North Sea noise in the Anthropocene: an impact study of human-made impulsive sounds on free-ranging cod

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Citation

Knaap, I. E. J. van der. (2023, January 25). *North Sea noise in the Anthropocene: an impact study of human-made impulsive sounds on free-ranging cod*. Retrieved from https://hdl.handle.net/1887/3513027

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PhD thesis, Leiden University, the Netherlands

ISBN: 978-94-6458-787-6
Cover design by Elisabeth van der Knaap & Wouter van der Knaap
Lay-out by Inge van der Knaap
Printed by Ridderprint, Ridderkerk

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Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op woensdag 25 januari 2023
klokke 16:15 uur

door

Inge Elisabeth Jacoba van der Knaap
geboren te Bunnik, Nederland

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If control is the problem, then, by the logic of the Anthropocene, still more control must be the solution

(Under a White Sky: The Nature of the Future, by Elizabeth Kolbert)

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General Introduction

All fishes have evolved various ways to detect sound and, although we have detailed knowledge on hearing capacity from only a few species, it is highly likely that all fishes use the aquatic soundscape to gain information that is vital for their survival (Putland et al., 2018). Hearing in the marine environment is very important for fish in relation to a wide variety of functions linked to, e.g., survival and reproduction. Fish use sound for orientation and communication, during migration, aggregation and spawning, but also for detection of prey and predators (Gordon et al., 2019; Popper and Hawkins, 2019; Wilson et al., 2014). It provides them with information about sources at short and long distances, in all types of environments, at any time of day and from all directions. Visual and chemical stimuli may also provide important information about the environment and animals are typically exposed to all at the same time. At close range in particular, sensory input from multiple senses may interact and affect auditory perception, but sound is likely to be the most prominent, especially in dark conditions, turbid waters, and over larger distances.

Approximately 33,500 species of fish exist today and only for a small number of species we know how they use and perceive sound (Popper and Hawkins, 2020). Understanding how fish hear has become increasingly important in light of the rapidly changing ocean soundscape and the increasing contribution of noise produced by human activity at sea, which can interfere with the ability of fish to detect and respond to biologically relevant sounds (Duarte et al., 2021). At short distances, very loud anthropogenic noise can lead to severe tissue damage or even death in fish. However, this only affects a relatively small proportion of animals that are within a couple of hundred meters from the sound source. Over larger distances, anthropogenic noise can still a) lead to temporary hearing impairment and increased stress levels, masking biologically important sounds for communication or orientation, b) deter fish from an area and c) affect normal behaviour, which can potentially impact a much larger proportion of animals (Slabbekoorn et al., 2010).

In this thesis, I investigated the effects of two man-made sound sources in relation to the movement behaviour of free-swimming Atlantic cod (*Gadus morhua*) in the Belgian part of the North Sea (BPNS): seismic surveying for the exploration of the seabed and pile driving during the construction of offshore windfarms. This is achieved by using acoustic telemetry, a tracking method that allows for fine-scale investigation of movement and activity. The results will help to better understand how a widely distributed fish species like cod responds to high-impact anthropogenic sound in its natural habitat, and what are the effects on the time spent in different behavioural states. Ultimately, these insights will bring us one step closer towards recognising the effects of anthropogenic noise on the behaviour of individual marine fish and the potential consequences this may have at population level.

The marine soundscape and sound perception by fish

To understand how anthropogenic noise can affect fish, we first need to understand which natural and anthropogenic sounds are present in the ocean soundscape, how sound behaves in water, and how fish perceive sound. The marine environment is filled with natural sounds originating from geological sources, such as earth quakes, tectonic plate movement, rain and wind, and biological sources, like sounds produced by animals living around a coral reef, fish choruses and marine mammal calls (Duarte et al., 2021; Gordon et al., 2019; Hatch and Wright, 2007; Slabbekoorn et al., 2010). Since the industrial revolution, anthropogenic sounds have become an increasingly larger part of the ocean soundscape (Duarte et al., 2021). The biggest contributors are shipping traffic, offshore construction and exploitation activities of oil and gas platforms and wind turbines, and exploration of the seabed by seismic surveys. Anthropogenic sounds can now be heard in every part of the ocean, and one of the most noisy marine areas in the world is the southern North Sea region. Just off the coast of the Netherlands and Belgium, sound levels reach 30 dB above the median sound levels measured in the North Sea (Hildebrand, 2009; Sertlek et al., 2019); however, also here relatively little is known about the effect of increasing anthropogenic noise on marine life (Slabbekoorn et al., 2010; Williams et al., 2015).

Sound propagation in the marine environment depends on the physical properties of water. Water is a much denser substance than air and sound therefore travels much faster and further in water than it does in air; at 10 °C sound travels at 331 m/s in air compared to 1500 m/s in salt water (Ainslie et al., 2009). Sound is a vibration of the fluid particles that propagates away from the source as a longitudinal pressure wave. The wave causes fluctuations in water particle velocity, fluid density and pressure. The strength of the received sound in water is characterized by two aspects: the sound pressure and particle motion (Ainslie et al., 2009). Sound pressure describes the sound wave that travels in all directions and has a magnitude, as well as a temporal and spectral characteristic. Particle motion describes the back-and-forth motion of particles and, as such, is a vector quantity with a direction. All fishes, including elasmobranchs, can detect particle motion (Nedelec et al., 2016; Popper and Hawkins, 2018) and some fishes, i.e. those with a swim bladder, can also detect sound pressure.

The role of natural sounds or the impact of anthropogenic sounds depends directly on what animals can hear. Fish detect sound with their inner ear and through their lateral line (Popper and Fay, 2011). The inner ear consists of three pairs of otoliths: Calciferous stones with a density higher than water or the fish itself, that are surrounded by sensory hair cells. In response to particle motion, the otoliths move at a different phase and amplitude, which is detected by the hair cells. The lateral line includes sensory hair cells that can pick up the low-frequency particle motion (Popper and Fay, 2011). Many fish also have a gas-filled swim bladder which resonates the sound pressure wave by compressing and expanding and thereby converting pressure into motion, making it possible for these fish to also detect the sound pressure component (Popper and Hawkins, 2019). How well fish detect sound pressure depends on the proximity of the swim bladder to the inner ear and on whether they have a structural connection between the two.

Anthropogenic sounds and their effects on fish

The effect of anthropogenic noise on fishes depends on their hearing sensitivity, received sound levels at the fish, as well as on sound characteristics. The sounds that most fishes hear best are within the lower frequency ranges between 50-500 Hz (Popper and Hawkins, 2020). Some species, like the European eel (*Anguilla anguilla*), can detect even lower frequencies (Jerkø et al., 1989), or higher frequencies, like herring (*Clupea harengus*) (Enger, 1967). Fish hearing ranges overlap with the majority of noisy human activities (fig. 1) (Duarte et al., 2021; Slabbekoorn et al., 2010), as the predominant energy of anthropogenic noise falls within 100-500 Hz (fig. 1). Under water, sound attenuates over distance and the received signal amplitude and frequency range will be different close to the source compared to further away from the source. In addition, the sediment type and water depth may also have a substantial effect on sound propagation through the water column. In the relatively shallow North Sea, the seabed is predominantly composed of soft medium-grain sediments (Brabant et al., 2013) and depths vary between 15-40 m. Here, the low-frequency component of sound pressure attenuates quicker through the seabed than the high-frequency components. The effect of the same noise source may therefore change depending on whether the noise source is closer to, or further away from, the fish.

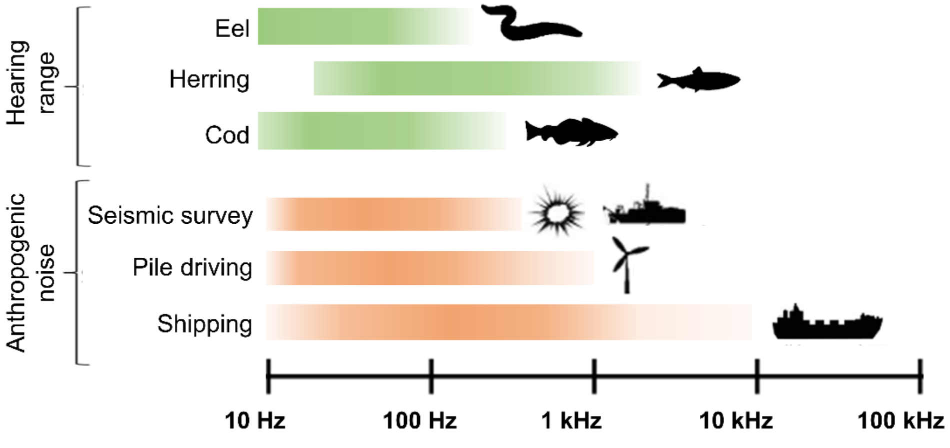

Figure 1. Top: the spectral hearing range of three fish species: eel (*Anguilla anguilla*) (Jerkø et al., 1989), herring (*Clupea harengus*) (Enger, 1967), and cod (*Gadus murhua*) (Chapman and Hawkins, 1973). Bottom: the range of three relevant anthropogenic noise sources: seismic exploration through surveying,

pile driving for e.g. wind turbines, and general shipping traffic (adopted from Duarte et al., 2021).

The temporal characteristics of anthropogenic noise can be continuous or impulsive, depending on the source. Shipping produces a continuous low-frequency sound that elevates ambient noise levels, which are particularly loud around shipping routes and harbours (Sertlek et al., 2016). This constant noise may mask biologically relevant sounds (Holles et al., 2013; Radford et al., 2014) and affect predator-prey relationships. Damselfish (*Pomacentrus amboinensis*), for example, have been found to respond less rapidly and less often to a simulated predator attack when exposed to boat noise (Simpson et al., 2016).

Anthropogenic sound production may also cause impulsive sounds. Among the most noisy, impulsive activities at sea is seismic surveying, commonly used to explore the seabed for fossil fuel deposits, such as oil and gas, and site selection for offshore wind farms and carbon sequestration (Carroll et al., 2014; Shogenov et al., 2017). Seismic surveys cause high-intensity, low-frequency (10 to 500 Hz, own data) acoustic pulses every 8-15 seconds (Sertlek et al., 2019), audible over hundreds of km^2 almost 24/7 (Dragoset, 2005; Gisiner, 2016; Nieukirk et al., 2012; Slabbekoorn et al., 2019). Another source of impulsive sounds is pile driving, which is the most commonly used method to construct foundations at sea for offshore platforms and wind turbines. During pile driving, a foundation pile is driven into the seabed using a hydrologic hammer that strikes the steel pile repeatedly at intervals of 2-4 sec, creating a series of loud impulsive sounds (Ainslie et al., 2020; Hildebrand, 2009). Pile-driving creates low-frequency (10 to 1000 Hz) sounds that travel downward as a conical wave from the pile wall into the water column and seafloor (Dahl et al., 2015; Martin and Barclay, 2019; Zampolli et al., 2013). Both seismic and piling sounds have a high potential to negatively affect marine wildlife, both on a lethal and sub-lethal level (Duarte et al., 2021; Popper and Hastings, 2009; Popper and Hawkins, 2019; Slabbekoorn, 2019; Slabbekoorn et al., 2010).

Potential effects of impulsive sounds produced by seismic airguns and pile driving on marine fish, range from physical injuries which may result in death, when fish are present at close proximity (5 - 300 m) from the sound source (McCauley et al., 2003; Popper et al., 2005), to more subtle effects on physiology (Davidsen et al., 2019a; Sierra-Flores et al., 2015) and behaviour, which may extend to tens of kilometres from the sound source (Carroll et al., 2017; Slabbekoorn et al., 2010). In an outdoor field experiment, juvenile sea bass (*Dicentrarchus labrax*) exposed to pile driving noise at 45 m from the source, reduced their oxygen consumption, an indication of stress (Debusschere et al., 2016). Hubert et al. (2020) found that playback of seismic sound pulses changed the movement behaviour of Atlantic cod (*Gadus morhua*) in a net-pen experiment, indicating a change in energy expenditure. Further from the source, field experiments have shown that fish might change their distribution over an area, or be deterred from an area, due to exposure to seismic sound. A number of studies have looked into commercial catch rates of fish in relation to seismic shooting and found that this can results in higher or lower catch rates, depending on the fish species and vessel distance (Bruce et al., 2018; Engås and Løkkeborg, 1996; Løkkeborg et al., 2012; Streever et al., 2016). Video observations found that reef fish that were active before, were hiding during seismic exposure (Paxton et al., 2017). Still, data on the effects of impulsive sound on fish exist for only very few species and are restricted in their scope.

Tracking and interpreting the movement of free-swimming fish

To study realistic effects of noise on free-swimming fish, we need to be able to observe the animals' movement and behaviour in their natural habitat. This remains a challenge as the visibility under water is often limited, due to high concentrations of suspended particles and sunlight reaching down to maximally 200 m. In addition, radio waves and GPS signals do not propagate well in salt water, which makes live tracking difficult (Hussey et al., 2015). Over the past decades, however, technologies have developed fast, resulting in a number of increasingly smaller, electronic tracking devices to remotely track wild fish (Evans et al., 2013; Hussey et al., 2015). Bio-logging devices range from very small passive PIT tags that are activated to transmit a signal when the fish passes an electrical detection field to monitor passage (Boarman et al., 1998), to large satellite tags that pop off from the animals after data collection (measurements of depth, temperature and/or light levels), and ascend to the water surface where they transmit the recorded information to a satellite (Myers et al., 2006). Another well-established method to study presence and movement of free-swimming fish is acoustic telemetry (Hussey et al., 2015). Using this technique, it is possible to obtain high-resolution behavioural information including 2D-positions, depth, temperature and acceleration of the tagged individual (Williams et al., 2020).

Passive acoustic telemetry uses an electronic tag, including a battery, optional sensors (measuring e.g. temperature, depth, or acceleration), and a transmitter that emits a signal which can be picked up by a stand-alone hydrophone-receiver. Tags transmit information on fish-id and sensor measurements at set intervals as a coded high-frequency (e.g. 69, 180, 307, 416.7 kHz) signal. The range over which the signal can be detected depends on the tag power output, transmission frequency, and the local environmental conditions. In a shallow habitat, sound is absorbed and reflected by the seabed and water surface and a signal will reach less far than in an open ocean environment (Ainslie et al., 2014). In addition, fish with smaller home-ranges are easier to track using this method than ones with larger home-ranges (Bruce et al., 2018).

For optimal information collection, acoustic receivers have to be strategically positioned in the habitat where the tagged animals reside. To record fine-scale 2D-positions of tagged fish, receivers are placed in an array so that their detection ranges overlap and the same tag-signal is picked up by multiple receivers. A hyperbolic positioning algorithm is then implemented to estimate positions at a spatial resolution of meters, using the Time-Difference-Of-Arrival (TDOA) (Biesinger et al., 2013; Espinoza et al., 2011; O'Dor et al., 1998; Skerritt et al., 2015; Voegeli et al., 2001) of a signal detected by three or more receivers in the array. By linking fish positions with recorded bio-logging sensor information, we can derive fine-scale behavioural information, like feeding events, mating and resting, from free-swimming fish (Abecasis et al., 2018; Hussey et al., 2015).

The derivation of behavioural information from recorded positions and sensor data-sets requires an analysis method that can deal with large amounts of data and estimate when and how long an individual spent in a particular type of behaviour. These types of analysis can be done using state space models that include both a behavioural/movement and a spatiotemporal component (Hooten et al., 2017). A Hidden Markov model (HMMs) is such a state-switching time series model (Zucchini and

Macdonald, 2016) which is increasingly used for movement analysis from animal tracking behaviour (Langrock et al., 2012). Used almost exclusively with position data before, their application to incorporate additional data streams from sensors like accelerometers and pressure sensors, was highlighted recently with promising results (Bacheler et al., 2019; Conners et al., 2021; Leos-Barajas et al., 2017a). HMMs model the time probability that an animal spends in certain, predefined, behavioural states using multiple types of data recorded in the field and have the ability to test the effect of predictor variables on state transition probabilities (Leos-Barajas et al., 2017b; McClintock and Michelot, 2018; Patterson et al., 2009). This makes them a promising tool to go from field data to behavioural classifications and estimate the effect of a disturbance, like anthropogenic noise, on the time spent performing these behaviours.

Atlantic cod in the North Sea

A species of fish that is well suited to study the anthropogenic sound impacts using acoustic telemetry, is Atlantic cod (*Gadus morhua*). Cod is a widely distributed, commercially and culturally important species for the fisheries industry in the North Atlantic including the North Sea region (Hutchings, 2004; Rose, 2004), while there have also been dramatic population declines of this species, related to overfishing and climate change (Engelhard et al., 2014; Pitcher et al., 2009; Worm et al., 2006). At least five different cod stocks exist in the North Sea (Neat et al., 2014), with different seasonal migration patterns, each with their associated home- and thermal-ranges (Righton et al., 2007; Righton et al., 2010). Adult individuals in the southern North Sea reside during prolonged periods in summer around (artificial) hard structures, such as wind turbine foundations (Reubens et al., 2013a; Reubens et al., 2013b; Winter et al., 2010). They use these areas to forage and for shelter, which is typically reflected in stereotypic diurnal cycles, with elevated local activity and feeding during dusk and dawn (Reubens et al., 2013b; Winter et al., 2010). During fall adult cod move away from offshore sites towards coastal areas for spawning.

Cod are sensitive to low-frequency sound and hear best up to 400 Hz (Chapman and Hawkins, 1973). They can sense both particle motion and sound pressure as they have a swim bladder that is positioned close to the inner ear (Hawkins and Popper, 2020). Cod are also known to produce sound and use it in a variety of behaviours including territorial interaction and mating (Hawkins and Picciulin, 2019). The grunts they produce are made using drumming muscles attached to the swim bladder; both females and males can produce grunts. Hearing is therefore a very important sense for cod and any interference with it may adversely affect their behaviour, individual fitness and population survival. A variety of studies in captivity have addressed their vulnerability to impulsive sounds and demonstrated the effects of seismic exposure on larval growth (Nedelec et al., 2015), a possible impact on physiological stress levels (Davidsen et al., 2019b; Sierra-Flores et al., 2015), and moderate changes in individual swimming trajectories in response to a scaled seismic sound source (Hubert et al., 2020). A risk assessment desk study showed that pile driving may negatively affect cod spawning behaviour (Hammar et al., 2014). However, little empirical data exist that would allow to assess impacts on actual free-swimming cod exposed to anthropogenic noise. In this thesis, I use acoustic telemetry to study the fine-scale movement of Atlantic cod around existing offshore wind turbines during two different impulsive sources of noise pollution: seismic survey sounds and pile driving strikes.

Outline of this thesis

In order to investigate the effects of impulsive sounds on cod movement, I first conducted a field study in which I looked at the effectiveness of two different receiver set-ups to calculate cod 2D-positions. In chapter 2, I describe the results from two different acoustic positioning receiver set-ups around two different wind turbines, located 50 km from the Belgian coast. To understand the effectiveness of the setup specifically for Atlantic cod, a number of individuals was caught and tagged (fig. 2a) and released within the receiver setups. I then analysed the contribution of each individual receiver to cod position calculation and explored which of the two setups suited our main goal best: to track fine-scale movement of cod around a wind turbine base.

After selection of our Acoustic Positional Telemetry set-up (fig. 2b, a circle of six receivers), I used this to conduct a field experiment in which free-swimming Atlantic cod were exposed to a full-scale three-and-a-half-day seismic survey. In chapter 3, I analyse and discuss the findings from this field study in which I tagged cod with acoustic tags (fig. 2a) and used both the calculated positions of cod as well as their Vector Dynamic Body Acceleration (VeDBA), recorded by the tags, to define three different behavioural states. Furthermore, to understand how seismic exposure affected cod presence, I used historic cod detection data from two previous years in the same wind farm, along with our study results.

In addition to analysing the effects of the seismic survey exposure, I performed a separate analysis, including yet another reference year of detection data, in which I specifically looked for any effect of an increase of water temperature on cod presence. Which is a major concern in relation to climate change. Results of this analysis can be found in chapter 4. Besides performing two field experiments, I also analysed an existing acoustic telemetry dataset. In chapter 5, the effects of another impulsive noise source: pile driving, on cod presence and local movement behaviour was analysed. Here, the results on cod presence and movement while exposed to 50 piling events occurring at different distances, over a period of three months, are presented.

Lastly, in chapter 6, I discuss the results presented in this thesis and I highlight several directions for further research.

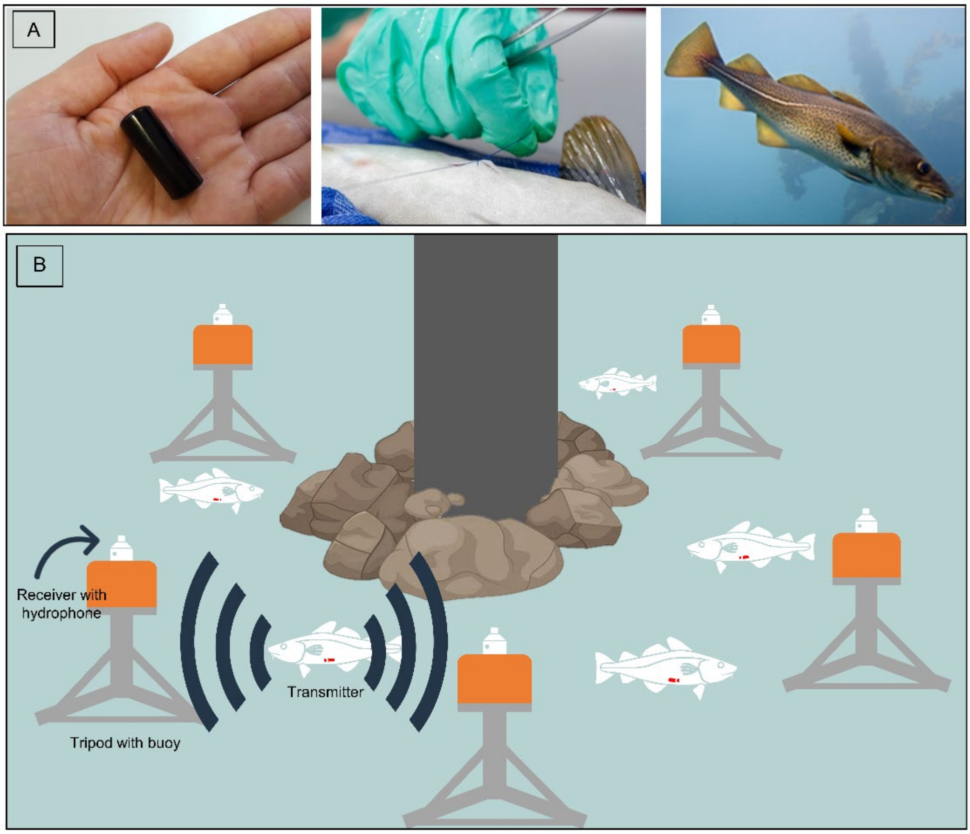

Figure 2. A) tagging of cod using V13 acoustic tags: first a 2-cm cut is made in the abdomen, which is large enough for the tag to slide into the abdominal cavity, after which the cut is sutured up using three stiches and the fish is released after recovery (pictures by Yoeri van Es). B) Schematic overview (not to scale) of the setup of retrievable acoustic receivers around an offshore wind turbine where Atlantic cod are tagged with acoustic tags that transmit information.

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Evaluating receiver contributions to acoustic positional telemetry: a case study on Atlantic cod around wind turbines in the North Sea

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Published in Animal Biotelemetry 2021

DOI: https://doi.org/10.1186/s40317-021-00238-y

Abstract

Background: The effect of individual acoustic receiver contributions to animal positioning is a crucial aspect for the correct interpretation of acoustic positional telemetry (APT). Here we evaluated the contribution of each receiver within two APT designs to the number of tag signals detected and the position accuracy of free-ranging Atlantic cod, through data exclusion of single receivers from the analysis. The two APTs were deployed around offshore (ca 50 km) wind turbines at which 27 individual cod were tagged.

Results: We found that the exclusion of data from an APT receiver that was positioned within the movement area of the individual fish reduced the number of tag signals detected and the position accuracy of the set-up the most. Excluding the data from a single receiver caused a maximum of 34% positions lost per fish and a maximum increase in core area of 97.8%. Single–receiver data exclusion also caused a potentially large bias in the reconstruction of swimming tracks. By contrast, exclusion of a receiver that was deployed within 50 meters from a turbine actually improved fish position accuracy, probably because the turbine can cause signal interference as a reflective barrier.

Conclusions: We recommend that an exploratory small-scale study like the one presented here be conducted before embarking on a larger-scale APT study. By excluding the data of single receivers from the positioning analysis, we were able to explore the suitability of a receiver set-up for the movement patterns of our target species. Furthermore, when a receiver is lost from an APT during deployment, the data should be treated with care as our results show that changes in triangulation outcome can lead to considerable differences in swimming tracks and home range estimates.

Keywords

Acoustic telemetry, position triangulation, behaviour, Atlantic cod, offshore structures, wind power

Background

Acoustic Positional Telemetry (APT) is an established method to study fine-scale fish behaviour and movement at large spatial and temporal scales (Donaldson et al., 2014; Hussey et al., 2015). In an open marine environment, the acoustic receivers that form an APT are often placed in regular squared and triangular grids (Gandra et al., 2018) or in a circular constellation (Leclercq et al., 2018; Neo et al., 2018), depending on the location and research questions (Heupel et al., 2006) and on the species-specific behaviour of the target animals. Some fish species exhibit a high residency and site fidelity, while others are more mobile (Ellis et al., 2019). Even within species, movement patterns may vary with life stage, personality traits and subpopulations (Harrison et al., 2015; Spiegel et al., 2017). The spatial deployment of receivers therefore influences the outcome and reliability of any APT study.

In addition, the performance of a receiver set-up in a marine open-water system will be affected by local habitat conditions (Steel et al., 2014) and by the dynamic nature of environmental factors (Kessel et al., 2014; Reubens et al., 2018). The difference in time of arrival of the transmitter signals at various hydrophones is used in the positioning algorithm. Any factor influencing the speed of sound and signal propagation in the water will therefore affect APT performance (Biesinger et al., 2013; Espinoza et al., 2011a). Habitat-specific features (e.g., vegetation type and density, bottom characteristics, the presence of rocks and man-made obstacles) can block signal propagation (Swadling et al., 2020). Furthermore, natural events such as currents and surface waves can influence receiver detection range (Huveneers et al., 2016; Reubens et al., 2018) and lead to signal interference through receivers getting temporarily buried or even lost (Reubens et al., 2013a). If this occurs, an APT set-up may suffer significantly in terms of the number of tag signals detected and position accuracy. The impact of losing a receiver in an APT study is usually unknown, since information stored on the receiver is no longer available. Furthermore, trade-offs exist between detection range (i.e., area covered by receivers that are able to detect animals) and costs, as acoustic telemetry studies are very expensive (Heupel et al., 2006). Before embarking on a large-scale study, exploring the effects of excluding the data from a receiver on the overall results can greatly facilitate the design of a cost-efficient ATP set-up and enhance understanding of the consequences of receiver loss.

Atlantic cod (*Gadus morhua*) at offshore wind farms in the North Sea are a suitable model system to explore the contribution of individual receivers in an APT design. During summer, cod spend several months residing close to a turbine's scour bed, which provides food and shelter (De Troch et al., 2013; Reubens et al., 2013b; Reubens et al., 2013c). They generally have a small home range around a single wind turbine (Reubens et al., 2013a), but occasionally make excursions to an adjacent one (Winter et al., 2010). Later in the season, cod move away from these offshore structures towards coastal areas for spawning (Righton and Mills, 2008; Righton et al., 2007). Additionally, fishing or shipping is not allowed in the wind farms in the Belgian Part of the North Sea (BPNS). This limits the risk of losing tagged fish or receivers to bottom-trawl fisheries compared to other wind farms in neighboring countries. There are no data yet confirming receiver network suitability for high-resolution spatial tracking of individual fish around a wind turbine, nor is there any exploration reported on the impact of losing or removing a receiver from an operational network.

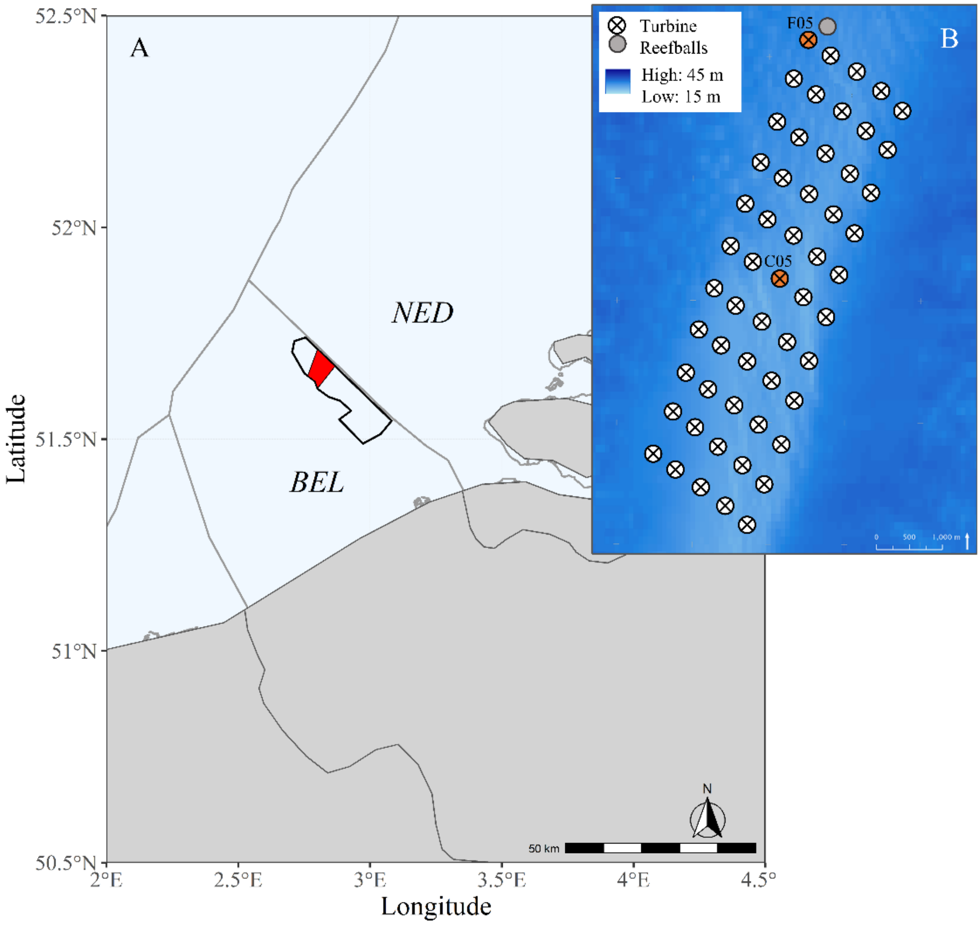
We evaluated the contributions of individual receivers to the detection rate and position accuracy within two acoustic positional telemetry (APT) designs for Atlantic cod at a wind farm in the BPNS. We aimed to answer the following questions: Do individual receivers contribute equally to the spatial data collection and position accuracy? Which local factors explain variation among individual receivers? To what extent is the reconstruction of fish swimming tracks affected by data exclusion from individual receivers? We addressed these questions by quantifying the effects of simulated data exclusion of single receivers on cod detection and positioning data. Our study results provide insights into how positional array designs can be improved for studies into the spatial behaviour and activity changes of fish in response to disturbance by anthropogenic noise (e.g. pile driving, seismic surveys) and reveal the consequences of removing or losing a receiver.

