

Effect of pile-driving sound on the survival of fish larvae.

Loes J. Bolle, Christ A.F. de Jong (TNO), Ewout Blom, Peter W. Wessels (TNO), Cindy J.G van Damme, Hendrik V. Winter

Report number C182/14



IMARES Wageningen UR

(IMARES - Institute for Marine Resources & Ecosystem Studies)

Client:

Ministry of EZ
T.a.v. Jasper Dalhuizen
PO Box 20401
2500 EK Den Haag

BAS code: BO-11-011.04-011

Publication date:

23 December 2014

IMARES vision:

- 'To explore the potential of marine nature to improve the quality of life'.

IMARES mission:

- To conduct research with the aim of acquiring knowledge and offering advice on the sustainable management and use of marine and coastal areas.

IMARES is:

- An independent, leading scientific research institute.

P.O. Box 68
1970 AB IJmuiden
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 26
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 77
4400 AB Yerseke
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 59
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 57
1780 AB Den Helder
Phone: +31 (0)317 48 09 00
Fax: +31 (0)223 63 06 87
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 167
1790 AD Den Burg Texel
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 62
E-Mail: imares@wur.nl
www.imares.wur.nl

© 2014 IMARES Wageningen UR

IMARES, institute of Stichting DLO is registered in the Dutch trade record nr. 09098104, BTW nr. NL 806511618

The Management of IMARES is not responsible for resulting damage, as well as for damage resulting from the application of results or research obtained by IMARES, its clients or any claims related to the application of information found within its research. This report has been made on the request of the client and is wholly the client's property. This report may not be reproduced and/or published partially or in its entirety without the express written consent of the client.

A_4_3_2-V14.2

Contents

Summary4

Samenvatting.....4

Background.....5

Assignment.....6

Reading Guide6

References.....7

Quality Assurance8

Justification.....9

Appendix: Manuscript "Do Pile-Driving Sounds Cause Mortality in Fish Larvae?" 10

Summary

Concern exists about the potential adverse effects of underwater sounds generated by pile-driving during the construction of offshore wind farms. Loud impulsive sounds, such as pile-driving sound, can cause fatal injuries in fish. Until recently, very little was known about the sound levels at which injuries or death occur in fish. We examined lethal effects of exposure to pile-driving sound in different larval stages of three fish species (common sole *Solea solea*, European sea bass *Dicentrarchus labrax* and herring *Clupea harengus*), representing different swim bladder developments. Controlled exposure experiments were carried out using the 'larvaebrator', a device that was developed to enable exposure of larval fish to pile-driving sounds in a laboratory setting. Recorded pile-driving sounds could be reproduced at zero-to-peak sound pressure levels up to 210 dB re 1 μPa^2 (zero to peak pressures up to 32 kPa) and single pulse sound exposure levels up to 186 dB re 1 $\mu\text{Pa}^2\text{s}$. The highest cumulative sound exposure level (SEL_{cum}) applied was 216 dB re 1 $\mu\text{Pa}^2\text{s}$ (999 strikes). Survival was monitored during a seven day (sole) or ten day (sea bass and herring) period. In two of the three larval studies (sole and sea bass), pilot experiments were carried out, which enabled a power analysis to determine the number of replicates required in the final experiments. The difficulty of obtaining herring larvae hindered pilot experiments for this species, and previous experiences were used to determine the number of replicates. The results of the larval studies showed no significant differences in mortality between the control group and the exposure groups for any of the species or larval stages.

Samenvatting

Er bestaat zorg over de mogelijk schadelijk effecten van onderwatergeluid gegenereerd gedurende het heien bij de aanleg van offshore windparken. Harde impuls geluiden zoals heigeluid kunnen dodelijke verwondingen veroorzaken bij vissen. Tot voor kort was er weinig bekend over de geluidsniveaus waarbij fysieke schade optreedt. Wij hebben letale effecten van blootstelling aan heigeluid onderzocht in verschillende larvale stadia van drie vissoorten (tong *Solea solea*, zeebaars *Dicentrarchus labrax* en haring *Clupea harengus*). De experimenten zijn uitgevoerd met de 'larvaebrator', een apparaat dat ontwikkeld is om larven bloot te kunnen stellen aan heigeluid in het laboratorium. Veldopnames van heigeluid konden gereproduceerd worden tot een piekniveau van 210 dB re 1 μPa^2 (maximale amplitude van de geluidsdruk van 32 kPa) en een enkele puls blootstellingsniveau van 186 dB re 1 $\mu\text{Pa}^2\text{s}$. Het hoogste cumulatieve blootstellingsniveau dat gebruikt is in de experimenten was 216 dB re 1 $\mu\text{Pa}^2\text{s}$ (999 klappen). Overleving werd gedurende zeven (tong) of tien (zeebaars en haring) dagen gemonitord. In twee van de drie studies (tong en zeebaars) zijn er pilot experimenten uitgevoerd, zodat een power analyse uitgevoerd kon worden om het gewenste aantal herhalingen in de definitieve experimenten te bepalen. Voor haring was dit niet mogelijk en is het aantal herhalingen gekozen op grond van de eerdere studies. Bij geen van de drie vissoorten zijn significante verschillen in mortaliteit waargenomen tussen de controle groep en de blootstellingsgroepen.

Background

In the Netherlands, pile-driving for the construction of offshore wind farms is limited to the period July - December. This precautionary management measure was installed partly because of potential adverse effects of pile-driving sounds on fish larvae. Very little knowledge is available on the effects of sound on fish, especially for the early life stages. Negative effects on fish larvae can have an impact on the abundance of juvenile fish in Natura 2000 areas, thus affecting food availability for birds and marine mammals.

Fish can suffer (lethal) injuries due to loud impulse sounds such as pile-driving sounds, but detailed dose-response studies are still scarce, especially for fish larvae. While juvenile and adult fish may actively swim away from a sound source, planktonic larvae are passively transported by currents and are therefore not capable of avoiding sound exposure.

For the Appropriate Assessment of Dutch offshore wind farms, a modelling study was carried out to estimate the effect of pile-driving sounds on the number of plaice (*Pleuronectes platessa*), common sole (*Solea solea*), and herring (*Clupea harengus*) larvae that reach the Dutch Natura2000 sites [1]. For this, an existing larval transport model [2] was expanded with an assumption on larval mortality caused by pile driving. Although it was recognised that insufficient scientific knowledge was available on the relationship between sound exposure and mortality, it was assumed that 100% mortality occurs up to a distance of 1 km from the pile-driving site [1]. This assumption was based on interim guidelines developed by the US Fisheries Hydro-acoustic Working Group [3]. The results of this modelling study indicated a reduction of 0-18% in the number of larvae that reach the Natura2000 sites due to pile driving on specific construction sites.

Subsequently, based on expert-judgment, the model results were extrapolated to other fish species and older life stages in an attempt to assess the effect of offshore pile-driving on the overall prey availability for birds and marine mammals in Natura2000 sites [4]. This extrapolation indicated that a reduction of more than 5% might occur for seven important prey species: plaice, flounder, herring, sprat, cod, whiting and smelt. These findings contributed to the decision for implementing a mitigation rule on the period of the year in which pile driving is allowed.

The Appropriate Assessment was hampered by lack of knowledge. Predominantly, little was known about the vulnerability of fish eggs and larvae to pile-driving sound and the spatial scale at which mortality or injury may occur. To address this important knowledge gap, larvae experiments were included in the 'Shortlist Masterplan Wind' (SMW) research programme (2010-2011) and the on-going 'Voortzetting Uitvoering Masterplan' (VUM) research programme (2012-2015), both financed by the Dutch government.

Assignment

The fish larvae experiments carried out in the SMW and VUM research programmes focussed on lethal effects of pile-driving sounds. As high intensity, low frequency impulse sounds are distorted in aquaria and small basins, a device (the 'larvaebrator') was developed specifically for the purpose of exposure of fish larvae to pile-driving sounds. During the SMW programme, experiments were carried out for 3 larval stages of sole (*Solea solea*). This work was continued during the VUM programme, in which 2 larval stages of sea bass (*Dicentrarchus labrax*) and 1 larval stage of herring (*Clupea harengus*) were examined. Sole and sea bass larvae were reared from eggs obtained from commercial hatcheries. Herring larvae were reared from eggs that were artificially fertilised during the herring larvae survey. The (different larval stages) of the 3 species represent different swim bladder developments and therefore potentially different vulnerabilities to sound exposure.

The VUM studies were performed within the EZ-program Beleidsondersteunend onderzoek (BO).

Reading Guide

The results of the sole larvae experiments carried during the SMW programme were documented in a report and a peer-reviewed paper [5-6]. The work done within the VUM research programme so far has been documented in progress reports [7-10]. This final report on lethal effects of pile-driving sounds in fish larvae consists of a draft manuscript, which will be submitted for publication in a peer-reviewed journal. The manuscript comprises all experimental work with fish larvae and is presented in the Appendix.

References

1. Prins TC, van Beek JKL, Bolle LJ (2009) Modellschatting van de effecten van heien voor offshore windmolenparken op de aanvoer van vislarven naar Natura 2000. Deltares rapport Z4832
2. Bolle LJ, Dickey-Collas M, van Beek JKL, Erfstemeijer PLA, Witte JIJ, van der Veer HW, Rijnsdorp AD (2009) Variability in transport of fish eggs and larvae. III. Effects of hydrodynamics and larval behaviour on recruitment in plaice. *Marine Ecology Progress Series* 390:195-211
3. Oestman R, Buehler D, Reyff JA, Rodkin R Technical Guidance for Assessment and Mitigation of the Hydroacoustic Effects of Pile Driving on Fish. San Francisco: Caltrans.
4. Bos OG, Leopold MF, Bolle LJ (2009) Passende Beoordeling windparken: Effecten van heien op vislarven, vogels en zeezoogdieren. IMARES rapport C079/09
5. Bolle LJ, de Jong CAF, Bierman S, de Haan D, Huijter T, Kaptein D, Lohman M, Tribuhl S, van Beek P, van Damme CJG, van den Berg FHA, van der Heul J, van Keeken O, Wessels P, Winter E (2011) Shortlist Masterplan Wind. Effect of piling noise on the survival of fish larvae (pilot study). IMARES report C092/11
6. Bolle LJ, de Jong CAF, Bierman SM, van Beek PJG, van Keeken OA, Wessels PW, van Damme CJG, Winter HV, de Haan D, Dekeling RPA (2012) Common sole larvae survive high levels of pile-driving sound in controlled exposure experiments. *PLoS ONE* 7(3):e33052
7. Bolle LJ, van der Sluis M, Blom E, van Damme CJG, de Jong CAF (2012) Effect of pile-driving noise on the survival of fish larvae. Desk study in preparation of future experiments. IMARES report C022/12
8. Bolle LJ, de Jong CAF, Blom E, Wessels PW, Bierman SM, van Damme CJG, Winter HV, Pennock I, Hoek R, Hinrichs C (2012) Effect of pile-driving sound on the survival of fish larvae. Progress report 2012 [Confidential]. IMARES report C120/12
9. Bolle LJ, de Jong CAF, van Damme CJG, Blom E (2013) Effect of pile-driving sound on the survival of fish larvae. Desk study on swim bladder resonance [Confidential]. IMARES report C074/13
10. Bolle LJ, de Jong CAF, Blom E, Wessels PW, van Damme CJG, Winter HV, Pennock I, Hoek R, Lohman M, Hinrichs C (2013) Effect of pile-driving sound on the survival of fish larvae. Progress report 2013 [Confidential]. IMARES report C194/13

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.

