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Citation

Kok, A. C. M., Bruil, L., Berges, B., Sakinan, S., Debusschere, E., Reubens, J., ...
Slabbekoorn, H. W. (2021). An echosounder view on the potential effects of impulsive noise pollution on pelagic fish around windfarms in the North Sea. *Environmental Pollution*, 290.
doi:10.1016/j.envpol.2021.118063

Version: Publisher's Version
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Downloaded from: <https://hdl.handle.net/1887/3277276>

Note: To cite this publication please use the final published version (if applicable).

Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

An echosounder view on the potential effects of impulsive noise pollution on pelagic fish around windfarms in the North Sea[☆]

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ARTICLE INFO

Keywords:

Anthropogenic noise
Fish schools
Good environmental status
Noise impact assessment

ABSTRACT

Anthropogenic noise in the oceans is disturbing marine life. Among other groups, pelagic fish are likely to be affected by sound from human activities, but so far have received relatively little attention. Offshore wind farms have become numerous and will become even more abundant in the next decades. Wind farms can be interesting to pelagic fish due to food abundance or fisheries restrictions. At the same time, construction of wind farms involves high levels of anthropogenic noise, likely disturbing and/or deterring pelagic fish. Here, we investigated whether bottom-moored echosounders are a suitable tool for studying the effects of impulsive – intermittent, high-intensity – anthropogenic noise on pelagic fish around wind farms and we explored the possible nature of their responses. Three different wind farms along the Dutch and Belgian coast were examined, one with exposure to the passing by of an experimental seismic survey with a full-scale airgun array, one with pile driving activity in an adjacent wind farm construction site and one control site without exposure. Two bottom-moored echosounders were placed in each wind farm and recorded fish presence and behaviour before, during and after the exposures. The echosounders were successful in detecting variation in the number of fish schools and their behaviour. During the seismic survey exposure there were significantly fewer, but more cohesive, schools than before, whereas during pile driving fish swam shallower with more cohesive schools. However, the types and magnitudes of response patterns were also observed at the control site with no impulsive sound exposure. We therefore stress the need for thorough replication beyond single case studies, before we can conclude that impulsive sounds, from either seismic surveys or pile driving, are a disturbing factor for pelagic fish in otherwise attractive habitat around wind farms.

1. Introduction

Many aquatic animals change their behaviour in response to increasing ambient noise levels (Cox et al., 2018; Slabbekoorn et al., 2018; Southall et al., 2019). 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 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). Increased noise levels

have been found to affect all trophic levels, from prey species, such as invertebrates (Hubert et al., 2018) to top predators, such as marine mammals (Southall et al., 2016). Specifically, changes in predator-prey interactions have high potential to translate to effects on the ecosystem as a whole (Kunc et al., 2016; Slabbekoorn et al., 2019; Soudijn et al., 2020).

The effects of noise pollution on marine animal behaviour vary significantly with the type of sound. Not only sound level, but also temporal structure, predictability, and amplitude fluctuation can play a role. The effect of intermittent sound can be stronger than that of

[☆] This paper has been recommended for acceptance by Eddy Y. Zeng.

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<https://doi.org/10.1016/j.envpol.2021.118063>

Received 9 March 2021; Received in revised form 24 August 2021; Accepted 26 August 2021

Available online 28 August 2021

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continuous sound (Neo et al., 2014), and intermittency can also change the relationship between sound level and the magnitude of the response (Kok et al., 2018). The amplitude fluctuation from a moving seismic sound source may also lead to slower behavioural recovery than a stationary pile driving sound source (Neo et al., 2014). Studies on effects of seismic survey sound on behaviour have so far shown mostly short-term displacement and startle responses in harbour porpoises and fish and changes in predator-avoidance responses in invertebrates (Carroll et al., 2017; Chapman and Hawkins, 1969; Day et al., 2016; Paxton et al., 2017; Pirodda et al., 2014; Sarnocińska et al., 2020; Thompson et al., 2013; Wardle et al., 2001). As most of the fish studies were performed with captive fish, the response of free-ranging fish could be quite different.

The effects of sound on pelagic animals have been studied sporadically. One benchmark study by Hawkins et al. (2014) indicated changes in cohesion and vertical displacement of pelagic fish and zooplankton when exposed to playback of an artificial, intermittent sound. Another experimental exposure study, with a single seismic survey, reported increased mortality in zooplankton 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 on changes in fisheries catch rates during and after noisy human activities (e.g. Skalski et al., 1992; Parry and Gason, 2006; Løkkeborg et al., 2012; Streever et al., 2016). Recent telemetry tagging studies on the impact of seismic survey and pile driving sounds all focussed on residential benthic fish species which guarantee a relatively high chance of sufficient telemetry data (van der Knaap et al., 2021; Iafate et al., 2016; Bruce et al., 2018). 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. Once operational, wind farms supposedly have little or even positive environmental impact (Ashley et al., 2014; Lindeboom et al., 2011; Raoux et al., 2017). Rocky scour beds surrounding the piles introduce an artificial reef, leading to a different and more diverse benthic community (Ashley et al., 2014; Lindeboom et al., 2011; Raoux et al., 2017), which may also affect the local pelagic community. However, in the pre-construction and construction phase, seismic surveys and pile driving activities can cause considerable acoustic disturbance (Carroll et al., 2017; De Jong and Ainslie, 2008; Hastie et al., 2019; Norro et al., 2013; Popper and Hawkins, 2019; Sertlek et al., 2019; Slabbekoorn et al., 2010), while in the exploitation phase, just a moderate, low-frequency noise from the rotor blades and some additional shipping remains (Madsen et al., 2006; Nedwell et al., 2007; Norro and Degraer, 2016).

