Shortlist Masterplan Wind
Effect of piling noise on the survival of fish larvae (pilot study)
Progress report

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Contents

1. Introduction

Fish can suffer lethal damage to swimming bladder or other organs due to extreme loud impulse sounds caused by e.g. pile driving (Popper & Hastings 2009). Juvenile and adult fish can actively swim away from a sound source, but planktonic larvae are not able to do this. As a result, fish larvae may suffer more from underwater noise than the older life stages. Despite the many indications for adverse effects, detailed information on the effect of different sound levels on fish is still scarce, especially for the early life stages.

Within the framework of the Appropriate Assessment of Dutch offshore wind farms, the effect of piling noise on the southern North Sea population of herring, sole, and plaice larvae was simulated (Prins et al. 2009). For this, an existing larval transport model (Bolle et al. 2005, 2009, Dickey-Collas et al. 2009, Erftemeijer et al. 2009) was expanded with crude assumptions on larval mortality caused by pile driving. The model results were extrapolated to other fish species and older life stages, based on "expert-judgment", in an attempt to assess the effect of offshore piling on the prey availability for birds and marine mammals in Natura 2000 areas (Bos et al. 2009). This assessment involved a large number of uncertainties. The first and most important uncertainty was the range around a piling site in which larval mortality occurs. It was assumed that 100% mortality occurs up to a distance of 1 km from the piling site. However, little is known about larval mortality rates in relation to the level of exposure to piling noise.

In general, there is an urgent need to obtain more knowledge on the effect of sound on fish (survival, distribution, and behaviour) during different life stages. More particularly, in view of the rapid extension of offshore wind farms, there is an urgent need to fill the knowledge gap on lethal effects of loud impulse noises caused by pile driving. The broader aim of the current project is to examine the effect of piling noise on the survival of fish larvae. However, within the limited resources and time frame of the Shortlist research programme it is not possible to carry out field experiments, nor is it possible to execute elaborate series of experiments. The first goal within the Shortlist programme is to examine the feasibility of laboratory experiments with pile driving noise and fish larvae. The second goal is to use the laboratory set-up in a pilot study aiming at determining the threshold at which mortality of fish larvae occurs.

This shortlist study is limited to laboratory experiments, lethal effects, larvae of 1 species (sole, *Solea solea*) and 3 series of experiments (trials). The study consists of exposure-effect experiments only; the effects of pile driving at the population level will not be modelled, nor will the results be extrapolated to other species or life stages.

The progress to date has been documented in a series of memo's. These memos are included in this report as Appendices and are summarised in the following sections.

2. Methods

2.1 Phase 1

The approach taken in this study is novel in 2 ways: sound exposure experiments with fish larvae and generating piling noise in a laboratory set-up. Therefore the project has been divided in 2 phases. During the first phase the feasibility of laboratory experiments with piling noise and fish larvae was examined. This phase was completed by a go/no-go decision before the second phase, the actual exposure experiments, was started.

The evaluation of the feasibility of the approach and a description of the preparations is presented in TNO memos 1-3 (Appendix A-C) and IMARES memo 1 (Appendix D).

TNO memo 1 (Appendix A) describes requirements for the simulated piling noise spectra and levels. In addition 3 options are discussed for a laboratory test set-up. It was concluded that the most promising option is to develop an exposure chamber, driven by an underwater loudspeaker. With this so-called larvaebrator (derived from an existing experimental set-up in the USA for larger fish), effects of pressure and particle velocity can be tested independently: by driving a rigidly enclosed chamber using a piston, the pressure is raised with negligible particle velocity, while by driving a semi-open chamber, the velocity is raised at negligible increase of pressure. The acoustic pressure is measured by pressure transducers, mounted flush in the wall of the chamber. The particle velocity is measured by a watertight accelerometer mounted on the surface of the piston of the projector.

TNO memo 2 (Appendix B) describes the practical design of the experimental test set-up. The 'larvaebrator' design consists of an LFPX-4 projector (underwater sound source, Figure 1 left panel) on which a compact chamber (Figure 1, middle panel) is placed. The chamber is filled with sea water in which the larvae are inserted. The piston of the projector is also the bottom of the chamber and can directly excite the water with a given signal. Depending on the required exposure, the top cover (Figure 1, right panel) of the chamber can be closed (pressure excitation) or released (velocity excitation).

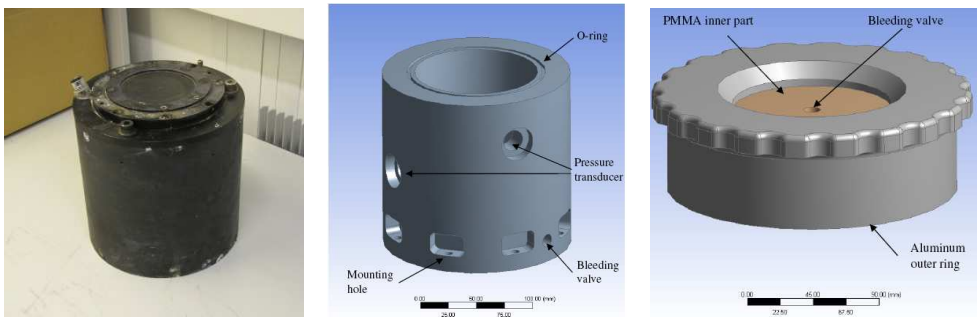

Figure 1. LFPX-4 projector (left), compact chamber for larvae (middle) and top cover (right).