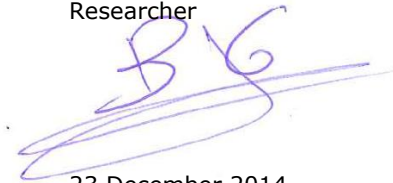
Justification

Report number : C182/14
Project number: 4308601076

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: Ir. A.B. Griffioen
Researcher

Signature:



Date: 23 December 2014

Approved: Drs. J.H.M. Schobben
Head of Fish Department

Signature:



Date: 23 December 2014

Appendix: Manuscript “Do Pile-Driving Sounds Cause Mortality in Fish Larvae?”

Version 23 December 2014

Loes J. Bolle^{1*}, Christ A.F. de Jong², Ewout Blom¹, Peter W. Wessels², Cindy J.G van Damme¹, Hendrik V. Winter¹

¹ IMARES, IJmuiden, The Netherlands

² TNO, Den Haag, The Netherlands

* Corresponding author

Email: loes.bolle@wur.nl

Abstract

Concern exists about the potential adverse effects of underwater sounds generated by pile-driving during the construction of offshore wind farms. Loud impulsive sounds, such as pile-driving sound, can cause fatal injuries in fish. Until recently, very little was known about the sound levels at which injuries or death occur in fish. We examined lethal effects of exposure to pile-driving sound in different larval stages of three fish species (common sole *Solea solea*, European sea bass *Dicentrarchus labrax* and herring *Clupea harengus*), representing different swim bladder developments. Controlled exposure experiments were carried out using the 'larvaebrator', a device that was developed to enable exposure of larval fish to pile-driving sounds in a laboratory setting. Recorded pile-driving sounds could be reproduced at zero-to-peak sound pressure levels up to 210 dB re 1 μPa^2 (zero to peak pressures up to 32 kPa) and single pulse sound exposure levels up to 186 dB re 1 $\mu\text{Pa}^2\text{s}$. The highest cumulative sound exposure level (SEL_{cum}) applied was 216 dB re 1 $\mu\text{Pa}^2\text{s}$ (999 strikes). Survival was monitored during a seven day (sole) or ten day (sea bass and herring) period. In two of the three larval studies (sole and sea bass), pilot experiments were carried out, which enabled a power analysis to determine the number of replicates required in the final experiments. The difficulty of obtaining herring larvae hindered pilot experiments for this species, and previous experiences were used to determine the number of replicates. The results of the larval studies showed no significant differences in mortality between the control group and the exposure groups for any of the species or larval stages.

Introduction

Sources of anthropogenic underwater sound are becoming more prevalent and powerful. There is a growing concern about the impact that these man-made sounds may have on marine fauna. With the increased construction of offshore wind farms, pile-driving activity has multiplied. Pile-driving generates loud, impulsive sounds, which have the potential to kill or injure fish [1]. Until recently, very little was known about the sound levels at which physical damage may occur in fish, especially in fish larvae [1,2]. Fish larvae are planktonic and have limited capabilities of avoiding sound and, therefore, may be more vulnerable to sound exposure.

Previously, interim criteria for non-auditory tissue damage in fish due to pile-driving sounds were formulated [3]. These criteria included a cumulative sound exposure level (SEL_{cum}) threshold of 183 dB re 1 $\mu\text{Pa}^2\text{s}$ for fish < 2 g and of 187 dB re 1 $\mu\text{Pa}^2\text{s}$ for fish \geq 2 g. Since then, several experimental studies have been carried out to examine the effects of pile-driving sounds on fish, indicating that the SEL_{cum} thresholds for injuries or death are considerably higher than the interim criteria.

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 five fish species with a swim bladder [4,5,6,7]. No injuries were observed in a flatfish species without a swim bladder exposed to 216 dB SEL_{cum} [5]. Recovery from injuries was examined in three species and evidence of healing was observed within 10-13 days post-exposure, for fish exposed to 207-217 dB SEL_{cum} [6,7,8]. Controlled exposure experiments in a laboratory setting showed no lethal effects up to seven days after exposure for common sole (*Solea solea*) larvae exposed to 206 SEL_{cum} [9]. In field experiments, no lethal effects were observed up to 14 days after exposure for early juvenile European sea bass exposed to 215-222 dB SEL_{cum} [10].

New insights were incorporated in recently published sound exposure guidelines [11]. For pile-driving sound, these guidelines propose a SEL_{cum} threshold >210 dB re 1 $\mu\text{Pa}^2\text{s}$ for mortality and potential mortal injury in fish eggs and larvae. The authors emphasize that the guidelines should still be treated as interim and more research is required. They specifically comment on the scarcity of data for fish eggs and larvae.

We examined lethal effects of exposure to pile-driving sound in different larval stages of 3 fish species (common sole *Solea solea*, European sea bass *Dicentrarchus labrax* and herring *Clupea harengus*), representing different swim bladder developments. The results for sole larvae have been published previously [9] and are summarised in this paper to present a comprehensive overview of laboratory experiments on lethal effects of pile-driving sound in fish larvae. The controlled exposure experiments were carried out using the 'larvaebrator', a device that was specifically developed to enable exposure of larval fish to low frequency, high intensity sounds in a laboratory setting [9]. We compared original (pile-driving) sound signals to playback signals in the larvaebrator and evaluated the consequences of differences in spectral content for our conclusions. Survival was monitored during a seven to ten days period.

Material and Methods

Fish larvae

Common sole

Common sole is a commercially important European flatfish species. It is a high value species in the fishing industry and, on a limited scale, it is exploited in the aquaculture industry.

Fertilised eggs were purchased from a commercial hatchery in the Netherlands (SOLEA) and reared to the required larval stage in large cylindrical cultivation chambers according to protocols developed by IMARES and SOLEA (pers. com. Ewout Blom). The water temperature was slowly raised from the temperature in the hatchery (12°C) to the ambient temperature in the laboratory (16°C). Three larval stages (expressed in DAH, days after hatching) were used in the experiments: 2 DAH (yolk-sac larvae, no swim bladder), 8 DAH (yolk-sac adsorbed, onset swim bladder development), 15 DAH (swim bladder fully developed, onset asymmetry). Photos of the larval stages used in the experiments were published in [9]. Gas-filled swim bladders were observed in almost all larvae at 15 DAH.

Sole only has a swim bladder during a short period of its larval life [12,13]. At the end of the larval life phase, when metamorphosis is completed (approximately 25 DAH when reared at 16°C, this study), the swim bladder is completely resorbed. A histological study on sole larvae [13] showed a dilated pneumatic duct (connection between swim bladder and gut) when the swim bladder begins to inflate, indicating passage of gas from the digestive tract to the swim bladder at initial inflation (i.e. physostomous swim bladder). The histological study [13] also revealed a gas gland, indicating that subsequent inflation may be realised by gas secretion.

European sea bass

European sea bass is an important species in both the fishing and aquaculture industry, mainly in southern Europe.

Sea bass eggs were purchased from a commercial hatchery in France (Ecloserie Marine de Gravelines) and reared to the required larval stage in large squared cultivation chambers, according to guidelines from the hatchery. The water temperature was slowly raised from the temperature in the hatchery (14°C) to the ambient temperature in the laboratory (17°C). Two larval stages were used in the experiments: 18-19 DAH and 38-39 DAH (Figure 1). Gas-filled swim bladders were observed in almost all larvae of both larval stages.

Sea bass has a physoclistous swim bladder (no connection with the gut) in the adult life phase. Like most physoclistous fish, sea bass has a pneumatic duct as larva and initially inflates the swim bladder by passage of air through this duct. Initial swim bladder inflation starts at 7 DAH and ends at 16 DAH when reared at 13-14°C [14].

Herring

Herring is both commercially and ecologically an important species. It is only exploited in the fishing industry, not in aquaculture. Its ecological significance is mainly due to its abundance; herring is an important predator and prey species in the pelagic and coastal ecosystems.

No commercial hatchery exists for herring. The procedures developed by the University of Bergen, Norway [15] for fertilisation of herring eggs and rearing of herring larvae were adapted to our situation in close communication with Audrey Geffen (University of Bergen). We collected mature and ripe herring in the southern North Sea (i.e. Downs herring) during the annual herring larvae survey [16]. On board of the research vessel, the eggs from females were stripped onto glass plates and fertilised with sperm from males. The glass plates with attached (benthic) eggs were transported to the laboratory and kept in a fast flowing water system. Shortly before hatching, the plates with eggs were placed in large squared cultivation chambers. The (planktonic) larvae were reared to the required larval stage. The water temperature was held at 10°C during the entire rearing process. We only had enough larvae to examine one larval stage with sufficient replication: 88-89 DAH (Figure 2). Herring larvae of this age ranged in size (total fish length) from 19 to 40 mm. Gas-filled swim bladders were observed in 50% of the larvae of this age. The size range of larvae with gas-filled swim bladders was 23 to 40 mm, those without filled swim bladders ranged from 19 to 30 mm.

Herring has a physostomous swim bladder throughout its life [17]. Clupeids, such as herring, have a unique inner ear structure. They have evolved a small gas bubble (bulla) that is connected to the swim bladder and presses against the labyrinth wall, helping propagate vibrations from the swim bladder to the ear [18]. When the bulla appears, the larvae do not have a swim bladder yet and pressure adaptation by gas exchange between the bulla and swim bladder is not possible [19,20].

Sound

Larvaebrator

The larvaebrator is a device that was developed to enable exposure of fish larvae to high intensity, low frequency impulsive sounds in a laboratory setting. It was inspired by an existing laboratory set-up for larger fish called the fishabrator or HICI-FT [4,21]. A detailed description of the larvaebrator is presented in [9].

Sound signals

Fish larvae were exposed to pile-driving sound in the larvaebrator by playing-back sound recorded in the field. The sound signal used in most experiments was recorded at 100 m distance from pile-driving for the OWEZ wind farm in the North Sea (4 m diameter steel monopile, at a water depth of ± 20 m, with a hammer strike energy of ± 800 kJ). Typical recorded SEL_{ss} spectra [22] show that the main energy of underwater pile-driving sound is generated in the 50 Hz to 1 kHz bands. The playback sound was limited to this frequency band, to avoid excitation of spurious resonances in the larvaebrator. The play-back level was 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 $\mu\text{Pa}^2\text{s}$) and cumulative sound exposure level (SEL_{cum} in dB re 1 $\mu\text{Pa}^2\text{s}$). Definitions of these sound metrics are given in [9,23]. Greater distances from the pile were simulated by downscaling the amplitude of the signal, thus reducing SEL_{ss} and L_{z-p} . SEL_{cum} was varied by changing the number of strikes.

