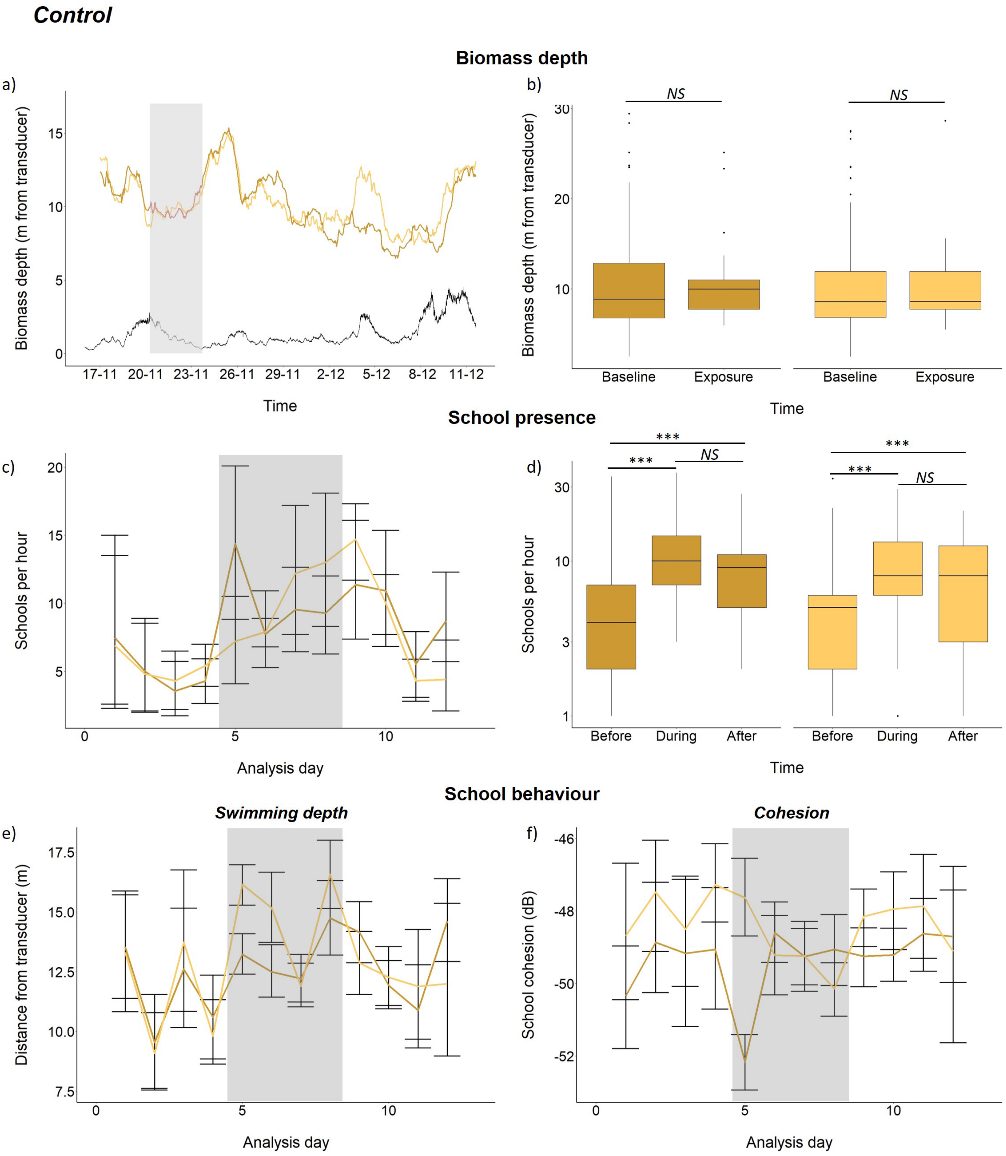

Figure 7: At wind farm CONTROL, there were no changes in a & b) biomass depth during the no-exposure period. The number of fish schools per hour (c & d) did change in the DURING period. Also, in the DURING period fish schools e) swam shallower inside the wind farm and f) were less cohesive. Dark coloured 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, f) depict bootstrapped 95% CI. Boxplots (b, d) 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.

ers 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 wind farm SEISMIC and wind farm PILE. The current data set thus provides an unreplicated proof of concept. Finding proof for causal explanations for the correlations between exposure conditions and associated changes in fish schooling behaviour requires replication at multiple sites.

