Population level consequences of seismic surveys on fish:

Final Progress Report, Phase II

"PCAD4Cod"

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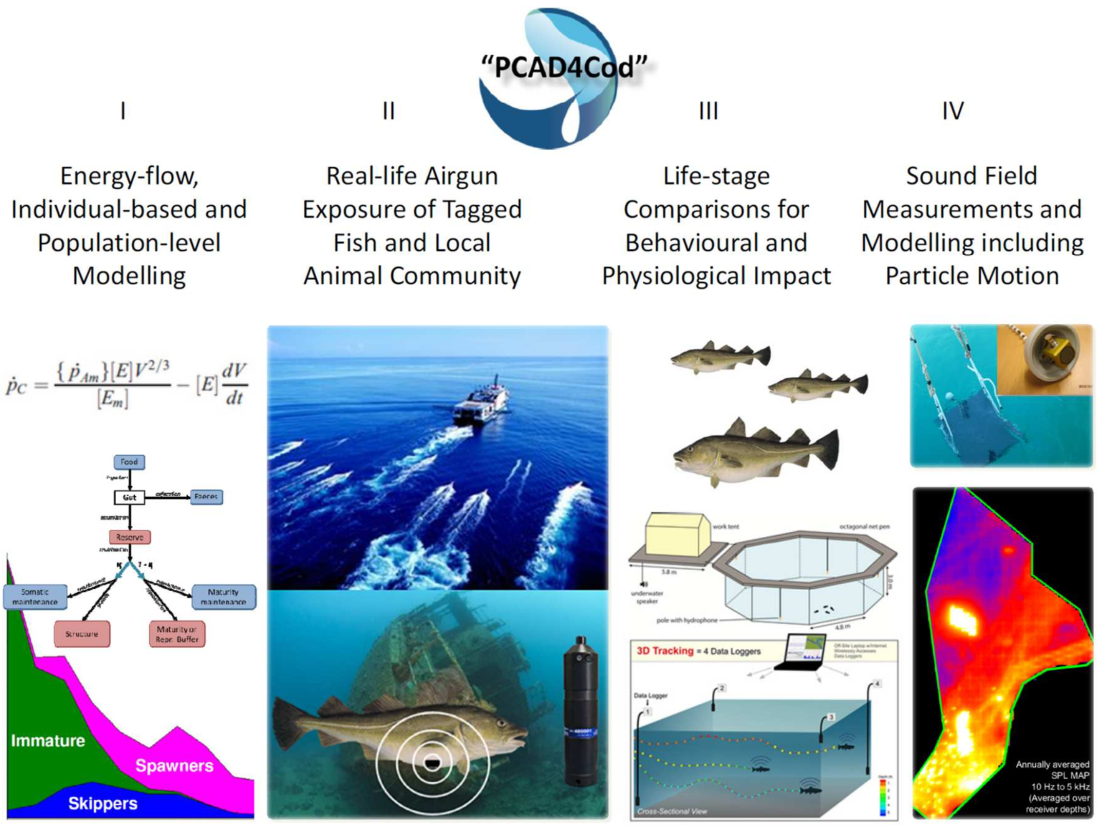
CONTENT

1. Project components with core people and their affiliations



I Modelling: Dynamic Energy Budget models: Floor Soudijn (WMR), Tobias van Kooten (WMR), and Andre de Roos (UVA); Individual Based Models: Lars Mortensen (DHI) and Frank Thomsen (DHI)

II Field work at sea: Jan Reubens (VLIZ) and Inge van der Knaap (GU), Dick de Haan (WMR), Benoit Berges (WMR) and Erwin Winter (WMR)

III Studies in captivity: Floating pen: Jeroen Hubert (IBL), James Campbell (IBL) and Hans Slabbekoorn (IBL); swim tunnel: Christian Tudorache (IBL) and Inge van der Knaap (GU)

IV Sound measurements and modelling: Peter Rogers (GT), James Martin (GT), James Campbell (IBL), Jeroen Hubert (IBL), Michael Ainslie (JASCO, Applied Sciences).

WMR = Wageningen Marine Research; UVA = University of Amsterdam; DHI = Danish Hydraulic Institute; VLIZ = Flandres Marine Institute; GU = Ghent University; IBL = Institute of Biology, Leiden University; GT = Georgia Tech; JASCO = Joint Assault Signal Company.

2. Summary of activities and achievements

2-0 General overview

Overview core work periods, mile stones, and plans:

Year 1

	JIP-approval of two-year project proposal
2017-Q2	Kick-off meeting
2017-Q3	
2017-Q4	Interim progress report
	Presentation IOGP-One Ocean workshop, St. Johns, New Foundland
	Presentation EAE, Annual Ethological Conference, Salvador, Brazil
2018-Q1	Q&A process Interim progress report
	SPAWNSEIS kick-off meeting Bergen, Norway

Year 2

2018-Q2	JIP-approval for 2nd year programme
2018-Q3	Presentation ESOMM-JIP joint meeting, The Hague, the Netherlands
	Presentation IAGC-IOGP joint HSSE forum, Stavanger, Norway
	Presentation NVG, Behavioural Biology Meeting, Egmond, the Netherlands
2018-Q4	First draft final report
2019-Q1	Q&A process final report
	Closing meeting
2019-2021	Further data processing and publishing in the peer-reviewed literature

Introduction

We began the PCAD4Cod project on the first of April 2017. We started the first year with modelling explorations for a top-down approach with the Dynamic Energy Budget (DEB) approach and theoretical explorations for an application of the individual-based modelling (IBM) approach. We also conducted a pilot experiment in the Belgian North Sea with tagging cod and trying out two designs for receiver stations around windfarm turbines. We gathered data on spatial behaviour, acceleration, and depth on a pilot sample of long-term data of 7 tagged cod and confirmed operational procedures and settings. We also completed a full series of behavioural observations on double-tagged cod in a net pen in the Dutch Jacoba harbour aiming at assessing behavioural variation data for accelerometer measurements. We also conducted an exposure experiment on crab and shrimp (cod food) foraging behaviour in the Jacoba harbour. Finally, we made a series of acoustic measurements in the Jacoba harbour to assess acoustic exposure conditions in the net pen and to better understand the acoustic world of fishes.

In the second year, we completed the DEB modelling and the results are written up in a first draft of a paper to be submitted to a peer-reviewed journal. We conducted a telemetric study with 57 tagged cod in the Belgian North Sea (yielding optimized data on presence and spatial data by triangulation of multiple receiver detections, activity data by accelerometers, and depth data by pressure recordings). The fish were followed for several weeks, including a period before, during and after three days of experimental seismic survey exposure for more than 30 individual cod. We failed in getting a spatial control data set in the Dutch North Sea, because of absence of sufficiently sized fish in the target area. All data on behaviour, sound, and abiotic conditions at the Belgian treatment site were collected and data processing is on its way on all aspects. We also started processing the net pen cod data and processed the foraging crabs and shrimps, which was written up, submitted and published in the peer-reviewed literature. Furthermore, we were unable to keep fish long enough in net pens in the Jacoba harbour to conduct a growth experiment, due to extreme temperatures in the summer. Instead, we completed complementary studies in (cooled) indoor basin conditions with video on natural foraging behaviour on cod (searching and eating crabs) and also succeeded in getting such data on an individual tagged with an accelerometer. Further acoustic measurements on seismic pulse recordings at various distances and sound propagation modelling efforts have taken place and are still in process to gain better understanding of the perceptual experience of fish during a seismic survey.

Planning and outreach

We are currently in full swing in processing data and integrating results and insights. This process is a long-lasting one, due to the amount of data collected and due to the fact that several basic processing and statistical procedures still have to be developed. All data will be processed and explored to answer all our questions to serve as core data to a series of papers to be written and submitted to the peer-reviewed literature in 2019 and 2020. During this period, all three PhD-students remain dedicated to the project, as well as the essential supervising staff. The contracts between IOGP and WMR and between IOGP and the VLIZ can end according to schedule. However, for administrative reasons, we propose to extend the contract end date between the IOGP and Leiden University until 30 June 2020. This will keep the IOGP in the loop for all progress in processing and publishing and allows the PCAD4Cod team to complete their scientific job.

The PCAD4Cod overall coordinator and spokesman, Hans Slabbekoorn, presented the work in progress and rational behind design and plans in 2017 and 2018 at several IOGP/JIP related public events in Canada, Norway, and the Netherlands, as well as at behavioural meetings in Brazil and the Netherlands. The review paper of phase I of the project has now been published in Fish and Fisheries as: *Slabbekoorn et al. Population-level consequences of seismic surveys on fishes: an interdisciplinary challenge.* The review also included the flow-chart of the PCAD-model for fishes, and is thereby officially published in the peer-reviewed literature (Figure 1). Before we report on progress and plans in data processing and interpretation and publication of results for all four of the PCAD4Cod project components, we like to mention a recent publication from a group of Australian colleagues who published on a telemetric fish study as we conducted during an actual seismic survey (Bruce et al. 2018). It provides insight into the current state of the art and into the challenging conditions for such a study at sea (a detailed description can be found as supplement to this report).

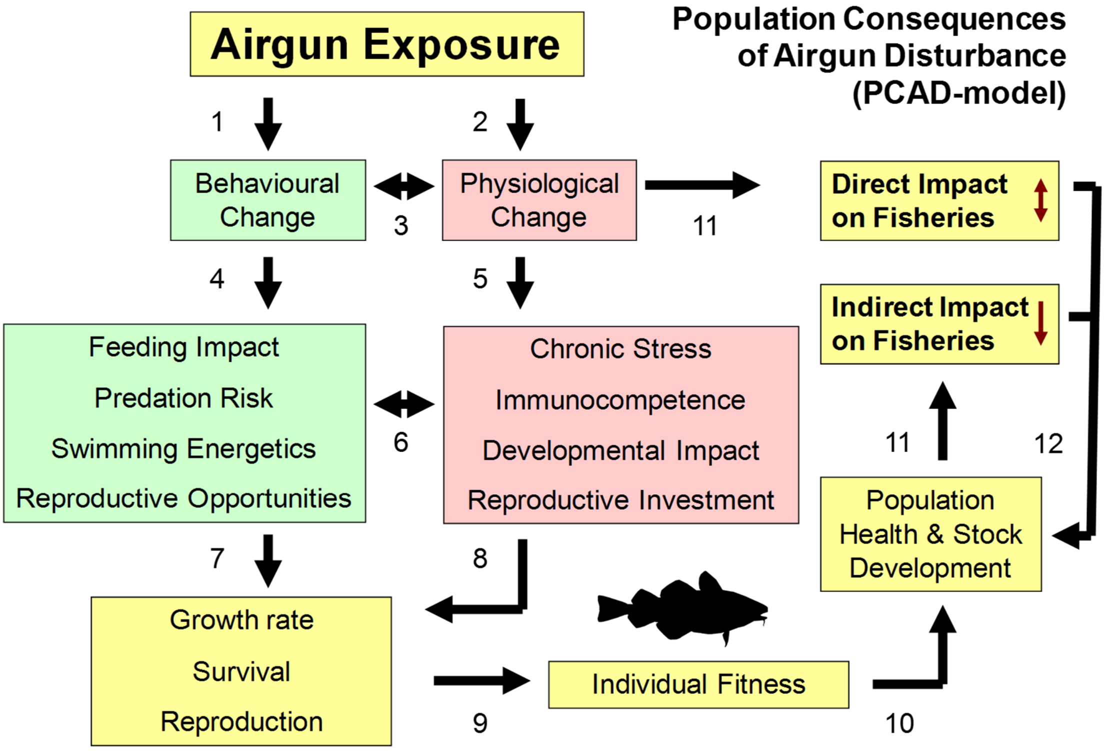


Figure 1: *A flow-chart of the Population Consequences of Acoustic Disturbance model concept tailored to airgun exposure of fishes (PCAD4Cod). Direct impact on fisheries, indicated in the top-right, is determined by positive or negative changes in catch rate due to fish movements during and after a seismic survey. Indirect impact on fisheries underneath is affected by behavioural (green) and physiological (pink) changes and their potentially negative effects for individual fitness, population health and stock development. Transfer functions that require critical evaluation are numbered 1-12 and explained in the paper). Note that individual fitness concerns life-time reproductive success which is the accumulation of vital rates at the individual level (growth, survival and reproduction). Furthermore, although transfer functions are depicted unidirectionally, the reversed pathway can also be relevant and important as population-level metrics such as abundance and shifts in predator-prey or competitor relationships may feedback from population to individual level (i.e. warranting bi-directional arrows) (Slabbekoorn et al. prepared for resubmission).*

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GENERAL NOTE ABOUT EXPERIMENTAL DESIGN

During the first phase of the PCAD4Cod-project, we stressed the importance of replication when addressing experimental design and global standardization of studies on the effect of seismic surveys on free-ranging aquatic animals, fishes in particular. We aimed for two replicates of two pairs of sites (one in Belgium and the Netherlands and the other one in Norway). Ideally we would get more replicates over a wider geographic range in diverse ecological settings. However, funding is an issue for experiments of this scale and we received funding for only one replicate pair. We therefore aim at getting replication in another way by global collaboration and to convince every other project to collect treatment and control site data in the same way as we planned. Due to unforeseen circumstances we were in the end not able to collect a proper spatial control ourselves and we here address in general terms what the consequences are for the current project and for the effort to get global replication.

We argued before and we still advocate that the treatment site (TS) and the control site (CS) should be as similar as possible in terms of ecology, time of sampling with respect to time of day, tidal fluctuations, season and year. Replicates pairs of both TS and CS are optimal replicates if they vary with respect to all of these. A proper CS site is as similar as possible to the TS, but also as independent as possible and therefore out of acoustic reach of the exposure (below ambient or inaudible to the target species at the CS). The CS should also be beyond a zone of ecological impact of the TS, meaning that there should for example be no fish arriving at CS after moving away from TS in response to the exposure (which will depend on target species).

In the current achievement of PCAD4Cod, we now lack the spatial control. However, the (unexpectedly large) number of fish tagged and yielding data provide sufficient replication for testing the current case of yes or no impact for this exposure, these fish, and this place. The lack of a spatial control makes our contribution to the global accumulation of data less than we would have liked, but has for the current project no serious consequences. The latter conclusion is also related to the lack of large numbers of fish leaving the treatment area and the lack of dramatic changes in behaviour. If these would have occurred a spatial control in which this not happened would have been more important. The current treatment site was the same as used before by Jan Reubens and for the 2017 pilot sampling, which does provide a temporal control, which will be integrated into the analyses. The successful exposure and number of tagged, free-ranging fish followed for an extensive period of weeks, overlapping with the days of exposure is unique and unprecedented, and will be a major step forward in our understanding of potential impact.

2-Ia Dynamic Energy Budget Model

Overview core work periods, mile stones, and plans:

Year 1

2017-Q2	Participation kick-off meeting (Tobias van Kooten and André de Roos)
2017-Q3	Start postdoc contract (Floor Soudijn) Setting up model
2017-Q4	Setting up model
2018-Q1	Testing methodology Initial model analysis

Year 2

2018-Q2	Participation kick-off meeting (Tobias van Kooten, André de Roos, Floor Soudijn) Model parameterization North Sea Cod
2018-Q3	Model parameterization North Sea Cod Model validation and analyses
2018-Q4	Model analyses and writing manuscript
2019-Q1	Finalize manuscript

Expected manuscript: Soudijn et al. Population level effects of acoustic disturbance in North Sea cod. – May 2019

F.H. Soudijn, H. Slabbekoorn, T. van Kooten, & A.M. de Roos

Bioenergetic modelling for PCAD4Cod

The effect of seismic airgun exposure on Atlantic cod is unclear and understudied. Seismic surveys may affect the North Sea cod population through a myriad of factors. It has been argued that sound disturbance may cause: stress, lower detection of prey and predators, changes in mating behaviour, changes in swimming behaviour, displacement from foraging grounds and displacement from spawning areas in marine animals (Cox et al. 2018, Slabbekoorn et al. 2010). It is unclear which of these factors has the strongest effect on the population level. A so-called top-down approach using a population model allows for a comparison of the consequences of these effects on the population level. This aids the decisions in prioritizing areas of further research of individual level effects of seismic survey exposure. Moreover, the effect of disturbances is usually defined at the individual level. For policy, the population level effect of disturbances is often relevant. A population model allows for the extrapolation of individual level effects to the population level (Kooijman, 2010; de Roos & Persson 2013; Martin *et al.* 2013).

In this study, we developed a life history model for North Sea cod (*Gadus morhua*) based on the model previously described by van Leeuwen *et al.* (2013). The model specifies relationships between feeding, energetic expenditure, growth, reproduction and mortality (Figure 2). The model considers size-dependent processes for energy uptake and energy use and is parameterized using individual-level data of North Sea cod. The model is validated based on field data of growth patterns and reproductive output of North Sea cod. We consider four potential effects of seismic surveys: 1. increased mortality, 2. lower reproductive success, 3. lower food intake, and 4. higher energetic costs. Using the model, we did a sensitivity analysis of the population growth rate and fisheries yield to changes in these four processes.

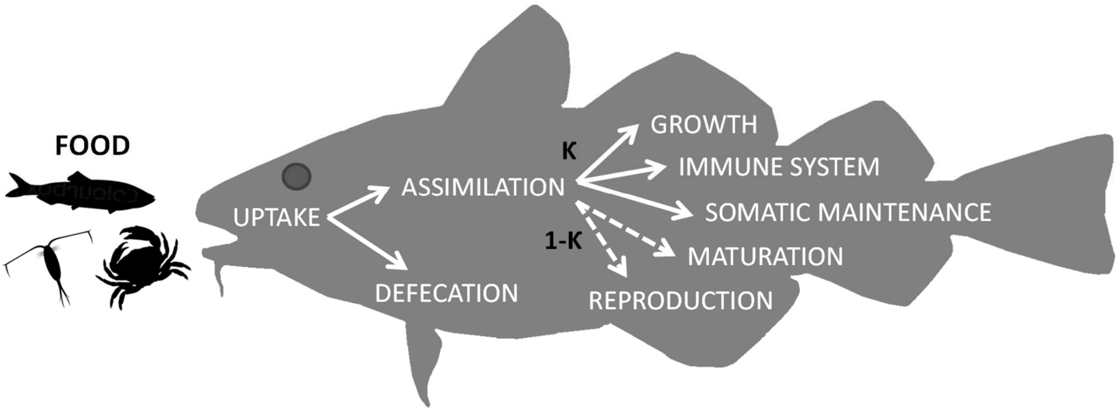

Figure 2: Structure of the Dynamic Energy Budget (DEB) framework. Food is assimilated into an energy reserve pool, from which a fixed proportion (Ƙ) is spent on growth, immune system and somatic maintenance, and deducted from the total (1- Ƙ) to get the energy available for maturation and reproduction (modified from Martin *et al.* 2013).

Summary of the results

The length-age trajectories that the life history model predicts are a good match to observed growth patterns of Atlantic cod in the North Sea (Figure 3). The high feeding level represents a situation of growth that is not limited by food availability. The growth trajectory that the model predicts for this feeding level falls within the upper range of observed growth patterns in the wild. The intermediate and low feeding levels are chosen such that they match the range of observed growth patterns. The cod population is relatively insensitive to changes in mortality and reproductive success (Figure 4). On the other hand, changes in food intake and energetic costs have a strong effect on the population. Both population resilience (population growth rate) and fisheries yield decrease significantly with an increase of energetic costs or decrease of food intake (Figure 4). Our study indicates that changes in for example foraging efficiency and swimming behaviour may impact population resilience and fisheries yield, if they result in a structural decrease in food intake or increase of energetic costs. The exact effect of seismic surveys on fish behaviour remains so far unresolved. Our results indicate that behavioural effects related to food intake and energetic costs have the strongest impact on the population level. These type of effects should be further investigated in empirical studies to allow for an assessment of the effect of seismic surveys on cod.

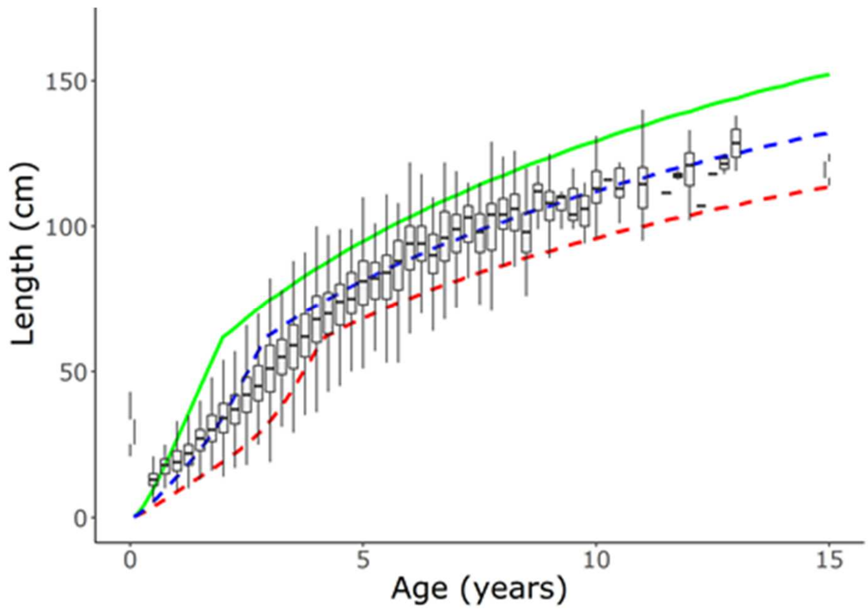

Figure 3: *Boxplot of length at age of North Sea cod from 1970-2016 (IBTS data) and length at age in the model output for three feeding levels (high, green; intermediate, blue; low, red).*

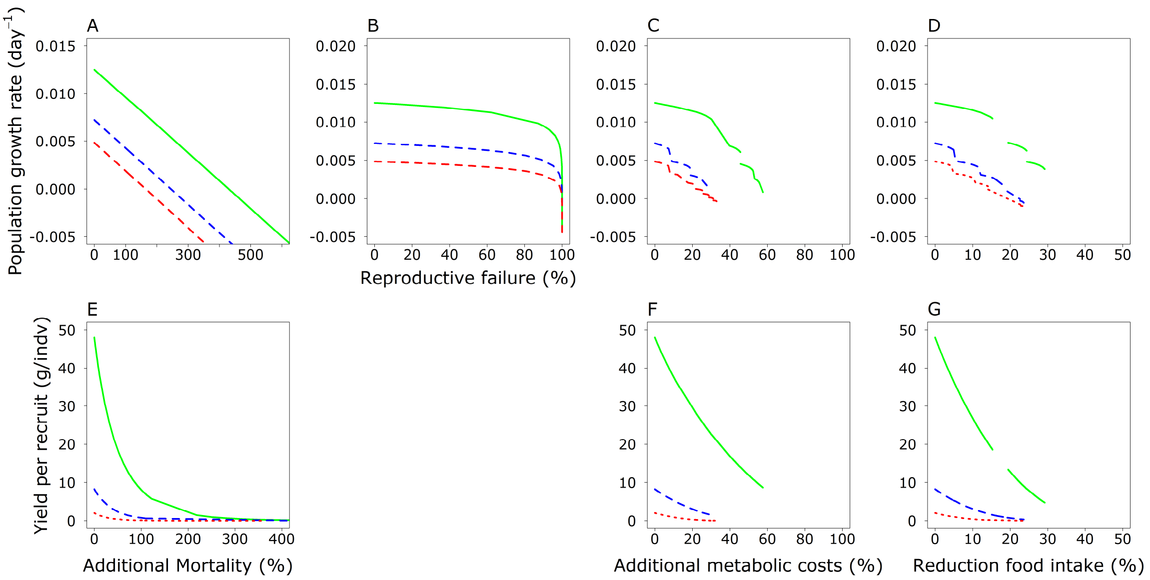

Figure 4: *Effect of acoustic disturbance for three feeding levels (high, green; intermediate, blue; low, red). The population growth rate is plotted as a function of additional mortality (A), reproductive failure (B), additional metabolic costs (C) and a reduction in food intake (D). Expected yield per recruit is plotted as a function of additional mortality (E), additional metabolic costs (F) and a reduction in food intake (G).*

To an integrated assessment

The follow-up of these results, reported above and the content of our first manuscript, would include a further integration with data from the field that are currently not available yet. We therefore outlined a 'wishlist' with data sets and processing steps needed for a more bottom-up population level assessment paper. We would perhaps have to adjust the current model slightly to allow for seasonal fluctuations in seismic exposure and food availability. This second model will require a new contract and more funding.

1. Individual level effects of seismic surveys on feeding and metabolism (and/or growth patterns).

2. Estimate of exposure level of North Sea cod population to seismic surveys, on both a temporal and spatial scale.

3. Combine 1 and 2 with the population model we have developed to estimate population level effects of seismic surveys in the North Sea on North Sea cod.

