CHAPTER 3

SWIM BLADDER BAROTRAUMA IN ATLANTIC COD WHEN *IN SITU* EXPOSED TO PILE DRIVING

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Abstract

In view of the rapid increase of offshore wind farms in the North Sea, and in order to further determine sound thresholds to be used in international guidelines, it is needed to acquire more knowledge on the effects of pile driving sounds on fish health. Therefore, in the summer of 2016, a field experiment was undertaken in the Nobelwind OWF on the BPNS to determine the direct effect of pile driving on the health status of Atlantic cod (*Gadus morhua*). Large netted cages, each holding 9 to 12 cod individuals (avg. size 31 cm), were submerged at 8 m under the water surface. The cages were placed at increasing distances (75 m, 400 m, 1400 m and 1700 m) from the sound source, being the offshore installation vessel *Vole au vent*. All cages were submerged for on average 16 hours before pile driving, after which all fish were exposed to one pile driving event (lasting on average 2 hours). A similar control experiment was repeated in the same period when no pile driving took place. Underwater sound levels were measured at different distances during pile driving, while background measurements were made to determine ambient sound levels. Average single strike sound exposure levels (SEL_{ss}) decreased from 175 dB re 1µPa²s at 400 m distance to 168 dB re 1µPa²s at 1700 m distance. Ambient sound pressure levels (SPL) varied between 114 and 138 dB re 1µPa. After retrieval of the cages onboard RV Simon Stevin, all cod individuals were evaluated for buoyancy in water tanks. Shortly afterwards, all fish were euthanized and examined for swim bladder barotrauma and internal bleeding. Overall, 11% cod were retrieved dead, most probably due to handling stress, as no direct relation could be found with distance to the sound source. On the other hand, a steep increase in swim bladder barotrauma was detected with decreasing distance to the pile driving source: no swim bladders were ruptured at 1700 m nor at the control treatments, 20% were ruptured at 1400 m distance, 40% at 400 m distance and up to 90% of the swim bladders were ruptured at 75 m distance. Although most fishes in the cages in the direct vicinity of the piling source (100 m distance) did survive this short term experiment, they all showed many multiple instances of internal bleeding and a high degree of abnormal swimming behavior, hinting towards a reduced survival rate on the longer term. However, these immediate detrimental effects seem to occur only locally, close to the high impulsive sound source, as swim bladder injuries rapidly decreased with increasing distance from the pile

driving source. Results of this *in situ* experiment provide valuable information to scientifically evaluate the current "critical sound limits" implemented in Belgium in the setting of the Marine Strategy Framework Directive.

1. Introduction

Underwater sound related to human activities is an increasing source of pollution in the marine environment (Hildebrand 2009). Although offshore wind farms (OWFs) do create green energy, they alter temporarily and permanently the marine ecosystem by introducing different types of underwater sound. Especially during the construction phase, high impulsive sound is generated when the steel foundation piles are driven into the sea bottom. Impulsive underwater sound can be detrimental to marine life. Several laboratory experiments on fish and marine mammals showed disturbance of behaviour, physiological stress, internal and external injuries, sometimes leading to mortality (Popper & Hastings 2009; Hawkins & Popper 2016). However, a recent *in situ* study in the Belgian part of the North Sea (BPNS) only showed short term physiological effects in larval and juvenile seabass (*Dicentrarchus labrax*) after exposure to high impulsive pile driving sound in the direct vicinity (< 50 m) of a real pile driving event (Debusschere *et al.* 2014; 2016). Still, during that field experiment adult whi ting (*Merlangus merlangus*) was seen floating at the surface at the moment of pile driving. Next to the need to further determine solid sound thresholds to be used in international guidelines, this anecdotal observation was the immediate reason for the current *in situ* experiment with Atlantic cod (*Gadus morhua*).

2. Material and methods

2.1. Study area

To examine the impact of pile driving on Atlantic cod, a field experiment was undertaken during construction of the Nobelwind wind farm, situated on the Bligh Bank (fig. 1). In total, 50 monopiles have been installed and each one was designed for its specific position in the wind farm, and varied in length, diameter and steel thickness. The monopiles

Table 1. Characteristics, date, pile driving time, number of strikes, energy and depth for each monopile

MP number	J05	J08	J07	I06
Measurement type	Cod exposure 1	Cod exposure 2	Sound measurement 1	Sound measurement 2
Date	5/07/2016	13/07/2016	12/07/2016	13/09/2016
Time of day (h)	01:35	10:18	08:56	15:50
Diameter (m)	5	5	5	4.5
Steel thickness (mm)	70	70	70	78
Length (m)	66.4	67.7	65.3	67.2
Depth in seafloor (m)	30	30	32	30
Total strikes	2985	2888	3606	3123
Total energy (kJ)	2488771	2380981	3020305	2017849
Total pile driving time (h)	2:18	2:03	3:11	1:52
Net hammering time (h)	1:14	1:17	1:38	1:12

were installed by using a hydraulic piling hammer (IHC Hydrohammer B.V.). During our exposure experiments, monopiles J05 (lat. 51.67223°, long. 2.86620°) and J08 (lat. 51.67255°, long. 2.84803°) were driven into the seabed. When sound was measured monopiles J07 (lat. 51.67005°, long. 2.85506) and I06 (lat. 51.65195, long. 2.84043) were installed (table 1, fig. 1).