Changes in fish school behaviour related to sound exposure

The number of fish schools per hour significantly decreased during exposure to the seismic survey. Earlier studies on reduction in catch rates and changes in fish abundance found varying results. Effects of seismic surveys on catch rates have been 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). 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 echosounder. However, proper replication is required for drawing any 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. At the same time, the mean depth of the total biomass went down in the water column during exposure to the seismic survey. Interestingly, our results on swimming depth of fish schools 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. 2014b; 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. 2015b). The discrepancy between the patterns found for fish schools and the total pelagic biomass 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. 2014b; Neo et al. 2014; Neo et al. 2015b). 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.

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 wind farm SEISMIC) 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).

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 wind farm PILE and wind farm CONTROL, but not for wind farm SEISMIC. 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. However, an alternative explanation is that wind farm SEISMIC was sampled first in the summer with long daylight periods, while wind farm PILE and wind farm CONTROL were sampled later in the autumn to winter, with already much shorter days and more distinct nocturnal parts of the day.

Using echo sounders to monitor long-term changes in fish behaviour

This study has shown the potential of echosounders for studying long-term changes in the behaviour of pelagic fish. The advantage of using bottom-moored echosounders 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. Depending on how long a school is in view of the echosounder, it might even be possible to study behaviour at the scale of seconds to minutes, as has been done previously with echosounders that were not bottom-moored but towed from a small boat (Hawkins et al. 2014b).

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 or false negative results, for example if a natural peak in the data coincides accidentally 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 echosounders placed in each wind farm were strikingly similar. This suggests that data from one echosounder may be representative of an area that is larger than the wind farm in which it is placed.

Further understanding of fish behaviour may be achieved when echosounder deployment is combined with bio-sampling to verify the species composition on site. Another consideration is the use of multi-beam echosounders or, for large water depths, an autonomous vehicle with a mounted echosounder (Chu 2011; Benoit-Bird et al. 2017). Multi-beam echosounders 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 echosounders, as used here, are very well capable of studying vertical movement behaviour of fish (an often observed change in response to sound) and long-term changes in fish school dynamics.

Conclusions

We have shown that bottom mounted AZFP-echosounders are a very suitable method to monitor fluctuations in time and space in pelagic fish communities. Fish exposed to the seismic survey and pile driving were less abundant and swam shallower in more coherent schools during the exposure days than before the exposure. However, we refrain from drawing strong conclusions about a causal relationship here and we stress that these data concern case studies and 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. We stress the importance of the well-replicated use of bottom-mounted echosounders for future studies, to gain a better understanding of the pelagic fish community, potential effects of wind farm ecology, and the impact of anthropogenic noise.

Acknowledgments

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Appendix

Table S1: overview of the measuring locations for the environmental variables: tide height, water temperature and wave height. All measurements were taken from www.waternet.nl (Rijkswaterstaat), an online governmental repository for (a)biotic measurements of Dutch water bodies. Data from measuring locations were chosen based on the proximity of the location to the wind farm, as well as similar conditions (e.g. a measuring location that was also in open water was taken for temperature measurements). For some variables, choice of the measuring location was restricted to a subsample of locations that had available data of the desired period.

Wind farm	Variable	Measuring location
Wind farm SEISMIC & Wind farm PILE	Tide	Euro platform
	Temperature	Euro platform
	Wave height	Schouwenbank Anchor South (wrakkenboei)
Wind farm CONTROL	Tide	Platform F16-A
	Temperature	Borkum Noord
	Wave height	Schiermonnikoog Noord

Table S2: GPS coordinates of deployment locations of AZFP echosounder frames at the three wind farms and distance between the outside and inside frames at each wind farm.

