		Inventaris Wob-verzoek W17-11								
			wordt verstrekt				**weigeringsgronden**			
nr.		**document NTS 20171085**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1		Origineel aanvraagformulier				x		x		
2		NTS	x							
3		Projectvoorstel				x		x		
4		Bijlage beschrijving dierproeven 1				x		x		
5		Bijlage beschrijving dierproeven 2				x		x		
6		Bijlage beschrijving dierproeven 3				x		x		
7		Bijlage beschrijving dierproeven 4				x		x		
8		DEC-advies				x		x		
9		Ontvangstbevestiging				x		x		
10		Verzoek om aanvullende informatie				x		x		
11		Adviesnota CCD		x						x
12		Beschikking en vergunning				x		x		

22 MEI 2017

1.

Centrale Commissie Dierproeven

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☒ Ja > *Vul uw deelnemernummer in* 10600
☐ Nee > *U kunt geen aanvraag doen*

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	Universiteit Leiden
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	27368929
Straat en huisnummer	Rapenburg 70
Postbus	9502
Postcode en plaats	2300RA Leiden
IBAN	NL78RABO0102468869
Tenaamstelling van het rekeningnummer	Universiteit Leiden / Faculteit W&N

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted]
Functie	
Afdeling	
Telefoonnummer	
E-mailadres	

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	
Functie	
Afdeling	
Telefoonnummer	
E-mailadres	

1.6 *(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.

(Titel) Naam en voorletters	[redacted]
Functie	
Afdeling	
Telefoonnummer	
E-mailadres	

1.7 Is er voor deze projectaanvraag een gemachtigde?

☐ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*
☒ Nee

2 Over uw aanvraag

2.1 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag > Ga verder met vraag 3
☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn
Vul uw vergunde projectnummer in en ga verder met vraag 2.2
☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn
Vul uw vergunde projectnummer in en ga verder met vraag 2.3

2.2 Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?

☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
☐ Nee > Ga verder met vraag 3

2.3 Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?

☐ Nee > Ga verder met vraag 3
☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1 Wat is de geplande start- en einddatum van het project?

Startdatum	0 - 9 - 2017
Einddatum	31 - 12 - 2021

3.2 Wat is de titel van het project?

'PCAD4Cod' - Impact of seismic survey sound exposure on fish

3.3 Wat is de titel van de niet-technische samenvatting?

Het effect van seismisch onderzoek op zoutwater-vissen

3.4 Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?

Naam DEC	DEC Leiden
Postadres	[redacted] Postbus 9600 2300 RC Leiden
E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

- ☒ Nieuwe aanvraag Projectvergunning € 1684,- Lege
- ☐ Wijziging € [redacted] Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

- ☐ Via een eenmalige incasso
- ☐ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

- ☒ Projectvoorstel
- ☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

- ☐ Melding Machtiging
- ☐

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam: [redacted]

Functie: [redacted] Faculteit Wiskunde en Natuurwetenschappen

Plaats: Leiden

Datum: 17-5-2017

Handtekening: [redacted]

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10600
1.2	Provide the name of the licenced establishment.	Leiden University
1.3	Provide the title of the project.	'PCAD4Cod' - Impact of seismic survey sound exposure on fish

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

x Basic research

☐ Translational or applied research

☐ Regulatory use or routine production

☐ Research into environmental protection in the interest of human or

x Research aimed at preserving the species subjected to procedures

☐ Higher education or training

☐ Forensic enquiries

☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Project background and stake holders

The proposed experiments concern the core investigations with living animals (marine fish) of a project that is funded by the [redacted], which supports

independent research to help increase understanding of the effect of sound on marine life generated by oil and gas exploration and production activity. Our research proposal and team was selected in an open competition of a global Request For Proposals, based on scientific content and expertise, respectively.

The target information and insights of the project are fundamental in nature, but also have applied value and are shaped by the explicit call from society. Our research and findings will play a critical role in legislation and authorization of noisy offshore activities and are thereby important for ecology and economy.

Before the current plans (Phase II), the JIP has also funded a one-year desk study (Phase I), which included several workshops in 2015 and 2016 with experts from all over the world. Phase I has led to a 95-page report on: "Population level consequences of seismic surveys on fish: *Knowledge gaps and research strategies"* ([redacted] October 2016). The report is approved by the PCAD4Cod advisory board and the scientific board of the JIP.

Noise pollution in an acoustic underwater world

The amount of marine anthropogenic noise has increased over the last decades, globally and in the North Sea. This has led to a growing concern about its effect on aquatic life including fish. The visibility in the aquatic environment is typically low, so fish make use of sounds for all kinds of activities including orientation, communication, predator avoidance and reproduction. Consequently, noise pollution may hinder, mask, disturb and deter, but we still have limited insight into potentially detrimental effects on individual fitness and population dynamics.

A potentially important anthropogenic sound source is the performance of marine seismic surveys. Seismic surveys are explorations of the geological structure beneath the seafloor, this includes the search to oil and gas resources. Marine seismic surveys are conducted using a vessel towing one or two arrays of airguns and one up to more than ten streamers of hydrophones (Slabbekoorn et al., 2010; Carroll et al., 2016).

The airguns (seismic sources) produce high intensity, low-frequency impulsive sounds at regular intervals (e.g. every 10 seconds, potentially for sequences of several hours and repeated for several days to weeks and even months). Most energy of the sounds produced by airguns falls within the 10 – 300Hz frequency range, which is within the sensitive hearing range of most – if not all – fish (Carroll et al., 2016).

The sound pulses of seismic surveys can affect fish in a multiple ways, this is highly depended on the distance of the fish from the seismic source. The sound pulses are so loud and the rise-time is so short that fish close to the source can suffer from physical injury and immediate death. The range in which physical injury poses a threat is however relatively small compared to the range in which the sound is audible and in which it might induce masking of biological relevant sounds and yield physiological and behavioural effects. These effects are more subtle but can affect many more individuals and species and are therefore more relevant for the consequences at population level (Popper et al., 2005; Slabbekoorn et al., 2010).

The majority of the studies on the effect and impact of seismic surveys on fish focussed on confined or caged fish, because tracking free-swimming fish involves observational challenges. Some papers describe reduced or increased catch rates after seismic surveys, underlying mechanisms are usually not clear but might involve energetic costs and opportunity loss in terms of feeding or spawning due to spatial deterrence, change in swimming depth, reduced foraging activity, and increased swimming activity.

To predict the consequences of anthropogenic noise on marine mammals 'Population Consequences of Acoustic Disturbance' (PCAD)-models have been developed (e.g. New et al., 2013). But little progress has been made on such models for fish. It is not the specific target of this project to make a PCAD-model for a fish species, but we used a PCAD-framework to design a set of experiments, complementary to various modelling activities, that ultimately will help to get insight in the population level consequences of seismic surveys on fish.

References:
Carroll, A.G., Przeslawski, R., Duncan, A., Gunning, M., and Bruce, B. (2016). "A critical review of

the potential impacts of marine seismic surveys on fish & invertebrates," Mar. Pollut. Bull., **114**, 9–24. doi:10.1016/j.marpolbul.2016.11.038
Metcalfe, J.D., Wright, S., Tudorache, C., and Wilson, R.P. (2016). "Recent advances in telemetry for estimating the energy metabolism of wild fishes," J. Fish Biol., **88**, 284–297. doi:10.1111/jfb.12804
Neo, Y.Y., Hubert, J., Bolle, L., Winter, H.V., ten Cate, C., and Slabbekoorn, H. (2016). "Sound exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure," Environ. Pollut., **214**, 26–34. doi:10.1016/j.envpol.2016.03.075
New, L.F., Moretti, D.J., Hooker, S.K., Costa, D.P., and Simmons, S.E. (2013). "Using Energetic Models to Investigate the Survival and Reproduction of Beaked Whales (family Ziphiidae)," PLoS One, , doi: 10.1371/journal.pone.0068725. doi:10.1371/journal.pone.0068725
Popper, A.N., Smith, M.E., Cott, P. A., Hanna, B.W., MacGillivray, A.O., Austin, M.E., and Mann, D.A. (**2005**). "Effects of exposure to seismic airgun use on hearing of three fish species," J. Acoust. Soc. Am., 117, 3958. doi:10.1121/1.1904386
Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., and Popper, A.N. (2010). "A noisy spring: The impact of globally rising underwater sound levels on fish," Trends Ecol. Evol., **25**, 419–427. doi:10.1016/j.tree.2010.04.005

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

Consequences of seismic surveys for fish

The main objective of this project is to gain insight in the consequences of seismic surveys for fish behaviour and physiology. Our sub-goals are gaining insight into the effects of seismic surveys on swimming patterns/spatial avoidance, foraging activity, habituation, energy metabolism and whether the effect of seismic surveys on fish can be reduced by adjusting temporal or spectral features of the procedure and sound source. We want to gain this insight by complementary studies in captivity (caged fish in an on-shore tank and in a floating pen) and in the wild (free-ranging fish in the North Sea).

The objective of this project can be achieved within the proposed time frame through our expertise and experience. All researchers and collaborators have ample experience working with fish, addressing questions related to bioacoustics and fish behaviour, in both indoor and outdoor conditions. All experiments will be conducted based on methods that are already applied in earlier experiments by at least one of the researchers or collaborators involved in this project. The research team is supported by an international advisory board, which includes global experts in all aspects of our studies.

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

The impacts of human activities on aquatic animals have been a major concern to scientists, conservationists, fisheries, and policy makers, since poorly regulated but noisy marine exploitations may cause irreversible damage to the environment or fish stocks. This study will improve our understanding on the relationship between seismic surveys and behaviour and physiology of fish, which may lead us towards prediction tools for population consequences of aquatic noise pollution.

As sound may be responsible for changes in fish behaviour, species interactions and declines in fish stocks, with consequences for people in fisheries, the outcomes of the research will be of interest to several scientific fields (including behavioural biology, marine ecology, bioacoustics and conservation biology). However, the outcomes are critical to the offshore industries, which rely on the biological insights for project permits by European and American Law, as insights into detrimental effects or potential for mitigation are concerned essential for sustainable exploitation of marine resources.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

Recognizing behaviour with accelerometers in captive fish monitored by video

Effects of acoustic variation on fish response to sound in a floating pen

Sound exposure effects on fish growth and stress physiology in a floating pen

Full seismic survey effects on tagged fish while free-ranging at sea

We will 1) test telemetry tools (tags and accelerometers) and collect reference data in captive fish monitored by video to later interpret detailed fish behaviour in semi-captive and free-ranging conditions; 2) explore the temporal and spectral acoustic features, as well as exposure conditions, that are critical for acoustic response tendencies for fish in a floating pen (taking age and species into account); 3) test for effects of sound exposure on growth and stress physiology in floating pen conditions; and 4) investigate effects of a full seismic survey on tagged fish while free-ranging at sea. We will use Atlantic cod (*Gadus morhua*) as the model species throughout (in 1-4) and compare (sub-)adults (in 2) to juveniles and to other types of fish such as mackerel (*Scomber scombrus*) and sole (*Solea solea*).

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

Recognizing behaviour with accelerometers in captive fish monitored by video (1): We will study a limited number of individual fish to link accelerometer (3d motion tracker) data of tagged cod to their behaviour (appendix 1). This study will be conducted in a large indoor, transparent fish tank so we can film the fish and link the behaviour with the accelerometer output. These results will provide fundamental insights into cod swimming patterns that can be linked to metabolic requirements and that will be used in the other studies with fish in the floating pen and in the wild, for which we will not have video images. Tags with accelerometers have only recently become commercially available and they provide opportunities to measure detailed movements, recognize specific behaviours such as foraging behaviour and swimming bursts and thereby estimate costs and benefits in terms of energy budget (Metcalfe et al., 2016).

Effects of acoustic variation on fish response to sound in a floating pen (2): We will do experiments in a floating pen in a harbour at the Oosterschelde, which we used before in 2014 and 2016 to study the effect of anthropogenic sounds on fish [redacted] We will do different experiments in which we expose groups of (sub-)adult cod to a seismic sound source or artificial sound stimuli that deviate to a variable extent from the seismic pulse (appendix 2). To determine how fish respond to the sound exposures, we internally tag the group of fish with acoustic tags (to continuously determine their position in the net over time). During some of the experiments we will also internally tag them with the accelerometers. We will conduct 5 experiments in which we typically have 20 trials: 20 groups of 4 fish exposed to multiple sessions of (modified) seismic sounds.

Sound exposure effects on fish growth and stress physiology in a floating pen (3): We will use similar floating pens as in the acoustic response studies for a more long-term study to assess the effect from relatively long-term seismic airgun exposures on growth and physiology of cod (appendix 3). Fish will not have to be tagged for telemetry, but will remain for periods of several weeks in the harbor environment to get individual measures of changes in body size. They will only get a small personal identity tag under the skin. We will determine cortisol levels to assess base line conditions and test for acute and chronic elevations. There will be treatment fish exposed to noise pollution and control fish exposed to ambient conditions during the same periods in the water (which is possible because of the shallowness of the harbor, restricting high amplitudes to a relatively small zone around the sound source).