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2-Ib Individual based model

Overview core work periods, mile stones, and plans:

Year 1

2017-Q2	Participation in kick-off meeting (Frank Thomsen and Magda Chudzinska)
2017-Q3	Development of conceptual approach to insert ABM into the PCAD framework (Magda Chudzinska)
2017-Q4	Further processing of ideas and development of model framework
2018-Q1	Follow-up meeting in Leiden on the possibility of integrating ABM with the dynamic energy budget model (Lars Mortensen)

Year 2

2018-Q2	Participation in progress meeting 12-13 April 2018 (Frank Thomsen and Lars Mortensen)
2018-Q3	Development of conceptual ABM on cod behaviour in relation to noise impacts. Outlining manuscript draft on ABM modelling within PCAD framework
2018-Q4	Report input
2019-Q1	Finalize manuscript

Expected manuscript: Mortensen et al. ABM-approach to evaluate cod response patterns and population level consequences of seismic surveys – March 2019

L. Mortensen, M. E. Chudzinska, H. Slabbekoorn, & F. Thomsen

Advisory role of DHI

DHI has provided the PCAD4Cod project with senior advice on agent-based modelling during the two years the project has been in operation. The advice has primarily been given at meetings, through e-mail and conference calls. Additionally, a concept note on the possibility of integrating Individual or Agent Based Models (IBM or ABM) into the PCAD framework has been developed (Figure 5), along with a draft manuscript on the benefits of ABM in PCAD. The DHI input into the kick-off meeting was twofold and in line with the agreed tasks. First, DHI participated actively in the discussion about the planned field experiment (data collection and data analysis of the airgun response study). DHI also presented some results from previous experiment exposing cod and sole to pile driving sounds (COWRIE study; see Mueller-Blenkle et al. 2010). This discussion was relevant as the COWRIE study used acoustic tracking of the fish similar to the planned experiments in PCAD4Cod. Second, DHI discussed the possibility to apply the ABM approach to gain information on the spatial redistribution of the agents due to airgun sounds exposure.

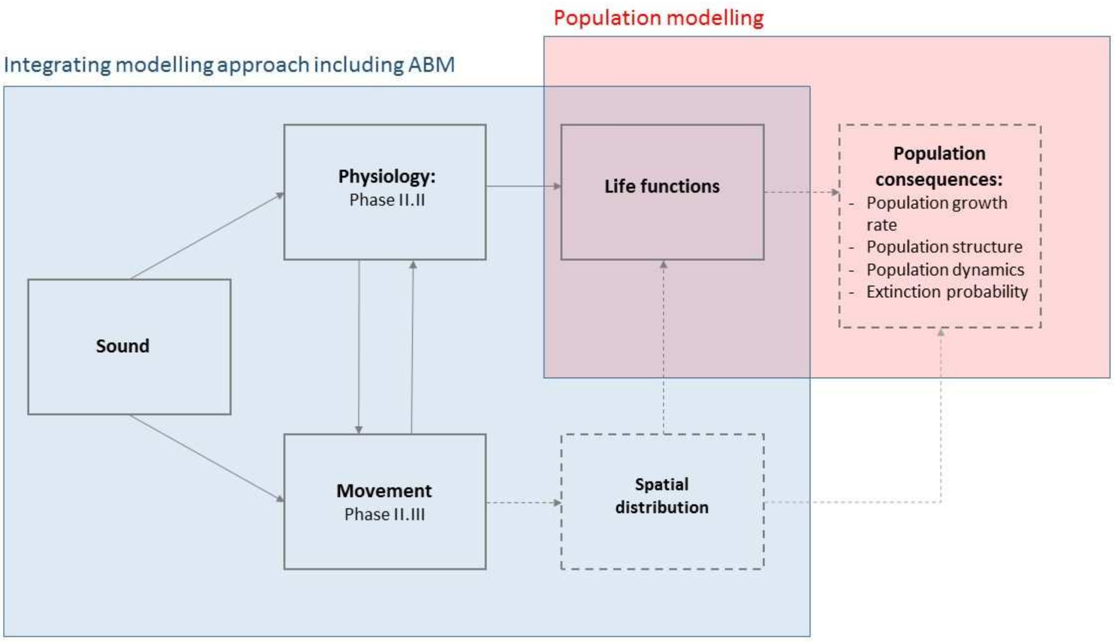

Figure 5: *Schematic overview of the integratotion of ABM and population modelling into the PCAD4Cod framework.*

Agent based models (ABM) are currently the best way to estimate impacts on groups of individuals and the translation of individual impacts to population level impacts (e.g. Scheffer et al. 1995; McLane et al. 2011; Sibly et al. 2013). The complexities of ecosystems are viewed in ABMs as emergent properties from variation in individual's properties and the dynamics between individuals. Thus, it is a so-called bottom-up approach, where the collective effect on individuals are raised to population effects. As ABMs also contain a spatial component, they allow for an estimation of spatial variation in effect. Thus, the benefit of using ABM to estimate population effects of noise is that it allows for a spatial quantification of effect, along with variation in effect among individuals.

Towards a conceptual paper

During an extra meeting in Leiden in January 2018, Lars Mortensen, Floor Soudijn, and Hans Slabbekoorn) discussed the possibility of integrating the dynamic energy budget model with potential output from an agent-based model to provide spatial content to the DEB model. Several methods were suggested, and it was agreed that using the ABM to estimate the proportion of the population affected by the seismic survey and the degree of impact, would be a plausible integration, adding extra benefits to the project. At the progress meeting in April 2018, DHI provided a talk on the possibilities for agent-based modelling in the context of the PCAD4Cod project. The talk started with a summary of the basic concepts of agent-based models and a subsequent review and discussion within the project team of how ABM could be integrated into the PCAD framework, using examples from a previous ABM study on mackerel (Heinänen et al. 2018).

The initial steps for developing and parametrising a cod model for the PCAD4Cod were taken. This entailed literature searches for research on cod behaviour and distribution data for habitat suitability index development and model verification (Humston et al. 2004; Righton et al. 2007; Neat et al. 2014; ICES 2014). Additionally, the conceptual basis for the model setup has been outlined and generally follows the approach used for the study on mackerel (Figure 6). The manuscript will provide insight into the potential for using ABM in the PCAD framework, based on a conceptual ABM of cod impacted by seismic surveys. The manuscript will contain an analysis of the theoretical output of the ABM aiming to quantify the benefits of for the PCAD. An initial draft of the manuscript was sent around to team members for feedback The revised draft is currently under production and is planned to be delivered at the end of the project, pending additional funds from within the project budget.

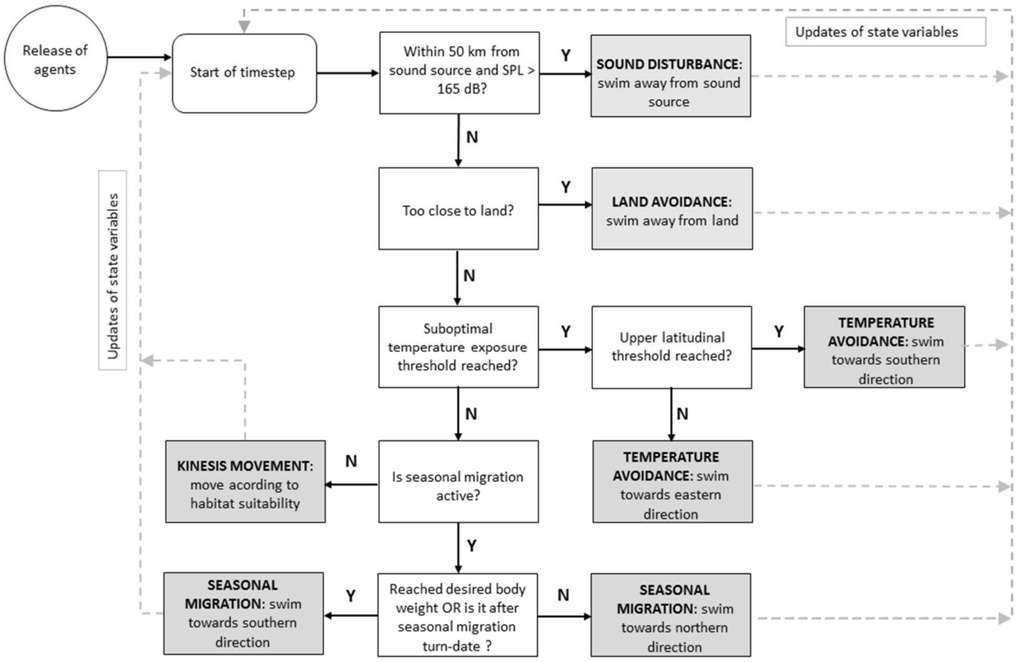

Figure 6: *Flow diagram describing general decisions of cod. Boxes with white background depict model evaluations made by each agent and with grey boxes depict resultant movement decisions (from Heinänen et al. 2018) .*

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ANSWERS TO ORIGINAL QUESTIONS I

Our main objective for this project component was to explore the potential for population level effects through top-down sensitivity analyses of vital rates with highest potential for detrimental impact from seismic survey sound exposure. Furthermore, realistic field data, critical to validate any model, would be integrated through bottom-up, energy-based modelling, individual-based extrapolation modelling, culminating in multi-trophic stock models. The top-down modelling has been completed, the bottom-up approach awaits the detailed field data. We therefore answered the postulated questions from the original proposal as far as possible at the moment:

1. How much behavioural or physiological effects are needed to negatively affect vital rates, such as growth, survival and reproduction, to such an extent that the population is affected?

ANSWER: Our top-down modelling approach does not allow a quantitative evaluation at this point, but indicated that additional mortality and effects on reproduction by seismic survey sounds are not realistic to expect to have any effect on cod populations. In contrast, energetic effects caused by seismic survey sounds through an impact on increased swimming activity and reduced food intake has the potential to affect population growth rate and warrants further investigation and future integration of field data of this kind into the model.

2. Can effects on prey or predator species and other community members modify the direct impact of seismic survey sounds on the target species?

ANSWER: Crabs and shrimps are prominent prey items for cod residing around scourbeds of wind turbines. In our experimental exposure experiment (MS01), we have shown that experimental sound exposure can affect the accumulation of both species at a prey item. The typical rise in crab numbers over time reduced under noisy conditions, while shrimps appeared to benefit from this and showed a pattern of noise-dependent increase in numbers, presumably by competitive release. The consequences of this kind of effect on the prey items or the cod predator are not investigated and not clear. These do not necessarily have to be negative for cod, but the data should raise awareness that moderate changes in acoustic conditions can cause shifts in species interactions with unknown consequences.

3. Are seismic survey related effects on vital rates and the population level robust and independent from fluctuations in weather, tide and other human activities?

ANSWER: Our top-down modelling approach did not address such fluctuations yet. Weather, tide, and other human activities may cause an elevation of ambient background noise levels to the exposure conditions of a seismic survey. We can currently not exclude that seismic survey effects on fish behaviour may be more severe through cumulative effects or less severe due to lower signal-to-noise ratios for the seismic exposure.

4. To what extent are potential effects determined by the spatial scale of a survey and the geographic area considered as habitat of the (meta-)population and how long-lasting are they?

ANSWER: Our top-down modelling approach does not depend on spatial scale and all interpretations apply independent of scale as it works with a proportion of the total being exposed and affected. However, spatial effects may obviously play a role. The work on individual based modelling may yield more insight about spatially explicit effects that may relate to the size and distribution of the population and the temporal and spatial scale of the seismic survey.

2-II Field work at sea

Overview core work periods, mile stones, and plans:

Year 1

2017-Q2	Identify possible telemetry positioning setups and setting options Order equipment
2017-Q3	Deployment of receiver for pilot VPS setup in Belwind (BEL) Tagging 27 Atlantic cod for pilot VPS in Belwind (BEL)
2017-Q4	Analysing pilot data
2018-Q1	Assessment of best receiver placement design for during seismic exposure Order extra equipment and tags

Year 2

2018-Q2	Deployment of receivers in Belwind (BEL) & Gemini (NL)
2018-Q3	Tagging of 57 Atlantic cod in Belwind (BEL) Catch failure at Gemini, switch to Belwind as exposure site Exposure to seismic survey at Belwind Delivery of Cruise report, MMO-report, and PAM-report
2018-Q4	Data recovery Data analysis and preliminary results
2019-Q1	Detailed results and first movement models (HMM)

Expected manuscripts: Van der Knaap et al. VPS receiver design: lessons learned from a pilot telemetry study in the North Sea VPS – March 2019; Van der Knaap et al. Telemetric data on behavioural responses of free-ranging cod during a seismic exposure experiment – Aug 2019; Kok et al. Echosounder potential for monitoring pelagic community changes before, during, and after anthropogenic noise exposure events – July 2019

I. van der Knaap, D. de Haan, E. Winter, L. Thomas, J. Campbell, J. Hubert, A. Kok, B. Berges, H. Slabbekoorn, J. Reubens.

Receiver array set-up and behavioural explorotion

We worked in the offshore wind farm ‘Belwind’ (51.670°N 2.802°E), located 46 kilometres off the coast of Zeebrugge in the Belgian part of the North Sea. The wind farm is made up of 55 monopole turbines (Figure 7) each with a 5 m diameter, filled with seawater, and surrounded by a rocky scour bed, measuring between 15-20 m across to protect the turbine base from erosion. The seabed surface in-between turbine scour beds, consists predominantly of fine-grained sand dune habitat and the area varies in depth between 15 – 37 m. Two arrays of receivers were deployed from July 4th until September 28th 2017 around two wind turbines: the northern F05 and the central C05 turbine. The setup around turbine F included 8 receivers, 6 placed in a circular shape with similar spacing, and 2 inner receivers at 50m from the turbine base. At turbine C, 10 receivers were placed in a triangular-grid configuration. The distance between receivers was based on a range test, performed by Reubens et al. (2018), in similar environmental conditions. Receivers were spaced at a maximum distance of 200 m from each other at which detection probability remained above 70%. We used VEMCO VR2AR (69kHz) receivers, with a built-in synctag and sensors monitoring the receiver tilt, water temperature, depth and environmental noise. Receivers were bottom moored using floats positioned ca 1 m above the hydrophone to avoid blocking of the hydrophones. No surface floats were used to avoid ship collisions.

In 2017, 27 Atlantic cod (size range 33 -43 cm, fork length), were caught and tagged to end up with 7 fish for which we harvested 10 to 30 days of continuous presence data (Figure 8). Fish were caught using hook and line from up to 30 m depth and reeled in slowly preventing barotrauma. Individuals were kept in a holding tank for observation. If fish displayed any sign of serious discomfort or abnormal behaviour (e.g. unable to keep buoyancy or swimming at the surface) they were excluded from the tagging process. Tagging took on average 5 minutes per fish and proceeded as follows. An individual was sedated in clove oil (0.03 ml/L). Upon losing buoyancy, the fish was placed in a holder on its back at a slight angle, keeping its mouth and gills submerged in oxygenated seawater. After scale removal, an incision (2 - 3 cm) was made on the ventral side through which the acoustic transmitter tag (Vemco V13AP) was slid into the abdominal cavity. The incision was closed using three sutures (monofilament). Next, fish were measured and additionally tagged with a T-bar flow tag in front of the dorsal fin for individual identification were the fish to be re-caught. After tagging, the animal was placed in a recovery tank. Upon resuming normal swimming behaviour, the cod was gently released close to the turbine at the catch site.

The V13AP transmitter tag includes a pressure and acceleration sensor, set to transmit at a ratio of 1:2. A transmission is a coded signal consisting of a unique tag identity code and sensor information measured by the tag (e.g., pressure or acceleration). Transmitters were divided in two groups, the first had a random transmission delay of between 40 - 80 s with an average of 60 s, and the second group a delay of between 30 - 60 s with an average of 45 s. As reference to their respective random delay times, the transmitter groups are referred to 60 s and 45 s transmitters respectively (Table 1). The duration of short transmission was 30 days. Therefore, per individual a maximum of 30 days of data on short delay could be collected. After these 30 days, all transmitters were programed to increase their random delay times to min 540 s and max 660 s making efficient use of the remaining battery life for detection beyond the scope of this study. First, 14 fish were tagged with 60 s transmitters. 30-48 days later, 13 fish were tagged with 45 s transmitters (table 1). To prevent signal collision with the 45 s transmitters, the 60 s transmitters were programmed to turn off for the duration of 60 days after the first 30 days of transmission. The data confirmed the suitability of this set-up to triangulate positions at high resolution and for long duration (Figure 9) and to assess detailed patterns of variation in activity over the day (Figure 10).

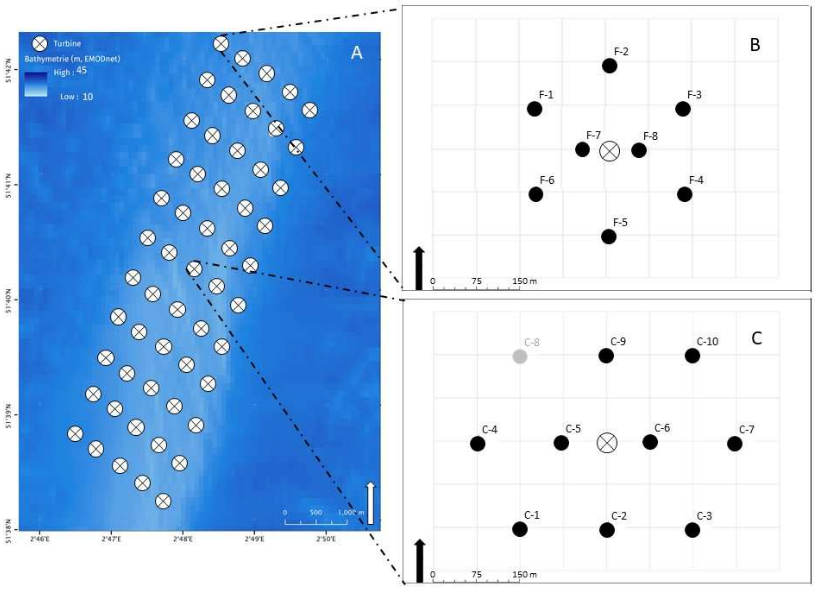

Figure 7: *Overview of wind turbine positions at Belwind Windpark in 2017 (a). Two receiver array setups at turbine F; circular grid (b) and turbine C; triangular grid (c). Receiver C-8 (grey) was lost on September 21st. Arrows indicate North, receiver depth varied between receivers from 18 m – 24 m.*

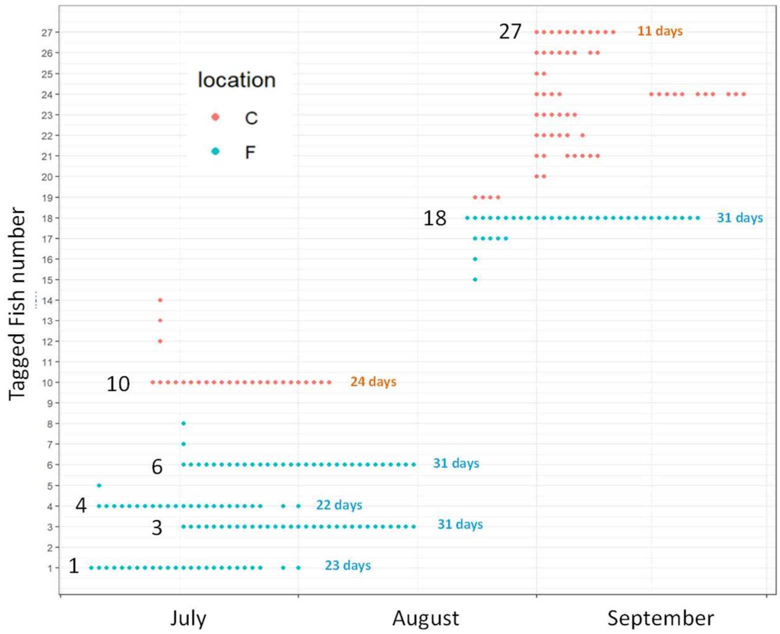

Figure 8: *Overview of tagged fish, tagging dates in 2017 and presence at any of the receivers around the two turbines at Belwind Windpark. Five cod in July/August and two in August/September were tracked for 10 days or more; five at turbine F (blue) and two at turbine C (red).*

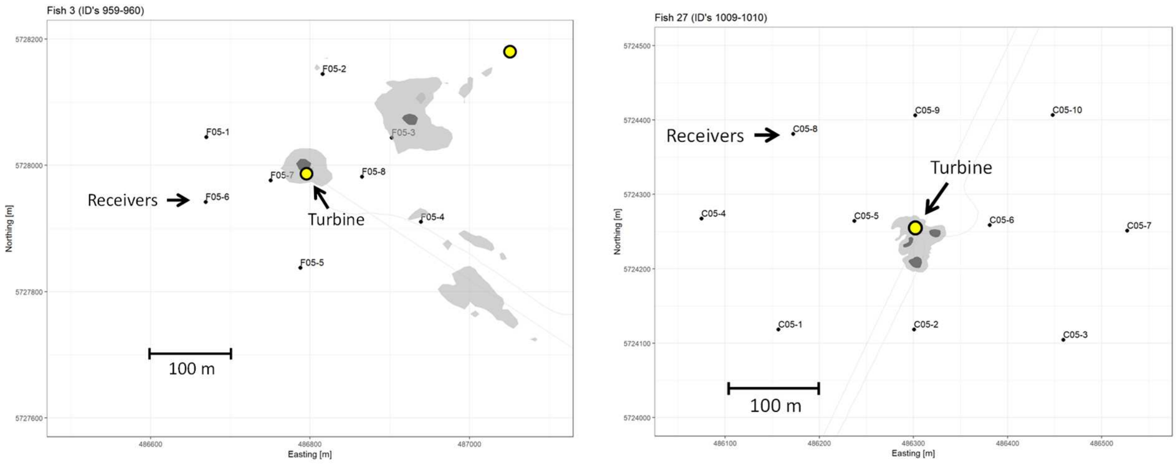

Figure 9: *Examples of successful triangulation of two individual fish: #3 at turbine F (31 days of data) and #27 at turbine C (11 days of data). Turbines are indicated with a yellow dot, receiver stations with a small black dot and identity number. The light shaded area indicates the area in which the fish is reported for 95% of the detections. The dark shaded area indicates the core area in which the fish resided for 50% of the time. Thin grey lines represent cables at the sea floor. Cod #3 had two favourite areas: around the turbine on the north side and about 120 meters north-east of that at a reef ball. Cod #27 remained within 50m of the turbine all the time and there seem to be three spots 20-45m apart at which it hangs out most.*

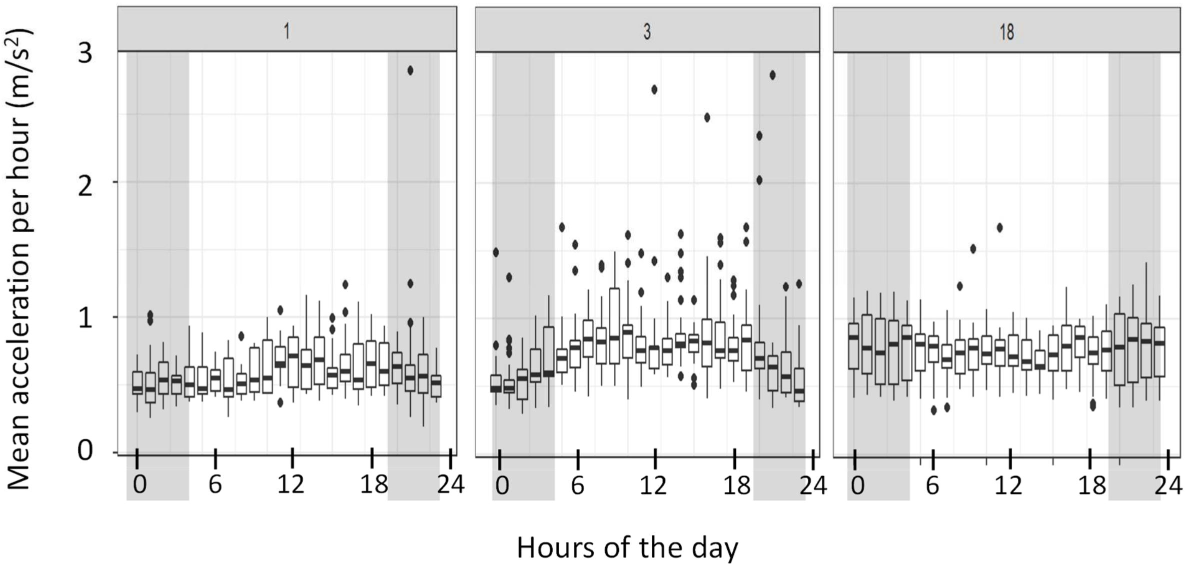

Figure 10: *Examples of fluctuations in activity level for three out of seven individual fish: #1 at turbine F (23 days of data); #3 at turbine F (31 days of data) and #18 at turbine C (31 days of data). The shaded areas indicate night time. Cod #1 shows variable data without a distinct pattern (like cod #27). Cod #3 has a gradual incline and decline over the day with many distinct outliers of high activity (like cod #6 and #10); Cod #18 seems to have similar median activity levels throughout the day, but more variability levels at night time (like cod #4).*

Seismic survey conditions and features

After the pilot season for cod telemetry and receiver placement around wind turbines in 2017, a full seismic survey was scheduled in coastal waters in 2018, originally with a treatment site at the Dutch Gemini wind farm and a control site at the Belgian Belwind wind farm. We had to cancel the spatial control at Gemini, due to failure of catching sufficiently large cod for tagging and therefore switched to the Belgian site as treatment location, with the data from 2017 and years before as long-term baseline data and the days and weeks before and after the seismic survey as their own control in time of the same site. We aimed at testing the effects of a seismic survey on the behaviour of free-ranging Atlantic cod using a controlled experimental exposure with a real-size seismic survey vessel, sailing in a pre-determined route relative to the fish tagging site at the Belwind wind farm and a predetermined shooting pattern, starting at long-range and passing by the tagging site at just over two kilometres (Figure 11).