2.2. Characteristics, catching and housing of Atlantic cod

Atlantic cod (*Gadus morhua*) is an important commercial species but due to overexploitation, it is classified as a vulnerable species on the IUCN list (http://www.iucnredlist.org/). Age I and II-group cod are known to aggregate around OWFs in the BNS (Reubens *et al.* 2013; 2014). Atlantic cod is a round fish with a closed swim bladder (physoclist), which makes it more vulnerable to swim bladder injuries. Physoclistous fish cannot rapidly change the volume of their swim bladder, but depend on gas secretion and absorption to regulate their buoyancy. Consequently, when exposed to high impulsive sound such as pile driving, the swim bladder acts as an air bubble which vibrates. These vibrations can cause damage to the swim bladder itself or to neighbouring organs (Halvorsen *et al.* 2012a and references herein).

The Atlantic cod used for this experiment were caught using hook and line gear (bait: *Arenicola marina*) near the gravity-based foundations of the C-Power wind farm (51°33´N, 2°56´E, WGS84) from RHIB Zeekat on 23 June 2016 and 7 July 2016. Depth around the foundations is around 23 m at mean low water spring (MLWS). In order to minimize the risk of barotraumas, fish were hauled very slowly to allow them to release excess gas and prevent swim bladder rupture. Fish ranged between 21 and 42 cm in total length, and were on average 31 cm (± SD 4 cm) (age I-group). 85% of the fish survived the angling, the other 15% died almost immediately because of barotrauma of the swim bladder. In total, 87 individuals (70 on 23 June, 17 on 7 July) survived to be used as test animal. After capture, the fish were kept on board RV Simon Stevin in an aerated flow-through seawater tank covered with wet blankets to create a shaded environment during transit to the land-based facilities. Transit at sea took between 6 and 8 hours.

Back on land in Ostend, the water tanks were immediately transported (5 minutes) to the Marine Station Ostend (MSO) of VLIZ, where the cod individuals were housed in two large, circular water tanks (4000 l, 2.5 m Ø and 1.2 m depth) for acclimatization. Each tank contained a maximum of 45 individuals. Both tanks were completely separated and were provided with aeration by a flow through of ozone sterilized seawater in a closed circulation system. Furthermore, each tank had its own filtration system: a biological filter tank and mechanical filters (drum filter and protein skimmer). The tanks were located in a climatic room (100 m²) with adjustable light and temperature regime. Conditions in the tank were kept as similar as possible to the natural conditions, which were a sea water temperature around 15.5° C, salinity of 30 PSU and a light regime (with dimmed light) of 16 h light/8 h dark. Each tank was equipped with a temperature sensor and a redox sensor. Mortality was checked every day: only one dead fish was observed and removed. Water quality (pH, NH_4^+/NH_3, NO_2^-, NO_3^-, O_2 % and kH) was checked on a near daily basis and when needed, part of the seawater in the circulation system was replaced by fresh sea water to restore the water quality. After an acclimatization period of 5 days, the cod were fed every two to three days with frozen fish, shrimps or lugworms (± 2% of estimated body weight/day).

The cod were kept in these aquarium units between 5 and 19 days before the experiment took place. At the day of the experiment, the cod were transferred to a mobile water tank, transported to RV Simon Stevin (5 minutes) and put on board. During