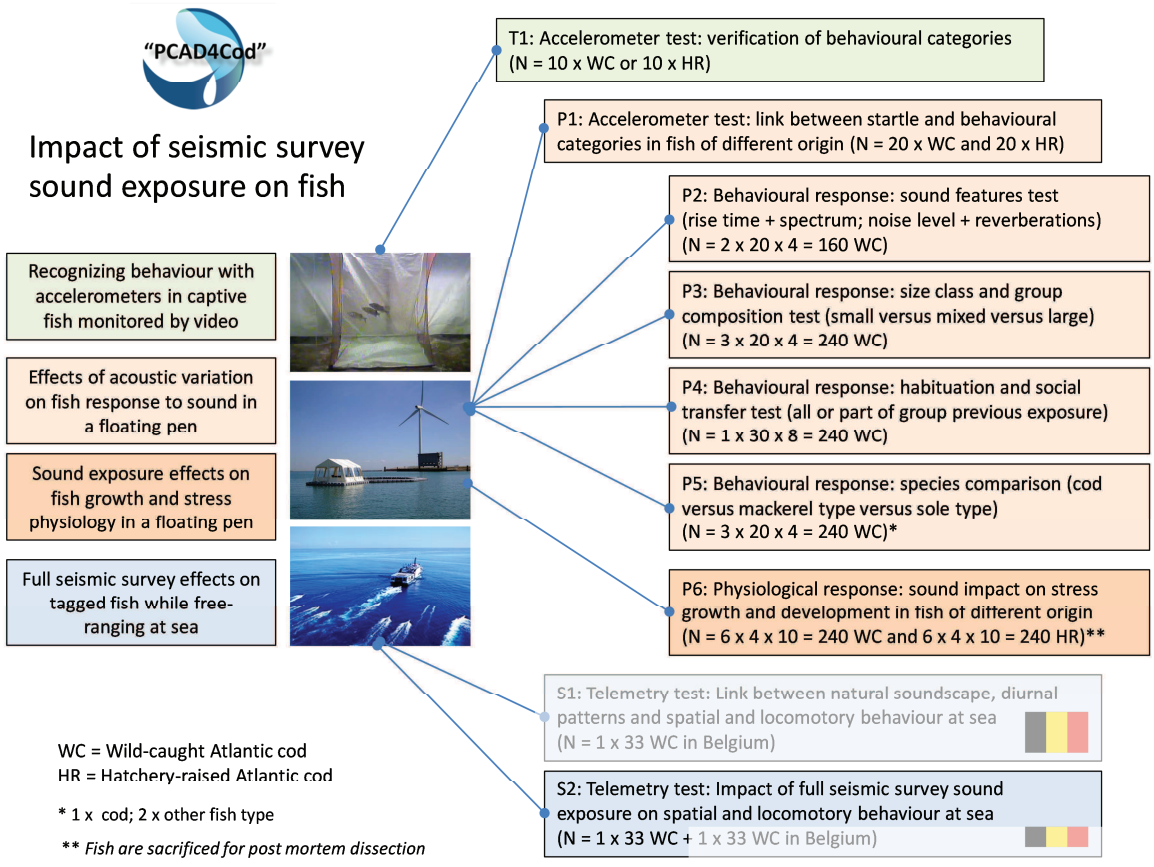
Full seismic survey effects on tagged fish while free-ranging at sea (4): We will do an experiment in the North sea, with wild, free ranging cod (appendix 4). We selected two sites (wind farms) in which we will tag cod with acoustic tags and accelerometers. These sites are in front of the Dutch and Belgian coast; very similar in ecology, distant enough to be acoustically independent, but close enough to have similar populations of cod and comparable weather conditions. Both sites are familiar to the research team and we have several years of experience with tagging cod at these sites. In 2017, we will conduct a small-

scale study in one site (Belgium) to fine-tune telemetry and accelerometer data collection (related to optimal set-up, preferred tag settings, technical procedures). In 2018, we will conduct the full seismic survey experiment: one of the sites will be exposed to a vessel with a full airgun array (Netherlands), the other site will serve as a control (Belgium). Detailed data on fish movements will be collected for several weeks before, several days during, and several weeks after the seismic exposure.

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

All four components are part of the overall JIP-project and are complementary in gaining insight into the effects of seismic survey sound exposure on fish populations (see overview below). Additional parts to the project concerns detailed measurement and modeling of sound field conditions in all tests (fish tank, floating pen and the North Sea) and energy budget modeling based on input parameters from the field data on behavioural responsiveness, intensity and duration as well as on physiological response measures of growth and stress hormone levels. All parts are expected to be done and published within four years from the start of this project on the 1st of April 2017 (experiments only start after CCD-approval).

The first indoor experiment (T1) will yield data that will also be used to interpret (part of) the data from accelerometers in the other experiments. The semi-natural experiments provide data in a more natural sound field than in the indoor fish tank and of higher resolution than is possible at sea (P1-P5). The floating pen also makes it possible to catch fish quickly after exposures, which enables monitoring growth and stress physiology (P6), which is not feasible at sea. Large numbers of fish can be tested in the floating pen for relative response differences to a variety of seismic and other artificial sounds. Also size and species comparisons can be done in these semi-captive conditions. However, the response data for a large number of acoustic features, ambient conditions and fish categories will only be put in a realistic perspective by the response of free-ranging cod to an actual seismic survey (S1 and S2, note that S1 and half of S2 will be carried out in Belgium and are not an explicit part of this Dutch application).



3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.	
Serial number	Type of animal procedure
1	Recognizing behaviour with accelerometers in captive fish monitored by video
2	Effects of acoustic variation on fish response to sound in a floating pen
3	Sound exposure effects on fish growth and stress physiology in a floating pen
4	Full seismic survey effects on tagged fish while free-ranging at sea
5	
6	
7	
8	
9	
10	

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

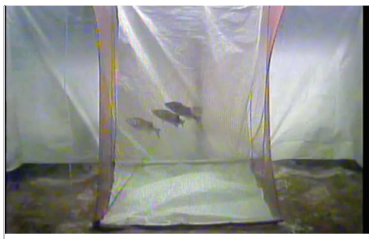
1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10600
1.2	Provide the name of the licenced establishment.	Leiden University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 Type of animal procedure: Recognizing behaviour with accelerometers in captive fish monitored by video

2 Description of animal procedures

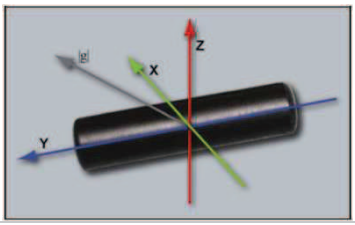
A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.



We here propose one experiment in which we will equip Atlantic cod with an accelerometer tags and record the cod on video to collect data on a variety of activities. We will provide multiple stimuli such as food and sound to gather data on movement characteristics related to feeding on particles in the water column or at the bottom and acoustically induced jerks or acceleration. We will also use this study to determine the recovery time of the tagging procedure.

In laboratory aquariums it is easy to video fish and thus to track and identify activities (Neo et al. 2015). However, the acoustic and behavioural validity is low in aquariums. The validity will be higher in outdoor settings, but there it is harder to get high-quality video on fish because of the low visibility underwater. A way to overcome this is to equip the fish with an acoustic tag that enables us to track the fish' position each second, in this way we can reconstruct swimming patterns but we cannot identify activities such as feeding or startle responses.



Previous work has shown, however, that data from accelerometer tags (motion trackers) can be used to identify activities (e.g. Broell e.a., 2013, see figure). To be able to identify specific categories of activities, we 'calibrate' the accelerometer-data by recording fish with accelerometers on video, in a fish tank with good visibility, and link activities to accelerometer data. This will yield reference data on movement patterns

and behavioural categories that we will use as reference in outside studies.

[redacted]

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

The fish will be caught in the North Sea from a research vessel using fishing rods. On the vessel, the fish will be kept in holding tanks, water in these tanks will regularly refreshed with North Sea water using a pump. After landing at the shore, the fish will be transported to the holding tanks at [redacted] Here, the fish can recover and acclimatize for at least 3 weeks before being used in the experiment. Two days before tagging, the fish will be transferred to a separate tank in which its behaviour can and will be recorded on video.

Right before tagging, the fish will be transferred into an anesthetics bath with 2-phenylethanol. Once anesthetised, the fish will be implanted with an accelerometers tag in its abdominal cavity with an incision of 2 cm and sutures (fig. 1). The size-dependent selection of the tag will result in a tag that is less than 5% of the fish body weight, and is not expected to alter the swimming patterns of the fish. The process (anesthesia and tagging) will take less than five minutes and the fish will start swimming again a few minutes after the surgery.

Right after the surgery, the fish will be transferred back into the tank in which the fish can be recorded on video. As in standard practice, no analgesics will be used after the surgical implantation. From our experience, fish do not show signs of pain after such minor surgeries. Research on analgesia in fish is still in its infancy and very little is known about the possibilities and consequences of its usage (Gräns et al., 2014; Sneddon, 2012; Stevens, 2008).

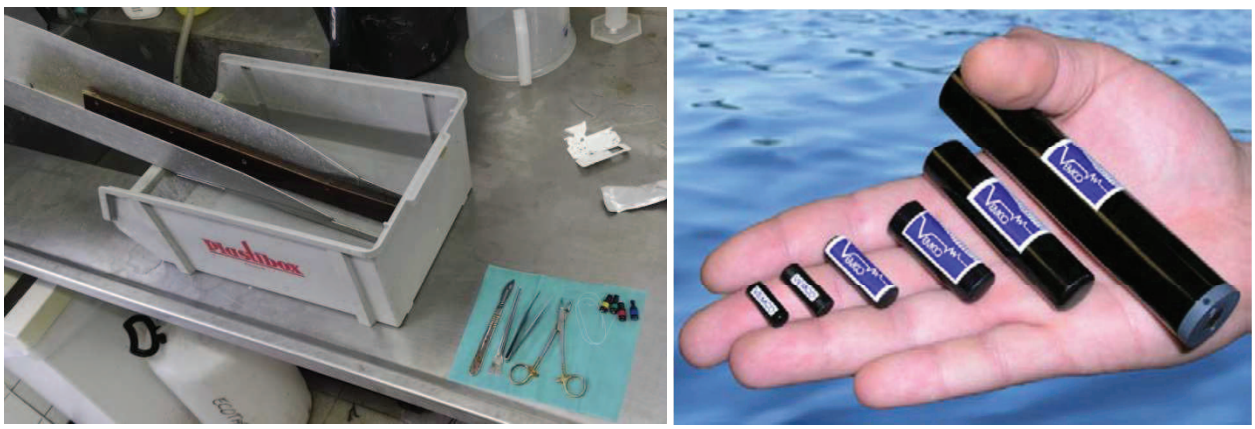


Figure 2: Tagging set-up at [redacted] (left) and tags of various sizes (right). The middle two classes are appropriate for our fish size classes.

We expect the fish behaviour to recover within two days (based on previous experiments that involved internal tagging in European seabass), but we will video the fish for a maximum of 5 days, and in this way assess the full recovery time. From day one, we will provide several food items to assess whether the fish resumes feeding and to track the behaviour with the accelerometers. On the last day we will playback several short sound stimuli to elicit startle responses to also track this with the accelerometers.

After the last video recording, the fish will be caught using a scoop net and its tag will be removed using a similar procedure as the tag implantation. The fish will be anesthetised in a bath with 2-phenylethanol.

Once anesthetised, the incision made for tag implantation will be opened again using an incision. Depending on whether the position of the tag inside has changed, the incision may be slightly bigger than the incision for tag implantation. We will remove the tag with tweezers. The process (anesthesia and tagging) will take less than five minutes and the fish will start swimming again a few minutes after the surgery. The fish will be kept in a recovery tank for at least a week before being released in the wild again.

References:

Gräns, A., Sandblom, E., Kiessling, A. and Axelsson, M. (2014). Post-surgical analgesia in rainbow trout: Is reduced cardioventilatory activity a sign of improved animal welfare or the adverse effects of an opioid drug? *PLoS One* **9**.

Sneddon, L. U. (2012). Clinical Anesthesia and Analgesia in Fish. *J. Exot. Pet Med.* **21**, 32–43.

Stevens, E. D. (2008). " Pain " and analgesia in fish : What we know , what we do not know , and what we need to know , before using analgesics in fish. In *Conference: Blue sky to deep water: the reality and the promise. ANZCCART Proceedings, At Auckland, New Zealand*, pp. 115–124.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

For this experiment, we need 10 Atlantic cod. The main goal of this study is to calibrate accelerometer tags, for this we do not just aim for significant differences within parameters, but we want to be able to identify and categorize activities. A similar study with great sculpins was done by Broell and colleagues (2013): for them 7 individuals were enough to calibrate the accelerometers resulting in near 80% correctly identified activities (at a 100hz sampling rate). We want to use 10 instead of 7 fish to be able to use the data from 3 individuals to test the calibration.