Methods

Study site

This study was performed in the offshore wind farm Belwind (51.670°N 2.802°E), situated on a sand bank ca 50 kilometres off the Belgian coast (fig. 1A, and Brabant et al., 2013). The 55 turbines (fig. 1B) in the wind farm have steel monopile foundations, surrounded by a scour bed protection layer consisting of stones of various sizes (information obtained from Van Oord Dredging & Marine Contractors). The monopile turbines and the scour beds have a diameter of 5 m and of ~40 m, respectively; the precise extent of the scour bed varies, but covers approximately 500 m² per monopile (Degraer et al., 2017). The seabed between turbine scour beds predominantly consists of medium-grained sand dunes, which are formed by the tidal currents. The water depth at the study site was between 20 – 30 m during our study, including tidal fluctuations (Brabant et al., 2013).

Figure. 1 (nest page) Location of Belwind wind farm in the Belgian part of the North Sea. A) Grey lines outline the country's land and water borders (e.g. EEZ's). Contours of the offshore area designated to wind farm construction are shown in black, while red indicates the location of the Belwind park. B) Overview of monopile turbine positions at Belwind. The two orange turbines are the ones around which APT's were deployed. Depth around the turbines varied between 20 -30 meters (bathymetry data obtained from "European Marine Observation and Data Network" (EMODNet)).



Experimental design

VR2AR (Innovasea, Halifax, N.S. Canada) acoustic receivers were used. Two APT designs were deployed from July 4th until September 28th 2017 around two wind turbines: the northern F05 and the more centrally located C05 turbine (fig. 2A and fig. 3A). The set-up around turbine F05 included eight receivers, six of which were placed in a circular shape with similar spacing (150 m on average), the remaining two inner receivers being positioned at approximately 50 m from the edge of the turbine base (fig. 2A). At turbine C05, ten receivers were placed in a triangular-grid configuration, with 150 m up to 200 m spacing between adjacent receivers (fig. 3A). Detection probability at these distances has been tested in the same environment and under similar environmental conditions (Reubens et al., 2018), and remained above 70%, even during harsh environmental conditions. During the deployment period, receiver C05-8 was accidentally lost on the 21st of September. All other 17 receivers remained functional for the entire deployment period. They were bottom-moored using a mooring weight of 60 kg natural stone and a buoy keeping the receiver upright approximately 1.5 m above the seabed (Reubens et al., 2018).

We caught and tagged 27 Atlantic cod (total length range 33-43 cm) with V13AP transmitters (Innovasea, Halifax, N.S. Canada) between July 4th and September 1st 2017 (Table 1). Fish were caught using hook and line from up to 30 m depth and slowly reeled in to prevent barotrauma. Individuals were kept in a holding tank for observation. If fish displayed any sign of serious discomfort or abnormal behaviour (e.g., being unable to keep buoyancy or swimming at the surface), they were not used for tagging. Fish were sedated using clove oil (0.03 ml/L). Upon losing equilibrium, they were placed on their back in a holder at a slight angle, keeping mouth and gills submerged in oxygenated seawater. An incision (2-3 cm) was made on the ventral side through which the acoustic tag was slid into the abdominal cavity. The incision was closed using three monofilament sutures. Fish were measured and tagged with an additional T-bar Floy tag in front of the dorsal fin to avoid double tagging in case individuals would be recaptured (which did not happen). The tagging procedure took on average five minutes, after which the animal was placed in a recovery tank. Upon resuming normal swimming behaviour, individuals were released at the catch site (i.e. turbine F05 or C05). We tagged fish in two rounds, with two groups of tags set at different transmission intervals (Table 1). A transmitter's interval delay was set for a period of 30 days to a random delay varying between 40-80 s or 30-60 s (Table 1). We used two different intervals to investigate if these would lead to differences in numbers of detections or positions. We found no such differences and therefore pooled the data from both groups for analyses.

Table 1. Number of cod tagged per turbine and signal transmission delay

Catch and tag date	Turbine	Cod tagged (#)	Random transmission interval (sec)
4-17 Jul 2017	F05	8	40-80
13 Jul 2017	C05	6	40-80
23 Aug 2017	F05	5	30-60
1 Sep 2017	C05	8	30-60

Data analysis

Data from the receivers were uploaded to the European Tracking Network (ETN) data platform (lifewatch.be/etn). Per positional set-up, a linear time correction of raw detections was performed (on the online Fathom Position platform, position.fathomcentral.com) to correct for differences in internal clock drifts of the different receivers. To calculate transmitter x-y position, we used a hyperbolic positioning algorithm that employed the Time-Difference of Arrival (TDOA) of a coded signal by three or more receivers. This yielded a dataset per APT set-up design (i.e., F05 and C05) containing information on the number of detections per fish and receiver, and triangulated positions with set-up-specific position accuracy estimates. The indicator of position triangulation accuracy provided is called the Horizontal Position Error (HPE) (Smith, 2013). HPE is a dimensionless estimate of position accuracy based on the relationship between theoretical position error sensitivities and observed measurement errors for synchronization tags (Smith, 2013), calibrated to the local environmental conditions (water temperature: 17-19 °C; salinity 33.2 ppt). HPE is set-up-specific and therefore can only be used to compare positions calculated through multiple receiver combinations within the same set-up (Brownscombe et al., 2019; Meckley et al., 2014). The lower the HPE, the higher the expected position accuracy.

We excluded all data from the first day after tagging to avoid possible impact of catching and tagging on fish behaviour. We scanned the dataset for stationary tags (i.e. tags remaining stationary for longer periods of time while acceleration remained 0 ms^{-2} as these would indicate an expelled tag) but found none. No prior position filtering was applied, based on set-up-specific accuracy (e.g. Horizontal Position Error (Smith, 2013)), since the contribution of each individual receiver on fish positions triangulation, is required this the analysis.

We evaluated the contribution of each receiver within the two APT arrays (i.e. eight for the APT at F05 and nine for the APT at C05) to the number of tag signals detected and position accuracy by excluding the data recorded by each receiver once from the position triangulation analysis. For every receiver exclusion, the cod positions with their associated HPE were recalculated (online Fathom Position platform). Cod were expected to reside close to the turbine and were therefore assumed to be detected by the APT when they were in the detection area. To understand the effect of excluding the data from a single receiver on the APT performance, we assumed that when all data from all receivers within each APT were included in the positional analysis, the number of fish positions detected was 100% and the triangulated positions were the "base" fish positions. This enabled us to determine two metrics for each APT performance for each single receiver exclusion (i.e. eight or nine for F05 and C05 respectively): position accuracy and set-up efficiency (Donaldson et al., 2014; Swadling et al., 2020). The latter was calculated as the proportion (%) of daily successfully calculated positions by the APT (i.e. [number of positions when one receiver is removed / number of positions when all receivers are included] *100). Position accuracy was the variability in Horizontal Position Error (HPE) and was the standard deviation of the mean daily HPE associated with all cod positions. A positive difference in HPE indicates a reduced position accuracy, and a negative difference in HPE an improved accuracy in comparison to the complete APT. These metrics were calculated and averaged to give a daily value for both receiver designs per single receiver exclusion for all fish (27 fish in total), including all days with at least 100 detections for that fish. All calculations were performed in R Studio (version 4.0.0).

To illustrate the effect of receiver loss, we evaluated the effect of data exclusion from single receivers on shifts in the triangulated positions of fish with a high residency. We identified fish with high residency by calculating their Residency Index (RI). The RI was defined by dividing the hours an individual fish was detected by the total number of hours between first and last day of detection (maximum of 30 days = 720 h). A value of 0 or 1 indicated no residency or permanent residency, respectively (La Mesa et al., 2012; Lee et al., 2015). Four fish (two at turbine F05 and two at turbine C05), that were detected for more than ten days, exhibited a high RI of >75%. Horizontal space use patterns (i.e., two-dimensional in meters) of these four resident cod were evaluated using 50% (core use area) and 95% (home range extent) kernel utilisation distributions (KUDs) (Worton, 1989). KUDs are a common approach to estimate the activity space of animals from telemetric tracking data (Heupel et al., 2004; Simpfendorfer et al., 2012). All calculations were performed in R Studio (version 4.0.0) using R package *ks* (Duong, 2015).

Results

Individual receiver contributions to APTs

Set-up efficiency was based on all derived cod positions: 76,743 at turbine F05 and 31,202 at turbine C05. To evaluate position accuracy, we used a sub-set of position data to include only positions that could be calculated in all single-receiver exclusion options, resulting in 62,240 and 23,916 positions for turbines F05 and C05, respectively.

Excluding the data from receivers from the APT around turbine F05 reduced the mean daily set-up efficiency to 78-93% of the total detections (fig. 2C), and position accuracy (change in HPE per position) by 0.06-0.94 HPE on average, depending on the receiver that was excluded (fig. 2D). Exclusion of receiver F05-8, one of the most central receivers with respect to fish spatial area use (fig. 2B), had the strongest impact on efficiency. The daily percentage of derived positions was reduced to 78±1.8% (mean ± SE) for this receiver. Excluding a receiver on the east side of the array (i.e. F05-3 or F05-4) lowered the number of derived positions to 85±1.4% and 84±0.8%, respectively (fig. 2A). The position accuracy was affected most when excluding receiver F05-3, which increased HPE to 0.94±0.099 (mean ± SE) per position, as this receiver was critical for many positions of fish that moved to and from the nearby reefballs (fig. 2B). Accuracy improved for 10 and 48% of the positions when one of the central receivers (i.e. F05-8 or F05-7) was excluded from the analysis (i.e., the HPE difference was negative for these positions, fig. 2D).

Excluding data from single receivers around the turbine CO5 reduced the percentage of positions that could be determined to 61-99% (fig. 3C) and position accuracy by 0.11-0.44 HPE (fig. 3D). Excluding the two central receivers (C05-5 and C05-6) had a pronounced effect on the daily set-up efficiency, lowering the percentage of triangulated positions to 61±3.7% (mean ± SE) or 75±2.6%, respectively. Likewise, excluding receivers on the south side of the turbine (C05-2 or C05-3) contributed significantly to the set-up efficiency, lowering the percentage of positions to 73±3.7% or 85±1.6%, respectively (fig. 3C). The receiver contribution to position accuracy revealed a similar pattern. When either of the two central receivers (C05-5 or C05-6) was removed, the HPE increased with 0.45±0.03 (mean ± SE) or 0.17±0.06 (i.e., the accuracy of positions were reduced) respectively. This occurred to a lesser extent when the southern C05-2 or northern C05-9 receivers were excluded (HPE increased by 0.22±0.03 or 0.16±0.01, respectively) (fig. 3D). This reflected the general proximity of fish to the turbine.

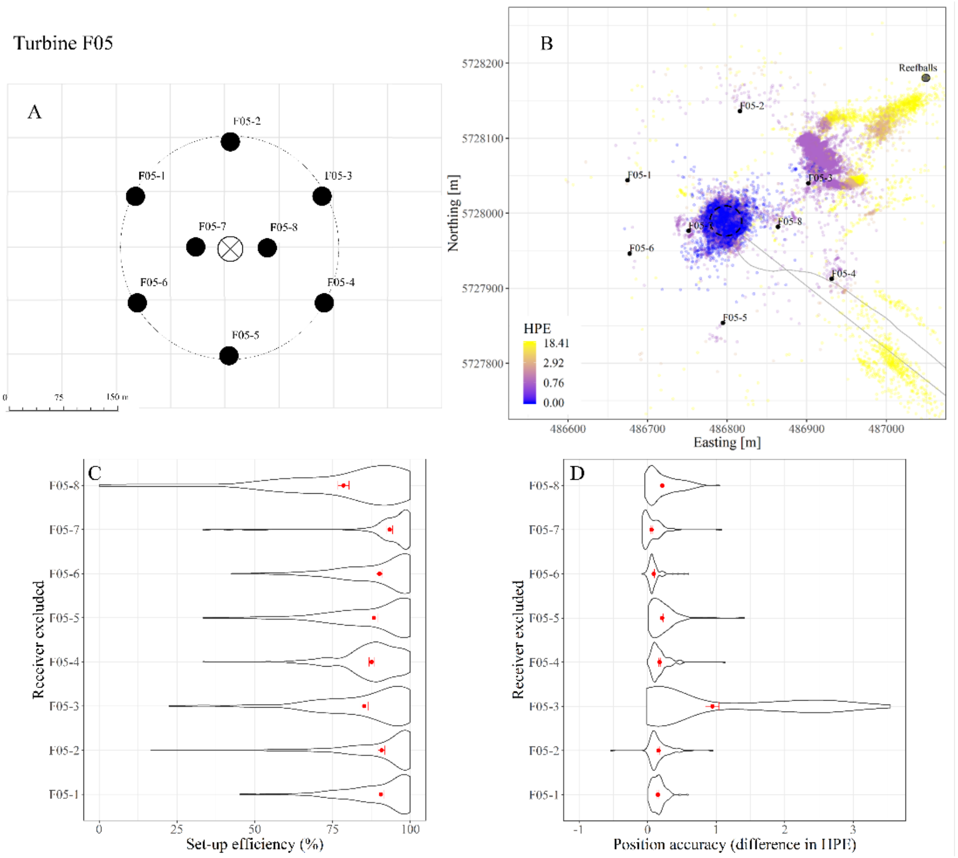


Figure. 2. Overview of the set-up and data from turbine F05. A) Overview of the complete APT set-up. In the data analysis, each receiver was excluded once from the triangulation analysis. B) Derived fish positions (based on all receivers included): colour indicates HPE associated with the position: the lower the HPE, the higher the expected position accuracy. Positions of receivers and reef balls are indicated with black dots and buried electrical cables are represented by grey lines. C) and D) Effect of the exclusion of single receivers from the position triangulation analysis on the spread of: C) the daily set-up efficiency (% of positions calculated) and D) the daily position accuracy (difference in HPE between the same fish positions). Violin plots illustrate the probability density and the red points with lines are the mean values with standard errors.

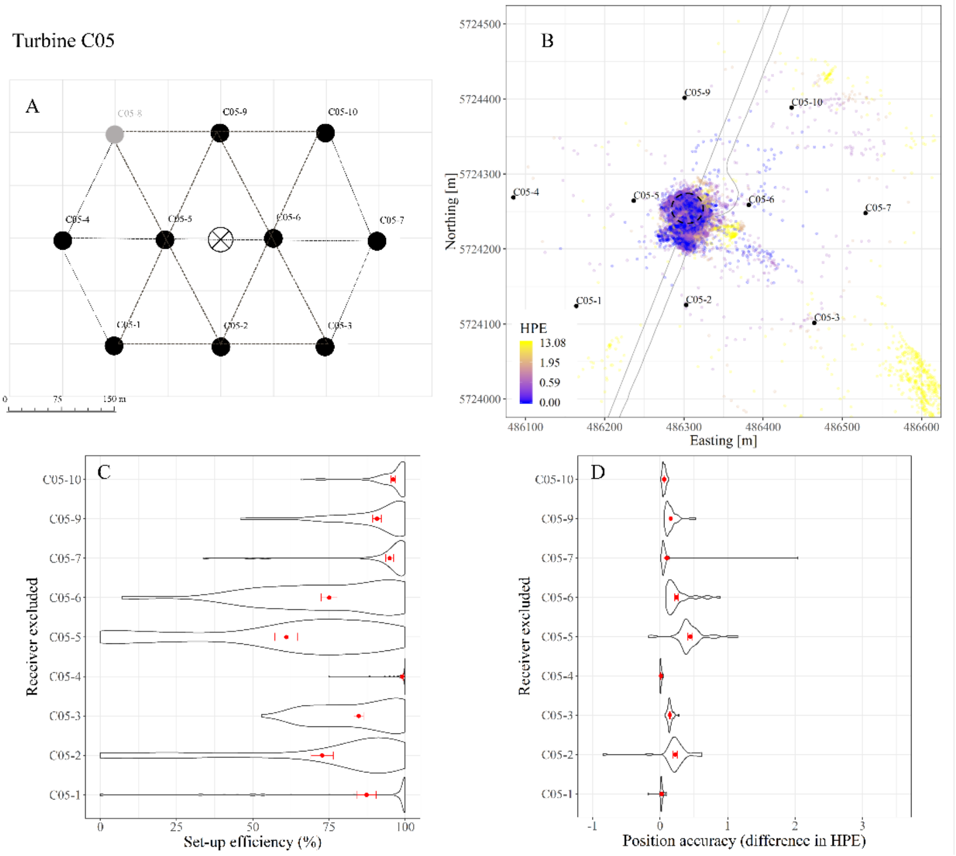


Figure. 3. Overview of the set-up and data from turbine C05. A) Overview of the complete APT set-up. In the data analysis, each receiver was excluded once from the triangulation analysis. B) Derived fish positions (based on all receivers included): colour indicates HPE associated with the position: the lower the HPE, the higher the expected position accuracy. Positions of receivers are indicated with black dots and burrowed electricity cables are represented by grey lines. C) and D) Effect of the exclusion of single receivers from the position triangulation analysis on the spread of: C) the daily set-up efficiency (% of positions calculated) and D) the daily position accuracy (difference in HPE between the same fish positions). Violin plots illustrate the probability density and the red points with lines depict the mean values with standard errors.

Effect of receiver loss on fine-scale fish tracks & KUD

Our results show that the proportion of the positions of the four fish with a high RI (Table 2) derived from the complete receiver set-up is reduced by a single receiver loss by 4-14% and 0-34% for APT's F05 and C05, respectively. The location of the triangulated position of a given fish showed a large shift when a particular receiver was excluded. For example, core area (i.e., 50% KUD) of fish 6 changed from 390 m^2 to 300-780 m^2 depending on which receiver was removed from the APT at turbine F05 (Table 2). Figure 4 uses the same 3-h fish tracks and 50% KUD to demonstrate the extent to which triangulated fish positions can shift in space with or without the data from a single receiver. Exclusion of some receivers caused a small change in core area, between 2.3-22.3 m^2 (Table 2), while exclusion of others caused a large change in core area, between 59.6-384.8 m^2 (Table 2), for the four resident fish (fig. 4).

Table 2. Effect of single receiver exclusion for four resident cod. RI = Residency Index. KUD = Kernel Utilization Distribution.

Fish with RI>75%	Wind turbine	Receiver removed	Positions (#)*	KUD 50% (m^2)*	KUD 95% (m^2)*
6	**F05**	**None**	**13908**	**393.3**	**3912.7**
6	F05	F05-1	-579	+22.3	-250.8
6	F05	F05-2	-814	-95.8	-1201.2
6	F05	F05-3	-1559	+384.8	+4138.1
6	F05	F05-4	-813	+31.2	-942.2
6	F05	F05-5	-307	-67.5	-464.5
6	F05	F05-6	-398	-31.6	-114.9
6	F05	F05-7	-321	+64.1	-228.7
6	F05	F05-8	-1862	-37.7	-342.5
18	**F05**	**None**	**28601**	**341.5**	**5393.9**
18	F05	F05-1	-1626	+50.4	-34.4
18	F05	F05-2	-816	-6.2	-1649.6
18	F05	F05-3	-572	+0.7	-61.1

18	F05	F05-4	-1271	+82.1	-542.3
18	F05	F05-5	-2306	+95.7	-199.3
18	F05	F05-6	-1638	+14.2	-257.1
18	F05	F05-7	-472	+56.5	-618.3
18	F05	F05-8	-1603	+10.5	-960.2
10	**C05**	**None**	**16427**	**194.5**	**1925.2**
10	C05	C05-1	-27	-9.8	-39.6
10	C05	C05-2	-1380	+59.6	+528.3
10	C05	C05-3	-2080	+30.1	-189.4
10	C05	C05-4	-14	+4.6	+18.4
10	C05	C05-5	-1977	+63	+583
10	C05	C05-6	-2143	-22.2	-625.2
10	C05	C05-7	-319	+19.4	-513.5
10	C05	C05-9	-341	+23.8	-59.4
10	C05	C05-10	-163	-5.7	-216.9
27	**C05**	**None**	**5197**	**336.6**	**2630.3**
27	C05	C05-1	-59	-8.6	-116.7
27	C05	C05-2	-464	+134.8	+245.4
27	C05	C05-3	-500	+55.7	+1.3
27	C05	C05-4	-48	-13	-126.2
27	C05	C05-5	-791	+51.7	+797.8
27	C05	C05-6	-724	-25.3	-430.7
27	C05	C05-7	-157	+2.3	-255.3
27	C05	C05-9	-389	+44	+228.7
27	C05	C05-10	-148	-39	-325

*+ or – indicates change from no receivers removed

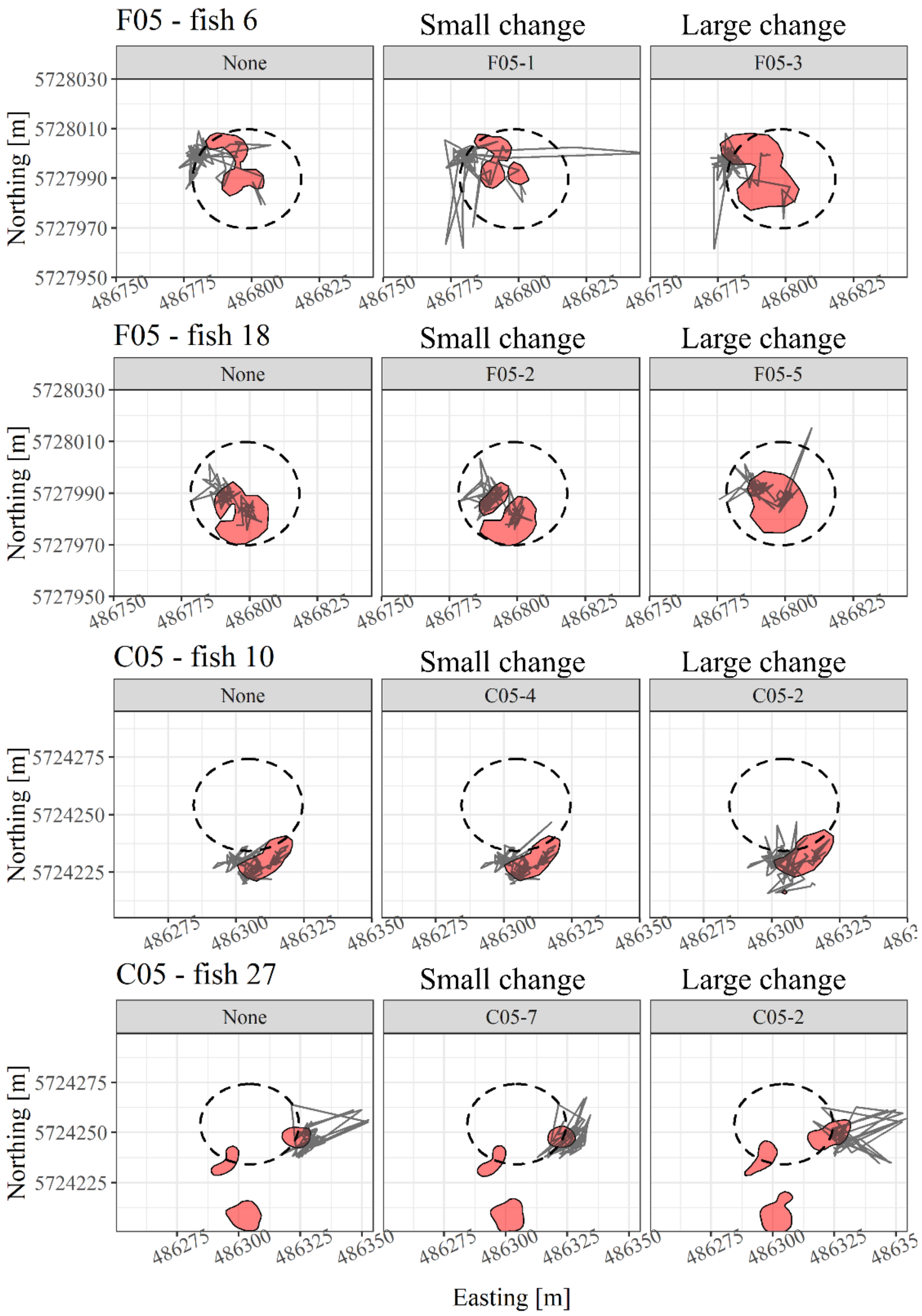

F05 - fish 6
Small change
Large change
None
F05-1
F05-3
F05 - fish 18
Small change
Large change
None
F05-2
F05-5
C05 - fish 10
Small change
Large change
None
C05-4
C05-2
C05 - fish 27
Small change
Large change
None
C05-7
C05-2
Northing [m]
5728030
5728010
5727990
5727970
5727950
5724275
5724250
5724225
486750
486775
486800
486825
486275
486300
486325
486350
Easting [m]

Figure. 4. (previous page) The same 3-hour tracks (grey lines) and 50% core Kernel Utilisation Distributions (KUD in red) of the four cod with a residency index (RI) > 75%, plotted when all receivers were used for the position triangulation (left column: None) and when a single receiver was excluded (simulated loss) from the analysis, which caused a small change in core area, between 2.3 - 22.3 m^2 (middle column), and a large change in fish core area, between 59.6-384.8 m^2 (right column).

Discussion

Our study revealed considerable variation in individual receiver contributions to position accuracy within two Acoustic Positional Telemetry (APT) designs. Critical factors explaining the variation of both outer-edge and inner-circle receivers were the location of the core area of fish activity, the direction of specific journeys outside the receiver set-up area, and the receiver proximity to the turbine. Receivers that overlapped with the spatial distribution of the tagged cod whose core area was concentrated at the turbine base contributed most to the APT's performance. Consequently, excluding the data from a receiver close to the turbine base reduced the percentage of positions derived the most. This was especially the case when the outer-edge receivers were positioned at a distance of more than 200 m from the turbine (as was the case for the outer receivers at turbine C05), and less so when this distance was around 150 m (as was the case for all receivers at turbine F05). Additionally, the presence of the reefballs north east of turbine F05 led to a high contribution of receiver F05-3 to both set-up efficiency and position accuracy, as exclusion of this receiver from the analysis removed positions detected outside of the APT array (fig. 2B). Excluding the data of a single receiver from the analysis, furthermore, resulted in variable changes of fish movement-tracks and spatial area use. Again, loss of the receivers closest to the core area with most fish positions had the largest effect on the fish swimming track and home range.

Lessons for an optimal APT design

The aim of most behavioural APT studies is to understand the movement behaviour of individually tagged animals at a fine scale (Donaldson et al., 2014; Hussey et al., 2015). However, the choice for a particular APT design will also influence how often the target animal is detected as well as the accuracy of positions, which might affect the inferred animal movement patterns. Our results showed distinct patterns in position distribution and cod movement between the two APT locations. Cod at turbine C05 constrained their movements mostly around the turbine, while cod at turbine F05 made frequent excursions towards the adjacent reefballs. Cod residing in offshore windfarms are known to occasionally move between turbines (Winter et al., 2010), and the relative proximity of the reefballs to turbine F05 (~250m) most likely resulted in a clear capture of this movement behaviour by the APT. This resulted in very different patterns in position accuracy and receiver contribution between the two APT arrays. When receivers are positioned too far from the animals' core area, they will be less likely to pick up tag signals (Kessel et al., 2014). Consequently, understanding how a target species will use a spatial area is fundamental to the study outcome and may accordingly require adjustments to juxtaposition of the receivers in the array. This underlines the need for studies similar to ours [c.f. 34] to understand the spatial use of the target species and receiver detection range before undertaking a full-scale project.

Our results furthermore demonstrate that exclusion of either one of the two receivers closest to turbine F05 (i.e., ≈50 m) improved HPE values for fish positions and thus position accuracy. This was not the case for either of the two receivers closest to C05, which were positioned further from the turbine (≈150 m). This difference is therefore also likely due to a proximity-dependent impact of the monopile on signal propagation. The monopile forms a reflective barrier in the middle of the receiver set-up that can cause tag signal reflections and result in multiple signals from the same tag at a nearby receiver (Smith, 2013). This phenomenon of signal reflection by barriers (e.g., water surface, air bubbles, sediment or obstacles) is called "Close Proximity Detection Interference" (CPDI) (Gjelland and Hedger, 2017; Kessel et al., 2015). Consequently, when designing an APT around a reflective barrier, position accuracy can be improved or stabilised by keeping the receivers as distant from the reflective barrier as possible.

Bathymetry can influence sound propagation (Özkan Sertlek and Ainslie, 2014) and therefore affect signal detection and receiver contribution to APT performance. We moored the receivers on a sand bank with dune-like bathymetry (depth may have varied by 1-3m, EMODNet). The structure and position of these dunes can vary under the influence of current direction and strength, and can cause acoustic shadow effects of receivers behind these structures by blocking part of a receiver's listening angle. Additionally, currents can affect the angle at which a receiver is standing (i.e., "tilt"), which can also directionally bias the receiver's listening angle. Tilt is one of the main influencers of detection range (Reubens et al., 2018) and, together with water flow noise, can hamper detectability (Huveneers et al., 2016). At our study site, the semi-diurnal flood and ebb currents flow to the north-east and south-west (respectively), with current speeds typically reaching up to 1 m/s during the turn of the tide (Degraer et al., 2013). Change in receiver listening angle could have influenced the spatial distribution of fish positions to be more towards the west side of both turbines (which is likely more sheltered from the current by the turbine), either because of a true spatial preference of the fish or because of a higher detectability of signals. Obviously, the receiver listening angle in environments with high current speeds can be improved by fixing the receiver in a solid construction above the seabed (Goossens et al., 2020).

