Sub-lethal effects of pile-driving sounds on juvenile sea bass

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Summary

Loud impulsive sounds such as pile-driving sound have the potential to injure fishes. There is an urgent need to acquire more knowledge on the effects of pile-driving sounds, in view of the rapid increase of offshore wind farms in the North Sea.

In this study, sub-lethal effects of pile-driving sounds were examined in juvenile European sea bass (*Dicentrarchus labrax*). Controlled exposure experiments were carried out using the larvaebrator, a device that was developed to enable exposure of larval and juvenile fish to pile-driving sounds in a laboratory setting. The focus of the study was to examine injuries in relation to different sound exposure levels. In addition, short-term survival (during a 13 day monitoring period) and recovery from injuries (after 13 days) were examined.

Injury assessments revealed barotrauma injuries in juvenile sea bass exposed to pile-driving sound. Quantitative analyses showed significant differences in the degree of damage between the control group and the group with the highest cumulative sound exposure level (SEL_{cum} = 215 dB re 1 μPa^2s). Post-exposure monitoring indicated that these injuries would not cause increased short-term mortality under laboratory conditions. Injury assessments after 13 days showed recovery from injuries.

Samenvatting

Vissen kunnen fysieke schade ondervinden door blootstelling aan harde impulsieve geluiden zoals heigeluid. Er is een dringende noodzaak om meer kennis te verwerven over de effecten van heigeluid, gezien de snelle uitbreiding van offshore windparken in de Noordzee.

In deze studie zijn sub-letale effecten van heigeluid onderzocht bij juveniele zeebaars (*Dicentrarchus labrax*). De experimenten zijn uitgevoerd met de larvaebrator, een apparaat die ontwikkeld is om larvale en juveniele vis bloot te kunnen stellen aan heigeluid in het laboratorium. Het onderzoek richtte zich primair op verwondingen in relatie tot verschillende geluidsniveaus. Daarnaast is er ook naar korte termijn mortaliteit (gedurende een 13 daagse monitoringperiode) en herstel van verwondingen (na 13 dagen) gekeken.

Barotrauma verwondingen zijn waargenomen bij vissen die blootgesteld waren heigeluid. Kwantitatieve analyse toonde aan dat er significante verschillen waren in de mate van beschadiging tussen de controle groep en de groep met de hoogste cumulatieve blootstellingsniveau (SEL_{cum} = 215 dB re 1 μPa^2s). Monitoring na de geluidsblootstelling gaf aan dat deze verwondingen niet tot kort termijn mortaliteit zullen leiden onder laboratoriumomstandigheden. Beoordelingen van de verwondingen na 13 dagen liet herstel van verwondingen zien.

1. Introduction

The rapid increase of offshore wind farms has led to an urgent need to acquire more knowledge on the ecological effects of offshore wind farm construction and operation (Inger et al. 2009). Concern exists about the potential adverse effects of sounds associated with these activities, in particular the loud impulsive sounds generated by pile-driving during the construction of wind farms. Loud impulsive sounds have the potential to kill or injure fishes (e.g. Caltrans 2001, Govoni et al. 2008, Popper & Hastings 2009).

In 2009, despite limited knowledge at that time (Popper & Hastings 2009), interim criteria were formulated for non-auditory tissue damage in fish due to pile-driving sounds (Oestman et al. 2009). These criteria included cumulative sound exposure level (SEL_{cum}) thresholds of 183 dB re 1 μPa^2s for fish < 2 g, and 187 dB re 1 μPa^2s for fish ≥ 2 g. Since then, several experimental studies have been carried out to examine the effect of pile-driving sounds on fish (larvae and juveniles). These recent studies indicate that the SEL_{cum} thresholds for injuries or death are considerably higher than the interim criteria.

Controlled exposure experiments in a laboratory setting showed no lethal effects up to 10 days after exposure for fish larvae exposed to 206-216 dB SEL_{cum}. This was examined in common sole (*Solea solea*), European sea bass (*Dicentrarchus labrax*) and herring (*Clupea harengus*) larvae (Bolle et al. 2011, 2012a, 2012b, 2013b, in prep.). Field experiments corroborated these findings; no lethal effects were observed up to 14 days after exposure for early juvenile European sea bass exposed to 215-222 dB SEL_{cum} (Debusschere et al. submitted).

Injury assessments have been carried out for juvenile fish exposed to pile-driving sound in a laboratory setting. These studies revealed onset of injuries at 204-210 dB SEL_{cum} for 4 fish species with a swim bladder: Chinook salmon (*Oncorhynchus tshawytscha*), lake sturgeon (*Acipenser fulvescens*), Nile tilapia (*Oreochromis niloticus*) and hybrid striped bass (*Morone chrysops x M. saxatilis*) (Halvorsen et al. 2012a, 2012b, Casper et al. 2013). No injuries were observed in a flatfish species without a swim bladder (hogchoker, *Trinectes maculatus*) exposed to 216 dB SEL_{cum} (Halvorsen et al. 2012b). Recovery from injuries was examined in 2 species: Chinook salmon and hybrid striped bass. Evidence of healing was observed within 10 days post-exposure, for fish exposed to 207-217 dB SEL_{cum} (Casper et al. 2012, 2013).