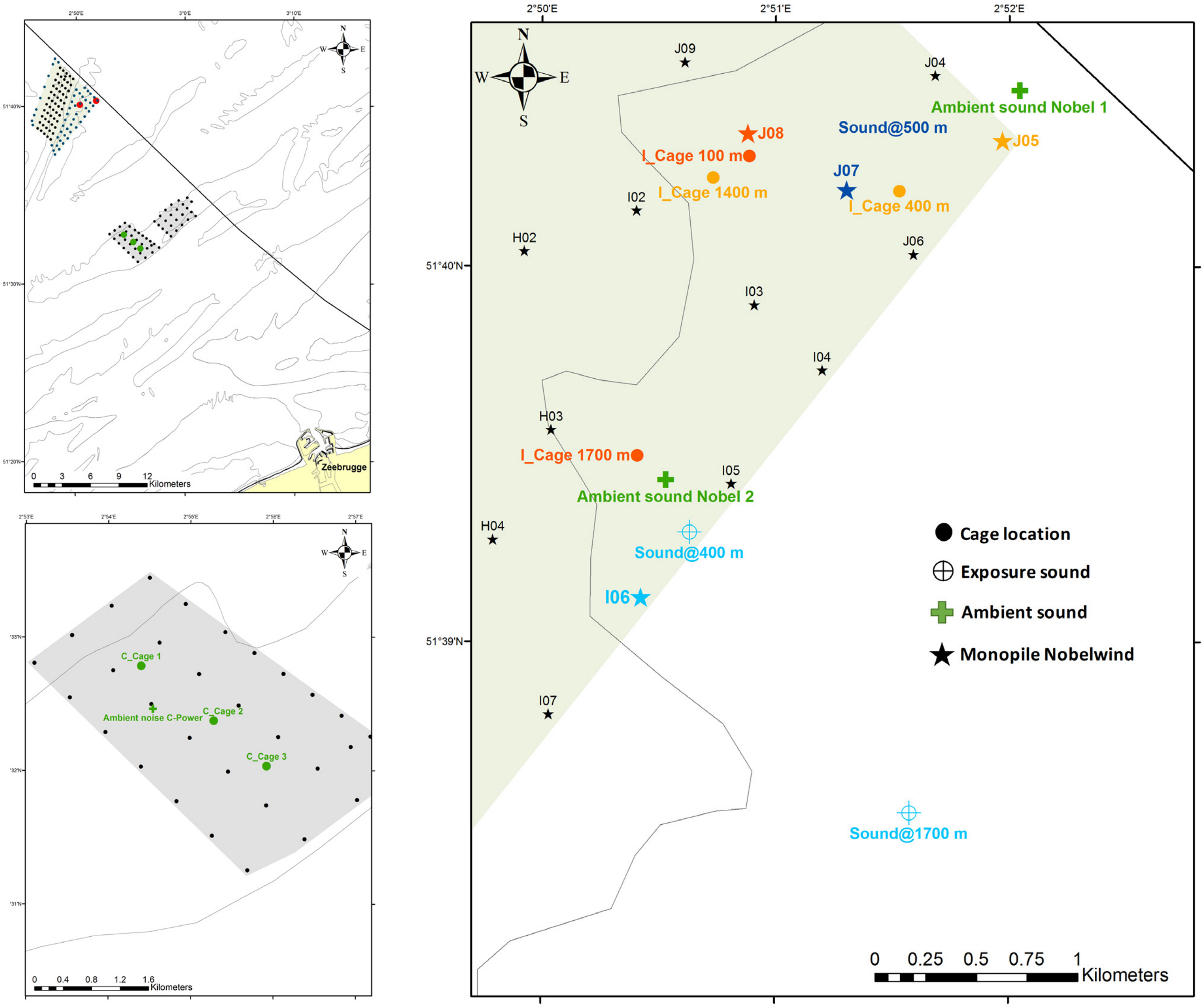

Figure 1. Top left: Overview of study area with indication of control locations (green dots) and exposure locations (red dots) in under construction wind farm Nobelwind (in blue). Below left: Locations of the three control cages in the C-Power wind farm and location of the background sound measurement for the control locations. Right: Zoom in showing 1) the monopiles driven in the seabed during cod exposure (orange J08 and yellow J05) with position of the exposure cages (same colour as mp) relative to the monopile; 2) the monopiles driven in during sound measurements (I06 and J07 in blue) with position of measurement locations (same colour as mp); 3) locations of the background sound measurements in the Nobelwind concession area when no pile driving took place.

transit to the *in situ* experimental location, tanks were provided with aeration and seawater flow through, and covered with wet blankets to create a shaded environment.

The experimental protocol was approved by the ethical committee of the Institute for Agricultural and Fisheries research (ILVO) (Permit Number: EC 2016/275 and recognition LA1300512 for temporal storage in MSO).

2.3. Cage design and experimental set-up

To expose cod, large netted cages (mesh size 2 x 2 cm, 1.5 m Ø and 6 m height), were submerged at an average depth between 7 and 14 m (fig. 2). The cages were kept in place by a weight of 600 kg at the seabed. Subsurface buoys were used to keep the cage open and a surface buoy was put in place to be able to relocate the cage position for pick-up (fig. 2). Cages were put in position and