The highest SEL_{cum} that has been applied in all larvaebrator experiments to date is 216 dB re 1 $\mu\text{Pa}^2\text{s}$. This was realised by 999 strikes of the OWEZ sound signal at 100 m. Although increasing the number of strikes is possible in theory, it has not been applied in practice due to the expected temperature increase in the test chamber

The highest SEL_{ss} that could be produced by the larvaebrator sound projector was 186 dB re 1 $\mu\text{Pa}^2\text{s}$. The associated L_{z-p} for the OWEZ pile-driving sound signal was 210 dB re 1 μPa^2 .

To increase L_{z-p} (at the same level of SEL_{ss}) a theoretical, exponential sound signal was used. An increase of L_{z-p} to 215 dB re $1 \mu Pa^2$ was achieved by using a positive exponential pulse; the L_{z-p} values measured in the larvaebator for a negative pulse were 4 dB lower.

The sound pressure time series, broadband metrics and frequency spectra were compared between the original and reproduced signals. The wav-files contained a single impulsive sound in a duration of 1 s. The time series (Figure 3) show the first 0.5 s of the signal duration of 1 s, the second 0.5 s are 'silent'. The reproduced time series closely corresponded to the original time series. For each pulse, we determined the broadband values of the unweighted sound exposure level (SEL_{ss}), the zero-to-peak pressure level (L_{z-p}) and the signal duration t_{90} [definition in 23]. The signals are reproduced well in the larvaebator, in terms of these broadband metrics. The 1/3-octave band spectra of the SEL are shown in Figure 4. In the low frequency range, the sound levels reproduced in the larvaebator are equal to those of the original signal, but above the 315 Hz third octave band, the reproduced sound levels are lower than the original levels. The possible consequences of this limitation of the device have been investigated theoretically.

Excitation of swim bladder resonance

The most likely mechanism for causing injury in fish due to sound exposure is tissue deformation under influence of sound pressure. The amount of deformation depends on the mobility of the fish body and in particular on the compressibility of the gas-filled swim bladder. Although most energy of pile-driving sound is in the low-frequency range [22], excitation of swim bladder resonance may occur in the higher frequency range. Excessive deformation at gas bladder resonances might be one of the mechanism causing (sub-)lethal effects in fish (larvae). That mechanism may not be replicated in the larvaebator due to the frequency limitation of the device. Therefore, the relevance of swim bladder resonance was assessed using a simplified theoretical model of the swim bladder as a gas bubble. The model is presented in the supporting information (file S1). The sound frequency at which resonance occurs decreases with bubble size from ± 100 kHz for a bubble of 0.05 mm (volume in equivalent bubble radius) to ± 1 kHz for a bubble of 5 mm (file S1).

Application of the bubble model on the spectral content of the OWEZ@100m signal (unfiltered) indicated that for swim bladders with an equivalent radius equal to or smaller than 2 mm, the deformation associated with resonant response of the swim bladder is less than the low-frequency response (Figure 5, top). Therefore, swim bladder resonances excited by the OWEZ@100m signal are not expected to be important (relative to the low frequency deformations) for swim bladders less than 2 mm. Theoretically, resonant effects of the OWEZ@100m signal may be important in a swim bladder larger than 2 mm (Figure 5, top). Note that the estimate for the amount of amplification of the deformation at resonance (Q-factor, see file S1) was chosen such that an overestimation of the resonant response is more likely than underestimation.

The predicted swim bladder deformation due to resonance is lower for the reproduced OWEZ@100m signal than for the original OWEZ@100m signal (Figure 5), because of the limited ability of the larvaebator to reproduce high frequency signals (Figure 4). Extrapolation of the model results to larger swim bladders indicated that potential resonant effects will not be reproduced in the larvaebator for swim bladders up to approximately 10 mm. For swim bladders larger than 10 mm, the resonant excitation frequency will be within the spectral range that is reproduced well by the larvaebator.

The bubble model was also applied to the spectral content of a synthetic exponential pulse, to examine resonant responses in a sound signal in which the higher frequency content (> 315 Hz) is relatively high (file S1). For this sound signal, resonance effects are not expected to be important for swim bladders less than 0.5 mm and will not be reproduced in the larvaebator for swim bladders between 0.5 and 10 mm.

Swim bladder height and length were measured for sea bass and herring larvae. Swim bladders are typically elongated in anterior-posterior direction (Figures 1 and 2) and volume was estimated assuming a cylinder shape.

For comparison with the results of the theoretical model, volume was converted into the radius of a bubble with an equivalent volume. Swim bladder volume, expressed in equivalent bubble radius, ranged from 0.06 to 0.17 mm for sea bass larvae 18 DAH, from 0.14 to 0.57 mm for sea bass larvae 38 DAH, and from 0.25 to 1.0 mm for herring larvae 88 DAH. For sole, only swim bladder length was measured and the maximum length observed was 0.4 mm. Given the generally elongated shape of swim bladders, it can be inferred that the corresponding maximum volume (in bubble radius) was less than 0.2 mm.

To our knowledge, very little has been published on swim bladder size in fish larvae. Most studies that addressed the swim bladder, focussed on inflation of the swim bladder and the developmental stage or larval size at which initial inflation occurs. We found only one study including swim bladder size estimates for fish larvae [17]. They measured swim bladder volumes in herring ranging from 27 mm (larvae) to 280 mm total fish length. Average values for larvae were taken from their figure and swim bladder volume in ml/g wet weight was converted into equivalent bubble radius. Herring larvae with a mean total length of 29 mm had a mean swim bladder volume (in equivalent bubble radius) of 0.62 mm. This closely corresponded to our results: mean total length was 28 mm and mean swim bladder volume (in equivalent bubble radius) was 0.60 mm.

The results of the theoretical study indicated that resonance effects excited by the OWEZ@100m signal are not important for swim bladders less than 2 mm (volume expressed in bubble radius). Swim bladders of fish larvae used in the larvaebator experiments were substantially smaller. The higher frequency content (>315 Hz) of pile-driving sound increases closer to the pile. The synthetic exponential pulse can be considered to be an extreme example of a near field signal. For this signal, the results of the theoretical study indicated that resonance effects may be important in swim bladders equal to or larger than 0.5 mm (volume expressed in bubble radius). This size is near the upper limit of the range observed for sea bass and well within the range observed for herring.

Experimental design

General procedures

The term treatment is used in broad sense; a treatment is one of several sound exposures, or no sound exposure (i.e. the control group). The control group underwent the same handling procedures as the exposure groups.

Each experiment consisted of a treatment followed by a monitoring period. The water in the test chamber was refreshed before each treatment. Water temperature in the test chamber was the same as in the cultivation chambers (this differed between species, see above). For each experiment, approximately 25 (sole and herring) larvae or 30 (sea bass) larvae were taken from the cultivation chambers and subjected to treatment. The exact numbers of larvae in each experiment were counted after the treatment. The duration of the treatment depended on the number of strikes (1 strikes per second) and the handling time depended on the species and larval stage (5-15 minutes). The duration of the control treatment was set to the longest duration of that series of experiments. After treatment, each batch of larvae was transferred to a separate 'batch-container' and held during the monitoring period. Temperature in the batch-containers was the same as in the cultivations chambers. The procedures for transferring larvae and maintaining larvae in the batch-containers differed between species; species-specific adaptations were required to optimise control group survival.

The number of live larvae in each batch were counted directly after the treatment and daily during the monitoring period. The duration of the monitoring period was seven days in the final experiments for sole and ten days in all other series of experiments. The batch-containers were coded and, except for the observations directly after the treatments, the person scoring survival was not aware of the treatment belonging to the code. The treatments within each replication round were applied in random sequence to avoid bias due to potential serial effects.

Pilot experiments

Pilot experiments with relatively many exposures and few replicates were carried out for sole and sea bass larvae (for each of the larval stages). Firstly, because very little was known about critical values for sound exposure with regard to larval survival. Secondly, to determine the number of replicates in the final experiments. The results of the pilot experiments were used in power analyses to estimate the number of replicates required for sufficient power (i.e. probability of detecting an effect significantly at the 95% level, given a certain sample size and experimental design) to detect a '50% effect'. The % effect was defined as $100\% \cdot (p_e - p_c) / (1 - p_c)$, in which p_e is the estimated mean probability of death in the exposure group and p_c is the estimated mean probability of death in the control group. In other words, the proportion of the larvae surviving 'natural' (control-group) mortality, that died due to sound exposure. Based on the power analyses, we decided on 15 replicates (with 25 larvae per replicate) for all larval stages of sole, and 10 replicates (with 30 larvae per replicate) for both larval stages of sea bass.

Sole and sea bass larvae were reared from eggs obtained from commercial hatcheries. As no commercial hatchery exists for herring, it is far more difficult to obtain herring eggs. We artificially fertilised eggs from wild females with sperm from wild males (see above). The scope for obtaining eggs this way is limited to the natural spawning season and ongoing surveys. Therefore no pilot experiments were carried out for herring. Based on the previous power analyses and the limited amount of herring larvae available, we decided on 10 replicates (with 25 larvae per replicate) for the herring experiments.

Final experiments

Two sound exposures were examined in three larval stages of sole: 100 strikes of the OWEZ signal recorded at 100 m distance from the pile (OWEZ@100m), and 100 strikes of this signal, with the amplitude downscaled by a factor 1.7, to represent the sound level at 200 m distance from the pile (OWEZ@200m). Three sound exposures were examined in two larval stages of sea bass: 100 strikes of the OWEZ@100m signal (highest exposure in the sole experiments) signal, 999 strikes of the OWEZ@100m (SEL_{cum} increased compared to sole experiments), and 100 strikes of the exponential positive pulse (L_{z-p} increased compared to sole experiments). Two sound exposures were examined in one larval stage of herring: 100 and 999 strikes of the OWEZ@100m signal (Table 1). The sound level values presented in Table 1 are the measurements in the larvaebator, averaged over all pulses and pressure transducers.