Knowledge on lethal and physical effects of pile-driving sounds in fish is rapidly increasing, but the number of species examined is still limited. To date, barotrauma injuries induced by pile-driving sounds have only been examined in 5 (mainly freshwater) fish species occurring in the United States. This study examined effects of pile-driving sounds in juvenile European sea bass. The primary goal was to assess injuries directly after exposure to pile-driving sound. In addition, short-term survival (during a 13 day monitoring period) and recovery from injuries (after 13 days) were examined.

2. Materials and Methods

2.1 Fish

European sea bass have a closed (physoclistous) swim bladder. Average fish length (total length) at the time of treatment was 104 mm (sd = 5 mm) (Figure 1).

Figure 1. European sea bass juvenile.

The fish were obtained from a commercial hatchery in France (Ecloserie Marine de Gravelines, Duinkerken) and acclimated to the conditions in the IMARES laboratory (IJmuiden, The Netherlands) prior to the experiments. The fish were divided over 3 squared tanks with rounded corners, sized 70x70x40 cm, and filled with natural seawater (salinity 34.3 ‰). A small RAS (Recirculated Aquaculture System) with a MBBR (Moving Bed Biofilm Reactor), sand filter, UV treatment and cooling was used to maintain good water quality. During the 14 days prior to the experiment, the temperature was gradually decreased from 16.0 °C (sd = 0.0 °C) to 13.2 °C (sd = 0.2 °C) to reduce oxygen consumption in the limited volume of the larvaebrator. The density per tank was approximately 8.5 kg/m^3 and oxygen satiation was around 90%. The fish were fed to saturation three times a day by hand.

2.2 Larvaebrator

Previously, a device was developed to enable controlled exposure of fish larvae to loud, low frequency impulsive sounds in a laboratory setting. This so-called larvaebrator was inspired by an existing laboratory set-up for larger fish called the fishabrator or the HICI-FT (Martin & Rogers, 2008). A detailed description of the larvaebrator is presented in Bolle et al. (2011, 2012a). The dimensions of the test chamber of the larvaebrator are such that small samples of juveniles can also be tested.

2.3 Sound

Exposure to pile-driving sound was realised by play-back of a recorded pile-driving signal. The signal, referred to as OWEZ@100m, was recorded at 100 m distance from a pile-driving event in the North Sea (OWEZ wind farm, 4 m diameter steel monopile, at a water depth of ±20 m, with a hammer strike energy of ±800 kJ). The play-back level was quantified in terms of zero-to-peak pressure level ($L_{z\text{-}p}$ in dB re 1 μPa^2), single-strike sound exposure level (SEL_{ss} in dB re 1 μPa^2s) and cumulative sound exposure level (SEL_{cum} in dB re 1 μPa^2s). Definitions of these sound metrics and further characteristics of the original and reproduced signals (such as frequency spectra) have previously been published by Bolle et al. (2011, 2012a, 2013a). SEL_{ss} and $L_{z\text{-}p}$ were varied by scaling the amplitude of the signal; SEL_{cum} was varied by changing the number of strikes.

The highest SEL_{ss} attainable by the larvaebrator sound projector is 186 dB re 1 μPa^2s. The associated $L_{z\text{-}p}$ for the OWEZ@100m sound signal is 210 dB re 1 μPa^2. An increase of $L_{z\text{-}p}$ to 215 dB re 1 μPa^2 was achieved by using a different sound signal, an exponential positive pulse. A positive pulse was chosen because the $L_{z\text{-}p}$ values measured in the larvaebrator for a negative pulse were 4 dB lower than for a positive pulse. The characteristics of the exponential sound signals were described in Bolle et al. 2012b.

The highest SEL_{cum} that has been applied in all larvaebrator experiments to date is 216 dB re 1 μPa^2s. This was realised by 999 strikes of the (unscaled) OWEZ@100m sound signal. Although a higher SEL_{cum} is possible in theory, it has not been applied in practice due to the expected temperature increase in the test chamber.

2.4 Pilot experiments

Pilot experiments were carried out for training and calibration purposes. Two treatments were included: 1 control and 1 sound exposure (Table 1). The exposure was the highest SEL_{cum} possible in the larvaebrator. The measured sound metric values were 1 dB lower than the expected values.

Table 1. Pilot experiments. Expected and measured sound exposures, quantified in terms of zero-to-peak pressure level ($L_{z\text{-}p}$ in dB re 1 μPa^2), single-strike sound exposure level (SEL_{ss} in dB re 1 μPa^2s) and cumulative sound exposure level (SEL_{cum} in dB re 1 μPa^2s), and the number of fish examined on the day of treatment (day 0) and 1 day post-exposure (day 1).

Treatment	Signal	Strikes	Expected			Measured (mean)			# fish	
			$L_{z\text{-}p}$	SEL_{SS}	SEL_{cum}	$L_{z\text{-}p}$	SEL_{SS}	SEL_{cum}	day 0	day 1
1	none (control)	-	-	-	-	-	-	-	9	7
2	OWEZ@100m	999	210	186	216	209	185	215	9	7

Sixteen replicates were carried out for each treatment. Only 1 fish was inserted in the test chamber per replicate. The control group underwent the same handling procedures as the exposure group, except for the exposure to sound. The duration of both treatments (including handling time) was 20 (±2) min. Water quality parameters (temperature, oxygen, pH) were measured in the test chamber before and after treatment.