TNO memo 3 (Appendix C) describes the performance validation test of the experimental test set-up. A new specification has been added to the design requirements of the test set-up: for both the velocity and pressure source test conditions, it has to be possible to introduce a static overpressure inside the chamber, varying between about 0.2 and a maximum of 3 bar (Figure 2). This overpressure should better simulate the variety of underwater conditions for the range of depths at which the larvae occur (see IMARES memo 1).

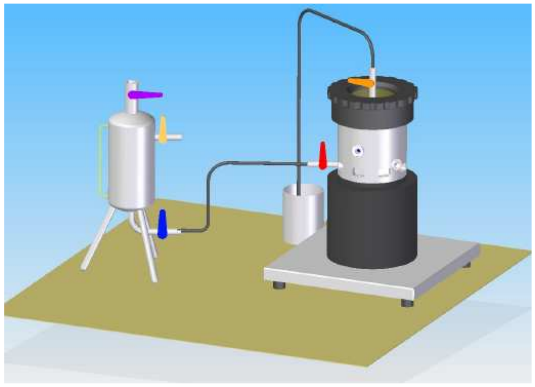


Figure 2. A 3D impression of the experimental test set-up (left) and laboratory test set-up with sound projector, larvae chamber, reservoir and pressure regulator (right).

Two measured noise signals are selected to excite the water in the chamber, one at 100m and one at 800m from a pile at the OWEZ wind farm. The amplitude will be varied in 4.5 dB steps, which roughly corresponds with doubling of the distance to the pile (see Table 1).

Table 1. Sound levels at different distances.

distance	peak pressure	SEL	peak velocity	integrated velocity	wav-file
m	dB re 1 μPa^2	dB re 1 μPa^2s	dB re 1 $(nm/s)^2$	dB re 1 $(nm/s)^2s$	
100	210	188	147	124	pressure_100m_filter.wav
200	205	183	142	119	pressure_100m_filter.wav
400	201	179	138	115	pressure_100m_filter.wav
800	196	174	133	110	pressure_800m_filter.wav
1600	192	170	129	106	pressure_800m_filter.wav
3200	187	165	124	101	pressure_800m_filter.wav

The sound field reproduced the original recorded wav-files quite accurately in case of pressure excitation. The pressure distribution in the chamber was very homogeneous in that configuration. The maximum achievable pressure levels for pressure excitation are about 1-2 dB higher than required for this study. In case of maximum velocity excitation, the pressure levels are 8-13 dB lower than in case of pressure excitation. Because the required velocity levels are about 8 dB lower than the maximum velocity levels, it follows that the pressure levels in case of velocity excitation are negligibly small, compared to the levels for pressure excitation.

IMARES memo 1 describes the preparations required for experiments with fish larvae: sources from which larvae can be obtained, DEC (Animal Experiments Commission) formalities, laboratory facilities, and procedures for handling larvae, maintaining larvae and scoring survival based on test trial experiences. Furthermore this memo presents an estimation of larval mortality without exposure to sound, biological arguments for choosing certain values for larval stage and water pressure, and a test scheme for the first trial with sound exposures.

Sole (*Solea solea*) larvae obtained from a hatchery in IJmuiden (SOLEA BV) were chosen for this pilot study, because of the high frequency of spawning episodes in this hatchery and for practical reasons (quick and easy delivery of larvae due to close connections with IMARES).

Several test trials, i.e. experiments without exposure to noise, have been carried during the first phase of the project. Primary goal of these test trails was to develop and optimise procedures for handling larvae, maintaining larvae and scoring survival. These procedures (described in detail in IMARES memo

1) were further optimised based on experiences obtained in the second phase of the project and an update of procedures is presented in IMARES memo 2 (Appendix F).

Larval mortality without sound exposure was estimated based on the test trials. Average mortality (test trial 3 results, 3 samples, 25 larvae per sample) was 4% (sd=4%) after 5 days and 11% (sd=12%) after 16 days. These mortality rates are considered to be low, i.e. much lower than natural mortality in the field. Although average mortality was low, variability between batches was high with no apparent explication.

The test trails were also used to address specific questions with regard to the (design of) the experimental set-up, such as vertical distribution of larvae in the test chamber and the effect of rapid changes in overpressure. The latter is reported in IMARES memo 2, as it wasn't possible to carry out the 4th (additional) test trial on the effects of changes in overpressure prior to the first trail with sound exposures.

2.2 Phase 2

The "go" decision for the 2nd phase of the project was taken on 20 September 2010. The 3 trials with sound exposures were carried out in October-December 2010. An overview of the sound exposures and the preliminary results of these trials are presented in TNO memo 4 (Appendix E) and IMARES memo 2 (Appendix F).

As little is known about the critical values for sound parameters with regard to larval survival, the aim of the first trial was to examine the sensitivity range. Hence we chose to maximise the number of exposures and minimise the number of replicates. A test scheme was designed in which each exposure depended on the results of the previous exposure (IMARES memo 1). This iterative approach is the most effective way to find critical sound exposure levels, but it depends on immediate visibility of the effects of sound exposure. Trial 1 consisted of 6 sound exposure and 2 control experiments in duplo (see Table 2 in IMARES memo 2).