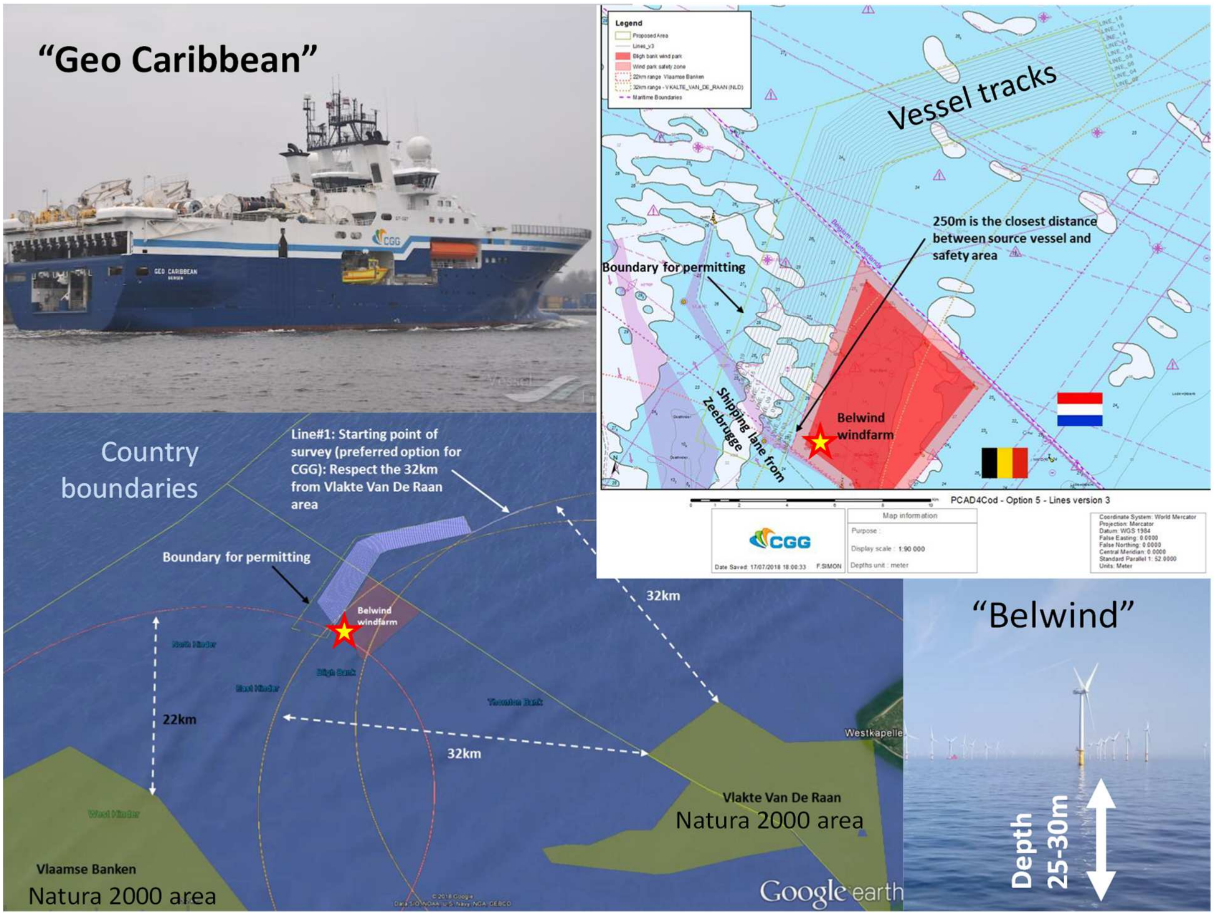

Figure 11: *The CGG seismic survey vessel, the "Geo Caribbean" and the location and details of the vessel track pattern, starting in coastal waters of the Netherlands and approaching the cod tagging site (red and yellow star) in the Belgian wind farm "Belwind". The parallel tracks were repeatedly crossing the Dutch-Belgian boundary and following a bended hockey stick track pattern.*

The CGG seismic survey vessel "*Geo Caribbean*" departed from Amsterdam on the 20th of July 2018 to the target site at the Dutch-Belgian boundaries. The seismic survey started on the 21th of July and ended on the 24th of July (see Table 1). The vessel used a set of 18 airguns in three sub-arrays. The experimental exposure concerned a total of 76 hours and 42 minutes of airgun activity, including 3 soft-starts and 2 source tests (Figure 12). Scheduled shooting interruptions of 1 or 2 minutes were carried out between transect lines. Ten slightly longer breaks (drop tests) of 4 to 6 minutes occurred on the northern loop ends to test air-pressure leakage with the source not firing. Furthermore, on two occasions the source had to be shut down briefly (less than 10 minutes) on entering a conservation exclusion zone (within 32 km from the Natura 2000 area "Vlakte van de Raan", The Netherlands).

In order to minimise operational impacts on marine mammals the JNCC (2017) mitigation measures were applied, using a dedicated JNCC trained Marine Mammal Observer. Watches were carried out throughout daylight hours and during hours of darkness, a passive acoustic monitoring system (PAM) was utilised to monitor vocal presence of marine mammals (see the Marine Mammal and Observers' (MMO) report and the PAM report). During the entire survey period, four marine mammal sightings were recorded in Dutch waters, all involving harbour porpoises (*Phocoena phocoena*). One involved an animal travelling slowly, one appeared to actively avoid the vessel, while two concerned decaying animals (cause of death unknown, but well before the onset of the seismic survey). The two living animals remained beyond 750 m from the seismic sound source and mitigation measures were not required.

Table 1: *Time table from the cruise report by Dick de Haan of the activities and operations related to the seismic vessel "Geo Caribbean" and its survey operations.*

Date	Time	Operation
20-07-2018	07:00	Kick-off meeting on board MV *GEO Caribbean.* Introduction participants and discussing the final survey plan and MMO procedures with ship's staff.
	10:00	Departure Amsterdam docks
21-07-2018	03:30	Primary Seismic source deployed. Start MMO mitigation, single cell test followed by a soft-start and full volume operation (2950 in^3).
	05:50	Start loop sequence line#01-#11
22-07-2018	00:00	Sequence LP-0312, 2nd repeat of line#01, LP-1501C
23-07-2018	00:00	Sequence 0001C
24-07-2018	00:00	Sequence LP-0619
24-07-2018	12:01	Single cell test secondary source and proceeding to full volume 2930in^3 operation towards line#0001D (fourth repeat).
	16:16	Final shot, equipment recovered and heading to IJmuiden, NL
25-07-2018	06:00	Sailing into the locks of Velsen.
	08:00	Arrival in Amsterdam at Alaska dock.

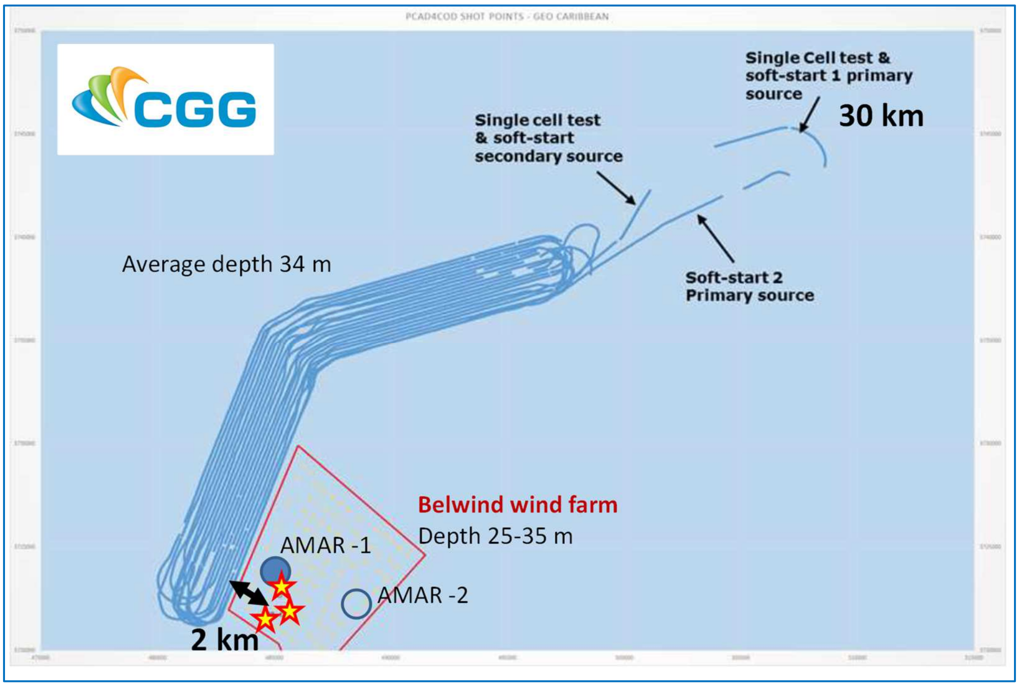

__Figure 12:__ Camera shot of the airgun array and Belwind wind farm (above) and map details of the vessel tracks and seismic pulse locations (below) relative to the three turbines (stars) with tagged fish and the AMAR recording stations of JASCO (AMAR-1 success; AMAR-2 failure).

Receiver deployment and tagging effort

Based on the results from the pilot study in 2017, in which both designs were capable of reporting typical spatial behaviour of cod around the turbines, we selected an optimal receiver placement design given the number of receivers available and increased the number of animals originally planned to be tagged in the exposure area. We thereby optimised detection probability and maximized the sample size. We also selected adjacent turbines and added three extra receivers, deployed by themselves, at three additional turbines locations, next to the turbines with each six receivers to detect more animals over a larger area (Figure 13). So, three turbines were provided with a full VPS setup (B10, C09 & B08), 6 receivers placed in a circle around the turbine at 150-250m apart from each other. At three other turbines surrounding the VPS turbines (C10, B09 & C08), we placed single receivers. All of the 21 receivers deployed, except for one of the single receivers (at turbine C08), could be recovered and generated data.

In 2018, well before the seismic survey, we caught cod of 35 cm or larger on six tagging days (24th to 27th of June and the 11th and 17th of July. In the end, a total of 57 individuals were caught, tagged and released, of which 52 were detected afterwards at our receiver stations at one of the five turbines (Figure 14). At the start of the survey, 32 of the 52 tagged cod were still present, of which 23 were tagged in June and 21 provide almost continuous presence data for more than three weeks prior to the exposure. For 16 individuals, we have presence for more than a week before, three days during, and more than a week after the exposure period. Of the 32 individuals present at the start, 28 had an almost continuous record of presence, and 26 of these stayed during and beyond the seismic survey, while two of these left (21, 48). Four individuals had a more scattered presence at one of the five turbines, and two of these stayed and also two left our detection range during the seismic survey (5, 15).

The presence data (Figure 14) indicates that most fish have a strong turbine fidelity (24 individuals out of 32 for which we have more than two weeks with more or less continuous presence), typically with brief visits to neighbouring turbines. The turbine of preference is often the turbine of capture and release. Only a rare individual seems to stick completely to a single turbine (11). Eight out of the 32 fish with a long record exhibit distinct lower turbine fidelity and make for example frequent switches among more than 2 turbines (e.g. 19, 56) or make a clear switch from one to another (6). Fifteen individuals have a relatively long continuous presence at one turbine and were then detected at another just before leaving our detection range, which provides some insight into the direction of leaving. Nine out of the 15 seem to have gone south (14, 18, 22, 29, 33, 34 36, 40, 41), three to the north (24, 28, 56), while two may have gone east (3, 4) and one west (39). This does not necessarily indicate a prominent dispersal direction, as from turbine B08, fish can only be detected to the south, and for B10, they can only be detected if they go north or east, while from C09, they could leave and be detected in any direction but east. Six of the nine south bound fishes came for example from turbine B08, from which there were also five other individuals that left with a similar trace record into unknown direction.

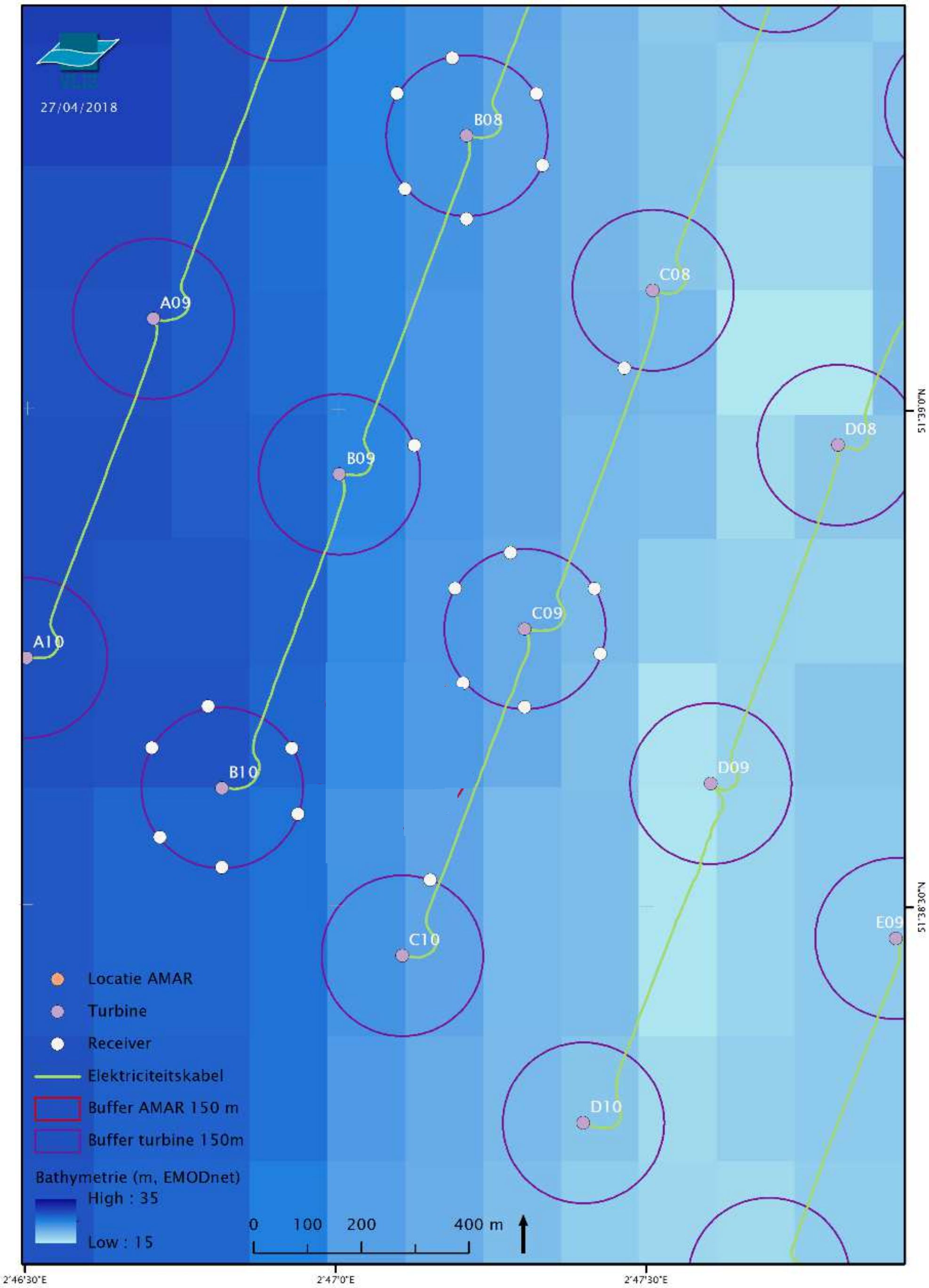

Figure 13: *Positions of the 21 acoustic receivers (VEMCO) at the exposure site in the Belwind wind park, in the Belgian part of the North Sea. Orange dots represent wind turbines, white dots indicate receivers and the purple circle is the 150m buffer zone from the turbine.*

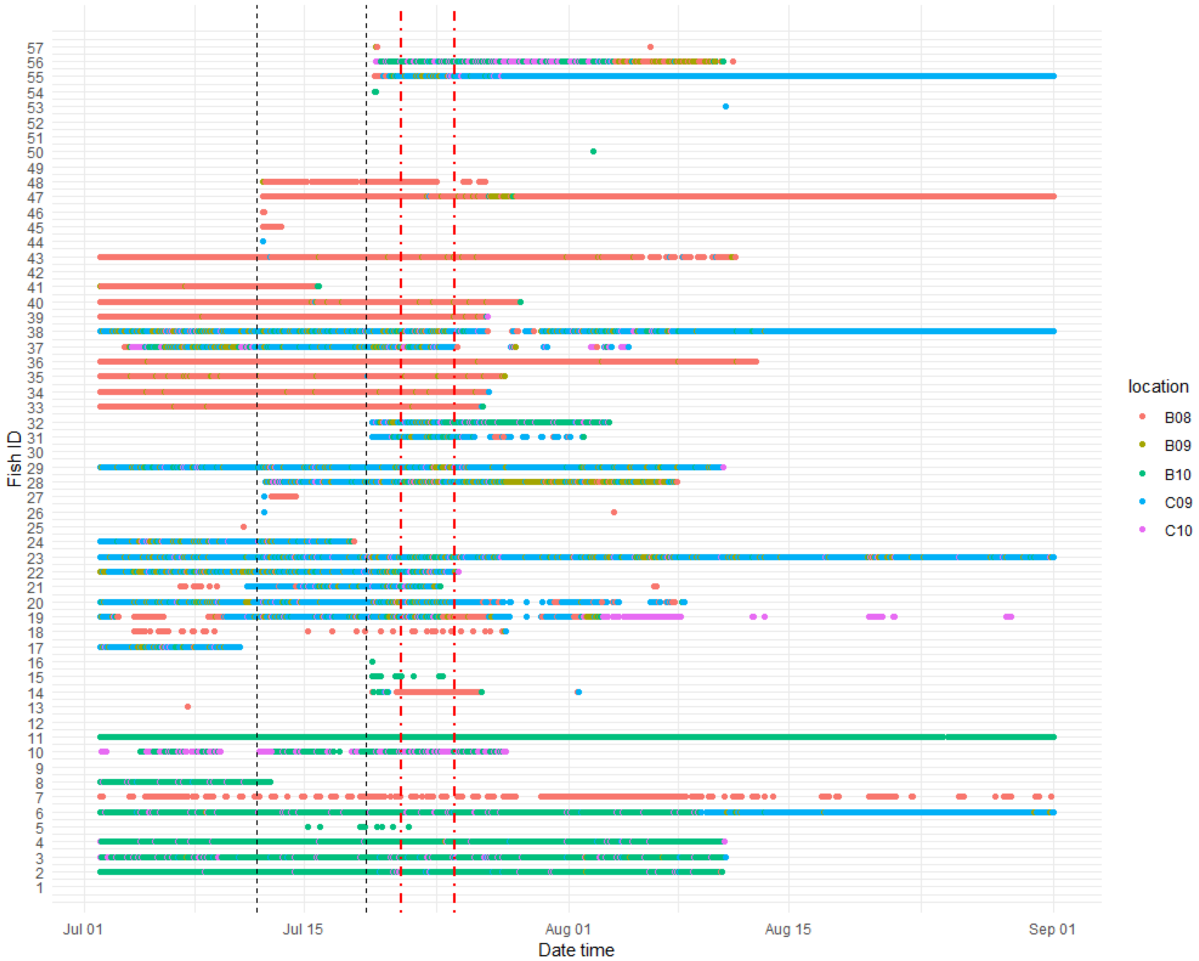

Figure 14: *Detection data overview per fish (individual fish identities are numbered from 1-57, from bottom to top, on the left), from the 1st of July 2018 until the 1st of September 2018. Vertical red dashed lines indicate the seismic survey period, while black dashed lines refer to the dates of tagging events. Colours of the horizontal lines indicate the turbine at which the fish were detected (green, blue and red are the three turbines with 6 receivers: B10, C09, and B08, and brown and pink are two of the originally three turbines (one receiver was accidentally released by a third person and got lost) at which we placed a single receiver: B09 and C10).*

Besides the information on presence, we can assess position of an individual if it is detected at multiple receivers at the same time, while the time series of position tracking allows for statistical reconstruction of swimming pathways. The electronic tags emitted an acoustic signal every 75 seconds. The signal considered either pressure data from which we derived depth information or acceleration data from which we derived insight into activity level. We are still in a preliminary phase of data processing, but provide a first look at what the data look like and describe the next steps to be taken in the data analyses. We have generated graphs for the % of emitted signals detected as a potential indicator for deterrence (Figure 15 & 16); for pressure as an indicator of vertical area use (Figure 17-19); and acceleration as an indicator for activity level and potentially for inferring behavioural categories (Figure 20-23). We also provide an example map of individual fish positions localized by triangulation from which the distance travelled (movement) over time can be calculated (Figure 24).

Detection

Using the % of emitted signals detected trough time, we can tell something about the presence of the animals within the detection area. In Figure 11, the percentage of signals that were detected per day up to 3 days before, during and after the survey, show reduced signal detection during the seismic period. To gain insight into the number of animals that stayed in the detection area after tagging, we plotted the percentage [%] of the individuals that were detected of the total number of tagged animals in the area at that moment (Figure 12). There is a gradual decline over time, as expected, and there does not seem to be a rise in animals disappearing from the area during or right after the seismic survey. There also was a larger percentage of animals remaining within the detection area as compared to last year's telemetry pilot data (Figure and also see "PCAD4Cod, Interim Progress report"). Data of last year will be included in further statistical analyses.

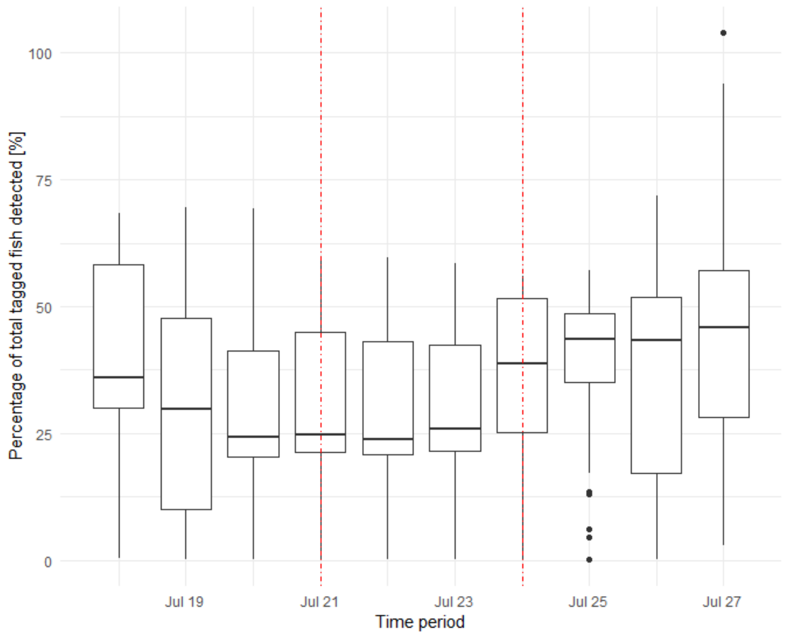

Figure 15: *Percentage [%] of tagged fish from which transmitted signals were received, by which continued presence at the site was confirmed, 3 days before, during and after the three-day seismic survey (21-24 July 2018). Vertical red dashed lines demarcate the exposure days in the centre of the figure.*

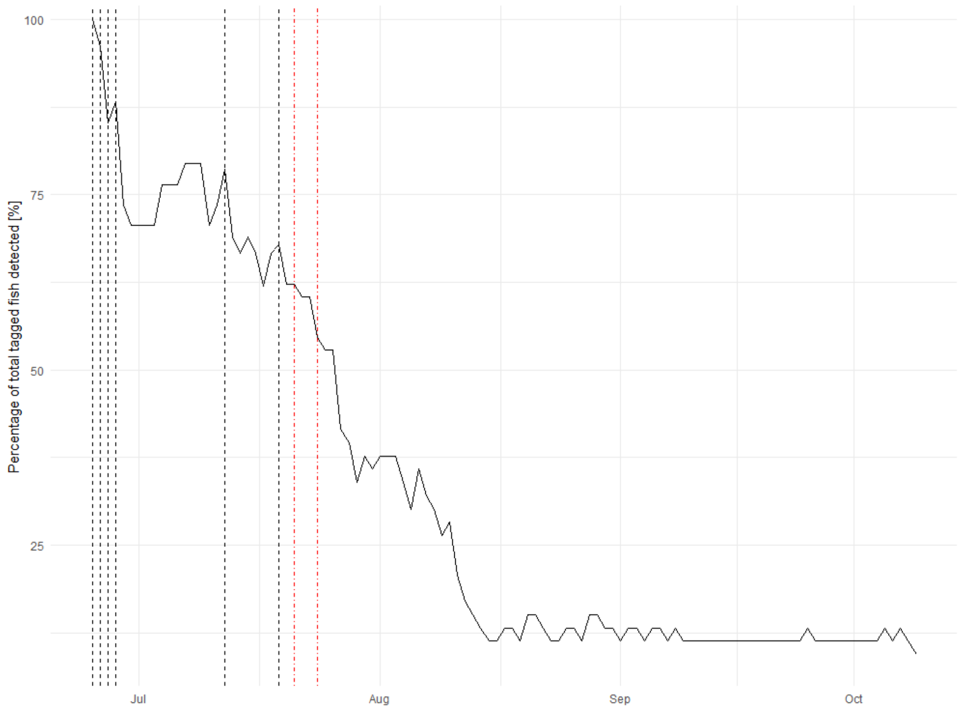

Figure 16: *Cumulative time series as overview for the percentage of tagged fish still detected at any one of the receivers. Vertical red dashed lines demarcate the exposure period again and vertical black dashed lines indicate the dates of tagging efforts.*

Pressure

Water depth fluctuates with the tide and varies among the turbines. This results in predictable and synchronized fluctuations in time as well as differences among individual fish dependent on the turbine, on top of which the variation in pressure data driven by individual swimming behaviour up and down the water column. To provide a first overview, values have been plotted for the three VPS turbines (Figure 14-16) for the time period of 18-27 July 2018. The tidal fluctuations are clearly visible and we will substract this pattern from the fluctuations in depth use per individual fish. Many fish show high similarity and synchrony in their depth fluctuations, with apparently little change during the survey. However, there are also some deviating patterns of individuals that suddenly go down towards the end or just after the survey (Figure 15).