Broell F., Noda T., Wright S., Domenici P., Steffensen J. F., Auclair J. P., Taggart C. T. (2013) Accelerometer tags: detecting and identifying activities in fish and the effect of sampling frequency. *J. Exp. Biol.* **216**: 1255−1264.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

We will use Atlantic cod (*Gadus morhua*) caught in the North sea using fishing rods. We want to use wild-caught cod because they might exhibit more natural behaviour (than fish from a hatchery). This preference is a direct consequence of the explicit target to address potential effects on wild-ranging fish and to use complementary data to gain insight from floating pen and open water studies. We also use hatchery fish in other project components as we plan explicit comparisons between wild caught and hatchery raised fish. These comparisons will make a better link to the physiological studies that benefit from prior knowledge of age and background of individual fish (unavailable for wild caught fish) and are therefore including hatchery raised fish.

We will use fish of both sexes. The size will be dependent on what we will catch, but we aim for subadult fish of about 35-40 cm, but large variation in size is not a problem in an experiment like this and might even increase the validity of the results ([redacted] We only bring in healthy individuals from our fishing activities and do not expect any dropouts due to housing or handling procedures.

We chose to use Atlantic cod because it is an important and abundant fish species with a large distribution. Also accelerometer-data of cod have been correlated to energy metabolism (in prep), which means that we will be able to link our accelerometer-data to energy metabolism under seismic survey conditions. Furthermore, cod's hearing ability and ecology are generally similar to several other important commercial fishes, such as European seabass (*Dicentrarchus labrax*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*) and pollock (*Pollachius pollachius*).

We will use 10 individuals for this experiment.

C. Re-use

Will the animals be re-used?

x No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

x No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
The behaviour of fish can only be studied in a living specimen. Modelling is no option as species and size specific data are needed to interpret movement effects on accelerometer data to allow comparisons to field data on the complementary studies in the other appendices 2 and 4. Replacement is not possible.

Reduction
We use a limited amount of fish to achieve two goals (1. Link accelerometer data to behaviour; 2. Study the recovery after tagging).

Refinement
We have chosen to use Atlantic cod for this project. This species is known to do well in captivity and thanks to a partnership with [redacted], we can make use of specialized facilities to keep the fish until the start of the experiment.

We use an experimental set-up that has yielded valuable results in previous years. Throughout the years, the set-up has been refined. Our experience with this effective set-up ensures that unnecessary animal suffering can be avoided.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

1) animal suffering, pain or fear
The aim of this study is to investigate behavioural categories and recovery after tagging in fish. Great care on the handling of fish to minimise stress is crucial in obtaining our objective. During the tagging process, 2-phenylethanol will be used as anesthesia to reduce mobility, sensory input and pain. The process is quick and the fish are expected to recover quickly after the procedure. We also allow ample time for the fish to recover from tagging and acclimatise to their new environment.

2) adverse effects on the environment
The experiment is conducted in a fish tank with fresh salt water directly from the nearby source (Oosterschelde). Water circulation and refreshment does not involve any chemical additives and therefore no adverse effects on the environment are to be expected.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

We have done an extensive review of the literature on the use of accelerometers in fish research. Also, we are being advised by – and collaborate with - fellow experts from all over the world and we visit conferences on topics that relate to these proposed studies. We think that there is an urgent need to do studies that ultimataly give new and critical insight into population consequences of seismic surveys.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

x No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☐ No > Continue with question H.

x Yes > Describe this establishment.

The animal procedures will be carried out [redacted] is specialised in applied research regarding marine aquaculture and ecology.

The fish will be kept in holding tanks of 14.4 m^3 which fits a maximum of 120 individuals. The stock and experimental fish tanks will have a sandy bottom and the water will be continiously refreshed with fresh seawater from the Oosterschelde. In this way, we ensure that water quality and temperature are simular to the natural enviroment of cod.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

[redacted] offers excellent research facilities for the housing, maintenance and care of different North Sea fish species. It is right next to our field site, making the transfer of fish between holding tanks and outdoor experimental pen very quick, thus minimizing transfer stress experienced by the fish.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

x Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

x Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Prior to the tagging procedure, the fish will be put into an anesthetic bath of 2-phenylethanol solution. 2-phenylethanol is known to be an effective anesthesia for fish with a short recovery time. As in standard practice, no analgesic is necessary after surgery implantation on fish as fish do not show signs of pain after the procedures (Stevens, 2008).

Reference:

Gräns, A., Sandblom, E., Kiessling, A. and Axelsson, M. (2014). Post-surgical analgesia in rainbow trout: Is reduced cardioventilatory activity a sign of improved animal welfare or the adverse effects of an opioid drug? *PLoS One* **9**,.

Sneddon, L. U. (2012). Clinical Anesthesia and Analgesia in Fish. *J. Exot. Pet Med.* **21**, 32–43.

Stevens, E. D. (2008). " Pain " and analgesia in fish : What we know , what we do not know , and what we need to know , before using analgesics in fish. In *Conference: Blue sky to deep water: the reality and the promise. ANZCCART Proceedings, At Auckland, New Zealand*, pp. 115–124.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Since the fish with be kept in tanks with re-circulating water from the sea, there is a potential that the fish may be infected by infectious diseases from seawater. The risk will be highest after tagging.

Explain why these effects may emerge.

For tagging we need to make an incision which we make the fish more viable for infectious diseases.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

We think we can decrease the likelihood to a minimum by only tagging fish that have good health and body condition. Also, we will prevent any water from entering the incision because the abdomen will be above water during the surgery. Fish housing facilities are provided by [redacted], who is very experienced in aquaculture and marine research.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

x Yes > Describe the criteria that will be used to identify the humane endpoints.

If there is visual evidence for lasting, abnormal behaviour, obvious disease or physical disorder, such as patches of scale loss and red or white spots, the fish will be euthanized with a percussive blow to the head to free them from any unnecessary suffering.

Indicate the likely incidence.

Stress caused by the transport from hatchery and potential infections. Based on our previous experiments, we expect very little likelihood that these occur.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Fish catching (at the North Sea) and transferring - mild
Surgical implantation of tag – moderate
Various food stimuli – none
Sound stimuli - mild
Surgical removal of tag – moderate
Overall – moderate

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

x No

☐ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☐ Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10600
1.2	Provide the name of the licenced establishment.	Leiden University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Effects of acoustic variation on fish response to sound in a floating pen

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

To examine the effects of seismic surveys on fish, we will expose Atlantic cod to sound sources (scaled air gun and/or underwater speaker) in an outdoor net pen. We will identify behaviour and position via telemetry and accelerometry (follow-up from previous indoor test); test response variation to acoustic variation; explore influence of fish size and group composition; and test habituation and effects of previous experience by all or part of the fish. We will also compare cod with two other fish types, such as mackerel and sole.

The behaviour of the fish in all behavioural tests will be tracked using 3D acoustic telemetry and in some tests also accelerometers (for the latter, we use the results from appendix 1). The telemetry will provide data on the swimming activity of the fish, from which we can quantify changes and recovery in swimming depth, swimming speed, group cohesion and spatial avoidance. The accelerometers will provide data about the detailed motion patterns of the fish, which will yield insight into their energy metabolism and enables the identification of more complex behaviours like foraging (see appendix 1). All parameters will help us to gain insight into the effect of seismic sources on fish.

P1: Accelerometer test: link between startle and behavioural categories in fish of different origin (N = 20 x WC and 20 x HR)

P2: Behavioural response: sound features test (rise time + spectrum; noise level + reverberations) (N = 2 x 20 x 4 = 160 WC)

P3: Behavioural response: size class and group composition test (small versus mixed versus large) (N = 3 x 20 x 4 = 240 WC)

P4: Behavioural response: habituation and social transfer test (all or part of group previous exposure) (N = 1 x 30 x 8 = 240 WC)

P5: Behavioural response: species comparison (cod versus mackerel type versus sole type) (N = 3 x 20 x 4 = 240 WC)

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

All fish will be caught in the North Sea from a research vessel using fishing rods. On the vessel, the fish will be kept in holding tanks, water in these tanks will regularly refreshed with North Sea water using a pump. After landing at the shore, the fish will be transported to the holding tanks at [redacted]. Here, the fish can recover and acclimatize for at least 3 weeks before being used in the experiments.

In total, we want to conduct 5 different experiments in the outdoor net-pen, that follow a similar design, but differ in (sound) stimuli, amount of sound exposures or species. The experiments sometimes require a different number of animals (table 1) and the fish will be equipped with either only an acoustic tags or both an acoustic and an accelerometer tag.

Tagging
Before any of the experiments can start, the animals need to be tagged. To do so, the fish will be transferred into an anesthetics bath with 2-phenylethanol. Once anesthetised, the fish will be implanted with a small acoustic tag (volume: 1.4 cm^3; weight: 4.6 g; Model 795-LG, HTI Inc, US) and/or a tag with accelerometers (fish size dependent, < 5 % of the body weight) in its abdominal cavity with an incision of 2 cm and sutures. Note that we will make sure that the total weight of both tag(s) will be less than 5% of the fish weight, and that they are not expected to alter the swimming of the fish.

The process (anesthesia and tagging) will take less than five minutes and the fish will start swimming again a few minutes after the surgery. After this, they will be placed in a recovery tank for at least two days before being transferred to the outdoor floating pen, where the experiments take place. Although we expect the fish behaviour to recover within two days (based on previous experiments that involved internal tagging in European seabass), we will examine twice a day for stress-related behaviours, such as freezing and bottom-swimming, to see if a longer period is needed for cod. The fish will only be used for experiments when they have resumed their normal swimming behaviour without signs of discomfort.

As in standard practice, no analgesics will be used after the surgical implantation. From our experience, fish do not show signs of pain after such minor surgeries. Research on analgesia in fish is still in its infancy and very little is known about the possibilities and consequences of its usage (Gräns et al., 2014; Sneddon, 2012; Stevens, 2008).

Experimental design
At the start of each experiment, the fish will be transferred to the floating pen in an artificial cove in the Oosterschelde. This cove ([redacted], fig. 1) is situated right next to [redacted]. After transfer to the floating pen (fig. 2), the fish will be allowed to acclimatise to the new environment for at least eight hours, before being exposed to sound of a seismic source or sound from an underwater speaker.

Data will be collected on swimming activity via the sound emitting tags. Using the acoustic tags, we can determine the position of all individuals every second. From these data, we can derive the swimming speed, swimming depth, group cohesion and distance from the sound source. Using accelerometer tags we can identify startle responses, feeding behaviour, lateral rotations, overall dynamic body acceleration and tail-beat frequency. The latter two will give insight into potential impact on the energy metabolism of the fish. We will use a before-during-after treatment design.

We will treat levels, of all of the above mentioned behavioural response parameters, 'before' the treatment as baseline behaviour and we will compare these levels for periods 'during' and 'after' the treatment (within-subject comparisons). This before-during-after design also enables us to determine recovery within a trial and reduced responsiveness between trials (habituation), by calculating how long it takes till certain levels return to baseline levels ([redacted]). This has proven to be a useful measure to compare different treatments (like we will do in experiment 2) and likely also to compare different life stages (as in experiment 3).

[redacted] "Sound

exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure," Environ. Pollut., **214**: 26–34. doi:10.1016/j.envpol.2016.03.075
[redacted] (2016). Swimming bass under pounding bass. Fish response to sound exposure. PhD-thesis Leiden University.

Figure 1: The [redacted] with the floating pen

Figure 2: Floating pen and office tent

Experiment P1: Accelerometer test: identification of behavioural categories in fish of different origin

In this experiment we want to gain insight into the behavioural response of Atlantic cod on single airgun exposures. We will equip the cod with acoustic tags to determine their swimming patterns and also equip them with accelerometers to gain insight in more complex behaviours such as foraging. We will expose the cod to two periods of sound exposures per day for two days (two days extension period possible if more replication of behavioural categories per individual are needed), a single exposure takes 30-60 minutes. Also, if necessary on top of natural availability, we will add food stimuli to identify additional behavioural categories and to assess the effect of airgun exposures on feeding behaviour. We will compare 20 individuals that are wild-caught and 20 hatchery-raised cod.

Experiment P2: Behavioural response: sound features test

In this experiment we plan to play back sounds that reflect seismic survey conditions at various distances from the source by modifying some of the acoustic parameters. In this way, we will gain insight into when and why fish may be affected and whether it is possible to decrease effects on cod behaviour. We will use 2 x 20 groups of 4 individuals (Atlantic cod) and the fish will be equipped with both acoustic and accelerometer tags. The groups will be exposed to two sound treatments of 30 – 60 minutes per day, for two days. One series of 20 groups will focus on the effect on response from rise time and spectrum, the other series will focus on noise level (ambient conditions as background to seismic pulse) and reverberations (accumulating echoes with distance).