Statistical analysis

A generalised linear mixed model was used to test the statistical significance of differences between exposure and control groups, and to estimate the probability of death by species, larval stage and treatment. The model treats the data (death or survival of a larva) as outcomes of binomial trials in which the probability of death is a function of treatment and random variation in mortality between batches ('batch effect'). It is necessary to account for such batch effects because, if present, the assumption (under the binomial distribution) that the outcomes of larvae are determined independently of one another is violated. The statistical model was formulated as follows:

- 1) The logit transformed probabilities of death $p_{i,j}$ (in treatment i and batch j) were modelled as a function of treatment and random batch effect (α_j): $\text{logit}(p_{ij}) = \text{treatment}_i + \alpha_j$.
- 2) The number of dead larvae in batch j from treatment i (k_{ij}) were assumed to be binomially distributed depending on the probability of death (p_{ij}) and the number of larvae at the beginning of the experiment (N_{ij}): $k_{ij} \sim \text{Bin}(p_{ij}, N_{ij})$.
- 3) The random batch effects (α_j) were assumed to be normally distributed with mean zero and variance σ^2 : $\alpha_j \sim N(0, \sigma^2)$.

The model was fitted and statistical significance tests were performed using the glimmix procedure (with the Kenward-Roger approximation for the degrees of freedom) in SAS (SAS/STAT software).

SAS Institute Inc., Cary, North Carolina, USA). The model was fitted separately to the data for each species, larval stage and for each of two monitoring periods (5 or 7 days for sole, 5 or 10 days for sea bass and herring). If, for a given species, larval stage and monitoring period, the variance of the batch effect was estimated to be (near) zero, then the model was reduced to a generalised linear model without a batch effect.

Ethics statements

The studies for sole, sea bass and herring were performed in accordance with Dutch law concerning animal welfare. The protocols were approved by the Animal Ethical Commission (DEC) of Wageningen UR (sole: experiment code 2010085 under application 2010063.c; sea bass: experiment code 2013025 under application 2013018.b; herring: experiment code 2013203 under application 2013171.b).

Results

Pilot experiments were carried out for sole and sea bass and the results were used to determine the number of replicates in the final experiments. This resulted in 15 replicates per treatment for sole and 10 for sea bass (Table 1). The difficulty of obtaining herring larvae hindered pilot experiments for this species. We aimed for 10 replicates per treatment for herring, as in sea bass, but did not have sufficient numbers of larvae. Therefore only four replicates were carried out for one of the two sound exposures (Table 1).

All species and larval stages were exposed to 100 strikes of the OWEZ@100m signal. The sound pressure levels measured in the larvaebator for this exposure varied slightly in the sole and sea bass experiments ($SEL_{cum} = 206 \pm 1$ dB, $L_{z-p} = 210 \pm 1$ dB, Table 1), but dropped in the herring experiments ($SEL_{cum} = 203$ dB, $L_{z-p} = 206$ dB, Table 1). A possible cause could be a reduced gain of the (old) power amplifier.

Exposure to 100 strikes of the OWEZ@100m signal was the highest sound pressure exposure in the sole experiments. As no significant differences between this exposure and the control were observed in the sole experiments, exposure levels were increased in the following series of experiments. In the sea bass experiments, SEL_{cum} was increased to 216-217 dB by increasing the number of strikes to 999, and L_{z-p} was increased to 217 dB by using a synthetic exponential pulse. The number of exposures was limited in the herring experiments due to number of larvae available. We choose 100 and 999 strikes of the OWEZ@100m signal (Table 1).

Mortality varied between species, larval stages and duration of monitoring (Figure 6). Differences between control and exposure groups within each species, larval stage and monitoring period were insignificant (Figure 6, Table 2). The 95% confidence interval for the difference between exposure and control indicates the power of the experiments. Estimates of the upper limit of the 95% confidence interval for effect ranged from 6 to 40% (Table 3). These values indicate the effect that could have been detected significantly (at the 95% level) with these experiments. The highest value (lowest power) was estimated for the herring larvae treatment with only four replicates (Table 1).

Discussion

No statistically significant differences in mean mortality were found between the control and exposure groups for any of the species or larval stages. Exposure of sole larvae to sound pressure levels up to $SEL_{cum} = 206$ dB re $1 \mu Pa^2 s$ and $L_{z-p} = 210$ dB re $1 \mu Pa^2$ did not result in increased mortality during the first 7 days after exposure. In the subsequent sea bass experiments, sound levels were increased to $SEL_{cum} = 216$ dB re $1 \mu Pa^2 s$ and (using a synthetic pulse instead of a pile-driving pulse) to $L_{z-p} = 217$ dB re $1 \mu Pa^2$, but no significant differences between control and exposure were observed during the first 10 days after treatment. The highest sound pressure levels applied in the herring larvae experiments were $SEL_{cum} = 212$ dB re $1 \mu Pa^2 s$ and $L_{z-p} = 207$ dB re $1 \mu Pa^2$; no significant effect of exposure was observed during the first 10 days after treatment.

The statistical power of the experiments differed between species and larval stages, depending on mortality in the control group, batch variance and the number of replicates. The highest power was achieved in the experiments with sea bass larvae 38-39 DAH; an effect larger than 6-8% could have been detected significantly (at the 95% level) in these experiments. The lowest power was attained in the herring experiments; the detectable effect was 29% for the highest sound exposure (10 replicates) and 40% for the lowest sound exposure (4 replicates).

The larvaebibrator can be used for exposing fish larvae and small fish to underwater sound by playing back recorded or synthesized sounds. These sounds can be reproduced accurately in the third-octave frequency bands between 10 Hz and 315 Hz. Higher frequencies are filtered out. Although most energy of pile-driving sound is in the low-frequency range [22], resonant excitation of the swim bladder may occur in the higher frequency range and this may be one of the mechanism causing physical damage in fish (larvae). A theoretical assessment indicated that, for the OWEZ sound signal recorded at 100 m distance from the pile, the effect of swim bladder resonance will be negligible in swim bladders less than 2 mm (volume expressed in equivalent bubble radius). The swim bladders of the larvae used in this study were considerably smaller. The relevance of swim bladder resonance will become greater with an increase of the swim bladder size or an increase of the high frequency content of the sound signal.

The recently published sound exposure guidelines [11] suggested a SEL_{cum} threshold >210 dB re $1 \mu Pa^2 s$ and a L_{z-p} threshold >207 dB re $1 \mu Pa^2$ for mortality and potential mortal injury in fish eggs and larvae due to pile-driving. The same thresholds were proposed for seismic airguns. The present study indicated that the thresholds might be even higher for fish larvae (assuming potential mortal injuries will lead to increased mortality within 10 days under laboratory conditions): $SEL_{cum} >216$ dB re $1 \mu Pa^2 s$ and $L_{z-p} >210$ dB re $1 \mu Pa^2$. This was corroborated by field experiments with early juvenile (68-115 DAH) sea bass [10]; no lethal effects were observed up to 14 days after exposure at a SEL_{cum} of 215-222 dB re $1 \mu Pa^2 s$ and a L_{z-p} of 210-211 dB re $1 \mu Pa^2$.

Two 'old' studies showed that exposure to loud impulse sounds can cause mortality and injuries in fish larvae [24,25]. The effects of seismic air gun sounds on eggs and larvae of cod (*Gadus morhua*), saithe (*Pollachius virens*), herring, turbot (*Psetta maximus*) and plaice (*Pleuronectes platessa*) were examined in field experiments [24]. Effect was related to the distance from the sound source and the corresponding L_{z-p} ranged from 220 to 242 dB re $1 \mu Pa^2$. Sound exposure levels were not reported. Significant effects were observed for some species, in some of the larval stages, including the yolk-sac stage of turbot (no swim bladder). Larval and small juvenile spot (*Leiostomus xanthurus*) and pinfish (*Lagodon rhomboides*) were exposed to blast shock waves in field experiments [25]. At the highest exposure levels all spot and most of the pinfish died or were injured, while none died or were injured in the control groups. Sound measurements of the highest exposures were zero to peak pressure = 278-866 kPa ($L_{z-p} \approx 229-239$ dB re $1 \mu Pa^2$) and energy flux density = 1.096-3.642 J m⁻² ($SEL_{ss} \approx 182-187$ dB re $1 \mu Pa^2 s$ assuming the impedance of the medium to be $1.53 \cdot 10^6$ kg/m²s). The L_{z-p} values were much higher in these two 'old' studies [24,25] than in the current study or other recent studies on the effect of pile-driving sound in which L_{z-p} was reported [4,10].

The presence and type of swim bladder (physoclistous or physostomous) is expected to determine the vulnerability for sound pressure exposure. Least susceptible to sound pressure induced injuries are fish with no swim bladder and most susceptible are fish with a physoclistous swim bladder [5,6]. In the recently published sound exposure guidelines [11] a distinction was made between no swim bladder, swim bladder involved in hearing and swim bladder not involved in hearing, each with different thresholds for mortal and potential mortal injuries. Fish with a swim bladder involved in hearing are expected to be most susceptible for mortal and potential mortal injuries. The whole range of swim bladder types is included in the present study: no swim bladder (sole larvae 2 DAH), physostomous swim bladder (herring larvae), physoclistous swim bladder (sea bass larvae), swim bladder not involved in hearing (sole and sea bass larvae) and swim bladder involved in hearing (herring larvae). No differences between the swim bladder types were observed at the sound levels applied in this study.

Acknowledgments

We thank Corrina Hinrichs, Tim Huijjer, Ruben Hoek, Marco Lohman and Ineke Pennock for their practical assistance. We are also grateful to Audrey Geffen from the University of Bergen for advice on artificial fertilisation and herring larvae rearing. We thank Martine Graafland, Paul Boers, Suzanne Lubbe and Joop Bakker for constructive feedback during the project, and Ben Griffioen for reviewing an earlier version of the manuscript.