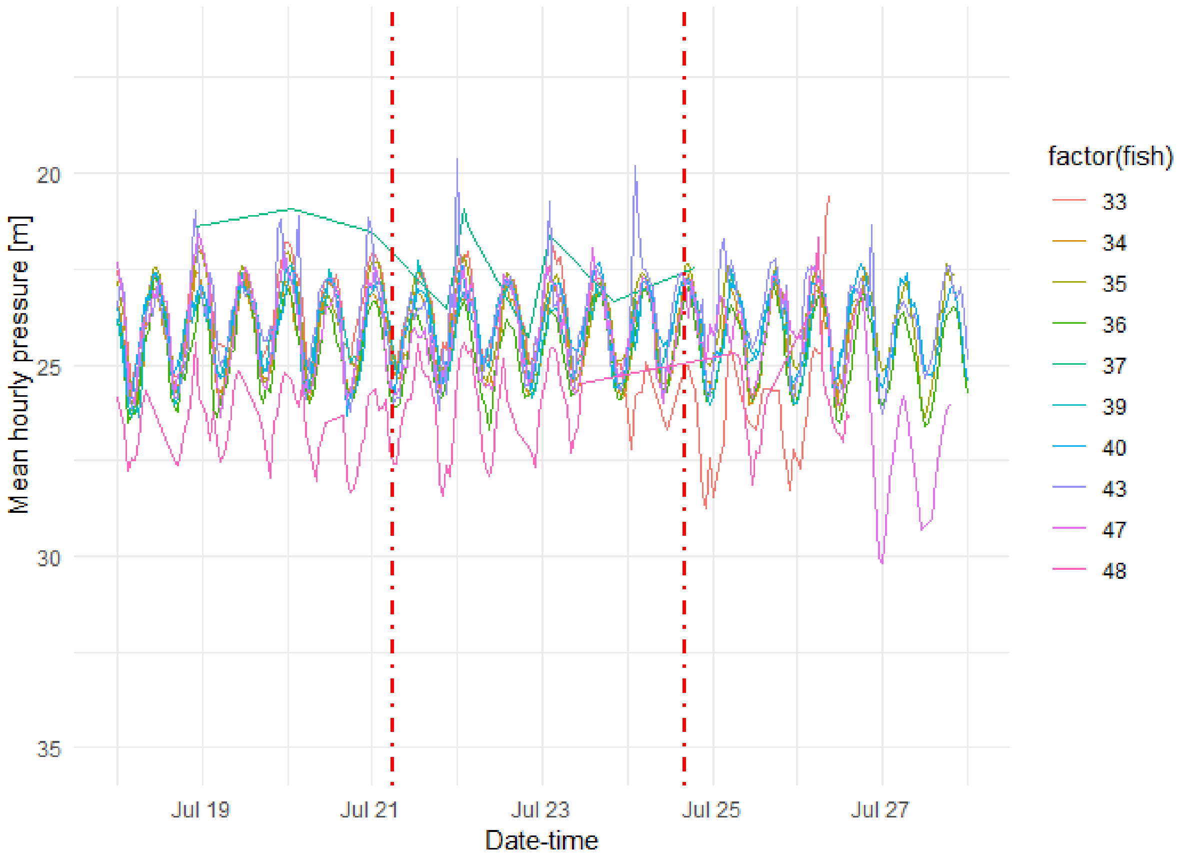

Figure 17: *Depth data based on pressure measurements transmitted by tags on individual fish. These data are from 10 individuals present at turbine B08 from 18-27 July, binned per hour. Individual fish are indicated by colours. The airguns at the seismic vessel were active during the three days between the vertical red dashed lines. General depth fluctuations reflect the tidal cycles. Note deviant swimming depth of fish 48, already before the seismic survey has started and change in patterns towards deeper water for fish 33 towards the end of the seismic survey and for fish 47 well after the seismic survey had stopped.*

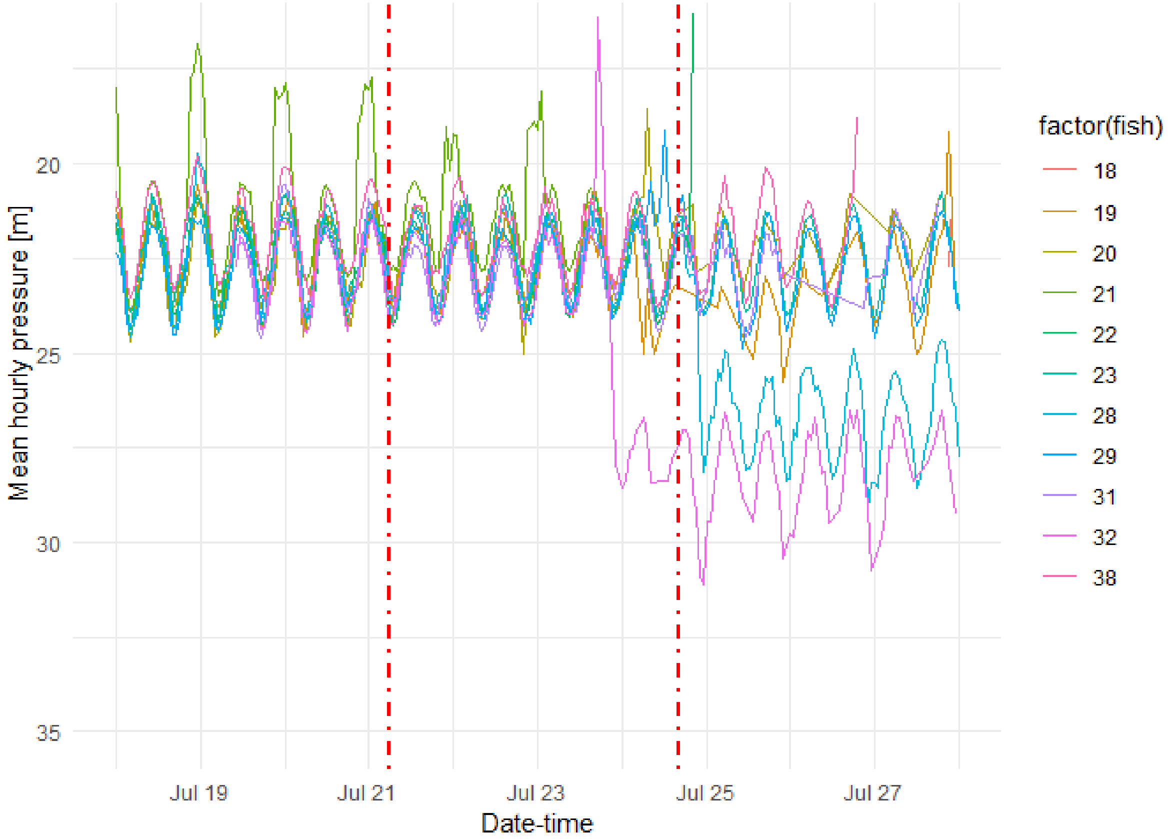

Figure 18: *Depth data based on pressure measurements transmitted by tags on individual fish. These data are from 11 individuals present at turbine C09 from 18-27 July, binned per hour. Individual fish are indicated by colours. The airguns at the seismic vessel were active during the three days between the vertical red dashed lines. General depth fluctuations reflect the tidal cycles. Note fish 21, who goes up in the water column at alternate low tides, from the start until halfway the seismic survey. Also note fish 32 and 28, who suddenly shift to deeper water towards the end or just after the seismic survey.*

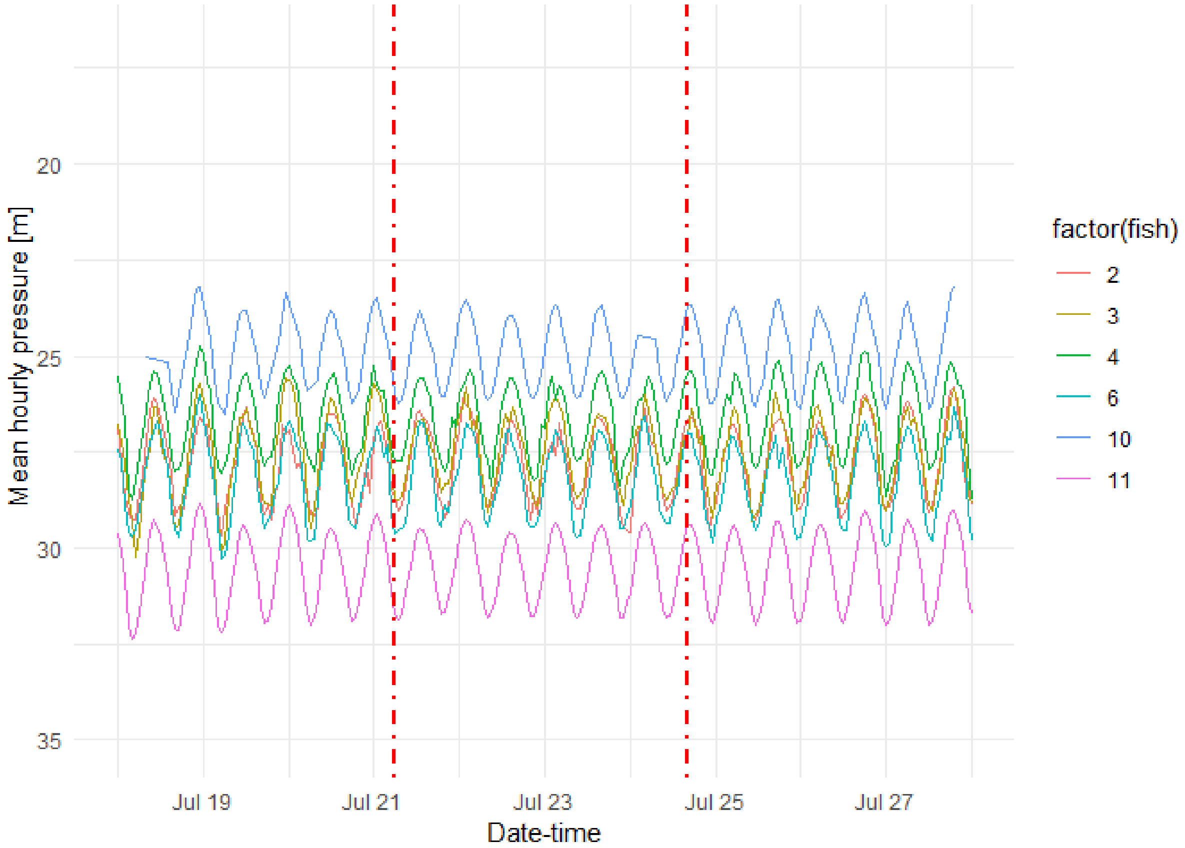

Figure 19: *Depth data based on pressure measurements transmitted by tags on individual fish. These data are from 6 individuals present at turbine B10 from 18-27 July, binned per hour. Individual fish are indicated by colours. The airguns at the seismic vessel were active during the three days between the vertical red dashed lines. General depth fluctuations reflect the tidal cycles. Note inter-individual variation and intra-individual consistency in swimming depth.*

Acceleration

Information on acceleration is transmitted as an averaged acceleration over the 3 axes: x, y & z, for a pre-set time period. Acceleration provides information, on top of the movement pattern inferred from spatial information, about the animal's activity over time. In Figure 20, we zoomed in to look at the hourly mean acceleration data for fish 3, 29 and 43 for the period in which the seismic vessel was active. Individuals show much variation in activity patterns over time, some of which are more or less synchronous patterns and some of which are more independent patterns per individual. Acceleration data can be compared and linked to pressure data in the previous figures. To provide insight into what the individual variation in acceleration data looks like over a longer time period and what kind of details will be analysed, we plotted the daily mean acceleration (plus standard deviation) for three example individuals at three differen VPS turbines (again for fish 3, 29 and 43) for the entire time period it was detected (Figure 21-23. All three individual fish show a different pattern in acceleration during the seismic period. Some may suggest a different behaviour during compared to before and after, or a change in behaviour after compared to before and during. However, such interpretation is premature and will depend on statistical analyses of all individuals and will take into account whether apparent pattern deviations during or right after the survey are indeed statistically deviant taking all fluctuations in time into account.

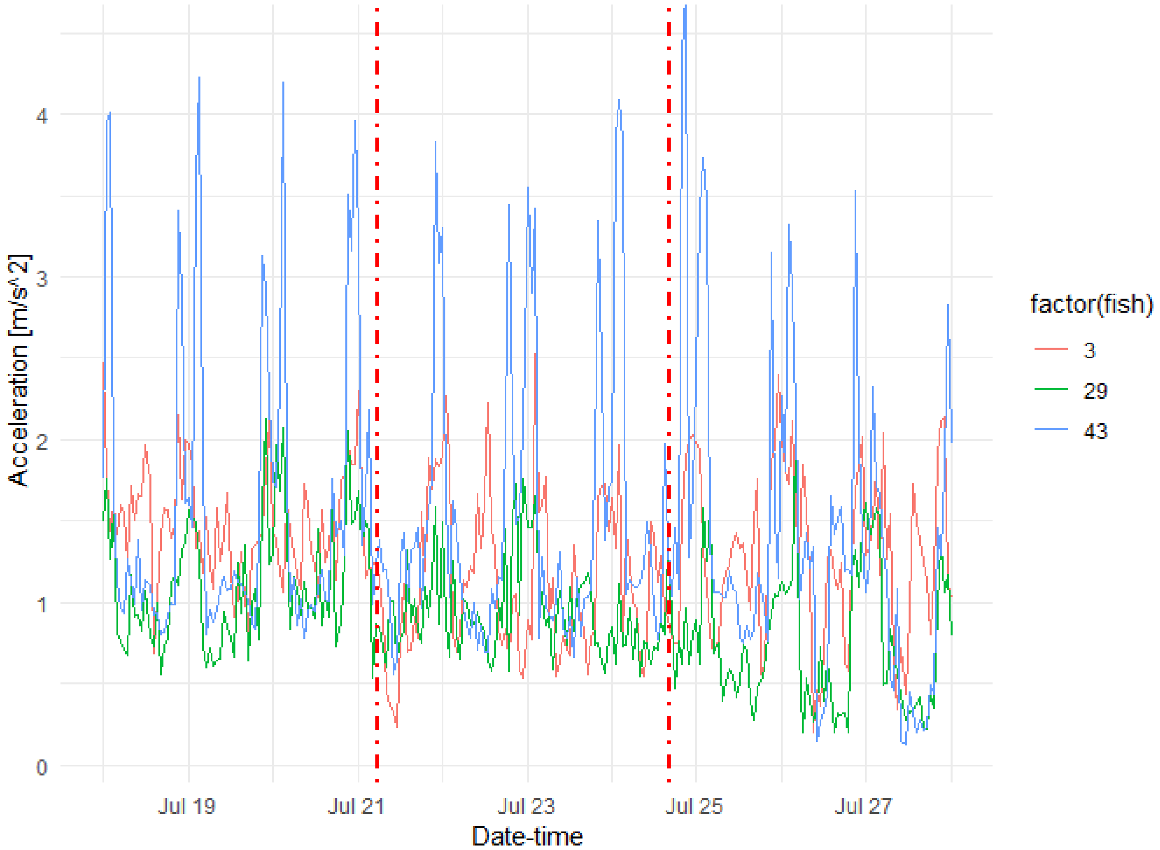

Figure 20: *Hourly mean acceleration data of three fish (individuals 3 from turbine B10, 29 from turbine C09, and 43 from turbine B08) zoomed in for the time period 18-27 of July. The airguns at the seismic vessel were active during the three days between the vertical red dashed lines. Note that there are many small-scale fluctuations, as well as larger-scale fluctuations, roughly coinciding with alternate tidal patterns, and an overall gradual decline in activity levels over these ten days of data. Note that the larger-scale fluctuations show some synchrony among the three individual fish, despite the fact that individual 43 has much higher peaks than the other two, which may indicate a different foraging style or different swimming style pattern any way. Note that individual 29 has a deviant pattern in swimming depth in figure 11, towards the end of the seismic survey, which coincides with apparent lower amplitude of its large-scale fluctuation in activity level in this figure.*

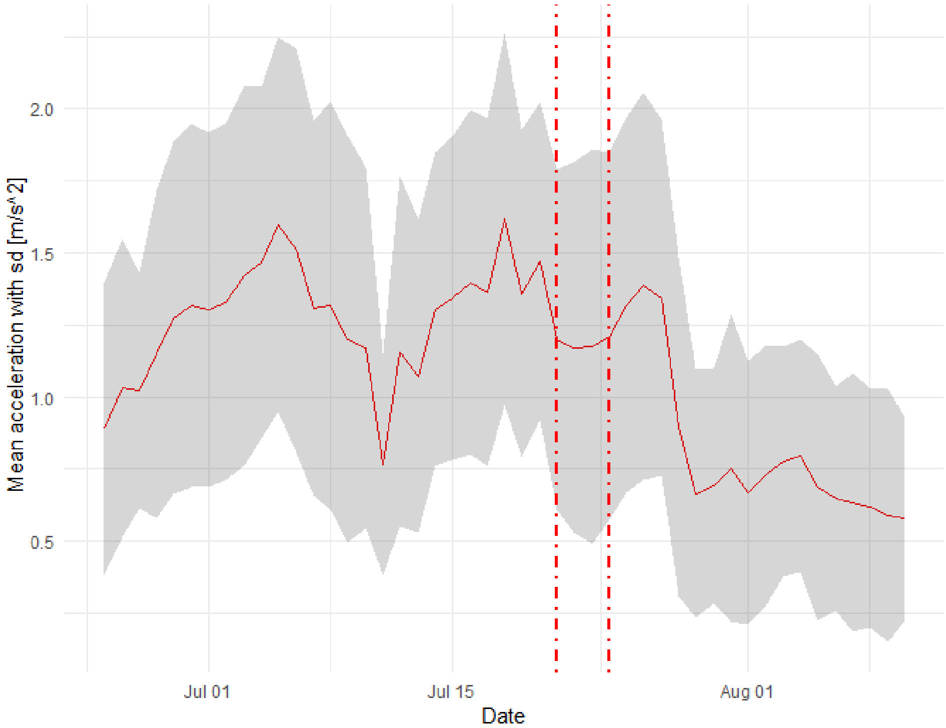

Figure 21: *Daily mean acceleration for fish 3 at turbine B10, for a 6 weeks period, with the standard deviation (sd) in grey. The airguns at the seismic vessel were active during the three days between the vertical red dashed lines. The fish seems slightly less active during the days of the seismic survey compared to the days before and after, while there are gradual and abrupt changes in the overall pattern that exceed the scale of the fluctuations around the survey period.*

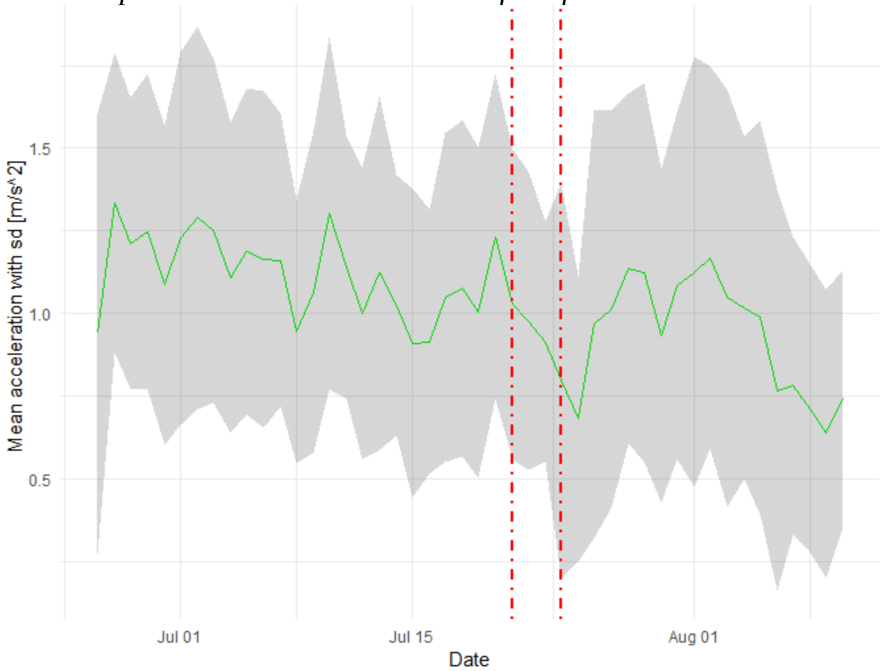

Figure 22 (page 33): *Daily mean acceleration for fish 29 at turbine C09, for a 6 weeks period, with the standard deviation (sd) in grey. The airguns at the seismic vessel were active during the three days between the vertical red dashed lines. The fish only shows moderate fluctuations and a gradual decline over the whole period. There is also a decline in activity around the survey period, but this started well before the onset and continued until after the airgun activity seized.*

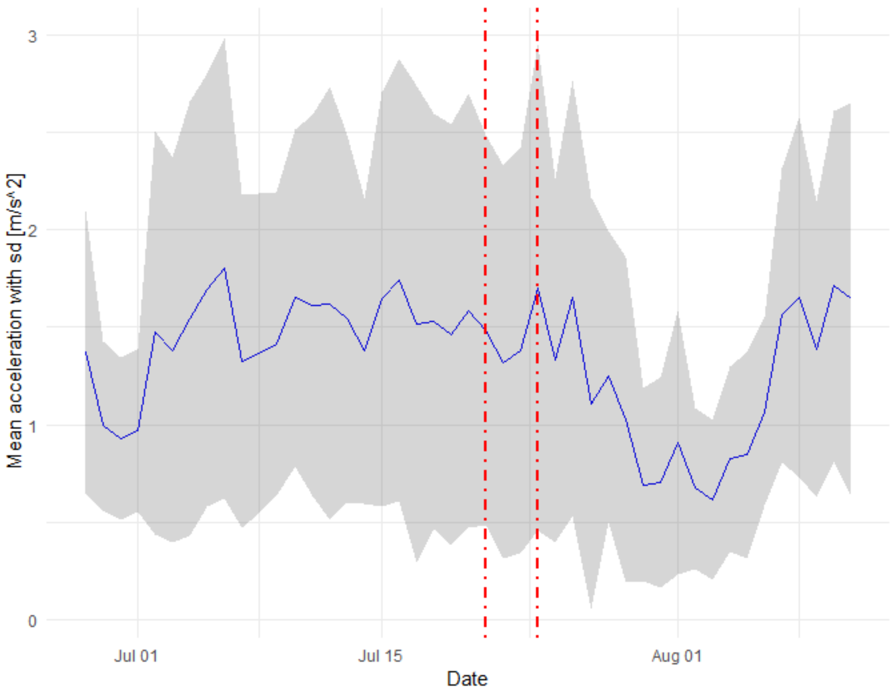

Figure 23: *Daily mean acceleration for fish 43 at turbine B08, for a 6 weeks period, with the standard deviation (sd) in grey. The airguns at the seismic vessel were active during the three days between the vertical red dashed lines. This fish also shows moderate fluctuations, but with several weeks of a steady level, followed by about a week of lower level activity, to move back up again after that, to the original level. The drop in activity appeared several days after the seismic survey period and appears not to be related.*

Positions

Finally, based on the timing that different receivers within the study area detected the individual fish, positions can be calculated over time. In Figure 24, we depicted the positions of one fish (blue dots are localizations based on pressure and red dots are based on acceleration signal transmissions) over the entire tagging period (similar to earlier examples from our pilot year in 2017, provided in the interim progress report). From this data, we will calculate individual home ranges (within the area) and time period dependent travel distances. From the combination of data on detection, depth, acceleration and positions of the tagged fish, we can derive detailed information about where the fish were and how active they were. The next step in the data analyses is to include information on the environment (tidal & circadian rhythm, water temperature, wind, etc.), and the detailed time line of anthropogenic activity (repeated approaching vessel yielding fluctuating seismic sound exposure conditions, turbine activity, shipping traffic, environmental noise, etc.), to get an understanding of the factors that might explain the variation in behavioural patterns of our large sample of free-ranging fish. We are working on the following analyses: animal movement models (McClintock et al. 2018), Mahalanobis distance analyses (de Ruiter et al. 2013) and home range analysis (Nilsen et al. 2008, Nanami et al. 2018).