Consequences of receiver loss

We mimicked receiver loss through the exclusion of data from single receivers in the APT array. Receiver loss is unfortunately quite common when receivers are deployed for long periods in offshore areas (Fontes et al., 2014; Reubens et al., 2013a), but also in more shallow coastal areas [41,42, current study]. Our results show that the effect of receiver loss depends on how well an APT is covering the spatial area used by the target species. If the array is larger than the home range of the species, losing one receiver may not have a very large effect on the information recovered. However, considerable shifts in the estimated swimming tracks, or direction of frequent journeys outside the set-up range, can occur if a receiver close to the core area of a tagged animal is lost. Our results provide insights into receiver network design and potential consequences of receiver loss for future studies into the spatial response and activity changes of fish due to human disturbance (e.g. effects of anthropogenic sounds on fish movement (Jacobsen et al., 2014; Payne et al., 2014)).

Conclusions

Our results confirmed that a set-up of receivers around a turbine, separated by distances tailored to local propagation conditions (detection range), can provide an APT array suitable for recording the movement and site fidelity of cod resident around wind turbines. We also show that increased resolution of fine-scale positioning can be achieved by placing additional receivers within the array. Locations in close proximity to the turbine should be avoided, however, as this will lower the position accuracy. Based on our findings, we advocate a circular APT design, with an additional receiver in the middle of the array, for future studies on the movement behaviour of fish with a high residency around hard substrates. We also recommend fixing the receiver in a solid construction above the seabed to reduce detection problems due to sand dunes and current-related receiver tilt. Furthermore, our results reveal that the loss of one receiver from an array can result in significant changes in triangulation data, which can lead to considerable shifts in measured swimming tracks and home ranges. Our results also show that a small-scale pilot study allows to test the suitability of a receiver array for the specific movement patterns and local tracking conditions of a particular target species and is recommended before embarking on any larger-scale APT study.

Abbreviations

BPNS: Belgian Part of the North Sea; CPDI: Close Proximity Detection Interference; ETN: European Tracking Network; HMM: Hidden Markov Models; HPE: Horizontal Positioning Error; KUD: Kernel Utilization Distribution; RI: Residency Index; TDOA: Time-Difference-Of-Arrival; VeDBA: Vector Dynamic Body Acceleration; VPS: Vemco Positioning System

Ethics approval and consent to participate

Catching and tagging treatment of free-ranging animals was performed under the approved ethical certificate number EC2017-080, in line with official guidelines for animal welfare in Flanders.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated during the current study are available through the European Tracking Network (ETN) repository, http://www.lifewatch.be/etn, upon request. Further information on the analyses used can be obtained from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

Funding for this research was provided through the 'PCAD4Cod' project granted by the E&P Sound and Marine Life Joint Industry Programme (JIP). Infrastructure for fieldwork (RV Simon Stevin, RHIB Zeekat and receiver deployment) were provided by Flanders Marine Institute and funded by the Research Foundation - Flanders (FWO) as part of the Belgian contribution to LifeWatch.

Authors' contributions

JR, HS, HW, TM and IvdK conceived the ideas and designed the methodology; JR, HW and IvdK performed the fieldwork and collected the data; IvdK analysed the data; IvdK led the writing of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

J. Hubert, J. Campbell, F. Soudijn, P. Verhelst, J. Goossens, J. Vermaut and T. Versteeg assisted with the set-up of the receivers, data collection and fish capture. We thank J. MacAulay and F. Smith for their help and advice on receiver data analysis and B. Lonneville for her help with the wind farm figure. We acknowledge Parkwind for their kind cooperation and for granting access to the offshore wind farm 'Belwind'. This work was supported by the E&P Sound and Marine Life Joint Industry Programme (JIP) as part of the 'PCAD4Cod' project. Infrastructure for fieldwork (RV Simon Stevin, RHIB Zeekat and receiver deployment) was provided by the Flanders Marine Institute and funded by the Research Foundation - Flanders (FWO) as part of the Belgian contribution to LifeWatch. This work was further supported by COST Action CA18102. Lastly, we thank all reviewers for their contributions that have greatly improved the paper.

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3

Effects of a seismic survey on movement of free-ranging Atlantic cod

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Published in Current Biology 2021

DOI: https://doi.org/10.1016/j.cub.2021.01.050

Highlights

- A full-scale seismic survey affected free-ranging cod behaviour in multiple ways
- Cod left the area earlier than expected, two days to two weeks after the survey
- Cod became less active during the seismic sound exposure, and likely foraged less
- Repeated passage of the survey vessel disrupted the diurnal activity cycle

In Brief

van der Knaap et al. show that exposure to a seismic survey caused delayed deterrence of free-ranging Atlantic cod. During sound exposure, cod became less active at dusk and dawn, interrupting their diurnal activity rhythm. These effects indicate potential for anthropogenic noise to affect energy budgets and to have population level consequences.

Keywords:

Acoustic telemetry; Airgun array; Good Environmental Status; Hidden Markov models; Noise pollution

Summary

Geophysical exploration of the seabed is typically done through seismic surveys, using airgun arrays that produce intense, low-frequency sound pulses (Sertlek et al., 2019) which can be heard over hundreds of km^2, 24/7 (Dragoset, 2005; Gisiner, 2016). Little is known about the effects of these sounds on free-ranging fish behaviour (Carroll et al., 2017; Slabbekoorn, 2016; Slabbekoorn et al., 2010). Effects reported range from subtle individual change in activity and swimming depth for captive fish (Davidsen et al., 2019; Hubert et al., 2020) to potential avoidance (Bruce et al., 2018a; Slotte et al., 2004) and changes in swimming velocity and diurnal activity patterns for free-swimming animals (Bruce et al., 2018b). However, the extent and duration of behavioural responses to seismic surveys remains largely unexplored for most fish species (Slabbekoorn et al., 2019). In this study, we investigated the effect of a full-scale seismic survey on the movement behaviour of free-swimming Atlantic cod (*Gadus morhua*). We found that cod did not leave the detection area more than expected during the experimental survey, but that they left more quickly from two days to two weeks after the survey. Furthermore, during the exposure, cod decreased their activity, with time spent being 'Locally active' (moving small distances, showing high body acceleration) becoming shorter, and time spent being 'Inactive' (moving small distances, having low body acceleration) longer. Additionally, diurnal activity cycles were disrupted with lower 'Locally active' peaks at dusk and dawn, periods when cod are known to actively feed (Reubens et al., 2013a; Winter et al., 2010). The combined effects of delayed deterrence and activity disruption, indicate the potential for seismic surveys to affect energy budgets and to ultimately lead to population-level consequences (Soudijn et al., 2020).

Results and Discussion

We examined the response behaviour of 37 free-ranging Atlantic cod to an experimental seismic survey using fine-scale acoustic telemetry (Hussey et al., 2015). Cod were caught, equipped with acoustic transmitters and released at the offshore wind farm 'Belwind' (Figure S1), in the Southern North Sea, 50 km offshore, at 20-30 m depth. A seismic survey vessel performed a standard survey by towing an array of airguns past the wind farm in continuous loops, with parallel tracks of about 25 km, over a period of 3.5 days, with a closest point of approach to the tagging location of 2.25 km (Figure S1). Fish detection, position and axillary sensor information, before, during and after the exposure, were used to answer the following questions: 1) Do cod move out of the study area in response to a seismic survey? 2) Does a seismic survey affect the spatial behaviour, overall activity pattern, and diurnal cycles of cod? And 3) Is there a relationship between sound level exposure and cod activity level (e.g. dose-response)? We analysed data on presence/absence and on the detailed, behavioural patterns in spatial use and accelerometer-based activity of cod using hidden Markov models (Bacheler et al., 2019; Langrock et al., 2012; Leos-Barajas et al., 2017; Mcclintock and Michelot, 2020), and used linear mixed models for the dose-response analysis.

We included daily presence/absence data for all individuals in 2018, with record periods up to three months. Additionally, we included reference data from two preceding years, 2016 and 2017, both including presence/absence data of 14 different individuals within the same wind farm, with variable record periods (Figure 1A). Hidden Markov model analysis found no evidence that more cod left during the survey than before or after (table S1, covariate 'seismic'). However, there was a higher probability of fish leaving up to two weeks after the end of the sound exposure, indicating a delayed effect on leaving or persistent exposure impact (Figure 1B). Of the exposure covariates tested, the ‘persistent exposure effect’ (comparing during and after exposure to before exposure) had the largest effect (table S1). The probability that cod remained onsite in 2018 went down significantly from 36% before, to 3% during and after the survey (significance was established when there was no overlap between the 95% confidence intervals (CI), Figure 1B). During and after the survey, the probability to remain onsite was significantly lower and the probability that cod switched from onsite to offsite significantly increased (Figure 1C).

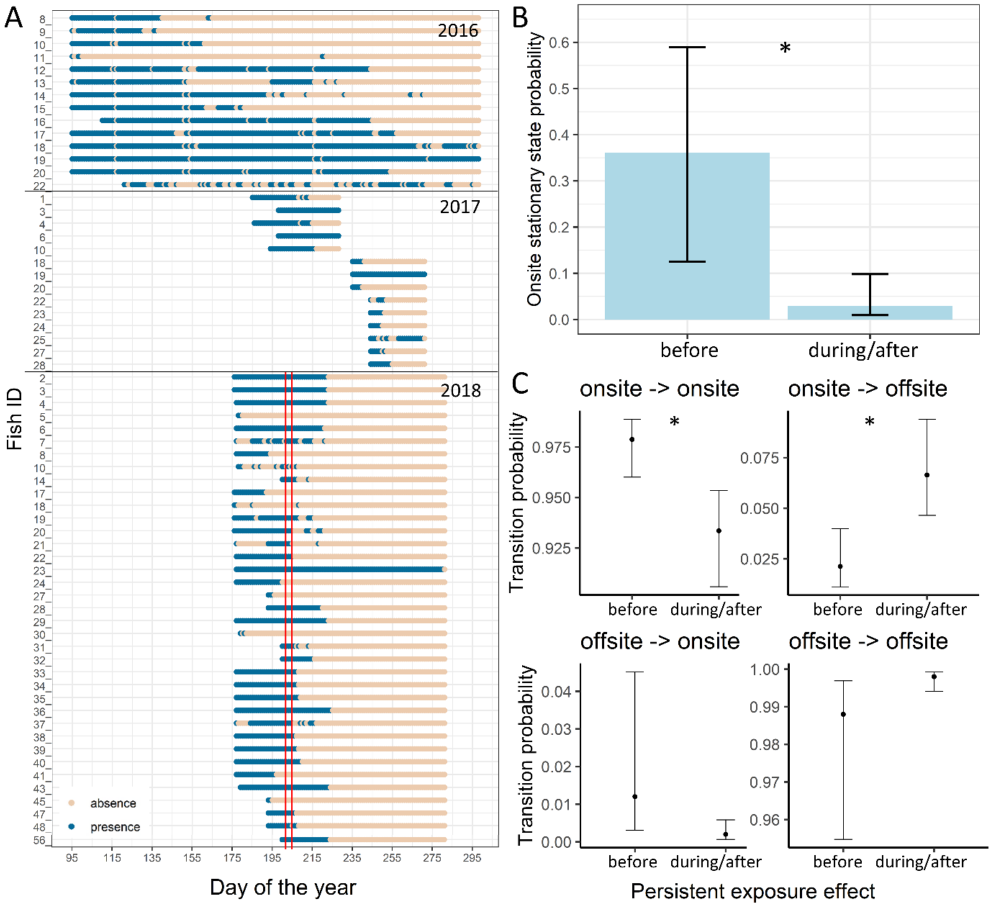


Figure 1. Presence/absence patterns of tagged cod in 2016 (n =14), 2017 (n =14) and 2018 (n = 37) at the Belwind wind farm.

A) Horizontal lines represent individual fish presence (blue)/absence (orange) data (note that fish ID does not correspond to the same individual across years), while the tag was active and detection stations were present, per day of the year (1 = 1 Jan). The vertical red lines outline the period during which in 2018, the seismic survey took place (21–24 July). B) Model-predicted onsite stationary state probabilities for the seismic survey 'persistent exposure effect': before exposure and during/after exposure. C) Predicted transition probabilities between states with their 95% confidence intervals for the 'persistent exposure effect' variable. An * indicates a significant difference in state probability. The other model covariates for the model predictions were set to those experienced during the survey period, e.g. year = 2018, mean temperature = 18.5 °C, and the mean of the time in days since release. See also Table S1.

Fish distance to the closest turbine gradually increased over the three four-day periods of analysis, i.e. from 'before' to 'during' to 'after' the seismic exposure (Figure 2A), with average distances from a turbine per individual of 21.5 m (SD: 13.6, number of positions: 3508), 22.6 m (SD 18.9, positions: 2950) and 26.7 m (SD: 30.1, positions: 2587). A linear mixed model demonstrated that 'after' was significantly different from 'before' (estimate: 0.05, post-hoc 'Tukey' $p < 0.001$; Figure 2B) and 'during' (estimate: 0.03, post-hoc 'Tukey' $p = 0.03$; Figure 2B) for the 19 cod included in the analysis. The model covariate current speed (m/s) also had an independent significant effect ($p < 0.001$), with higher current speeds (related to changing tides) correlated with further distances from a turbine (Figure 2B).

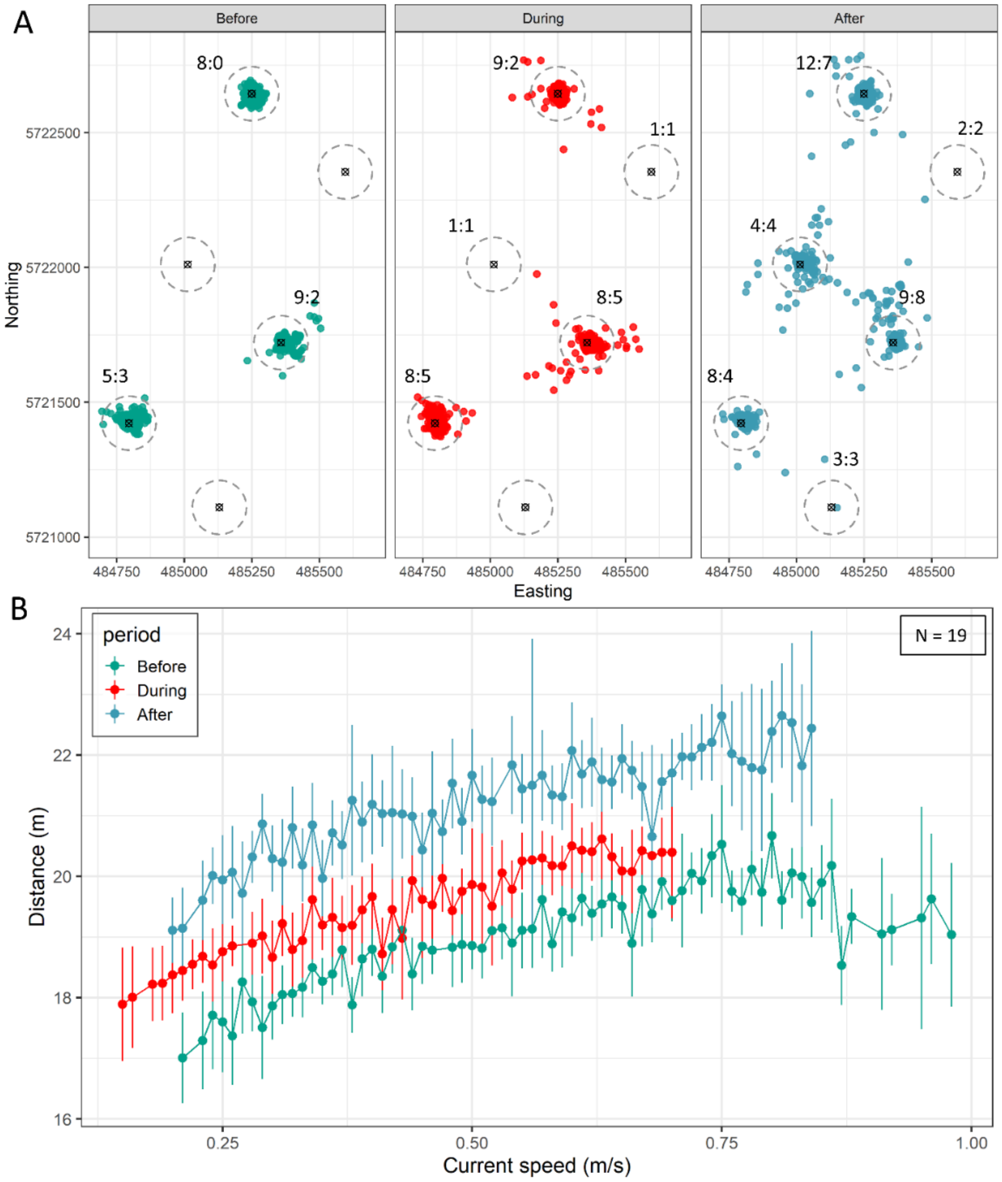


Figure 2. Distance to the closest turbine during the four days before (green), during (red), and after (blue) the seismic survey.

A) Spatial distribution of all triangulated cod positions for the 19 fish used in this analysis. Dashed circles outline areas with a radius of = 100 m around each wind turbine. Numbers indicate the number of individuals detected around a turbine (left of the colon) and how many of those individuals were detected outside of the 100 m radius (right of the colon), during each period. B) Mean and standard deviation of the modelled distance of cod to the nearest turbine, related to current speed (m/s) as predicted by the linear mixed model. Fitted log distance was transformed back to meters. Significant differences in distance were found between the periods During-After ($p = 0.03$) and Before-After ($p < 0.001$).

Between 27 June and 8 August 2018, 24 cod provided enough position data to derive 313 tracks with between 20 and 735 consecutive half-hour positions per track. Hidden Markov models (HMMs) were used to define three behavioural states based on the observed step length between consecutive half-hour fish positions and the mean vector dynamic body acceleration (VeDBA) (Wright et al., 2014). Behavioural states (BS) were then defined as follows: BS1 'Inactive' (small-step, low VeDBA), BS2 'Locally active' (small-step, high VeDBA), and BS3 'Transit' (large-step, low VeDBA) (Figure 3 and table S2). State transition probabilities were modelled as functions of: fish length (cm), current speed (m/s), tidal height (m), hour of day (between 0 and 24) and sea water temperature (°C). We included the covariate seismic on/off (where 'on' refers to during the survey period) to model the effect of the survey (table S3).

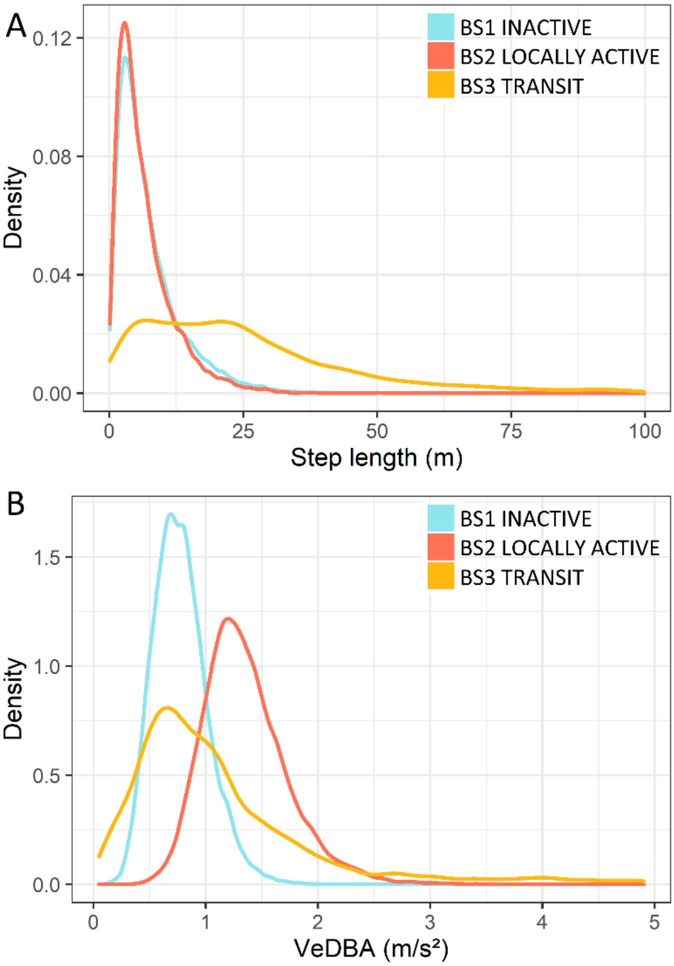


Figure 3. Distribution and classification of two data streams used for the behavioural hidden Markov model (HMM).

A) step length in m between positions (left) and B) averaged vector of the dynamic body acceleration in m/s^2 (right), over the three behavioural states: BS1 'Inactive' (blue), BS2 'Locally active' (red), BS3 'Transit' (yellow). See also Table S2.

Overall, fish spent 47% of the time being ‘Inactive’, 41% being ‘Locally active’ and 12% being in ‘Transit’. Covariates which affected the behavioural states were ‘seismic exposure’ (on/off) and ‘time since survey start’ (0 until start of survey, then increasing with time) (table S3). The fish were significantly less likely to be ‘Locally active’ and significantly more likely to be ‘Inactive’ (no 95% CI overlap Figure 4A) during the survey. The hourly HMM state prediction (Figure 4B) furthermore showed a distinct pattern of diurnal activity cycles before and after the seismic survey. Before the sound exposure, the probability of cod being ‘Locally active’ and in ‘Transit’ increased every evening and night (between 19:00-5:00), while during the day (between 6:00-18:00), cod were more likely to be ‘Inactive’. This diurnal rhythm was disrupted during the seismic exposure, when fish became overall more ‘Inactive’ (Figure 4C).

Finally, we performed an analysis with only the VeDBA, as this was available at a finer time scale than the half-hour position averages, for the 24 fish present during the seismic exposure period. We examined whether there was a dose-response relationship between cod activity and the seismic survey related variation in local Sound Pressure Levels (SPLs, within the 100 and 200 Hz frequency band). Linear modelling showed no effect of SPL on VeDBA ($p = 0.43$ and $p = 0.78$, for 100 Hz and 200 Hz frequency bands, respectively). Only temperature had a significant effect on the activity of the cod: they became less active at higher temperatures ($p < 0.001$).

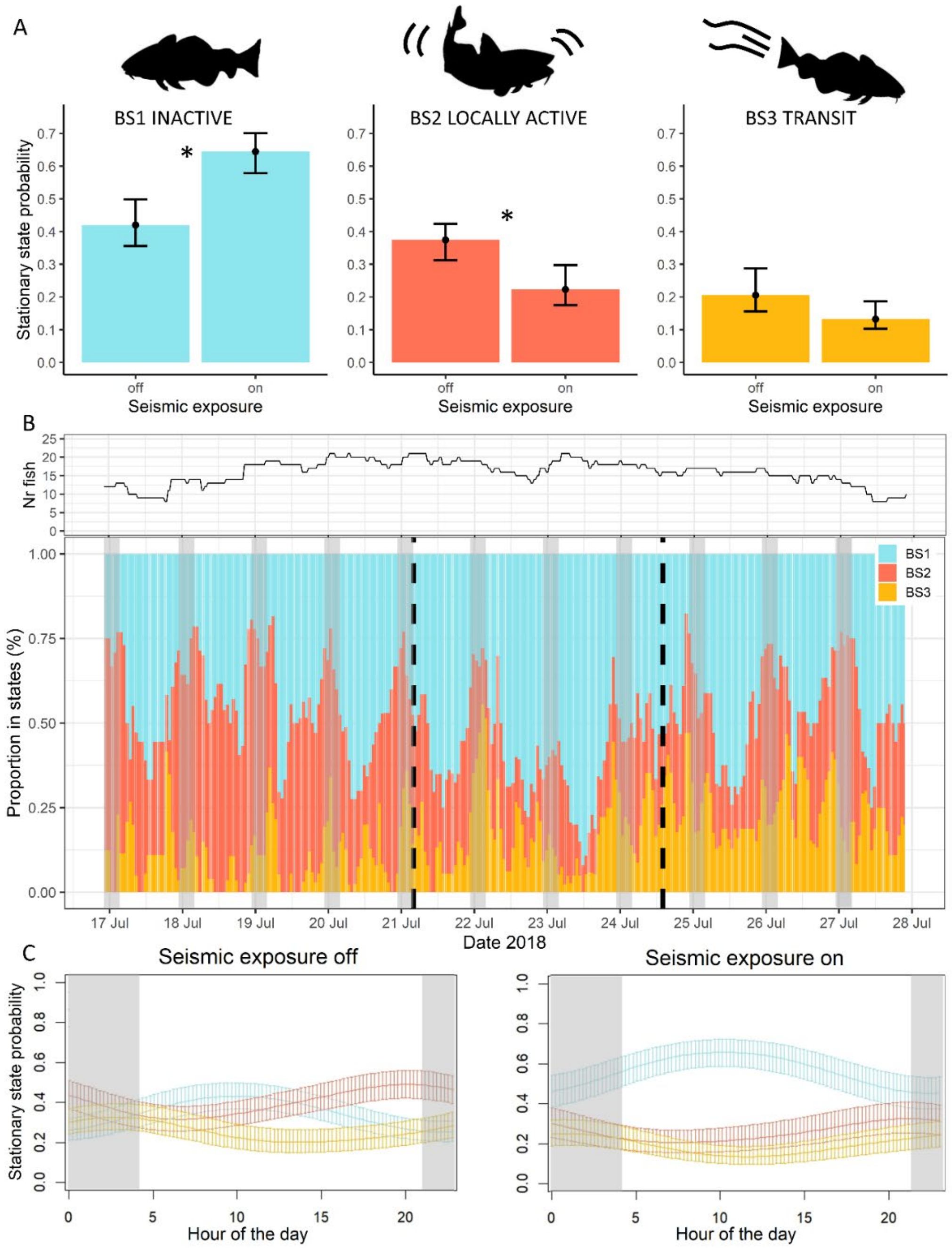


Figure 4. Hidden Markov model (HMM) output for the behavioural states of cod: BS1 in blue: 'Inactive'; BS2 in red: 'Locally active'; and BS3 in yellow: 'Transit'.

A) The stationary state probability for the 24 fish in each of the three behavioural states between seismic exposure off and seismic exposure on. Vertical lines represent the 95% confidence intervals (CI), an * indicates a significant difference (no 95% CI overlap) between exposure off and on in state probability. B) Derived proportion of the time cod spent in the three behavioural states between 17 and 28 July; the number of animals present during that time is plotted on top. Night is shaded and start and end of the experimental seismic survey are indicated with vertical dashed lines (i.e. on 21 and 24 July). C) Stationary state probability with 95% CI for the covariate 'hour of the day', acquired through the HMM when seismic exposure = off (left) and seismic exposure = on (right). Night is shaded (time zone UTC). For all probabilities presented, the values of the other covariates were set to their mean during the sound exposure period: e.g. fish length 39 cm, current speed 0.46 m/s, time since start 1.7 (days), temperature 18.7°C and (for A only) hour 12:00. See also Figure S2 and Table S3.

Our results demonstrate that exposure to a seismic survey had an effect on Atlantic cod movement behaviour during and after the sound source had passed. In the Southern North Sea, cod is a seasonally resident demersal fish (Reubens et al., 2013b; Righton et al., 2007). However, whereas just 22-86 % of cod left their habitat in the reference data from 2016, 2017, and pre-survey in 2018, all but one individual of the 37 tagged cod had left within two weeks after the end of the seismic survey sound exposure. If animals leave their feeding or breeding grounds earlier than usual, or change their migratory behaviour and/or routes, in response to an acoustic disturbance, there may be population-level consequences (Hawkins et al., 2014; Slabbekoorn et al., 2010). Moreover, if fish stay in an area despite a disturbance, there can still be behavioural and population-level effects. Iafrate et al. (Iafrate et al., 2016) showed that resident reef fish that remained on site during pile driving sounds were susceptible to behavioural effects during the exposure.

In line with these results (Iafrate et al., 2016), we found several behavioural changes during and after the seismic survey period that can be attributed to acoustic disturbance. Cod gradually, but significantly, increased their distance to the nearest wind turbine after the sound exposure period compared to before and during exposure. At the same time, more individuals were observed at more than one wind turbine, indicating that the larger distances from a turbine may be associated with increased roaming behaviour and turbine switching (Winter et al., 2010). This could be an indication of deviant movement behaviour eventually leading up to departure from the detection area.

Importantly, the movement analysis revealed an impact of the seismic sound exposure on the behaviour of cod: during the survey, cod spent a significantly larger portion of their time being 'Inactive'. Moreover, the clear daily activity cycle they exhibited in reference periods, being 'Locally active' for most of the time during dusk and dawn, was disrupted during the seismic sound exposure. Based on stomach analysis, Reubens et al (Reubens et al., 2013a) demonstrated that cod at wind turbines feed mostly at dusk and dawn. Indeed, in the present study, the behavioural state 'Locally active' was associated with high VeDBA values, which are considered to be a good proxy for movement activity (Broell et al., 2013; Brownscombe et al., 2014; Metcalfe et al., 2015; Wright et al., 2014), indicating that feeding behaviour occurred mostly in this behavioural state (Brownscombe et al., 2014).

Overall, our results demonstrate that cod became less active during the entire sound exposure period, which may have important repercussions for their foraging time and food intake. Previous reported responses of cod to acoustic stressors in captive studies were at a scale of minutes to hours (Davidsen et al., 2019; Hubert et al., 2020; Kastelein et al., 2008). Here, we observed disruption of activities and daily rhythm over a much longer period, throughout the 3.5 day survey period, and a possible delayed deterrence effect up to two weeks after the exposure.

The results from the current study are an important step forward in providing quantitative field data on individual fish for population-level effect studies of seismic surveys (Slabbekoorn et al., 2019; Soudijn et al., 2020; van Leeuwen et al., 2013). Behavioural changes may affect the energy an individual spends on growth and reproduction, if they lead to changes in metabolic maintenance rates or food intake rates. Such changes have been shown to be more influential at the population level in the long term, than direct changes in reproductive output or mortality (Soudijn et al., 2020). In the current study, we found potential changes in both: cod activity levels, as well as the diurnal rhythm of behavioural states that include feeding. We now need additional information to quantify energy expenditure per behavioural state, and how food intake is affected by the change in diurnal rhythm, to adequately model population-level effects (Griffiths et al., 2018; Langrock et al., 2012; Pirotta et al., 2018; Soudijn et al., 2020).