References

1. Popper AN, Hastings MC (2009) The effects of anthropogenic sources of sound on fishes. *J Fish Biol* 75: 455–489.
2. Popper AN, Hastings MC (2009) The effects of human-generated sound on fish. *Integr Zool* 4: 43–52.
3. Oestman R, Buehler D, Reyff JA, Rodkin R Technical Guidance for Assessment and Mitigation of the Hydroacoustic Effects of Pile Driving on Fish. San Francisco: Caltrans.
4. Halvorsen MB, Casper BM, Woodley CM, Carlson TJ, Popper AN (2012) Threshold for Onset of Injury in Chinook Salmon from Exposure to Impulsive Pile Driving Sounds. *PLoS ONE* 7: e38968
5. Halvorsen MB, Casper BM, Matthews F, Carlson TJ, Popper AN (2012) Effects of exposure to pile-driving sounds on the lake sturgeon, Nile tilapia and hogchoker. *Proc. R. Soc. B* 279: 4705–4714
6. Casper BM, Halvorsen MB, Matthews F, Carlson TJ, Popper AN (2013) Recovery of Barotrauma Injuries Resulting from Exposure to Pile Driving Sound in Two Sizes of Hybrid Striped Bass. *PLoS ONE* 8
7. Bolle LJ, Blom E, Halvorsen MB, Woodley CM, de Jong CAF, Wessels PW, van Damme CJG, Hoek R, Winter HV (submitted) Barotrauma Injuries in European Sea Bass Due to Exposure to Pile-driving Sounds. *PLoS ONE*
8. Casper BM, Popper AN, Matthews F, Carlson TJ, Halvorsen MB (2012) Recovery of Barotrauma Injuries in Chinook Salmon from Exposure to Pile Driving Sound. *PLoS ONE* 7: e39593
9. Bolle LJ, de Jong CAF, Bierman SM, van Beek PJG, van Keeken OA, Wessels PW, van Damme CJG, Winter HV, de Haan D, Dekeling RPA (2012) Common sole larvae survive high levels of pile-driving sound in controlled exposure experiments. *PLoS ONE* 7: e33052
10. Debusschere E, De Coensel B, Bajek A, Botteldooren D, Hostens K, Vanaverbeke J, Vandendriessche S, Van Ginderdeuren K, Vincx M, Degraer S (2014) *In Situ* Mortality Experiments with Juvenile Sea Bass (*Dicentrarchus labrax*) in Relation to Impulsive Sound Levels Caused by Pile Driving of Windmill Foundations. *PLoS ONE* 9: e109280

11. Popper AN, Hawkins AD, Fay RR, Mann DA, Bartol S, Carlson TJ, Coombs S, Ellison WT, Gentry RL, Halvorsen MB, Løkkeborg S, Rogers PH, Southall BL, Zeddies DG, Tavolga WN (2014) ASA S3/SC1.4 TR-2014 Sound Exposure Guidelines for Fishes and Sea Turtles: A Technical Report prepared by ANSI-Accredited Standards Committee S3/SC1 and registered with ANSI. ASA Press Springer, New York
12. Palazzi R, Richard J, Bozzato G, Zanella L (2006) Larval and juvenile rearing of common sole (*Solea solea* L.) in the Northern Adriatic (Italy). *Aquaculture* 255: 495–506
13. Boulhic M, Gabaudan J (1992) Histological Study of the Organogenesis of the Digestive-System and Swim Bladder of the Dover Sole, *Solea solea* (Linnaeus 1758). *Aquaculture* 102: 373–396
14. Moretti A, Pedini Fernandez-Criado M, Cittolin G, Guidastrì R (1999) Manual on hatchery production of seabass and gilthead seabream. Volume 1. FAO, Rome, 194p. Available: <http://www.fao.org/docrep/005/x3980e/x3980e08.htm#TopOfPage>. Accessed November 2014
15. Folkvord A, Hoie H, Johannessen A, Solbakken T (2009) Effects of prey concentration, light regime, and parental origin on growth and survival of herring larvae under controlled experimental conditions. *ICES J Mar Sci* 66:1702-1709
16. ICES (2014) Report of the Working Group of International Pelagic Surveys (WGIPS), 20-24 January 2014. ICES CM 2014/SSGESST:01
17. Blaxter JHS, Batty RS (1984) The herring swimbladder - loss and gain of gas. *J Mar Biol Ass UK* 64: 441-459
18. Popper AN, Fay RR (2011) Rethinking sound detection by fishes. *Hearing Research* 273: 25-36
19. Hoss DE, Blaxter JHS (1979) Effect of rapid changes of hydrostatic-pressure on the Atlantic herring *Clupea harengus* L. 1. Larval survival and behaviour. *J Exp Mar Biol Ecol* 41: 75-85
20. Colby DR, Hoss DE, Blaxter JHS (1982) Pressure sensitivity of Atlantic herring, *Clupea harengus* L, larvae. *Fish Bull* 80: 567-574
21. Martin JS, Rogers PH (2008) Sound exposure chamber for assessing the effects of high-intensity sound on fish. *Bioacoustics* 17: 331–333.
22. Ainslie MA, de Jong CAF, Dol HS, Blacquièrre G, Marasini C (2009) Assessment of natural and anthropogenic sound sources and acoustic propagation in the North Sea. TNO-DV 2009 C085. Available: <https://www.tno.nl/downloads/Assessment%20of%20natural%20and%20anthropogenic%20soundsources%20and%20acoustic%20propagationin%20the%20North%20Sea.pdf>. Accessed November 2014.
23. Ainslie MA (Ed.) (2011) Standard for measurement and monitoring of underwater noise, Part I: physical quantities and their units. TNO report TNO-DV 2011 C235 (2011). Available : http://www.noordzeeloket.nl/images/Standard%20for%20measurement%20and%20monitoring%20of%20underwater%20noise%20Part%20I_648.pdf. Accessed November 2014.
24. Booman C, Dalen J, Leivestad H, Levsen A, van der Meeren T et al. (1996) Effekter av luftkanonskyting pa egg, larver og yngel. Undersokelser ved Havforskningsinstituttet og Zoologisk Laboratorium UIB. Rapport Fisken og Havet Nr. 3-1996. Bergen: Havforskningsinstituttet.
25. Govoni JJ, West MA, Settle LR, Lynch RT, Greene MD (2008) Effects of underwater explosions on larval fish: Implications for a coastal engineering project. *J Coast Res* 24: 228–233.

Tables

Table 1. Treatments, mean measured sound levels and number of replicates in the final experiments.

Species	Age (DAH)	Sound signal	Strikes	Sound pressure levels			Replicates
				L_{z-p}	SEL _{ss}	SEL _{cum}	
Sole	2	none (control)	0	0	0	0	15
		OWEZ @ 200 m	100	205	181	201	15
		OWEZ @ 100 m	100	210	186	206	15
Sole	8	none (control)	0	0	0	0	15
		OWEZ @ 200 m	100	205	180	200	15
		OWEZ @ 100 m	100	209	185	205	15
Sole	15	none (control)	0	0	0	0	15
		OWEZ @ 200 m	100	205	181	201	15
		OWEZ @ 100 m	100	209	185	205	15
Sea bass	18-19	none (control)	0	0	0	0	10
		OWEZ @ 100 m	100	210	186	206	10
		OWEZ @ 100 m	999	210	186	216	10
		Exponential positive	100	217	187	207	10
Sea bass	38-39	none (control)	0	0	0	0	10
		OWEZ @ 100 m	100	211	187	207	10
		OWEZ @ 100 m	999	211	187	217	10
		Exponential positive	100	217	187	207	10
Herring	88-89	none (control)	0	0	0	0	10
		OWEZ @ 100 m	100	206	183	203	4
		OWEZ @ 100 m	999	207	182	212	10

L_{z-p} = zero to peak sound pressure level (in dB re 1 μPa^2), SEL_{ss} = single strike sound exposure level (in dB re 1 $\mu\text{Pa}^2\text{s}$), and SEL_{cum} = cumulative sound exposure level (in dB re 1 $\mu\text{Pa}^2\text{s}$). Approximately 25 (sole and herring) or 30 (sea bass) larvae were used for each replicate.

Table 2. Analysis of variance of the probability of death modelled as a function of treatment and random batch effect.

Species	Age (DAH)	Duration (days)	Chi ² /DF	Random effect variance	Fixed effect			
					Num DF	Den DF	F-value	p-value
Sole	2	5	0.82	0.000	2	42.00	0.31	0.73
		7	0.68	0.000	2	42.00	0.09	0.91
Sole	8	5	1.00	0.140	2	41.90	0.48	0.62
		7	1.00	0.057	2	41.67	0.40	0.67
Sole	15	5	0.99	0.034	2	42.00	0.03	0.97
		7	0.99	0.000	2	42.00	0.10	0.90
Sea bass	18-19	5	1.02	0.231	3	33.95	0.74	0.53
		10	1.01	0.196	3	34.99	0.03	0.99
Sea bass	38-39	5	1.08	0.000	3	36.00	1.42	0.25
		10	1.02	0.019	3	34.95	0.11	0.95
Herring	88-89	5	1.01	0.019	2	20.27	0.25	0.78
		10	1.03	0.128	2	19.84	0.19	0.83

Table 3. Estimated probability of death and estimated effect of exposure, with the upper limits of the 95% confidence interval.

Species	Age (DAH)	Duration (days)	Sound signal	Strikes	Probability of death		Effect (%)	
					mean	upper limit	mean	upper limit
Sole	2	7	none (control)	0	0.55	0.61		
			OWEZ @ 200 m	100	0.54	0.59	-3%	14%
			OWEZ @ 100 m	100	0.54	0.59	-3%	13%
Sole	8	7	none (control)	0	0.21	0.26		
			OWEZ @ 200 m	100	0.24	0.30	4%	14%
			OWEZ @ 100 m	100	0.22	0.27	1%	10%
Sole	15	7	none (control)	0	0.31	0.36		
			OWEZ @ 200 m	100	0.31	0.36	0%	10%
			OWEZ @ 100 m	100	0.30	0.35	-2%	8%
Sea bass	18-19	10	none (control)	0	0.38	0.47		
			OWEZ @ 100 m	100	0.39	0.48	2%	23%
			OWEZ @ 100 m	999	0.38	0.47	0%	21%
			Exp. positive	100	0.39	0.48	2%	23%
Sea bass	38-39	10	none (control)	0	0.06	0.10		
			OWEZ @ 100 m	100	0.07	0.11	1%	7%
			OWEZ @ 100 m	999	0.07	0.11	1%	8%
			Exp. positive	100	0.06	0.10	0%	6%
Herring	88-89	10	none (control)	0	0.49	0.58		
			OWEZ @ 100 m ^a	100	0.53	0.67	9%	40%
			OWEZ @ 100 m	999	0.51	0.60	5%	29%

^a Insufficient number of replicates (see Table 1 and text)

Figures



Figure 1. European sea bass (*Dicentrarchus labrax*) larvae 18 (top) and 38 days after hatching (bottom).



Figure 2. Herring (*Clupea harengus*) larvae 89 days after hatching.