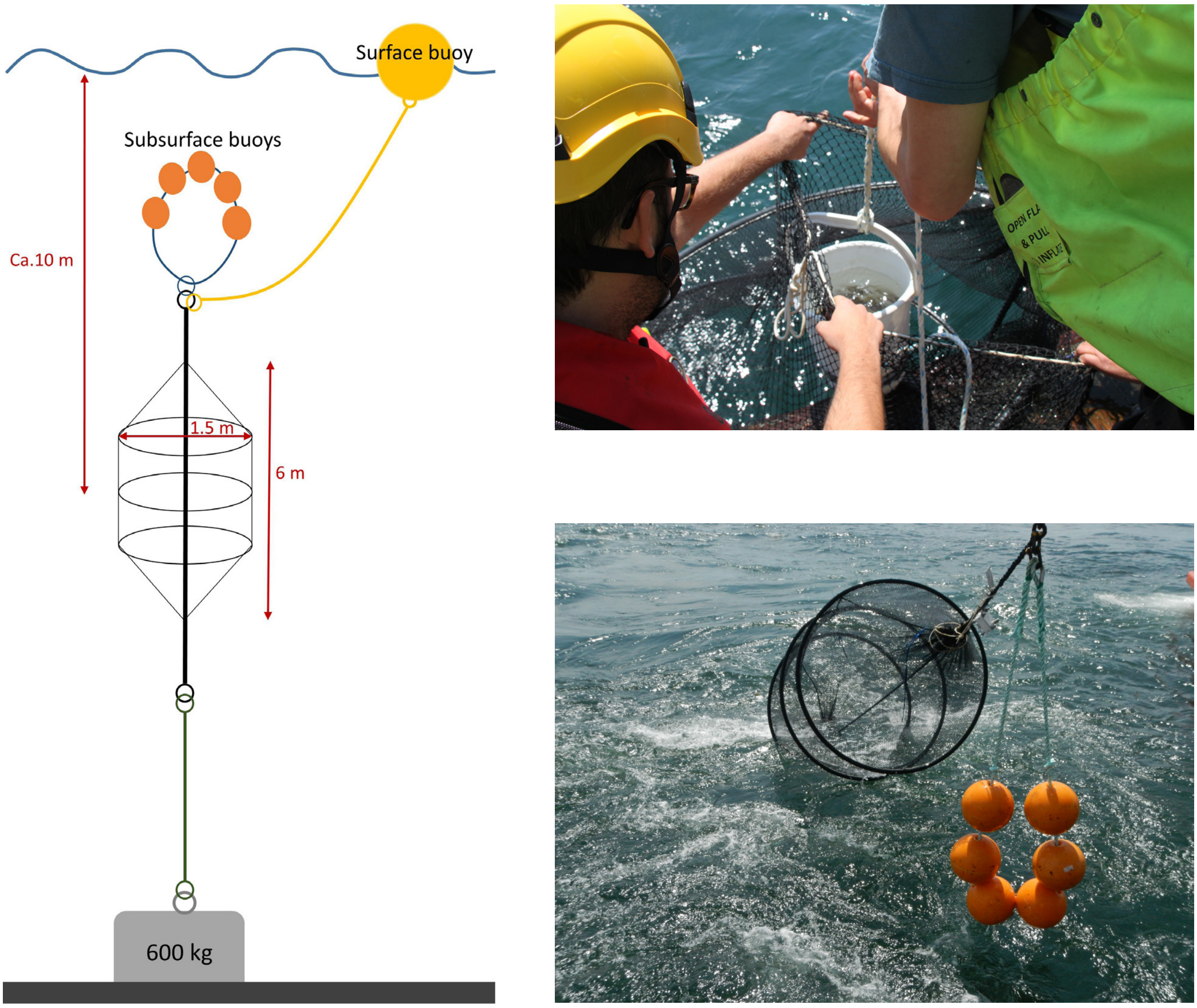

Figure 2. Left: schematic drawing of the set-up of the cages used for the exposure and control treatments. Right top: picture of cage. Right down: putting cod in the cage.

picked up by RV Simon Stevin at moments with sea state ≤ 3.

Lowering of the cages was executed with the winch of the ship's A-frame. Once the cage was partly submerged, fish were gently put in the cage with a bucket filled with seawater. After closing the cage, it was lowered further until the mooring weight touched the bottom. Length of the ropes (cage to weight; cage to surface buoy) was adjusted to the depth of the seabed at location to make sure that cage depth was similar for all cages.

The study set-up consisted of two treatments: exposure and control. Exposure cages, each holding 10 or 12 age I-group cod, were placed at increasing distances (100, 500, 1400 and 1700 m) from the sound source, being the offshore installation vessel *Vole au vent* (fig. 1). This was done at two different moments in time, when pile driving was predicted to occur. At each date, two cages were put out (table 2, fig. 1). Cages were put in place at least 12 hours before the start of pile driving in order for the fish to adapt to the pressure at depth and acquire neutral buoyancy. It was intended to retrieve cages after 24 hours (cf. control treatment), but due to bad weather conditions at both exposure occasions, this was not possible. Cages were only retrieved after 45 hours (table 2).

Table 2. Metadata on timing, depth, fish length and exposure time for each experimental set-up (Exp = exposure)

Experiment	**Exposure 1 (Nobelwind)**		**Exposure 2 (Nobelwind)**		**Control (C-Power)**		
Treatment	**Cage@500 m**	**Cage@1400 m**	**Cage@100 m**	**Cage@1700 m**	**Cage 1**	**Cage 2**	**Cage 3**
Monopile	J05	J05	J08	J08	/	/	/
Number of cod individuals	10	10	12	12	9	9	9
Average length ± SD (cm)	30 ± 4	30 ± 3	30 ± 5	31 ± 5	31 ± 4	33 ± 5	30 ± 3
Date in	4/07/2016	4/07/2016	12/07/2016	12/07/2016	6/07/2016	6/07/2016	6/07/2016
Time in (h)	12:30	13:00	15:02	14:15	14:30	14:55	15:15
Date out	6/07/2016	6/07/2016	14/07/2016	14/07/2016	7/07/2016	7/07/2016	7/07/2016
Time out (h)	12:05	12:55	12:52	12:20	11:40	12:15	13:11
Date piling	5/07/2016	5/07/2016	13/07/2016	13/07/2016			
Total time in H_2O (h)	47:35	47:55	45:50	45:55	21:10	21:30	21:56
Time in H_2O before exp (h)	13:05	12:35	19:03	18:15			
Time in water after exp (h)	32:12	33:02	24:31	23:59			
Avg depth cage (m)	10	12	8	8	14	7	11
Depth location (m)	33	35	33	33	24	22	21

For the control treatment, a similar experiment was repeated in C-Power (located at 15 km from Nobelwind), when no pile driving took place on the BPNS (fig. 1). Three replicate cages, each holding 9 cod individuals, were put out for 21 to 22 hours under similar conditions as the exposure treatments in order to be able to measure the cage effect (table 2).

Retrieval of the cages occurred with RHIB Zeekat and RV Simon Stevin. The RHIB could get close to the surface buoy to attach the winch rope of RV Simon Stevin onto the cage. When attached to the vessel, winching started very slowly in order to allow the cod to release excess gas, and not to rupture the swim bladder. Both cages of exposure 1, control cage 2 and exposure 2 cage@100 m were not retrieved ideally due to collapse of the circular hoops of the cages whilst winching up. Because of these retrieval issues, these fish got probably extra stress.