Experiment P3: Behavioural response: size class and group composition test

Noise impact studies – including the other experiments in this appendix - are usually (highly) controlled and therefore use uniform (groups of) subjects. However it might be that cod of different life stages (sizes) respond different to sound exposures. This is also important for determining population consequences. Because of that, we will compare the responses of two life stages (sizes) of Atlantic cod to seismic airgun exposures. We will compare the two life stages via 20 groups each with 4 individuals. In addition, to test whether size classes affect each other in mixed composition groups, we will have another 20 groups of 4 fish in which 2 are small and 2 are large. The fish will only be equipped with acoustic tags because we have calibrated the accelerometers for a single life stage (see appendix 1) and two tags might be too much for small fish. All groups will be exposed again to two sound treatments of 30 – 60 minutes per day, for two days.

Experiment P4: Behavioural response: habituation and social transfer test

It might be that cod initially respond strongly but that they habituate over time. This is highly relevant because seismic surveys in a specific area can take months and continue with pulse sequences that are hours long. We want to gain insight into short- and long-term habituation (intra- and inter-trial recovery) and into a phenomenon labelled as social transfer: experienced fish may transfer their response tendency to naïve fish. We will therefore test 3 x 10 groups of 8 fish. The first 10 will all 8 be in the floating pen for two days of repeated exposure. The second group will have 4 fish exposed to repeated sequences on day 1 and 4 naïve fish will join for exposures on day 2. The third group will have all fish in on day 1, but without exposure, making them all naïve to repeated exposure on day 2. Repeated exposure will include a maximum of 8 half-hour periods per day for two days.

Experiment P5: Behavioural response: species comparison (cod versus mackerel type versus sole type)

This project mainly focusses on cod as the prime model species, but we also want to explore to what extent the effects of seismic surveys on cod are comparable to the effects of seismic surveys on other abundant fish species in the North Sea. For that reason, we want to make a species comparison among cod, mackerel and sole as representatives of benthopelagic (living close to the bottom), pelagic (living across the water column) and benthic fish (living at or in the bottom). We will test again 20 groups with 4 individuals for all three species (in total 60 groups), they will be exposed to airguns for 30-60 minutes only once. The fish will be equipped with an acoustic tag. As this study includes a species that makes

explicit use of a sandy bottom, we will adjust our test conditions to have a few centimeters of sand on a bottom plate for the fish to lay down and to cover themselves.

Table 1: Summary of the behavioural experiments (P1-P5) planned for a period of four years. WC=wild-caught; HR=hatchery-raised.

Experiment	Individuals + origin (WC/HR)	Tags (acoustic/accelerometer)	Number of exposure days
P1 behaviours	20WC + 20HR = 40	Both	2-4
P2 sound features	2 x 20 x 4 = 160WC	Both	2
P3 size classes	3 x 20 x 4 = 240WC	Acoustic tag only	2
P4 habituation	3 x 10 x 8 = 240WC	Acoustic tag only	2
P5 species comparison	3 x 20 x 4 = 240WC	Acoustic tag only	2
Total:	900WC + 20HR		

After the last trial, the fish will be caught using a scoop net and its tag will be removed using a similar procedure as the tag implantation. The fish will be anesthetised in a bath with 2-phenylethanol. Once anesthetised, the incision made for tag implantation will be opened again using an incision, depending on the position of the tag we might have to make the incision slightly bigger than for implantation. We will remove the tag with tweezers. The process (anesthesia and tagging) will take less than five minutes and the fish will start swimming again a few minutes after the surgery. The fish will be kept in a recovery tank for at least a week before being released in the wild again.

References:

Gräns, A., Sandblom, E., Kiessling, A. and Axelsson, M. (2014). Post-surgical analgesia in rainbow trout: Is reduced cardioventilatory activity a sign of improved animal welfare or the adverse effects of an opioid drug? *PLoS One* **9**,.

Sneddon, L. U. (2012). Clinical Anesthesia and Analgesia in Fish. *J. Exot. Pet Med.* **21**, 32–43.

Stevens, E. D. (2008). " Pain " and analgesia in fish : What we know , what we do not know , and what we need to know , before using analgesics in fish. In *Conference: Blue sky to deep water: the reality and the promise. ANZCCART Proceedings, At Auckland, New Zealand*, pp. 115–124.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We study fish in groups because cod are social (shoaling) species that show natural behaviour only in groups. Moreover, some behavioural changes of interest, such as group cohesion (inter-individual distance) can only be studied in groups. The choice of four fish is based on minimising fish number while maintaining natural group dynamics. See table 1 for the number of fish that is needed for this experiment, in total 920 fish from which 740 Atlantic cod, and two other fish species with both 80 individuals. We typically use 20 groups as our replication sample (per experimental group i.e. life stage or species), this is slightly more than in our previous experiments using the same set-up. Reason for this is that the variation was slightly higher than expected based on indoor experiments. The number of 20 groups was determined using a one-sided t-test power analysis that was executed in R using the package *pwr*. We set the statistical power at 80% and p-value at 0.05, and expecting a difference of 0.2 m (primary parameter of interest: swimming depth) with a standard deviation of 0.34 m.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

We will use Atlantic cod (*Gadus morhua*) as our prime model species. We will catch the cod in the North Sea using fishing rods. We want to use wild-caught cod because they might exhibit more natural behaviour (than fish from a hatchery). We also plan to test hatchery-raised cod for some comparisons on behaviour. We will use fish of both sexes. The size and variation in size will depend on what we will catch, but we aim for subadult fish of about 35-40 cm. For the life-stage comparison in experiment 3 of this appendix, we will aim for a group of fish of about 25-30 cm and a group of 45-50 cm.
We chose to use Atlantic cod because it is an important and abundant fish species with a large distribution. Also accelerometer-data of cod have been correlated to energy metabolism, meaning that

our accelerometer-data will also yield insight into energy metabolism under seismic survey conditions. Furthermore, cod's hearing ability and ecology are relatively well-studied and generally similar to several other species, many of which are important commercial fishes, such as European seabass (*Dicentrarchus labrax*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*) and pollock (*Pollachius pollachius*). At last, several cod stock populations collapsed in the 1990's, many others are at risk and therefore industries will be interested to gain insight in the effects of seismic surveys on cod.

In experiment 5 of this appendix, we also want to use two other fish species. In this way we can compare cod as benthopelagic (mainly inhabiting water just above the sea bottom) with fish of more pelagic and more benthic life styles.

For the number of individuals, see table 1 in section A.

C. Re-use

Will the animals be re-used?

x No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

x No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
The behaviour of fish can only be studied in living specimens. Relative response to acoustic stimuli is not well-known enough to be able to study using modelling. A replacement is not possible.

Reduction
By exposing the fish to a series of multiple treatments without incurring additional harm, we reduce the number of fish while obtaining maximal research gain. Moreover, we choose a minimum group size of four where fish can still show natural aggregational behaviour. A further reduction in the number of animals is not possible.

Refinement
We have chosen fish that are known to be able to live in captivity and thanks to a partnership with [redacted], we can make use of specialized facilities to keep the fish until the start of the experiment. We use an experimental set-up that has yielded valuable results in previous years. Throughout the years, the set-up has been continually refined. Our experience with this effective set-up ensures that unnecessary animal suffering can be avoided.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

1) animal suffering, pain or fear
The aim of this project is to investigate how sound stimuli causes stress and behavioural changes in fish. Great care on the handling of fish to minimise stress is inherently crucial in obtaining our objective. During the tagging process, 2-phenylethanol will be used as anesthesia to reduce mobility, sensory input and pain. The process is quick and the fish are expected to recover quickly after the procedure. We also allow ample of time for fish to recover from tagging and acclimatise to their new environment.

2) adverse effects on the environment
The sound levels that we expose in the floating pen are not high enough to cause tissue injuries or

hearing loss. Although the sound itself may be heard by nearby aquatic animals, they may swim away from the sound temporarily to avoid auditory masking or physiological effects. We expect to aquatic animals to resume their distribution and normal behaviour either during or right after the sound exposure.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

We made an extensive literature review on research that has been done on the effects of seismic surveys of fish. Also, we are being advised by fellow experts from over the world and we visit conferences on topics that relate to these proposed studies. We think that there is an urgent need to do studies that ultimataly give insight in population consequences of seismic surveys.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

x No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☐ No > Continue with question H.

x Yes > Describe this establishment.

The animal procedures will be carried out at [redacted] research institute at [redacted] in [redacted] is specialised in applied research regarding marine aquaculture and ecology.

The fish will be kept in holding tanks of 14.4 m^3 with maximum 120 individuals. The basins will have a sand bottom and the water is continiously refreshed with seawater from the Oosterschelde. In this way, we ensure that waterquality and temperature are simular to the natural enviroment of cod.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

[redacted] offers excellent research facilities for the housing, maintenance and care of different North Sea fish species. It is right next to our field site, making the transfer of fish between holding tanks and outdoor experimental pen very quick, thus minimizing transfer stress experienced by fish.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

x Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

x Yes > Indicate what relieving methods will be used and specify what measures will be taken

to ensure that optimal procedures are used.

Prior to the tagging procedure, the fish will be put into an anesthetic bath of 2-phenylethanol solution. 2-phenylethanol is known to be an effective anesthesia for fish with a short recovery time. As in standard practice, no analgesic is necessary after surgery implantation on fish as fish do not show signs of pain after the procedures (Stevens, 2008).

Reference:

Gräns, A., Sandblom, E., Kiessling, A. and Axelsson, M. (2014). Post-surgical analgesia in rainbow trout: Is reduced cardioventilatory activity a sign of improved animal welfare or the adverse effects of an opioid drug? *PLoS One* **9**,.

Sneddon, L. U. (2012). Clinical Anesthesia and Analgesia in Fish. *J. Exot. Pet Med.* **21**, 32–43.

Stevens, E. D. (2008). " Pain " and analgesia in fish : What we know , what we do not know , and what we need to know , before using analgesics in fish. In *Conference: Blue sky to deep water: the reality and the promise. ANZCCART Proceedings, At Auckland, New Zealand*, pp. 115–124.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Since the fish with be kept in tanks with re-circulating water from the sea, there is a potential that the fish may be infected by parasites or bacteria from seawater. The risk will be highest after tagging.

Explain why these effects may emerge.

For tagging we need to make an incision which we make the fish more viable for infectious diseases.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

We think we can decrease the likelihood to a minimum by only tagging fish with a very good health and condition. Also, we will prevent any water from entering the incision because the abdomen will be above water during the surgery. To prevent water from entering the incision after stitching the incision, we will add some surgical/tissue glue on top the incision.
Fish housing facilities are provided by [redacted], who is very experienced in aquaculture and marine research.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

x Yes > Describe the criteria that will be used to identify the humane endpoints.

If there is visual evidence of consistent abnormal behaviourm, disease, physical disorder such as patches of scale loss and red or white spots, the fish will be euthanized with percussive blow to the head to free them from any unnecessary suffering.

Indicate the likely incidence.

Stress caused by the transport from hatchery and potential infections. Based on our previous experiments, we expect very little likelihood that these occur.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Fish catching (at the North sea) and transferring - mild
Catching and fish transfer from maintenance tank to outdoor pen - mild
Surgical implantation of acoustic tag - moderate
Sound exposure – mild
Surgical removal of tag – moderate
Overall – moderate

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Only the 20 hatchery-reared fish will be sacrificed after the procedures because we cannot release them in the wild and we do not have facilities to keep them. The 900 wild-caught cod will be released into the wild.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10600
1.2	Provide the name of the licenced establishment.	Leiden University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: Sound exposure effects on fish growth and stress physiology in a floating pen

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We want to study the effect of long-term sounds exposures to growth and stress levels in cod. To do this we will keep groups of cod in floating pens, either in noisy conditions or in ambient control conditions, for a time period of 6 weeks. Long-term anthropogenic noise including sounds from airguns might induce increased stress levels, disrupt foraging, decrease feeding success, and increase energy metabolism. This might accumulate to a reduction of growth and a decreased immune response.

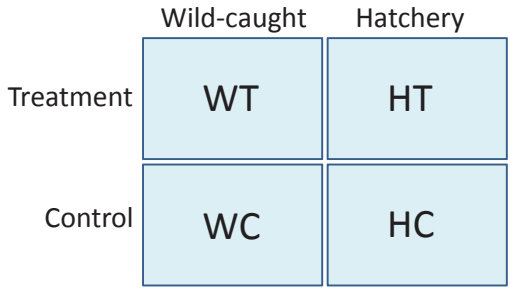
The noisy conditions will be created using a single airgun or an artificial sound source that is able to mimic and vary seismic exposure features. We will weigh and measure the fish before and after the 6-week period. We will also measure stress hormone levels (cortisol) in blood samples before and after the end of this period. After the exposure period, we will sacrifice these fish to assess body condition (fat ratio) and maturation (gonad development).

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Half of the fish will be caught in the North Sea from a research vessel using fishing rods and half of the fish fish will be delivered as a batch from a foreign hatchery. The fish will be kept in holding tanks and transported within hours to the facilities of [redacted]. Here, the fish can recover in a continuous flow-through system of natural sea water and acclimatize to captivity for at least 3 weeks before being used in the experiment. Each fish will be used in only one experiment and be part of either

treatment or control group.