The delayed deterrence and persistent effect in leaving the area may also have consequences for energy expenditure and food intake, as the cod might spend more time swimming and less time feeding at their preferred foraging grounds. To quantify how leaving affected the energy balance, more information is still needed on where the animals went and what feeding conditions they experienced at those places. The tagged cod may have moved towards the coast, as they are expected to do later in the season (Righton and Mills, 2008; Righton et al., 2007), or even just to another similar nearby site at a turbine or shipwreck (Winter et al., 2010). Such information cannot be deduced from our current dataset, and will require the use of tags which can track fish over larger distances (Hussey et al., 2015). These tags are, however, inevitably larger than conventional acoustic tags, and they need to be recovered or have to transmit their data to a satellite, both of which are still important bottlenecks for data acquisition (Hussey et al., 2015; Lennox et al., 2017).

The vessel track, going back and forth past the wind farm, was designed to optimally mimic an actual survey (apart from the absence of towed streamers with hydrophones) and to establish sound level related behavioural changes (e.g. create a dose-response). However, the analysis on VeDBA data at high time resolution revealed no indication of a sound level-dependent dose-response of cod activity. This lack of a significant result in the short-term analyses, in combination with the significant results in the longer-term analyses (delayed effect of more fish leaving after the survey and increased inactivity during the survey) may reflect the pace of response patterns for this type of marine fish. Cod may not have responded instantaneously to the airgun sounds, but they may have changed their behaviour more gradually over a longer time period. We believe that this should be an important alert, for any noise impact study, to be aware of species-specific timing of behavioural response patterns.

The current dataset provides an extensive case study, but drawing conclusions on causal relationships should be done with caution, as our study is a single event and results could potentially be influenced by other confounding factors. Examples of such factors could be the unobserved arrival of a particular predator (Link et al., 2009), or increasing water temperatures reaching above certain thresholds for the local cod stock (Høyer and Karagali, 2016; Righton et al., 2010). Rising water temperatures due to global warming are known to affect welfare of local populations and to drive changes in species distributions in the North Sea (Kirtman et al., 2013), and fish are thereby likely dealing with an accumulation of multiple anthropogenic stressors. Therefore we do believe that replication would be valuable (Hubert et al., 2020; Slabbekoorn et al., 2019), especially to shed light on effects of the interplay of multiple stressors on single species as well as on species interactions and the local ecosystem (Kunc et al., 2016; Link et al., 2009). Furthermore, cod vocalizations can be interrupted and masked during the sound exposure (Stanley et al., 2017), which could be an additional response measure and impact factor, respectively, and should be investigated in future studies.

Understanding how anthropogenic stressors like noise pollution affect fish populations is important if we want to achieve a 'Good Environmental Status (GES)' (European Parliament, 2008) of our seas, a goal set for all member states in the marine strategy framework directive by the European Union (Griggs et al., 2013). The North Atlantic is heavily impacted by human activities and the soundscape is dominated by noise from shipping and seismic surveys (Sertlek et al., 2019). Seismic explorations of the seabed are still needed for future offshore developments, related to oil and gas, or for renewable energy sources, such as offshore wind farms, and CO_2 deposition (Carroll et al., 2014; Shogenov et al., 2017). The results of our empirical study on Atlantic cod, in combination with the theoretical exploration of likely causes of population-level effects (Soudijn et al., 2020), suggest that exposure to seismic survey sounds could affect the GES of the North Sea through an impact on the cod population. We therefore believe that replication of the current study, in the same or at other places and with the same or other species, is warranted, but that we also need to follow-up with additional information on movement related oxygen use (de Almeida et al., 2013), behavioural state related feeding rates (van Leeuwen et al., 2013), and prey nutritional values (Soudijn et al., 2020). The insights from our study underline the applied relevance of further investigations into the impact of seismic airgun sounds, and also stress the general validity of conservation concerns about anthropogenic noise pollution in the marine environment.

Acknowledgements

F. Soudijn, P. Verhelst, J. Goossens, O. van Keeken, J. Vermaut and T. Versteeg assisted with the set-up of the receivers, data collection, and fish capture. A special thanks to J. Campbell for his help on all aspects of the data collection, processing and analysis. We thank D. de Haan for his contribution in overseeing the execution of the seismic survey. T. Michelot and the crew of CREEM for their help and advice regarding the data analysis. P. Rogers, for his sound advice, C. Tudorache for his insights in fish physiology, D. Van den Eynde for the environmental data, and Parkwind for their kind cooperation and access to the offshore wind farm ‘Belwind’. This work was supported by the E&P Sound and Marine Life Joint Industry Programme (JIP) as part of the ‘PCAD4Cod’ project. Infrastructure for fieldwork (RV Simon Stevin, RHIB Zeekat and receiver deployment) were provided by Flanders Marine Institute and funded by the Research Foundation - Flanders (FWO) as part of the Belgian contribution to LifeWatch. This work is supported by COST Action CA18102.

Author Contributions

IvdK, JR, HVW and HS were involved in the concept development and experimental design of the study. IvdK, JR, HVW, JH and HS collected the data. IvdK preformed the analysis with input from LT, MA, JH and BM. IvdK led the manuscript writing, and all auteurs edited the paper.

Declaration of Interests

The authors declare no competing interests.

STAR Methods

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Cod acoustic telemetry detection data including sensor information	European Tracking Network	https://lifewatch.be/etn/
Data analysis and code	This paper	https://doi.org/10.14284/438
Software and Algorithms		
RStudio	R Code Team	https://www.r-project.org/
momentuHMM: R package for analysis of telemetry data	McClintock and Michelot (2020)	https://cran.r-project.org/web/packages/momentuHMM/index.html
nlme package	CRAN	https://cran.r-project.org/web/packages/nlme/index.html
MuMIn package	CRAN	https://cran.r-project.org/web/packages/MuMIn/index.html
multcomp package	CRAN	https://cran.r-project.org/web/packages/multcomp/index.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Inge van der Knaap (iej.vanderknaap@gmail.com).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The datasets and code generated during this study are available at the Marine Data Archive: https://doi.org/10.14284/438 and the raw detection datasets can be requested through the European Tracking Network: https://www.lifewatch.be/etn/

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study site and telemetry setup

This study took place in the Belgian and Dutch part of the North Sea between 27 June and 1 October 2018. Fish were tracked in the Belgian offshore Belwind/Nobelwind wind farm (51.670°N 2.802°E; Figure S1A), situated on the Bligh Bank, approximately 50 km offshore from the coastal harbour of Zeebrugge. The water depth at the wind park area varied between 15 – 37 m, including tidal fluctuations (and at our study site this was 3 m); currents in the wind farm predominantly run from northeast to southwest (Brabant et al., 2013).

Acoustic telemetry was used to record presence and track movement of Atlantic cod at the study site. We deployed a total of 21 VR2AR receivers (Innovasea) from 1 June until 9 October around six wind turbines (Figure S1B). A circle (r = 150 m) of six receivers was deployed around three of these turbines (i.e. B8, C9 and C10) in a positioning array, intended for fine-scale positioning of the acoustically tagged fish. Around the other three turbines (i.e. C8, B9 and B10), lone standing receivers served to detect presence/absence of the tagged fish (Figure S1B). The receivers were moored using customised anchoring with the receiver placed on top of a 1.5 meter tall stainless steel tripod (weighing 80 kg) (Goossens et al., 2020).

Fish tagging

A total of 51 Atlantic cod (total length between 32.0-56.0 cm, with an average of 39.3 cm) were caught, tagged, and released within 2 m from one of the 6 experimental turbines. Catching and tagging of free-ranging animals was ethically approved under certificate number EC2017-080, in line with official guidelines for animal welfare in Flanders (Belgium). We tagged and released individual fish on six different days prior to the experimental seismic survey: 25-28 June 2018, 12 and 19 July 2018 (Figure 1A), using the same procedures as van der Knaap et al. (van der Knaap et al., 2020) to surgically insert a V13AP (Innovasea) tag in the abdominal cavity of the fish. The tags were set to transmit at random intervals between 50-100 s, at which point they transmitted a signal lasting ~5 s at 69 kHz. The V13AP tags included an accelerometer and pressure sensor, allowing collection of information on body acceleration and depth. The tags alternatingly recorded and transmitted the information from each sensor. The accelerometer sensor measured acceleration in three directions and provided cumulative means over 37 sec in the three dimensional Vector Dynamic body Acceleration (VeDBA),

$$\text{VeDBA} = \sum_{i=1}^{T} \frac{\sqrt[2]{x_i^2 + y_i^2 + z_i^2}}{T}$$

where x, y, and z are the acceleration values recorded from each axis at sample i over the sample period T.

METHOD DETAILS

Experimental seismic survey

The seismic survey was conducted at the study site from 21-24 July 2018 (by the MV Geo Caribbean, contracted through CGG, Norway). The airgun-array consisted of 36 airguns (G-Gun II Sercel) with a total volume of 2950 in^3 (48.3 L), which fire every 10 seconds during operation. This airgun configuration and firing sequence are standard for a real seismic survey, and the track lines and firing procedures were also realistic for an actual survey. Unlike in an actual survey, streamers and hydrophones were absent. The vessel track consisted of 11 passes alongside the wind farm, crossing the Dutch-Belgian border (Figure S1A). The exposure started approximately 30 km North of the wind farm with a gun test and ramp-up (lasting 40 min), passed by the wind farm 11 times with a closest point of approach of 2.25 km, and ended approximately 25 km North-East from the wind farm (Figure S1A), while maintaining an average speed of 2.2 m/s. During the entire survey period, the airguns fired 2352 times.

Sound measurements and processing

Sound measurements were collected using a moored hydrophone (AMAR G3), at 22 m depth, attached to a 60 kg mooring anchor, and positioned in the middle of the receiver area (Figure S1B). The recording period lasted from 13 July to 3 September 2018, covering sufficient time before, during and after the seismic survey sound exposure experiment. The AMAR was equipped with a hydrophone (M36) and three orthogonally oriented, low-sensitivity particle motion sensors (microelectromechanical systems, MEMS), both sampling at a rate of 32 kHz. Recordings were converted to sound pressure level (SPL) and sound particle acceleration level (PAL) respectively in decidecade (ddec) bands (ref: ADEON soundscape spec (Ainslie et al., 2020)) with centre frequencies ranging from 10 Hz to 16 kHz. Our acoustic terminology follows ISO 18405.

Ambient sound pressure level (SPL, re 1 μPa^2) fluctuates with time and at our site the median SPL (60 s temporal observation window (TOW (Ainslie et al., 2020)) was 116 dB (inter-quartile range (IQR) 5 dB) in the 40 - 400 Hz ddec bands (band filter selected to match cod hearing) for all ambient recordings (table S4). During the exposure period, the median SPL rose by 7 dB to 123 dB (IQR 14 dB). At the closest point of approach (at 2.25 km), SPL averaged over 60 seconds was 147.2 dB re in the 40 - 400 Hz ddec bands (Figure S3A and table S4 for a more in-depth description of the ambient levels).

The self-noise particle acceleration level (PAL, re 1 $(\mu m/s^2)^2$ had a median value of 70 dB (60 s TOW) with IQR 0.4 dB in the 40 - 400 Hz ddec bands (Figure S3B and table S5). PAL reached above self-noise levels when the vessel came within 6 km from the study site, the median value increased to 79 dB, with IQR 5 dB when the vessel was within 2.5 km of the study site. At the closest point of approach (at 2.25 km) PAL was 90.0 dB (Figure S3B, and table S5, for further detailed particle motion description).

Single pulse sound exposure level (SELss) was calculated for the 40-400 Hz and 10 Hz-16 kHz ddec bands, from the 60 s sound exposure divided by the number of actual airgun shots (between 5-7 shots) within that time period (Figures S3C and S3D). The cumulative sound exposure level (SELcum, re 1 $\mu Pa^2 s$) over the 3.5 day survey period at the receiver position was 186.3 dB in the 40 - 400 Hz band.

QUANTIFICATION AND STATISTICAL ANALYSIS

Fish position triangulation and filtering

After recovery of equipment, corrections for receiver clock drift of the receivers during the deployment period were done by applying a linear time correction over the entire sampling period (VUE, Innovasea). We subsequently transformed the detection data into 2-dimensional locations through time difference of arrival (TDOA) positioning [50], using a web interface hosted by Innovasea (i.e. 'VPS lab'). The Innovasea's TDOA algorithm applies weighted averaging of positions from subsets of receivers for a given tag transmission to reduce overall positioning error, although the exact error terms used for weighting and position error estimates are not provided to the end user. The position was only determined when the tag signal was detected by at least three receiver stations.

Positions were filtered based on an associated horizontal positioning error (HPE) of below 8.9 which removed 2% of the positions (van der Knaap et al., 2020); HPE is a unit-less error metric used in Innovasea systems. This cut-off removed positions outside of the positioning arrays that had a clear linear direction bias known to be associated with large positioning error. Furthermore, we removed tags from the dataset if: tagged fish left the detection area within 24 h after tagging, if tags failed to emit any signal (fish 11 and 55), or if tags provided static positions indicative of tag loss or fish mortality (fish 6, 38 and 47).

Data analysis

We used two discrete-time hidden Markov models (HMMs) to determine fish presence/absence and behavioural states from the telemetry data. HMMs are suited for multivariate datasets with temporal autocorrelation, such as the current dataset, and return the probabilities of an animal being in each of a set of mutually exclusive behavioural states (17, 26). HMMs were applied in R (version 4.0.0) using the 'momentuHMM' package (version 1.5.1).

To assess whether the seismic survey sound exposure had an effect on the presence of cod in the area, we combined the newly collected data of 2018 with detection data of Atlantic cod tagged in the same wind farm ('Belwind') in 2016 and 2017. Data from fish that were detected for less than 24 h after tagging and stationary tags (i.e. fish dying or losing the tag) were again removed from the analysis. We only used data from individuals from 2016 and 2017 that were within the size range of the individuals from 2018 (32 -58 cm, total length), resulting in a total of 14, 14 and 37 fish for 2016, 2017, and 2018, respectively. Next, the presence or absence of individuals was defined for each day during the entire study period: from the moment of tagging until receiver recovery. Fish were considered present if they were detected for more than 1 h per day (Figure 1A). We applied a non-spatial hidden Markov model (HMM) to examine if the experimental seismic survey increased the probability that individuals would leave the study area during the survey period or whether there was a decaying or persistent effect, starting from the survey onset and including part or the whole period after the survey. Daily observations of presence/absence (Bernoulli distribution) per individual (65 fish in total) were fitted to the states 'on-site' and 'off-site'. Covariates included in the model were: year, decay after tagging (exponential decay over time since a fish was tagged), and temperature (°C). To model the effect of the survey, the covariates seismic on/off (where on just refers to during the survey period), decay survey (value of 1 during the survey followed by an exponential decay after the end of the survey), and seismic persistent (where on refers to both during and after the survey period) were examined (table S1). The fitted model included all presence/absence data per individual as a single track [51].

Fish positions from four days before until four days after the survey period (17 to 28 July 2018) were selected to examine the effect of the seismic survey sounds on the distance of the fish to the turbines. The four day period was selection to balance the data between the three different periods: before, during and after. The fish that were tagged two days before the survey, and fish that left the area before the end of the survey period, were excluded from this analysis (yielding a sample size of n = 19 for this analysis). Positions were projected to UTM (zone 31) and averaged over half-hour bins per individual. The distance to the closest turbine was calculated as the Euclidean distance in meters. The minimum distance between two turbines was 450 m, and consequently, if a position was calculated to be further than 225 m from a turbine at the centre of a receiver array, it was excluded from the analysis. Distance measurements were positively skewed; therefore, a log-transformation was applied to normalise the distribution. We applied a Linear Mixed Model, which accounted for temporal autocorrelation (AR(1), R package 'nlme' version 3.1), and in which fish ID was a random variable and the fixed effect covariates were: the survey period (before, during and after), hour of the day (cosinor of hour), current speed (m/s), temperature (°C), and tidal change between consecutive points (m). Model selection was done based on AIC comparison using model dredging ('MuMIn' version 1.43). The best model (lowest AIC) was: log(distance) ~ period + current speed + tidal + (1|fish). To examine whether distance of fish to the turbine differed between periods, a host-hoc test was preformed (Tukey HSD 'multcomp' version 1.4).

We applied spatially explicit HMMs to examine if the seismic survey had an effect on the movement behaviour of the study animals. For this analysis, individuals were included that were present for a sufficient amount of time, before, during and after the exposure period, resulting in a sample size of 24 fish. The number of animals present varied over time between 8 and 21, because some fish were temporarily not detected or left the detection area. As raw detection data from the receivers frequently included missed detections and low accuracy on the calculated positions, time-difference-of-arrival locations were averaged over 30-minute bins. Individual movement paths were only retained if they had at least twenty subsequent positions. Observed data streams consisted of step length (Euclidean distance between two subsequent positions) and the associated mean VeDBA transmitted by the tag's acceleration sensor, per 30 minute time period. As no robust, numerical selection criteria for choosing the number of states for HMMs exist [52,53], we based the number of states on the expected number of biologically meaningful behavioural types that could be distinguished in the data (c.f. (Hubert et al., 2020), resulting in: 'Inactive' (Behavioural State 1, BS1), 'Locally active' (BS2), and 'Transit' (BS3) (Figure 3 and table S2). Individual variation was accounted for in the model by including fish length as a covariate. State transition probabilities were modelled as functions of: fish length (cm), current speed (m/s), tidal height (m), hour of day (as cos(2πt /24 h) and sin(2πt /24 h), where t is the time of the observation between 0 and 24 h) and sea water temperature (°C). To model the effect of the survey, the covariates: seismic on/off (only "on" during survey period), time since start survey (value of 0 until the start of the survey after which it increased with time) decay survey (value of 1 during the survey followed by an exponential decay after the end of the survey) and seismic persistent (on during and after the survey period) were examined (table S3). Model selection was based on AICs (table S3).

Potential short-term response patterns to noise exposure levels were examined based on all VeDBA measurements, transmitted by the tags of the 24 fish present during the seismic survey period. The VeDBA data were available as mean acceleration data measured over periods of 37 s and accumulated for all three directions, at a mean resolution of 18.8 ± 14.0 (SD) minutes. We applied a Linear Mixed Model, which accounted for temporal autocorrelation (AR(1), R package 'nlme' version 3.1), and in which the fixed effect covariates were: sound pressure level (SPL of the 100 or 250 Hz frequency band), current speed (m/s), temperature (°C), and tidal change (m). Model selection was done based on AIC comparison, using model dredging ('MuMIn' version 1.43). The best model (lowest AIC) did not include SPL and was: VeDBA ~ temperature.

SUPPLEMENTS

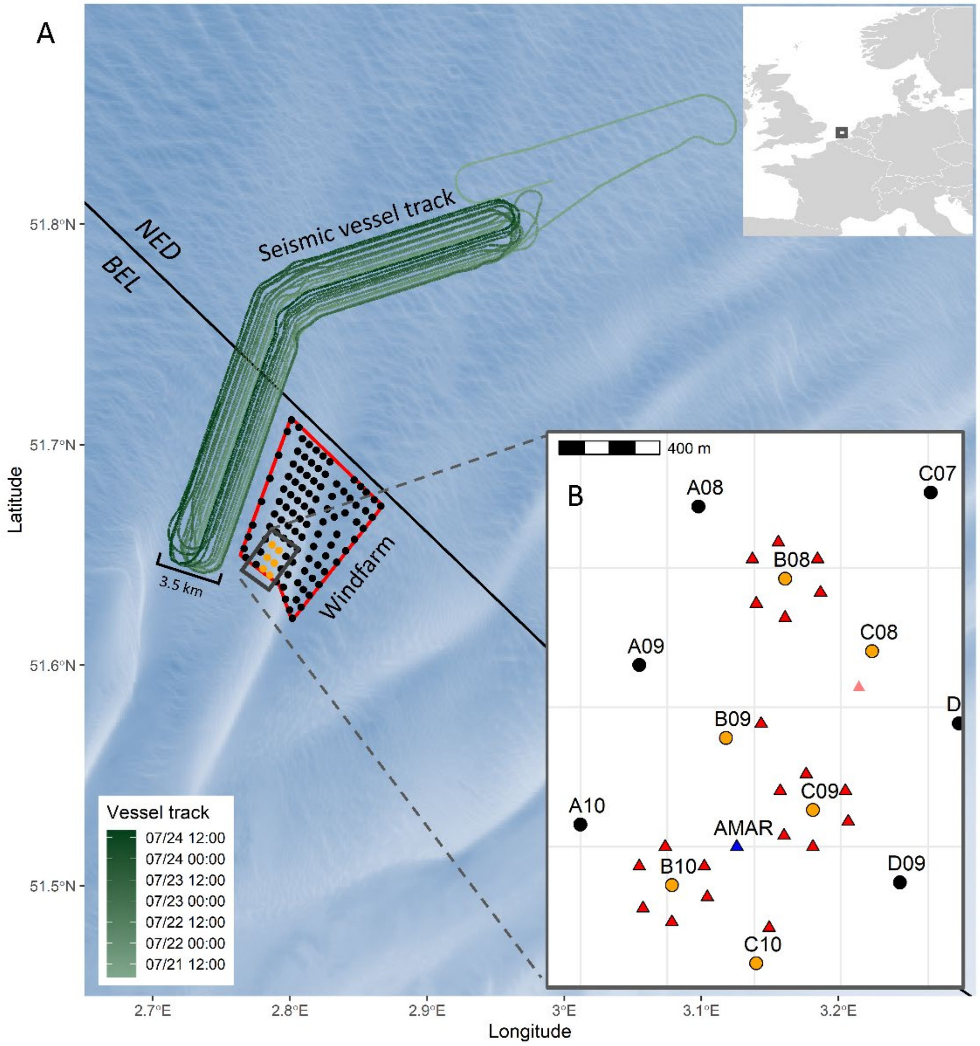
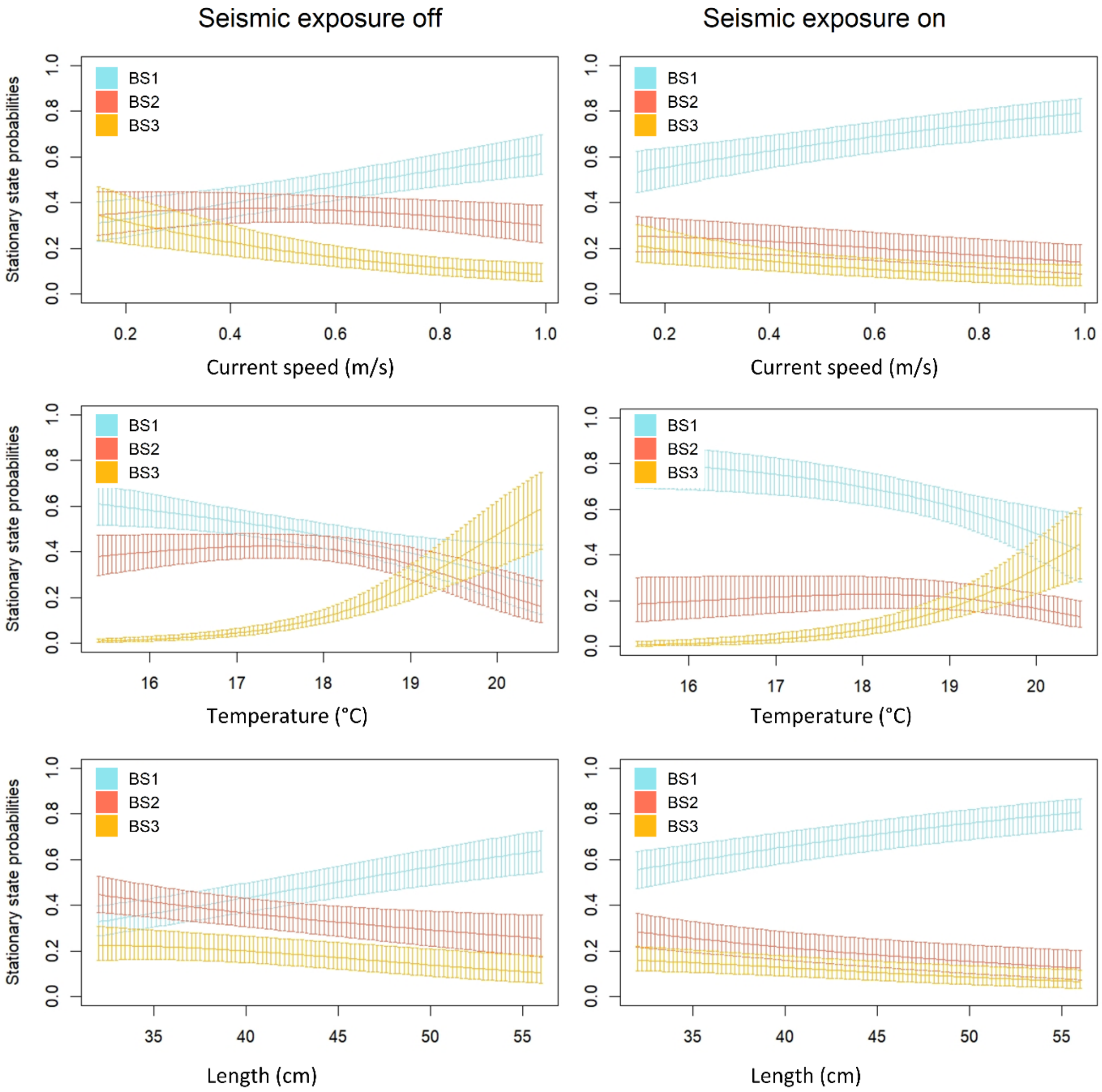


Figure S1 Study location and setup, related to STAR Methods

A) Location of Belwind/Nobelwind offshore wind farm in the Belgian waters next to the Dutch maritime border. The track of the seismic survey vessel is indicated in green. The colour gradient indicates the date and time of the marked survey track positions. Closest point of approach was 2.25 km and varied per passing loop. The red lines outline the wind farm concession area with locations of the wind turbines indicated as black dots. Yellow dots represent the six turbines around which cod were tagged. Local water depth varied between 15-37 m (bathymetry downloaded from EMODNET). B) Positions of the acoustic receivers (red triangles) and the AMAR recording device (blue triangle) relative to the wind turbine positions. Around turbines B10, B08 and C09, six receivers were anchored in a circular array for position triangulation of detected cod, and around turbine B09, C10 and C08, 1 receiver was anchored for presence/absence detection. The receiver at C08 was lost on 22 August 2018. No data were recovered from this device.

Figure S2. **Effects of model covariates on the distribution of behavioural states (e.g. BS1 in blue: 'Inactive'; BS2 in red: 'Locally active'; and BS3 in yellow: 'Transit'), related to Figure 4. (Next page)**

Stationary state probabilities for the three covariate values: current speed (m/s), temperature (°C) and fish total length (cm). State probabilities were predicted for seismic exposure is off (left) and on (right). Increased current speeds, affected the time spent in BS2 and BS3 negatively and increased the time cod spend in BS1. When water temperature increased, cod increased the time they spent in behavioural states 1 and 3 and reduced time spent in BS2. Lastly, larger fish (longer than 35 cm) spent more time in BS1 than smaller fish, and this pattern was reversed for the time spent in BS2. For these three covariates, the state intercepts between seismic exposure off and on changed slightly for all behavioural states. The values of the other covariates were set to their mean during sound exposure period to make these graphs: e.g. fish length 39 cm, current speed 0.46 m/s, time since start 1.67, temperature 18.7 and hour 12:00


Seismic exposure off
Seismic exposure on
Stationary state probabilities
BS1
BS2
BS3
0.0
0.2
0.4
0.6
0.8
1.0
Current speed (m/s)
Temperature (°C)
16
17
18
19
20
Length (cm)
35
40
45
50
55

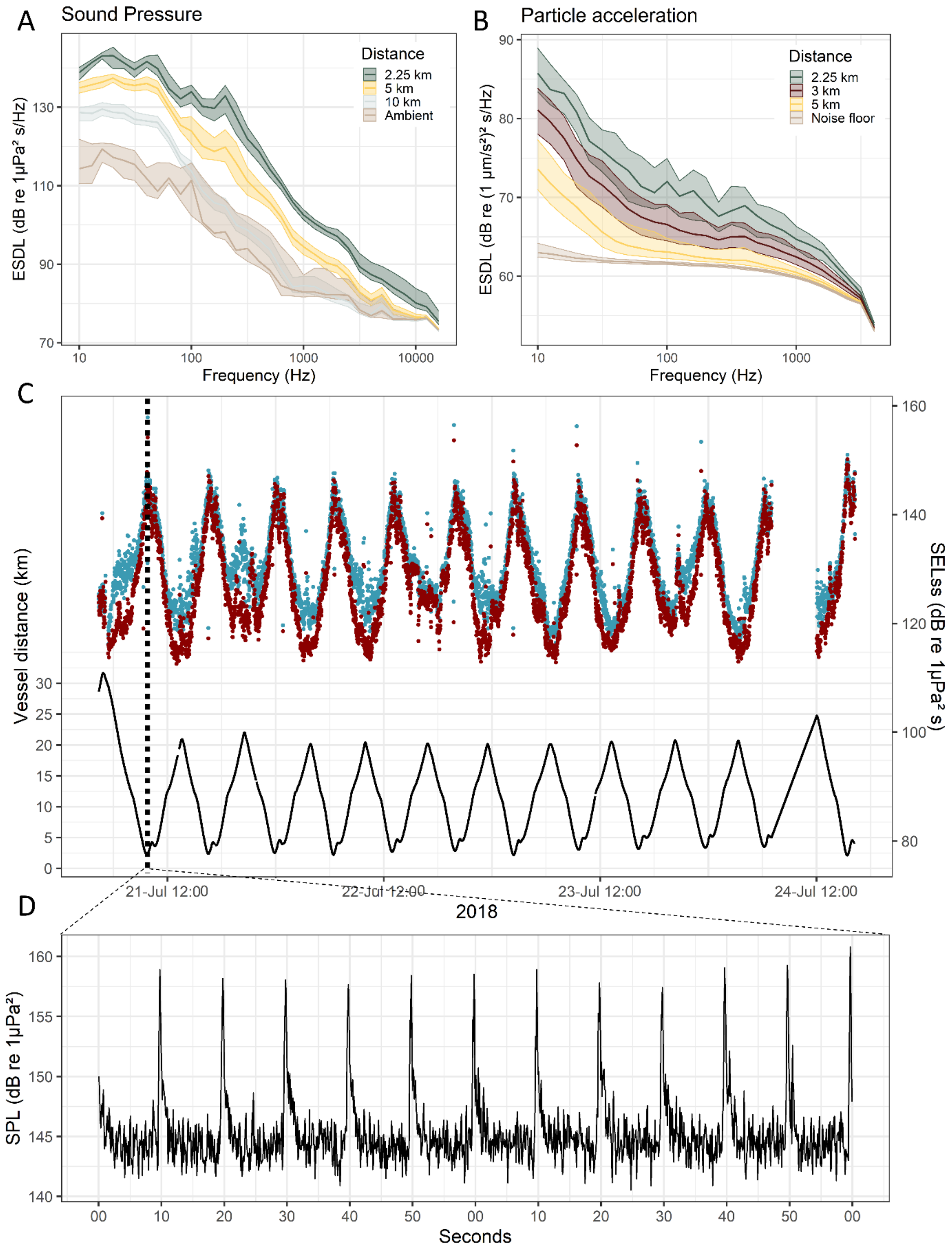


Figure S3. Sound exposure during the seismic survey 21-24 July, related to STAR Methods. The graphs depict 60 s effective energy spectral density levels (ESD) in decidecade bands of A) sound pressure and B) sound particle acceleration (5-7 shots per minute), as the median value of the sound energy with lower 25 and upper 75 quartile ribbon, when the towing vessel was at different distance and for ambient (A) or system noise floor (B) conditions. C) Seismic vessel distance from study site and the single shot sound exposure level (SELss) for the frequency band 10 Hz -16 kHz (red) and 40 - 400 Hz (blue) frequency band, which is considered to be the optimal hearing range of Atlantic cod (50, 51). The vessel made 11 loops parallel to the wind farm with one final and closest passage at the end of the survey. Raw sound files were analysed per decidecade band. D) SPL in the band 10 Hz – 16 kHz of 12 airgun shots in two minutes (TOW = 120 ms).