2.4. Cod necropsy

Upon retrieval of the cages, the cod individuals were, with help of a hand net, fished out of the cages when these surfaced, and placed onboard in water tanks with flow through and aeration. Necropsy was started two to three hours after retrieval of the fish from the cages, so they had time to adjust their buoyancy to atmospheric pressure.

Just before necropsy, buoyancy status of each fish was judged and noted by two persons. Buoyancy status of a fish was evaluated by observing its swimming behaviour during 5 minutes. Behaviours identified were: normal swimming near the bottom, on side swimming, belly-up swimming, abnormal swimming which is all behaviour different from the above (*e.g.*, struggling to get down or up, very passive…) and dead. Afterwards, the fish was euthanized in an overdose anesthetic (5 g benzocaine dissolved in 25 ml acetone and 1 l sea water), total length and wet weight were measured, the fish was coded and a picture taken. Fish were taken randomly from the different cages at each experimental day, and handed over to a person performing the necropsy, who was unaware of the cage treatment. All necropsies were done by the same person.

The necropsy was focused on potential swim bladder (SB) injuries. For each fish, inflation or deflation of the SB was noted; the presence of ruptures or small holes in the SB; was noted as well; and it was also written down whether air was trapped in the body wall and if so, what the volume of the air bubble was. Each necropsy was documented with at least one photograph. The protocol followed was outlined after personal communication with Michele Halvorsen (CSA Ocean Science).

2.5. Acoustic equipment and sound recordings

Sound pressure was measured using two Brüel & Kjaer hydrophones (type 8104, voltage sensitivity 47.7 µV.Pa^{-1}, charge sensitivity 0.391 pC.Pa^{-1}, 10 m cable and 50 m cable). The 10 m cable hydrophone was connected to the charge channel of a Brüel & Kjaer portable amplifier (Nexus type 2690-0S). The 50 m cable hydrophone was connected to a Brüel & Kjær amplifier (Nexus type 2692-0S4) The measurement chain was completed resp. with a multi-channel portable recorder (Tascam DR-680) and an audio MARANTZ Solid State Recorder (type PMD671). The signal was recorded in 1-channel WAVE format (.wav) on Compact Flash cards of resp. 16 GB (SanDisk Ultra) and 2 GB (Sandisk Ultra II) with a sampling rate of 44,100 Hz at 24 bit. To standardize the recorded signals, a reference signal together with the output sensitivity was used. Batteries powered all equipment. Hydrophones were deployed at 10 m depth for all sound recordings.

Recordings of pile driving sound were performed at two occasions (MP J07 on 12 July 2016 and I06 on 13 September 2016). The nearby measurements were made from a drifting RHIB (Zeekat) with motor turned off and the further away measurement from the anchored RV Simon Stevin (table 3, fig. 1). Background measurements were made to measure ambient sound at two occasions and at both the exposure location and the control location (fig. 1). See table 3 for details on the sound recordings.

Table 3. Detailed information (date, duration, ship, sensitivity) of all sound recordings performed

	Pile driving 1	Pile driving 2		Background		
	Sound@500 m	Sound@400 m	Sound@1700 m	Nobel 1	Nobel 2	C-Power
Date	12/07/2016	13/09/2016	13/09/2016	12/07/2016	13/07/2016	13/07/2013
Ship	RHIB Zeekat	RHIB Zeekat	RV Simon Stevin	RHIB Zeekat	Geosurveyor X	Geosurveyor X
Moving/Anchored	drift	drift	anchored	drift	anchored	attached to turbine
Recording duration	7'	21'	53'	36'	38'	35'
Number of strikes	283	945	1228	0	0	0
Sensitivity	100 µV Pa^{-1}	100 µV Pa^{-1}	1 mV Pa^{-1}	31.6 mV Pa^{-1}	10 mV Pa^{-1}	10 mV Pa^{-1}

The sound pressure metrics, zero-to-peak sound pressure level (L_{z-p}), average sound pressure level (SPL), single strike and cumulative sound exposure level (SEL_{ss} and $SEL_{cum,p}$) were calculated using Matlab R2012b (version 8.0). In addition, the sound pressure metrics were calculated per 1/3 octave band, resulting in the highest energy over the frequencies. More details on the sound pressure parameters and how these were calculated can be found in Debusschere *et al.* (2014).

3. Results

3.1. Sound parameters

The pile driving sound levels that were measured at two occasions at 10 m water depth reached an average SEL_{ss} of 175-176 dB re1 µPa².s at 400-500 m distance and 168 dB re1 µPa².s at 1700 m distance from the sound source (table 4). The L_{z-p} rose to 196-199 dB re1 µPa at 400-500 m distance and 188 dB re1 µPa at 1700 m distance, while $SEL_{cum,p}$ reached resp. 210-212 dB re1 µPa².s and 203 dB re1 µPa².s (table 4). The dominant energy during exposure (SEL_{ss}) was present in the range 125-200 Hz, although no steep decline was recorded towards the higher frequencies (fig. 3). The ambient SPL during the background sound measurements varied between 114 dB re1 µPa (in Nobelwind) and 138 dB re1 µPa (in C-Power) (table 4).

3.2. Buoyancy status

In total, 8 out of 71 (11%) cod individuals died during the field study. Dead fish occurred in both the control and the exposure treatments (fig. 4). These fish probably died due to handling stress, as no direct relation could be found with distance to the sound source.