All fish were examined for injuries; 9 fish per treatment (18 in total) were examined on the day of the treatment, and 7 fish per treatment (14 in total) were examined the day after (~24 hours post-exposure). The person scoring injuries was not aware of the treatment.

2.5 Final experiments

The final experiments consisted of 5 treatments: 1 control and 4 exposures (Table 2). Treatment 2 presented the highest SEL_{cum} possible in the larvaebrator, as was done in the pilot experiments. Treatment 3 reduced the number of strikes with a factor ~10, which lowered SEL_{cum} by 10 dB compared to treatment 2. This exposure was included in previous studies on lethal effects of pile-driving sound in fish larvae (Bolle et al. 2011, 2012a, 2013b). Treatment 4 scaled down the amplitude of the sound signal, thereby reducing SEL_{ss} and L_{z-p} by ~10 dB compared to treatment 2 and 3. Treatment 4 sound levels aimed to correspond to the reported levels of injury onset for Nile tilapia, the most sensitive of the 4 species examined by Halvorsen et al. (2012a,b). Treatment 5 used a different sound signal to increase L_{z-p} as compared to treatment 2 and 3. The measured sound metric values were 1-2 dB lower than the expected values (Table 2).

Table 2. Final experiments. Expected and measured sound exposures, quantified in terms of zero-to-peak pressure level (L_{z-p} in dB re 1 μPa^2), single-strike sound exposure level (SEL_{ss} in dB re 1 μPa^2s) and cumulative sound exposure level (SEL_{cum} in dB re 1 μPa^2s), and the number of fish examined on the day of treatment (day 0) and 13 days post-exposure (day 13).

Treatment	Signal	Strikes	Expected			Measured (mean)			# fish	
			L_{z-p}	SEL_{SS}	SEL_{cum}	L_{z-p}	SEL_{SS}	SEL_{cum}	day 0	day 13
1	none (control)	-	-	-	-	-	-	-	19	21
2	OWEZ@100m	999	210	186	216	209	185	215	19	21
3	OWEZ@100m	100	210	186	206	209	185	205	19	0
4	OWEZ@100m	999	201	177	207	199	175	205	19	0
5	exp. pos. pulse	100	217	187	207	215	186	206	19	0

Nineteen replicates were carried out for each treatment, resulting in a total of 95 experiments. Three fish were inserted in the test chamber per replicate. The control group underwent the same handling procedures as the exposure groups, except for the exposure to sound. The duration of the treatments (including handling time) was 20 (±2) min for treatments 1, 2 and 4 (999 strikes and control), and 6 (±1) min for treatments 3 and 5 (100 strikes). Water quality parameters (temperature, oxygen, pH) were measured in the test chamber before and after treatment.

The experiments were carried out over 3 consecutive days. The replicates per treatments were circa evenly divided over these 3 experiment days. Day 0 refers to the day of treatment. Consequently, the date of day 0 is not the same for all replicates.

One of the 3 fish per experiment was randomly selected for injury assessment directly after the treatment (day 0), giving a total of 19 fish per treatment (Table 2). The other 2 fish were transferred to flow-through aquaria for a 13 day survival monitoring period (see section 2.7). After this period (day 13), 21 fish from treatment 1 and 2 (i.e. the control group and the highest SEL_{cum} exposure) were randomly selected for injury assessment. The person scoring survival or injuries was not aware of the treatment.

2.6 Injury assessments

The injury assessment procedure used to examine effects of pile-driving sounds in Chinook salmon (Halvorsen et al. 2012a), lake sturgeon, Nile tilapia and hogchoker (Halvorsen et al. 2012b) was adapted and applied to European sea bass. This procedure was developed by Christa Woodley and Michele Halvorsen and is described in detail in Halvorsen et al. (2011).

The investigators in this study were trained by Halvorsen and Woodley to detect, examine and evaluate injuries. The training was done using several untreated fish and the control and exposed fish from the pilot experiments (Table 1).

The fish were euthanized (using an overdose of the anaesthetic 2-phenoxy ethanol) and subsequently examined for external and internal injuries. Basic biological parameters (such as length and weight) were recorded and the presence or absence of 85 injuries or abnormalities was scored. Not all parameters were sound pressure induced, therefore the data were scrutinised to distinguish between possibly barotrauma and other (perhaps handling induced) injuries or abnormalities, thus paring down the final assessment to 76 parameters. The parameters were assigned to weighted trauma categories: 1 for mild, 3 for moderate and 5 for mortal types of injury, according to the definitions by Halvorsen et al. (2011, 2012a). The weighted summation of the injuries is called the Response Weighted Index (RWI). This weighted summation procedure enables complex and variable data to be reduced to a single value for each fish.

2.7 Survival monitoring

Two fish of the 3 fish per experiment were held after the treatment for survival monitoring. The fish from each treatment and from each of the 3 consecutive experiment days were held separately, resulting in 3 replicates per treatment.

The 15 groups of fish (5 treatments * 3 replicates) were held in 15 squared glass tanks with rounded corners, sized 70x70x18 cm, filled with seawater (salinity 33.1 ± 0.5 ‰). The tanks were connected to a RAS (Recirculated Aquaculture System) consisting of a drum filter, a sedimentation tank and a trickling filter to maintain good water quality. The water in the tanks had an average temperature of 15.9 ± 0.1 °C and was refreshed every two hours. The fish were fed at a ration of 2% body weight per day (Inicio plus 1.1mm, Biomar) with a belt feeder.