Formula for transition probability 2018/2017/2016 presence/absence data per day	AIC	Delta AIC
M ~ seismic persistent + decay after tagging + year + temp	**2265.894**	**0**
M ~ decay seismic + decay after tagging + year + temp	2271.501	5.607
M ~ seismic + decay after tagging + year + temp	2274.954	9.06
M ~ decay after tagging + year + temp	2277.809	11.915
M ~ decay after tagging * year + temp	2282.102	16.208
M ~ temp + year	2288.803	22.909
M ~ decay after tagging + temp * year	2294.228	28.334
M ~ temp	2295.738	42.622
M ~ year	2310.723	57.607
M ~ decay after tagging	2328.556	62.662
M ~ 1	2337.749	84.633

Table S1. Hidden Markov model selection based on AICs for cod presence/absence, related to Figure 1.

We tested three scenarios for seismic survey impact: only during the survey period (seismic), during the survey and then decaying after to zero (decay seismic), and during the survey and then persisting until the end of the study period (seismic persistent). Delta AIC is the difference between model AIC score and that of the best fit model which is printed in bold.

Behavioural state	Step length mean (m)	Step length sd (m)	VeDBA mean (m s^{-2})	VeDBA sd (m s^{-2})
BS1: Inactive	7.14	5.53	0.78	0.24
BS2: Locally active	6.44	4.97	1.35	0.37
BS3: Transit	31.68	31.24	1.12	0.73

Table S2. Data distribution per behavioural state, related to Figure 3.

Behavioural states were based on Step length (m) and vector of the dynamic body acceleration (VeDBA) (m s^{-2}).

Formula for transition probability 2018 step length and acceleration	AIC	Delta AIC
M ~ length + seismic * time since start + temp + hour + current speed	**180318.6**	**0**
M ~ length + period + temp + hour + current speed	180353.1	34.5
M ~ length + seismic persistent + temp + hour + current speed	180359.1	40.5
M ~ length + seismic decay + temp + hour + current speed	180366.6	48
M ~ length + seismic + temp + hour + current speed	180380.9	62.3
M ~ length + temp + hour + current speed	180386.9	68.3
M ~ temp	180536.1	217.5
M ~ time since start	180592.6	274
M ~ length	180679.9	361.3
M ~ hour	180723.5	404.9
M ~ current speed	180747.2	428.6
M ~ seismic	180753.2	434.6
M ~ 1	180782.4	463.8

Table S3. Hidden Markov model selection based on AICs for cod movement patterns, related to Figure 4.

We tested for a behavioural change: during the survey (seismic), between before, during and after the survey (period), from the survey onset and with a decay period after the survey (seismic decay), and from the survey onset and during the whole period after the survey (seismic persistent). Delta AIC is the difference between model AIC score and that of the best fit model which is printed in bold.

Frequency band	airgun sound	TAW / d	L5 / dB	L25 / dB	L50 / dB	L75 / dB	L95 / dB	IQR / dB
band40_400	ON	3.5	112.7	117.4	122.8	131.5	138.8	14.1
band10_16000	ON	3.5	117.4	122.3	127.2	134.8	140.8	12.5
band40_400	OFF	44.5	95.5	113.0	115.6	118.3	124.5	5.3
band10_16000	OFF	44.5	110.2	116.0	119.4	125.1	135.8	9.1

Table S4. Statistics of SPL (re 1 uPa^2), related to STAR Methods.

Airgun sound 'ON' correspond to statistics for the analysis window corresponding to the 3.5 d exposure duration. Airgun sound 'OFF' are averaged over all other times in the 48 day period 2018-07-13T01:00Z to 2018-08-30T00:00Z. TAW = temporal analysis window, IQR = inter-quartile range.

Frequency band	Distance (km)	TAW / h	L5 / dB	L25 / dB	L50 / dB	L75 / dB	L95 / dB	IQR / dB
band40_400	mainly self-noise	8.4	69.3	69.5	69.7	69.9	70.8	0.4
band10_4000	mainly self-noise	8.4	76.3	76.5	76.6	76.8	77.2	0.3
band40_400	<2.5 km	1.0	72.8	76.0	78.8	81.1	85.3	5.1
band10_4000	<2.5 km	1.0	78.5	80.4	82.3	83.6	86.8	3.2

Table S5. Statistics of effective PAL (re 1 (um/s^2)^2), related to STAR Methods.

Based on the 11 closest points of approach (CPA) of the towing vessel to the fish exposure site. A passage exist of 20 minutes before and after the CPA during which the vessel distance was between 10 km – 2.25 km. Period 2018-07-21T08:25Z to 2018-07-24T16:16Z.

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4

Effects of anthropogenic noise in warm waters: southern cod respond to seismic survey sound but persist at high temperatures

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Made ready for publication as a short note

Summary

As a consequence of increased human activity, marine fish may be affected by a combination of anthropogenic stressors such as an increase in anthropogenic noise as well as the consequences of climate change. It is therefore important to understand multi-stressor effects. Here, we looked at the potential effects of elevated water levels in combination with exposure to seismic survey sound, on the presence of Atlantic cod in the Belgian part of the North Sea. This analysis was done as an addition to our findings in Chapter 3, where we concluded that cod departed the study area earlier in the summer than expected after exposure to a seismic survey. This exposure study however, happened to take place during an extraordinarily warm summer in 2018, when water temperatures reached 19 ˚C. Since then, new cod tagging data have been collected form the same area during another warm year in 2020, in which water temperatures also reached 19 ˚C, but without seismic activity. We took this opportunity to compare the cod presence data from these two years and looked deeper into the potential effect of warming water temperatures. We considered both the effect of a continuous, i.e. linear, and a threshold effect, i.e. nonlinear, of temperature as a covariate. Our results, again, did not indicate that high water temperatures lead to an earlier departure of cod from the study area in summer.

Background, Methods, Results and Discussion

Humans have introduced a wide variety of anthropogenic noise to the ocean soundscapes (Duarte et al., 2021) and climate change has led to increased water temperatures worldwide (Alfonso et al., 2021; Doney et al., 2012). Both ecological changes can affect marine life, and if we want to understand how, we need to consider both and their potential interaction. In 2018, we conducted an experimental exposure study aimed at understanding the effects of sound from a full-scale seismic survey on swimming patterns and area departure in free-ranging Atlantic cod (*Gadus morhua*). Our results showed a significant change in the tagged individuals with respect to spatial behaviour, activity level, and diurnal rhythm, during the seismic survey, relative to before and after the exposure period (Van der Knaap et al., 2021). We also found that cod left the study area earlier than expected based on baseline data from 2016 and 2017, and pre-exposure data from 2018. We did not aim to test an effect of extreme temperatures, but that year turned out to be a particularly warm year.

Here, we went back to our study results and, by adding new data from 2020, another warm year, looked deeper into the potential effects of the extreme water temperatures of 2018 on the presence of cod at our study site. If cod departure was effected by temperature, this might either have been due to a build-up of continuously increasing temperature, or because the water temperature reached a certain threshold value. In our additional analysis we therefore considered both a continuous (i.e. linear), and a threshold function (i.e. nonlinear), for temperature as covariate. In our original models (Chapter 3), we included temperature as a continuous explanatory covariate, which did not preclude finding a significant effect of the seismic exposure (Van der Knaap et al., 2021). In 2018, only one individual remained throughout the whole observational period, but visual examination of that fish's activity pattern suggests continuation of normal activity patterns during all temperature fluctuations (Fig. 1). However, since most cod had departed the study area in 2018 before the water temperatures had reached above 19 °C, and temperatures in the two baseline years (2017 and 2018) did not reach above this height, we were unable to test for a nonlinear (threshold) effect of temperature on the departure of cod using our original dataset. In 2020, another warm year in which temperature reached above 19 °C, but without nearby seismic survey activity, an additional eleven cod were tagged of similar length in the same area. Four of the tagged cod had a sufficient dataset for further analysis. These four animals departed considerably later in the year and stayed in the area during the warmest period of that year when temperatures were above 19 °C (Fig. 1). To search for a possible nonlinear effect of temperature on probability of departure from the study site, we re-analysed all our daily presence/absence data (including the 2020 data) adding nonlinear candidate models for temperature as covariate, to identify certain temperature levels above which departure became more likely (cubic B-spline R package *splines2,* version = 0.4.3). The AIC-best model was the same as we had found previously – i.e., with a linear effect of the temperature covariate and a significant effect of the seismic survey (table 1).

Figure 1. Data from receivers and tags used to track Atlantic cod in wind farm 'Belwind' (Belgium). (next page). A) The mean daily temperatures recorded by the bottom moored receiver stations (blue 2018 and yellow 2020, pale shading around lines indicates +/- 1 SD) and the departure data, in boxplots, of acoustically tagged cod in 2018 (blue, black dots indicate exceptionally early and late departures, n = 37) when we conducted the experimental seismic survey, and 2020 (yellow, n = 4) when there was no seismic survey. B) The hourly mean (+/- 1 SD in grey) of the vector dynamic body acceleration (m/s^2) of one cod (fish 23) tagged in 2018, and present for the whole 97-days recording period and all temperatures. Vertical dashed lines indicate the period in which our experimental seismic survey took place (in 2018 only).

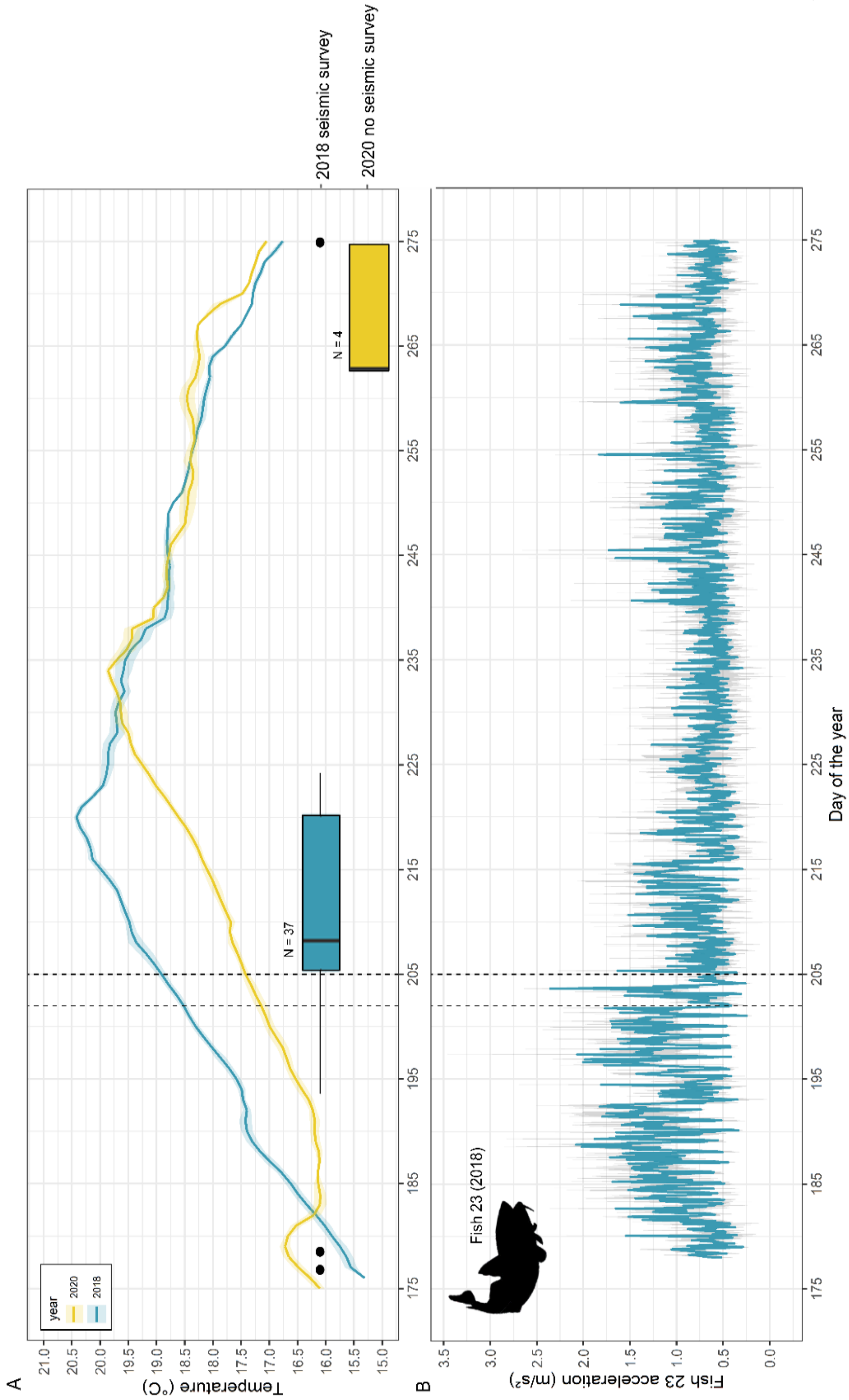
A
Temperature (°C)
21.0
20.5
20.0
19.5
19.0
18.5
18.0
17.5
17.0
16.5
16.0
15.5
15.0
year
2020
2018
N = 37
N = 4
2018 seismic survey
2020 no seismic survey
B
Fish 23 acceleration (m/s²)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Fish 23 (2018)
175
185
195
205
215
225
235
245
255
265
275
Day of the year

Table 1. Hidden Markov model selection based on AICs for cod presence/absence. We tested whether the AIC-best model included the covariate indicating a persistent effect of seismic survey on presence/absence ("seismic_persistent") after including either a linear effect of temperature ("temp", on the logit link scale) or a non-linear effect (cubic bSpline with 2 internal knots, and so 5 degrees of freedom, "s(temp)"). The current analysis included new data from four cod in 2020 (in addition to the 65 from previous years). Delta AIC is the difference between model AIC score and that of the best fit model which is printed in bold.

Formula for transition probability 2020/2018/2017/2016 presence/absence data per day	AIC	Delta AIC
M ~ seismic_persistent + decay_after_tagging + year + temp	**2394.318**	**0**
M ~ seismic_persistent + decay_after_tagging + year + bSpline(temp, df=5)	2396.789	2.471
M ~ decay_after_tagging + year + bSpline(temp, df=5)	2397.496	3.178
M ~ decay_after_tagging + year + temp	2405.955	11.637
M ~ year + bSpline(temp, df=5)	2412.519	18.201
M ~ 1	2469.82	75.502

In a recent report from the Intergovernmental Panel for Climate Change (IPCC), they predict that the global warming of 1.5 °C will happen within the next ten years and that this will have extreme consequences for the worlds ecosystems (Masson-Delmotte, V., P. Zhai et al., 2021). Concerns about increasing temperature trends caused by climate change have led to several studies on individual- and population-level responses of fish species. World-wide redistributions of species attributed to climate change are reported (Pecl et al., 2017), and northward shifts are expected and reported for Atlantic cod (Engelhard et al., 2014). However, different stocks have been identified for Atlantic cod inhabiting different regions and experiencing different temperature regimes (Neat and Righton, 2007). The Southern North Sea stock has the highest records for temperature tolerance and a temperature limit has not been identified yet. A study by Neat and Righton (Neat and Righton, 2007) showed that free-swimming cod in the southern region did not leave an area with temperatures that were considered too high for optimal growth of captive individuals from a more northern stock (Lannig et al., 2004). Studies on free-ranging cod in a northern stock have found vertical displacement of cod to cooler waters when temperatures increased (Freitas et al., 2021), but again they did not observe horizontal migration.

These studies indicate that increasing water temperature due to global warming, may not necessarily lead to individual departure and that the observed redistribution of cod towards more northern waters may be driven by variable rates of reproduction and mortality of local populations (Neat and Righton, 2007). The Southern cod stock, which was tested in our sound exposure study (Crain et al., 2018), inhabits a well-mixed system, shallow waters (depths ~25m), and resides predominantly close to the seabed. Vertical migration to cooler water is not an option in this area as fish would need to travel far to reach cooler, deeper waters. In addition, once cod in this area reach their summer feeding grounds they generally have low movement rates (Reubens et al., 2013; Van der Knaap et al., 2021). These insights further strengthen our confidence in that the unexpected early departures of cod in 2018 were caused by the preceding experimental seismic exposure and not due to an extremely hot year.

Marine life has to deal with an increasing amount of anthropogenic stressors and more studies are warranted on cumulative effects and multi-stressor exposures (Boyd and Hutchins, 2012; Crain et al., 2008; Gunderson et al., 2016; Orr et al., 2020), including elevation of temperature (Alfonso et al., 2021; Farrell et al., 2008; Schultz and Bertrand, 2011) and sound levels (Duarte et al., 2021; Slabbekoorn, 2019). Experimental approaches are a good strategy to test for causal mechanisms underlying observational patterns, and well-replicated studies on captive animals are best followed up with challenging experiments on free-ranging individuals. However, given natural variation in environmental conditions, variety in local fish communities, potential for local adaptations in target species, experience with anthropogenic noise pollution, and novel temperature levels related to global warming, we did and do stress that replication is required even for strong experimental case studies like ours (Crain et al., 2018).

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5

Effects of pile driving sound on local movement of free-ranging Atlantic cod in the Belgian North Sea

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Published in Environmental Pollution 2022

DOI: https://doi.org/10.1016/j.envpol.2022.118913

Abstract

Offshore energy acquisition through the construction of wind farms is rapidly becoming one of the major sources of green energy all over the world. The construction of offshore wind farms contributes to the ocean soundscape as steel monopile foundations are commonly hammered into the seabed to anchor wind turbines. This pile driving activity causes repeated, impulsive, low-frequency sounds, reaching far into the environment, which may have an impact on the surrounding marine life. In this study, we investigated the effect of the construction of 50 wind turbine foundations, over a time span of four months, on the presence and movement behaviour of free-swimming, individually tagged Atlantic cod. The turbine foundations were constructed at a distance ranging between 2.3-7.1 km from the cod, which resided in a nearby, existing wind farm in the southern North Sea. Our results indicated that local fish remained in the exposed area during and in-between pile-driving activities, but showed some modest changes in movement patterns. The tagged cod did not increase their net movement activity, but moved closer to the scour-bed (i.e. hard substrate), surrounding their nearest turbine, during and after each piling event. Additionally, fish moved further away from the sound source, which was mainly due to the fact that they were positioned closer to a piling event before its start. We found no effect of the time since the last piling event. Long-term changes in movement behaviour can result in energy budget changes, and thereby in individual growth and maturation, eventually determining growth rate of populations. Consequently, although behavioural changes to pile driving in the current study seem modest, we believe that the potential for cumulative effects, and species-specific variation in impact, warrant more tagging studies in the future, with an emphasis on quantification of energy budgets.

Keywords

Pile driving, Atlantic cod, acoustic telemetry, wind farm, movement behaviour

Introduction

The number and size of offshore wind farms is growing worldwide with potentially diverse consequences for aquatic wildlife during the construction and operational phases (Gill, 2005; Gill et al., 2020; Lindeboom et al., 2011; Nabe-Nielsen et al., 2018). Predictions are that around 70 GW of Europe's energy will come from offshore wind turbines by 2030 (Nghiem and Pineda, 2017). By 2050, this could expand to 450 GW, 380 GW of which is to be produced in the North Sea (Freeman et al., 2019). Wind turbine foundations are anchored to the seabed, typically surrounded by a scour-protection layer of rocks or other hard substrate, which creates artificial reef structures (Ashley et al., 2014; Petersen and Malm, 2006; Reubens et al., 2013a). Especially invertebrate animal communities benefit from these new artificial reef habitats. These communities include bivalves, anemones and crustaceans (Mavraki et al., 2020), which in turn attract a variety of fish species (De Mesel et al., 2015; Paxton et al., 2020; Reubens et al., 2014). However, construction and operation of wind farms also bring changes to the acoustic environment, the impact of which on marine wildlife is as yet insufficiently understood and may not always be beneficial (Gill et al., 2020).

Once the wind farm is operational, turbine vibrations, altered flow conditions around turbines, and wind park maintenance vessels all contribute to a growing ambient sound level (Madsen et al., 2006; Popper and Hawkins, 2019a; Sertlek et al., 2019; Slabbekoorn et al., 2010; Tougaard et al., 2009; Tougaard et al., 2020; Wahlberg and Westerberg, 2005; Duarte et al., 2021). However, the most dramatic acoustic events are associated with the park's construction phase (Dahl et al., 2015; Lippert et al., 2018). Elevated sound levels usually start long before actual construction, as a seismic survey and multi-beam sonar is often required for seabed mapping. When the seabed at an offshore location is considered suitable, the preparation phase is initiated with seabed preparations (i.e. deposition of hard substrate to reduce sediment erosion and flattening of the seabed), followed by pile driving. After foundations have been anchored, the turbines and blades are attached, which is then followed by the operational phase.

Especially the sound event train with long periods of loud series of impulsive sounds associated with pile driving for turbine foundation placements have a high potential to negatively affect marine wildlife (Duarte et al., 2021; Popper and Hastings, 2009; Popper and Hawkins, 2019; Slabbekoorn, 2019; Slabbekoorn et al., 2010). The most common type of offshore turbine foundation is a steel monopile, that ranges in diameter from 4 – 8 m and is piled into the sediment from a rigid platform using a hydraulic hammer. Piling one monopile 30 m into the seabed typically takes over 3500 strikes, lasting around 1.5-4.5 hours, depending on the density of the sediment. Hammer strikes are associated with low-frequency (100 to 1000 Hz) sound, reaching levels above 200 dB re 1 µPa close to the source, including sharp pulse rise times and inter-pulse intervals of 2-4 seconds (Ainslie et al., 2020; Hildebrand, 2009). Strikes create downward sound waves that spread cylindrically from the pile wall into the water column and seafloor (Dahl et al., 2015; Martin and Barclay, 2019; Zampolli et al., 2013). The total duration of the construction phase depends on the size of the wind farm but usually takes between 2 to 6 months.

Fish can be directly affected by pile driving through immediate physical and behavioural effects, or indirectly through the effect of piling on their predators (Thompson et al., 2020) and prey (Roberts et al., 2016). Exposure effects from multiple impulsive pile strikes at close range have been investigated in captivity and include damage to internal organs, including the inner ear, swim bladder, liver, kidney, and gonads (Casper et al., 2017, 2013; Halvorsen et al., 2012), which may eventually result in fish death (Slabbekoorn et al., 2010; Popper and Hawkins, 2019). Acoustic modelling has recently demonstrated that lethal and sub-lethal injury could occur at distances as far as 1.8 and 3.1 km from the pile driving location, respectively (Ainslie et al., 2020). In addition, indoor studies with exposure to moderate levels of impulsive sound have found increased gill ventilation rates, indicative of a stress response, in several but not all fish species (Bruintjes et al., 2016; Spiga et al., 2017).

Similar to stress responses, behavioural effects of impulsive sounds may occur at a large spatial scale (Duarte et al., 2021; Popper and Hawkins, 2019; Slabbekoorn et al., 2010). Groups of captive seabass (*Dicentrarchus labrax*) in a small indoor basin increased their swimming depth and group cohesion, and swam faster, in response to both continuous and impulsive sounds, but during the latter, they took twice as long to return to their baseline levels (Neo et al., 2015, 2014). Impulsive sound exposure in a larger outdoor pen, in shallow harbour conditions, revealed stronger effects from exposure at night compared to daytime conditions, and showed a potential for habituation to repeated exposure, at least for seabass (Hubert et al., 2020b; Neo et al., 2018, 2016). Pile-driving playback on schooling behaviour of juvenile seabass, again in lab conditions, demonstrated that groups became less cohesive, less directionally ordered, and individuals became less correlated in speed and directional changes (Herbert-Read et al., 2017). Captive exposure and response patterns may not accurately reflect free-ranging conditions qualitatively nor quantitatively, but observations and analyses in captivity may provide important complementary insights to studies in the wild (Hubert et al., 2020b).

Examining the behaviour of tagged individuals during pile driving at sea is more challenging than observing fish in captivity, but such *in situ* studies provide better insight into potential consequences of noisy events in nature. Iafrate et al. (2016) reported that individual sheepshead (*Archosargus probatocephalus*) were little affected by 35 days of pile driving at a busy wharf in Florida, but four grey snappers (*Lutjanus griseus*), with high prior site fidelity, had relatively low day-time residency during piling, and two left the area within the first three days of the noisy activities. In a recent study, we showed that impulsive sounds from another loud anthropogenic source (an experimental seismic survey) resulted in delayed deterrence from a wind farm area, interrupted diurnal activity cycles, and likely reduced foraging activity in free-ranging Atlantic cod (*Gadus morhua*) (van der Knaap et al., 2021a). However, we are unaware of any published data on effects of pile driving on tagged individuals for this species.

During summer, Atlantic cod stay several months around the hard-bottom structures of wind turbine foundations in the southern part of the North Sea (Reubens et al., 2013a, 2013c; Winter et al., 2010). They use these structures to forage and seek shelter (Reubens et al., 2013b; van der Knaap et al., 2021a; Winter et al., 2010). Atlantic cod also play an important role in the local food web (Reubens et al., 2014), are commercially and culturally important for fisheries (Hutchings, 2004; Rose, 2004), and have shown dramatic population declines, related to overfishing and climate change (Engelhard et al., 2014; Pitcher et al., 2009; Worm et al., 2006). Consequently, Atlantic cod at wind farms are a feasible and important model species for sound impact studies (van der Knaap et al., 2021a; 2021b). Moreover, a captive study (Thomsen et al., 2010) as well as a theoretical evaluation (Hammar et al., 2014) both reported that impulsive sounds from pile driving could be a potential risk to them.

In this study, we examined the presence and movement of free-swimming Atlantic cod at an existing offshore wind farm (OWF) in the Belgian North Sea, in response to nearby pile driving in 2016. Within a period of four months, 50 monopiles were hammered into the seabed for a new wind farm, adjacent to an existing wind farm. This provided a unique opportunity to monitor cod presence and movement *in situ* during offshore piling operations. We used acoustic telemetry to monitor individual presence and movement in the area to answer the following questions: 1) Do cod move out of the study area in response to pile driving? And 2) Does piling affect the general spatial behaviour of cod, their distance to the nearest turbine (indication for the distance to the scour bed), and their distance to the pile-driving location, taking into account the time interval between consecutive piling events?

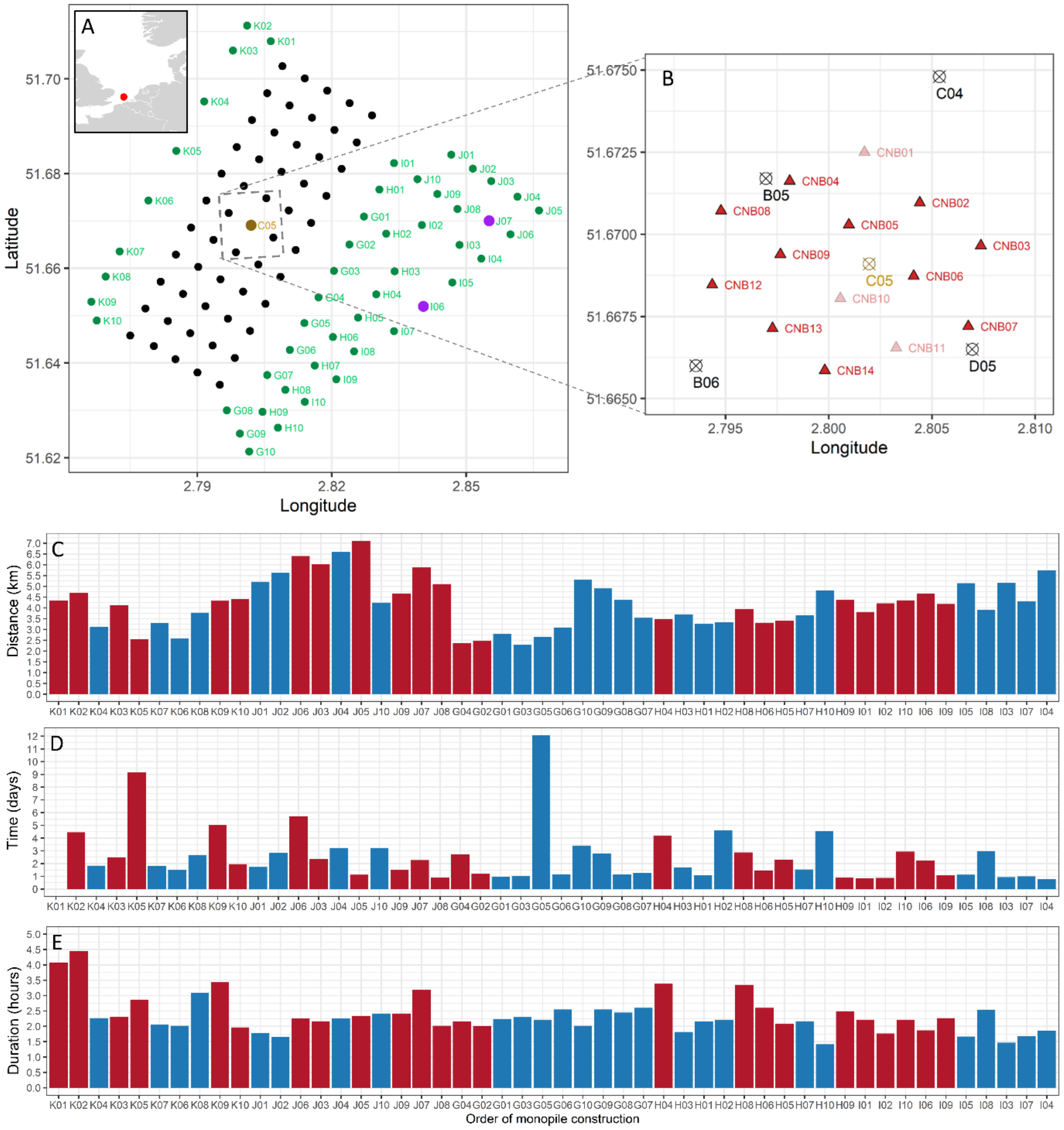
Methods

Study site

Fifty steel monopile wind turbine foundations were hammered into the seabed using pile driving between May 16 and September 22, 2016 (Table S1) for the construction of the wind farm 'Nobelwind' at the North-Eastern edge of the Belgian Part of the North Sea. The farm was built around the existing wind farm 'Belwind' (completed in 2013) situated on the Bligh Bank, about 50 km from the coastal harbour of Zeebrugge (fig. 1A). The water depth at the study site varied between 15 – 37 m, including tidal fluctuations. Main currents in the wind farm run from northeast to southwest (Brabant et al., 2013). Steel monopiles were piled into the seabed using a hydraulic piling hammer (IHC Hydrohammer B.V.) from a rigid platform. Each pile strike creates downward energy, forcing the pile into the seabed. As a by-product this creates a low-frequency, high-intensity acoustic energy wave that radiates into the air, water and seabed (Lippert et al., 2018).