A way to study long-term presence and behaviour of pelagic fauna during the pre-construction, construction, and operational phases of wind farm development could be through the use of bottom-moored active acoustic systems, or echosounders (Simmonds and MacLennan, 2005). Because of their non-invasiveness and inaudibility to fish, echosounders are widely used to assess fish biomass, identify individual species, and to observe changes in fish school cohesion and swimming depth (Fraser et al., 2018; Gerlotto et al., 2004; Guillard et al., 2010; Hawkins et al., 2014; Weber et al., 2009). Active acoustic monitoring does not rely on animals making sound and therefore extends beyond the soniferous community. As disturbance and deterrence are likely reflected in school cohesion and swimming depth (Hubert et al., 2020; Neo et al., 2014; Sarà et al., 2007), echosounders seem an excellent tool to assess responses of pelagic fish to seismic surveys and pile-driving (Benoit-Bird et al., 2017; Colbo et al., 2014; Lawson et al., 2001).

Here, we explored the effects of two types of impulsive anthropogenic noise on pelagic fish around wind farms in the Southern North Sea, while testing whether bottom-moored echosounders are a suitable tool for this. We repeatedly deployed two echosounders for about a month, at three locations in three subsequent periods. We conducted two before-during-after tests of noise impact at different time scales. Test 1: We

investigated whether there were long-term changes (days) in pelagic fish presence and behaviour correlated to a 4-day exposure with seismic survey sounds and compared this to a sham exposure spatial control. And, test 2: we investigated whether there were short-term changes (hours) in pelagic fish presence and behaviour correlated to pile driving events lasting a few hours per turbine. We expected that general predator avoidance behaviour in response to anthropogenic noise would yield fewer, more cohesive, and deeper swimming fish schools.

2. Materials and methods

2.1. Study locations

The AZFP echosounders were placed at two wind farms in the Belgian part of the North Sea and one wind farm in the Dutch part of the North Sea: 1) Belwind – an offshore wind farm situated on the Bligh bank (38 m depth), 40 km off the Belgian coast, 2) C-Power – an offshore wind farm situated at the Thornton bank (25 m depth), 27 km off the Belgian coast and 3) Gemini, located 85 km off the Dutch coast (33 m depth), north of Schiermonnikoog (Fig. 1). Belwind wind farm was exposed to a four-day experimental seismic survey (Fig. 2; PCAD4Cod project, Van der Knaap et al., 2021; Rogers et al. *in review*). C-Power wind farm was exposed to 12 separate days of pile driving during the AZFP deployment period, for the construction of the nearby offshore wind farm Norther (2–5 km). Gemini wind farm was not exposed to any particular anthropogenic activity, other than shipping noise from local maintenance traffic and a nearby shipping lane and functioned as control site for the seismic exposure at Belwind.

To investigate differences between the pelagic fish inside and outside the wind farm, at all three locations, one AZFP echosounder was placed inside the farm, 150 m from a wind turbine in the centre of the turbine field, while the second AZFP was placed outside the wind farm, 700 m away from the edge of the turbine field. The AZFPs were placed in the three wind farms consecutively: first at Belwind wind farm (seismic exposure site), next at C-Power wind farm (pile driving events), and finally at Gemini wind farm (seismic control site (Table S1)).

Water temperature, wave height and tide records were taken from the Dutch Ministry of Infrastructure and the Environment (waternet.nl, Rijkswaterstaat) from measuring stations at ~50 km distance to the wind farms, except for one tide measuring station that was ~125 km from Gemini. Water temperatures were quite constant per wind farm: 16.8–18.7 °C at Belwind, 17.3–19.2 °C at C-Power, and 9.0–10.8 °C at Gemini. Day time periods were longest for Belwind (summer), and shortened with the seasons for C-Power (autumn) and Gemini (winter). Distances between the inside and outside locations for the AZFP-frames were 2.3–3.0 km. The locations inside Belwind and C-Power were 15.52 km apart, while the location inside Gemini was 333 km (in a straight line) from the others.

2.2. Echosounders

Two Acoustic Zooplankton Fish Profilers (AZFPs, ASL Environmental Sciences, Canada) were deployed consecutively at three wind farms. Both AZFP echosounder sets emitted narrowband signals at four frequencies, of which three were shared between units (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. Both AZFPs were moored on a frame at the seafloor, at a depth of 24–38 m, and had a vertical upward beam (Table S1). The AZFPs recorded 27 to 33 consecutive days per location with a ping rate of 1 Hz (sound pulses emitted by the echosounder). Data were extracted after retrieval of the AZFPs, using an acoustic release (Edgetech PORT). The 125 kHz channels, for both echosounders, were calibrated after the deployments in a test tank with a 38.1 mm Tungsten Carbide sphere (standard sphere method, Demer et al., 2015).