Experimental design



Each experimental round consists of 4 groups with 10 fish (see 2x2 design), of which 2 are treatment groups (seismic sound exposure) and 2 are control (ambient noise) and 2 are wild-caught (treatment and control) and 2 hatchery-raised (again one treatment and one control). At the start of each experiment, the four groups of fish will be implanted with a PIT tag and transported to the outdoor floating pens. The pens are located inside an artificial cove in the Oosterschelde, this cove ([redacted]) is situated right next to [redacted]. The very shallow nature of the cove enables us to make a noisy and quiet area within the same cove (the low frequencies audible to fish have long wave lengths and just do not transmit across the cove from experimental to control site).

Before the fish are placed inside the outdoor net-pens, they will be individually weighted and measured (length and body condition). This procedure will be repeated for each individual fish at the end of the study period. We will also implant the fish with a PIT tag, this is a very small transponder (well below 1 grams) that allows individual recognition. We will implant it with an injection needle in the dorsal muscle.

After transfer, the fish will acclimatise to the new outside environment for at least two days, after that we will catch the fish to collect blood samples from the tail vein to determine baseline levels of cortisol. We will wait another two days to allow recovery and then expose two of the four groups to sound from a seismic source/speaker for 30-60 minutes. Right after the first exposure we will collect another blood sample to determine the short time response in stress (cortisol) level. After that we will continue exposing half of the groups to sound from a seismic source/speaker twice per day for maximum 6 weeks. After the last trial we will again catch the fish and collect another blood sample to determine the long-term stress levels. Also, all fish will be sacrificed by a percussive blow on the head. This enables us to sample the muscles (for fat ratio) and gonads (for gonad development). This experimental design will be repeated 6 times, every time using 4 new groups of fish, the locations will be swapped to control for position of the pens and the interaction between position and time of the year.

[redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We will use a total 24 groups of cod (total 240 individuals), 12 groups per treatment. In this way we will be able to detect a significant difference in growth of the group-means if the growth between groups differs for more than 10% and has a standard deviation of less than 10% of the growth. This is based on a power analysis executed using R and the package pwr, with a statistical power of 80% and a p-value at 0.05.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

We will use Atlantic cod (*Gadus morhua*), both wild-caught in coastal area of the North Sea as raised at a hatchery. We want to use wild-caught cod because they are the explicit target model for which we aim to get a better understanding. They might exhibit more natural behaviour and may have a different

physiology to some extent from hatchery-raised fish. We also want to use hatchery-raised fish as they provide the opportunity to have a homogenous group of known age and background, which is critical for measures of development. The replication with both wild-caught and hatchery-raised fish will yield complementary data and added value for interpreting studies on captive and/or hatchery-raised fish in general.

We will catch the North sea cod using fishing rods as this yields the least physical damage to the fish and is the best technique to collect fish that are healthy and still in good condition after capture and transport. Hatchery-raised cod will be ordered from a foreign company as there are no hatcheries in the Netherlands (details of the selected company will be shared before buying). We chose to use Atlantic cod because it is an important and abundant fish species with a large distribution. Furthermore, cod hearing ability and ecology are relatively well known and similar to several important commercial fishes, such as European seabass (*Dicentrarchus labrax*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*) and pollock (*Pollachius pollachius*). At last, several cod stock populations collapsed in the 1990's, many others are at risk and therefore off-shore industry and fisheries should be interested in the effects of seismic surveys on cod.

In total we will use 240 cod; 120 hatchery reared and 120 wild caught fish.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
Fish physiology in response to sound exposures can only be studied in living specimens. Relative response to acoustic stimuli is not well-known enough to be able to study using modelling. A replacement is not possible.

Reduction
We did a power analysis to determine the minimum amount of fish needed for this experiment. We will tag the fish with PIT tags to be able to use parametric tests and reduce the amount of fish needed. Expected differences in growth and standard deviation are based on experiments in which the effect of sound from filters was assessed.

Refinement
We have chosen the Atlantic cod. This fish species is known to do well in captivity and thanks to a partnership with [redacted], we can make use of specialized facilities to keep the fish under optimal conditions until the start of the experiment. We will use an experimental set-up that has yielded valuable results in behavioural studies in previous years. Throughout the years, the set-up has been continually refined. Our experience with this effective set-up ensures that unnecessary animal suffering can be avoided.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

1) animal suffering, pain or fear

The aim of this project is to investigate how sound stimuli causes stress and physiological changes in fish. Great care on the handling of fish to minimise stress is critical in obtaining our objective. We will expose the fish to sound levels that are not physically harmful to the study objects.

2) adverse effects on the environment
The sound levels that we expose the fish to in the floating pen are not high enough to cause any physical injuries or permanent hearing loss. Although the sound itself may be heard by nearby aquatic animals, they may swim away from the sound temporarily to avoid auditory masking or physiological effects. We expect aquatic animals to resume their distribution and normal behaviour either during or right after the sound exposure.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

We have done an extensive literature review on studies of the effects of seismic surveys on fish. Also, we are being advised by fellow experts from all over the world. We also visited conferences on topics that relate to these proposed studies. The current state of the art is an urgent need to do studies on the more long-term exposure and long-term effects that will give insights into potential population consequences of seismic surveys.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☐ No > Continue with question H.

X Yes > Describe this establishment.

The animal procedures will be carried out at [redacted], research institute at the [redacted] is specialised in applied research regarding marine aquaculture and ecology.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

[redacted] offers excellent research facilities for the housing, maintenance and care of different North Sea fish species. It is right next to our field site, making the transfer of fish between holding tanks and outdoor experimental pen very quick, thus minimizing transfer stress experienced by fish.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

X No > Continue with question I.

☐ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

The fish that are exposed to the sound exposure might induce increased stress levels and consequently also affect the immune response and growth negatively.

Explain why these effects may emerge.

Sound can induce stress and stress can induce reduced immune response and growth.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Fish housing facilities are provided by [redacted], who is very experienced in aquaculture. marine research.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

If we spot fish that are swimming just below the surface, with their gills wide open, this means that the fish is quite ill and probably will not recover, we will catch and euthanize the fish with percussive blow to the head to free them from any unnecessary suffering.

Indicate the likely incidence.

Based on our previous experiments with tagged fish, which were more short-term but many, we expect the likelihood that this occurs to be small.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Fish catching (at the North sea) and transferring - mild
Weighing, measuring and transferring the fish from holding tank to outdoor pen – mild
Implanting PIT tag – mild
Taking blood sample (3 times) - mild
Weighing and measuring the fish at the end of the experimental period - mild
Sound exposure - mild
Overall - moderate

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

The long-term effects on body condition (fat ratio) and maturation (gonad development) can only be done by dissection for which the fish need to be sacrificed.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10600
1.2	Provide the name of the licenced establishment.	Leiden University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 4 Type of animal procedure: Full seismic survey effects on tagged fish while free-ranging at sea

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We want to study the effects of a full seismic survey on free-ranging fish. This experiment will be conducted in an offshore wind farm in the Belgian North Sea and an offshore wind farm in the Dutch North sea. The site in the Dutch North sea will be exposed to the seismic survey and the site in the Belgian North Sea will serve as a control site. We will also develop our methodology and collect base line data in Belgium, one year before the actual exposure. Within this appendix we will apply for approval for the part of the experiment that will be conducted in the Netherlands, for the Belgian part we will (have to) apply in Belgium. Nevertheless, we will here explain the rationale and set-up for the full experiment.

Within this project we most often study captive fish in a net pen. This approach allows the fish more space than in an indoor fish tank, also provides conditions of a more natural sound field than indoor tests, and yields excellent possibilities for behavioural measurements of high resolution and replication with individuals of known history and background [redacted]). However, the behaviour of fish in an enclosure might not be representative for free-ranging fish in the wild and information on the real thing (an actual seismic survey) in the wild is the explicit call from society (policy makers responsible for sustainable exploitation of marine resources, off-shore industry that requires permission for noisy operations). Parallel studies that allow comparisons between captive and free-ranging fish and natural and artificially manipulated sound sources are therefore needed and the optimal approach to gain fundamental and applied insights [redacted] Previous experiments in both the Netherlands and

Belgium have shown that Atlantic cod have a relatively high site fidelity around wind farms in the period from June until October (Winter et al. 2010; Reubens et al., 2013). This makes cod (in wind farms) ideal for studying noise impact on wild free-ranging fish.

Reubens, J.T.; Pasotti, F.; Degraer, S.; Vincx, M. (**2013**). Residency, site fidelity and habitat use of Atlantic cod (Gadus morhua) at an offshore wind farm using acoustic telemetry. *Mar. Environ. Res.* 90: 128–135. hdl.handle.net/10.1016/j.marenvres.2013.07.001
Slabbekoorn, H. (**2014**). Aiming for progress in understanding underwater noise impact on fish: complementary need for indoor and outdoor studies. In: Popper, A.N., Hawkins, A.D. (Eds.), The Effects of Noise on Aquatic Life, Budapest Conference Proceedings. Elseviers.
Winter, H.V., Aarts, G. & van Keeken, O.A. (**2010).** Residence time and behaviour of sole and cod in the Offshore Wind farm Egmond aan Zee (OWEZ). *IMARES Report C038/10*, *NoordzeeWind Report OWEZ_R_265_T1_20100916*.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

We will catch the fish with a fishing rod from or close to the research vessel. The fish will then be put into an anesthetics bath with 2-phenylethanol. Once anesthetised, the fish will be implanted with a small acoustic tag with accelerometers (volume: 5.8 cm^3; weight : 12.3 g; V13AP, Vemco or similar type of other brand) in its abdominal cavity with an incision of 2 cm and sutures (fig. 1). The tags are less than 5% of the fish body weight and is not expected to alter the swimming dynamics of the fish. The handling process (anesthesia and tagging) will take less than five minutes and the fish will be back in the water for recovery within a few minutes after surgery.

The fish will be placed in a recovery tank for 0.5 to 2 hours before being released in the sea again. An individual will be released once it resumed normal swimming behaviour, this recovery time can differ per individual, but we aim to keep this time as short as possible. We will use a special release cage by which we set the fish free at their usual swimming depth close to the bottom, which has proven to be a successful method. We expect the fish behaviour – in the sea - to recover back to normal patterns within two days. As in standard practice, no analgesics will be used after the surgical implantation. From our experience, fish do not show signs of pain after such minor surgeries. Research on analgesia in fish is still in its infancy and very little is known about the possibilities and consequences of its usage (Gräns et al., 2014; Sneddon, 2012; Stevens, 2008).

Figure 1: Tagging at the research vessel

Experimental design
Cod on both locations (Belgian and Dutch North sea) will be equipped with tags that alternate between emitting information on their position or averages of accelerometer-data (from three axis) every 20

seconds as long as the fish is in proximity of the receiver stations. The tag-batteries last for 7 weeks, but we will track the behaviour at least for 5 weeks to make sure that the absence of a signal is not due to empty batteries. The data from the first or 2 days will be excluded to minimize the influence of tagging-recovery on baseline levels. The first two weeks will be used to determine baseline levels for parameters such as absence/presence, location, swimming depth, speed, overall dynamic body acceleration (linked to energy metabolism). After these two weeks there will be a full seismic survey of maximum 4 days around the Dutch site, the Belgian site serves as a control. After the survey we will track the behaviour for another two weeks to assess whether – if fish changed their behaviour – return to their baseline levels and whether fish that left the area return after the survey. The data will be retrieved from the receiver stations and not from the tags in the fish, still, the data can only be retrieved after the entire 5 weeks.

References:

Gräns, A., Sandblom, E., Kiessling, A. and Axelsson, M. (2014). Post-surgical analgesia in rainbow trout: Is reduced cardioventilatory activity a sign of improved animal welfare or the adverse effects of an opioid drug? *PLoS One* **9**,.

Sneddon, L. U. (2012). Clinical Anesthesia and Analgesia in Fish. *J. Exot. Pet Med.* **21**, 32–43.

Stevens, E. D. (2008). " Pain " and analgesia in fish : What we know , what we do not know , and what we need to know , before using analgesics in fish. In *Conference: Blue sky to deep water: the reality and the promise. ANZCCART Proceedings, At Auckland, New Zealand*, pp. 115–124.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

In total we want to use 99 Atlantic cod for this experiment; 33 for a pilot in Belgium; 33 for the actual experiment in Belgium and 33 for the actual experiment in the Netherlands. We cannot apply for an experiment in the Belgium waters via this application, so with this application we apply for the use of 33 cod in the Netherlands. We actually need data from 30 fish per site and the pilot instead of 33, but we take into account that maximum 3 fish will not recover from tagging and will not be released in the North sea again (based on previous experience 1 or 2 non-recovering individuals per 30 fish is most likely). Since we will tag wild, free-ranging fish, there is some uncertainty about the data that we will collect, because fish can swim away and never return. We have taken this into account for the two power analysis' (p-value at 0.05) we made.