High 'batch variability' (variability between batches with the same treatment) was observed in the previous trials. Therefore the number of replicates for each treatment was increased in the 2nd trial, at the expense of the number of exposures. The iterative approach was reduced to 1 exposure representing 100m and 1 stroke and 2 follow-up scenario's. Trial 2 consisted of 5 sound exposure and 2 control experiments in 4-fold (see Table 3 in IMARES memo 2).

The same approach was chosen for trial 3, i.e. 1 exposure representing 100m and 1 stroke and 2 follow-up scenario's. The number of replicates for each treatment was further increased. Trial 3 consisted of 5 sound exposure and 2 control experiments in 5-fold (see Table 4 in IMARES memo 2).

Different larval stages were used in the 3 trials: stage 1 (yolk-sac stage) in trial 1, stage 2 in trial 2, and stage 3 (swim bladder maximally inflated) in trial 3. Batch-size for each experiment was 25 (±2) larvae in trial 1 and 2, and 28 (±2) in trial 3. All experiments were carried out with no or a low (0.5 bar) overpressure.

The acoustic measurements of trial 1 are presented in TNO memo 4.

3. Preliminary results

Mortality rates were scored directly after the experiment and daily until 10-12 days after the experiment.

No instantaneous effects were observed in any of the 3 trials. The mortality rate directly after the experiment was 0% for all experiments, except 4 experiments in trial 1 (1 dead larva)

In trial 1, no clear differences were observed between the different treatments 1-12 days after the experiment. Differences at T=12 are statistically insignificant, but the statistical power of 2 replicates is limited given the large variability between batches with the same treatment.

In trial 2, the highest pressure exposure, corresponding to a distance of 100m and 100 strokes, appeared to have an effect on mortality after 5-10 days. A cumulative mortality rate of 80% after 10 days was observed for this exposure ,compared to 60% in the control group (Figure 3). A difference of this magnitude, i.e. 50% of the larvae which survive 'natural mortality' are killed due to noise, is relevant. The difference, however, was not statistically significant. A larger number of replicates is necessary to be able to assess the statistical significance of a difference of this magnitude, given the large variability between batches with the same treatment.

The monitoring results for trial 3 are not yet available.

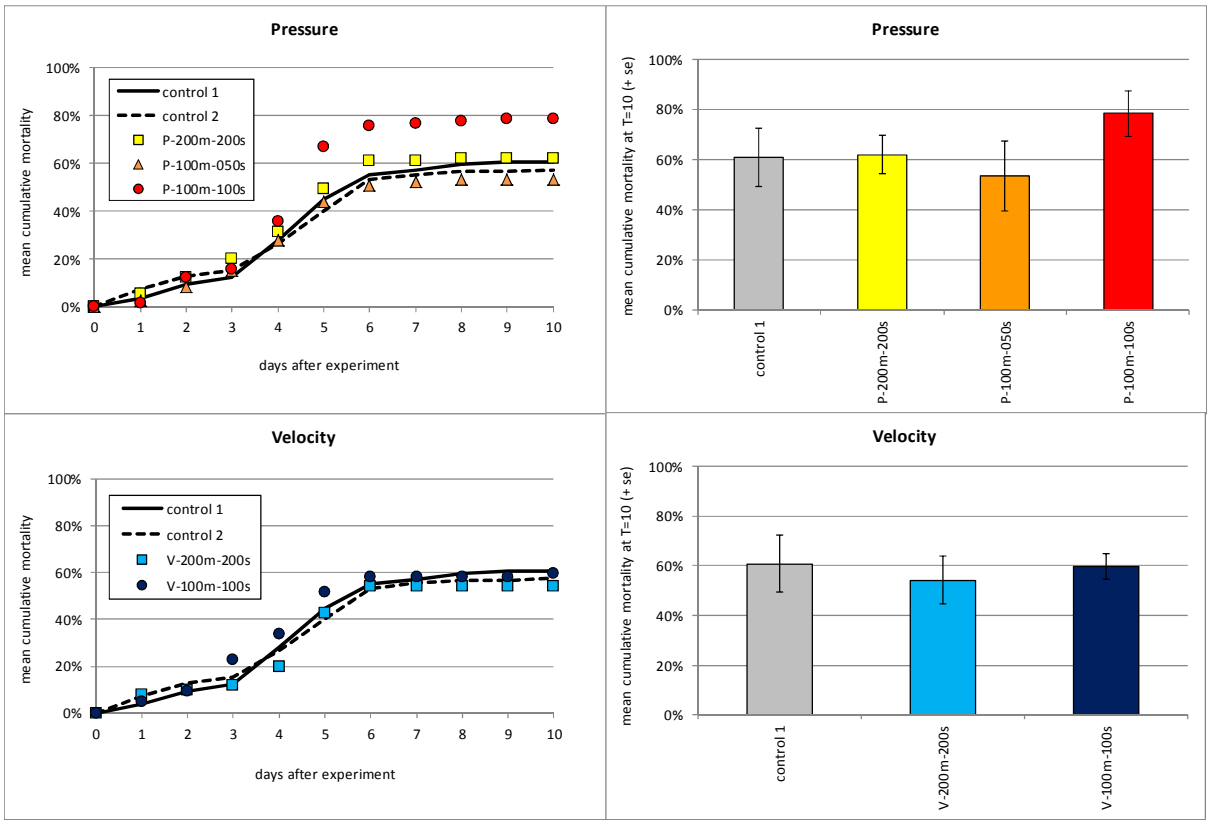

Figure 3. Trial 2 results. Left: mean cumulative mortality rate for each treatment 0-10 days after experiment. Right: mean cumulative mortality rates (± se) for each treatment 10 days after the experiment (95% confidence limit = 3.2*se at n=4).