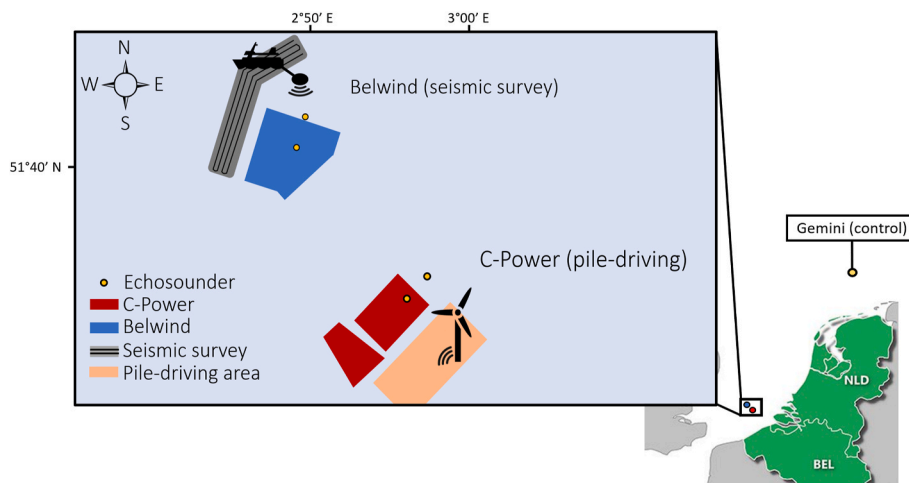


Fig. 1. Schematic overview of the placement of the AZFP echosounders (yellow dots in inset) at the two wind farms in the Belgian North Sea. Note the roughly equal distance of the AZFPs to the track of the seismic survey (Belwind) and to the pile driving area (C-Power). At Gemini, there were no periods of impulsive anthropogenic noise during these measurements in the Dutch North Sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

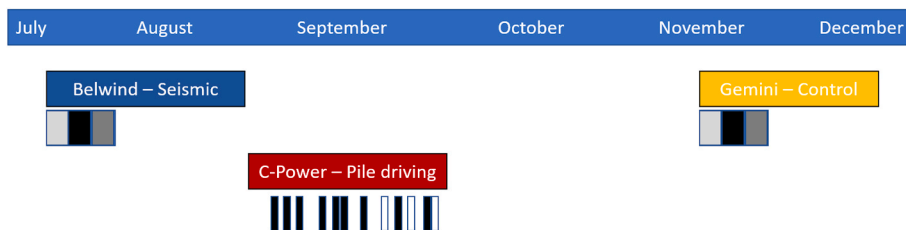


Fig. 2. Schematic representation of the study periods. Echosounders were first placed at Belwind (seismic exposure), next at C-Power (pile driving events), and finally at Gemini (control). Pelagic fish biomass was measured for the entire recording period (coloured, named boxes). For the test of seismic impact, 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), for both the real experimental seismic survey at Belwind and the sham

exposure control at Gemini. We conducted a separate test for the piling impact. At C-Power pile driving exposure took place on 12 separate days, during the echosounder deployment period, but three of these concerned nocturnal events, which were not included in the analyses (black boxes: included exposure events; white boxes: not included exposure events).

2.3. Natural and exposure sound levels

Ambient sound pressure levels for Belwind fluctuated in phase with tidal currents (detailed in Rogers et al. *in review*), and were on average 95–110 dB re 1 $\mu\text{Pa}^2/\text{Hz}$ in the 10–500 Hz frequency range (Rogers et al. *in review*). For C-power, ambient average sound pressure levels during the pile driving period were 78–109 dB re 1 $\mu\text{Pa}^2/\text{Hz}$ at 10–500 Hz (15 min sample measurement on 09-07-2018, measured with BK-8104 hydrophone, BK-Nexus 2692-0S4 amplifier and MARANTZ-PMD671 recorder at 10 m depth (Norro, 2019)). Ambient sound levels at Gemini were not measured during the study period, but have been reported for 2013 and then ranged from 80 to 100 dB re 1 $\mu\text{Pa}^2/\text{Hz}$ at 10–500 Hz (Lucke, 2015).

A full-scale airgun array was used for an experimental seismic survey at Belwind from 21–24 July 2018 (Fig. 2, Table S2). Sound levels at the echosounder ranged from 123 to 195 dB re 1 μPa SPL_{z-p} (Van der Knaap et al., 2021; Rogers et al. *in review*). 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. A soft-start procedure of 20–40 min was used for the first line.

Pile-driving was carried out next to C-Power during the construction of additional wind turbines in an adjacent plot (Norther: 51° 32' N, 3° 2' E). Sound levels for one pile driving period were on average 172 dB re 1 μPa SPL_p at both AZFPs. This was calculated by back-propagating the received levels from the recorder to the sound source, assuming cylindrical spreading. A total of 20 turbines were built during the period from

6 August to September 25, 2018, with twelve days of actual pile-driving, separated by one or more quiet days. Nine days with daytime sound exposure were used for the behavioural analysis (Fig. 2, Table S2). The average pile-driving duration was 148 min (range 95–180 min). As observed in previous studies (Axenrot et al., 2004; Fréon et al., 1996), the diel school structure was variable with highly dispersed fish during night time. No analysis could be carried out for night time recordings, as highly dispersed fish school layers were then mixed with plankton and suspended particles.