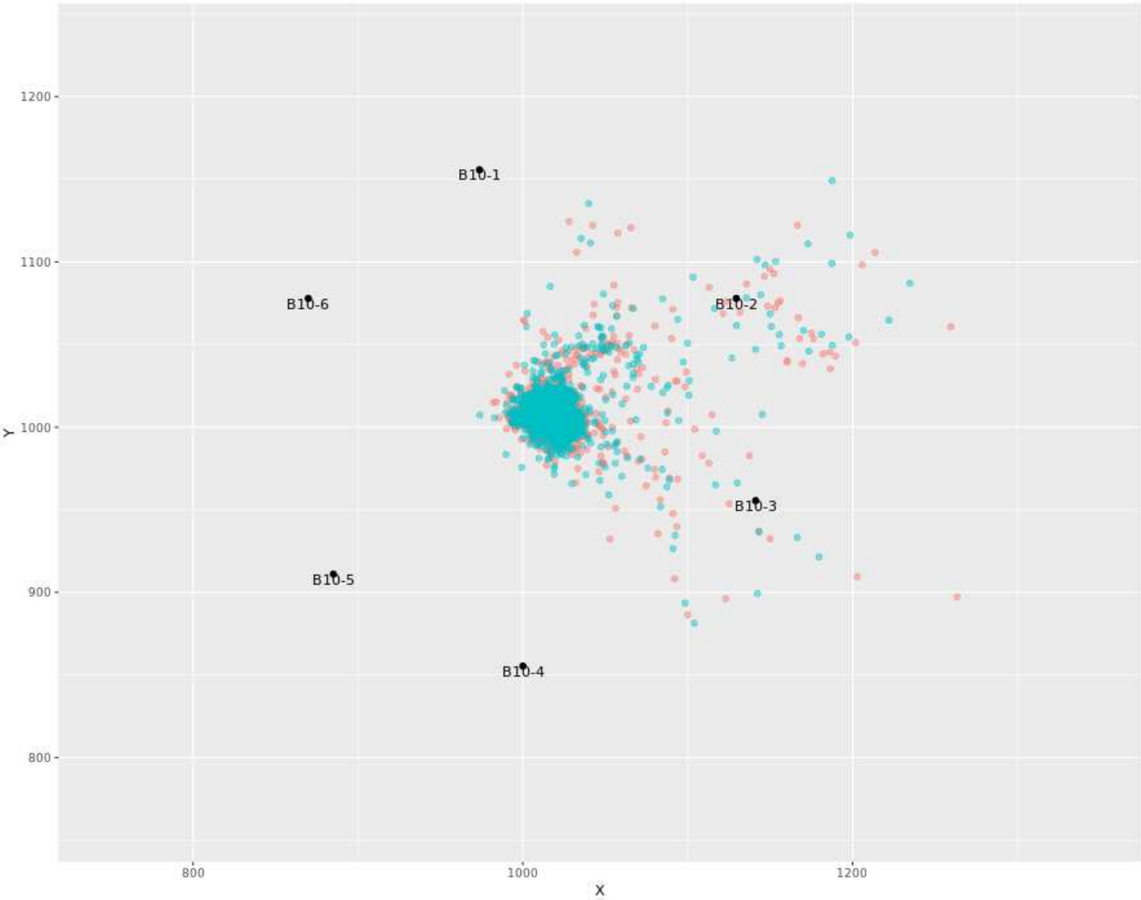

Figure 24: *Example plot for positions calculated for one individual fish (3) over the entire detection period. Red dots indicate moments when the tag emitted acceleration information and blue dots indicate pressure emissions. Both types are used for the localization by triangulation.*

Animal movement models, Mahalanobis distance, home ranges

Animal movement models are used to analyse changes in behaviour of individuals over time. These models exploit data on the animals position, depth usage and acceleration to define different behavioural states (e.g. resting, foraging or migrating) and can then predict when changes in state occur in relation to other variables like sound, turbine activity but also tidal and circadian rhythm. Positioning of our fish within the VPS covered area, is done through the online user platform provided by VEMCO. Apart from VEMCO's positioning analyses, we are exploring possibilities of performing this analysis ourselves, giving us more insight into the underlying errors. In part III of the report "Floating pen studies" we provide a more in-depth description of this kind of analysis. Movement modelling will be performed trough a discrete Hidden Markov Model, the R package momentuHMM (https://cran.r-project.org/) provides this model specifically for telemetry data (McClintock et al. 2018).

We will perform Mahalanobis distance analyses to define when statistically significant changes in behaviour (DeRuiter et al. 2013). This analysis summarizes all relevant parameters on behaviour and can be overlaid by acoustic data on sound exposure. Results from this analysis will provide another look at the changes over time in behaviour and test whether they are affected by the changes in sound conditions during the seismic survey exposure. Furthermore, we will analyse the 'home range' of the individuals within the VPS detection area. This analysis will inform us on the area's used by different individuals and what percentage of time they spend in certain areas. The home range analysis uses a kernel density estimator of the utilization distribution (Nilsen et al. 2008, Nanami et al. 2018).

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ANSWERS TO ORIGINAL QUESTIONS II

Our main objective for this project component was to do an explorative study in 2017 into tagging cod and receiver design to assess how cod activity relates spatially to the turbine and to confirm that we can trace our tagged fish sufficiently for quantifying detailed behaviour during the experimental exposure to seismic survey sound in 2018. We succeeded in deploying and retrieving all receivers for two array designs in 2017 and 7 fish (out of 27) provided sufficient insights into the suitability of tags, receivers and design features. In 2018, a larger sample size of tagged fish and a larger number of receivers at adjacent turbines yielded a very large data set with for more than 30 individuals weeks of detailed data on whereabouts by triangulation, overall activity patterns by acceleration, and depth by pressure. Only a small part of the large data set has been analysed in detail, but the following answers can be provided to the questions raised in the original proposal:

1. Is the spatial range, swimming speed, and depth of free-ranging adult cod, different before, during and after a seismic survey?

> *ANSWER: Although we have not tested the lack of statistical significance yet, there is no obvious departure by a large number of tagged cod, away from the area within reach of our receivers, from before to during the seismic survey. There may be more subtle changes in spatial behaviour, swimming energetics or depth, but these data have not been completely analysed. First explorations indicate, however, that there are no dramatic and synchronous changes in cod behaviour associated with the passing by of the seismic vessel of our experiment. Confirmation of these statements depends on current processing and current development of statistical procedures.*

2. Do potential prey or predator species and other community members of adult cod vary in density or activity over these periods?

> *ANSWER: Although our behavioural test on crabs and shrimps (certain prey items of cod) in the Jacobaharbour indicated that prey availability may be affected by the presence of seismic survey sounds, we have not assessed such effects in the North Sea during the experimental survey in the current project. In a parallel project, two bottom-mounted echosounders have recorded the presence of individuals and schools of pelagic fishes (potential prey items of cod) and the preliminary data from this resource indicate a potential effect of the seismic survey for a period of about 10 hours after the on-set of sound pulse production.*

3. Are airgun related changes in behaviour and community distinct from fluctuations with weather, tide or other human activities?

ANSWER: The weather during the experimental exposure with seismic survey sounds in July 2018 was calm, warm, and optimal for our experimental requirements, but the lack of variation prevents any remark about the influence of weather on fish behaviour relative to the survey sounds. The tidal fluctuations induced clear fluctuations in water column height, reflected in pressure variation, and current speed, reflected in variation in ambient noise levels. We are exploring the impact of both factors on fish behaviour and take this into account in our analyses of base line conditions versus potential changes during the days of the treatment period. Very modest presence of sound from other human activities (vessels at nearby shipping lanes or from surveyance or maintenance vessels by the windfarm operators) is unlikely to have affected our results much, but will be explored and evaluated. In the complementary study, we not only assessed the impact of the experimental seismic survey on the pelagic fish community by bottom-mounted echosounders, but also of pile driving two months later in a nearby windfarm.

4. Are there any lasting differences in cod behaviour after the seismic survey?

ANSWER: The persistence of our tagged individuals in the area before, during, and after the survey, and the relatively modest behavioural changes (at least as suggested by the preliminary exploration of data) do not exclude lasting effects (physiologically, ecologically, or delayed behaviourally), but makes them less likely.

2-III Outdoor net pen and indoor basin studies

Overview core work periods and mile stones:

Year 1

2017-Q2	Preparing ethical application for cod-studies
2017-Q3	Preparing floating pen study with cod + conducting crab and shrimp study (n = 49)
2017-Q4	Conducting floating pen study with cod (n = 20)
2018-Q1	Processing spatial data floating pen study with cod

Year 2

2018-Q2	Preparing and conducting cod-growth study (this failed because of unusually high summer temperatures). Preparing and conducting accelerometer calibration study.
2018-Q3	Conducting a foraging study with cod (n = 6) and a t-maze study with crab (n = 230). Conducting accelerometer calibration study.
2018-Q4	Processing cod foraging study and crab t-maze study
2019-Q1	Processing spatial + accelerometer data floating pen study with cod. Processing data from accelerometer calibration study.

Completed Manuscript: Hubert, J., Campbell, J., van der Beek, J., den Haan, M., Verhave, R., Verkade, L., & Slabbekoorn, H. 2018. Effects of broadband sound exposure on the interaction between foraging crab and shrimp – A field study. *Environmental Pollution*, 243, 1923-1929.

Expected manuscripts: Campbell et al. spatial and behavioural tracing of cod; from video to field May 2019; Hubert et al. Behavioural categories for accelerometer data on cod – Sep 2019; Hubert et al. The effect of seismic sound exposure on individual cod in a net pen – Apr 2020.

Behavioural studies in a net pen

Behavioural studies in a net pen do not provide insight into actual dose-response thresholds of free-ranging fish, nor do they provide insight into natural escape behaviour (Neo et al. 2016; 2018). However, it is an excellent complementary method to study the variation in effect among different sound stimuli and different exposure patterns. Furthermore, if fish have sufficient space, immediate responses in altered swimming patterns can be studied and, if tagged with accelerometers, related to tag-data of free-ranging fish. For PCAD4Cod, we studied whether the playback of seismic airgun sound disrupts swimming patterns and activities of individual Atlantic cod (n = 20), sequentially exposed in a floating pen in the Jacoba harbour, the Netherlands. The Jacoba harbour is a man-made cove in the Oosterschelde and about 200 m wide, 300 m long and 2 to 5 m deep depending on tides. It has a level and muddy bottom. The area is sheltered, which makes the water relatively calm, and there is no external boat traffic allowed within 2 km of the cove, making it quiet and ideal location for noise impact studies. In the middle of the Jacobahaven, we built a floating island consisting of two platforms: one for the net pen and one for our "office tent". The octagonal platform for the net pen was Ø 11.5-12.5 m with a custom-made octagonal net of a volume of 334m^3 (Neo et al. 2016; 2018).

We equipped the cod with an acoustic tag (to track their swimming pattern) and an accelerometer tag (to detect activity patterns and to provide us with a proxy for energy metabolism). Preliminary processing of the swimming patterns revealed large variation between individual fish regarding changes in swimming patterns during the onset of sound exposure. The intra-individual variation in time has typically shown a dip (swimming down upon sound exposure) in other fish (nursery raised seabass (*Dicentrarchus labrax)* tested in groups) that have been exposed to this kind of experimental treatment with other sounds (Neo et al. 2016; 2018). However, we did not find significant changes for horizontal displacement (distance to net at speaker side, Figure 25), swimming depth (Figure 26), or horizontal speed (Figure 27) for our wild-caught cod. The next step in our analysis will be to apply more sensitive animal movement models.

Animal movement models

Animal movement models allow for the correction of measurement error based on prior assumptions about the animal's movement (Baktoft et al. 2017; McClintock et al. 2018). They also directly model the latent behaviour states of the animal rather than the observable movements related to a behaviour state. By reducing the measurement error in our spatial dataset we can increase the statistical power of our analyses. These models have been widely used on other species to deal with low GPS accuracy on large spatial scales, but they have not yet been applied to fish. So far, we've successfully adopted YAPS, a 2D time-of-arrival-localization method based on a simple continuous time animal movement model (Baktoft et al. 2017). We have expanded YAPS to work on our 3D dataset and have modified some model assumptions which are specific to our study site. Figure 28 shows one of our early implementations of YAPS on our dataset and it shows a promising increase in accuracy as compared to conventional time-of-arrival-localization methods.

We are further planning to expand the model by incorporating accelerometer data to the position estimates, fine-tuning the behaviour assumptions, and removing intermittent tag clock errors in our dataset which are currently the biggest roadblock to the performance of YAPS. If we are able to tune YAPS to perform efficiently on our dataset, we will explore expanding it into a full continuous time, animal movement model which will be capable of modelling a latent behaviour state (such as stress or activity level) to examine the effect of seismic survey sounds. If the performance of YAPS on our dataset is not good enough to expand into a full animal movement model, we will use it only as a localization method and a simpler discreet time animal movement model from the recent R package momentuHMM will be applied (https://cran.r-project.org/, McClintock et al. 2018).

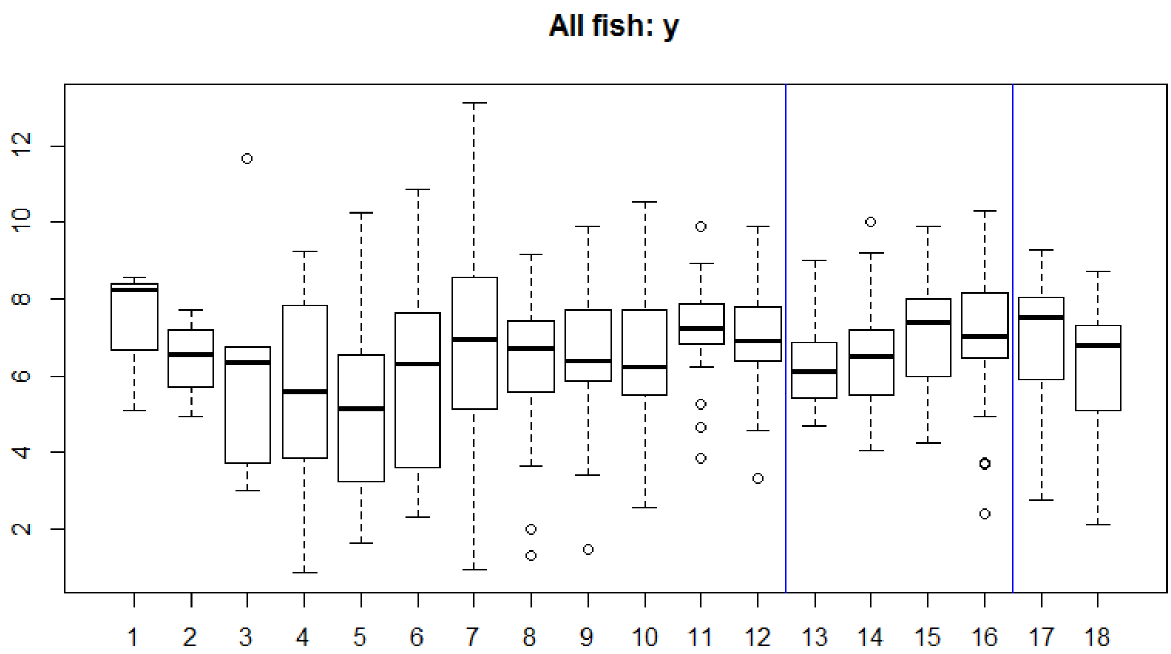

Figure 25: *Distance from the net that is closest to the speaker. Every boxplot represents 15 minutes. The blue lines indicate the start and end of the sound exposure, respectively. We did not analyse the data from all trials yet (the first 8 bins are partial datasets).*

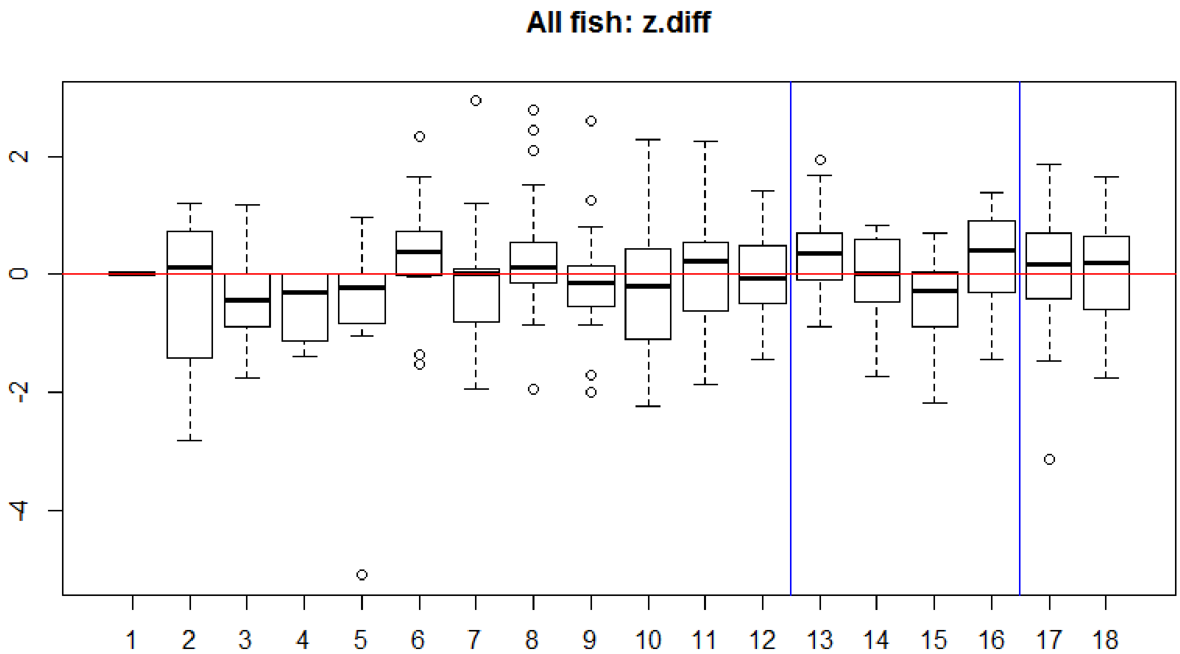

Figure 26: *Change in depth (compared to the previous 15-min-bin). The blue lines indicate the start and end of the sound exposure, respectively. We did not analyse the data from all trials yet (the first 8 bins are partial datasets).*

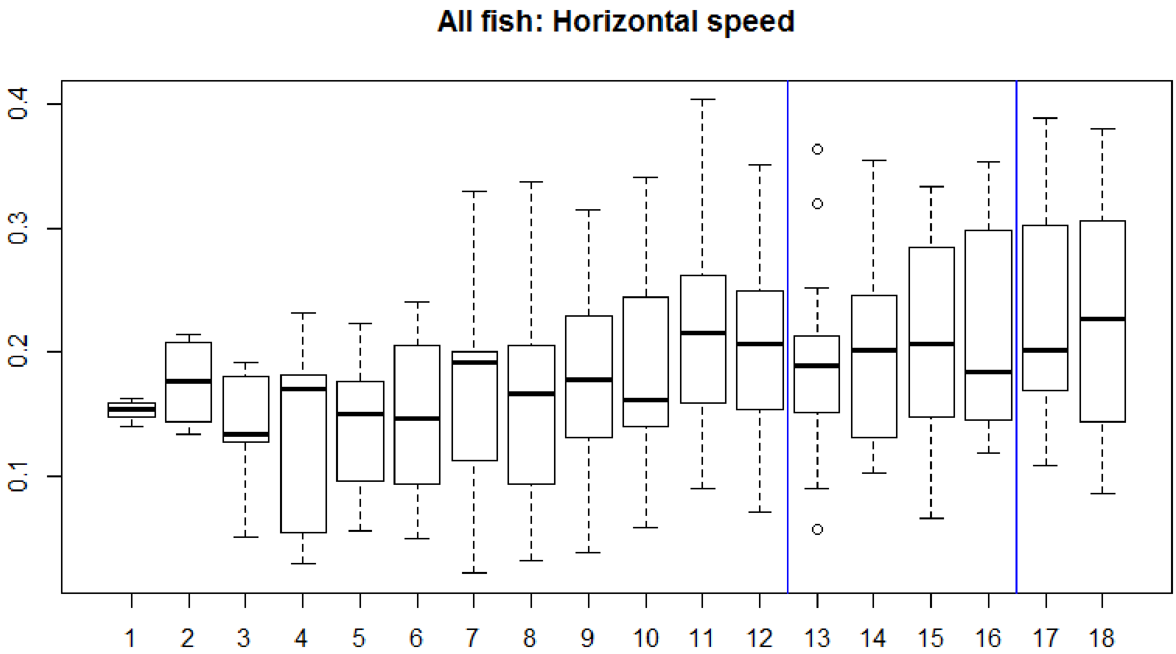

Figure 27: *2D speed (m/s). The blue lines indicate the start and end of the sound exposure, respectively. We did not analyse the data from all trials yet (the first 8 bins are partial datasets).*

Exploring energy intake and use through captive cod

The sensitivity analyses by Floor Soudijn et al. (in preparation) showed that changes in energy budgets of cod have most potential to result in population consequences (as compared to other causes such as reproductive success). For this reason, we designed a study that examined proxies for energy use (swimming behaviour) and energy intake (foraging behaviour). Several studies already examined the effects of sound on foraging behaviour in fish but often on a short time scale and with unnatural food items (Purser & Radford 2011; Shafiei Sabet *et al.* 2015). Our wild-caught cod, however, showed a lot of natural foraging behaviour in tanks with crabs (a major food source for cod). We therefore examined changes in swimming and foraging behaviour of wild-caught cod in an experimental basin aiming at reflecting shallow water costal environments.

Behavioural trials were conducted in two cylindrical tanks (Ø 3.5 m, depth 1.2 m) in which we exposed groups of two Atlantic cod to repeated 1-hour exposures of recorded seismic survey sounds (Figure 29). We selected cod that differed in size so we could distinguish them on camera footage. Before introducing the cod to the experimental tank, we performed a netting stress test and then allowed them to acclimatize overnight. In the following days, the cod were exposed to two 1-h seismic survey sound exposures for three consecutive days and three consecutive days of silence, the order of this resulted in a partial counterbalance experimental design (Figure 30). We filmed the cod from 10 am till 17 pm using five GoPro cameras to facilitate automated 3D tracking of swimming patterns. Foraging behaviour and feeding strikes had to be scored manually, thus we made a script that extracted 20 s clips every 5 minutes and stitched the different cameras into one screen so an observer (blind to the treatment) could view the entire tank in a single video file. After six days of observation, we caught the fish from the basin and performed another netting stress test. Because of disappointing catch rates after the heatwave this summer, we had a limited stock of wild cod and were only able to conduct three trials. Preliminary results of manually scoring the activities of the fish revealed a potential effect of sound treatment on swimming behaviour, but further analyses is required on the swimming track details before we can draw any more conclusions on this small but high resolution data set.