4. Preliminary conclusions

This study has showed that it is possible to examine the effects of loud impulse sounds, such as pile driving noise, on the survival of fish larvae in the laboratory. Major advantage of laboratory experiments compared to field experiments is that variables (both sound parameters and co-variables such as static pressure) can be controlled, allowing investigation of the critical variables and processes causing mortality. Furthermore, insights obtained from laboratory experiments will facilitate future field experiments.

The pilot experiments so far are not conclusive on the threshold at which larval mortality occurs. The results indicate that exposure to sound causes mortality at 207 dB cumulative SEL (corresponding to the sound of 100 strokes at a distance of 100m from a 'typical' piling site). No effect is observed at a 3 dB lower exposure level (50 strokes at 100m). These findings are tentative. Additional experiments are required to prove the statistical significance. However, based on these results, the validity of the hypothesis of 100% mortality up to 1000m from the pile driving site (assumption Appropriate Assessment) appears to be unlikely.'

The indicative mortality threshold at 207 dB cumulative SEL is 24 dB (~ 250 times) higher than the interim criterion for injury to fish less than 2 grams from pile driving activities, as agreed by the US Caltrans Fisheries Hydro acoustic Working Group (Oestman et al. 2009). This discrepancy raises the question whether the results for sole larvae can be extrapolated to other fish species. Furthermore, additional experiments to determine dose-effect relationships taking into account relevant sound parameters (e.g. peak pressure versus cumulative SEL, signal shape) and co-variables (e.g. static pressure) are required. Finally, field experiments are necessary to confirm the results obtained in laboratory experiments.

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Justification

Rapport C176/10
Project Number: 4302501504

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

Approved: Tobias van Kooten
Researcher

Signature:

Date: 20 December 2010

Approved: Jakob Asjes
Head of Department

Signature:

Date: 20 December 2010

Appendix A. TNO memo 1

TNO Science and Industry

Memorandum

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From
Christ de Jong

Copy to
Pieter van Beek, Dick Kaptein, Erwin Jansen, Frans Staats, Frank van den Berg

Subject
The effect of piling noise on the survival of fish larvae - pilot experiments - memo-1: definition of acoustic signals and suggestions for experimental set-up

Date
2 September 2010

Our reference
MON-MEM-2010-02243

Direct dialling
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SUMMARY
This memo describes the first worked-out thoughts for the design of an experimental set-up to study the effect of piling noise on the survival of fish larvae. Several options for generating representative signals in a laboratory environment are evaluated. It is concluded that the most promising option is to develop an exposure chamber, driven by an underwater loudspeaker.

1 Introduction
This is the first memorandum in the preparation of pilot experiments for determining the effect of underwater noise due to pile driving on the survival of fish larvae. It addresses the definition of the acoustic signals that the larvae will be exposed to in an experimental set-up and discusses how representative of pile driving noise these signals can be made in an experimental set-up. This memo addresses several issues that were originally planned for the second memorandum, because of the strong connection between the definition of the acoustic signals and the design of the experimental set-up.

2 Background
A tentative conclusion of the study towards an appropriate assessment for the environmental impact of the offshore wind farms by Prins et al [1] was that pile driving may have a significant impact on the number of fish (plaice, sole and herring) larvae reaching Natura 2000 sites *Noordzeekustzone* and *Waddenzee*. Model calculations of the transport of eggs and larvae under influence of the impact of pile driving noise, assuming that mortality occurs up to 1000 m from a pile driving site, indicate that the number of fish reaching the Natura 2000 sites may decrease by 3 to 9%. The assumed mortality radius is not based on evidence. Actually, there is a large uncertainty about the vulnerability of fish eggs and larvae to piling noise (impulsive sound) and the spatial scale at which mortality or injury will occur [2].

To mitigate this important gap in the knowledge, a pilot study is proposed in the framework of the 'Masterplan short list' studies for the NL Ministry of Transport, Public Works and Water affairs. Further studies in this field were proposed in a ZKO project. The pilot studies are proposed to accelerate the knowledge development, to meet the time line driven by the offshore wind plans.

3 Objective

The objective of the proposed pilot study is to determine whether levels of underwater noise from piling activities can result in immediate mortality or injury to fish larvae (i.e. to lethal or sub-lethal effects). The piling noise should be representative at distances from 100 m to 2 km from the piling installation in an offshore environment.

4 Characterizing underwater noise due to pile driving

Piling noise in connection with the impact on marine life is usually quantified in terms of Sound Exposure Level (SEL in dB re 1 μPa^2s; per strike and/or cumulative) and peak sound pressure (value in µPa or level in dB re 1 μPa^2). Other possible measures (particle velocity, impulse, rise time, peak to peak sound pressure, kurtosis, etc.) are sometimes suggested, but the associated dose-response relations are even less clear than for SEL and peak pressure. Hence, other measures are not primarily considered, because the author is not aware of any references in which these are clearly related to effects.