Echosounder	Latitude	Longitude	Distance outside-inside (m)
Wind farm SEISMIC Outside frame	51.68821	2.84921	3031
Wind farm SEISMIC Inside frame	51.66522	2.82558	
Wind farm PILE Outside frame	51.58611	3.01083	2347
Wind farm PILE Inside frame	51.56972	2.98944	
Wind farm CONTROL Outside frame	54.0102	5.94827	2443
Wind farm CONTROL Inside frame	54.01033	5.91089	

Table S3: Exposure periods for analysing biomass data and fish school data.

Wind farm	Actual exposure		Exposure period biomass data		Exposure period fish school data	
	Start	*End*	*Start*	*End*	*Start*	*End*
SEISMIC	21-07 04:22	24-07 16:16	Actual exposure		21-07 00:00	24-07 23:59
PILE	26-08 06:00	26-08 08:25	Actual exposure		26-08 00:00	26-08 23:59
	28-08 13:40	28-08 16:40			28-08 00:00	28-08 23:59
	30-08 10:30	30-08 13:15			30-08 00:00	30-08 23:59
	03-09 12:10	03-09 13:50			03-09 00:00	03-09 23:59
	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				
CONTROL	None		20-11 06:30	23-11 17:00	20-11 00:00	23-11 23:59

Table S4: Settings for automatic school detection with Echoview, as used to process data from the two echosounders 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

Table S5: Distributions used for modelling the effect of sound on biomass depth, schools per hour, school size, school depth and school cohesion.

Response variable	Wind farm	Model distribution
Biomass depth	All	Gamma
Schools per hour	All	Zero-inflated negative binomial
School size	All	Log_{10} transformation
School depth	SEISMIC	normal
	PILE	Square transformation
	CONTROL	Log_{10} transformation
School cohesion	All	Gamma

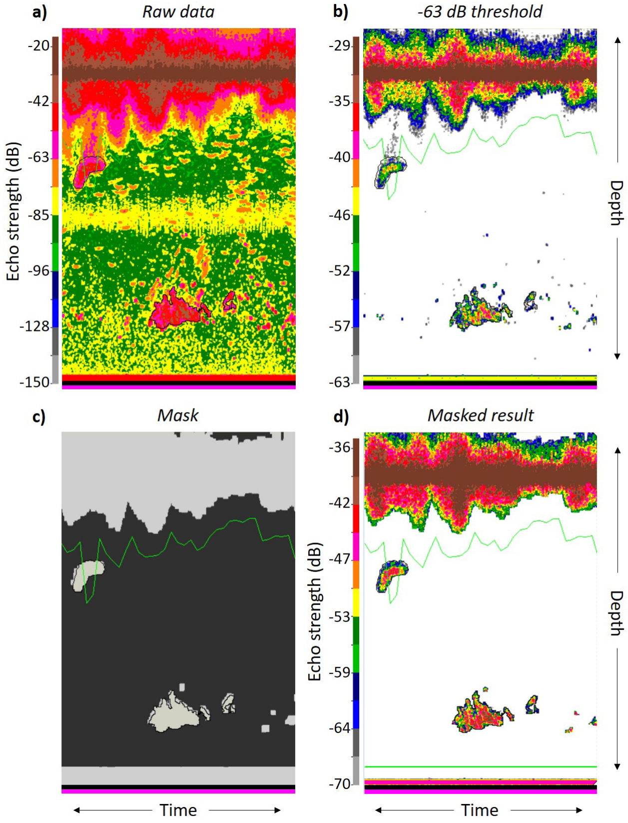

Figure S1: The raw echosounder data (a) was pre-processed to reduce noise and enhance the detectability of schools. Data of all echosounder frequencies were filtered with a -63-dB threshold (b) and combined to create a maximum strength echogram. Next, the combined data was eroded and dilated so only 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 analysis (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.