Figure 1. (next page) Overview of location and study setup. A) Location of Belwind (black dots) and Nobelwind (green dots) wind turbines in the Belgian part of the North Sea (red dot in overview map). The turbine C05 (golden dot) in Belwind is the centre of our fish tagging site. Purple dots in Nobelwind indicate locations of sound measurements during construction. B) Receiver setup around Belwind turbine C05. 14 acoustic receivers (red triangles) were deployed on

4 April 2016 around turbine C05 (orange dot). During the deployment period, 3 receivers were lost (shaded red triangles). C), D) and E) order in which the Nobelwind turbines were constructed and the distance of the piling event (C), time since last event (D), and the duration of an event (E). The red bars indicate the piling events that were included in the movement analysis.



Sound pressure was recorded using a hydrophone (Brüel & Kjaer hydrophones type 8104) suspended at 10m depth from a drifting Zodiac workboat. Ambient sound levels were recorded at three locations without construction activities (on 12 and 13 July 2016 see Degraer et al., 2017), and sound recordings during construction were made at 400m and 1700m from monopile I06 (lat. 51.65195, long. 2.84043, 13 September 2016 fig. 1) and at 500m from monopile J07 (lat. 51.67005, long. 2.85506, 12 July 2016 fig. 1). Ambient sound pressure levels (SPL) varied between 114 dB and 138 dB (re1 µPa) on average. During piling operations, the zero-to-peak SPL reached 199, 196 and 188 dB at 400, 500 m and 1700 m distance from the sound source, respectively. Single strike sound exposure levels (SELss) were on average 176, 175, and 168 dB (re1 µPa^2.s), respectively (adopted from Degraer et al., 2017).

Experimental setup

In 2016, 14 acoustic receivers (12 VR2AR and 2 VR2Tx, Innovasea) with an internal sync-tag were deployed around the Belwind turbine C05 to form a network of receivers that allowed to locate the positions of the tagged fish (fig. 1B) (Smith, 2013). The deployment period ran from April 4 to October 25. Nine receivers were operational during the whole period of deployment and provided continuous presence and movement data for the tagged cod after retrieval. Three receivers (CNB01, CNB10, CNB11) were lost and no data was retrieved, while two receivers (CNB07 and CNB14) stopped recording before the end of the study (18 September and 8 August, respectively) (fig. 1B). Overall, 64.9% of synctag transmissions were logged on 3 or more receivers.

Fifteen Atlantic cod, with a total length range of 40-49.5 cm (table S2), were internally tagged with V13P tags (Inovaseas) on April 4, as per

Reubens et al. (2013c; c.f. van der Knaap et al., 2021a; 2021b). They were caught using hook and line, all close to the wind turbine C05 (fig. 1A). Soon after tagging, fish were released again at the catch site. Acoustic tags had a pressure sensor and transmitted a coded signal with information on tag ID. The tags measured pressure at a random delay between 140-220 seconds. Catching and tagging of free-ranging animals was performed after ethical approval (certificate number LA1400452) and in line with official guidelines for animal welfare in Belgium.

Data analysis

After recovery of the receivers, corrections for internal clock drift of the receivers during the deployment period were done by applying a linear time correction over the entire sampling period (VUE, Innovasea). Of the 15 tagged cod, one was not detected after release and therefore excluded from further analysis (table S2). Additionally, we excluded the first 24 hours of data from the first day after the animals were tagged, as it is expected that cod take a variable amount of time to resume their natural behaviour, but typically within this time period (Hubert et al., 2020a). We furthermore scanned the data for any stationary tags which could indicate fish that died or lost their tag during the study. No such stationary tags were found.

Presence/absence

We determined half hour presence/absence over the entire study period for the 14 cod (fig. 3A, tag ID's numbers ranged from 8-22). Fish were considered present if they were detected at least once within a half hour bin. We applied a discrete-time hidden Markov model (HMM) to analyse the effect of piling on the presence/absence of the fish. HMMs intrinsically deal with temporal autocorrelation and are able to predict the probability at which animals are in a certain mutually exclusive behavioural state, based on telemetric detection data (Langrock et al., 2012; McClintock and Michelot, 2020; Hubert et al. 2020a). Half hourly observations of presence/absence (Bernoulli distribution) per individual (14 fish in total) were fitted to the states 'Onsite' or 'Offsite'.

We tested whether cod had spent more time in the Offsite state, during piling; soon after each piling event (50 in total); or soon after the first or last piling event in particular, as a previous study on the impact of impulsive sound on cod indicated a delayed leaving effect (van der Knaap et al., 2021a). We therefore included a piling decay variable, which was 1 during piling, after which it exponentially decayed to 0 over a 24-h period. This piling decay variable tested whether the chance that cod would depart was highest during piling and exponentially became smaller afterwards. We then also included a piling order variable with four levels: no-piling, first piling event (K01), all middle piling events, and last piling event (I04). We checked for collinearity between these two variables (piling-decay and -order) by excluding them one-by-one from the HMM and looking at the model results. If the model results did not change, we assumed that collinearity was not an issue for the significance of our model. If they did change, one of the covariates causing the collinearity was left out.

Other covariates considered in the departure probability analyses were: day of the year (as $\cos(2\pi t / 365\ d)$ and $\sin(2\pi t / 365\ d)$), current speed (ms^{-1}), tidal elevation (m), sun angle (degrees, relative to the hours in a day), and fish length (cm)). Current speed and tidal elevation were estimated based on a hydrodynamic model (COHERENS, operated by RBINS

Belgium). Model selection was done based on AIC comparison using model dredging (‘MuMIn’ version 1.43, table S3). The best model (lowest AIC, Table S3) was: presence/absence ~ decay after each piling event + piling order + fish length + day of the year + current speed. HMMs were applied in R (version 4.0.4) using the ‘momentuHMM’ package (version 1.5.1).

Movement behaviour

The individual detections were converted into 2-dimensional positions (latitude, longitude) through time difference of arrival (TDOA) triangulation (Voegeli et al., 2001) (Innovasea). A position could only be determined when the tag signal was detected by at least three receiver stations (van der Knaap et al., 2021b). The associated horizontal positioning error (HPE, a unit-less error metric Innovasea) was then used to filter out the top 2% most erroneous positions (Smith, 2013). This excluded one fish whose positions were at the edge of, or outside our detection area and therefore had a high HPE. Furthermore, we removed fish 11 which had only very few positions. To understand how a piling event affected cod movement, we defined three fish movement metrics (table 1), i.e. step length (distance moved) (fig. 2A), distance to the scour bed (fig. 2A) and relative distance to the piling event (fig. 2B).

Movement metric	Calculated as
Step length (m)	Linear distance between consecutive average half hour fish positions (fig. 2A)
Distance to turbine scour-bed (m)	Distance of each fish position to the centre of the scour-bed of the closest turbine (fig. 2A)
Relative distance to piling event (m)	Relative distance of each fish position to the piling event corrected for the distance of the event to the C05 turbine (i.e. centre turbine of the study site, fig. 2B)

Table 1. Cod movement metrics

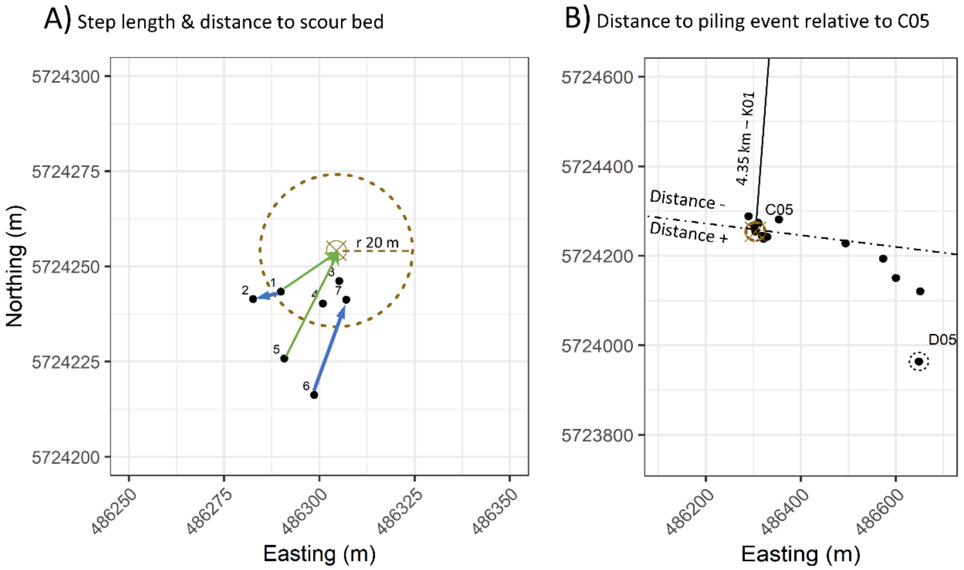


Figure 2. An example of how cod movement metrics are assessed. Black dots in both panels represent fictitious cod positions. A) Black dots indicate seven fictitious half-hour consecutive positions around Belwind turbine C05. The crossed circle at the centre is the turbine C05 and the dashed line is the edge of the scour-bed protection layer at 20 m from the turbine. The calculation of step length between consecutive positions and cod distance to the centre of the scour bed (i.e. position of the closest turbine) are indicated by blue and green arrows, respectively (both in meters). B) Distance of a cod position to the pile driving location, before, during and after each event, was calculated for all cod positions relative to the distance between the piling events and the centre of C05. Black dots represent fictitious fish positions and the dashed-dotted line served as a reference line to determine whether the relative distance changes of the fish to the piling event were positive (moved further from the piling event) or negative (moved closer to the piling event).

We investigated the effect of a piling event on these three movement metrics by filtering the dataset to include positions before, during, and after each piling event. The duration of each sound exposure varied per piling event and we set the before and after period to match each exposure period, which led to equal periods for comparison per event (fig. 1E). Piling operations took place during day and night and it took an average of 3353 blows to fully anchor a turbine foundation ca 30 m into the seabed, which lasted between 1.65 and 4.45 hours (fig. 1E). We only included piling events and fish if we had data for all three periods (before, during, and after). This resulted in 24 piling events with sufficient data, including 11 fish with data for 1-18 piling events per individual. We then analysed the effect of piling on cod movement, averaging the movement metrics over half hour time bins, taking the following parameters into account: piling period (before, during, and after), distance of the piling event (km between piling location and turbine C05, range 2.3-7.1 km (fig. 1C)), and time since last piling event (hours since end of last event, range 0-220 h (fig. 1E)). Other variables included: current speed (ms^{-1}), tidal elevation (m), sun angle (degrees), and we added fish length (cm) to the movement models to account for variation attributable to the individual.

We then used linear and generalised mixed models (LMM and GLMM) to model the effect of piling on cod movement behaviour. Fish ID (fish ID) and piling location (piling ID) were set as the random variables in all movement models. We checked for temporal autocorrelation and when found, included a correlation for a continuous time covariate (autoregressive process (AR(1)). Model selection from the full model (table S4) was done based on AIC comparison using model dredging ('MuMIn' version 1.43, table S4). We used the R package 'lme4' (version 1.1) for both LMMs and GLMMs. Cod half-hour step length was log-transformed (log10) to obtain a normally distributed dataset. The best model (lowest AIC, table S4) for cod step length was an LMM and included: log(step length) ~ sun angle + tidal elevation + current speed + (1|piling ID) + (1|fish ID). For the distance to the scour-bed protection layer, the best model was an LMM including: distance scour-bed ~ period + distance to piling event + sun angle + tidal elevation + current speed + period*distance to piling event + (1|piling ID) + (1|fish ID). The top model for cod relative distance to the piling location was a GLMM (distribution gamma, link = log) including: relative distance piling ~ period + distance to piling event + hours since last event + tidal elevation + current speed + period*distance to piling location + tidal elevation*current speed + (1|piling ID) + (1|fish ID).

Results

Cod presence within reach of our receiver network fluctuated over the study period (fig. 3A). However, we found no significant effect of pile driving on the probability that cod moved out of the study area, with or without the 24 hour decay period after each piling event (which means that the probability that cod spent less time in the Onsite state did not change significantly over the exponential decay 1-0, fig. 3B). In addition, we also did not find any significant differences in time spent in the Onsite state (the 95% confidence intervals (CI) overlapped between the four levels of the piling order variable), from before to after the first or last piling event in comparison to events in the middle or moments when there was no piling activity (fig. 3C). Day of the year strongly affected the departure from the area, as over time more cod are expected to leave (Reubens et al., 2013c; Winter et al., 2010) and spend less time in the Onsite state (fig. S1). After the first week of August, day 220, 50% of the tagged fish had left the area (fig. 3A).

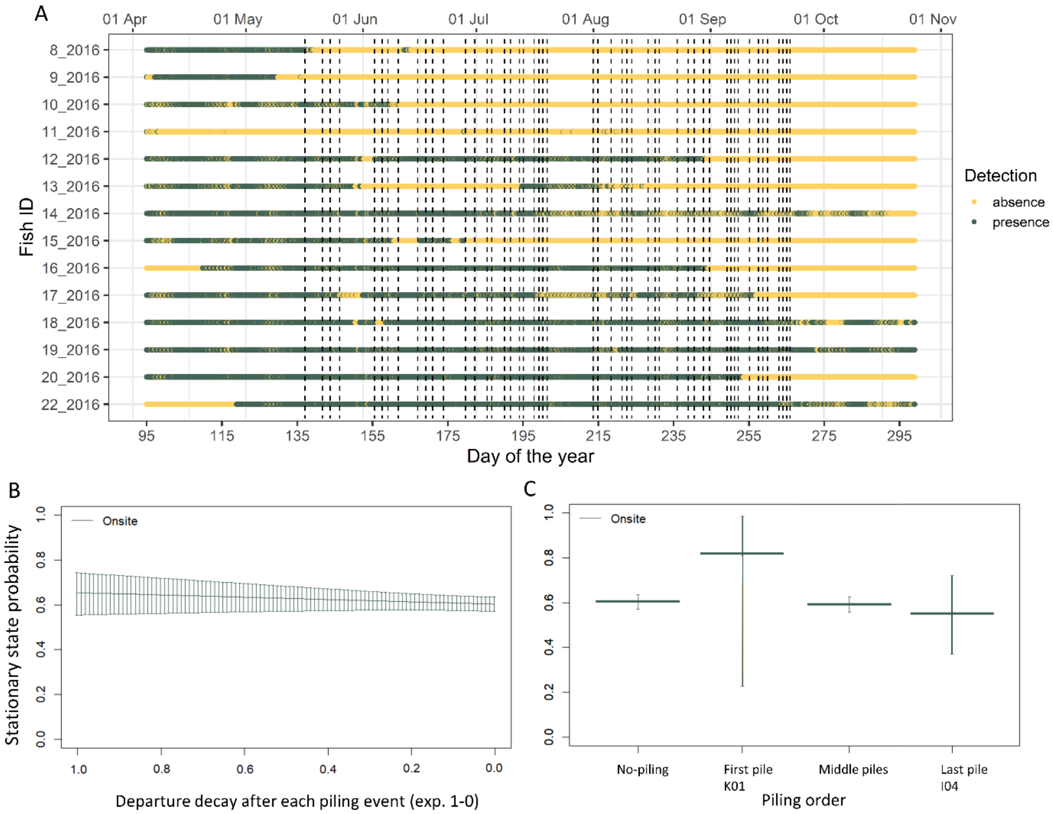
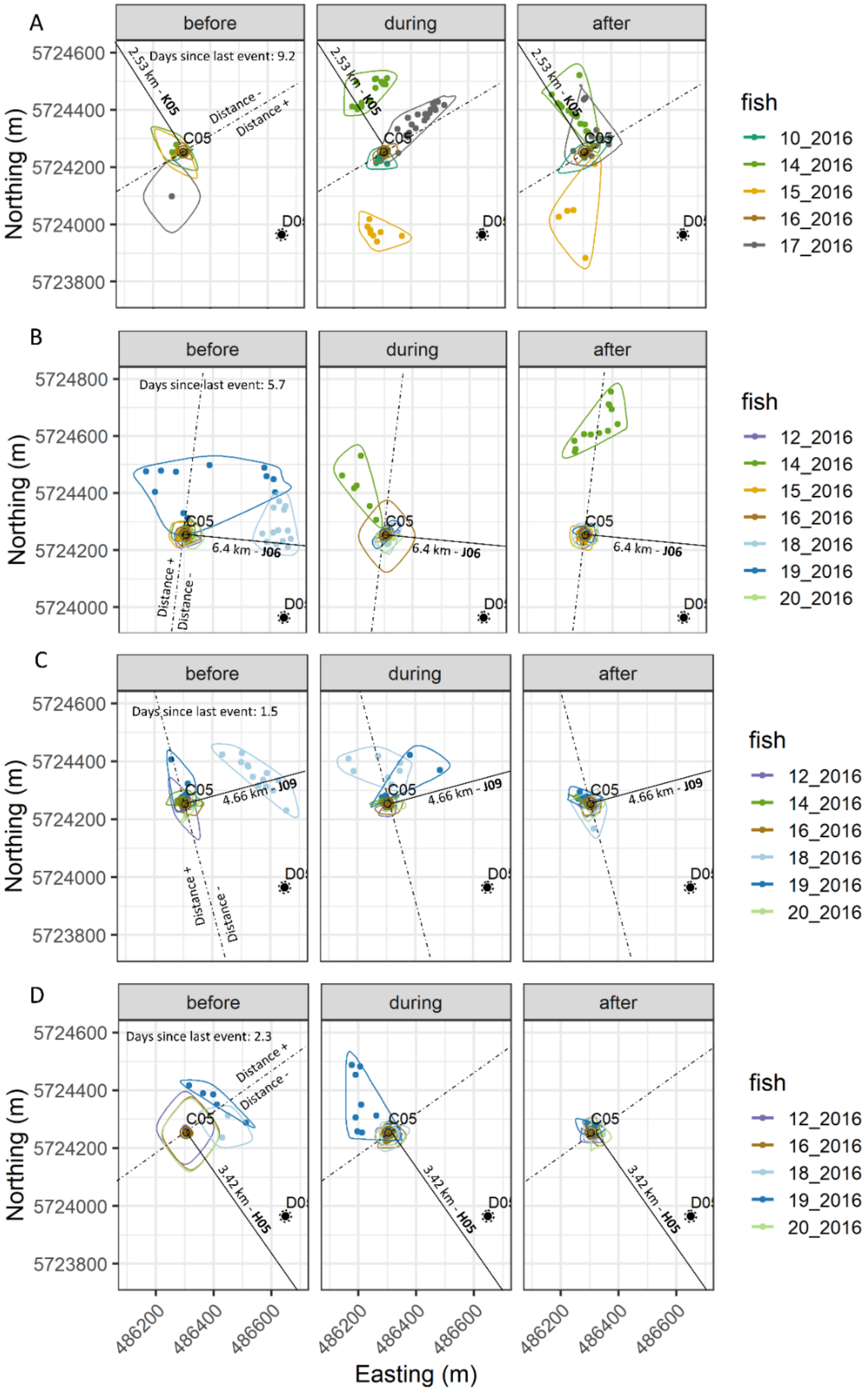


Figure 3. A) Half hour presence/absence of individual cod (fish ID on the y axis) over the entire study period (5 April – 30 October 2016). The dotted vertical lines represent the 50 piling events during which monopile foundations were piled into the seabed for the construction of the Nobelwind wind farm. Hidden Markov model (HMM) predicted stationary state probabilities of cod in the Onsite behavioural state including the 95% confidence intervals (CI). B) Effect of pile driving on Onsite probability, including a 24h exponential decay after the end of each piling period (value of 1 during piling followed by an exponential decay to 0 (representing 24 hours later) after each individual piling period ended). C) Effect of piling order on Onsite probability, including a level for the first, last and middle piling events and for all non-piling moments.

Movement behaviour

Our data revealed unprecedented detail in individual tracks of free-ranging fish during piling events. The individual tracks and positions of the tagged cod around turbine C05 revealed highly variable movement patterns per individual (Fig. 4). For example, fish with the tag ID numbers 12, 16 and 20, when present during these four piling events, were always very close to the turbine before, during, and after piling. Fish 18 and 19, when present, tended to move around at some distance from the turbine before piling in three of the events, but always moved closer to the turbine during and especially after piling, typically also moving away from the direction of the sound source. Fish 14 was close to the turbine in two of the examples before the piling started, and moved closer to and further from the turbine when the sound source was nearby (fig. 4A) and when it was further away, respectively (fig. 4B). Fish 15 was also close before the piling started in the same two examples, but moved away when the piling source was nearby (fig. 4A) and stayed close to the turbine during and after the piling at a more distant location (fig. 4B).

Figure 4. (next page) Examples of cod positions before, during and after four piling events in chronological order of construction, for wind turbines A) K05, B) J06, C) J09, and D) H05. The distance in kilometres and time since the previous piling event in days, are indicated in the panels. Individual fish are colour-coded and the turbines are at the junction of the direction line towards the piling event and the distance reference line (dashed-dotted lines). The contour around each individual fish illustrates the distribution of positions and does not represent an area use quantification.


A
B
C
D
before
during
after
Days since last event: 9.2
Days since last event: 5.7
Days since last event: 1.5
Days since last event: 2.3
2.53 km - K05
6.4 km - J06
4.66 km - J09
3.42 km - H05
Distance -
Distance +
C05
D0
Northing (m)
Easting (m)
5724800
5724600
5724400
5724200
5724000
5723800
486200
486400
486600
fish
10_2016
12_2016
14_2016
15_2016
16_2016
17_2016
18_2016
19_2016
20_2016

In the best model for step length period, distance and time between piling events were not included and we were therefore unable to further investigate any significant effects of these factors. We did plot the step length against piling period, distance, and time for visual comparison and saw that step length was fairly similar among periods (fig. 5A). Environmental variables that influenced the step length significantly were: sun angle ($p < 0.0001$, estimate: -0.005), step lengths were largest during night time; current speed ($p < 0.0001$, estimate: -0.82), step lengths increased with increasing current speed; and tide ($p < 0.03$, estimate: 0.722), step lengths were largest during absolute low tide.

We also found significant variation in cod distance to the scour-bed among the piling periods (fig. 5B). Cod significantly reduced their distance (m) to the closest scour-bed from before to during ($p < 0.03$), and from during too after ($p < 0.002$) the piling, and were thus moving closer towards the turbine (fig. 5B left panel). In addition, this effect showed an interaction with the distance to the piling location ($p < 0.001$, fig. 5B middle panel), with a relatively stronger effect at large distances (~4-7km) before pile driving and a relatively weaker effect at large distances after pile driving. Other variables included in the model that had a significant effect were: sun angle ($p < 0.0001$, estimate: -0.006), during day time fish were closer to the scour-bed; tide ($p < 0.0001$, estimate: -0.162), distance fluctuated with tidal changes; and current speed ($p < 0.0001$, estimate: 1.286), when current speeds increased, so did fish distance to the scour-bed.

Piling also had a significant effect on the relative distance of cod to the piling location; cod were positioned closer to a piling location before piling started than during ($p < 0.0001$) or after ($p < 0.0001$) piling (fig.

5C). The trend of cod moving away from the piling location was strongest at large absolute distances of the pile driving (fig. 5C middle panel). At these piling events, fish were positioned closer to the pile driving location before the pile driving started (relative distance was less than 0) and the effect of movement away from the piling sources was strongest for these animals, resulting in a significant interaction with piling period (fig. 5C middle panel). There was no effect of the time since the previous piling event on cod relative distance alone. There was, however, a significant interaction between the change in relative distance to the piling location and the length of the preceding interval between consecutive piling events, with cod positioning themselves closer to the piling location in the hours before the event started after a longer interval (fig. 5C). Other variables influencing relative distance were: tide ($p < 0.0001$, estimate: 11.504), during absolute low tide fish were further from the turbine C05 than during high tide; and current speed ($p < 0.0001$, estimate: -34.581), during low current speeds the fish were closer to the turbine C05.

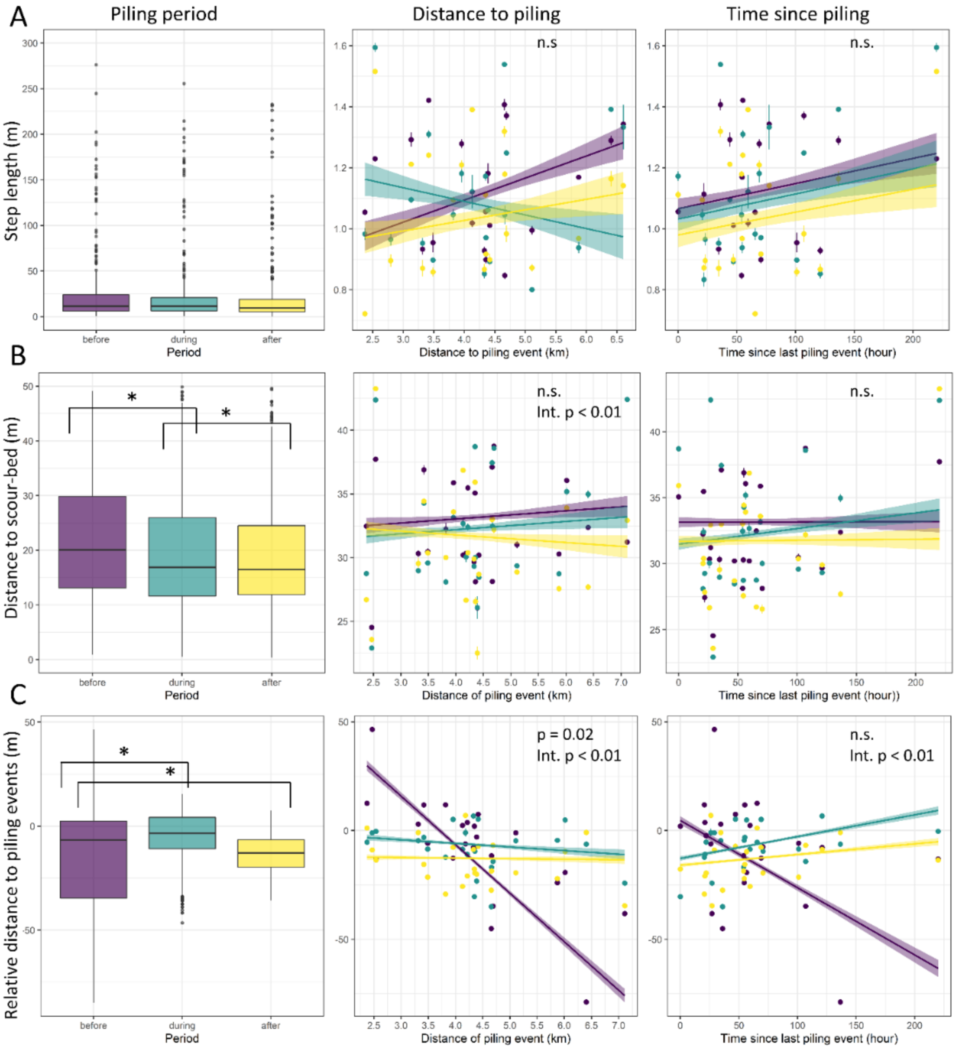


Figure 5. From left to right the effect of: piling period, distance to the piling event and time since last event, on A) cod step length (m) (not included in the best model and therefore no measurable effect), B) cod distance to scour-bed (m) and C) cod relative distance to the piling event (m). For piling period the significant differences are indicated with an asterisk (*, indicating a p<0.05). For the two interactions with period (of distance of piling event and time since last event), the significance of the overall trend is indicated (n.s. = not significant) as well as that of the interaction (Int.).

Discussion

Our results indicate that: 1) pile driving at distances between 2.3-7.1 km did not cause cod to leave the detection area during exposure, but that 2) there were several more subtle effects of piling on cod spatial behaviour. Over the six-and-a-half-month study period, cod left the detection area over time, however, this was statistically unrelated to the piling activity. Cod moved closer to the local turbine scour-bed during and after each piling event compared to the periods before pile-driving. Cods' relative distances to the pile driving location were correlated with the absolute distance to the location of the event: when piling took place at larger distances, the fish were positioned relatively closer to the sound source before the piling started and moved to an area further away from the source during and after piling. Time since last piling event in itself had no effect on cod movement, but a significant interaction indicated that with longer time intervals cod were positioned relatively closer to the sound source before piling started, which caused the effect of moving away during and after piling at large absolute distances to be stronger.

Disturbance-related departure decisions

We found no indication that cod left the area sooner than expected when exposed to pile driving sounds. Iafrate et al. (2016) found that tagged grey snappers (*Lutjanus griseus*) were more likely to leave an area when they were exposed to piling sounds. However, that effect was species-specific, as the same researchers found no effect of piling on the residency of sheepshead (*Archosargus probatocephalus*) (Iafrate et al., 2016). They argued that this difference in response could be caused by a general species-specific difference in site fidelity. From previous work on the residency of cod at offshore wind farms, we know that during summer months, cod often stay close to one turbine base for several months, only making occasional excursions to other turbines (Reubens et al., 2013c; Winter et al., 2010). This site fidelity of cod during the summer could explain why we did not find a change in presence. In line with the data on sheepshead (Iafrate et al., 2016), species with long residence periods may thus be less easily deterred by loud sounds. Our model did show that over time, cod moved out of the detection area. We expected to find this pattern as cod could move to another turbine outside of our detection range during the summer (Winter et al., 2010) and in addition, at the end of the summer, many move away from the offshore areas towards coastal areas (Reubens et al., 2013c).