(1) power to detect differences in proportion of fish leaving between control and experimental site
We also do not anticipate fish leaving the experimental site, but this could be a possible response to the seismic experiment. If fish do leave, one simple statistical test to determine if the proportion leaving is greater than at the control site is a test for differences in proportions (Cohen, 1988, Chapter 6). Figure 1 shows how power changes as probability of leaving the experimental site increases, assuming probability of leaving the control site is zero, and n=30 fish at both control and experimental sites. The horizontal line indicates a target power of 0.8, which is achieved with a probability of leaving of 0.13 – this corresponds to 3.9 of the 30 fish leaving. Hence, we conclude that if the experiment causes an average of 4 or more fish to leave the experimental site, we should have good power to reject the null hypothesis of no difference between control and treatment using a simple statistical test.

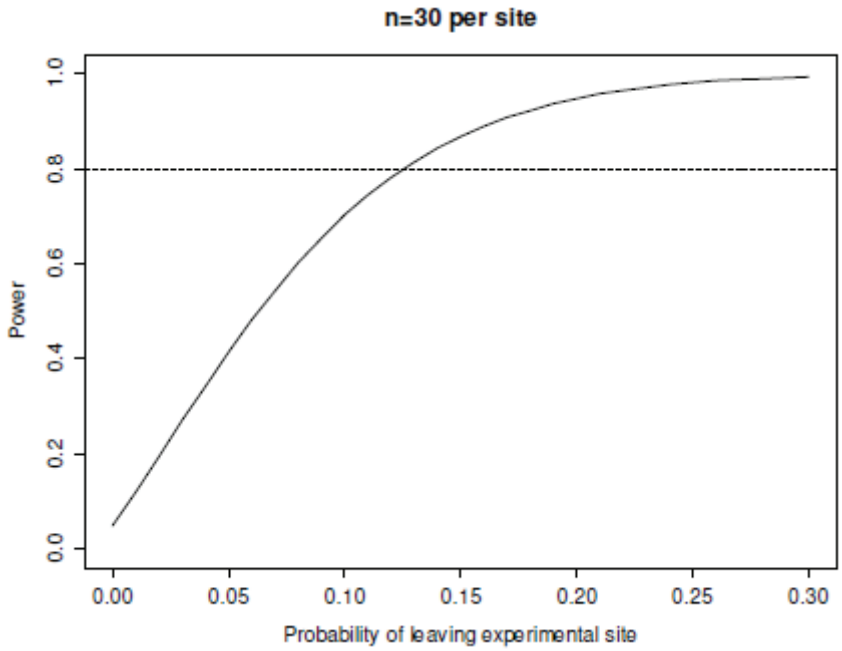


Fig. 5. Power analyses for 30 tagged Atlantic cod per site to assess the statistical threshold to be able to state whether or not more individuals leave the treatment site than the control site (plot by Len Thomas).

(2) power to detect changes in fish depth associated with the experimental protocol, as a function of sample size
We will look into a lot of different parameters such as spatial use of the environment and energy metabolism. For this section, we will focus on the potential change in depth, as an example, which will be based on the pressure sensor and/or 3d position from the tags.

Fish swimming depth changes due to other factors than sound exposure, such as time of day, so we have to take other factors into account by using control baseline data. A simple test for a decrease in depth from conditions without to conditions with sound exposure is a one-sided t-test (Cohen 1988, Chapter 2), comparing the mean depth in the baseline period to the mean depth during the experimental period. To undertake the power analysis, we again chose a target power of 0.8, and determined the mean decrease in depth that was detectable with this target power given sample sizes ranging from 10 (a pessimistic number, assuming two thirds of experimental fish are lost) to all 30 (the target number). To obtain the standard deviation of change in depth between fish, we analysed an actual sample dataset of depths of 14 tagged Atlantic cod individuals in the Belwind wind farm off the Belgian coast (data from Jan Reubens) measured intermittently on multiple Vemco (VR2AR) receiver stations (2016-data).

We found that depth changes periodically over the day, as expected, with the standard deviation of depth ranging from 1.1 to 2.8 m. We took the mean of these measures, 1.53m, as a crude indication of the standard deviation of possible fish responses to the experiment. Results are shown in Figure 2. Given the target sample size, a decrease in depth of 0.7m is detectable with high power; with only 10 fish, the detectable change of depth increases to 1.3m. We may anticipate losing some fish during the experiment, but a few losses (say 5 or less) will have little effect on the statistically detectable decrease in depth. We could do this analysis for depth as we had already data on this, but we expect similar power results for other spatial positioning data.

We anticipate using more sophisticated analyses on the real experimental data, and expect that detectable effect sizes will be smaller than those given above. In addition, we plan to ask more sophisticated questions (see e.g., Harris et al. 2016), using multivariate responses, using the time-series nature of the data (accounting for temporal autocorrelation), and looking at dose vs response (c.f. Miller et al. 2014) as well as dose vs response intensity (c.f. DeRuiter et al. 2013).

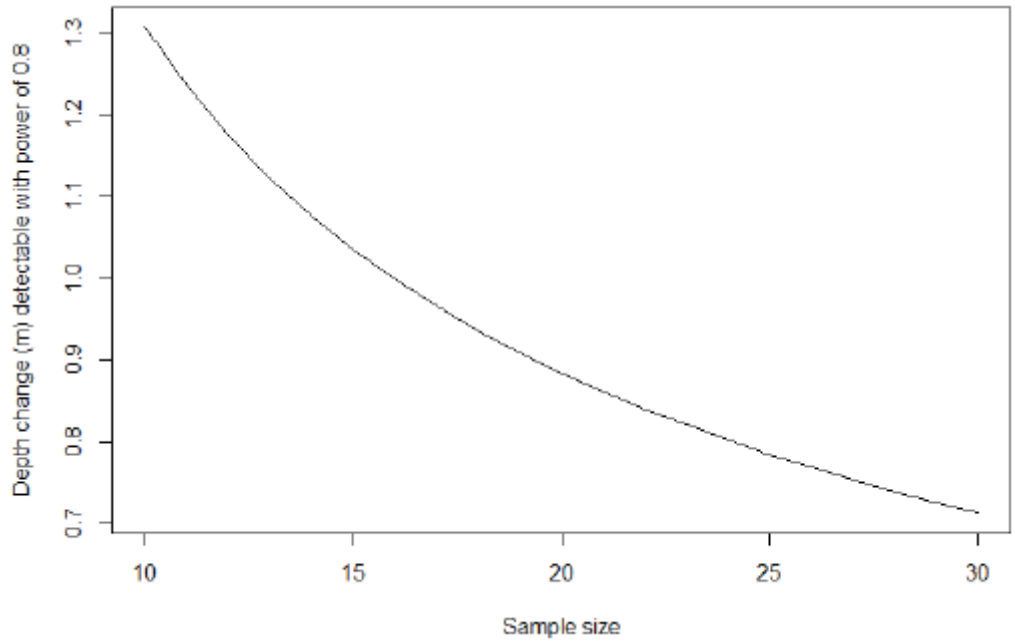


Fig. 2. Power analysis for 10 to 30 tagged Atlantic cod individuals at the treatment site to assess the statistical detection threshold for depth changes from before to during exposure to seismic survey sound pulses (plot by Len Thomas).

Cohen, J. 1988. Statistical power analysis for the behavioural sciences. Second Edition. Elsevier, ISBN: 978-0-12-179060-8.

DeRuiter, S.L., Southall, B.L., Calambokidis, J., Zimmer, W.M.X., Sadykova, D., Falcone, E.A., Friedlaender, A.S., Joseph, J.E., Moretti, D., Schorr, G.S., Thomas, L. & Tyack, P.L. 2013. First direct measurements of behavioural responses by Cuvier's beaked whales to mid-frequency active (MFA) sonar. *Biology Letters* 9: 20130223.

Harris, C.M., Thomas, L., Sadykova, D., DeRuiter, S.L., Tyack, P.L., Southall, B.L., Read, A.J. & Miller, P.J.O. 2016. The challenges of analyzing behavioural response study data: An overview of the MOCHA (Multi-study OCean acoustics Human effects Analysis) project. Pages 399-407 in Popper, A.N. and A. Hawkins. (Eds), The Effects of Noise on Aquatic Life II. *Advances in Experimental Medicine and Biology* 875. ISBN 978-1-4939-2981-8.

Miller, P.J.O., Antunes, R.N., Wensveen, P.J., Samarra, F.I.P., Alves, A.C., Kvadsheim, P.H., Kleivana, L., Lam, F.-P.A., Ainsle, M.A., Tyack, P.L. & Thomas, L. 2014. Dose-response relationships for the onset of avoidance of sonar by free-ranging killer whales. *Journal of the Acoustical Society of America* 135: 975-993.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

We will use Atlantic cod (*Gadus morhua*) for this experiment. We will catch the cod in the North sea using fishing rods and release them were they were caught. We will use wild-caught cod because they are known to be residential in the wind farm (previous experiment showed them to be so) and wild cod might exhibit more natural behaviour than fish from a hatchery. Also, it is against the law to release hatchery-reared Atlantic cod into the wild, especially since we will not be able to catch the fish back again after the experiment.

In total we want to use 99 Atlantic cod for this experiment; 33 for a pilot in Belgium; 33 for the actual experiment in Belgium and 33 for the actual experiment in the Netherlands (assuming that maximum 3 individuals per group do not recover from tagging and will be sacrificed, we need data from 30 fish per group). We cannot apply for an experiment in the Belgium waters via this application, so with this application we apply for the use of 33 cod in the Netherlands. For the 66 fish that will be tagged in Belgium, we will apply for ethical approval via the authorities in Belgium.

We chose to use Atlantic cod because it is an important and abundant fish species with a large distribution. Besides, cod is residential in wind farms (based on own experiences). Also accelerometer-data of cod have been correlated to energy metabolism (publication in prep), meaning that our accelerometer-data will also yield insight into energy metabolism under seismic survey conditions. Furthermore, cod hearing ability and ecology are similar to several important commercial fishes, such as European seabass (*Dicentrarchus labrax*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*) and pollock (*Pollachius pollachius*). At last, several cod stock populations collapsed in the 1990's, many others are at risk and therefore the offshore industry and fisheries should be interested in the effects of seismic surveys on cod.

C. Re-use

Will the animals be re-used?

x No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

x No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
The behaviour of fish can only be studied in living specimens. Response behaviour to acoustic stimuli is not well-known enough to be suitable for studies that solely use modelling. A replacement is therefore not possible.

Reduction
We use a limited amount of fish to yield information on several parameters that will yield insight on how free-ranging wild cod cope with seismic surveys. Numbers of fish that we can use are mainly limited by technical limitations in the tagging system. Also, we make use of only two locations (one treatment and one control site). Reason for this is the extremely high costs for studies like this. However we expect – based on statistical (power) analysis - that the numbers of fish are high enough to yield valuable results (see answer to last question of section A for statistical support on this).

Refinement
We have chosen to use Atlantic cod for this experiment because a previous experiment using Atlantic cod with a similar design has yielded valuable results. The Belgium site will also be the same as in the previous experiment. Multiple researchers of our team have experience with fishing, tagging and processing data from acoustic tags.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

1) animal suffering, pain or fear
The aim of this project is to investigate how sound stimuli cause stress and behavioural changes in fish. Great care on the handling of fish to minimise stress is critical for obtaining our objective. During the tagging process, 2-phenylethanol will be used as anesthesia to reduce mobility, sensory input and pain. The process is quick and the fish are expected to recover quickly after the procedure. We also allow ample of time for fish to recover from tagging and acclimatise to their new environment.

2) adverse effects on the environment
The sound levels that we expose the fish to are not high enough to cause physica injuries or hearing loss. Although the sound itself may be heard by nearby aquatic animals, they may swim away from the sound

We expect aquatic animals to resume their distribution and normal behaviour either during or right after the sound exposure.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

We have done an extensive literature review on the effects of seismic surveys on fish. Also, we are being advised by fellow experts from all over the world and we visit conferences on topics that relate to these proposed studies. We think that there is an urgent need to do studies that ultimataly give insight into population consequences of seismic surveys. We are aware of studies that report decreased catch rates and fewer fish on echosounders after seismic surveys. But we are not aware of any study that followed individual wild free-ranging fish before, during and after seismic surveys.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

x No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☐ No > Continue with question H.

x Yes > Describe this establishment.

Catching and tagging will be done from/on a research vessel. The vessel contains three tanks, one for catched fish (in case multiple fish are caught at the same time); one with the aneasthesia and where the surgery takes place; and one recovery tank. The seawater in these tanks will regurly be refreshed using a pump. After the fish resume normal swimming behaviour, the fish is released where we caught it at the appropriate depth.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

We are interested in the natural behaviour, the best way to achieve this is to study wild animals in the wild.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

x Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

x Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Prior to the tagging procedure, the fish will be put into an anesthetic bath of 2-phenylethanol solution. 2-phenylethanol is known to be an effective anaesthesia for fish with a short recovery time. As in standard

practice, no analgesic is necessary after surgery implantation on fish as fish do not show signs of pain after the procedures (Stevens, 2008).