In the control treatments, on average 81% of all fish were evaluated with normal swimming behaviour versus 55% in the exposure treatments. The lowest percentage (33%) of normal swimming behaviour was noted for the cage@100 m, which consequently had also the highest percentage of swimming behaviour deviating from normal (42%). The other exposure cages showed a normal swimming behaviour between 50-60% (cage up to 1400 m) and 75%

Table 4. Sound pressure metrics measured at different distance from the sound source during pile driving and background metrics when no pile driving took place

Exposure sound metrics	**Sound@500 m**	**Sound@400 m**	**Sound@1700 m**
Total number of strikes	3606	3123	3123
Total strikes measured	283	945	1228
SEL_{ss} mean (dB re1 µPa^2.s)	176	175	168
L_{z-p} (dB re1 µPa)	199	196	188
$SEL_{cum,\,p}$ (dB re1 µPa^2.s)	212	210	203
1/3 octave band with most energy (Hz)	125	160	200
Background sound metrics	**C-Power**	**Nobel 1**	**Nobel 2**
SPL (dB re1 µPa)	138	120	114
1/3 octave bands with highest energy (Hz)	25	50	125-200

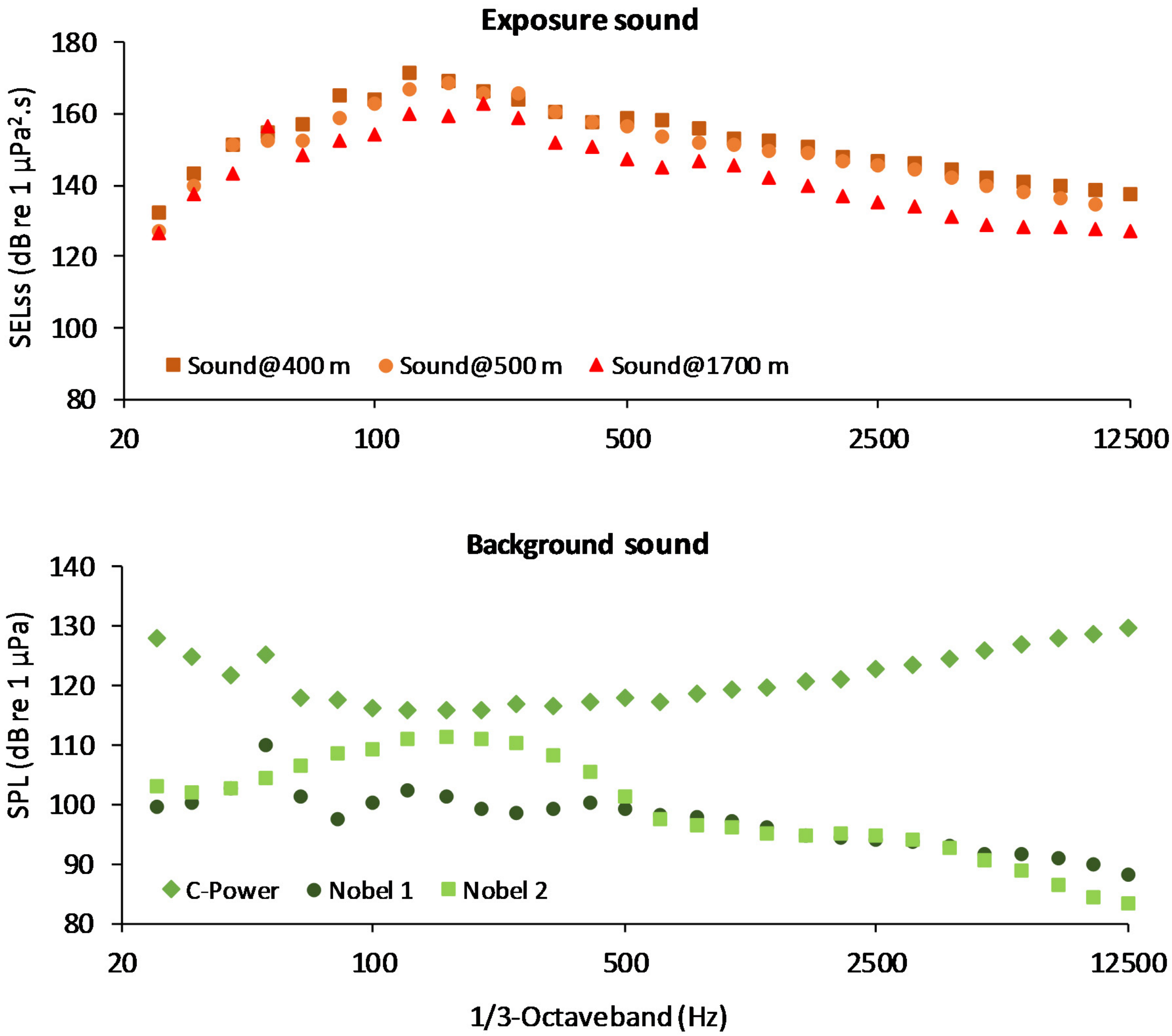

Figure 3. Measured frequency spectra in the presence (upper graph) and absence (lower graph) of pile driving. Mean SELss of the total recorded piling strikes versus 1/3 octave bands for exposure sound and SPL versus 1/3 octave bands for the background sound.