> *Peak sound pressure* is here defined as the maximum absolute value of the unweighted instantaneous sound pressure in the measurement bandwidth. *Peak sound pressure level* is ten times the logarithm to the base 10 of the ratio of the square of the peak sound pressure to the square of the reference sound pressure of 1 µPa.
>
> *Sound Exposure* is defined as the time integral of the time-varying square of the unweighted instantaneous sound pressure in the measurement bandwidth over the duration of a single piling impact. *Cumulative Sound Exposure* is the sound exposure summed over multiple piling impacts. *Sound Exposure Level* (SEL) is ten times the logarithm to the base 10 of the ratio of the sound exposure to the reference sound exposure of 1 μPa^2s.

In 2008, the US Caltrans Fisheries Hydro-acoustic Working Group has issued an Agreement in Principal for Interim Criteria for Injury to Fish from Pile Driving Activities [3]. The agreed criteria identify maximum received peak sound pressure levels of 206 dB re 1 μPa^2 and 187 dB re 1 μPa^2s accumulated SEL for all listed fish except those that weigh less than 2 g, for which the threshold for the accumulated SEL is 183 dB re 1 μPa^2s. No frequency weighting is mentioned in relation with dose-response relationships for fish.

5 Available information of underwater noise due to pile driving

TNO has measured the underwater noise during the piling for the Q7 offshore wind farm [4,5]. At a distance of 1 km from the hammering of a 4 m diameter pile in about 20 m water depth with a sand bottom, the broadband SEL per stroke was about 172 dB re 1 μPa^2s and the zero-to-peak pressure level ('peak level') about 195 dB re 1 μPa^2.

The dominant noise occurred at frequencies between circa 50 Hz and 1 kHz. In UK measurements [6] at a distance of 57 m from a 2 m diameter pile the observed SEL was 178 dB re 1 μPa^2s and the peak level 208 dB re 1 μPa^2. Both measurements were carried out for piling with the same hydraulic hammer at approximately the same stroke energy. The sediment into which the pile was driven was different (Q7: sand; UK: chalk), as was the water depth (Q7: 20-23 m, UK: 10-15 m). Scaling of sound levels with pile diameter, stroke energy, water depth, sediment properties, etc. is currently unknown. This will be investigated under another Masterplan WIND short list study. However, a comparison was made between various measurements of pile driving noise in [7]. Table 4.2 (from the Errata with [7]) provides an overview of the measurement data, with a scaling to a distance of 500 m from the piling location. At a distance of 500 m, scaled values of SEL vary between 155 and 178 dB re 1 μPa^2s and peak levels vary between 180 and 200 dB re 1 μPa^2. Using the same scaling to estimate the levels at 100 m distance would lead to values that are about 10 dB ($=15\log_{10}(500/100)$) higher, i.e. SELs between 165 and 188 dB re 1 μPa^2s and peak levels between 190 and 210 dB re 1 μPa^2.

In a large survey of underwater noise due to pile driving in shallow water [3] levels were scaled to 10 m from the pile. Impact driving on steel piles (of diameter larger than 1 m) in these studies (Table I.2-1) led to SEL values between 180 and 195 dB re 1 μPa^2s and peak levels between 208 and 220 dB re 1 μPa^2. Scaling these to 100 m distance, assuming a worst case scenario with a cylindrical spreading loss (10logR-scaling) leads to estimated SELs between 170 and 185 dB re 1 μPa^2s and peak levels between 198 and 210 dB re 1 μPa^2. These are close to the estimations based on the North Sea piling noise measurements.

For piling noise impulses, the difference between the numerical values of the peak pressure level and SEL is in the order of 20 to 25 dB, where the higher differences (shorter pulses) occur at positions closer to the pile. Each simulated pile driving signal should exhibit a similar level difference to be representative. The difference of peak pressure level and SEL has the dimension of dB re 1 s^{-1}. It is related to signal duration. The larger this difference, the shorter the signal, hence it is a measure of the 'impulsiveness' of the signals.

Particle velocity

Measurement data of particle velocity due to pile driving is very scarce. Some data can be found in [8]. This concerns impact driving of 76 cm diameter, 2.4 m long steel piles in a water depth of 10 m. At 10 m distance (and 5 m depth) the average peak pressure level was found to be 204 dB re 1 μPa^2 and the SEL[1] 178 dB re 1 μPa^2s. The measured peak velocity level was 141 dB re 1 $(nm/s)^2$ and the 90% RMS velocity level was 129 dB re 1 $(nm/s)^2$. At larger distances, the acoustic particle velocity and acoustic pressure levels are approximately related through the characteristic impedance of the medium, i.e. the velocity level in dB re 1 $(nm/s)^2$ equals the pressure level in dB re 1 μPa^2 minus $20\log_{10}\{\rho c \cdot (10^6/10^9)\} \approx 64$ dB. This includes a correction for the factor that accounts for the different reference units.

[1] This SEL is derived from the 90% RMS SPL plus $10\log_{10}(T_{90}$ signal duration), both provided in the report. The SEL value given in the report seems to be 6 dB too high.

Hence the measured peak pressure would correspond with a free field peak velocity of 141 dB re 1 $(nm/s)^2$ and the rms pressure corresponds with rms velocity level 129 dB. These are close to the measured values, which means that use of the free-field relationship does not result in large errors in this case.