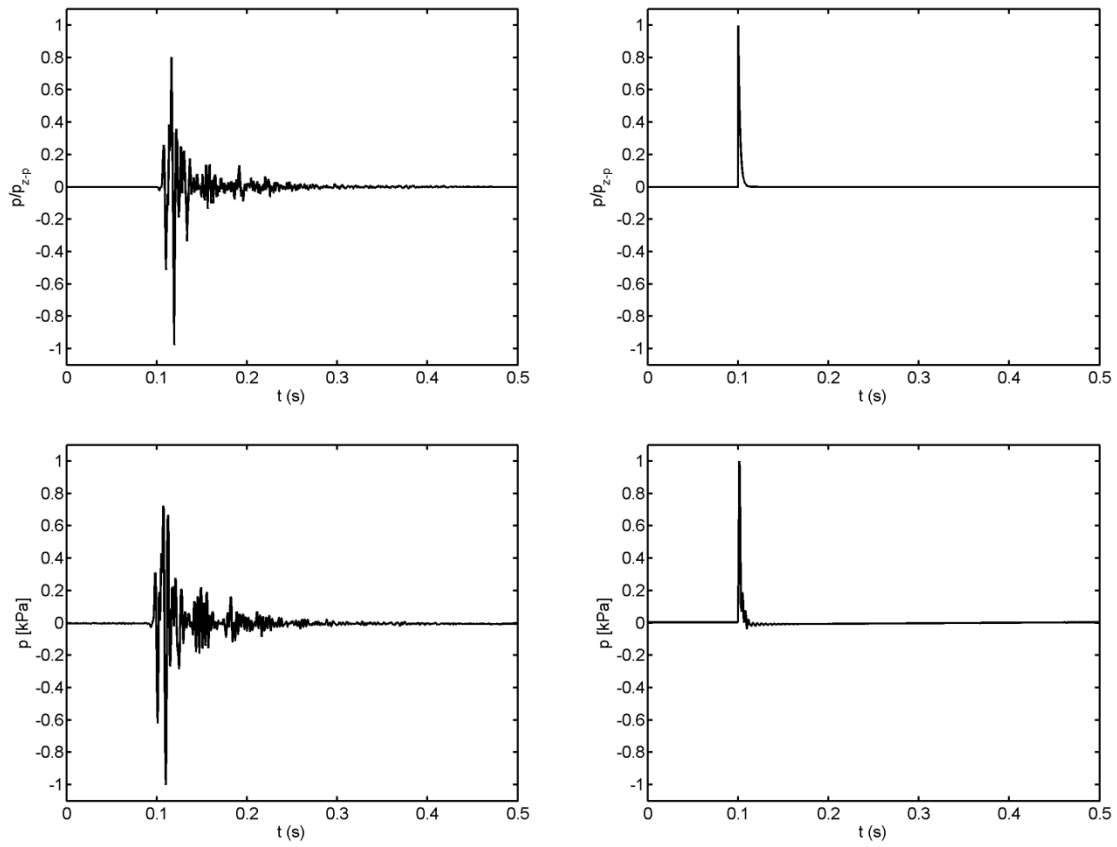


Figure 3. Original (top) and reproduced (bottom) sound pressure time series of the OWEZ@100m pulse (left) and the positive exponential pulse (right).

The original signals are amplitude scaled to the maximum absolute value of the instantaneous sound pressure (p / p_{z-p}).

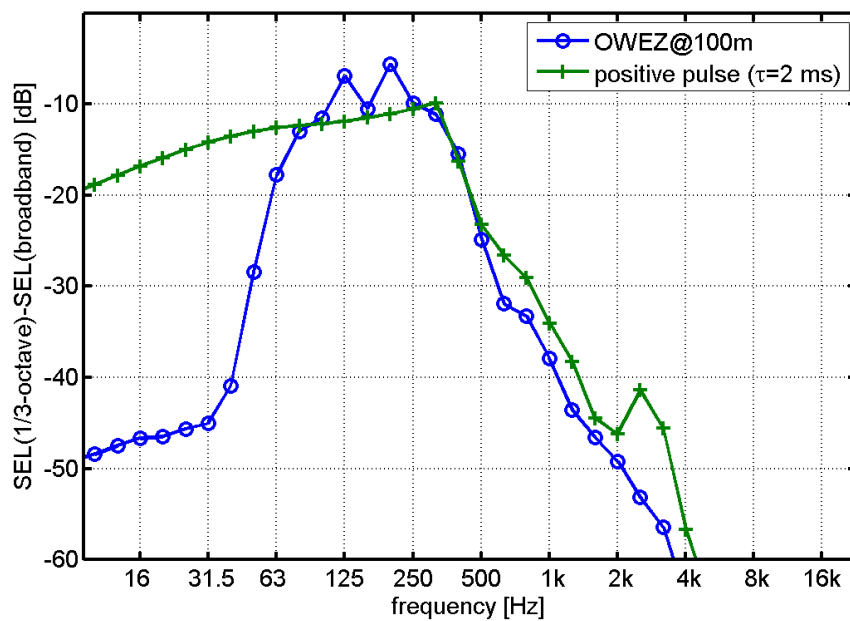
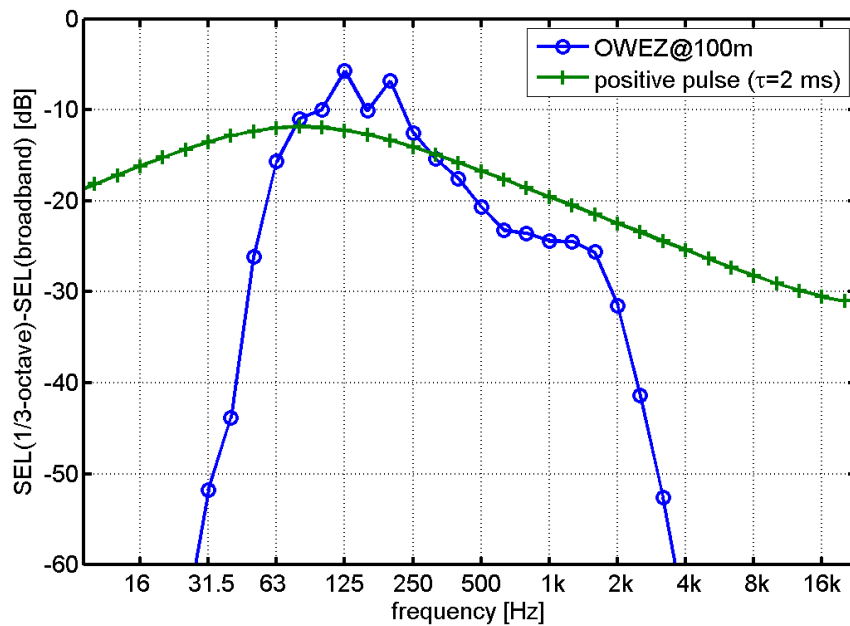


Figure 4. Original (top) and reproduced (bottom) 1/3-octave band spectra of SEL of the OWEZ@100m pulse and the positive exponential pulse.

1/3-octave band spectra of SEL are scaled to broadband SEL. Frequency band filtering is applied to the OWEZ signal (50 Hz – 1 kHz).

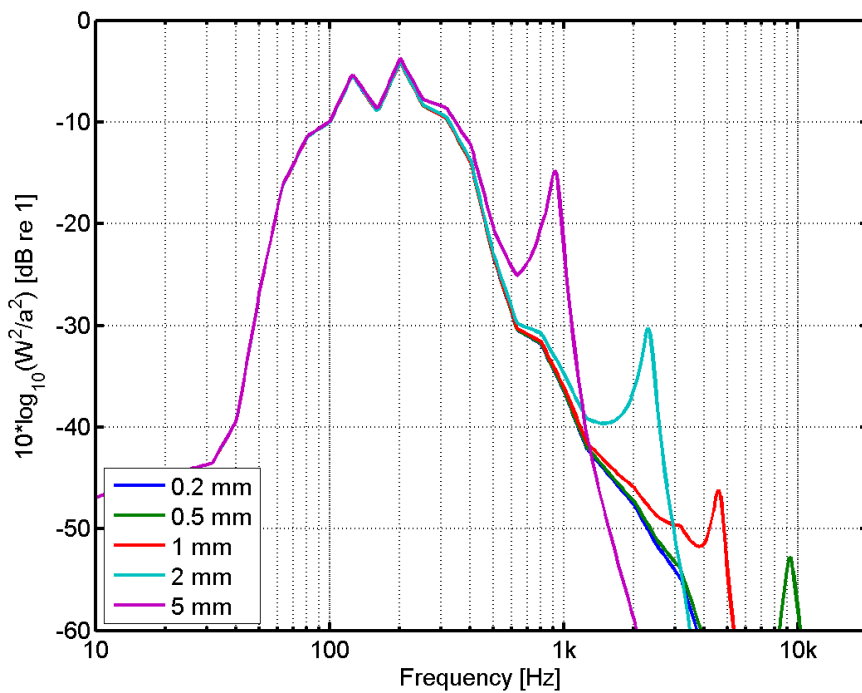
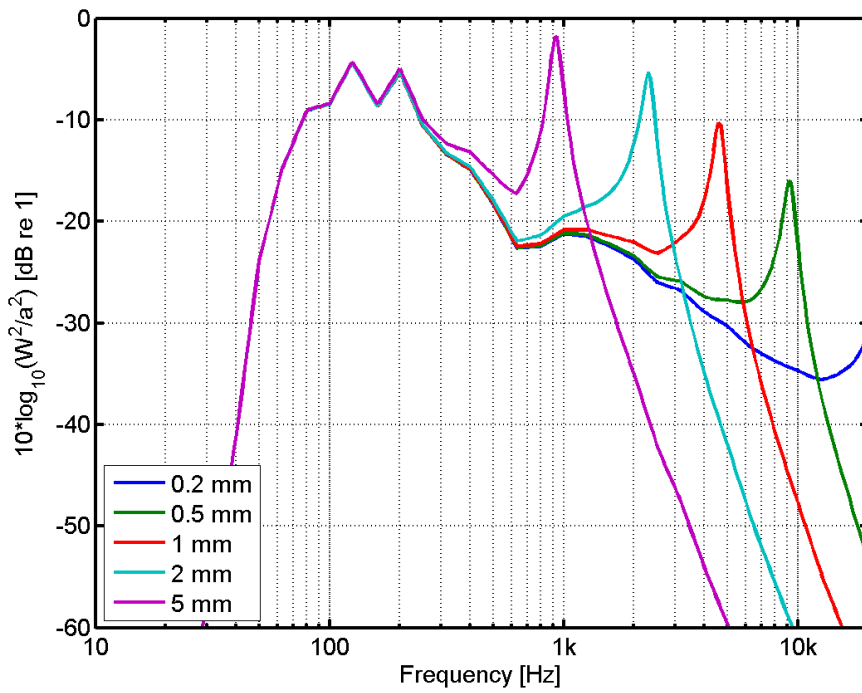


Figure 5. Forced response of air bubbles with different radii under influence of the sound pressure level spectrum of the OWEZ@100m signal, as measured in the field (top), and as measured in the larvaebtrator during play-back of the filtered (50 Hz - 1 kHz) signal (bottom).