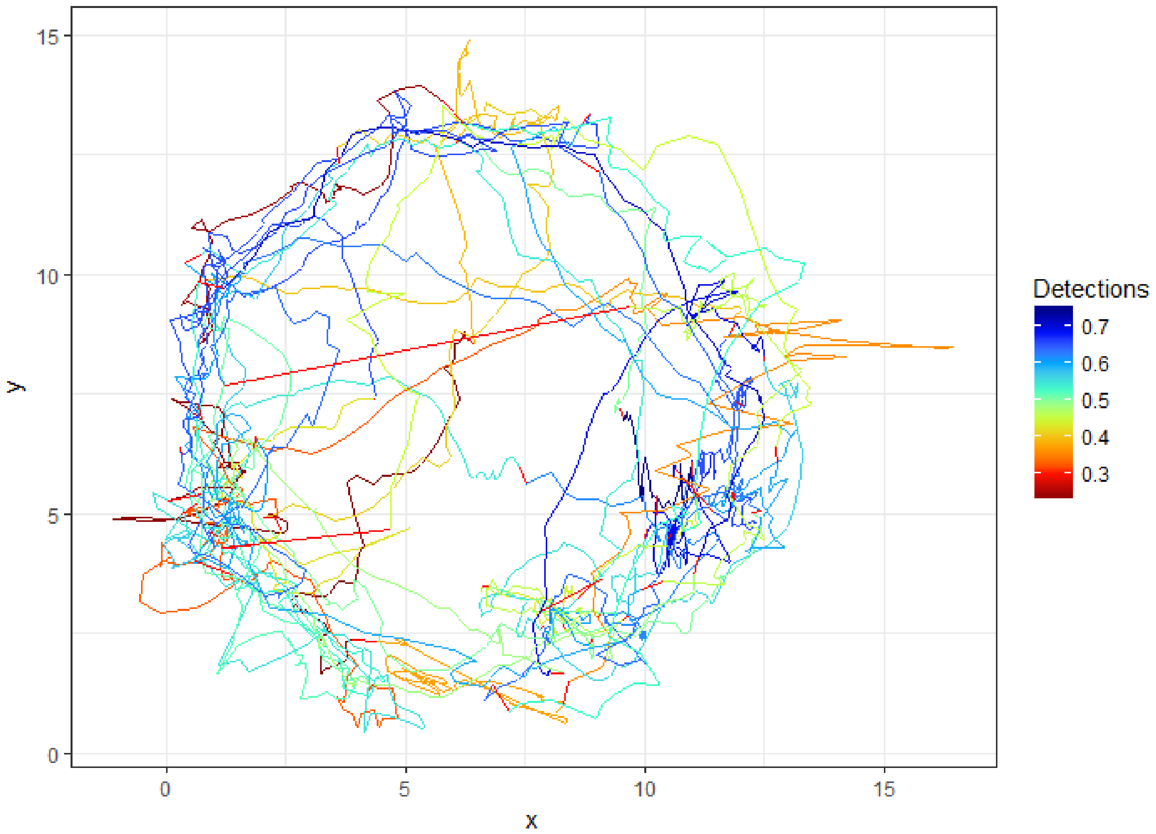

Figure 28. *An implementation of YAPS on our 3D acoustic telemetry dataset. YAPS is currently applied to 60-120 second segments of our telemetry data which are stitched together after each is independently computed, hence why segments are color coded with respect to the number of localizations within each. By incorporating accelerometer data and cleaning tag clock errors, we aim to apply YAPS on entire trials, removing the need to "stich together" independently computed segments and improving the accuracy of all positions.*

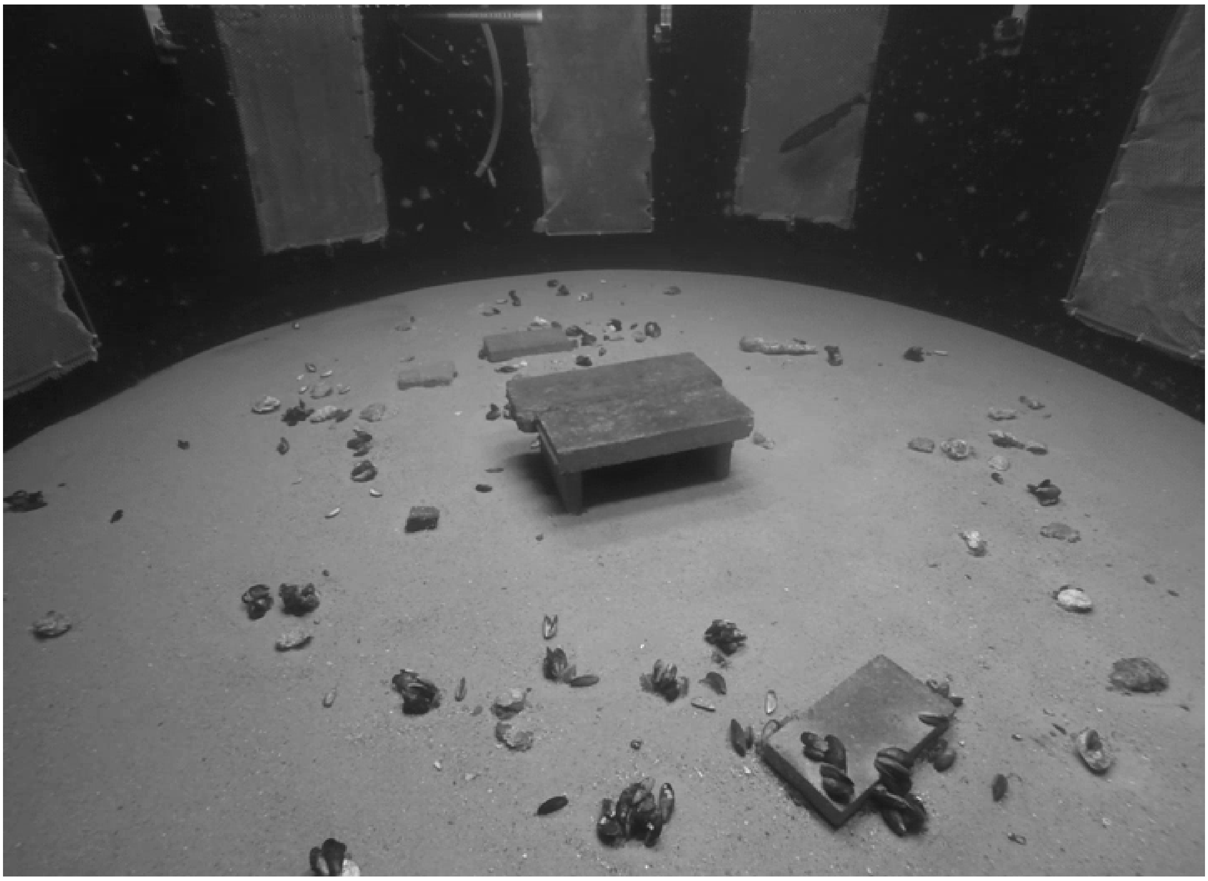

Figure 29: *A still frame from a trial of the accelerometer calibration experiment. The bottom of the 3.5m diameter basin is covered in sand, mussels, oysters, and live crab who have access to natural-like refuges. The set-up aims to mimic natural conditions in North Sea sandy shoreline habitats. The white panels in the basin help automated individual tracking when the fish is swimming high in the water column and close to the basin walls.*

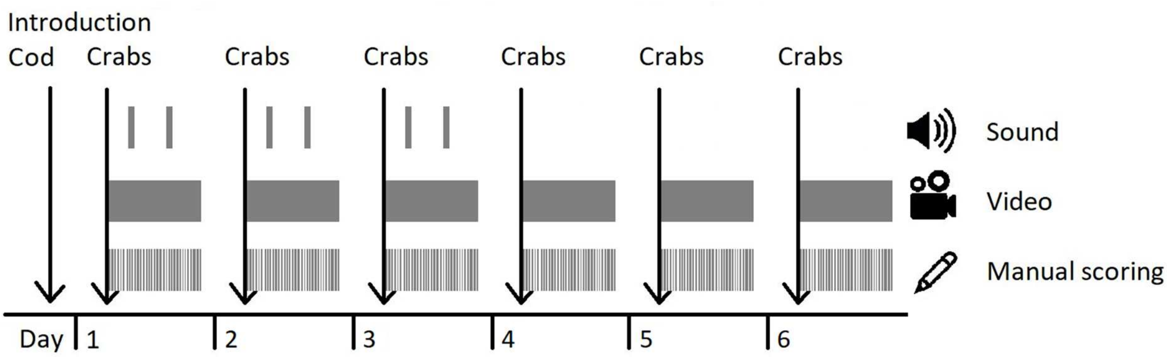

Figure 30*: Timeline for an experimental trial. We conducted three trials where each trial consisted of 6 days, 3 of which involved two hour-long sessions of seismic survey playbacks.*

Calibration of accelerometer dataloggers

Accelerometer dataloggers were used in the 2017 net pen study. In 2018, 10 individuals were used in a study paradigm consisting of two days of baseline swimming behaviour, 3 days of post-tagging behaviour, totalling 5 days per individual. Each of the 10 individuals were tagged with a datalogger accelerometer recording at 50Hz in the morning of the third day with a battery suitable for logging data for at least 2 days. Throughout the whole experiment, 5 GoPro cameras recorded videos of swimming behaviour for 7 hour sessions per day. Within the experimental basin of 3.5m diameter, we provided again a constant supply of live shore crabs and natural enrichment such as live mussels and oysters over a sandy bottom to mimic a North Sea shallow water costal habitat.

The analysis of the accelerometer calibration experiment is due to be analysed in the first and second quarter of 2019 (see Figure 31 for an example of a tri-axial behavioural pattern). Our goals are to use feeding events observed on the videos to train a random forest machine learning classifier to detect these feeding events using only the accelerometer data. We intend to apply this feeding classifier to the 2017 net pen study. Based on stomach contents of the 2017 study, we know that the cod were feeding (on crab) while inside the net pen. By using the accelerometer to identify feeding events from the 2017 study, we can gain insight into how feeding behaviour was altered in response to the seismic survey playback. Additionally, we can also use the experiment to correlate average accelerometer values with average swimming activity form the videos which can further give us insight into the 2017 accelerometer dataset.

In the beginning of the 2018 season, we tested the accelerometer calibration setup using three pairs of stereoscopic 3D cameras. We later switched to 5 single GoPro cameras due to concerns over the low visibility in periods when the natural water supply was turbid and bubbles and marine life attached to the cameras were obstructing the view. Additionally, using 5 cameras gave us a more robust set-up as we could still analyse our data if one, or even two, cameras were to fail during a recording session.

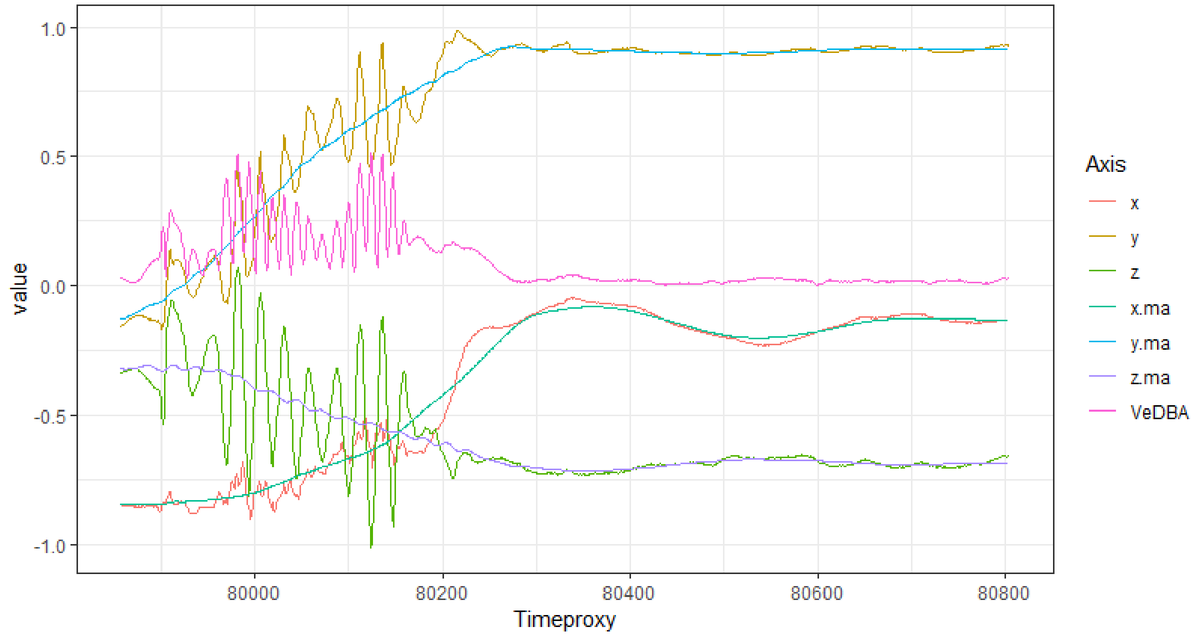

Figure 31. *Example track of the acceleration values for the three accelerometers. Those heavily fluctuating (y and z) are the ones that sense the tailbeat most, while the forward-backward axis shows less dramatic fluctuations (x). The smooth line through each more fluctuating line represents the component in the associated signal attributed to gravity. The pink line is the accumulation of all three axes and represents a measure of overall activity level by tri-axial acceleration (VeDBA).*

Crab and shrimp foraging behaviour in situ

As crabs were found to be a prominent food source in wild-caught fish foraging in the net pen In the man-made cove of the Jacoba harbour, and because we used crab in our foraging experiments with captive cod, we also studied crab behaviour in ambient cnditions and experimentally elevated sound conditions. The experiment was performed from the floating platform of the net pen, but without using the net (Neo et al., 2016; 2018). From the floating platform, we lowered bait stations which contained a cooked mussel and a waterproof camera. The cameras were aimed to film the sea floor and a cooked mussel (*Mytilus edulis*) that was connected to the crate using an iron wire. For each trial, we lowered a camera to the cove bottom from one of the 10 corners of the platform. After two minutes we started the playback of either silence (control) or white noise using an underwater speaker. We used two bait stations to conduct paired trials at the same time where the locations were at least 5.5 m apart from each other. The position of the speaker was fixed and the distance from the trial-location to the speaker varied between 3 to 14 m. We analysed 49 recordings, 27 control trials and 22 white noise trials. Every 10 seconds we scored the number of crabs and shrimps present and the number of crabs and shrimps that were eating the bait. Our results were published in 2018 and demonstrated that artificial broadband sound alters crab aggregation around the food item (Hubert *et al.* 2018). During trials with white noise treatments, less crabs came to the offered food item while no similar pattern was observed for shrimp. However, we did observe that the number of shrimps present was negatively correlated with the number of crabs present.

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ANSWERS TO ORIGINAL QUESTIONS III

Our main objective for this project component was to gain insight into the importance of particular acoustic features of the airgun sound pulse for triggering a response and to compare fish of different age classes for their responsiveness in terms of both behaviour and physiology. We have not been able to test variation in sound features in our experiments, nor to test physiology directly. However, we succeeded in testing wild-caught cod in the floating pen for behavioural responsiveness to scaled air gun sounds and to collect data to explore the behavioural categories associated with the multi-dimensional accelerometer data. We also gained insight into potential effects on energy budgets indirectly via the impact of sound exposure on swimming and fouraging behaviour (both relevant as indicated by the outcome of our modelling paper: MS02).

Below answers to the proposed questions as far as we can at this moment:

1. Are behavioural changes triggered by airguns similar in nature for free-ranging tagged fish and captive fish in a floating pen?

> *ANSWER: We have collected a large amount of data on both free-ranging fish in the North Sea and captive fish in the floating pen. The responsiveness appears to be moderate for both in this species. Detailed analyses and comparisons await further processing and statistical steps that are on their way.*

2. Do loud and impulsive sounds trigger both behavioural and stress-physiological responses? Are pulse rise time and reverberations critical parameters for these responses?

> *ANSWER: Our results indicate the potential for behavioural changes induced by sound exposure, although the scale of these appears to be moderate. The continuation or quick recovery of base line behavioural patterns also suggest no or modest physiological effects, but this statement awaits confirmation by studies that actually measure indicators of physiological change.*

3. Does repeated exposure lead to fading of behavioural responses? And is there acute but no chronic stress-physiological response? Or are growth rate and maturation affected?

> *ANSWER: We have not conducted long-term studies required for answering these questions. We do have behavioural data from free-ranging fishes over multiple days on the effect of seismic survey sounds fading in gradually and fading out gradually determined by the vessel track pattern, with the vessel coming closer and going further away repeatedly during the experimental survey.*

4. Do different life stages differ in response tendency, both behaviourally and physiologically? What is the impact of coping style?

> *ANSWER: We have not tested different age classes or life stages of cod in this study. We explored individual variation by assessing proxies for coping styles and we may gain insight into the role of coping styles on the behavioural tendencies in the floating pen.*

2-IV Sound measurements and modelling:

Overview core work periods and mile stones:

Year 1
2017-Q2
2017-Q3: Jim Martin, James Campbell, and Jeroen Hubert made propagation measurements in the floating pen setup and Peter Rogers analysed the data and provided us with an acoustic description of our study area.
2017-Q4
2018-Q1: Work on the seismic survey manuscript begins: Curating seismic survey recordings from other researchers.

Year 2
2018-Q2: Continuing collecting seismic survey recordings.
2018-Q3: Continuing collecting seismic survey recordings.
2018-Q4: Preliminary analysis done on seismic survey dataset. Began work on a supplementary simulated analysis.
2019-Q1: Manuscript complete

Expected manuscripts: Campbell et al. Seismic sound recordings from the fish perspective – April 2019; Rogers et al. Soundscape model of cod auditory perception during seismic exposure Oct 2019.

James Campbell, Peter Rogers, James Martin, Michael Ainslie, Bruce Martin, Hans Slabbekoorn

Acoustic variability of seismic survey pulses

In early 2018, we started collecting recordings of seismic surveys from other researchers. The aim was to build a dataset of recordings large enough to broadly describe how the acoustic characteristics of seismic surveys can vary within and between different shallow water locations. This dataset would also provide context to our North Sea experimental seismic survey. Preliminary measurements have been taken from a data set of recordings from a seismic survey off the east coast of Scotland (Thompson et al. 2013). Sonograms of seismic pulses illustrate variation in pulse characteristics after propagation over various distances (Figure 32), which will be compared to those of our own experimental seismic pulse recordings in the Belgian North Sea. We measured a replicated sample of pulses from this data set and plotted the variation in various features against distance from the source. Pulse amplitude declined steadily over the first 15 km, the peak frequency (frequency at maximum cumulated energy) showed a rise in range from about 100 Hz at 2 km to 100-300 Hz between 15 and 25 km, while pulse duration was very brief close at the source and longer and more or less constant over the rest of the range, and also pulse rise time did not vary predictably over distance from the source (Figure 33).

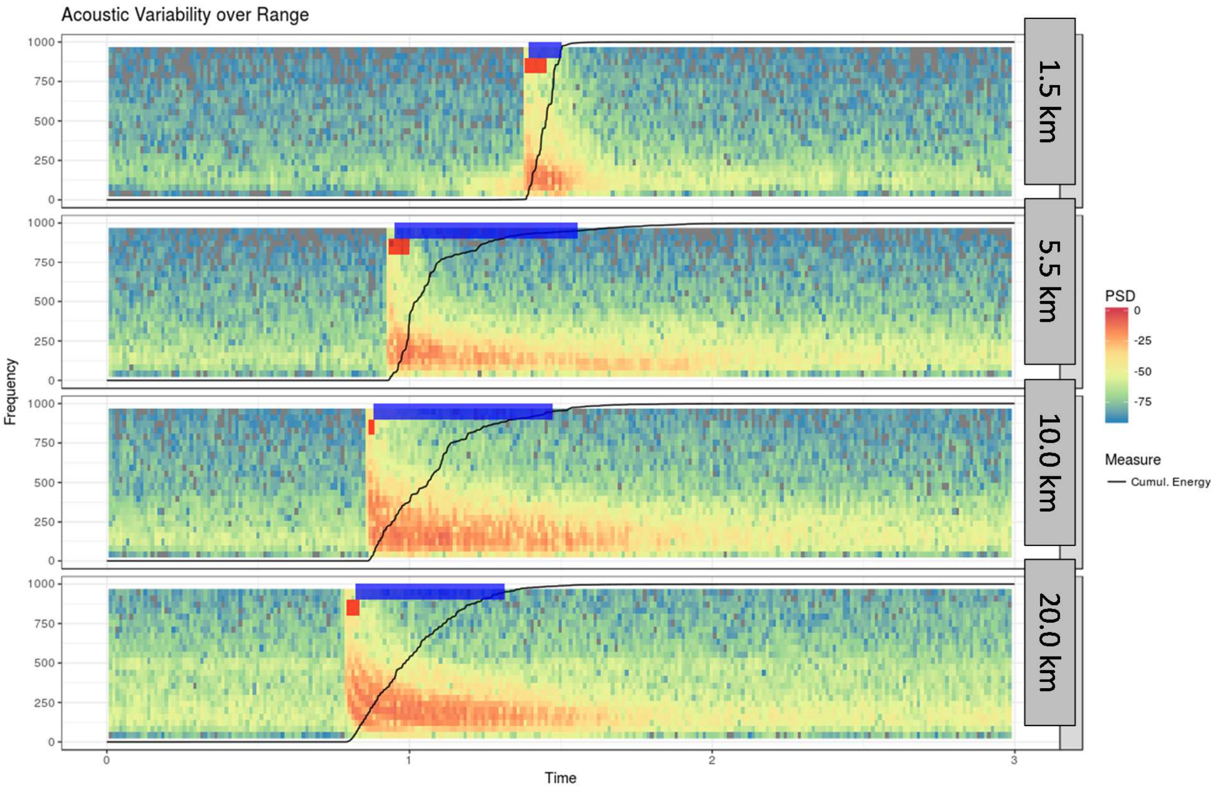

Figure 32: *Example sonograms of seismic pulses for different distances from the source for a survey conducted off the east coast of Scotland (Thompson et al. 2013).*

In addition to the actual recordings, James Campbell has begun exploring options for taking a simulated approach to gain more insight (Smith 2010; Jensen et al. 2011). Currently, we are using an implementation of Michael Collins' RAM parabolic equation propagation model to generate simulated impulse responses for shallow water environments which vary in range dependant depth and sediment type. The RAM PE model was chosen due to its computational efficiency, availability of source code, its suitability for low frequency shallow water propagation, and reputation in the literature for having a reasonable accuracy when compared to computationally expensive benchmark models such as the coupled normal mode model KrakenC. We are planning to convolve these impulse responses with seismic survey sounds to produce a simulation of what seismic surveys would sound like after they have propagated across different shallow water environments (also see Sertlek 2016; Sertlek et al. in preparation).

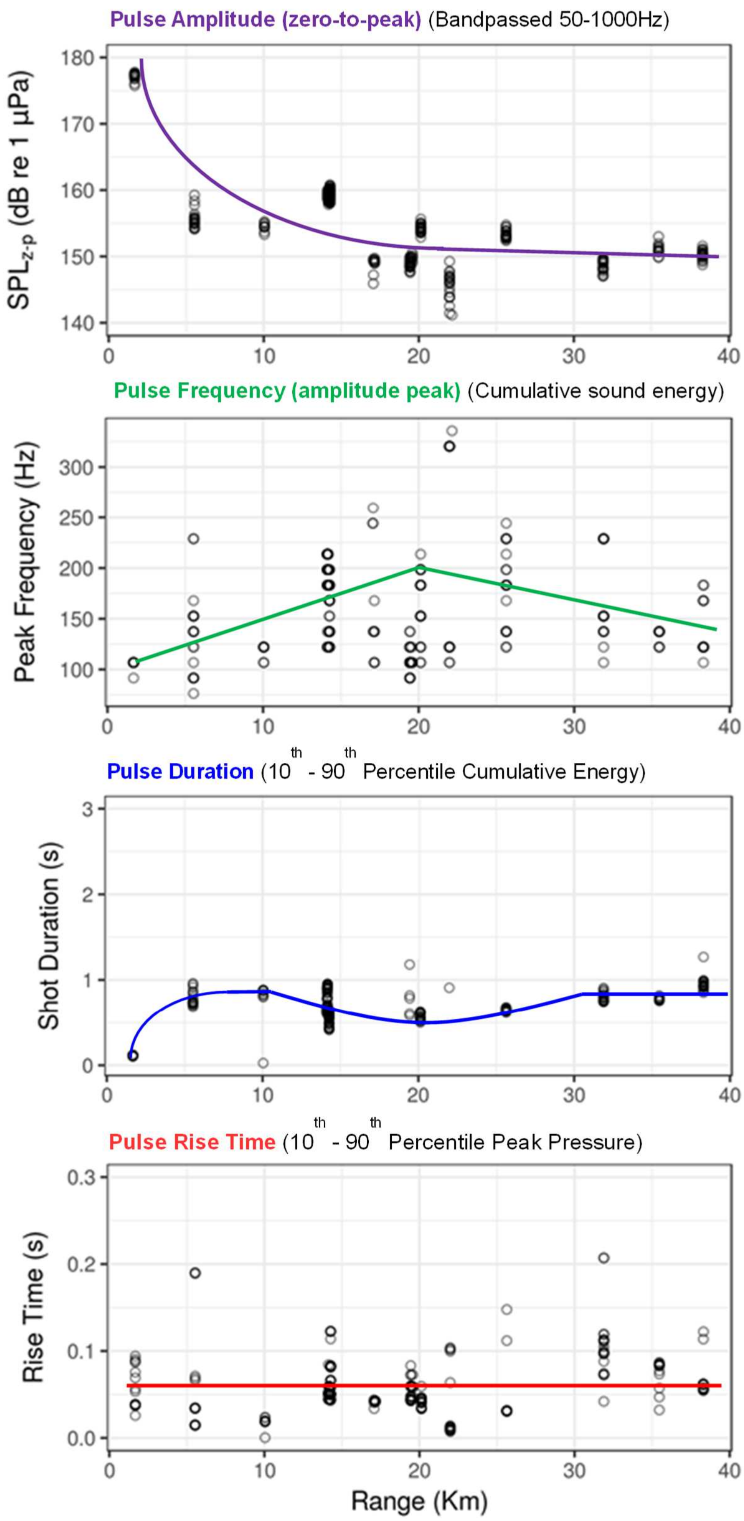

Figure 33: *Overview of acoustic variation in seismic pulse features over distance from the source for a survey conducted off the east coast of Scotland (Thompson et al. 2013).*

Sound recordings from the experimental seismic survey

On the 12th of July, we deployed two AMAR recording stations at the Belwind wind farm. Both concerned recording devices for pressure and particle motion and were prepared and delivered by JASCO with full support in deployment guidance and basic data processing. The start of recordings was set to the 13th of July 2018 (00:00 UTC). One station (AMAR-1) was placed at turbine B08, right were the tagged fish were (Figure 13), which was the original treatment station to be placed in the GEMINI wind farm in the Netherlands. Due to our change of plans, we decided to still exploit the access to two stations and collect a double set of recordings at the Belgian survey site. The second station (AMAR-2) was the original control station and was placed at a location a bit further away from, and perpendicular to, the seismic vessel tracks (Figure 13). However, for unknown reasons, this second recording station failed completely and there was no delivery of data for this second set. Both AMAR recording stations were successfully recovered on the 31st of August 2018 despite a problem with lost release systems.