The behavioural results of the current study differ from those observed during an earlier study on the effects of seismic survey sounds on Atlantic cod within the same wind farm (van der Knaap et al., 2021a). In that study, cod left the area shortly after the experimental exposure had ended (van der Knaap et al., 2021a). This difference may relate to the different nature of sound exposure, as pile driving and seismic surveys differ from each other in a number of ways. Sound exposure periods of a single piling event are relatively short: ca 1.5-4.5 hours, in comparison to a full seismic survey: continuously over four days in (van der Knaap et al., 2021a), but often weeks to months for actual surveys (Day et al., 2017; Slabbekoorn et al., 2019). The pulse rate intervals also differ, with 2-4 sec for pile driving and 8-15 sec for seismic surveys. In addition, pile driving occurs at a fixed location, with subsequent pile driving events making discrete steps in space, while a seismic survey typically moves back and forth across an area for longer periods of gradual changes in space. We obviously need more data on more seismic survey and pile driving events to gain general understanding about which parameters are critical for fish departure tendencies. We also need data on more species at the time, as species may interact and vary in their residence tendency, and on the energetic trade-offs associated with abandoning local resources (Iafrate et al., 2016; Kok et al., 2021; Soudijn et al., 2020; van der Knaap et al., 2021a).

Scour-bed related positioning

We found that cod moved closer to the scour-bed during and after the piling periods, from an average distance of 20 m before to 16 m during and after. Around the turbine base, a scour-bed rocky protection layer (r=20m) is deposited, which provides fish with food and hiding places (Reubens et al., 2013a). In our study area in the Belgian part of the North Sea, cod have a diurnal activity cycle (Reubens et al., 2013c; van der Knaap et al., 2021a) and are most active during twilight periods when they move around searching for food (Reubens et al., 2013b). This diurnal activity pattern is probably caused by a trade-off between light-dependent foraging success (Reubens et al., 2013b) and predation pressure (Scheidat et al., 2011; Todd et al., 2009). In the current study, piling events occurred both during day and night and we found a general effect of time of day (i.e. sun angle), in which cod were positioned on top and closer to, the centre of the scour bed during the day and moved towards the edges during night time, which could reflect a diurnal cycle in food searching behaviour.

The effect size of the response is only a few meters but could be related to some sort of general anxiety response. A typical response of fish to impulsive sounds is moving down the water column and forming tighter aggregations (e.g. Hawkins et al., 2014; Neo et al., 2018). Our tags included a pressure sensor, but with insufficient resolution (±0.5m at 30m depth (Innovasea)) for the small range in depth variation. Cod in the southern North Sea live in relatively shallow water and are closely associated with the seabed (Reubens et al., 2013c), which makes vertical avoidance by downward movement an unlikely response for this species here and any changes in depth inherently small. Additionally, the area around the turbines' scour-beds is sandy bottom that does not provide many hiding places. Cod movement towards the scour-bed during and after piling could therefore be indicative of cod seeking shelter closer to the turbine base and in-between the rocks.

This result of moving towards the scour-bed again differs from the findings in van der Knaap et al. (2021a), where cod moved away from the scour-bed during and after a four-day impulsive seismic survey (van der Knaap et al., 2021a). Those earlier results were in line with the observed gradual departure of cod from the detection area after the sound exposure period in that study. In the present study, however, cod did not leave the area. The benefits of staying in a noisy area may outweigh those of moving to a more silent one. Schools of herring that were exposed to seismic sounds did not cease their feeding activity (Pena et al., 2013). Pena et al. (2013) explained their finding by a strong motivation of the school to feed, a lack of suddenness of the exposure and an increased level of tolerance to the type of sound. Atlantic cod around offshore wind turbines are highly motivated to stay for food during summer, but apparently either the disturbance was different for the experimental seismic survey (van der Knaap et al. 2021a) and the pile driving (current study), or the consequences of leaving differed among the years of study, leading to the differences in their spatial behaviour relative to the scour bed.

Horizontal displacements relative to the sound source

Another interesting finding of the current study is that cod were positioned closer to the location of the sound source before piling started irrespective of the direction of the piling activity to the location of the fish. This was particularly so when the source was further from the fish and when the time since the last event was longer. This could be an indication that sounds from preparatory activities before the onset of piling may already be somehow deterrent when nearby, but that the very same sounds, when from larger distances and fainter, could be attractive and elicit phonotaxis. Indeed, certain sounds or sound levels can attract fish (Gordon et al., 2019; Neo et al., 2016). Radford et al. (2011) demonstrated how juvenile reef fish find their way back to their reef using environmental sound cues. In addition, man-made sounds can also attract fish as Chapman et al. (1974) observed how different fish species were attracted to the low-frequency sounds of scuba diving equipment.

Most studies investigating the movement of fish in response to impulsive sounds are done in captivity, which hampers a proper assessment of horizontal displacement (Davidsen et al., 2019; Neo et al., 2016). Potential horizontal displacements have been observed for free-swimming pelagic fish species in response to seismic sound (Engås and Løkkeborg, 1996; Løkkeborg et al., 2012). In the current study, we were unable to observe directional swimming behaviour but did find that fish were positioned closer to the piling location before piling started, in particular when piling preparations were happening at larger absolute distances. The type and level of sounds produced by these activities (vessel manoeuvring, on-board handling of heavy materials) could potentially have attracted the fish into the direction of the piling location before the actual start of pile driving. The displacements are only several meters, but since the area use of the cod is restricted to the source-bed surrounding the turbine base (r=20m), they indicate that the cod stay above the scour-bed during and after the piling exposure more than before.

We also observed distinct individual positioning and movement patterns of cod around the turbine. Individual personalities have been identified for Atlantic cod and have been linked to different response styles to environmental changes (Villegas-Ríos et al., 2017; Villegas Ríos et al., 2018), which might also apply to the responsiveness to anthropogenic sounds. Individual body condition may also affect response patterns, as Harding et al. (2020) showed that damselfish (*Chromis viridis*) with a poorer body condition startled later at shorter distances due to a predator threat during boat noise exposure, than did fish with a good body condition. We did not investigate the effect of individual differences further in our data set, but inter-individual variation could be an important factor in understanding animal responses to sound and has also been recognised as an important factor in translating behavioural effects to population dynamics in fish (Bolnick and Ballare, 2020).

Potential effects of habituation

Cod were exposed to a total of 50 piling events spread over four months. Although the interval between consecutive piling events varied from 1 to 12 days, we found no effect of time since last piling event on cod step length or their distance to the scour-bed. We only found an interaction between piling period and time since last piling event for relative distance to the piling site. The repetitive nature of the sound exposure may also have contributed to the lack of impact on departure, as over accumulating exposure time, fish may become more tolerant to the sound levels. Nedelec et al. (2016) demonstrated that juvenile reef fish (*Dascyllus trimaculatus*) exposed to boat noise reduced their hiding behaviour as well as their respiration rates (indicator for stress) after two weeks of exposure. Repeated exposure to impulsive sound has also been shown to reduce the disturbance response in seabass (*Dicentrarchus labrax*) over time (Radford et al., 2016).

Each piling event, in the current study, was preluded by a period of increased human activity at the piling site which might have served as some sort of ramp-up scenario, i.e. slowly increasing sound levels, providing animals with a warning signal, before sound exposure levels rise to the high levels of pile driving. We found that cod did not leave during or after the piling events, which might also be due to these slowly increasing sound levels, which may have accommodated habituation to the repeated occurrence of piling. However, we still know very little about the effect of fluctuating sound levels (Neo et al., 2014), and a net-pen study demonstrated little or no effect of a ramp-up exposure scenario on responsiveness in seabass (Neo et al., 2016).

Conclusions

The current study revealed that exposure to pile driving sounds at relatively close range of a few kilometres did not cause free-ranging cod to leave an area. We were able to show, however, several more subtle response patterns in their movement behaviour: they moved a couple of meters closer towards the scour-bed of the nearest turbine and also moved away from the sound source location. Spatial positioning before pile driving started suggested phonotactic approach behaviour in response to preparatory sounds at relatively large distances. Such changes in behaviour seem modest but can lead to changes in energy expenditure, which could potentially accumulate to population-level consequences (Soudijn et al., 2020). Offshore energy acquisition through the construction of wind farms is part of the EU's plans to move towards becoming climate-neutral by 2050 (European Commission, 2018), which will result in more piling in the North Sea in the coming years. Further studies are therefore needed to understand whether subtle behavioural changes like the ones we observed also occur with alternative construction strategies such as vibropiling, or while taking mitigation measures into account, such as bubble screens or acoustic deterrent devices. We also need to know whether the behavioural changes have any consequences for individuals or populations, whether there are any cumulative effects of co-occurrence of multiple stressors, and whether our findings can be extrapolated to other species. We therefore believe that raised awareness and more studies into anthropogenic under-water noise are warranted. More insight may allow us to detect and prevent unwanted detrimental effects of green alternatives to fossil energy resources on conservation of marine fish species like cod as well as their coastal habitat.

Acknowledgements

P. Verhelst assisted with the set-up of the receivers, data collection, and fish capture. We thank L. Thomas for help and advice regarding the data analysis and C. ten Cate for feedback on the manuscript. Parkwind is acknowledged for their kind cooperation and for allowing access to the offshore wind farm Belwind. This work was supported by the E&P Sound and Marine Life Joint Industry Programme (JIP) as part of the 'PCAD4Cod' project. Infrastructure for fieldwork (RV Simon Stevin, RHIB Zeekat and receiver deployment) were provided by Flanders Marine Institute and funded by the Research Foundation - Flanders (FWO) as part of the Belgian contribution to LifeWatch. This work is supported by COST Action CA18102. We thank the reviewers for their constructive feedback.

Supplements

Table S1. Start dates, pile driving duration, and number of strikes for all turbines involved in the construction of Nobelwind wind farm.

Nobelwind turbine	Start date and time (dd/mm/yyyy hh:mm)	Duration (hh:mm)	Number of strikes	Distance to C05 (km)
K01	16/05/2016 14:11	03:48	3539	4.3
K02	21/05/2016 05:02	04:26	3510	4.7
K04	23/05/2016 05:23	02:16	3610	3.1
K03	25/05/2016 19:06	02:19	3426	4.1
K05	04/06/2016 01:08	02:50	3211	2.5
K07	05/06/2016 23:50	02:04	3598	3.3
K06	07/06/2016 13:37	01:54	2740	2.6
K08	10/06/2016 07:15	03:05	4222	3.8
K09	15/06/2016 11:10	03:25	3921	4.3
K10	17/06/2016 13:42	01:58	3672	4.4
J01	19/06/2016 09:04	01:46	3222	5.2
J02	22/06/2016 07:07	02:06	2515	5.6

J06	28/06/2016 01:15	02:14	2894	6.4
J03	30/06/2016 12:22	02:05	2991	6.0
J04	03/07/2016 20:20	02:05	3123	6.6
J05	05/07/2016 01:35	02:17	2985	7.1
J10	08/07/2016 09:16	02:25	3181	4.2
J09	09/07/2016 23:37	02:36	3435	4.7
J07	12/07/2016 08:56	03:16	3606	5.9
J08	13/07/2016 10:18	02:03	2888	5.1
G04	16/07/2016 05:43	02:09	3015	2.3
G02	17/07/2016 12:54	02:05	2990	2.5
G01	18/07/2016 13:30	02:14	3328	2.8
G03	19/07/2016 16:43	02:11	3032	2.3
G05	31/07/2016 20:56	02:11	3215	2.6
G06	02/08/2016 03:09	02:31	4213	3.1
G10	05/08/2016 15:59	02:00	3089	5.3
G09	08/08/2016 12:44	02:32	4057	4.9
G08	09/08/2016 18:58	02:27	3380	4.4
G07	11/08/2016 03:12	02:37	3776	3.5
H04	15/08/2016 10:35	03:25	3955	3.5
H03	17/08/2016 06:15	04:31	2582	3.7
H01	18/08/2016 10:09	02:08	3297	3.3
H02	23/08/2016 03:08	02:11	3098	3.3
H08	26/08/2016 02:20	03:19	3625	4.0
H06	27/08/2016 16:04	02:46	2753	3.3
H05	30/08/2016 01:32	02:05	3123	3.4
H07	31/08/2016 16:53	02:09	3696	3.7
H10	05/09/2016 08:45	02:15	3603	4.8
H09	06/09/2016 08:24	02:28	4146	4.4
I01	07/09/2016 07:23	02:12	2989	3.8
I02	08/09/2016 06:50	01:39	2676	4.2
I10	11/09/2016 07:28	02:06	3620	4.4
I06	13/09/2016 15:50	01:50	3123	4.7
I09	14/09/2016 19:31	02:15	3754	4.2
I05	16/09/2016 00:50	01:39	2825	5.1
I08	19/09/2016 01:52	02:28	4406	3.9
I03	20/09/2016 02:50	01:27	2513	5.2
I07	21/09/2016 04:06	01:41	3235	4.3
I04	22/09/2016 00:47	01:50	2490	5.7

Table S2. Information on size, first and last detection date, and number of detections of all tagged fish in the current study.

Fish ID	Fish length (cm)	First detection date (dd/mm/yyyy)	Last detection date (dd/mm/yyyy)	Number of acoustic detections
8	40	04/04/2016	08/09/2016	24305
9	50	04/04/2016	16/05/2016	18415
10	46	04/04/2016	10/06/2016	23965
11	45.5	04/04/2016	01/09/2016	703
12	41	04/04/2016	30/08/2016	59396
13	43.5	04/04/2016	14/08/2016	34174
14	44.5	04/04/2016	19/10/2016	67614
15	49	04/04/2016	28/06/2016	37651
16	45.5	19/04/2016	31/08/2016	72933
17	49	04/04/2016	12/09/2016	74835
18	43	04/04/2016	05/11/2016	115303
19	48	04/04/2016	31/10/2016	129678
20	49.5	04/04/2016	09/09/2016	92984
21	69	-	-	0
22	39	28/04/2016	25/10/2016	75262

Table S3. Model selection for cod presence/absence using a hidden Markov model (HMM). Top five models and the null model, ranked based on the delta Akaike Index Criteria (ΔAIC) and that of the best fit model which is printed in bold.

Formula for transition probability HMM presence/absence per half hour	AIC	Delta AIC
M ~ piling + decay after tagging + decay after start + decay after end + pile order + fish length + day + current speed	**44667.3**	0
M ~ decay after tagging + decay after start + decay after end + pile order + fish length + day + current speed	44669.5	2.2
M ~ piling + pile order + fish length + day + current speed	44674.6	7.3
M ~ decay after piling + pile order + fish length + day + current speed	44676.5	8.2
M ~ decay after piling + decay after tagging + decay after start + decay after end + fish length + day + current speed	44680.7	13.4
M ~ 1	47061.3	2394

Table S4. Results of mixed model selection (ranked by ΔAIC) of the top models and full model, for all behavioural response variables. The marginal R2 (R2m) indicates the proportion of variance explained by the fixed effects, the conditional R2 (R2c) shows the proportion of variance explained by the entire model and AIC is the Akaike Index Criteria of the model. ΔAIC ≥ 2 indicates a significant difference between the models, if ΔAIC < 2 we picked the model with the least covariates indicated in bold.

Response variable	Mixed model	R^2m	R^2c	AIC	ΔAIC
Step length (m)	**~ sun angle + current speed + tidal elevation + (1|Piling ID) + (1|Fish ID)**	**0.09**	**0.36**	**1132.4**	**0**
	~ sun angle + current speed + tidal elevation + current speed*tidal elevation + (1|Piling ID) + (1|Fish ID)	0.09	0.36	1136.0	3.6
	~ sun angle + current speed + (1|Piling ID) + (1|Fish ID)	0.07	0.34	1136.7	4.2
	~ sun angle + current speed + tidal elevation + distance piling to C05 + (1|Piling ID) + (1|Fish ID)	0.09	0.35	1139.0	6.6
Full	~ fish length + piling period + distance to piling + hours since last event + sun angle + tidal elevation + current speed + period*distance to piling + period*hours since last event + tidal elevation*current speed + (1|Piling ID) + (1|Fish ID)	0.10	0.38	1180.4	48
Relative linear distance to piling event (m)	~ fish length + piling period + distance to piling + hours since last event + tidal elevation + current speed + period*distance to piling + period*hours since last event + tidal elevation*current speed + (1|Piling ID) + (1|Fish ID)	0.06	0.15	39168.3	0.0
	~ piling period + distance to piling + hours since last event + tidal elevation + current speed + period*distance to piling +	**0.06**	**0.16**	**39169.0**	**0.7**

Full	**period*hours since last event + tidal elevation*current speed + (1|Piling ID) + (1|Fish ID)**				
	~ fish length + piling period + distance to piling + hours since last event + tidal elevation + current speed + period*distance to piling + period*hours since last event + tidal elevation*current speed + (1|Piling ID) + (1|Fish ID)	0.06	0.16	39173.5	5.18
	~ piling period + distance to piling + hours since last event + tidal elevation + current speed + period*distance to piling + period*hours since last event + tidal elevation*current speed + (1|Piling ID) + (1|Fish ID)	0.06	0.15	39174.1	5.8
	~ fish length + piling period + distance to piling + sun angle + tidal elevation + current speed + period*distance to piling + tidal elevation*current speed + (1|Piling ID) + (1|Fish ID)	0.06	0.16	39189.1	20.8
Distance to scour-bed (m)	~ piling period + distance to piling + sun angle+ current speed + tidal elevation + distance to piling*period + current speed*tidal elevation + (1|Piling ID) + (1|Fish ID)	0.10	0.29	27437.9	0
	~ piling period + distance to piling + hours since last event + current speed + sun angle + tidal elevation + current speed*tidal elevation + distance to piling*period+ (1|Piling ID) + (1|Fish ID)	0.12	0.30	27438.0	0.1
	~ piling period + distance to piling + current speed + sun angle + tidal elevation + distance to piling*period+ (1|Piling ID) + (1|Fish ID)	**0.10**	**0.29**	**27438.9**	**1**
Full	~ piling period + distance to piling + hours since last event + tidal elevation + current speed + period*distance to piling + (1|Piling ID) + (1|Fish ID)	0.12	0.30	27438.9	1.1

	~ fish length + piling period + distance to piling + hours since last event + tidal elevation + current speed + period*distance to piling + period*hours since last event + tidal elevation*current speed + (1|Piling ID) + (1|Fish ID)	0.11	0.30	27442.7	4.8

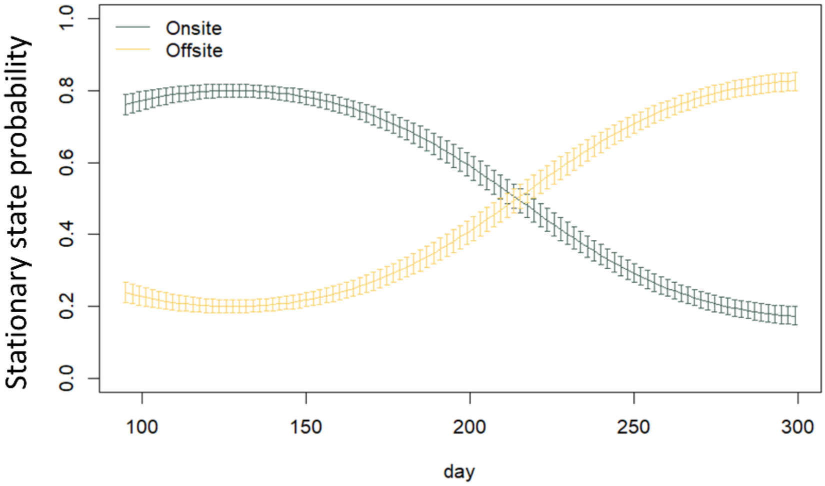


Figure S1. Stationary state probabilities of cod in the onsite and offsite behavioural state, predicted by a Hidden Markov model (HMM), including the 95% confidence intervals (CI). Effect of day of the year: the number of cod off-site increased at the expense of cod on-site, reflecting the cumulative tendency to have left the area over time.

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6

General discussion and conclusion

Over the past decades, human-made noise has become an increasingly large part of the ocean soundscape (Duarte et al., 2021), while we still know very little about the effect of underwater noise on marine life. The aim of my thesis was to answer pressing questions related to the effects of two anthropogenic sound sources on the behaviour of a widely distributed North Atlantic fish species: Atlantic cod (*Gadus morhua*). I studied the effect of impulsive sounds produced during a seismic survey (chapter 3) and pile driving (chapter 5) on the movement of free-swimming cod in their natural habitat. To study fish at an offshore location requires a robust study setup and I therefore first performed a pilot study in which I tested the effectiveness of two different acoustic telemetry setups (chapter 2). The behaviour of free-ranging animals is determined by many different external factors, in addition to the effect of human-made noise. As global warming and rising water temperatures are considered to be among the most serious threats to aquatic ecosystems, I also performed a separate analysis aimed at testing the effects of increasing water temperatures on cod movement (chapter 4). My results do not only provide new insights into the effects of two types of anthropogenic noise on individual cod behaviour, but also provide stepping stones towards studying the potential consequences of noise at a population level, by quantifying observed behaviours in relation to energetic costs. Below, I will discuss and integrate the conclusions from each of my chapters and will put them in a broader general perspective.

Cod behaviour during and after impulsive sound exposure

My main aim was to investigate if cod changed their tendency to leave an area and/or their movement behaviour, during exposure to seismic sound or pile driving (hereafter called anthropogenic sounds), and if these effects last beyond the sound exposure period. During exposure to anthropogenic sounds, cod did not leave the area more than expected (Chapters 3 and 5). However, after the seismic survey, more cod than expected, based on two years of baseline data, departed from the detection area (Chapter 3). Such an effect was not found in response to piling events, where cod did not leave the area more than expected after each individual event or after the end of the entire piling period (four months, Chapter 5). In both study designs, detection of tagged cod was limited to the detection range of the receivers that covered the area. Thus, while I could establish that an individual was no longer detected and therefore assumed to have left the detection area, I was unable to show where the animal went.

When the animals were present within the receiver setup, I was able to collect information on fine-scale movement of cod and found that exposure to anthropogenic sounds affected their movement (Chapters 3 and 5). During the 3.5 day seismic survey, cod moved slightly further from its closest turbine and spent significantly more time being inactive. In addition, their diurnal activity cycle was affected: when no seismic sound was present, cod were active during dusk and dawn. This diurnal activity cycle was disrupted during seismic exposure (Chapter 3). When free-swimming cod were exposed to piling events, the animals moved closer to the nearest turbine from before to during exposure and were positioned closer to the sound source before in comparison to during and after a piling event (Chapter 5). Thus, although both sources of impulsive sounds affected the movement of individual fish, the changes in movements or behaviour were slightly different per sound source, and piling resulted in significant but just small spatial changes (meters).

A possible explanation why the tendency to leave and change in behaviour were different in response to the two types of impulsive noise sources, could be the difference in sound characteristics. The acoustic characteristics of a sound source are defined by a number of different sound components that result in a particular sound. The focus of many noise impact studies lies on the sound pressure level (SPL) aspect of the exposure. However, in addition to SPL, particle motion (providing directional information) (Nedelec et al., 2016) and the impulsiveness (Müller et al., 2020) of anthropogenic noise can also be relevant for the response tendency of an animal. In addition, the sounds produced during seismic surveys or pile driving, which are both described as impulsive (European Parliament, 2008), differ in that a seismic vessel is a moving source, while piling is static over time, and that the interval between strikes is shorter during piling than during seismic. Piling event may also last several hours, after which there is often a break of one or two days, before the next piling event, while a seismic survey typically goes on for days to weeks, more or less continuously (Carroll et al., 2017). This differences might all be factors driving the difference in leaving tendency and movement behaviour of cod observed during the two noise exposure studies (Chapter 3 and 5).

A fish's response to sound

If and how a fish responds to sound not only depends on the type of sound and sound characteristics, but also on the interaction of different external factors and the species-specific ecology and associated behavioural traits (fig. 1). At least three main external factors seem to determine the potential for a behavioural response of an individual fish:

1) its ecotype and thereby the abiotic and biotic habitat conditions the animal is exposed to; 2) seasonal circumstances at that moment and place; and 3) anthropogenic-related changes (i.e. global warming, pollution, construction, noise).

How abiotic and biotic habitat conditions can influence fish behaviour in a fleeing-for-danger context was demonstrated by Christensen and Persson (1993), who studied the predatory behaviour of perch (*Perca fluviatilis*) on two juvenile fish species in relation to different habitat complexities. When the abiotic habitat complexity was low, i.e. no bottom structures were present, the predator pursuit speed was higher, and the attack strategy varied between short and long attacks depending on the type of prey, juvenile roach or perch (Christensen and Persson, 1993). Another example of an effect of abiotic conditions concerns differential response tendency to sound exposure in sea bass during day or night time (Neo et al. 2018). Abiotic habitat conditions, such as the food availability and predator pressure, may in addition influence the potential for an animal to stay or leave an area in response to a anthropogenic sound exposure.

Seasonality also plays an important role in the behaviour of fish. Spawning for example happens only during a certain time of the year and is predominantly triggered by changes in water temperature (Pankhurst and Munday, 2011). Some fish species perform seasonal migration, inhabiting a different area during one period of the year and migrate to another, where they might behave differently (Jansen and Gislason, 2011; Lelièvre et al., 2014). For example, cod studied in this thesis, inhabit the offshore areas during summer and migrate to coastal waters for spawning during the fall and winter (Fahay et al., 1999; Lelièvre et al., 2014; Reubens et al., 2013).

The influence of human presence on the marine environment is diverse and impacts almost every component of the environment, from water quality, chemical composition, and temperature, to light and sound in the environment (Aronson et al., 2011; Auta et al., 2017; Naser, 2013; Tornero and Hanke, 2016). Ambient noise levels around harbours of shipping lance are elevated All these external factors will influence fish responsiveness to an additional anthropogenic noise source in the marine environment.

Internal characteristics like a fish's hearing abilities, and therefore it's sensory dependency on hearing, as well as behavioural types will also affect how individuals and populations respond to sound exposure (fig. 1). Of the >111 species of fish for which audiograms exist (Ladich & Fay, 2013), hearing capabilities overlap largely with the frequency range of anthropogenic noise from noisy human activities (10–500 Hz; (Slabbekoorn et al., 2010)). However, while it is generally assumed that fishes with better hearing abilities are more likely to show behavioural responses to sound than less sensitive species, this may not always be the case. Comparisons of laboratory responses of zebrafish (*Danio rerio*) and Lake Victoria cichlids (*Haplochromis piceatus*), showed that both species exhibited a significant reduction in swimming speed in the first minute of sound exposure, which was not obviously related to differences in their species-specific hearing abilities (Shafiei Sabet et al., 2016). Similarly, Hawkins et al. (2014) showed that, in response to playback of impulsive sound at similar sound levels (i.e. 163 dB re 1 µPa), the schooling behaviour of both wild sprat (*Sprattus sprattus*) and mackerel (*Scomber* *scombrus*) were affected, despite major differences in their hearing abilities (Hawkins et al., 2014).

The behavioural phenotype of an individual fish, which can vary among and within species, may also affect acoustic reactions. For cod, it is known that different individuals in the same area may show individually distinct behavioural patterns (Villegas-Ríos et al., 2017). A fish with a smaller home-range may, for example, not move to another area as quickly as an individual that already uses a larger homing range.

In addition, of the fish that inhabit the North Sea region, a rough division can be made between demersal (species that spend most of their live close to or on the seabed) and pelagic species (fish that predominantly live in the rest of the water column), which may affect how a fish responds to noise. Roberts et al. (2016) examined the responses of a number of free-ranging demersal species, using baited remote underwater video (BRUV), to the playback of pile driving sounds and found responses including startle behaviour and directional avoidance (Roberts et al., 2016). And similar thresholds for responses but different response types in different species may not be rare. While exposure levels of playback piling at which responses occurred were similar to that from the study by Hawkins et al. (2014), the response of pelagic sprat and mackerel in the latter study were qualitatively different. Sprat schools were more likely to disperse and mackerel schools were more likely to change depth. In summary, what the repose triggering potential is for an animal does not only depend on the type of sounds but also on the local biotic and abiotic circumstances, species characteristics, and the individual.

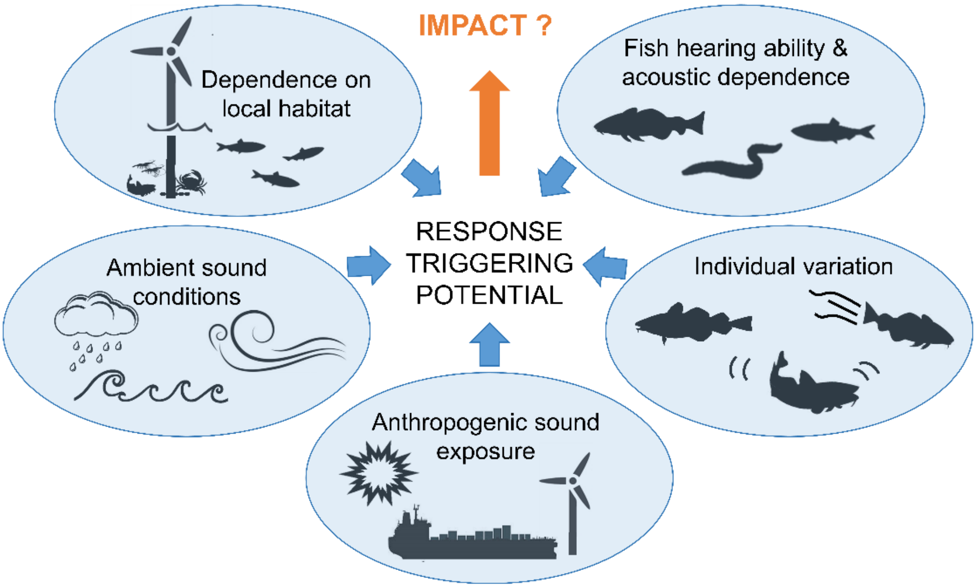

Figure 1. The potential to trigger a response in a fish in relation to sound is the result of the interaction of many different factors. Here, an overview of the most important factors is given. Clockwise ovals: 1) A species hearing ability and their dependence on their hearing will determine how the fish perceives and response to a sound, 2) Individual variation in behaviour may result in different response to the same sound source, 3) The type and sources of anthropogenic sound: e.g. explosions, seismic surveys, pile driving, also influences the response, 4) Local ambient sound conditions form the background to the sound exposure, 5) Fish dependency on the local habitat will in addition effect the response potential, which differs per habitat, fish species, and season. A response triggered by an anthropogenic sound will have an impact on the individual and potentially the population.