2.4. Echosounder data processing

All AZFP-echosounder 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; *c. f.* 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 varies among species (Demer et al., 1999). By taking the maximum echo strength for all frequencies, we made sure that species type did not affect detection probability. Low-level 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.

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 then filtered with a threshold of -70 dB. Finally, surface and bottom echoes were considered noise (i.e. waves, sediment particles) and were excluded from the data.

2.5. Fish school detection and biomass measurements

Fish schools were detected automatically using a built-in school detection function of Echoview (detection settings in Table S3). Detection settings were based on a comparison of automatically and 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 by the following criteria: 1) at least three separate traces of potential fish reflections, present in the same ping (sound pulse from the AZFP-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. S2a&b) 3) school need to be visible for at least 3 pings.

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 min intervals at a frequency of 125 kHz (MacLennan et al., 2002). 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 = 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), this variable was resampled to one 10-min bin every 3 h for Belwind and Gemini. The data were resampled to one 10-min bin every half hour for C-Power, since the consecutive exposure duration was 1–3 h.

2.6. Fish school behaviour and presence

We measured schooling fish presence as the number of schools present per hour for Belwind and Gemini, or per 10 min for C-Power, as well as the total biomass of these schools (schooling fish biomass) in NASC calculated per school (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 overweighing of larger schools. These values were then summed over each hour/10 min.

We took behavioural measurements from the detected schools based on reported responses of fish to intermittent sounds (Fewtrell and McCauley, 2012; Hawkins et al., 2014), including 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 potentially relates to an increase in the school density and thus potentially indicates a smaller

distance between individuals, i.e. a higher school cohesion.

2.7. Statistical analyses

2.7.1. Modelling framework

We investigated whether sound exposure influenced fish biomass and behaviour. 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 for Belwind and Gemini (test 1: long-term seismic exposure and control) were either linear or generalised linear models (Table S4). For C-power (test 2: short-term pile driving events), mixed models were used to accommodate the repeated-measures design of each day. 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). All statistical analyses were performed using R (version 3.5.2). 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.

2.8. Model parameters

The statistical models were constructed using fish biomass depth, school presence, schooling fish biomass, school depth, and school cohesion as response variables. Location (inside or outside the wind farm), wave height and tide were the 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 further 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.

School presence was evaluated in two ways: first, by applying a rotation test (tagtools package; DeRuiter and Solow, 2008) examine whether the pattern of school detection was similar during exposure and in the before and after periods. This rotation test was applied to the entire 12 days of data for both Belwind and Gemini, and to individual exposure days of C-Power separately, to account for the discontinuity of the pile driving events. Second, we investigated if the number of schools per hour, or per 10 min, changed during exposure, using a Hurdle model that consisted of two parts. The first part 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 were zero and only modelled the number of schools that were present (negative binomial distribution). With the latter part, we could then tell if the number of schools present was explained by the explanatory variables.

2.9. Test 1: Evaluating seismic noise impact

To investigate changes in pelagic fish abundance and behaviour between seismic exposure and control conditions, we analysed biomass and school characteristics from the echosounder data and compared those for Belwind (exposure) and Gemini (control). Since we expected natural variability in the data, we tested for both temporal variability, using a before-during-after design, as well as for spatial variability, by using the same before-during-after design without exposure in the control wind farm, Gemini. Total biomass was calculated per 1 m depth and 10 min bins for the entire survey period. The exposure period for the total biomass was the entire duration of seismic sound exposure, with a

control period of sham exposure of equal duration for the control site (Gemini, Table S2). 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 four day-periods before, during and after the exposure at Belwind (before, during and after). The four-day analysis period was selected based on the duration of the seismic survey exposure (Table S2). The first twelve days of deployment were arbitrarily selected as the 'before', 'during', and 'after' periods for Gemini.

2.10. Test 2: Evaluating pile driving noise exposure impact

To investigate the changes in pelagic fish abundance and behaviour in relation to pile driving, we performed a similar analysis of biomass and school characteristics from echosounder data for C-Power. Pile driving took place in 1-3-h periods on 12 separate days and the before and after periods were chosen to match the exposure duration (during) of that day. In some instances, one or more of the periods took place during a time of day when fish were showing nocturnal behaviour (i.e. did not school). These periods were then shortened to the data record length for daylight hours. If pile driving sound exposure fell completely outside daylight hours, this day was removed from the analysis, leaving 9 days with diurnal exposure.