6 Requirements for simulated piling noise levels.

Based on the overview in the previous section, signals representative of pile driving noise at distances from 100 m to 2 km from the piling installation, have broadband peak pressure levels up to about 210 dB re 1 μPa^2 (i.e. 32 kPa) and broadband single impulse SEL up to 188 dB re 1 μPa^2s. Assuming that the broadband propagation loss varies with circa 15log(distance), the corresponding levels at 2 km distance are about 20 dB lower (i.e. SEL 168 dB re 1 μPa^2s and peak level 190 dB re 1 μPa^2). The corresponding broadband peak particle velocity levels should be between 127 and 147 dB re 1 $(nm/s)^2$ and the broadband integrated velocity exposure levels between 104 and 124 dB re 1 $(nm/s)^2s$.

7 Requirements for simulated piling noise spectra.

Some typical piling underwater noise SEL spectra are given in Figure 1, see the properties in Table 1. The spectra of the noise measured at the Q7 site are similar. This shows that the main (unweighted) energy is generated in the 50 Hz to 1 kHz bands.

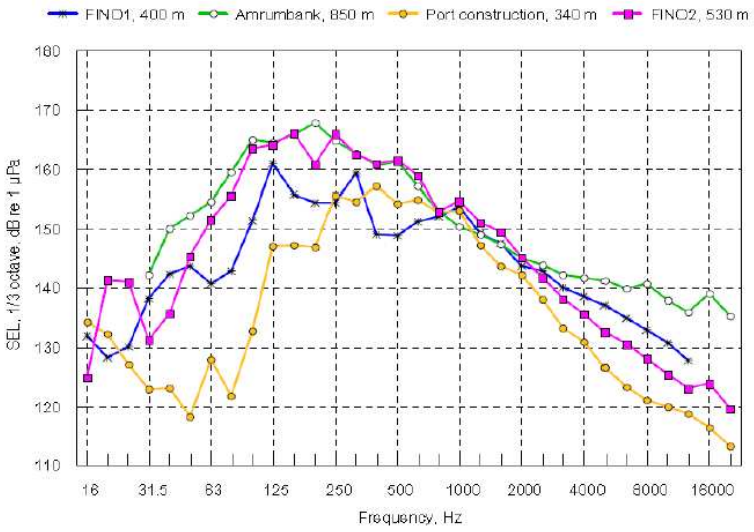

Figure 1 Third-octave band spectra of the single stroke SEL of some of the pile-driving operations, from Nehls *et al.* (2007), see also Table 1.

Table 1 Summary of measurement results for different pile driving operations, from [7]. The 'normalized' levels are scaled to a distance of 500 m in 20 m water depth.

Project	Pile diameter [m]	Water depth [m]	Measuring depth [m]	Measuring Distance [m]	Blow energy [kJ]	Peak Level [dB re 1 µPa^2]	SEL [dB re 1 µPa^{2}s]	Normalized Peak Level [dB re 1 µPa^2]	Normalized SEL [dB re 1 µPa^{2}s]
Jade port construction, Germany, 2005	1.0	11	5	340	70-200	190	164	186	160
FINO 1, Germany, 2001	1.6	30	10	750	80-200	192	162	196	166
FINO 2, Germany, 2006	3.3	24	5	530	300	190	170	191	171
Amrunbank West, Germany, 2005	3.5	23	10	850	550	196	174	200	178
Test Pile, UK, 2006	2.0	8-15	?	57	800	208	178	193	163
Test Pile, UK, 2006	2.0	8-15	4-7	1850	800	188	164	195	171
Q7 site, NL, 2006	4.0	20-25	8-15	890-1200	800	195	172	200	177

A closer investigation of wave form an spectral content for a typical piling stroke signal confirms that it is sufficient to reproduce the piling noise is the frequency range between 50 Hz and 1 kHz. This analysis is done for piling stroke signals, recorded at the North Sea site (depth about 20 m) at a distance of 100 m from the pile. Figure 2 shows the recorded wave form and the resulting wave form after applying a cosine-tapered (Tukey) band-pass filter (1050 points, with 50 Hz taper to zero).

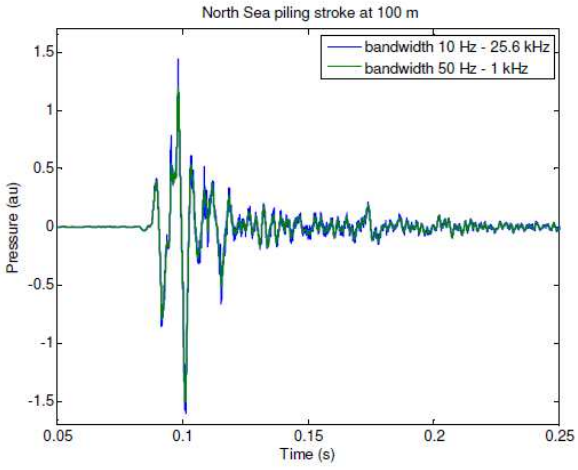

Figure 2 Underwater noise signal for a single piling noise stroke, recorded at the North Sea site at a distance of 100 m from the pile, for two different bandwidths. The amplitude scale in not calibrated ('au'='arbitrary unit').

It can be seen that the waveform is not significantly affected by the filtering. The resulting SEL and peak levels for the two different bandwidths differ less than 1 dB. Note that the peak level is determined by the negative peak just after 0.1 s.