Reference:
Gräns, A., Sandblom, E., Kiessling, A. and Axelsson, M. (2014). Post-surgical analgesia in rainbow trout: Is reduced cardioventilatory activity a sign of improved animal welfare or the adverse effects of an opioid drug? *PLoS One* **9**,.
Sneddon, L. U. (2012). Clinical Anesthesia and Analgesia in Fish. *J. Exot. Pet Med.* **21**, 32–43.
Stevens, E. D. (2008). " Pain " and analgesia in fish : What we know , what we do not know , and what we need to know , before using analgesics in fish. In *Conference: Blue sky to deep water: the reality and the promise. ANZCCART Proceedings, At Auckland, New Zealand*, pp. 115–124.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

1) The tagging-incision might get infected.
2) The physical presence might interfere with abdominal organs.

Explain why these effects may emerge.

1) The environment in which the tagging is done is not completely sterile (a research vessel).
2) The insertion of the tag might be hindered physically by internal organs.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

1) We will make the environment as clean as possible and use sterile materials. We will make sure that both during and after the tagging, no seawater can enter the incision.
2) We will only push a tag through the incision if it goes very smoothly.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

x Yes > Describe the criteria that will be used to identify the humane endpoints.

If a fish does not resume normal swimming behaviour, the fish will be euthanized with a percussive blow to the head to free them from any unnecessary suffering.

Indicate the likely incidence.

Based on earlier experience with tagging wild cod on a research vessel, we expect that one or two (maximum 3) individuals will not recover from the anesthesia and tagging procedure. So far, we have never experienced this during earlier studies with European seabass.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Catching the fish - moderate
Surgical implantation of acoustic tag - moderate
Sound exposure - mild
Overall - moderate

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

x No

☐ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☐ Yes

7.

DEC-advies

A. Algemene gegevens over de procedure

1. Aanvraagnummer: AVD1060020171085

2. Titel van het project: 'PCAD4Cod' - Impact of seismic survey sound exposure on fish.

3. Titel van de NTS: Impact van seismisch onderzoek op kabeljauw.

4. Type aanvraag:
   - ✓ nieuwe aanvraag projectvergunning
   - ☐ wijziging van vergunning met nummer

5. Contactgegevens DEC:
   - naam DEC: DEC Leiden
   - telefoonnummer contactpersoon: [redacted]
   - e-mailadres contactpersoon: [redacted]

6. Adviestraject (data dd-mm-jjjj):
   - ✓ ontvangen door DEC: 06-04-2017
   - ✓ aanvraag compleet: 06-04-2017
   - ✓ in vergadering besproken: 28-04-2017 & 12-05-2017
   - ☐ anderszins behandeld
   - ✓ termijnonderbreking(en) van 04-05-2017 t/m 05-05-2017
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   - ✓ aanpassing aanvraag: 05-05-2017
   - ✓ advies aan CCD: 16-05-2017

7. De IvD geeft aan dat de aanvrager de aanvraag met de IvD heeft afgestemd en dat deze de instemming heeft van de IvD

8. Eventueel horen van aanvrager
   N.v.t.

9. Correspondentie met de aanvrager
   - Datum: 04-05-2017
   - Strekking van de gestelde vragen:
     De DEC heeft bij de aanvrager aanvullende informatie ingewonnen met betrekking tot de go/no-go momenten, statistiek, gebruik wildvang en analgesie.
   - Naar aanleiding van deze vragen is het projectvoorstel inclusief bijlages en de NTS naar tevredenheid door de aanvrager aangepast.

10. Eventuele adviezen door experts (niet lid van de DEC)
    - Aard expertise: Statistiek
    - Deskundigheid expert: medische statistiek
    - Datum verzoek: 02-05-2017
    - Strekking van het verzoek: berekening groepsgrootte
    - Datum expert advies:16-05-2017
    - Advies expert: De gegeven berekening is correct.

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet)

2. De aanvraag betreft een nieuwe aanvraag.

3. De DEC is competent om over deze projectaanvraag te adviseren. De benodigde expertise op dit wetenschappelijke terrein is aanwezig binnen de DEC.

4. Geen van de DEC leden is betrokken bij het betreffende project.

C. Beoordeling (inhoud)

1. Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. De aanvraag komt overeen met voorbeeld 4B uit de handreiking 'Wat is een project': De verschillende subdoelen zijn niet uitkomstafhankelijk van elkaar maar zijn allemaal noodzakelijk om de hoofddoelstelling te behalen. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan. De DEC is er van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden. Gezien bovenstaande is de DEC van mening dat de aanvraag toetsbaar is en voldoende samenhang heeft.

2. Voor zover de DEC kan beoordelen is er geen sprake van tegenstrijdige wetgeving die het uitvoeren van de proef in de weg zou kunnen staan.

3. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.

   Belangen en waarden

4. Het directe doel van dit project is inzicht verkrijgen in de effecten van seismische surveys op zwempatronen/ruimtelijke vermijding, foerageeractiviteit, gewenning en energiemetabolisme en kan het effect van seismische surveys op vissen worden verminderd door aanpassing van temporale of spectrale eigenschappen van de procedure en geluidsbron. Het uiteindelijke doel is het verkrijgen van inzicht in de gevolgen van seismische surveys op het gedrag en de fysiologie van vissen. Het betreft hier fundamenteel onderzoek. De DEC is van mening dat er een duidelijke relatie is tussen het directe en uiteindelijk doel. De aanvrager heeft helder gemaakt wat de status is van het onderzoeksveld en wat de bijdrage van dit project aan het onderzoeksveld zal zijn. De hoeveelheid lawaai onder water, veroorzaakt door mensen, is de laatste decennia in de Noordzee, maar ook wereldwijd toegenomen. Dit heeft geleid tot een groeiende zorg over het effect hiervan op aquatisch leven, inclusief vissen. Er is echter nog weinig inzicht in de mogelijke nadelige effecten op de individuele gezondheid van vissen en op hun populatie dynamiek. Er is daarom dringend behoefte aan meer kennis over de relatie tussen seismische surveys en het gedrag en de fysiologie van vissen. De DEC is van mening dat het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld.

5. De belangrijkste belanghebbenden in dit project dat gericht is op het verkrijgen van inzicht in de gevolgen van seismische surveys op het gedrag van vissen en hun fysiologie zijn de proefdieren, de wetenschappers, de vispopulatie, de visserij en natuurbeschermers. Waarden die voor proefdieren in het geding zijn: De integriteit van de dieren zal worden aangetast, de dieren zullen beperkt worden in hun

natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Waarden die voor de wetenschappers bevorderd worden: De wetenschappers zullen kennis verkrijgen. Ook zullen de carrièremogelijkheden van de wetenschappers verbeteren door publicaties. Waarden die voor de vispopulatie en de visserij bevorderd worden: meer inzicht in de gevolgen van geluid kan bijdragen aan het in stand houden van de vispopulatie. Waarden die voor de natuurbeschermers worden bevorderd: het onderzoek en de bevindingen zullen een cruciale rol spelen in de wetgeving en het vergunnen van luidruchtige offshore activiteiten.

6. Hoewel het geluid gehoord kan worden door andere aquatische dieren in de buurt is er voor zover de DEC kan beoordelen geen sprake van substantiële milieueffecten.

Proefopzet en haalbaarheid

7. Het onderzoeksvoorstel en het team zijn geselecteerd in een open mondiale competitie op basis van wetenschappelijke inhoud en expertise. Naar de overtuiging van de DEC beschikt de aanvrager over voldoende expertise en voorzieningen om de projectdoelstelling met de gekozen strategie binnen de gevraagde termijn te realiseren. De onderzoeksgroep heeft veel expertise op het gebied van experimenteel onderzoek met vissen en met name over de relatie tussen vis gedrag en bio-akoestiek. In de afgelopen jaren zijn volgens vergelijkbare strategieën en aanpak belangrijke wetenschappelijke resultaten behaald.

8. De DEC is er van overtuigd dat het projectvoorstel aansluit bij recente wetenschappelijke inzichten en geen hiaten bevat die de bruikbaarheid van de resultaten in de weg zullen staan. De voorgestelde experimentele opzet en uitkomstparameters zijn logisch en helder gekozen en sluiten aan bij de aangegeven doelstellingen en de gekozen strategie en experimentele aanpak kunnen naar de mening van de DEC leiden tot het behalen van de doelstelling binnen de looptijd van het project.

Welzijn dieren

9. Er wordt gebruik gemaakt van zowel wildvang als gekweekte vissen. De onderzoeker motiveert duidelijk waarom voornamelijk wildvang gebruikt zal gaan worden. Wildvang heeft de voorkeur omdat er verschillen kunnen zijn met kweekvis in gedrag en reacties op geluid. Gebruik van kweekvis zal een extra vertaalslag opleveren om straks de resultaten naar het veld te extrapoleren. Wildvang is ook nodig om de data van de floating pen met die van de tagged, free-ranging fish te vergelijken. De vissen worden, na vangst gehuisvest bij een stichting die beschikt over uitstekende faciliteiten voor huisvesting en verzorging van verschillende Noordzee vissoorten. Er is geen sprake van bedreigde diersoorten, niet-menselijke primaten, en/of zwerfdieren. De toegepaste methoden voor anesthesie zijn conform de Richtlijn.

10. De DEC is ervan overtuigd dat de dieren gehuisvest en verzorgd worden op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de richtlijn. De vissen worden gehuisvest bij een stichting die beschikt over uitstekende faciliteiten voor huisvesting en verzorging van verschillende Noordzee vissoorten.

11. De DEC heeft zich ervan verzekerd dat de aanvrager al het mogelijke heeft gedaan om het eventuele ongerief voor de proefdieren te identificeren, te verminderen en waar mogelijk te voorkomen. De DEC schat dat de dieren als gevolg van de het implanteren en verwijderen van de tag cumulatief maximaal matig ongerief zullen ervaren. Deze inschatting is in overeenstemming met het niveau van het cumulatief ongerief ingeschat door de onderzoekers.

12. De integriteit van dieren wordt fysiek aangetast doordat de dieren een tag geïmplanteerd krijgen. De integriteit zal ook gedragsmatig worden aangetast. Gedurende het project worden de dieren namelijk beperkt in hun bewegingsvrijheid. Hierdoor zullen de dieren minder natuurlijk gedrag kunnen vertonen.

13. Naar mening van de DEC zijn de humane eindpunten zorgvuldig beschreven en is de inschatting van de incidentie met betrekking tot het bereiken van een humaan eindpunt eveneens zorgvuldig beschreven in de projectaanvraag.

3V's

14. In het project wordt de keuze voor de diermodellen duidelijk onderbouwd. De betrokken dieren en het gekozen diermodel zijn het meest geschikt voor deze studieopzet. Atlantische kabeljauw is een belangrijke en overvloedige vissoort met een grote verspreiding. Tevens is het gehoor en de ecologie van kabeljauw vergelijkbaar met andere belangrijke commerciële vissen zoals zeebaars, schelvis, wijting en koolvis. De desbetreffende dierproef berokkent de dieren het minste pijn, lijden, angst of blijvende schade. De DEC is ervan overtuigd dat er geen alternatieven beschikbaar zijn voor het voorgestelde gebruik van intacte dieren om de doelstelling van dit project te realiseren.

15. In het project wordt optimaal tegemoet gekomen aan de vereisten van vermindering van dierproeven. Zo worden de dieren aan een reeks van meervoudige behandelingen blootgesteld zonder dat dit extra schade veroorzaakt. De onderzoeksgroep heeft jarenlange ervaring met dit soort experimenten. Bovendien beschikt de onderzoeksgroep over een team van biotechnici die de benodigde ervaring hebben met proefdieronderzoek. De DEC is ervan overtuigd dat het onderzoek ethisch verantwoord zal worden uitgevoerd. De DEC acht het maximale aantal te gebruiken dieren realistisch geschat.

16. De uitvoering van het project is in overeenstemming met de vereisten van verfijning van dierproeven en is zo opgezet dat de dierproeven met zo min mogelijk ongerief worden uitgevoerd. Bij de opzet van dit onderzoek wordt rekening gehouden met dierenwelzijn door het gebruik van adequate anesthesie waar nodig. De DEC is ervan overtuigd dat de beschreven dierproeven zo humaan mogelijk zullen worden uitgevoerd.

17. Het betreft hier geen wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. De aanvrager zal in het project in gebruik maken van zowel mannelijke als vrouwelijke vissen.

19. Een deel van de dieren wordt in het kader van het project gedood. Door middel van dissectie kunnen lange termijn effecten op vet ratio en gonade ontwikkeling worden bepaald. Het merendeel van de dieren wordt in leven gelaten en terug gezet in het wild. Kweek vissen zullen na afloop van het experiment worden gedood omdat deze niet terug gezet kunnen worden in het wild. Het doden van de dieren gebeurd volgens een voor de diersoort passende dodingsmethode die vermeld staat in bijlage IV van richtlijn 2010/63/EU.