3. Results

3.1. General patterns

The AZFP-echosounder data provided insight into the presence and behaviour of a large number of pelagic fish schools, before, during, and after sound exposure periods, with a few biological (i.e., no nocturnal schooling) and methodological (i.e., masking of schools close to bottom and surface) restrictions. 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. S2). Because for C-Power and Gemini the fish did not have clear school structure at night-time, it was decided to exclude the data between dusk and dawn from the analyses for these wind farms (roughly 19:00–04:00 h for C-Power and 16:30–06:20 h for Gemini). At Belwind, 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. The median number of schools inside and outside the wind farm was roughly equal for Belwind (2 schools per hour inside and outside, N hours = 573, range = 0–53 schools per hour) and Gemini (6 schools per hour inside, 7 schools per hour outside, N hours = 251, range = 0–37 schools per hour). For C-Power, there were considerably more schools outside the wind farm (17 schools per hour) than inside (7 schools per hour, N hours = 246, range = 0–106 schools per hour). Total biomass in the water column was highest for Gemini with a median NASC of 22.72 m²/nm² for Belwind, 19.51 m²/nm² for C-Power and 48.88 m²/nm² for Gemini.

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 S5). Wave height led to deeper swimming schools (possibly due to masking of schools at lower depths due to mixing of the water), as well as influencing fish biomass depth, the number of schools per hour and school cohesion, although patterns for these other variables were not always consistent between wind farms (Fig. S3; Tables S5–S14). The third abiotic characteristic, tide, influenced all

variables (Fig. S3; Tables S5–S14). Tidal influences were consistent for fish school presence, which was higher at low tide than at high tide for both Belwind and Gemini.

3.2. Test 1: Changes during seismic sound exposure but also in the control

We found several apparent effects of the seismic survey on pelagic fish presence and behaviour. During the seismic sound pulse exposure, the biomass depth at Belwind was significantly deeper than during the baseline (Fig. 3a; Table S5). Fish school presence also changed during the exposure by the seismic survey (Fig. 3c and d; Table S7): fewer fish schools were present than before or after the exposure, although the total schooling fish biomass did not change significantly: fish were present in fewer but larger schools during the survey (Table S13). While we observed variation in the number of schools per hour, there was no change in the number of hours with schools present per day. This was confirmed by the non-significant result of the rotation test ($p > 0.1$). The fish schools that were present during exposure tended to swim at shallower depths (non-significant trend; Fig. 3e; Table S9), and schools inside the wind farm were more cohesive (Fig. 3f; Table S11). 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 shallower (Fig. 3e and f).

We also found, unexpectedly, significant changes in the sham exposure period for the control site. At Gemini, the biomass depth was not significantly different during the exposure compared to baseline, inside or outside the wind farm (Fig. 3b; Table S5). However, the number of fish schools significantly increased in the 'during' period (Fig. 3c and d; Table S7). 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 biomass of schooling fish tended to be lower 'during', but not 'after' (non-significant trend, Table S13). 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. Schools also swam shallower 'during' compared to 'before' as well as 'after' (Fig. 3e), and tended to be less cohesive (non-significant trend, Fig. 3f; Table S9 & S11). School cohesion was not different 'after' compared to 'before'.

3.3. Test 2: Effects of pile driving sound and interaction with weather

As opposed to the previous test, in which there was a period of four days of continuous seismic noise treatment, exposure to pile driving noise at the C-Power site was discontinuous. Therefore, the exposure period in this test corresponds to the nine 100–180 min periods with noise exposure, while before and after periods are the same duration as the exposure period they surround (e.g. an exposure period of 100 min has a before and after period of 100 min each). At the C-Power wind farm, the biomass depth during the exposure to pile driving was significantly different from baseline (Fig. 4a; Table S6). On each day of exposure, the probability of school detection in the actual hours of exposure (during period) did not differ from the probability in the other hours of that day (rotation tests > 0.1). However, the number of fish schools per 10 min was generally higher during, but not after, pile driving, especially for the pile driving events with a large number of schools observed (Figs. 4b and 26/08, 28/08 and 30/08; Table S8).

During and after pile driving, schools were present at shallower depths than before the exposure event both inside and outside the wind farm (Fig. 4c; Table S10). This effect depended on wave height, with fish being lower in the water column during high waves, an effect that was stronger during exposure than before and after exposure. School cohesion was significantly lower during pile driving compared to before, and decreased further after the exposure, a trend that was stronger outside the wind farm than inside (Fig. 4d; Table S12). School cohesion also depended on wave height, with fish schools being more cohesive with higher waves during and after pile driving, while they were less cohesive