Survival was scored on a daily basis during a 13 day monitoring period. This period was chosen to be at least the same duration as the monitoring period during previous studies on larval survival (7-10 days). When counting the fish each day, swimming and feeding behaviour were observed (anecdotally) to obtain qualitative information on potential differences between the groups.

2.8 Swim bladder measurements

Swim bladder measurements were carried in 2 additional samples of juvenile sea bass. These measurements could not be done during the injury assessments because complete uncovering of the swim bladder causes to much damage to the fish.

Total fish length and the length, posterior width and anterior width of the swim bladder were measured. The first sample consisted of 50 fish and the measurements were done using a microscope. The second sample consisted of 20 fish and the measurements were done using X-ray photographs. The photographs were taken by the department of veterinary medical imaging and small animal orthopaedics, faculty of veterinary medicine, University of Ghent.

The length and mean (of anterior and posterior) width measurements were converted to swim bladder volume, assuming that the swim bladder is cylindrically shaped.

2.9 Statistical analysis

For the data collected directly after the treatment (day 0), estimates of RWI by treatment, and statistical significance of differences between control and exposure groups were calculated using a generalised linear mixed model. As the RWI data are count data (non-negative integers), the Poisson regression was chosen. Despite the calibration exercises during the training period, RWI (mean and variance) estimates differed between investigators. We are interested in the general effect of treatment on RWI and not the effect by investigator. Therefore, we chose to include investigator as a random effect rather than a fixed effect. In the Poisson regression it is assumed that the variance is equal to the mean. To avoid violation of this assumption (overdispersion), it was necessary to include an additional random effect at the observation level. Ignoring overdispersion will cause underestimation of standard errors and *p*-values.

The statistical model was formulated as follows:

- The RWI for observation *i* of investigator *j* was assumed to be Poisson distributed with mean μ_{ij}:
  $RWI_{ij} \sim Poisson(\mu_{ij})$
- The RWI_{ij} estimates (for observation *i* of investigator *j*) were modelled as a function of treatment (as factor), random investigator effect (a_j) and random effect at the observation level (b_i):
  $E[RWI_{ij}] = \mu_{ij} = \exp(\alpha + \beta * treatment_{ij} + a_j + b_i)$
- The random effects (a_j and b_i) were assumed to be normally distributed with mean zero and variance σ_a^2 and σ_b^2:
  $a_j \sim N(0, \sigma_a^2)$; $b_i \sim N(0, \sigma_b^2)$

The model was fitted and statistical significance tests were performed using *lmer* from the lme4 package in R.

Only 2 of the 3 investigators participated in the injury assessments 13 days after the treatment (day 13), and 1 investigator did most of the assessments (37 out of 42). Consequently, variance related to investigator is nil for the data collected on day 13. Therefore, the model for day 13 was reduced to a generalised linear model (i.e. without random effects). Poisson regression exhibited overdispersion, therefore the dispersion parameter was modelled and corrected for (quasi-Poisson).

The statistical model was formulated as follows:

- The variance is assumed to be k times the mean (in which k is the dispersion parameter):
  $E(RWI_i) = \mu_i$ and $var(RWI_i) = k * \mu_i$
- The RWI_i estimates were modelled as a function of treatment (as factor):
  $E(RWI_i) = \mu_i = \exp(\alpha + \beta * treatment_i)$

The model was fitted and statistical significance tests were performed using *glm* from the stats package in R.

2.10 Ethics statement

This study was performed in accordance with Dutch law concerning animal welfare. The protocol was approved by the Animal Ethical Commission (DEC) of Wageningen UR (experiment code 2013083.c for pilot experiments; experiment code 2013127.a for final experiments).

3. Results

3.1 Pilot experiments

The primary goal of the pilot experiments was to train and calibrate the investigators. Initial differences in the detection and evaluation of injuries decreased during the training period. After the pilot experiments (and additional training days) the investigators were confident that they were consistent with themselves and each other, although a certain degree of subjectivity in the rating of injuries is inevitable.

Data collected during the pilot experiments was not suitable for quantitative analyses of the effects of exposure to sound. Firstly, because variations in observations were partly related to the investigators' learning curve and, secondly, because the number of replicates per treatment was low.

The pilot experiments provided valuable information and experience to optimise the injury assessment for sea bass. Small variations in morphology exist between species, requiring species specific adaptations of the procedures. For example, the procedure to remove the swim bladder was adapted for sea bass to prevent damage due to dissection; this step enables examination of the organs underneath (dorsal to) the swim bladder. Furthermore, the list of injuries to be scored was adapted specifically for sea bass.

3.2 Injury assessments

In the final experiments, a total of 95 fish (19 per treatment, 5 treatments) were examined directly after the treatment (day 0).

The occurrence of injuries was higher in treatment group 2 (highest SEL_{cum}) than in the other groups. Some injuries (such as damaged blood vessels) occurred in all groups but were observed more frequently in treatment group 2, while other injuries only occurred in some of the treatment groups. For example, swim bladder rupture (Figure 2), which is a severe injury with a clear link to pressure changes, occurred twice in treatment group 3 and 8 times in treatment group 2.