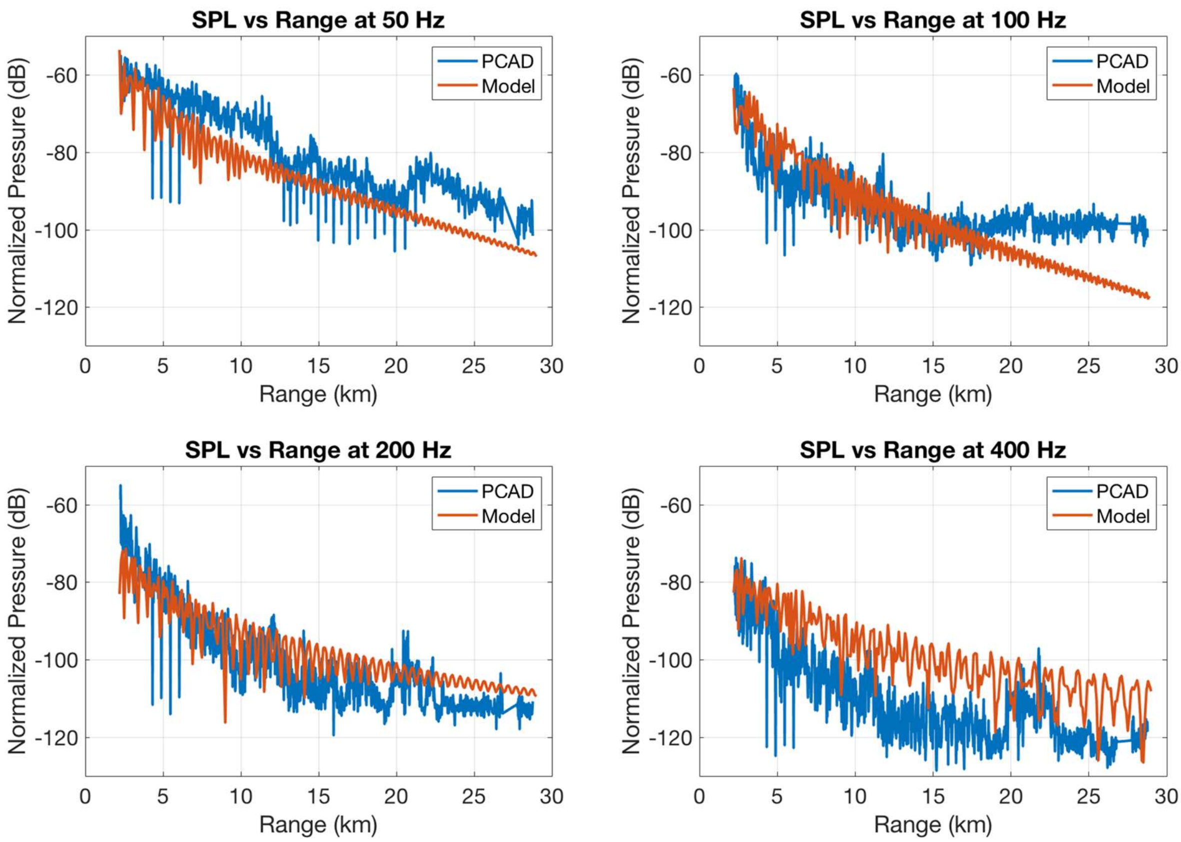

Figure 34: *Comparison of sound pressure measurements from AMAR-1 (in blue) to data from a Pekeris normal mode propagation model (in orange) for the first vessel track during the experimental seismic survey from 30 km away until the closest point of approach at 2.2 km for different frequencies (50, 100, 200 and 400 HZ), which are all relevant in terms of the auditory range of cod and biological sounds potentially interesting to cod.*

We are still busy with basic processing, exploration and integration of the large sample of sound recording tracks of AMAR-1. Both Bruce Martin at JASCO and Peter Rogers at Georgia Tech are working hard on making the data available and allowing data integration and interpretation of fluctuations in sound level and potentially related fluctuations in behavioural patterns of the tagged fish. The pulse shape variation versus range between sound source and tagged dish has been explored through comparing the pressure measurements from the first vessel track approach to data from a so-called Pekeris normal mode propagation model (Figure 34 and 35). Peter used a constant depth set at 34 m, for the average depth of the first pass from 30km away to the closest approach at 2.2km from turbine B08. The bottom features were taken as fine sand (c_s = 1.13 c_w sound speed in the sediment; ρ_s =1875 density; K_s = .51 sediment loss factor). Several cut-off frequencies were within the band of interest so several normal modes were usually required. Both time domain and frequency domain calculations were included (see Smith 2010; Sertlek & Ainslie 2015).

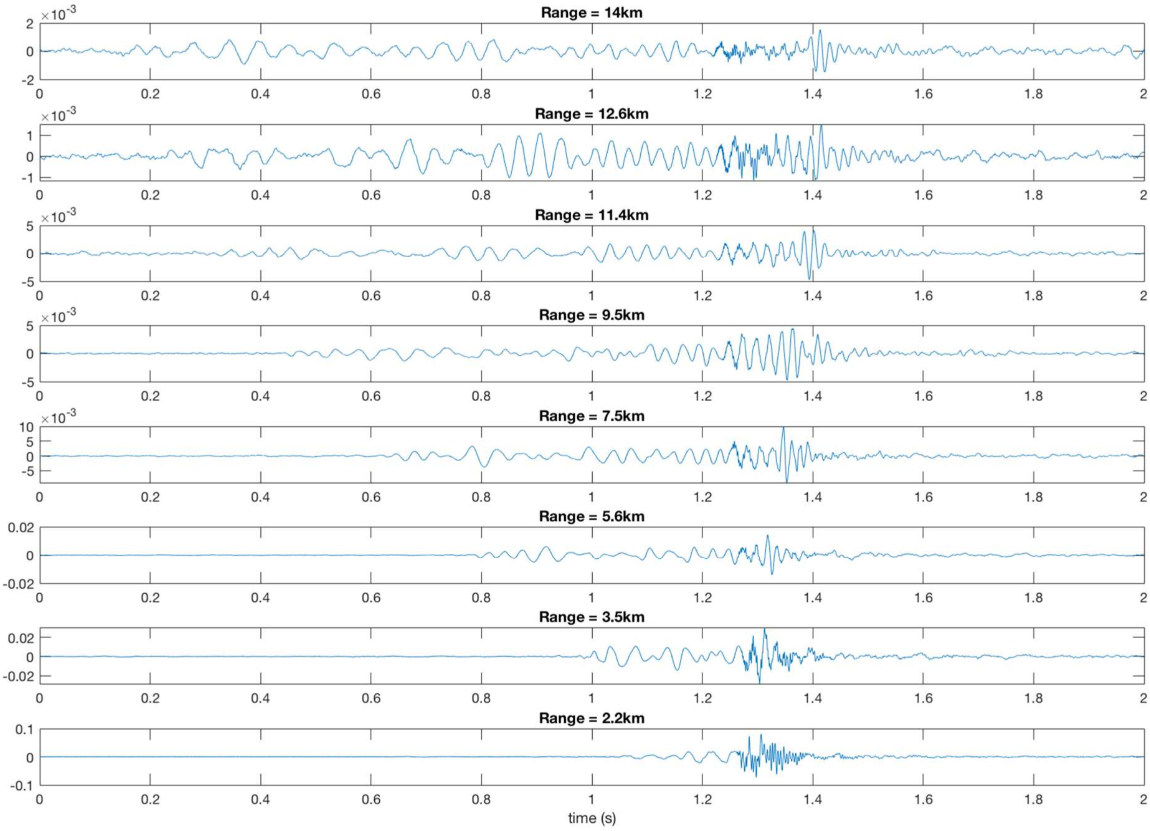

Figure 35: *Variation in pulse pressure amplitude patterns as a function of distance between sound source and hydrophone. The pulses all have a relatively abrupt ending and become longer with increasing distance. The likely explanation for this is that the ground wave of the 1st mode precedes the main pulse. For an example of the spectral shape of such a propagated pulse, see Figure 36.*

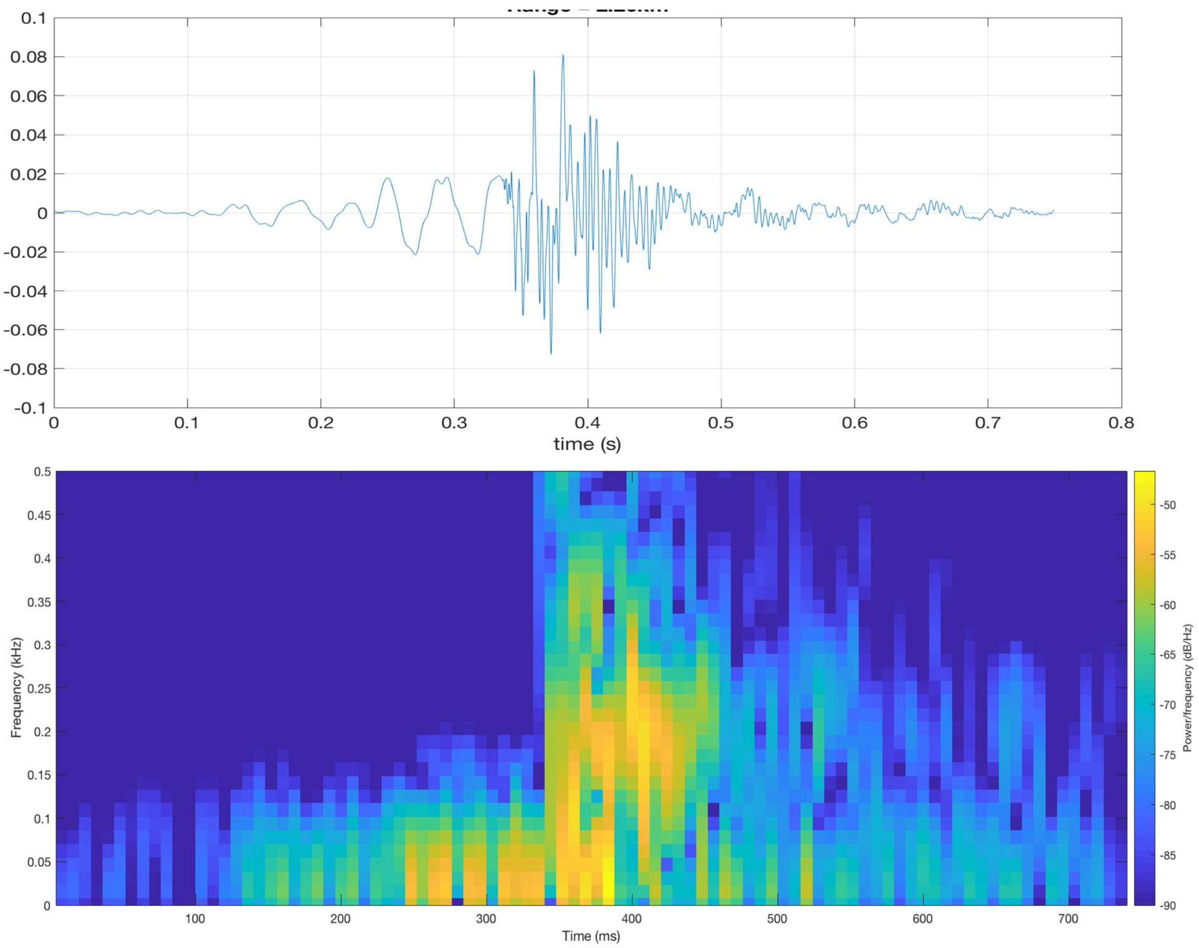

Figure 36: *Example of a pulse pressure amplitude pattern and a sonographic representation of the spectral shape of the pulse after propagation over the closest approach distance of the tagged fish and hydrophone site at turbine B08.*

Further explorations are on its way (Figure 37) and will provide more insight into the actual, measured sound conditions during the whole survey at the tagged fish site, as well as an indication of acoustic variation in the whole area in which fish were swimming around, stayed or left from. We not only measured and processed sound pressure, but also recorded acceleration in three directions. We will process these data as well to complete the picture of exposure conditions as much as possible to related the acoustic data to abiotic factors such as tidal fluctuations and weather conditions, but also to relate bot pressure and particle motion to potential behavioural responsiveness. Investigations into the details of the sound field around the fish, analysed from the fish perspective, will take the directionality of particle acceleration into account and the effect of angle on signal-to-noise ratio (Figure 38). The signal and noise could be the seismic pulse and the ambient sound respectively, or a biologically relevant signal to cod, such as a conspecific sound, and the reverberative seismic pulse respectively. Preliminary analyses revealed a low-frequency precursor wave just prior to the pulse and reverberations that fill up inter-pulse intervals up to considerable distances from the source (Figure 39).

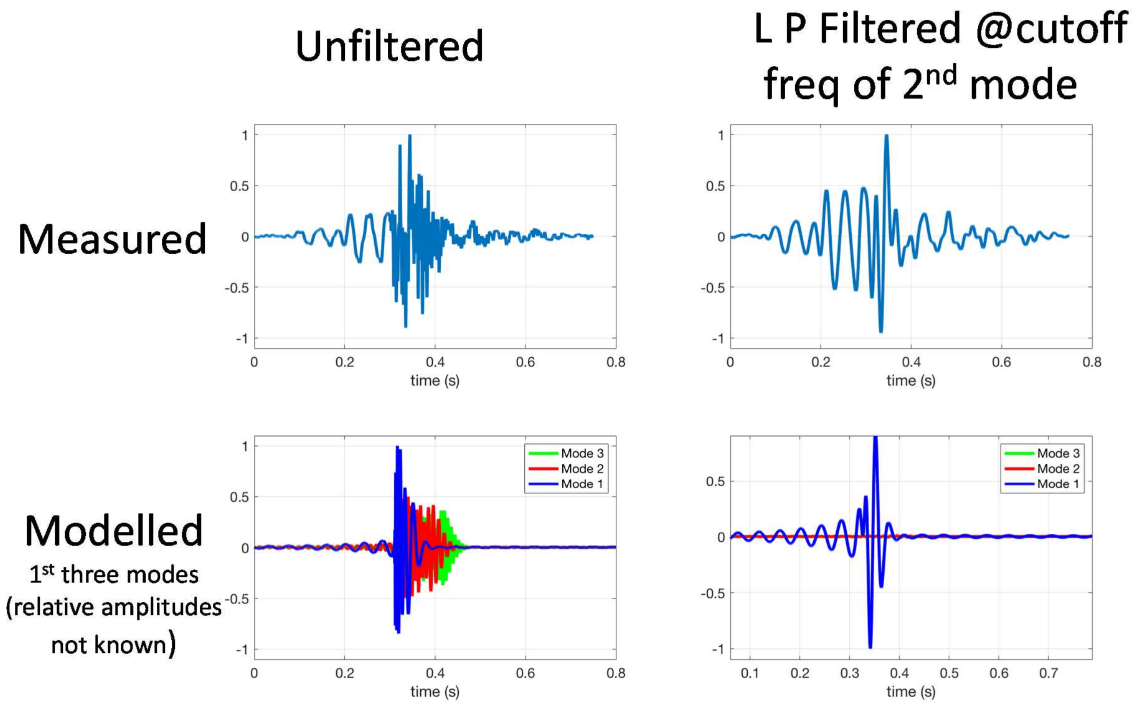

Figure 37: *Exploration of unfiltered and filtered representations of the seismic pulse at the closest approach distance for three modes combined or a single (2nd) mode extracted.*

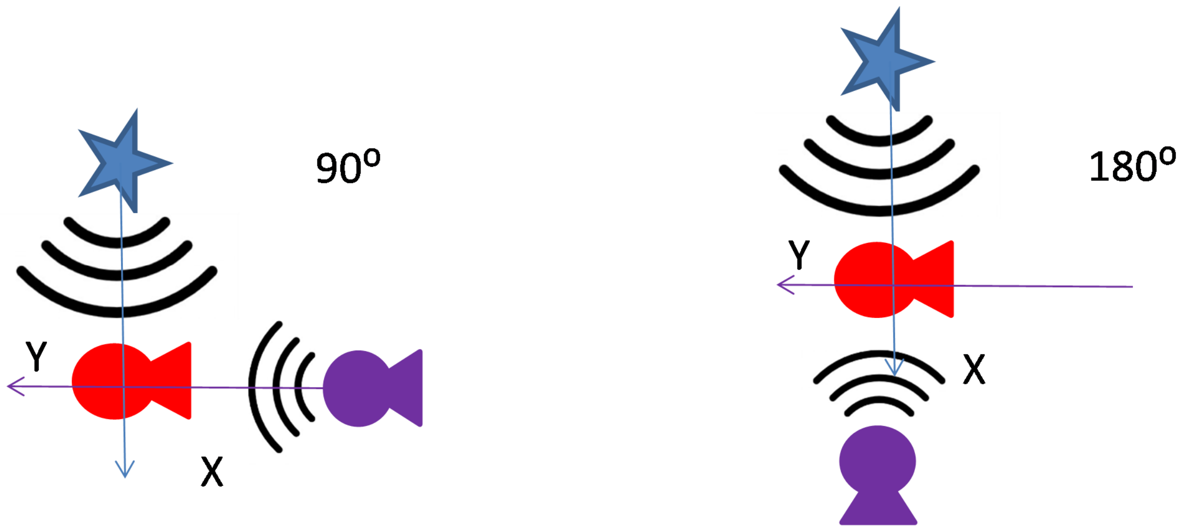

Figure 38: *Schematic illustration of the concept behind the planned modelling efforts. The star represents the seismic sound source at a certain distance. The red fish is the target fish receiver and the purple fish is a calling conspecific at certain distance, and at different angles from the source: 90° on the left and 180° on the right. Signal to noise ratios for the cod signal will be relatively straightforward for sound pressure and not depend on angle to the sound source. Signal-to-noise ratios for particle motion will vary dramatically with angle.*

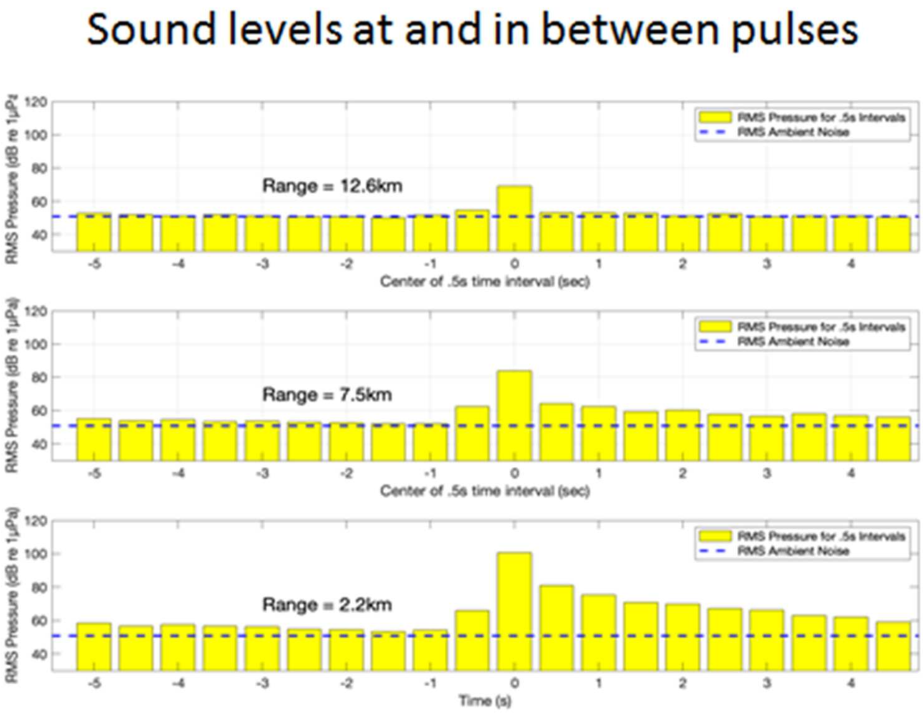

Figure 39. *A preliminary analyses of actual recordings at the tagging site during the experimental seismic survey for when the vessel was on its first track towards the tagged fish. Each bar represents the sound level (RMS Pressure in dB re 1uPa) in 0.5s bins and 0 is the time onset of the seismic pulse. Together all bars reflect the whole interval period from one pulse to the next.It is interesting to see that there is a clear precursor wave present prior to the pulse onset, which concerns a low-frequency ground wave traveling faster than the pulse spectrum through the water. At 12.6 km sound levels in the interval start to get above ambient level, while all bins are above at 2.2 km.*

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ANSWERS TO ORIGINAL QUESTIONS IV

Our main objective for this project component was to measure and model relevant sound field characteristics for both experimental set-ups; with captive fish in the floating pen and with free-ranging fish before, during and after the seismic survey. We assessed the sound energy distribution patterns across the area covered by the experimental sound exposure and explored detailed conditions around the exposed fish, in terms of sound pressure and particle motion, theoretically. We aim to answer the following questions:

1. How do tri-axial particle motion levels vary relative to pressure, in space and time, across the water column before, during and after an airgun sound pulse?

> *ANSWER: We have measured and modelled pressure and motion components of sound for the fish in the floating pen as well as for the tagged fish at the turbines. We have been able to elucidate the acoustic complexity in terms of both spectrum and reverberative temporal pattern.*

2. How do sea state and biotic cycles of sound-generating animals affect ambient particle motion conditions around fish?

> *ANSWER: We have assessed and explored ambient noise levels to address signal-to-nosie levels and illustrate the variable conditions for pressure and motion dependent on the vessel distance and angle relative to the site with our tagged fish.*

3. To what extent are fluctuations in tri-axial particle motion levels affected by the bottom or objects in the water nearby fish?

> *ANSWER: This question has not been addressed sufficiently yet and will be subject for further exploration.*

4. Are anthropogenic sounds distinct in both pressure and particle motion? And how do signal-to-noise ratios fluctuate for both during a seismic survey?

> *ANSWER: This question has not been addressed sufficiently yet and will be subject for further exploration.*

3. Budget overview and explanation

Contract budget and proposal for a partial extension

The original PCAD4Cod Phase II contract concerned a single contract between the IOGP and the three institutes IBL (Leiden University, the Netherlands), WMR (IJmuiden, the Netherlands) and VLIZ (Oostende, Belgium) and included the anticipated costs for the seismic vessel. We decided to split these in order to put the seismic vessel budget separate from the rest and to make European tenders for a seismic survey vessel not a potential issue for the PCAD4Cod team. We have now spent most of our budget and the expenses until the contract ended on 31st of March 2019 are clear (Table 2). We have overspent for the following reasons:

- Acoustic measurements (TNO/JASCO): we explored improvement of the VEMCO-telemetry signal detection and we hired the recording equipment with basic processing, which we had left out of the original budget.
- Salary costs (IBL/WMR): extra time had to be spent on negotiations with vessel companies after we lost the offer from GSO, which resulted in the much better deal with CGG in the end.
- Salary costs: extra time had to be spent on negotiations for permit requests after we had to change plans from a treatment site on the west-coast of the Netherlands to the north close to the German border and finally, to the Belgian site.
- Vessel expenses which we had not put on the budget for the right scale of this project and which became higher due to the GEMINI site being further offshore (WMR/VLIZ).
- Research expenses due to unforeseen difficulty in catching cod for the pen experiments and unforeseen heat in the Jacoba harbour, which made us have to switch some of our plans to cooled indoor conditions (IBL).
- Currency exchange rates fluctuated over the contract period, which made the budget amount in USD less value at variable rates in Euros (Figure 40).

We sought for sources that could at least partly cover these extra expenses and have been able to get the management of each of the three institutes willing to make an in-kind contribution (share investment costs in equipment/tools) and we received large benefits from the Belgian government waving vessel costs (accumulated € 297,000.-). We still end up with modest, but serious deficits on the three sub-budgets that amount to €127,329.-. The seismic vessel costs turned out to be a good deal in the end, leaving a positive balance of €348,000.- on this part of the budget. At this point, we also like to request for extra funding (€220,671.-) in order to complete the project successfully and process the unexpectedly large amounts of data that we collected in the first two years.

Work schedule extra budget explanation for the PCAD4Cod-team, including subcontractors:

Inge van der Knaap & Hans Slabbekoorn: We have budgeted (€115,730 direct costs and €28,941 as indirect costs for Inge van der Knaap, who moved to Leiden University to finish her last two years of the PhD-project. The first two years she spent at Ghent University and we had anticipated that we would be able to get additional funding for her at Ghent, but we did not. At Leiden University, Hans Slabbekoorn will now serve as her promotor and guarantee that she will finish her thesis. Inge has been the key player for the data collection at sea and coordinated and supervised all tagging and shipping and deployment of receivers and recording equipment together with Jan Reubens. The 2018 success with the large numbers of fish that remained in the area also requires extra processing time and we are currently investing in developing procedural and statistical methodology to enable this with Len Thomas and all three PhD-students. The funding will guarantee that all field data will be processed and planned papers will be written and submitted.

Len Thomas: We have budgeted €20,000 for his involvement (€5,000 within the current contract period and €15,000 within the extended contract period), which gives us 15.1 days of his time for consultancy on the project. Beyond the project review meetings, he has already been involved in videoconference meetings with the PhD students. The three PhD-students are visiting St. Andrews in the first week of June 2019 to work closely with Len. They will likely visit again later in the project, or Len will come to Leiden to work with them. He will be involved in advising on all statistical analyses, as well as helping to draft the papers listed above and providing feedback where required on the other papers, and in our reports.