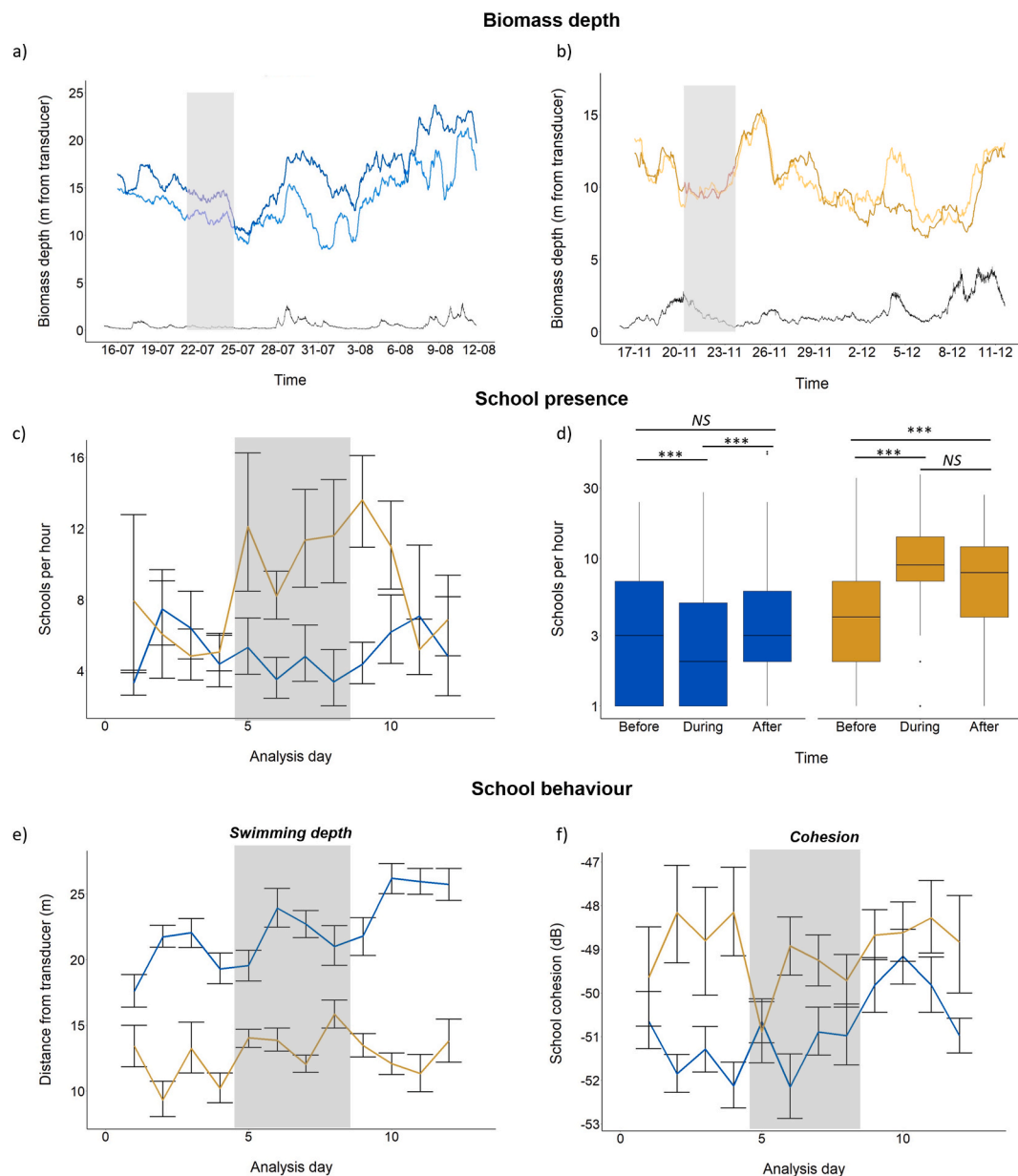


Fig. 3. The seismic survey correlated with changes in a) biomass depth, while the control showed no changes in biomass depth during the no-exposure period (b). The number of fish schools per hour changed for both the seismic survey and the control in the ‘during’ period (hours without schools were excluded) (c & d), as well as the: e) school swimming depth and f) school cohesion. Biomass depth is depicted as rolling mean (window length: 24 h), with wave height (black line). Blue coloured lines and boxes represent the AZFPs inside and outside Belwind (seismic exposure), yellow coloured lines and boxes represent the AZFPs inside and outside Gemini (control). 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. Note: for visualization purposes, the data for inside and outside locations were combined per wind farm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

with high waves before the events (Table S12). Schooling fish biomass did not change during exposure (Table S14). For all variables, there were considerable differences between exposure days, with a few days with high numbers of fish schools present driving the statistical direction of the change (28 and 30 August).

4. Discussion

The AZFP-echosounder data revealed changes in the abundance, schooling behaviour, and swimming depth of pelagic fish during exposure to both a seismic survey and pile driving sound. The results of the seismic survey exposure showed a number of significant exposure-

related changes that were in line with our expectations: a deeper biomass centre of gravity (outside the wind farm), higher school cohesion (inside the wind farm) and lower school numbers (both inside and outside the wind farm). Unexpectedly, we also found significant effects at our seismic control site (Gemini) with no sound exposure. During the sham-exposure period at the control site more schools were present, which swam shallower and which were less cohesive. The results of the pile driving exposure also showed a number of significant changes that were partly in line with our expectations: we found upward shifts in water column use and less cohesive schools during the pile driving events than before and after the events.