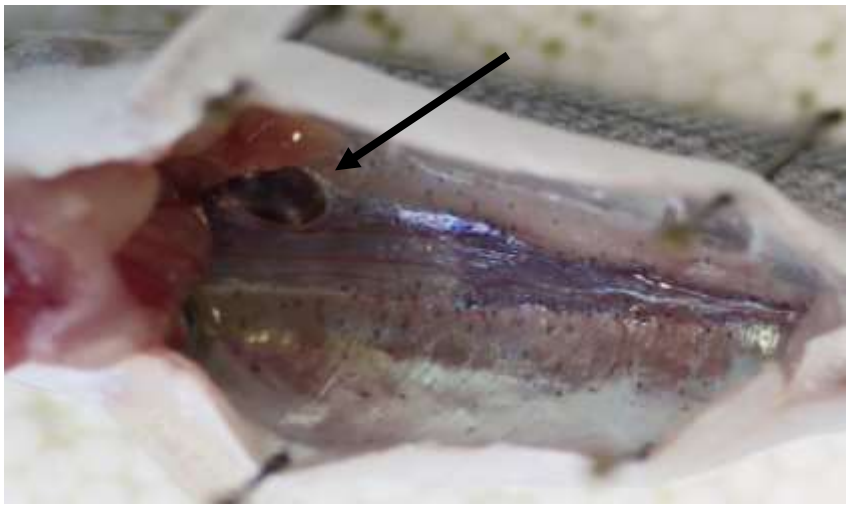

Figure 2. Sea bass juvenile with a ruptured swim bladder. Head is to the left and the body is ventral side up.

The highest occurrence of injuries in treatment group 2 was reflected by the RWI (Figure 3). Fish with zero injuries occurred in all treatment groups, but the highest RWI values were scored for treatment group 2. The estimated mean (and variance) decreased from treatment group 2 to 5, the lowest mean

and variance were observed in the control, treatment 1 (Figure 3). RWI was significantly higher in treatment group 2 than in the control group. For the other exposure groups, RWI was not significantly different from the control (Table 4).

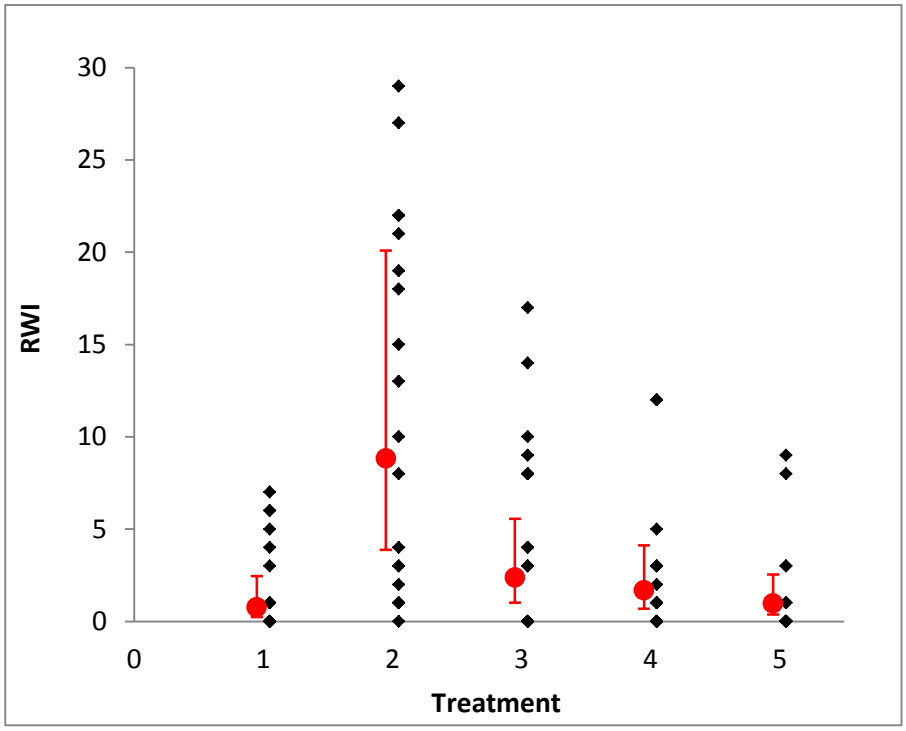

Figure 3. RWI by treatment on day 0. The red circles and the error bars present model estimates of the mean and the 95% confidence intervals. The black diamonds show the RWI values per fish (19 fish per treatment, symbols partly overlapping).

Table 4. RWI on day 0. Analysis of variance and estimates for RWI modelled as a function of treatment and random effects (Poisson generalised linear mixed model).

Analysis of Variance (drop 1)	**df**	**AIC**	**LRT/Chi²**	***p*-value**	
<none>		235.9			
treatment	4	260.6	32.7	<0.001	*
observation	1	348.3	114.4	<0.001	*
investigator	1	261.1	27.2	<0.001	*
Random effects	**n**	**variance**			
observation	95	0.97			
investigator	3	0.74			
Fixed effects	**estimate**	**se**	***z*-value**	***p*-value**	
intercept	-0.27	0.60	-0.46	0.65	
treatment 2	2.18	0.42	5.20	<0.001	*
treatment 3	0.87	0.43	2.00	0.05	
treatment 4	0.52	0.46	1.14	0.25	
treatment 5	-0.03	0.49	-0.07	0.95	

3.3 Survival monitoring

A total of 190 fish (38 per treatment, 5 treatments) were maintained during a 13 day survival monitoring period. All fish, from all treatment groups, survived. Sound exposure appeared to have no effect on short-term (13 day) survival, despite the level of injuries observed directly after exposure.