20. Er worden voor dit projectvoorstel geen niet-humane primaten, honden, katten of landbouwhuisdieren gebruikt.

NTS

21. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd. De NTS voldoet daarmee aan de eisen zoals gesteld in artikel 10.a.1.7 van de Wod.

D. Ethische afweging

1. Rechtvaardigt het verkrijgen van inzicht in de effecten van seismische surveys op zwempatronen/ruimtelijke vermijding, foerageeractiviteit, gewenning en energiemetabolisme en kan het effect van seismische surveys op vissen worden verminderd door aanpassing van temporale of spectrale eigenschappen van de procedure en geluidsbron met als uiteindelijk doel is het verkrijgen van inzicht in de gevolgen van seismische surveys op het gedrag van vissen en hun fysiologie, het ongerief dat de dieren wordt aangedaan?

2. Project gericht op het verkrijgen van meer kennis over de relatie tussen seismische surveys en het gedrag en fysiologie van vissen.
   Waarden die voor proefdieren in het geding zijn: matig nadeel.
   Waarden die voor wetenschappers bevorderd worden: gering voordeel.
   Waarden die voor de vispopulatie, visserij en dierbeschermers bevorderd worden: groot voordeel.
   De DEC is van mening dat de belangen voor de vispopulatie in het algemeen zwaarder wegen dan de belangen/waarden van de proefdieren. Vissen maken gebruik van geluid voor allerlei activiteiten, bijvoorbeeld voor oriëntatie, communicatie, foerageren, het ontwijken van roofdieren of het vinden van een partner. De hoeveelheid lawaai onder water, veroorzaakt door mensen is in de laatste decennia zowel wereldwijd als in de Noordzee beduidend toegenomen. Sommige geluidsimpulsen zijn zo hard dat vissen dichtbij de bron lichamelijk letsel kunnen oplopen of zelfs dood gaan. Dit heeft geleid tot een groeiende zorg over het effect hiervan op aquatisch leven, inclusief vissen. Er is echter nog weinig inzicht in de mogelijke nadelige effecten op de individuele gezondheid en populatie dynamiek. De DEC acht het daarom van essentieel belang dat er meer kennis wordt verkregen over de gevolgen van geluidsimpulsen op vissen. Het is aannemelijk dat de doelstelling behaald zal worden. Hiertoe zullen dieren worden gebruikt. De onderzoekers doen er echter alles aan om het lijden van de dieren te beperken, waardoor het ongerief van de dieren zo veel mogelijk beperkt blijft.

3. De DEC is overtuigd van het belang van de doelstelling van dit project. De DEC is van mening dat de waarden die voor de doelgroep bevorderd kunnen worden zwaarder wegen dan de waarden die voor de proefdieren in het geding zijn. Het project is goed opgezet. De DEC is bovendien van mening dat de voorgestelde experimentele opzet en uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstelling en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De DEC is er verder van overtuigd dat de onderzoeksgroep voldoende ervaring heeft met de gekozen onderzoeksstrategie en met de voorgestelde dierproeven om de doelstelling te behalen en dat de aanvrager voldoende maatregelen treft om zowel het ongerief van de dieren alsmede het aantal benodigde dieren tot een minimum te beperken. De DEC onderschrijft dat de doelstelling niet zonder het gebruik van proefdieren behaald kunnen worden en acht het gebruik van het aantal dieren en het daarmee samenhangende ongerief bij de dieren gerechtvaardigd.

E. Advies

1. Advies aan de CCD
   - ✓ **De DEC adviseert de vergunning te verlenen.**
   - ☐ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden
     - ☐ Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.
     - ☐ Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
     - ☐ Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...
   - ☐ De DEC adviseert de vergunning niet te verlenen vanwege:
     - ☐ De vaststelling dat het project niet vergunning plichtig is om de volgende redenen:…
     - ☐ De volgende doorslaggevende ethische bezwaren:…
     - ☐ De volgende tekortkomingen in de aanvraag:…

2. Het uitgebrachte advies is gebaseerd op consensus.

3. Er zijn tijdens de beoordeling van dit projectvoorstel geen echte knelpunten en of duidelijke dillema’s naar voren gekomen.

Centrale Commissie Dierproeven

8.

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Leiden
[redacted]
Postbus 9502
2300 RA LEIDEN

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD1060020171085

Bijlagen
2

Datum 18 mei 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted],

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 18 mei 2017. Het gaat om uw project "PCAD4Cod' - Impact of seismic survey sound exposure on fish". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD1060020171085. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
18 mei 2017
Aanvraagnummer:
AVD1060020171085

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
18 mei 2017
Aanvraagnummer:
AVD1060020171085

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	10600
Naam instelling of organisatie:	Universiteit Leiden
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	27368929
Straat en huisnummer:	Rapenburg 70
Postbus:	9502
Postcode en plaats:	2300 RA LEIDEN
IBAN:	[redacted]
Tenaamstelling van het rekeningnummer:	Universiteit Leiden/ Faculteit W&N

Gegevens verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	PhD-candidate
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Datum:
18 mei 2017
Aanvraagnummer:
AVD1060020171085

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	PhD-candidate
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens verantwoordelijke uitvoering proces

Naam:	[redacted]
Functie:	PhD-candidate
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 april 2017
Geplande einddatum:	31 december 2021
Titel project:	PCAD4Cod' - Impact of seismic survey sound exposure on fish
Titel niet-technische samenvatting:	Het effect van seismisch onderzoek op zout:: ater-vissen
Naam DEC:	DEC Leiden
Postadres DEC:	[redacted] LUMC, Postbus 9600 2300 RC Leiden
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.684,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting
Overige bijlagen:	[x] DEC-advies

Ondertekening

Naam:	[redacted]
Functie:	directeur
Plaats:	Leiden
Datum:	17 mei 2017

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Leiden
[redacted]
Postbus 9502
2300 RA LEIDEN

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD1060020171085

Bijlagen
2

Datum 18 mei 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 18 mei 2017
Vervaldatum: 17 juni 2017
Factuurnummer: 171085

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD1060020171085	€ 1.684,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

9.

[redacted]

Van: [redacted]
Verzonden: dinsdag 30 mei 2017 17:22
Aan: Info-zbo; [redacted]
Onderwerp: RE: vraag bij de behandeling van aanvraag AVD1060020171085

Categorieën: Dossier: [redacted]

Dank [redacted],
Indien ik van dienst kan zijn ergens mee of als ik nodig ben voor de procedure dan hoor ik het graag.
Ik hoop namelijk dat we onnodige vertraging zo veel mogelijk kunnen voorkomen :)
Vriendelijke groet,

[redacted]

Van: Info-zbo [info@zbo-ccd.nl]
Verzonden: dinsdag 30 mei 2017 15:21
Aan: [redacted]
CC: [redacted]
Onderwerp: RE: vraag bij de behandeling van aanvraag AVD1060020171085

Beste [redacted]
Dank voor de aanvulling. De aanvraag zal aanstaande vrijdag door de CCD besproken worden,
Groeten [redacted]
Namens **Centrale Commissie Dierproeven**
www.centralecommissiedierproeven.nl
.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl (let op: nieuw emailadres!)

Van: [redacted]
Verzonden: dinsdag 30 mei 2017 14:20
Aan: info@zbo-ccd.nl; [redacted]
CC: [redacted]
Onderwerp: RE: vraag bij de behandeling van aanvraag AVD1060020171085
Beste [redacted]
Wij hebben de NVWA in februari 2017 op de hoogte gebracht van het huisvesten van vissen op de locatie [redacted] (tezamen met de registratiegegevens). Deze locatie is derhalve geen nieuwe locatie waar proefdieren worden gehuisvest en deze locatie valt onder de instellingsvergunninghouder Leiden Universiteit. Vraag G in de appendix 1-3 had derhalve met nee beantwoord moeten worden. In appendix 4 klopt het antwoord op de vraag, dit omdat het niet mogelijk is om een niet nader te duiden locatie op open zee als proefdierlocatie aan te duiden. Ik hoop dat de aanvraag met deze extra informatie toch in behandeling kan worden genomen en we zien graag de vergunning tegemoet.
Met vriendelijke groet,

[redacted]

From: Info-zbo [mailto:info@zbo-ccd.nl]
Sent: dinsdag 23 mei 2017 12:12
To: [redacted]
Subject: vraag bij de behandeling van aanvraag AVD1060020171085
Geachte [redacted],
U heeft bij de CCD een aanvraag tot projectvergunning gedaan. Het betreft uw project: 'Impact of seismic survey sound exposure on fish' met aanvraagnummer AVD1060020171085. In uw projectvoorstel beschrijft u dat een deel van het onderzoek wordt uitgevoerd bij de [redacted] . U geeft aan dat het onderzoek plaatsvind in een instelling die niet onder een instellingsvergunning valt. U bent wel verplicht deze nieuwe locatie aan te melden bij de NVWA voordat de dierproeven aanvangen. Op deze wijze valt de locatie dan onder de instellingsvergunning van vergunninghouder 10600. U kunt de locatie aanmelden via chd@nvwa.nl
Vriendelijke groet, [redacted]
Uit de memorie van toelichting bij de dierproevenregeling
2.1.1 Direct melden van wijzigingen met betrekking tot de instellingsvergunning
Na verlening van een instellingsvergunning zal de vergunninghouder wijzigingen die betrekking hebben op de instellingsvergunning onverwijld door moet geven aan de Minister. Het gaat daarbij om wijzigingen in één van de personen die op grond van artikel 6, derde lid, van de wet in de instellingsvergunning worden vermeld, en elke significante wijziging van de structuur of de werking van een inrichting die het dierenwelzijn negatief kan beïnvloeden. Hierbij moet worden gedacht aan gevallen waarbij op basis van de gewijzigde situatie mogelijk anders op een aanvraag om een instellingsvergunning zou zijn beslist, dan wel wijzigingen die bekend moeten zijn bij de toezichthouder met het oog op inspecties. Hieronder valt in ieder geval elke nieuwe locatie waar proefdieren worden gehuisvest of dierproeven worden verricht, grootschalige verbouwingen op deze locaties en reorganisaties. De vergunninghouder geeft deze wijzigingen door aan de Minister. De wijzigingen moeten worden doorgegeven zodra de vergunninghouder hier redelijkerwijs van op de hoogte kan zijn.

Centrale Commissie Dierproeven
www.centralecommissiedierproeven.nl
.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl (let op: nieuw emailadres!)

10.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Leiden
[redacted]
Postbus 9502
2300 RA LEIDEN

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD1060020171085
Bijlagen
1

Datum 6 juni 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted],

Op 18 mei 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "PCAD4Cod' - Impact of seismic survey sound exposure on fish" met aanvraagnummer AVD1060020171085. Wij hebben uw aanvraag beoordeeld.

Op 23 mei 2017 heeft u uw aanvraag aangevuld. Wij hebben u een vraag gesteld over de locatie waar de dierproeven worden uitgevoerd. Uw antwoorden hebben niet geleid tot aanpassing van de aanvraag.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
Met het oog op artikel 10a, lid 1, zijn er algemene voorwaarden gesteld.

U kunt met uw project "PCAD4Cod' - Impact of seismic survey sound exposure on fish" starten. De vergunning wordt afgegeven van 6 juni 2017 tot en met 31 december 2021.

Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC Leiden gevoegd. Dit advies is opgesteld op 16 mei 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de

Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Datum:
6 juni 2017
Aanvraagnummer:
AVD1060020171085

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Centrale Commissie Dierproeven
namens deze:

[redacted]

ir.
Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Universiteit Leiden
Adres:	Postbus 9502
Postcode en plaats:	2300 RA LEIDEN
Deelnemersnummer:	10600

deze projectvergunning voor het tijdvak 6 juni 2017 tot en met 31 december 2021, voor het project "PCAD4Cod' - Impact of seismic survey sound exposure on fish" met aanvraagnummer AVD1060020171085, volgens advies van Dierexperimentencommissie DEC Leiden. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is PhD-candidate. Voor de uitvoering van het project is PhD-candidate verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 18 mei 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 18 mei 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 18 mei 2017;
c Advies van dierexperimentencommissie d.d. 16 mei 2017, ontvangen op 18 mei 2017.
d De aanvullingen op uw aanvraag, ontvangen op 23 mei 2017

Aanvraagnummer:
AVD1060020171085

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 Recognizing behaviour with accelerometers in captive fish monitored by video				
	Andere vissen (andere Pisces) / Atlantische kabeljauw	10	100% Matig	
3.4.4.2 Effects of acoustic variation on fish response to sound in a floating pen				
	Andere vissen (andere Pisces) /	920	100% Matig	
3.4.4.3 Sound exposure effects on fish growth and stress physiology in a floating pen				
	Andere vissen (andere Pisces) /	240	100% Matig	
3.4.4.4 Full seismic survey effects on tagged fish while free-ranging at sea				
	Andere vissen (andere Pisces) /	33	100% Matig	

Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van

het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

Aanvraagnummer:
AVD1060020171085

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.

Locatie
De vergunning wordt verleend voor een project waarbij dierproeven geheel of gedeeltelijk worden verricht buiten een inrichting van een gebruiker (artikel 10g van de wet).