Generally, we found very similar patterns within and outside the

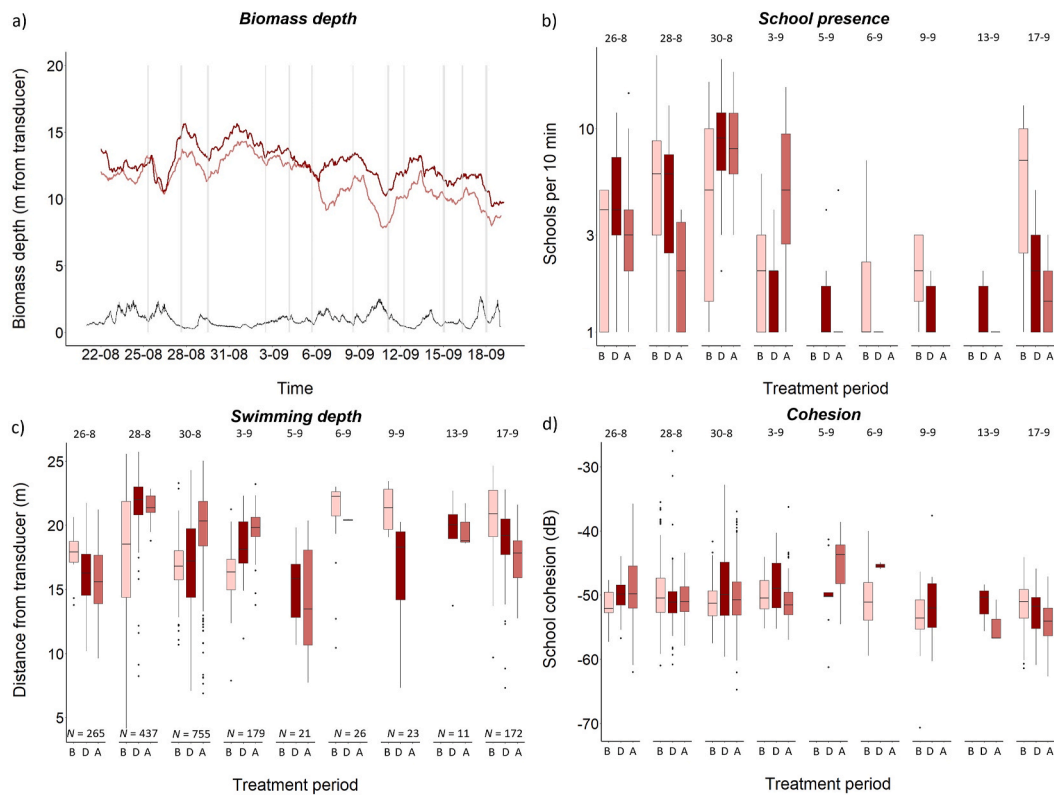


Fig. 4. When fish were exposed to pile driving, there were changes in a) biomass depth and b) number of fish schools per 10 min (excluding periods without schools). After exposure, the number of fish schools per 10 min changed back to baseline. During exposure, school behaviour changed with schools c) swimming shallower and d) being less cohesive. Note that there was considerable variation between days, with days with many fish schools present leading the trend. a) Biomass depth is depicted as rolling mean (window length: 24 h), with wave height (black line). Dark coloured lines represent the AZFP outside C-Power, light coloured lines 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). b-d) Data are shown for inside and outside locations combined. The before (B), during (D), and after (A) periods are coloured light pink, dark red, and coral pink, respectively. 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 ranges (whiskers). Dots represent any data point outside of this range. Date of exposure is noted above the plots, while number of schools per day is noted below the boxplots. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

wind farm. This suggests that the environmental conditions of the AZFP locations inside and outside the wind farm were not different enough to 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. This could be related to changes in fish behaviour, or changes in observability of fish schools due to bubble formation in high wave conditions. Alternatively, it could be that not the wave height, but increasing noise from the working wind turbines in windier conditions, was affecting fish behaviour. The current data set shows that bottom-moored AZFP-echosounders are suitable to assess patterns of variation in sufficient detail to detect sound event related changes in abundance, group cohesion, and swimming depth in the pelagic fish community. However, we like to stress that the current data set just provides a proof of concept. Finding proof for a causal relationship between exposure conditions and associated changes in fish schooling behaviour requires sufficient replication at multiple sites, as was illustrated by the seismic control site data.

4.1. Pelagic fish behaviour correlated with seismic sound exposure

During the exposure to the seismic survey, there were fewer but larger schools. Earlier studies have reported fleeing patterns, reductions in catch rates, and changes in fish abundance (Løkkeborg et al., 2012; Skalski et al., 1992; Streever et al., 2016). However, effects of seismic surveys on catch rates were sometimes also difficult to disentangle from the inherent variability in catch rates due to natural fluctuations (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). Our study is the first with bottom-moored echosounders, and suggests that pelagic fish biomass does not change, but that fish aggregate in fewer and larger schools. However, proper replication and knowledge of the species composition at all sites is required for drawing any firm conclusions about sound event related deterrence and stereotypic schooling responses.

Two recent telemetry tagging studies provided complementary data on the effects of seismic surveys on free-ranging benthic fish species. Bruce et al. (2018) found some evidence for behavioural changes in eight tiger flatheads (*Neoplattylus richardsoni*), inferred from shifts of diurnal activity patterns and general swimming speed. Atlantic cod that were tagged for the same experimental seismic survey as described in this study did not leave the area during the exposure, but did show a delayed response with a significantly elevated probability to leave in two days to two weeks afterward (Van der Knaap et al., 2021). The fish that stayed during the survey switched from being locally active (likely including foraging behaviour) to being more inactive. Like the flatheads in Bruce et al. (2018), the diurnal activity pattern of cod was also disrupted during the sound exposure. There was no significant change in swimming depth in the cod study, which was likely due to the fact that cod were already close to the bottom most of the time.