Hence the simulated piling noise signals in the proposed study should fulfil the following criteria to be representative:

1. Broadband peak sound pressure level between 190 and 210 dB re 1 μPa^2
2. Broadband SEL value per pulse at least 22 dB below the Peak Level value (i.e. SEL between 168 and 188 dB re 1 μPa^2s)
3. Broadband peak particle velocity level between 127 and 147 dB re 1 $(nm/s)^2$
4. Broadband integrated velocity exposure levels between 104 and 124 dB re 1 $(nm/s)^2s$
5. Main energy between 50 Hz and 1 kHz

The difference between the peak level and SEL accounts for the impulsiveness of the signals. Note that the lower frequency of 50 Hz is probably connected with the cut-off frequency for shallow water sound propagation. For piling in deeper water the lowest frequency of interest may be lower.

8 Definition of acoustic signals

The criteria that are described in the previous section can be fulfilled by various acoustic signals. Since the actual underwater sound due to pile driving will vary for different piling activities in different environments and also between different piling strokes and at different measurement locations relative to the pile, it is considered sufficient, for the proposed exposure tests, to select specific representative acoustics signals, which fulfil the above criteria. These signals can be actual recordings of piling noise or synthesized or mechanically generated impulsive signals. Actual recordings have the benefit that the signals also represent signal characteristics that are not covered by the proposed criteria. The options for generating signals are considered in the following sections, in connection with proposals for the experimental set-up.

Each trial of the proposed exposure study will consist of 4 sound exposures and 1 control group. These 5 treatments will be repeated during a 2nd and 3rd trial. The first trial will be used to crudely examine the sensitivity range of larvae to various acoustic parameters. The results of the first trial will be used to focus on relevant parameters during the second trial. Each batch of 50 larvae will be exposed only once, so a trial consists of a single acoustic exposure.

Signals representative of pile driving noise at distances of 100 m and 2 km from the piling installation differ about 20 dB in level. It is proposed to carry out the first trial at the highest level and to select the levels for the following trials on the basis of the initially observed effects on the larvae. If mortality is observed, the next trial could be carried out at e.g. a 10 dB lower level. The selection of the four test signals is still open for discussion with IMARES.

9 Options for pilot experiments in a laboratory setting

Due to budget and time limitations, which prohibits full scale experiments during actual offshore piling activities, it was decided to execute pilot exposure experiments in which fish larvae are exposed to underwater acoustic signals that are representative for piling noise, in a laboratory setting. Three options are considered:

1. experiments in a water tank or basin
2. experiments in a pipe wave guide
3. experiments in a compact chamber

Option 1 is based on the experience obtained at SEAMARCO with behavioural response studies with harbour porpoises, harbour seals and fish in the SEMARCO facilities in Wilhelminadorp.

Option 2 is based on publications from Mardy Hastings and colleagues [9], who developed a pipe test arrangement to expose fish to sound.

Option 3 is based on publications by Lewis et al [10], who developed a so-called 'fishabrator' sound exposure chamber for assessing the effects of high-intensity sound on fish.

Unfortunately, we have found just one publication [9] in which the test pipe was used to study exposure effects and no publications of studies carried out with the 'fishabrator'. The authors have not (yet?) responded on questions posed via email.

10 Option 1: Experiments in a water tank or basin

In a tank, the sound field is influenced by reflections at the walls and at the water surface [11]. At the lowest frequencies (determined by the smallest dimension of the tank and the acoustic wavelength in water), sound propagation away from the source is strongly attenuated. At intermediate frequencies the sound field is characterized by resonances in the tank and at higher frequencies, the resonance frequencies are so closely spaced that the reverberant sound field in the tank becomes homogeneous, with the direct field of the source, subject to spherical spreading, superimposed on it. To avoid excessive 'colouring' of the sound by resonant modes, the minimum size of the tank should be larger than the acoustic wavelength at the lowest frequency of interest. For piling noise at frequencies larger than 50 Hz, the minimum size should be larger than 30 m. In shallower tanks, the low frequency components of the piling noise decrease exponentially with distance.

For experiments in a tank, the Lubell LL1424HP projector (recently acquired by SEAMARCO) is the most powerful loudspeaker that could be made readily available. It operates in the range between 200 Hz and 9 kHz, with a maximum rms output of 197 dB re 1 μPa^2m^2 at a single narrowband frequency near 600 Hz (172 dB @ 200 Hz, 190 dB @ 1 kHz). This does not give direct information about the achievable peak and SEL levels. However, with the smaller Lubell 916 (max output 180 dB re 1 μPa^2m^2 at 1 kHz) we have been able to produce impulsive signals with peak level 177 dB re 1 μPa^2 and SEL 145 dB re 1 μPa^2s at a distance of 1 m from the projector.

This suggests that the maximum achievable levels for the LL1424HP are about 17 dB higher: 194 dB re 1 μPa^2 and SEL 162 dB re 1 μPa^2s. These are bout 16 dB too low compared with the requirements for the fish larvae experiments.

It can be concluded that experiments in a tank are not appropriate for studies of mortality of fish larvae due to piling noise.

11 Option 2: Experiments in a water filled pipe

In a pipe, the sound field is one-dimensional and plane sound waves propagate along the pipe axis without losses due to spatial spreading. To avoid propagating higher-order acoustic modes in the pipe, the diameter of the pipe should be smaller than 0.586 times the acoustic wavelength in water [12]. For frequencies up to 1 kHz, this condition is met for diameters smaller than 0.88 m.