Anecdotal information on swimming and feeding behaviour showed no clear differences between the 15 groups of fish during the monitoring period. None of the fish appeared to have buoyancy problems.

3.4 Recovery from injuries

A total of 42 fish (21 per treatment, 2 treatments) were examined for injuries after the 13 day survival monitoring period. Only the control group (treatment 1) and the highest exposure in terms of SEL_{cum} (treatment 2) were included in this assessment.

The occurrence of injures was low in both groups (Figure 4). Swim bladder distension and swim bladder connective tissue damage occurred more often in the exposure group than in the control group. However, the overall RWI was not significantly different between the 2 groups (Table 5).

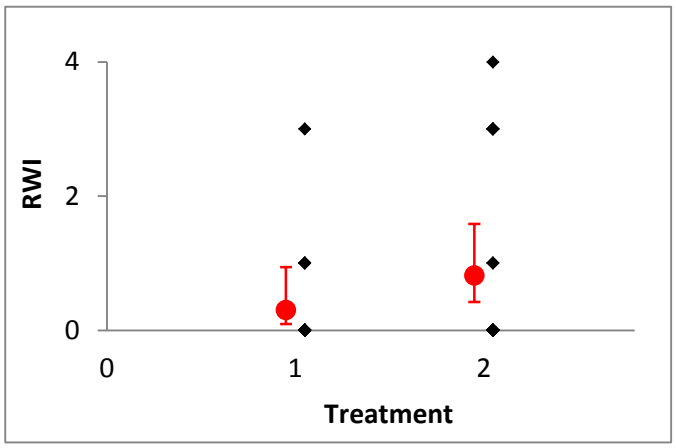

Figure 4. RWI by treatment on day 13. The red circles and the error bars present model estimates of the mean and the 95% confidence intervals. The black diamonds show the RWI values per fish (21 fish per treatment, symbols largely overlapping).

Comparison of treatment 2 with the control showed clear and significant differences on day 0, but not on day 13. This indicates that the fish are capable of recovery from injuries within 13 days. In several fish from treatment group 2, scar tissue on the swim bladder and connective tissues was observed on day 13 (Figure 5). This observation supports the conclusion of recovery from injuries.

Table 5. RWI on day 13. Analysis of variance and estimates for RWI modelled as a function of treatment (quasi-Poisson generalised linear model).

Analysis of Variance (drop 1)	**df**	**deviance**	***F*-value**	***p*-value**
<none>		65.7		
treatment	1	70.9	3.16	0.083
Dispersion parameter	**k**			
	2.05			
Estimates	**estimate**	**se**	***t*-value**	***p*-value**
intercept	-1.20	0.58	-2.06	0.05
treatment 2	1.00	0.67	1.49	0.145

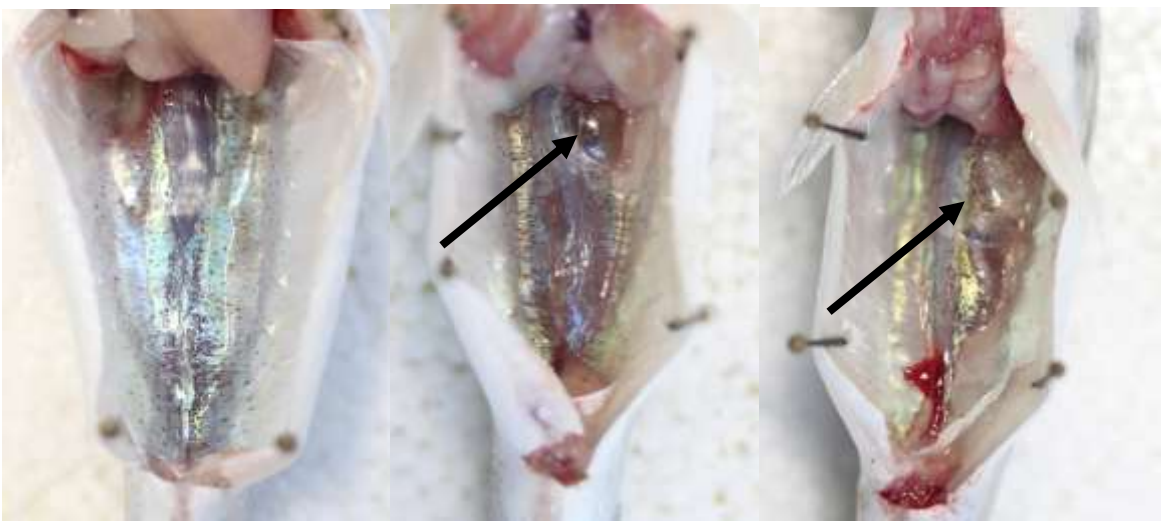

Figure 5. From left to right: an intact swim bladder, a damaged swim bladder and a swim bladder with scar tissue.