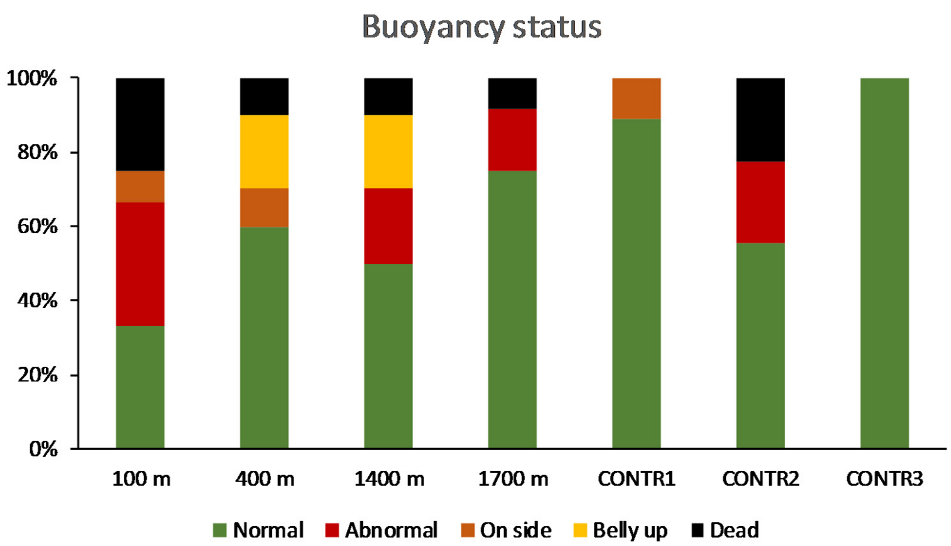

Figure 4. Relative occurrence of swimming behaviour for each control and exposure cage.

(cage@1700 m). Only 4 belly-up swimmers and 3 side swimmers were observed in all cages, mainly in individuals from exposure cages. For the control cages, only control cage @2 (which was not retrieved in the best circumstances) showed a slightly higher percentage of abnormal swimming behaviour (22%).

3.3. Swim bladder injuries and internal bleeding

A steep increase in swim bladder barotrauma was detected with decreasing distance to the pile driving source: no swim bladders were ruptured at 1700 m nor at the control treatments, 20% were ruptured at 1400 m distance, 40% at 400 m distance and more than 90% of the swim bladders were ruptured at 100 m distance (fig. 5). At most cages SB inflation was mostly 100%, while at the cage@100 m, a high percentage of deflated SBs (75%) was observed (fig. 5).

Concerning internal bleeding, the highest percentage of fish with multiple instances of internal bleeding (92%) was again detected for the cage@100 m, while at the exposure cages further away from the pile driving source, percentage of fish with internal bleeding still ranged between 20 and 50%. At control cages, on average 7% of fish with internal bleeding was observed (fig. 5).

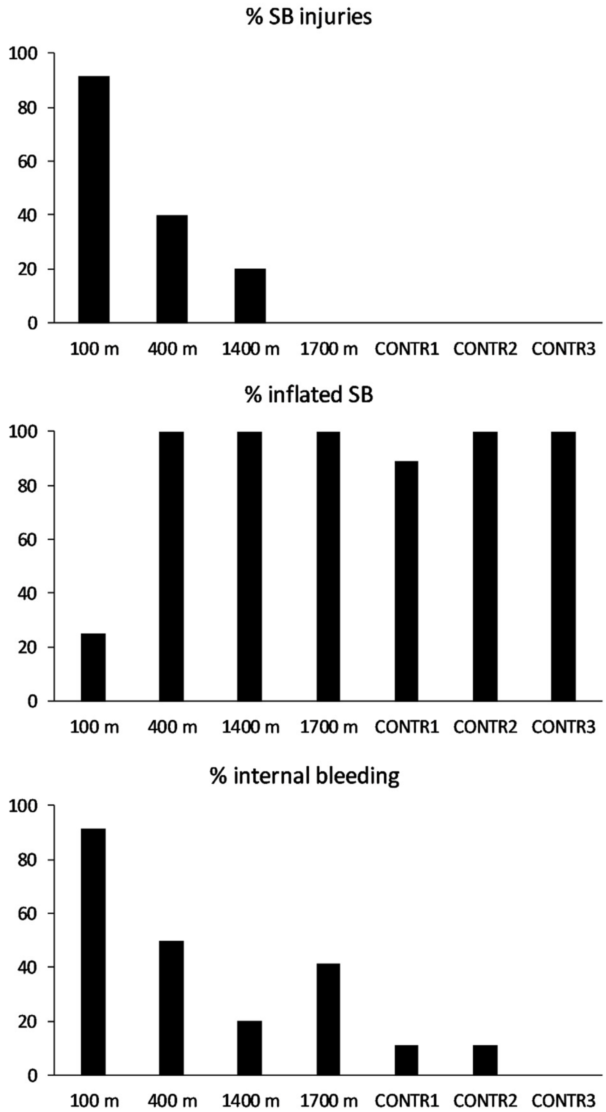

Figure 5. Percentage of swim bladders (SB) with barotrauma (upper graph), of inflated/deflated SBs (middle graph) and of fish with internal bleeding (lower graph) for each control and exposure cage.