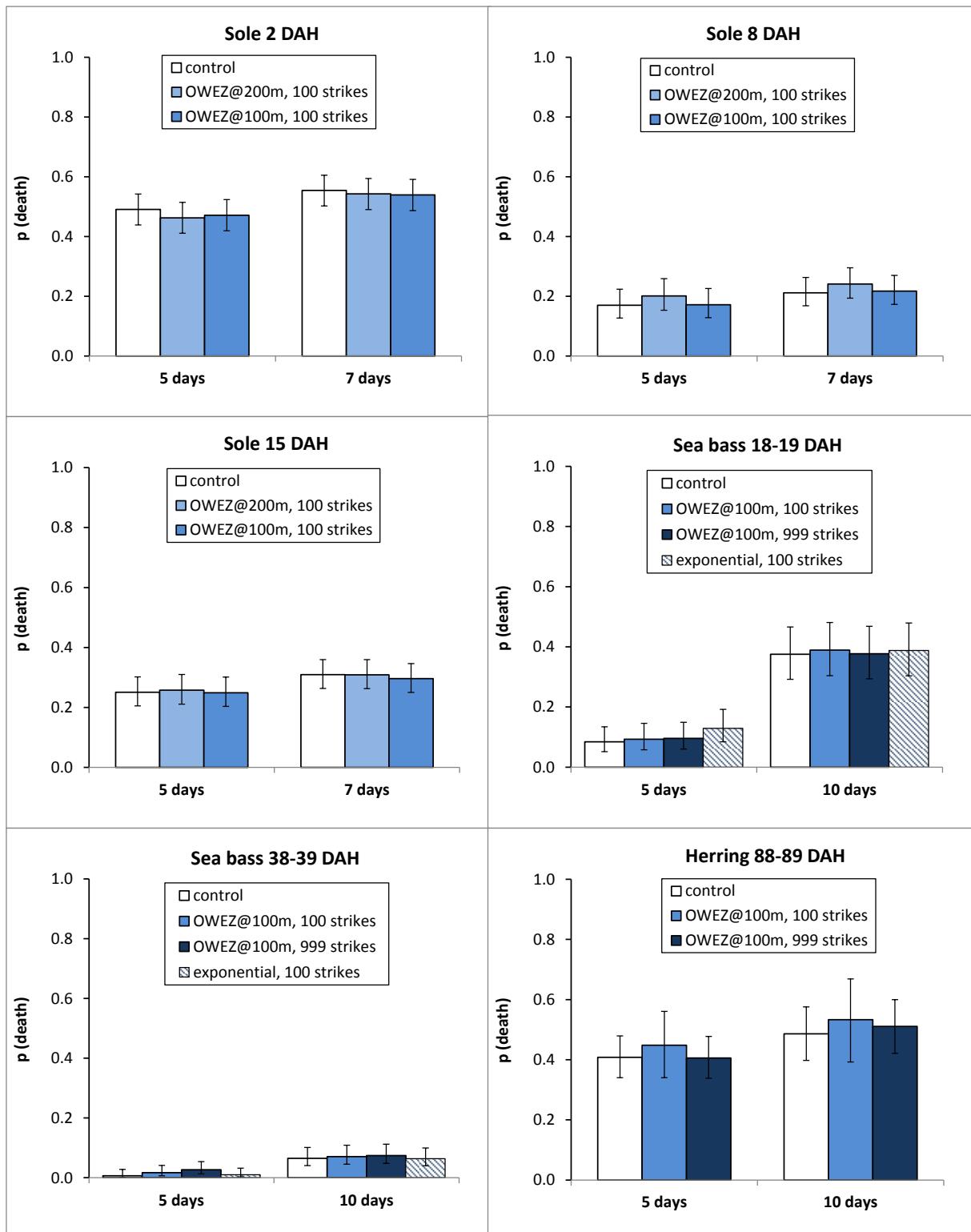


Figure 6. Mean probability of death (with 95% confidence interval) after 5 days and at the end of the monitoring period, for each species, larval stage and treatment.

The sound pressure levels and number of replicates (by species, larval stage and treatment) are presented in Table 1.

Supporting information

Theoretical model to examine swim bladder resonance in relation to swim bladder size and spectral content.

Swim bladder modelled as an oscillating bubble

A first order assessment of the potential impact of sound on fish larvae with a swim bladder can be made from the assumption that the main damaging mechanism is associated with deformation of the swim bladder. If one assumes that this bladder can be modelled as a gas bubble, the bubble pulsations can be estimated¹ as follows.

In a pulsating gas bubble of radius a , the external surface acoustic pressure $p(a)$ and the radial velocity \dot{W} of the bubble surface are related. In the low frequency limit $k_g a \ll 1$ (with $k_g = \omega/c_g$ the acoustic wave number of the gas in the bubble, c_g the sound speed and ω the radian frequency), the bubble behaves as a dynamic spring of stiffness per unit area² K_g :

$$\frac{p(a)}{\dot{W}} = \frac{-iK_g}{\omega} \approx \frac{-i3\rho_g c_g^2/a}{\omega} \quad (1)$$

Here ρ_g is the density and sound speed of the gas in the bubble. The bulk modulus of the gas $\rho_g c_g^2$ is a linear function of the hydrostatic pressure P , hence:

$$K_g \approx \frac{3\gamma P}{a} \quad (2)$$

When the bubble is also 'acoustically small', i.e. in the limit $k_f a \ll 1$ (with k_f the acoustic wave number of the water), the fluid accelerated by the pulsating bubble behaves like a mass M_f per unit area³:

$$M_f \approx a\rho_f \quad (3)$$

Hence, the natural frequency of a fluid-loaded bubble is:

$$f_0 = \frac{1}{2\pi} \sqrt{\frac{K_g}{M_f}} \approx \frac{1}{2\pi a} \sqrt{\frac{3\gamma P}{\rho_f}} \quad (4)$$

For example: an air bubble ($\gamma=1.41$) of radius $a=1$ mm at 10 m depth ($P=200$ kPa) in water ($\rho_f=1000$ kg/m³) has a natural frequency $f_0 \approx 4.6$ kHz, with $K_g \approx 846$ MPa/m and $M_f \approx 1$ kg/m².

For a real fish bladder⁴ the resonance frequency can be higher due to the additional tension from flesh, which must be added to the hydrostatic pressure in eq.(4). Also, the estimation of the resonance frequency must be corrected for the non-spherical shape of the bladder.

¹ Note that this analysis does not provide more than an estimation of the order of magnitude of the fish bladder behaviour. Prediction of bubble behaviour depends on a wide range of assumptions, see Ainslie & Leighton 2011 *Review of scattering and extinction cross-sections, damping factors, and resonance frequencies of a spherical gas bubble*. J. Acoust. Soc. Am. 130 (5), Pt. 2, 3184–3208

² Junger & Feit 1986 *Sound, structures, and their interaction*. MIT Press, eq.(2.47)

³ Junger & Feit 1986 *Sound, structures, and their interaction*. MIT Press, eq.(2.43)

⁴ Ainslie 2010 *Principles of Sonar Performance Modelling*. Springer-Praxis, p.219

To first order, this correction amounts to replacing the bubble radius a in eq.(4) with an equivalent radius a_{eq} that incorporates the volume V_b and surface area S_b of the bladder:

$$a_{eq} = \sqrt{\frac{3V_b}{4\pi S_b}} \quad (5)$$

Far below the natural frequency, the radial displacement W due to pulsation pressure $p(a)$ is approximately equal to $p(a)/K_g$, which means that the displacement will be of the order of magnitude of the bubble radius at a sound pressure $p \approx K_g a \approx 850$ kPa. That means that the maximum zero-to-peak sound pressure of about 70 kPa that can be generated in the larvaebrator thus could cause an 8% strain of the tissues surrounding the bubble. It seems not unrealistic that such a strain causes damage.

At and near to the bubble resonance, the response is governed by damping. The radial displacement W of the outer surface oscillating bubble model for the fish bladder can then be approximately written as:

$$\frac{W}{p(a)} = \frac{1}{M_f \sqrt{(\omega_0^2 - \omega^2)^2 + (\omega \omega_0 / Q)^2}} \quad (6)$$

Here, 'quality-factor' Q describes the amplification of the radial displacement at the resonance, which is inversely proportional to the damping. The Q -factor for a fish bladder in shallow water is dominated by flesh damping⁵, which is an order of magnitude larger than thermal and radiation effects. A typical value for the Q of a fish bladder at atmospheric pressure is 3, which may increase to 9 at larger depths. For the further analysis we assume a Q -factor 10, to get a conservative estimate, i.e. an overestimation of the radial displacement response, of the possible contribution of the bladder resonance.

Figure S1 illustrates the response (radial displacement due to excitation pressure) of bubbles of radii (actual or 'equivalent', eq.(5)) between 0.05 and 5 mm with a Q -factor 10 at 10 m below the water surface. For a frequency-independent Q -factor 10, the resonant response is 10 times larger (i.e. 20 dB higher) than the response at lower frequencies, at the same excitation pressure.

The curves in Figure S1 rely on the following two conditions:

1. The gas 'bubble' is compact, which means that the largest dimension of the bubble is much smaller than the acoustic wavelength. At the upper range of the graph (100 kHz) this wavelength is about 15 mm, so this condition is fulfilled for the bubble sizes in this study.
2. The Q -factor is frequency-independent. This is probably not true for real fish bladders, where the Q -factor is influenced by flesh damping, thermal damping and radiation damping. However, the Q -factor 10 was intentionally chosen to overestimate the resonant response. Though a more realistic model for the damping behaviour would lead to a different shape of the response curve at frequencies close to resonance, it is unlikely that this would influence the general conclusions about the relevance of the resonant response.

⁵ Ainslie 2010 *Principles of Sonar Performance Modelling*. Springer-Praxis, p.221

Forced response

The swim bladder deformation under influence of impulsive sound is a combined effect of the bladder response and the sound pressure excitation. If the time signal of the excitation pressure is known it can be convolved with the impulse response of the bubble (i.e. the inverse Fourier transform of the frequency domain response given in eq.5) to calculate the time signal of the bladder wall response. Here a simpler approach is chosen to get a first impression of the relative importance of the swim bladder resonance for the forced response of the swim bladder. This approach is based on adding the spectrum of the response (Figure S1) to the sound pressure excitation spectrum, assuming that the total deformation is proportional to incoherent sum of the spectral components of the resulting displacement spectrum.

The approach is first illustrated for the excitation spectrum of the OWEZ piling sound that was played back in the sole larvae studies, both the original offshore recording and the recording of the playback signal in the larvaebator. Figure S2 shows the calculated spectrum of the forced radial displacement of the bubble when excited by the sound pressure as measured at a distance of 100 m of piling for the OWEZ wind farm. Only for the largest bubble (5 mm radius, i.e. 925 Hz resonance frequency, eq.(4)) the resonant peak exceeds the non-resonant bubble response in the 60 to 400 Hz frequency range. For the 5 mm equivalent radius bubble, the broadband response (obtained by integrating W^2 over the frequency range from 10 Hz to 20 kHz) increases by 40% (or 1.5 dB) due to the resonant response.

The calculated spectrum of the forced radial displacement of the bubble when excited by the sound pressure as measured during playback of the OWEZ piling sound, is presented in Figure S3. Because the playback spectrum drops off above the 315 Hz band, the resonant response does not contribute to the total deformation in this case. Comparison of Figure S2 and Figure S3 suggests that it is unlikely that the absence of the high frequency content in the playback influences the results of exposure studies with fish larvae with swim bladder equivalent radii smaller than 2 mm.