3.5 Swim bladder measurements

The mean length (± sd) of the sea bass juveniles used in the final experiments was 104 (± 5) mm. Juveniles in this size range have a swim bladder of 4 -7 mm (volume expressed in equivalent bubble radius, Figure 6).

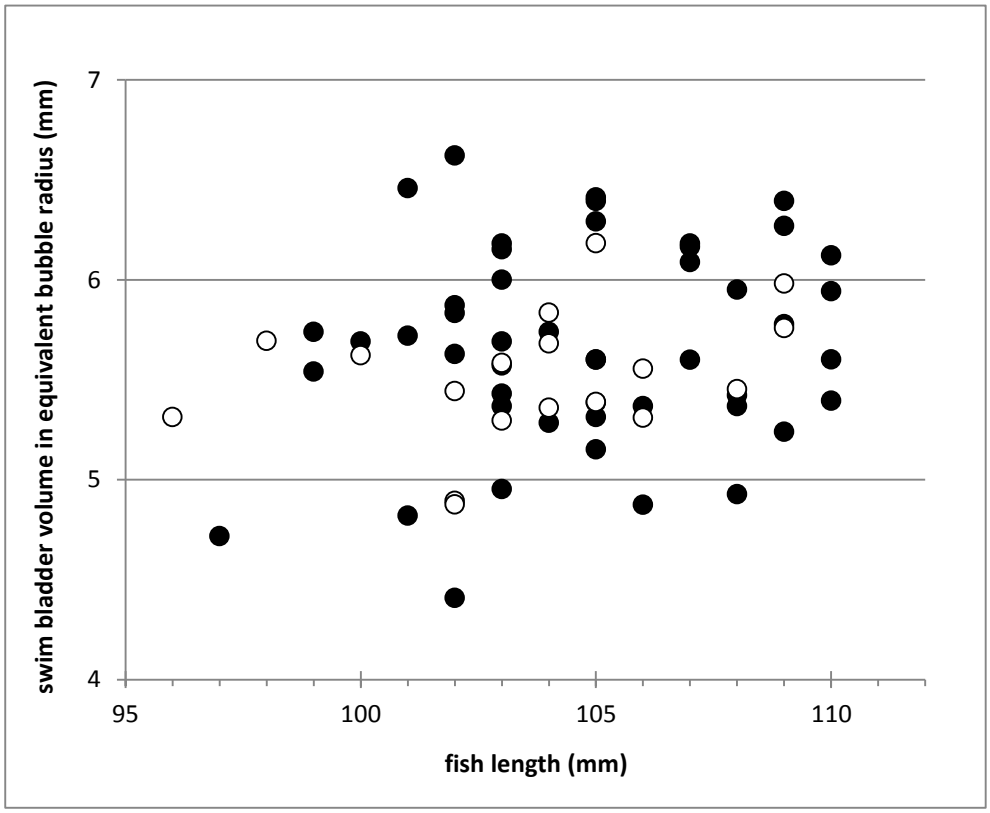

Figure 6. Swim bladder volume (expressed in equivalent bubble radius) for European sea bass juveniles (closed symbols = microscope measurements, open symbols = X-ray measurements).

4. Discussion

The injury assessments directly after treatment showed a significant effect of exposure to pile-driving sound at a SEL_{cum} of 215 dB re 1 μPa^2s (treatment 2). No significant differences between control and exposure were observed for the other 2 pile-driving sound exposures (treatments 3-4), which both had a SEL_{cum} of 205 dB re 1 μPa^2s. The mean RWIs for treatment 3 and 4 appeared to be higher than the mean RWI for the control group, suggesting the onset of injuries at a SEL_{cum} of 205 dB, but differences were not significant. This might be due to limited sample size; increase of sample size is required to determine if these patterns reflect 'true' differences or random variation.

The RWI approach, to integrate multiple injury parameters, was first used in a pile-driving impact study by Halvorsen et al. (2011, 2012a). They suggested a RWI value of 2 as threshold of physical injury to Chinook salmon. They also state that this threshold is specific to (Chinook) salmon of a certain size, due to differences among species, life stages and water quality. In line with this statement, we caution to compare absolute values of RWI between case studies. Besides differences between species and life stages, there may also be differences between investigators. We encountered a significant investigator effect. More training of, and calibration between, investigators would probably have reduced this effect, but a certain degree of subjectivity is inevitable.

In previous studies, onset of barotrauma injuries was observed between 204 dB re 1 μPa^2s SEL_{cum} (hybrid striped bass, Caspar et al. 2013) and 210 dB re 1 μPa^2s SEL_{cum} (Chinook salmon, Halvorsen et al. 2012a) for fish species with a swim bladder. The observed interspecific differences support the hypothesis that fish with closed (physoclistous) swim bladders are more susceptible to sound pressure than fish with open (physostomous) swim bladders (Halvorsen et al. 2012b, Caspar et al. 2013). European sea bass has a physoclistous swim bladder and, based on previous studies, the onset of injuries may be expected at approximately 205 dB re 1 μPa^2s SEL_{cum}. Although slightly increased RWIs were observed at 205 dB re 1 μPa^2s SEL_{cum} compared to the control group, the differences were statistically insignificant. Barotrauma injuries clearly occurred at 215 dB re 1 μPa^2s SEL_{cum}, but the data set was too limited to statistically determine an accurate SEL_{cum} threshold for tissue damage.