It is important to ultimately understand what the consequences of behavioural effects are in terms of individual vital rates (growth, maturation, survival and reproduction), which can accumulate to population level consequences (National Research Council, 2005; New

et al., 2014; Slabbekoorn et al., 2019; Soudijn et al., 2020). We still lack sufficient empirical data for this, independent of whether we address echosounder data on pelagic species or telemetry data on benthic fish species. For fish that are leaving an area, we need to know what the elevated costs are in terms of swimming energetics and predation risk and how foraging opportunities in the new area relate to those where they would otherwise have remained. For fish that change their behaviour, we also need to know the consequences for the shifts in energy expenditure and uptake.

4.2. Pelagic fish response to pile driving events

We also found several significant changes in behaviour in response to pile driving events, one of which was the shallower swimming of fish schools. The same patterns were found with the exposure to the impulsive sounds in Belwind, although fish schools also shifted upwards during the control at Gemini and the mean depth of the total pelagic biomass went down for the seismic exposure at Belwind. These results do not follow the general pattern found in literature of fish diving to deeper water upon acoustic disturbance (Doksæter et al., 2012; Fewtrell and McCauley, 2012; Hawkins et al., 2014; Neo et al., 2014; Slotte et al., 2004). However, there are also some studies that report fish swimming shallower, either during or immediately after exposure (Chapman and Hawkins, 1969; Neo et al., 2015; Sarà et al., 2007). Furthermore, we know very little about pelagic fish behaviour in turbid waters, which might be different than in the clear waters of earlier experimental studies. The discrepancy between the patterns found for fish schools and the total pelagic biomass 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.

We know of only one other study in which free-ranging fish were followed before, during and after a series of pile driving exposure events (Iafraite et al., 2016). Two benthic fish species, with high site fidelity, were tagged: sheephead (*Archosargus probatocephalus*) and grey snapper (*Lutjanus griseus*). Just 1 out of 13 sheephead and 2 out of 4 grey snappers left their residential area during a 10-day period of piling activity. Furthermore, there were some indications of reduced residence times during the exposure days compared to baseline, but these were rarely significant. Iafraite et al. (2016) therefore, does not provide much evidence for a strong effect on these species. At the same time, these sheepheads and snappers are again typical benthic species that likely remained close to the bottom throughout the before, during, and after periods.

School cohesion became higher during the seismic survey, 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., 2015, 2014). 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 hours to 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. School cohesion is likely to affect vital rates as it can not only affect predation probability (Benoit-Bird et al., 2017), but also foraging success (Wolf, 1987), and swimming efficiency (Hemelrijk et al., 2015).

4.3. Effect of abiotic conditions

Despite the large variation in fish schools as a response variable, fish school behaviour was affected by wave height and water temperature, as well as tide in some cases. Fluctuations in wave height affected swimming depth and school cohesion, with fish shifting down and (at Belwind) schools becoming less coherent in rough weather. Although we

cannot exclude the possibility that these results are caused by changes in observability of fish schools due to bubble formation, they may be true as such weather dependent patterns have been reported before (e.g. Kaartvedt et al., 2017) and have been explained as a response to decreased visibility (Tsuda et al., 2006) and a destratification of the water column (Secor et al., 2019). These patterns have also been correlated to increased wind speed (Lagardère et al., 1994) and a drop in barometric pressure (Heupel et al., 2003).

Another interesting pattern in our data was the distinct variation in schooling behaviour between day and night. Typical nocturnal behaviour with a drop in clustering of schools to spread out individually across the water column was found for C-Power and Gemini, but not for Belwind. 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 Belwind was sampled first, still in the summer, with longer daylight periods. 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. 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 swam shallower and changed their school cohesion during the exposure days compared to before 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 therefore stress the importance of well-replicated use of bottom-moored 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.

Credit author statement

Annebel C.M. Kok: Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization, Lisa Bruil: Formal analysis, Investigation, Writing – review & editing, Visualization, Benoit Berges: Methodology, Data curation, Writing – review & editing, Serdar Sakinan: Methodology, Data curation, Writing – review & editing, Elisabeth Debusschere: Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Project administration, Jan Reubens: Conceptualization, Methodology, Writing – review & editing, Dick de Haan: Methodology, Investigation, Writing – review & editing, Alain Norro: Resources, Writing – review & editing, Hans Slabbekoorn: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank all the people involved in data collection, especially the crew of RV Simon Stevin and RV Belgica. Furthermore, we would like to thank Echoview Inc. For providing us with a temporary license of Echoview 9, which was incremental for analysing the data. This project was funded by Rijkswaterstaat (ViSiON project) and by VLIZ as part of the Flemish contribution to LifeWatch. The seismic survey exposure was undertaken as part of a larger project, PCAD4Cod,

which was funded by the E&P Sound and Marine Life Joint Industry Programme (contract reference JIP22-III-15-10).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.118063>.

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