A second, more extreme, example is the bubble response due to a (positive or negative) theoretical exponential pulse. This pulse is sharper than any pulse that would be experienced in real life (probably more realistic for the pulse close to an underwater explosion than for pile driving sound). Figure S4 shows the calculated spectrum of the forced radial displacement of the bubble when excited by a theoretical negative exponential pulse with a 2 ms decay time. The resonant peaks exceeds the non-resonant bubble response in the 60 to 400 Hz frequency range for equivalent bubble radii of 0.5 mm and greater. The resonance increases the broadband response (the integral of W^2 over the frequency range from 10 Hz to 20 kHz) by 10% (0.4 dB) for a bubble of 0.5 mm equivalent radius, by 20% (0.8 dB) for a 1 mm bubble, by 40% (1.5 dB) for a 2 mm bubble and by 100% (3 dB) for a 5 mm equivalent bubble radius. Figure S5 shows the calculated spectrum of the forced radial displacement of the bubble when excited by the playback of the theoretical negative exponential pulse in the larvaebator. Like for the playback of the OWEZ signal, the resonant response does not contribute to the total deformation. Even for this extreme excitation, the predicted effect of the swim bladder resonance on the response of fish larvae seems very small.

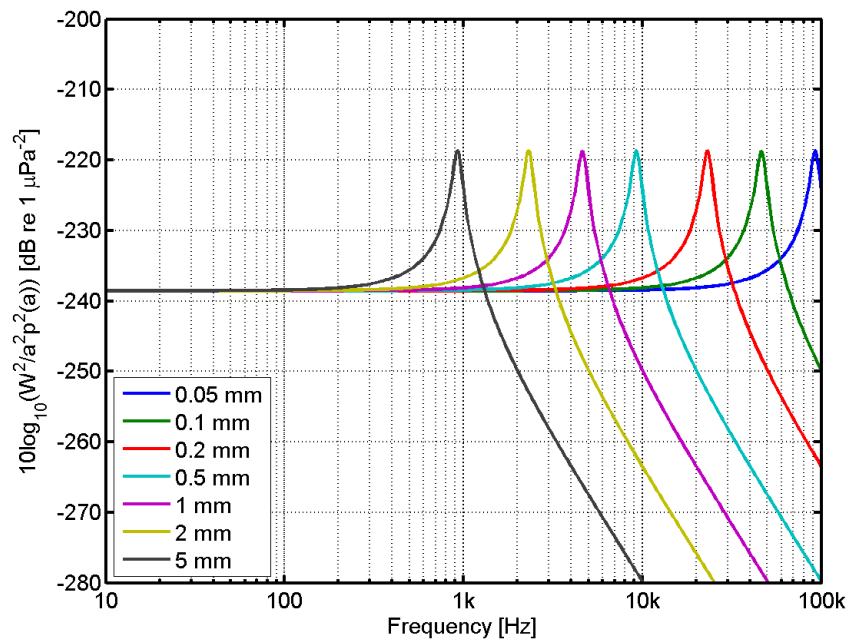


Figure S1. Oscillating bubble response (radial displacement of the bubble wall normalized on the bubble radius, per unit applied pressure), for air bubbles in water at 10 m depth, with different ('equivalent', eq.(5)) bubble radii (in mm) and $Q=10$.

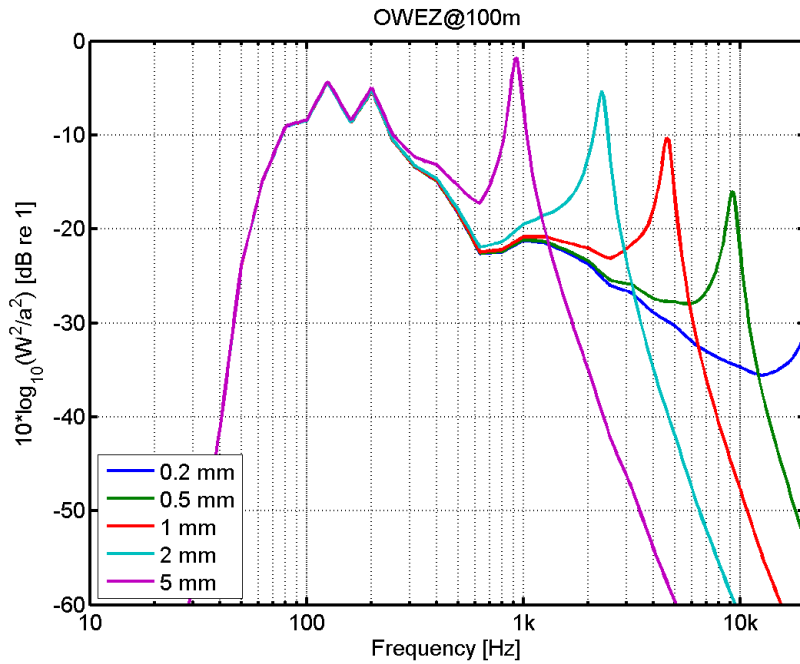


Figure S2. Forced response of air bubbles in water at 10 m depth, with different bubble radii (in mm) and $Q=10$, under influence of the sound pressure level spectrum as measured at a distance of 100 m of piling for the OWEZ wind farm.

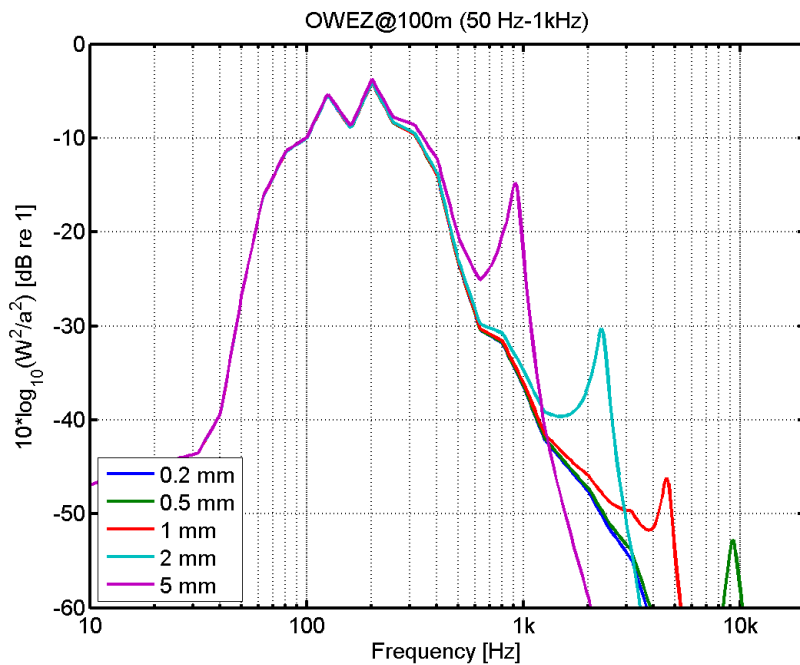


Figure S3. Forced response of air bubbles in water at 10 m depth, with different bubble radii (in mm) and $Q=10$, under influence of the sound pressure level spectrum of the playback of the OWEZ piling sound in the larvaebtrator.

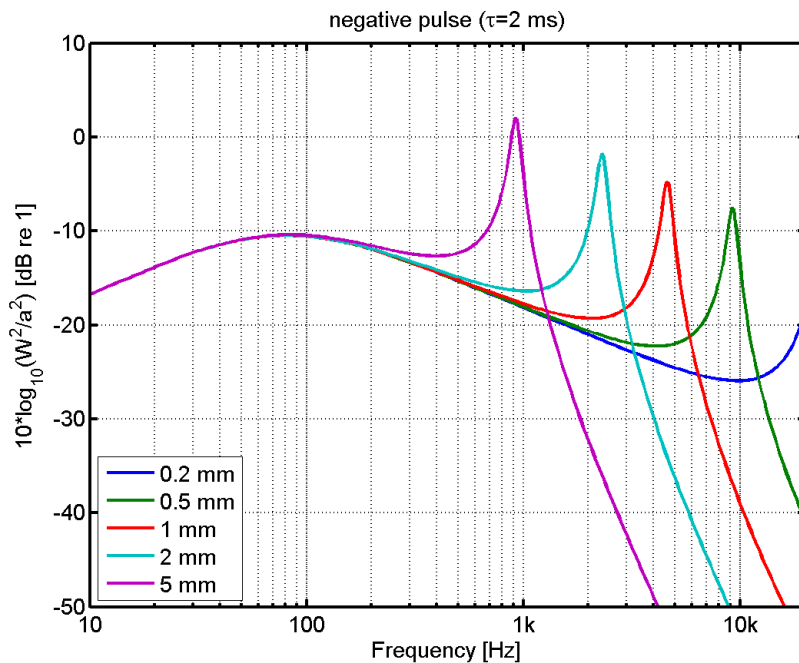


Figure S4. Forced response of air bubbles in water at 10 m depth, with different bubble radii (in mm) and $Q=10$, under influence of the sound pressure level spectrum corresponding with a theoretical negative exponential pulse with a 2 ms decay time.

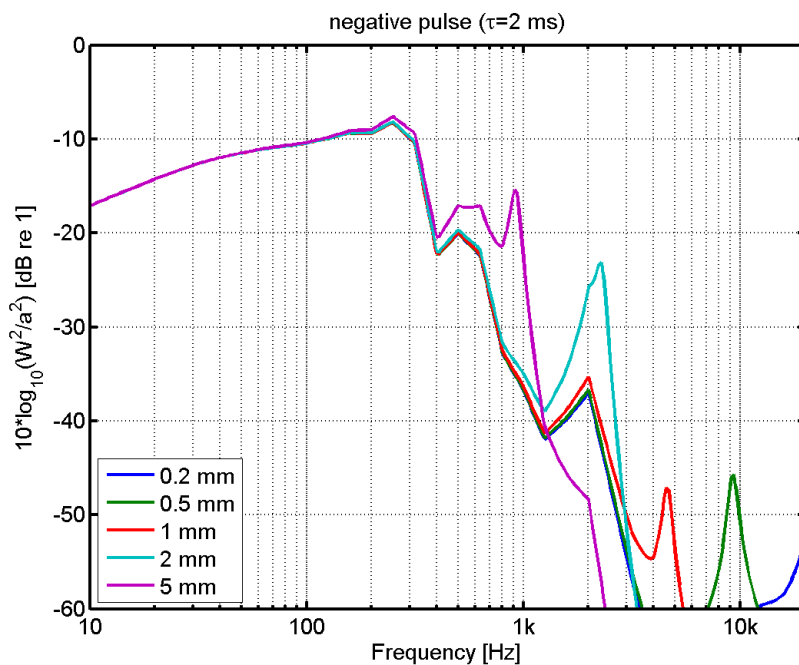


Figure S5. Forced response of air bubbles in water at 10 m depth, with different bubble radii (in mm) and $Q=10$, under influence of the sound pressure level spectrum of the playback of theoretical negative exponential pulse in the larvaebrator.