For treatment 5, there was no indication of increased RWI when compared to control. The OWEZ@100m pile-driving signal was used in all exposures except treatment 5; an exponential positive pulse was used instead. This sound signal was included to enable an increase of $L_{z\text{-}p}$ compared to treatment 2. However, this (positive) signal only reflected effects of compression of the swim bladder due to sound pressure, while the negative going phase of the pulse creates the shock wave and is thus believed to be more injurious to fish tissues. In any case, it is clear that the effects of both parameters need to be teased apart and better understood. These data are the first to document a positive going pulse.

Most studies use SEL_{cum} to quantify sound exposure in relation to effects on marine fauna. In this study, sound exposure was quantified in terms of zero-to-peak pressure level ($L_{z\text{-}p}$), single-strike sound exposure level (SEL_{ss}) and cumulative sound exposure level (SEL_{cum}). Our experiments showed a significantly higher RWI for treatment 2, whereas RWIs in treatments 3-5 were not significantly different from the control group. This corresponds well with SEL_{cum}; the highest RWI for the treatment with the highest SEL_{cum} (215 dB) and a more or less equally low RWI for treatments with a lower SEL_{cum} (205-206 dB). No clear correspondence between effects and exposure was found for $L_{z\text{-}p}$ or SEL_{ss}. Although our data set is too limited to truly disentangle effects in relation to different sound characteristics, our results do support the use of SEL_{cum} to quantify sound exposure in relation to physical harm.

The sound projector of the larvaebrator cannot accurately reproduce the higher frequency content of sound signals (Bolle et al. 2013a, in prep.). Most energy of pile-driving sounds is in the low-frequency

range, but resonant excitation of the swim bladder may occur in the higher frequency range. Resonant excitation might be one of the mechanisms contributing to (sub-)lethal effects in fish. The sound frequency at which resonant excitation occurs depends on the size of the swim bladder. The results of a theoretical study (Bolle et al. 2013a, in prep.) indicated that the larvaebrator may not be able to reproduce potential resonant effects of pile-driving sound for fish with a swim bladder between 2 and 10 mm (volume expressed in equivalent bubble radius). The swim bladder sizes measured for juvenile sea bass fall within this range. Hence, the effects of pile-driving sound might be underestimated in this study.

Sound exposure did not affect short-term mortality of juvenile European sea bass; zero mortality was observed in the control group and all exposure groups during a 13 day survival monitoring period. This indicated that the injuries observed directly after the treatment would not cause death within 13 days. At least not under laboratory conditions, with absence of predators, *ad libitum* food and minimal energetic expenditures.

Injury assessments at 13 days post-exposure suggested that recovery from injuries is likely to occur for fish exposed to 215 dB re 1 μPa^2s SEL_{cum} (treatment 2). The occurrence of injuries was low and the significant difference between control and treatment 2 at day 0, had disappeared by day 13. Furthermore, scar tissue on the swim bladder and connective tissues indicated healing of injuries.

Casper et al. (2012, 2013) examined recovery from injuries in Chinook salmon and hybrid striped bass up to 10 days post-exposure. They found evidence of healing in Chinook salmon exposed to 217 dB re 1 μPa^2s SEL_{cum} and hybrid striped bass exposed to 207-213 dB re 1 μPa^2s SEL_{cum}. At lower sound levels, the occurrence of injuries on day 0 was low and the post-exposure decrease in injures was insignificant.

The extent of the injuries observed directly after exposure raises the question whether recovery from injuries and absence of lethal effects, as observed in laboratory experiments, would also be observed in the wild. A dysfunctional swim bladder, for example, may seriously hamper swimming performance, which is key to feeding success and avoidance of entrainment and predators. For sea bass, no studies on healing rates of ruptured swim bladders are available. Field studies on behaviour of the physoclist species Atlantic cod (*Gadus morhua*) and Pacific cod (*Gadus macrocephalus*) showed that swim bladder functionality is much less compromised after swim bladder rupture than is often presumed. These species show rapid healing of ruptured swim bladders and have special mechanisms to quickly restore buoyancy control post-rupture (Nicol & Chilton 2006, van der Kooij et al. 2007, Midling et al. 2012). Van der Kooij et al. (2007) suggested that the development of these special mechanisms most likely are the result of evolutionary selection and suggest that cod also naturally experience extreme pressure reductions resulting in barotrauma as a consequence of sudden ascents during, for example, foraging or predator avoidance behaviour. If this is also the case for other physoclist species such as sea bass is unknown. If the same is true for sea bass, it would well explain our monitoring and 13 days post-exposure results.

The studies to date warrant the need to look at long-term effects and to examine effects in a (more) natural situation. The controversy between high levels of injury and high (short-term) survival emphasize the need for field experiments. The injury assessments may not cover all physical aspects that might influence survival or fitness in the long-term. For instance, given the size and developmental stage of gonads of juvenile sea bass, we were unable to assess damage using visual observations. More detailed observations using microscopy or bioassay would enable examination of the potential effects on fecundity, which manifests, depending on species, 6 month to 3 years after pile-driving exposure.

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Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

Justification

Report number: C111/14
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The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: Edward Schram
Researcher

Signature: 

Date: 21 juli 2014

Approved: John Schobben
Head of department Vis

Signature: 

Date: 21 juli 2014