Michael Ainslie: We have budgeted €15,000 for the extended involvement of Michael, and if needed Bruce Martin, who did the sound data processing for JASCO. Beyond the project review meetings, he has been involved from the start and has been the core advisor on acoustic measurements and modelling. He will attend progress/academic exchange meetings focussed on underwater sounds from the fish perspective and he will also be coordinating and advising on all acoustic analyses, as well as helping to draft the papers listed above and providing feedback where required on the other papers, and in our reports.

Peter Rogers: We continued to budget €30,000 for the extended involvement of Peter Rogers, and if needed James Martin, who have both been involved in discussing acoustic aspects of the project as well as propagation measurements and modelling in the Jacoba harbour (Peter has been very relaxed about declaring salary for his time and effort put into the project so far, which explains most of his unspent budget). He will be working on a modelling effort to explore the theoretical consequences of variation in signal-to-noise ratios in terms of pressure and the tri-axial directions of particle motion, which will provide insight into the fish perspective of seismic survey sound exposure for cod at our exposure site in the Belgian North Sea. He will also be advising on all acoustic analyses, as well as helping to draft the papers listed above and providing feedback where required on the other papers, and in our reports.

Frank Thomsen: We have budgeted €15,000 for the extended involvement of Frank and Lars Mortensen, who spearheaded a first draft of a conceptual paper about agent-based modelling application to sound impact assessments for cod in the North Sea. They both will continue to be advisor on project components that involve data integration for modelling and visualization applications. They will also have as key task to finish and submit the draft of the agent-based modelling paper listed above and provide feedback where required on the other papers, and in our reports.

As the contracts with each of the three institutes officially ended on the 31st of march 2019, and all practical work is done (except for plans with Peter Rogers), we like to propose to close the contracts with WMR and VLIZ according to schedule, but extend the contract between IOGP and IBL until 30-6-20 (as discussed with Jürgen Weissenberger in the communication about an earlier draft of this final report). This would cover the costs for the rest of the project and allow the three PhD-students (Jeroen Hubert and James Campbell already hired by Leiden University and Inge van der Knaap hired from the 1st of march by Leiden University) to receive supervision and advice from all team members and sub-contractors within the PCAD4Cod-project. We now provide a detailed overview of deliverables in terms of publications and scheduled submission dates (see below).

Currency exchange issues

Since the PCAD4Cod Phase I and the proposal associated with the contracts for phase II, the currency exchange rate between the US dollar and the Euro has fluctuated quite a bit. Since the start of the PCAD4Cod Phase II contract, on the 1st of April 2017, the rate went up from 1.07 (1.05 in proposal) to 1.25 and then down again to about 1.15 (Figure 35). Taking a mean shift in the exchange rate into account from 1.05 to 1.15, we should expect a detrimental budget difference of about 58, 46, and 30 kEuro for the three institutes (IBL, WMR, VLIZ) respectively.

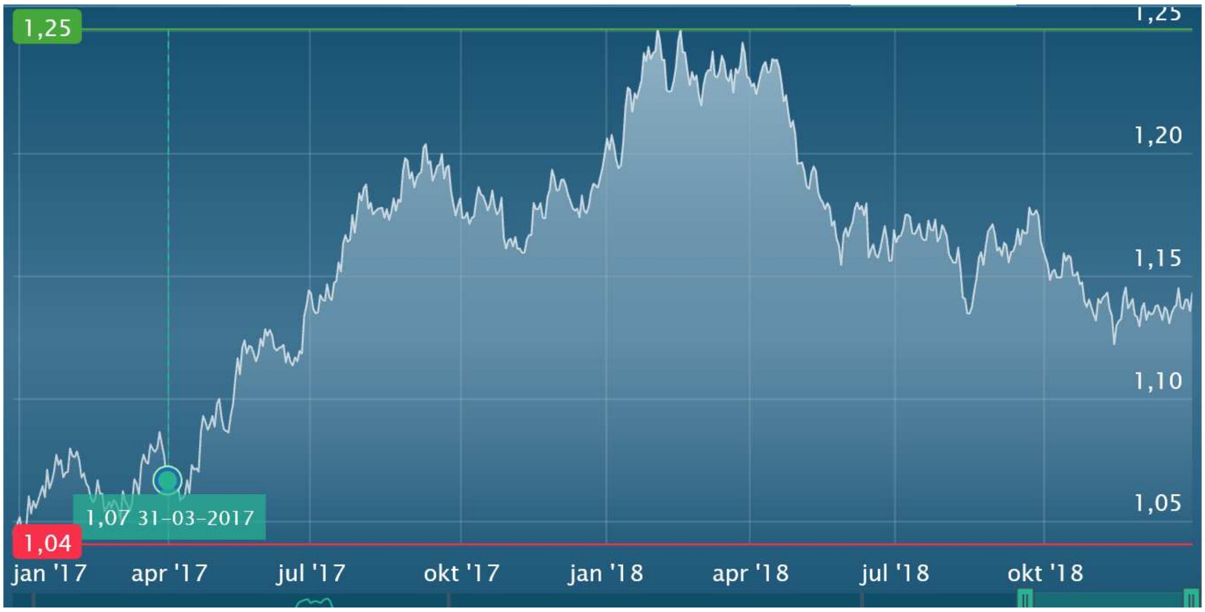


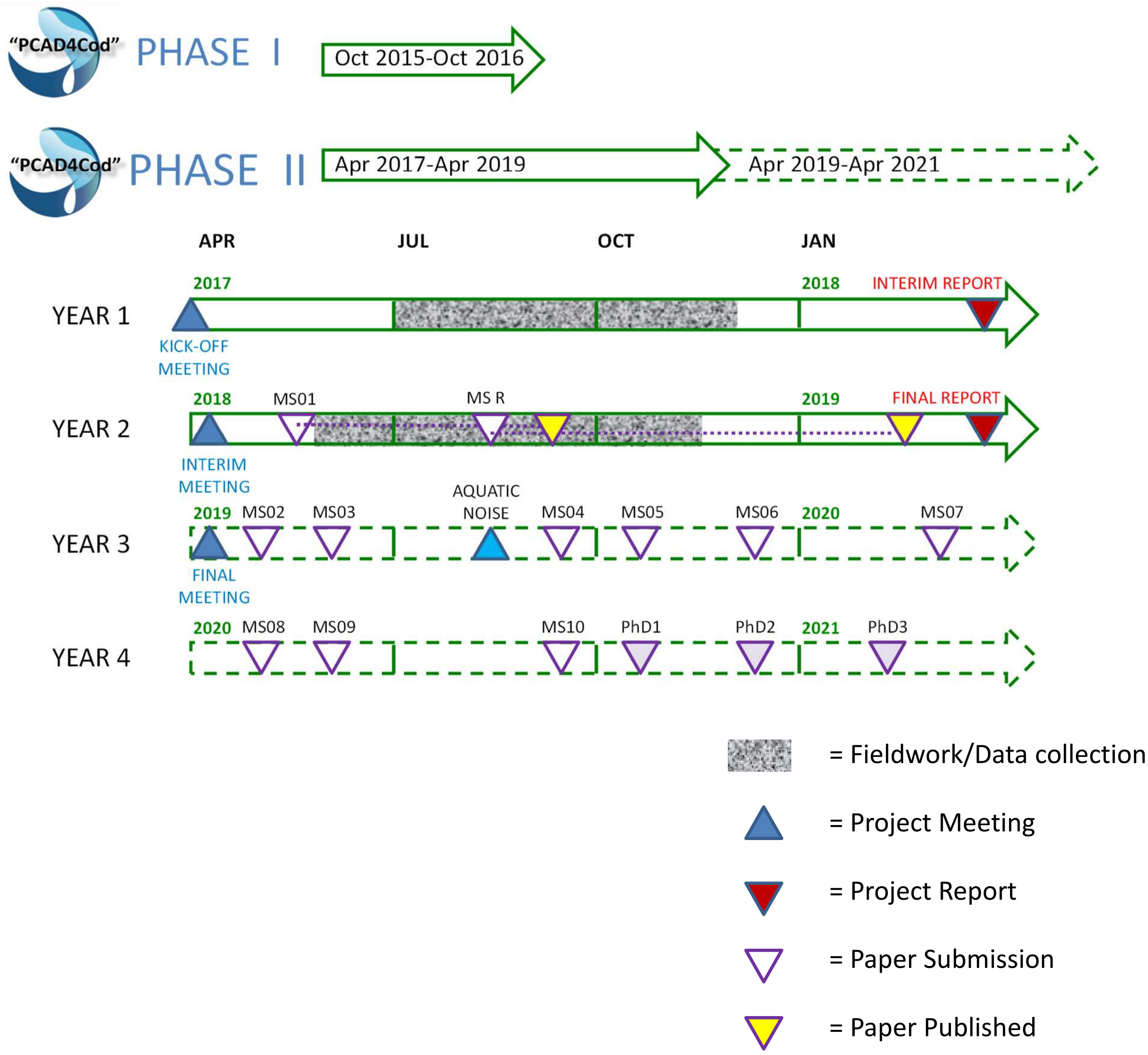
Figure 40: *Graph depicting Euro-US Dollar rate fluctuations between January 2017 and December 2018 (source https://www.valuta.nl/). The proposal budget had numbers in Euros and*

USDs at a rate of 1.05. The contract started on the 1st of April 2017, when the rate was 1.07. Over the contract period, the rate went up to 1.25 and down again to 1.1472 on the 28th of December 2018.

Table 2: *Financial overview of all three budgets of the three main institutes (IBL, WU, and VLIZ) involved. Under the three years of the contract period, all costs declared, or still to declare, are reported until 31-03-2019, the end of the current contract. To the right of these columns, the original budget is used to calculate what is left over and what the budget result will be, taking the contributions in kind into account from the three institutes (accumulated € 297,000.-). Each institute still spent more than the original budget, for reasons explained in the text. We spent much less in the end on the seismic vessel (at bottom part in yellow). The overall budget deficit (Budget result 1) concerns €127,329.- (including currency exchange differences). The positive balance on the seismic vessel budget concerns €348,000.-. The extra budget requested concerns €220,671.-, which would make us not go beyond the originally allocated funding.*

Budget holder 1:	Contract period			Original budget	Left over	Contribution	Budget result	Extended contract period			Budget result
IBL budget	**2017**	**2018**	**2019**	2017-2019	01/01/2018	in kind	1	**2019**	**2020**	**2021**	2
Direct costs	**€ 91,775**	**€ 122,366**	**€ 48,524**	**€ 262,665**	**€ 0**	**€ 0**	**€ 0**	**€ 77,154**	**€ 38,576**	**€ 0**	€ 115,730
Indirect costs	**€ 22,944**	**€ 30,592**	**€ 12,130**	**€ 65,666**	**€ 0**	**€ 0**	**€ 0**	**€ 19,294**	**€ 9,647**	**€ 0**	€ 28,941
Equipment and permits	€ 73,000	€ 96,500	€ 6,000	€ 50,000	-€ 125,500	€ 38,000	-€ 87,500	€ 0	€ 0	€ 0	€ 0
Advisory board - Thomas	€ 0	€ 0	€ 5,000	€ 20,000	€ 15,000	€ 0	€ 15,000	€ 10,000	€ 5,000	€ 0	€ 15,000
Subcontract VT - Rogers	€ 9,535	€ 0	€ 0	€ 60,543	€ 51,008	€ 0	€ 51,008	€ 20,000	€ 10,000	€ 0	€ 30,000
Subcontract TNO - Ainslie	€ 55,402	€ 0		€ 60,543	€ 5,141	€ 0	€ 5,141	€ 0	€ 0	€ 0	€ 0
Subcontract JASCO - Ainslie	€ 0	€ 50,000		€ 0	-€ 50,000	€ 0	-€ 50,000	€ 15,000	€ 0	€ 0	€ 15,000
Subcontract DHL - Thomsen	€ 27,384	€ 18,320		€ 45,704	€ 0	€ 0	€ 0	€ 15,000	€ 0	€ 0	€ 15,000
Consumables + Travel	€ 8,000	€ 9,000	€ 3,000	€ 20,000	€ 0	€ 0	€ 0	€ 500	€ 500	€ 0	€ 1,000
IBL Totals	**€ 288,040**	**€ 326,778**	**€ 74,654**	**€ 585,121**	**-€ 104,351**	**€ 38,000**	**-€ 66,351**	**EXTEND CONTRACT TILL 30-6-2020**			€ 220,671
					Total to declare:		**€ 651,472**				
Budget holder 2:											
WU budget											
Direct costs	**€ 82,385**	**€ 109,846**	**€ 84,366**	**€ 276,596**	**€ 0**	**€ 0**	**€ 0**				
Indirect costs	**€ 20,596**	**€ 27,462**	**€ 21,091**	**€ 69,149**	**€ 0**	**€ 0**	**€ 0**				
Equipment and permits	€ 6,500	€ 91,000	€ 0	€ 80,000	-€ 17,500	€ 38,000	€ 20,500				
Consumables + Travel	€ 2,500	€ 3,000	€ 1,750	€ 15,000	€ 7,750	€ 0	€ 7,750				
Vessel costs	€ 0	€ 98,000	€ 0	€ 20,000	-€ 78,000	€ 0	-€ 78,000				
WU Totals	**€ 111,981**	**€ 329,308**	**€ 107,207**	**€ 460,745**	**-€ 87,750**	**€ 38,000**	**-€ 49,750**	**CLOSE CONTRACT ON 31-3-2019**			
					Total to declare:		**€ 510,495**				
Budget holder 3:											
VLIZ budget											
Direct costs	**€ 54,234**	**€ 72,312**	**€ 27,490**	**€ 154,036**	**€ 0**	**€ 0**	**€ 0**				
Indirect costs	**€ 13,560**	**€ 18,078**	**€ 6,871**	**€ 38,509**	**€ 0**	**€ 0**	**€ 0**				
Equipment and permits	€ 38,478	€ 91,000	€ 0	€ 80,000	-€ 49,478	€ 38,000	-€ 11,478				
Consumables + Travel	€ 2,500	€ 3,000	€ 1,750	€ 7,500	€ 250	€ 0	€ 250				
Vessel costs	€ 51,000	€ 152,000	€ 0	€ 20,000	-€ 183,000	€ 183,000	€ 0				
VLIZ Totals	**€ 159,772**	**€ 336,390**	**€ 36,111**	**€ 300,045**	**-€ 232,228**	**€ 221,000**	**-€ 11,228**	**CLOSE CONTRACT ON 31-3-2019**			
					Total to declare:		**€ 311,273**				
All three budgets combined							**-€ 127,329**				
Seismic vessel costs	**€ 0**	**€ 252,000**	**€ 0**	**€ 600,000**	**€ 348,000**	**€ 0**	**€ 348,000**				
						€ 297,000	€ 220,671				

Overview of deliverables



MS-R Slabbekoorn, H., Dalen, J., de Haan, D., Winter, E., Radford, C., Ainslie, M.A., Heaney, K.D., van Kooten, T., Thomas, L. & Harwood, J. (2019). *Population level consequences of seismic surveys on fishes: an interdisciplinary challenge.* Fish and Fisheries: https://doi.org/10.1111/faf.12367.

MS01 Hubert, J., Campbell, J., van der Beek, J.G., den Haan, M.F., Verhave, R., Verkade, L.S., & Slabbekoorn, H. (2018). *Effects of broadband sound exposure on the interaction between foraging crab and shrimp - A field study.* Environmental Pollution 243: 1923-1929.

MS02 Soudijn, F., Slabbekoorn, H., van Kooten, T., de Roos A. (in prep – May 2019). *Theoretical potential for population level effects of acoustic disturbances in North Sea cod: a continuously size-structured energy-budget model.*

MS03 Campbell, J., Hubert, J., Rogers, P., Ainslie, M. & Slabbekoorn, H. (in prep – Jun 2019). *Acoustic variation of seismic sound pulses from a fish perspective: acoustic measurements and propagation modelling.*

MS04 van der Knaap, I., Campbell, J., Hubert, J., Slabbekoorn, Winter, E., Thomas, L., Reubens, J. (in prep – Sep 2019). *Telemetric measurements on North Sea cod at a windfarm: receiver matrix design and fish movement analyses.*

MS05 Hubert, J., Campbell, J., van der Knaap, I., Winter, E. & Slabbekoorn, H. (in prep – Oct 2019). *Behavioural patterns of cod in a floating pen during sound exposure: telemetry and accelerometry.*

MS06 Mortensen, L.O., Chudsinska, M.E., van der Knaap, I., Slabbekoorn, H. & Thomsen, F. (in prep – Dec 2019). *Potential effects of seismic sound exposure on cod populations: an agent-based modelling approach.*

MS07 Rogers, P., Campbell, J., Hubert, J., Ainslie, M. & Slabbekoorn, H. (in prep – Feb 2020). *Acoustic sound field variation around a fish during an experimental seismic survey: signal-to-noise ratios for pressure and motion.*

MS08 van der Knaap, I., Hubert, J., Campbell, J., Slabbekoorn, H., de Haan, D., Ainslie, M., Rogers, P. Winter, E., Thomas, L., Reubens, J. (in prep – May 2020). *Behavioural activity of free-ranging cod before, during and after and experimental seismic survey in the North Sea.*

MS09 Campbell, J., Hubert, J., van der Knaap, I., Winter, E., Reubens, J., Slabbekoorn, H. (in prep – Jun 2020). *Behavioural categories in dynamic accelerometer profiles from swimming, foraging, and resting cod.*

MS10 Slabbekoorn, H., van der Knaap, I., Campbell, J., Hubert, J., Kok, A., Soudijn, F., van Kooten, T., de Roos A., Rogers, P., Ainslie, M.A., Mortensen, L.O., Thomsen, F., de Haan, D., Berges, B., Winter, E., Reubens, J. & Thomas, L. (in prep – Sep 2020). *Predicting potential for population level consequences of seismic surveys on fishes: from field data to modelling implications.*

PhD1, PhD2, and PhD3 refer to the thesis defenses of the three PhD-students.

SUPPLEMENTARY MATERIAL:

Recent publication on related study by Bruce et al. 2018:

Marine Environmental Research 140 (2018) 18–30

Contents lists available at ScienceDirect

Marine Environmental Research

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

ELSEVIER

Quantifying fish behaviour and commercial catch rates in relation to a marine seismic survey

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Bruce et al. (2018) tagged 87 fish of three species, which were released between 30th March and 1st April 2015 at a treatment and control site in the western Gippsland Basin between the coast of Southern Australia and Tasmania. At the treatment site, a 2-D seismic survey was undertaken between 9-18 April 2015 investigating potential CO2 geosequestration sites. The M.V. Duke vessel was used to tow a single 2530 cubic inch airgun array (BOLT Long Life Array), comprised of 16 airguns towed at 6 ± 1m depth. The array had a working pressure of 2000 psi, with a lower acceptable limit of 1800 psi. Acoustic receivers (VR2W and VR2AR, Vemco-Amirix), tuned to detect frequencies of 69 kHz, were deployed in an array configuration within the experimental and control zones. Each array comprised 20 acoustic receivers arranged in five rows of four receivers each, spaced at 1000m intervals. Every second row was offset by 500 m, providing acoustic receiver coverage of an approximate 20 km2 area of seafloor. The control was more than 10 km from airgun operations and both treatment and control areas were in 50–60m water depth.

Behavioural changes in response to the survey were assessed by telemetry estimating displacement (the distance (metres) travelled 'between' receivers within each of the arrays) and movement (speed of movement in metres/second, determined by the accelerometer data). Data for the first two days after release were excluded to allow for a recovery period after the release of tagged fish. For displacement, a centre of activity (COA) was estimated for each fish following the methods of Simpendorfer et al. (2002). The COA is a weighted average of a fish's position over a time interval (Δt) based on the locations of all receivers that detected it, and weighted by the number of detections registered by each receiver. In addition to the telemetric data on three fish species, fisheries catch data (kg whole weight) on 15 species were extracted from a database of the Australian Fisheries Management Authority (AFMA) for two gear types (Danish seine and gill-net). A variable and inconsistent pattern of increased and decreased catch rates per species came out of this analysis.

The first two species of the telemetric data were two elasmobranch sharks, but did not yield any data on potential effects of the seismic survey because of low success rate in detecting them at the receivers. There were 33 gummy sharks (*Mustelus antarcticus*), of which 20 were released at the treatment site and 12 at the control site. Only 11 (35%) were reported by the receivers beyond the first two days after tagging and only 3 individuals had been present during the seismic survey period: 2 at the treatment and one at the control site. The two individuals that were reported at the treatment site during the survey had returned to the area just prior to or after a few days of seismic pulse activity. They left the area after being detected on one or two days, but were reported again later, one at the control site about a month later, and one at the treatment site, about two months later. There were 43 swell sharks (*Cephaloscyllium laticeps*), of which 24 were released at the treatment site and 19 at the control site. Only 13 (30%) were reported by the receivers beyond the first two days after tagging, and only 1 individual had been present during the seismic survey period at the control site. Two individuals moved from the control site to the treatment site, one even within the first two days after tagging to move back to the control site a week after the seismic survey had ended. Another individual, originally released at the treatment site, was two months after the survey reported at the control site and more than a week later at the treatment site again.

The third species with telemetric data was a ray-finned teleost and concerned 11 tiger flatheads (*Neoplatycephalus richardsoni*) which were all released at the treatment site. Nine individuals (81%) were reported by the receivers beyond the first two days after tagging (Figure 2). There were 8 individuals detected in the experimental array during the seismic survey, of which four were present during the entire survey period and four that left the area during the survey. Of these latter four individuals, one had been present on five days prior and six days into the 10-day survey period and another had been present four days prior and four days into the survey. The other two fish detected during the seismic survey arrived on the first day of the survey to stay for five days or arrived on the second day to leave again before the next day. The four that departed the treatment site during the seismic survey were not recorded to return in the four months of monitoring that followed.

The analyses of displacement and movement of the tiger flatheads revealed that the time of day at which the fish were most active was affected different for the different periods of this study. There were generally two peaks in activity over the day, which turned out to be later during the seismic survey than before the seismic survey: they shifted from about 8.00 to between 10.00 and 13.00 and from between 1700 and 1800 to between 1800 and 1900. The fish also moved more frequently after than before or during the survey period and had a higher average speed during than before or after. The latter was attributed to possible disturbance effects in startle responses or events of erratic swimming. The data on the behaviour of the nine tiger flatheads, of which eight recorded during the survey, concerns a single survey event at one location at one moment in time, but is consistent with a possible response to the seismic survey operations. The response is not one of large spatial displacement, but one of moderate changes in local activity as evident from variation in diurnal patterns and swimming speed. Energetic consequences of increased investment in movement or decreased opportunity to feed were beyond the resolution of the current data set. Physiological stress was not investigated and any potential impact cannot be excluded.

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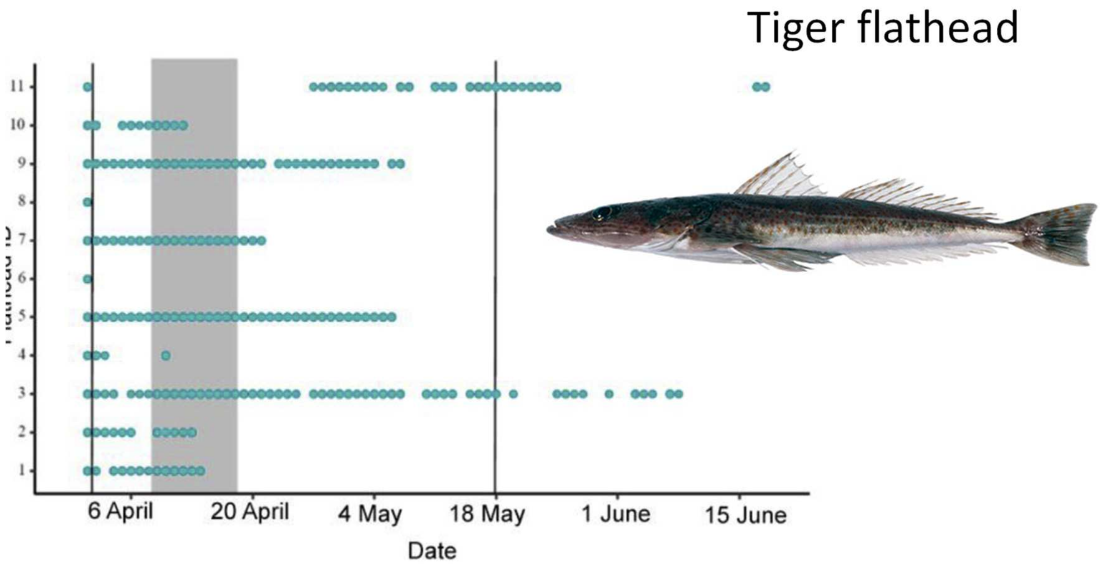

Figure x: *Daily presence of tagged tiger flatheads across the study periods in the area of the acoustic receiver array. The grey shaded area denotes the seismic survey period. The vertical bars define the period over which tag data were considered for fish behavioural analyses being two days post-release to one month after the end of the seismic survey (from Bruce et al. 2018).*