4. Discussion

Pile driving for offshore wind farm construction causes ruptured swim bladders and internal bleeding in age I-group cod (avg. total length 31 ± 4 cm). However, these internal injuries decreased rapidly with increasing distance from the pile driving source, and consequently with decreasing sound level. The immediate detrimental effects seem to be restricted, occurring only close to the high impulsive sound source. At 100 m distance of the pile driving source, over 90% of the swim bladders were ruptured while at a distance of 1700 m, no ruptured swim bladders were found anymore, only a few internal bleeding and most fish showed normal swimming behaviour. Furthermore, this field experiment represents a "worst-case" scenario: fish were caged and had no chance to swim away if they would have wanted; and cod is a representative for fish with a closed swim bladder (*i.e.*, physoclist), which are most sensitive to swim bladder injuries (Halvorsen *et al.* 2012b).

Most cod survived on the short term, but since they all showed numerous multiple instances of internal bleeding and a high degree of abnormal swimming behaviour, their survival chances on the longer term would probably be reduced. Most of these fish at 100 m had deflated swim bladders due to the large ruptures in the swim bladder, and although it is shown that these injuries might heal over time (Casper *et al.* 2013), the time needed for healing makes them more vulnerable to predation and other threats in the wild.

Although, we had the intention to measure pile driving sound simultaneously with cod exposure, we did not succeed due to weather and logistical issues. For similar exposure experiments in the future, we strongly recommend to use smart digital autonomous hydrophones which can be deployed together with the cages. This would increase the robustness of the results, and reduce the demanding logistical organization. Nevertheless, we were able to measure underwater sound during pile driving from two different monopiles and at three different distances. The sound metrics presented here are serving as proxies. However, Debusschere *et al.* (2014) has shown that sound metrics during pile driving do not differ a lot between different monopiles with similar characteristics, driven in the seafloor to a depth of 30-33 m and in a similar sandy environment. So, we trust our sound measurements to be valuable, and not to diverge too much from what the fish experienced during exposure.

The sound parameters measured at 400 and 500 m distance were very similar, with values a bit higher at 500 m, but this could probably be explained by the larger pile diameter (5 m compared to 4.5 m), since sound pressure level increases with increasing diameter (Nehls *et al.* 2007 and references therein). Additionally, at 500 m distance we also measured the last strokes of the pile driving event which contain more energy.

Pile driving sound showed a frequency peak between 125 and 200 Hz which is right in the middle of the hearing range of cod that is between 30 and 470 Hz with greatest sensitivity in the range between 60 to 310 Hz (Chapman & Hawkins 1973). Since this was a short-term study, we chose to focus on swim bladder barotraumas, and did as such not look for injuries at the inner ear. Possibly, some of the abnormal swimming behaviour observed at the further distance cages could be related to potential inner ear damage. For future studies, it would be interesting to investigate whether potential inner ear injuries at further distances of the sound source influence fish behaviour on the short- and/or the long-term.

Halvorsen *et al.* (2012a) showed that the severity of injuries is not only owing to the total energy level of exposure (SEL_{cum}); the energy level of exposure of one single impulse (SEL_{ss}), and the number of impulses are as important. Therefore, it is important to include these parameters in measures to manage the activities generating impulsive sounds (Halvorsen *et al.* 2012a). Based on the results of our *in situ* exposure experiment, where we observed no ruptured swim bladders at 1700 m distance from the sound source for Atlantic cod, swim bladder barotrauma in Atlantic cod could be prevented at SEL_{ss} values of 165 dB re1 µPa^2.s and SEL_{cum} values of 200 dB re1 µPa^2.s or lower. Zero-to-peak levels ($SPL_{z\text{-}p}$) should not exceed 185 dB re1 µPa in order to prevent swim bladder barotrauma. The current “critical sound limit” implemented in Belgium in the setting of the Marine Strategy Framework Directive is 185 dB re1 µPa at 750 m (Rumes *et al.* 2015), no sound thresholds for SEL_{ss} or SEL_{cum} are in place at the moment. Our results indicate that with the current sound limits, swim bladder barotrauma can occur in physoclistous fish like Atlantic cod when they are within a radius of 750 m distance around the sound source during pile driving. This is, however, a small-scale effect, and it seems unlikely to cause significant effects at the population level. Nevertheless, in order to investigate what the observed effect means on a wider scale, the individual impact can provide the basis for a population impact assessment. This is outside the scope of this manuscript, but it is important to consider when deciding on management or mitigation measures. The information gathered during our *in situ* exposure experiment contributes to the knowledge base on effects of impulsive sound, and can be used to scientifically evaluate and potentially modify existing sound limits.

5. Conclusion

This field experiment was a logistic and organizational challenge, and although the design could be criticized as no replicate cages were submerged at the different distances and sound measurements were not taken simultaneously with exposure of the fish, the obtained results are valuable because they increase the available knowledge of sound pressure effects on physoclistous fishes and help to evaluate current sound thresholds. To our knowledge, this is the first *in situ* experiment in which age I-group cod is exposed to pile driving in the field, and it scientifically underpins the anecdotic observation of whiting floating at the surface during pile driving, which was the immediate motivation of our experiment. This experiment proved that it should be repeated to answer further research questions relating inner ear injuries, long-term survival rate, etc.; this time, however, with small, autonomous digital hydrophones (*e.g.*, icListen HF-X2) that can be deployed together with the cages. Ideally, particle motion is also measured, since this is an important second component of sound, and its role in the effects of impulsive sound on fish needs further investigation.

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