Translation and extrapolation of case study results should always be done with great care. In my field studies, I found that Atlantic cod were affected by impulsive noise, which may indicate that the behaviour of other demersal species with comparative hearing abilities, under similar conditions, could be affected by seismic surveying and piling noise as well. Yet, to be able to say anything about how fish respond to anthropogenic noise sources in general, more long-term, field-based studies are required, that include a diverse range of fish species and that look into the cumulative and synergistic effects of the local circumstances. It is important to carry out such studies in the wild, where there are no constraints like tank walls or netting and where the sound propagation conditions are natural. To be able to collect this type of data on fish in the wild, more advanced tracking methods are needed.

Tracking fish in the wild: methodological challenges

To understand how anthropogenic noise affects free-ranging fish, we need to be able to investigate the individual behaviour and be able to interpret the observed behaviour in terms of energy expenditure. Different techniques exist that enable remote tracking of fish movement. The most commonly used techniques include: pop-up satellite tags, active radio tracking, and passive acoustic tags (Lennox et al., 2017), potentially with sensors logging bioenergetics (Cooke et al., 2016). In the field studies discussed here, I used acoustic telemetry (Hussey et al., 2015) to triangulate the positions of individual fish in combination with sensors embedded in the tags measuring acceleration and pressure. How well this method performs depends on the local environmental circumstances like, depth, turbidity and vegetation (Kessel et al., 2014; Reubens et al., 2018; Thiemer et al., 2022). During the pilot study, I tested two receiver array designs (Chapter 2) and found that, under the local circumstances, receivers spaced at 200m in a circle around the turbine, provide good detection coverage. Per study setup, the technique and setup required may be different and should be adapted to the species and local circumstances.

For acoustic telemetry, there are a number of factors that limit the efficiency, such as the scale of the area relevant for investigation. Whether the fish of interest has a large or small home range, will ultimately define if the fish tag signal can be picked up efficiently by a static receiver. A study by Bruce et al. (2018) used acoustic telemetry to track the 2D-movement of three fish species: gummy shark (*Mustelus antarcticus*), swell shark (*Cephaloscyllium laticeps*), and tiger flathead (*Neoplatycephalus richardsoni*). Their detection area covered approximate 20 km^2 area of seafloor, however, both gummy and swell shark were too wide-ranging and departed from their detection area after two days and were only detected occasionally afterwards, while most of the more sedentary tiger flatheads were detected consistently throughout the study period (Bruce et al., 2018).

Another limiting factor to the application of telemetry is the sensor information transmitted by acoustic tags. The tag signal emission is short and can only hold a limited amount of data. Therefore, a sensor that registers specific behaviours of fish, for example a feeding strike (de Almeida et al., 2013), or one that measures tri-axial acceleration, which can be linked to energy expenditures of specific behaviours (Bouyoucos et al., 2017; Wilson et al., 2020; Wright et al., 2014) can be highly useful. However, these do need to be calibrated for the species of interest in lab studies first (Bouyoucos et al., 2017). Once we have successfully obtained fine-scale information on fish movement combined with sensor data, we often still do not know what this movement means in terms of energy gain or expenditure. A combined approach of controlled exposure experiments in both the lab and field could tell us what the movements observed in the field mean in term of energetic costs in response to an anthropogenic disturbance like noisy human activities (Slabbekoorn, 2016; Slabbekoorn et al., 2019).

By transmitting the data, as well as storing it, information would also be collected when an animal leaves the detection area. However, to retrieve this data the fish either needs to be re-caught or the tag has to wash up on a beach after the death of an animal. How long a tag can store and transmit data for, depends on battery size which makes taking small animals over a longer period of time difficult. Battery size is at the moment the most important limiting factor for tag innovation (Lennox et al., 2017). Once smaller batteries become available, the possibilities to track behaviour and record sensor data on wild fish using acoustic telemetry, will increase. This will make more long-term and quantitative field studies on the effects of anthropogenic disturbances more feasible.

Cumulative effects in the Anthropocene

The Anthropocene is characterized by an increasing presence of human-generated sounds in natural soundscapes. However, the presence of humans on earth affects the natural soundscapes also through changes in the abiotic and biotic environment (Duarte et al., 2021). For example, climate change directly affects the water temperature and stratified heat content of the ocean. As sound travels faster in a warmer ocean, this results in oceans where anthropogenic noise can spread faster and further: a phenomenon termed 'sound of climate change' by Munk (2011). Besides sound traveling faster in warmer waters, the changes caused by climate change may also affect fish hearing directly. Ocean acidification, caused by elevated levels of $C0_2$ emitted by humans, has been linked to effects on the hearing sensitivity of fish, both by directly affecting the growth of otoliths, and indirectly through changes in auditory perception and associated behaviour (Radford et al., 2021; Simpson et al., 2011). Juvenile snapper (*Chrysophyrs auratus*), for example, raised under predicted CO_2 levels for the near future, were shown to be less sensitive to low-frequency sounds and altered their previously described hearing-related behaviour (Radford et al., 2021).

Apart from rising water temperature and elevated levels of $C0_2$, marine fish will have to deal with an accumulation of multiple stressors related to climate change and pollution, such as extreme weather, chemical spills and plastic waste (Aronson et al., 2011; Auta et al., 2017; Naser, 2013; Tornero and Hanke, 2016). It is therefore important to not only study single-stressor effects, but also cumulative effects and potential interactions of multiple stressors. During the summer of 2018 the North Sea experienced an unusually warm period: sea surface temperatures rose to 20 °C. This was also the year the seismic survey exposure experiment was performed. I therefore wanted to understand if and how these exceptional sea water temperatures affected the presence of cod at the offshore site. I performed an additional, separate analysis in which the 2018 data was compared to cod presence data from 2020, another year with extreme water temperatures reaching 20 °C. I found no evidence for cumulative effects or interaction in linear or non-linear correlations of water temperature and cod departure rates (Chapter 4). Nevertheless, I would like to emphasise here the need to take such potential effects into account in future studies and to be aware of the complexity of anthropogenic noise assessments with the variable diversity of stressors present in the Anthropocene.

From impact assessments to legislations

Currently, legislations to mitigate anthropogenic noise in the marine environment are in place for only a few regions and sound types, and only aim to mitigate the effects for a few marine species. In German waters, for example, pile driving levels should remain below 190 dB re 1 μPa^2 (SPLpeak) for harbour porpoises (Dekeling et al., 2021) and a ramp-up has to be used during a seismic survey to minimise the risk to injure marine mammals (JNCC, 2017). The European Union is working towards a sustainable marine future and the EU Marine Strategy Framework Directive (MSFD) has included noise produced during anthropogenic activities in the marine environment as pollution in Descriptor 11 (European Parliament, 2008). Each member state is required to develop a strategy for its marine waters in order to achieve so-called Good Environmental Status by 2020.

For anthropogenic noise, this means establishing threshold levels below which the noise levels should not adversely affect the marine environment. To establish what these levels are, the spatial distribution, temporal patterns, and sound level, for impulsive and continuous anthropogenic noise, have to be considered. The technical group on underwater noise (TG Noise) focusses on the assessments of impacts of noise and the development of thresholds in relation to these indicators. In their reports, the focus is mainly on marine mammals and sound levels are referred to as the sound pressure level (Dekeling et al., 2021), while for fish the particle motion sound component is equally, if not more, important for their hearing (Nedelec et al., 2016; Popper and Hawkins, 2018). A better understanding on how different levels of both sound pressure and particle motion affect fish is therefore important (Rogers et al. 2021).

However, there are only a few field studies, with a limited number of fish species, that have looked at the levels of anthropogenic noise that reach a fish and what levels are acceptable. Anthropogenic noise can have many different effects, from mortality, to physiological and behaviour responses, while most field studies only address one effect at the time (Popper and Hawkins, 2019). Debusschere et al (2014), for example, looked at the effect of piling on the mortality of Juvenile European sea bass (*Dicentrarchus labrax*), kept in nets while piling occurred at close range (exposure levels of SPLpeak = 210– 211 dB re 1 µPa^2) and found no differences between mortality in the treatment and control group (Debusschere et al., 2014). In a later experiment using a similar setup, Debusschere et al (2016) looked into the sea bass stress response and found significant reductions in oxygen consumption rate and low whole-body lactate concentrations during repeated piling exposure (Debusschere et al., 2016). This underlines the complexity of defining which effects to monitor and what sound levels should be considered to avoid negative effects on fish.

Interpreting the results from behavioural studies for the overall impact on fish may be even more complex. Results from my own field studies (Chapters 3 and 5) confirm that individual free-swimming cod, >50 km from the coast, are affected by anthropogenic noise. However, although these effects were found, the results do not provide information on how the effects ultimately impact fish fitness, nor could threshold levels for impact in sound pressure or particle motion be established. Once apparent effects of a disturbance from anthropogenic noise are observed, it is important to investigate if the effects are consistently and causally related to anthropogenic disturbance and not just natural fluctuations without severe consequences. It may be crucial for understanding the consequences of a disturbance to find out whether the response of a particular individual is similar to when a predator is present and how often such a situation would occur naturally (Miller et al. 2022).

As mentioned before, a next step in investigating noise impact would be to combine field movement data with visual observation data, using a video camera or tank studies in which the same movements, in the field and on camera, can be related to energy expenditure (Cooke et al., 2004). These data could then provide quantitative insights into consequences for individual growth, reproduction and individual and population survival through dynamic energy budget models and Population Consequence of (Acoustic) Disturbance models (PCAD or PCoD), (Pirotta et al., 2018; Van Leeuwen et al., 2008). PCAD models have been successfully implemented for marine mammals (e.g. DeRuiter et al., 2017), and recently also for fish. Soudijn et al. (2020) presented a size-structured energy budget model for Atlantic cod, modelling the potential effects of sound disturbance on cod energy budgets and the consequences for population growth. Using such a model, the effects of different acoustic disturbances can be simulated and threshold levels for management of anthropogenic noise can be established.

Conclusions

The North Sea is an intensely used area (Sertlek et al., 2016) and the anthropogenic activities taking place are not likely to decrease in the near future. These activities, introduce high levels of sound into the marine environment. Although noise mitigation measures are being developed, such as seismic airguns that are less loud in critical frequency ranges, and pile driving methods using a water yet instead of a hammer or using bubble curtains to reduce the spreading of sound, traditional and noisy methods are often still the preferred method to use (Acteon, Van Oord). Therefore, marine fish inhabiting these areas are, and will likely still remain, exposed to a wide and still increasing variety of anthropogenic sounds (Slabbekoorn, 2019; Duarte et al., 2021). If we want to understand how we can effectively mitigate our impact, studying how these different sounds affect fish is crucial.

The study results indicate that seismic survey and pile driving activities, both produce sounds that can affect the behaviour of free-swimming Atlantic cod. Still many questions remain about the implications of the results on individual cod fitness and overall population impact. Besides anthropogenic noise, marine fish are exposed to a variety of external and internal cues that determine their behaviour. Currently, rapid changes are taking place as a result of climate change, and understanding how the interaction of different factors, like boat noise and an increase in water temperature, affects fish are therefore becoming more and more important. In addition, technology is developing fast, making it possible to use smaller tags with longer life spans and larger detection areas. This is therefore the time to gather more information like the information presented here, on a variety of fish species and anthropogenic noise sources, in order to provide information for effective conservation and mitigation measures. Science-based understanding is the only way forward towards healthy marine ecosystems and a good environmental status.

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Nederlandse samenvatting

Alle vissen kunnen horen. Ook al weten we van maar een paar soorten hoe goed ze kunnen horen. Hoogst waarschijnlijk gebruiken alle vissen het onderwatergeluidslandschap om informatie in te winnen. Geluid gaat met een snelheid van 1500 meter per seconde door zeewater, dat is 5 keer sneller en verder dan door lucht. Dit terwijl licht in water, en dus ook zicht, maar een beperkte reikwijdte heeft. Voor onderwaterdieren is gehoor daarom van groot belang. Via geluidsdetectie krijgt een dier informatie over de geluidsbron: is deze bijvoorbeeld afkomstig van een soortgenoot, roofdier of prooi, én op welke afstand bevindt de bron van het geluid zich? Onderwater is geluid daarom van groot belang voor onder andere oriëntatie en communicatie, maar ook voor overleving en tijdens reproductie. Naast de informatie die vissen via hun gehoororganen binnen krijgen, krijgen ze ook informatie vanuit visuele en chemische prikkels. De interactie tussen de informatie die binnenkomt via verschillende sensoren bepaalt hoe een dier reageert op een geluidsbron, maar gehoor is waarschijnlijk de meest dominante sensor, zeker op langere afstand en in donkere, troebele wateren.

De oceanen zijn niet stil en onder water is veel geluid te horen. Dit wordt veroorzaakt door onder andere het bewegen van de aardkorst, regen en wind en door dieren zoals walvissen en garnalen. De laatste decennia is hier een geluidsbron bij gekomen: menselijk geluid. Dit wordt ook wel antropogeen geluid genoemd. De toename wordt met name veroorzaakt door wereldwijde scheepvaart, seismische exploratie en constructieactiviteiten langs de kust en op zee. Seismische exploraties van de zeebodem worden gedaan om te weten te komen wat er zich onder de bodem bevindt. Dit gebeurt middels het gebruiken van een seismisch luchtgeweer dat met regelmaat een drukgolf richting de zeebodem afschiet. De weerkaatsing van dit geluid wordt door hydrofoons (onderwatermicrofoons, die achter het schip aan lange kabels worden meegesleept) geregistreerd en geeft informatie over de samenstelling van de bodem. Zit er bijvoorbeeld olie of gas óf is het een geschikte bodem om de fundering van windturbines op te kunnen plaatsen? De meest gebruikte methode om een fundering voor een windturbine op zee te plaatsen is door een metalen paal meer dan 30 meter de bodem in te heien. Als bijproduct wordt er tijdens het heien een geluidsgolf geproduceerd door de waterkolom en de bodem.

Bij zowel seismische exploratie als heien op zee komt dus met regelmatige interval een luid laagfrequent geluid vrij dat kan worden gehoord door mariene fauna. Op korte afstand kunnen luide geluiden ernstig weefselletsel veroorzaken en zelfs leiden tot de dood van vissen. Dit vormt echter alleen een gevaar voor de dieren die zich binnen enkele meters tot een paar honderd meter van de geluidsbron bevinden. Het overgrote deel van de dieren bevindt zich op grotere afstand, waar antropogeen geluid kan leiden tot het maskeren van relevante biotische of abiotische geluiden, wegjagen van dieren uit een gebied en het verstoren van gedrag. Omdat geluid in water zich snel verplaatst reikt het ook verder dan in lucht en kunnen deze verreikende geluiden potentieel gevolgen hebben voor een groot deel van het mariene leven.

Hoe groot de impact van antropogeen geluid is op het gedrag van zeedieren is mede afhankelijk van hoe goed een dier kan horen. Vissen kunnen de beweging die geluid in het water teweeg brengt registeren met hun binnenoor en via de laterale lijn. Daarnaast hebben veel vissen ook een interne zwemblaas die resoneert met de geluidsdrukgolf die door het water gaat. Hoeveel de zwemblaas bijdraagt aan de geluidsdetectie hangt af van hoe dicht hij bij het binnenoor ligt en of er een verbinding is tussen beide. Vissen kunnen voornamelijk laagfrequente geluiden goed horen: tussen de 50-500 Hz. Dit overlapt met de piekfrequenties van zowel seismische exploratie als heien.

Om de effecten van geluid op de bewegingen en het gedrag van vrij-zwemmende vis te kunnen bestuderen is het belangrijk om ze in hun natuurlijke habitat te observeren. Dit is een uitdaging omdat zicht in zee vaak beperkt is door hoge concentraties deeltjes en het feit dat zonlicht meestal maar tot zo’n 200m diepte reikt. Hierdoor is visuele observatie vaak lastig. Daarnaast is het niet mogelijk om vissen, zoals op land gebruikelijk is, te voorzien van een GPS zender om ze te volgen omdat het GPS signaal niet door water gaat. De afgelopen jaren zijn er echter grote en snelle ontwikkelingen gaande op het gebied van technologie, zo ook op het gebied van elektronische zenders die het mogelijk maken om op afstand vis te kunnen volgen. Één van deze methodes is akoestische telemetrie.

Akoestische telemetrie werkt middels een akoestische zender en ontvanger. De zender wordt ingebracht of vastgemaakt aan de vis en zendt een akoestisch signaal uit op hoge frequentie. Dit signaal bevat een unieke code om de vis te identificeren en eventuele extra informatie, gemeten door o.a. diepte, temperatuur en acceleratie sensors in de zender. Zodra de gezenderde vis in de buurt komt van de ontvanger (binnen een straal van 500-1000 meter afhankelijk van omgevingsvariabelen), wordt het signaal opgevangen en opgeslagen. De ontvanger is losstaand en bevat een hydrofoon, batterij en opslaggeheugen, en wordt op een vaste plek verankerd (op de bodem of aan het wateroppervlak). Door meerdere ontvangers (minimaal 3) in een netwerk te plaatsen, is het daarnaast mogelijk om, via triangulatie, een 2D posities van de gezenderde vis te berekenen en zo met hoge resolutie zwemtracks te reconstrueren. Middels akoestische telemetrie kunnen vrij-zwemmende vissen zo toch gevolgd worden in hun natuurlijke omgeving.

Een vissoort waar al veel onderzoek naar is gedaan met behulp van akoestische telemetrie is de kabeljauw (*Gadus morhua*). Kabeljauw is een belangrijke vissoort met zowel een economische als culturele waarde. Veel landen hebben een traditioneel kabeljauwgerecht en het is een vis waar van oudsher op gevist wordt. Helaas gaat de totale populatie van kabeljauw hard achteruit door overbevissing en klimaatverandering.

In het zuiden van de Noordzee zijn kabeljauwen het hele jaar door te vinden en met name in de zomermaanden verblijven ze enkele maanden op één locatie. Dit is vaak bij harde structuren, zoals een scheepswrak of de fundering van windturbines, waar ze foerageren en zich kunnen verschuilen. Kabeljauwen zijn gevoelig voor laagfrequente geluiden, ze horen met name goed tot 400 Hz. Deze vissoort kan ook geluid produceren tijdens onder andere territoriaal gedrag en in de paringstijd. Gehoor is daarom van groot belang voor individuen. Verstoring in omgevingsgeluiden kan leiden tot veranderingen in gedrag, individuele fitness en uiteindelijk veranderingen op populatieniveau.

Studies in gevangenschap hebben aangetoond dat laagfrequente pulserende geluiden met een vaste interval, zoals geproduceerd tijdens seismische exploratie en heien, het gedrag van kabeljauw kunnen beïnvloeden. Wat ontbreekt is onderzoek naar vrij-zwemmende vissen. Om meer te weten te komen over het effect van dit type geluid op het gedrag van vrij-zwemmende kabeljauw, is het project PCAD4Cod opgezet. Dit project bracht een consortium van experts bij elkaar die, sinds 2017, drie PhD studenten en een postdoc hebben geadviseerd. Mijn thesis is onderdeel van het PCAD4Cod project en hierbinnen heb ik akoestische telemetrie gebruikt om het gedrag van vrij-zwemmende kabeljauwen op hoge resolutie rond windturbinefunderingen op zee te volgen, terwijl ze werden blootgesteld aan twee antropogene geluidsbronnen: seismische exploratie en het heien van windturbinefunderingen.

Het hoofddoel van mijn onderzoek was om er achter te komen of kabeljauwen eerder een gebied verlaten en/of ze hun bewegingen aanpassen tijdens blootstelling aan deze twee antropogene geluidsbronnen, en of er veranderingen waren in het gedrag die ook na de blootstelling nog meetbaar waren. Om dit te kunnen onderzoeken heb ik twee veldstudies opgezet, uitgevoerd en geanalyseerd en de data van nog twee andere veldstudies meegenomen in het onderzoek. Tijdens alle studies zijn vrij-zwemmende kabeljauwen in het windpark 'Belwind' in België voorzien van een akoestische zender en voor enkele maanden gevolgd op een netwerk van ontvangststations voor 2D positionering.

Om het netwerk van ontvangers op zee te optimaliseren, en zo nauwkeurig mogelijke 2D posities voor kabeljauw te krijgen, heb ik eerst een pilotstudie gedaan waarin ik twee verschillende netwerk ontwerpen (één cirkelvormig en één vierhoekig) van ontvangststations heb getest (Hoofdstuk 2). Hieruit bleek dat een netwerk waarin de stations 200 m uit elkaar geplaatst waren in een cirkel rond de windturbine een goed resultaat gaf (Hoofdstuk 2). Dit ontwerp heb ik daarna voor mijn volgende veldstudie gebruikt om het effect van een seismische exploratie op kabeljauwgedrag te onderzoeken (Hoofdstuk 3). In 2016 is al eerder een veldonderzoek gedaan in het zelfde windpark maar dan naar het effect van heigeluid op kabeljauw. De gegevens van dit onderzoek heb ik verder uitgewerkt en geanalyseerd (Hoofdstuk 5). Daarnaast zijn er in 2020 opnieuw een aantal kabeljauwen gezenderd in 'Belwind'. Deze gegevens heb ik gebruikt voor een vergelijkende analyse waarin ik heb gekeken naar het mogelijke effect van watertemperatuur op zwemgedrag (Hoofdstuk 4).

Uit de analyse van de blootstellingsexperimenten bleek dat, zowel tijdens seismische als heiactiviteiten, kabeljauw in het gebied bleven gedurende de blootstellingsperiode (Hoofdstuk 3 en 5). Ná de blootstelling aan seismisch geluid, verlieten de vissen het gebied wel eerder dan verwacht in vergelijking met twee referentiejaren (Hoofdstuk 3). Een soortgelijk effect was niet aanwezig na het heien van funderingen, zowel niet net na iedere heiperiode van enkele uren van één fundering als na de gehele periode van vier maanden (Hoofdstuk 5). Tijdens beide studies was de detectie van de individuele vissen beperkt tot het bereik van de ontvangststations. Hierdoor kon ik alleen vaststellen dat de vissen het gebied verlieten maar niet waar ze vervolgens naartoe zwommen.

Via triangulatie van de detecties van de viszenders, was het mogelijk om op hoge resolutie 2D posities van vissen te berekenen wanneer de gezenderde vissen aanwezig waren in het detectiegebied van de ontvangststations. Op basis hiervan bleek dat beide antropogene geluidsbronnen effect hebben op de beweging van de kabeljauw (Hoofdstuk 3 en 5). Tijdens de 3,5 dagen durende seismische exploratie bewogen de dieren verder weg van de dichtstbijzijnde turbine en waren ze minder actief. Daarnaast was hun dagelijkse activiteitscyclus beïnvloed (Hoofdstuk 3). Wanneer er geen seismisch geluid was waren de vissen extra actief tijdens schemering, en wanneer er wel seismisch geluid was waren ze juist inactief tijdens deze periodes (Hoofdstuk 3). De kabeljauwen bevonden zich tijden het heien juist dichter bij de dichtstbijzijnde turbine dan voor het heien begon en ze waren dichterbij de geluidsbron voordat het heien begon dan tijdens of na de blootstelling (Hoofdstuk 5). Beide geluidsbronnen hadden dus effect op de beweging van kabeljauw hoewel de reacties van de vissen anders waren.

Naast geluid zijn er natuurlijk nog andere externe factoren die van invloed kunnen zijn op het gedrag van vissen, zoals temperatuur, het weer, $C0_2$ niveaus in het water en vervuiling. Het is daarom belangrijk om niet alleen naar het effect van één factor te kijken maar ook naar het gezamenlijk effect en de interactie van verschillende factoren. Het jaar van het seismische exploratie experiment, 2018, was een extreem warm jaar: de watertemperatuur kwam boven de 20°C uit. Om het mogelijke effect hiervan op de aanwezigheid van kabeljauw in het gebied beter te begrijpen, werd een extra analyse uitgevoerd waarin de aanwezigheid van kabeljauw in 2018 is vergeleken met die van 2020, ook een jaar met extreem warme temperaturen (Hoofdstuk 4). Hieruit bleek echter dat temperatuur niet de verklarende factor was voor het moment waarop kabeljauw het gebied verliet. Desalniettemin is het belangrijk rekening te houden met de interactie van verschillende externe factoren, zeker in het Antropoceen, waarin wij als mensheid veel verschilde stressoren hebben toegevoegd aan de omgeving van vis.

Uit de resultaten van de twee geluidsblootstellingstudies blijkt dus dat het gedrag van kabeljauw in een windpark voor de Belgische kust, wordt beïnvloed door zowel seismisch- als heigeluid. Daarnaast bleek ook dat de vissen het gebied eerder verlieten ná een 3.5 dagen durende seismische survey en dat verhoogde watertemperatuur hierop niet van invloed was. De Noordzee is een intensief gebruikt gebied vol menselijke activiteit. Het is niet waarschijnlijk dat deze activiteit de komende jaren zal afnemen, wat betekent dat ook het geluid wat hierbij wordt geproduceerd niet snel zal verminderen. De dieren die in de Noordzee leven zullen dus waarschijnlijk blootgesteld blijven aan een kakafonie aan menselijke geluiden. Daarnaast is hun én onze omgeving, door klimaatverandering, snel aan het veranderen wat ook weer effect kan hebben op hoe geluid wordt ervaren. Technologie ontwikkelt zich op het moment snel en akoestische zenders worden steeds kleiner met langere batterijduur. Dit is daarom hét moment om meer informatie te verzamelen, zoals die in dit proefschrift, over het effect van verschillende antropogene geluiden op verschillende vissoorten. Alleen met een goed onderbouwde wetenschappelijke basis kunnen weloverwogen beslissingen worden genomen voor beheer en behoud van soorten en habitat, zodat we het onderwaterleven ook voor toekomstige (vis)generaties kunnen veiligstellen.

Acknowledgments

How do you decide to start a PhD on fish and sound? For me this journey started when I got the chance to do field work on just this topic in British Colombia. Thank you Rob for giving me a job without ever having met in person and introducing me to the wonderful world of fish and underwater sound.

My journey continued in Gent and Leiden, where I started my PhD in 2017 on the PCAD4Cod project together with two other PhD students. During my time as a PhD here (and afterwards), I could always turn to my supervisors, Jan R, Tom, Hans and Carel, whenever I wanted to discuss fieldwork, data, planning, writing, or any general life questions. Both the biology groups at Gent and Leiden University made me feel at home and there was always time for a coffee or an in-depth discussion. I want to specially thank, Pieterjan & Jolien, Floor, Annebelle, Judith, James, Peter, Temp, Jing, Ning, and of course Jeroen, my cod buddy! You have all been an amazing support throughout the years. A big thanks to Erwin, my unofficial supervisor, you were always in for a chat, serious or not, about fish, diving, sharks, the sea, and on who's fresh insights on the data interpretation and visualisation I could always count.

Field work would not have been possible without the fantastic crew of the Simon Stevin and the support from Jan V. at VLIZ. It was always a pleasure to go out with you to sea, in both good and bad weather, and make the very most of the time we had to get everything done. Thanks to Yoeri, Sofie, Olvin and Pieke from WMR for helping me catch (or more often not catch) cod. Data analysis would not have been possible without Len, who hosted Jeroen, James and me and helped us finding our way through the mountains of fish position data and analysis methods we could use to find out what it all meant. Thanks to Michael and Bruce, who patiently explained the concepts of sound and propagation.

Of course my life in Belgium would not been half as good without Loes. You were my home in Gent and my Belgian sister. Thank you for being there and for wanting to be my paranymph. Thanks to my friends who I could call when I needed to talk and who's questions changed from 'when is your defence?' into 'how is the thesis going?'. Lisa, thank you for your support and for being my paranymph.

My mom and dad, thank you for always having my back. When it got a little too much, I could come home for some tea (or wine) in the garden, good food and a warm bath, to get me back on my feet. My brothers and my extended Hoffer-family all supported me so much during these years. And of course, Emiel, you have simply been amazing throughout the entire process. You have shared my dreams but reminded me that there is also a life out there. I can take on any future journey with you.

Curriculum Vitae

Inge van der Knaap was born in Odijk, the Netherlands, on May 17th 1989 to parents Gina and Jos van der Knaap. She completed a Biology Bachelor at the Radboud University Nijmegen in 2012 after which she started an Erasmus Mundus master in Marine Biodiversity and Conservation. This master program led her to study one year at the University of Algarve in Faro (Portugal 2012-2013) and one year at Galway Mayo Institute of Technology in Galway (Ireland 2013-2014). For her master thesis, she worked together with Dr. Rob Williams on the effects of boat noise on fish. She performed two field seasons at the Salmon Coast research Station (Broughton Archipelago, British Colombia), where she investigated the effects of noise from pleasure boats on juvenile salmon and herring. After completing her master in 2014, she worked for one year at the Pelagic Freezer trawler Association as a fisheries researcher where she gathered fisheries catch data from the pelagic fishing industry on a.o. horse mackerel to help inform stock assessments. At that time Inge got interested in starting a PhD-project on the effects of humans on marine fish, which came together for her in the PCAD4Cod project, where she became one of the three PhD students between 2017-2021. The first two years of her PhD were conducted at Gent University (Belgium) in conjunction with Flanders Marine Institute (VLIZ) through which most of the fieldwork took place. During the second two years of the PhD-project, Inge was associated with the University of Leiden (the Netherlands), the place of her PhD-thesis defence. During her time in Gent, she got involved in a European COST action (2019-2023) connecting fish researchers and tracking systems to enlarge the European network of fish tracking scientists and data. Her role in this action is as one of the two Data-Management working group leaders, working toward data integration in a central database. Over the years, Inge has become increasingly interested in fish behaviour and the impact of different anthropogenic stressors, like noise. In 2021, she started a job at Van Hall Larenstein, University of Applied Sciences (the Netherlands), where she now works as a fish researcher investigating how human-made obstacles affect the migration of different diadromous fish species.

List of Publications

Reubens, J., Verhelst, P., **van der Knaap, I.,** Deneudt, K., Moens, T. and Hernandez, F. (2018). Environmental factors influence the detection probability in acoustic telemetry in a marine environment: results from a new setup. *Hydrobiologia* 1–14.

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