Samples of 50 larvae are to be kept in a compact volume of about 1 litre of water. Assuming that this volume should be contained by a cylindrical tube over a length approximately equal to the diameter, the internal diameter should be at least 0.11 m. Of course, the test section in which the larvae are kept could be bigger than the pipe diameter, but this will introduce additional reflections that are better avoided.

The pipe could be made of e.g. (transparent) PolyMethyl MethAcrylate (PMMA; alos know as 'Plexiglas' or 'Perspex'). Such a test arrangement has been used by Hastings [9] to expose fish to sound, see FIG.1 below. In their setup the Plexiglas pipe had an inner diameter of 0.12 m and a length of 15 m.

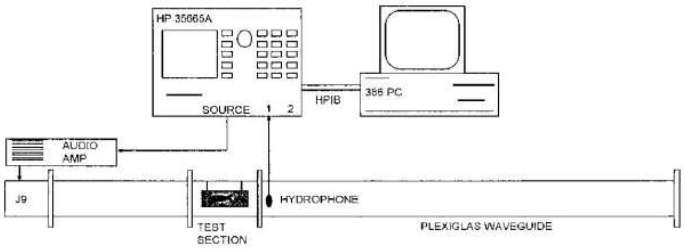

FIG. 1. Schematic diagram of the experimental setup showing the 15-m-long Plexiglas® waveguide, the removable section of the waveguide in which the fish was located, and the position of the J-9 projector that was used as the sound source.

Advantages of using PMMA are the possibility to observe the larvae, relatively easy machining and available components and shorter wavelengths and higher damping than e.g. steel, which reduces the effects of resonances in the pipe, as explained in the following paragraphs.

In a pipe, the sound field may be influenced by reflections at the pipe ends. These can lead to standing waves in which sound pressure and particle velocity are strongly position dependent.

Effects of reflections at the far end may be reduced by reducing the reflection coefficient (e.g. by shaping the pipe end into a horn, or by applying sound absorbing constructions/materials at the pipe end) and by increasing the pipe length, so that the reflected waves are attenuated by the losses in the pipe wall.

The attenuation depends on the wavelength and the loss factor for the plane waves in the pipe. The wave speed for plane waves in a flexible pipe is lower than the sound speed in unbounded water [12]. Taking for the PMMA a modulus of elasticity of 3200 MPa and a mass density of 1200 kg/m^3, the plane wave speed (modified by the flexibility of the wall) in a pipe of 120 mm internal diameter and 5 mm wall thickness is about 355 m/s. In the setup of Hastings [9], reflections were significant at 60 Hz in a 15 m long Plexiglas pipe. The measurement results show that the plane wave attenuation was about 2 dB/m at 300 Hz (i.e. absorption coefficient ~0.043).

For the current experiments, the setup should consist of a pipe of at least 15 m length. (Note: A greater pipe length, or horn-shaped end, connected to a water tank would be beneficial.) This would create a well defined acoustic environment in which the particle velocity (in the frequency range between 50 Hz and 1 kHz) can be estimated from sound pressure measurements (e.g. using 3 hydrophones, B&K8103, or pressure transducers, available at TNO).

Note, that the ratio between sound pressure and particle velocity in this Plexiglas pipe differs from that in plane waves the sea. At the same sound pressure, the plane wave particle velocity in the pipe will be about 5 times greater than in unbounded water, because the wave speed is about 5 times lower.

The particle velocity at the location of the larvae can be determined by means of the 'two microphone method' [12]. This uses the signals of two pressure measurements at an axial distance d. It can be used in range $0.08\pi \leq kd \leq 0.8\pi$, which spans a decade of frequencies for a fixed distance d. For the range of 100 Hz to 1 kHz, with a wave speed of 355 m/s, the distance d should be 14 cm, or 28 cm for the range from 50 Hz to 500 Hz. Hence the frequency range between 50 Hz and 5 kHz can be covered by three pressure sensors at 14 cm distance.

Note that the above analysis is based on preliminary estimations of the material properties of the PMMA. It is recommended to obtain a more accurate estimation of these properties before the final design of the set-up (pipe lengths, transducer positions, etc.)

Excitation by an underwater sound projector

In the pipe experiment, Hastings [9] used a USRD J9 sound projector. The maximum produced SPL was about 180 dB re 1 µPa^2 at 300 Hz, which is too low for our purpose. It is not clear whether they could have generated higher pressures in their setup.

TNO has a somewhat bigger USRD J11 sound projector available, which could be used in such a setup. It operates in the frequency range between 20 Hz and 12 kHz.

The maximum free field source level (SL) of this source (between 50 Hz and 1 kHz) is about 150 dB re 1 μPa^2m^2. To estimate the maximum output when driving the fluid in a pipe, we assume that the radiation impedance that is experienced by the piston of the J11 remains approximately the same in both cases. That means that the volume velocity produced by the J11 remains the same. The volume velocity Q can be estimated from the free field source level: $Q = 4\pi 10^{(SL-120)/20}/\rho c \approx 265000\ \mu m^3/s$. If the piston drives a long PMMA pipe with a diameter of 15 cm with this volume velocity, the corresponding plane wave rms pressure is about 22.5 kPa, i.e. 207 dB re 1 μPa^2. Since the peak levels are at least 3 dB higher, this suggests that it should be in principle possible to use the J11 to generate the required levels in the pipe, in the required frequency range.

The advantage of using a sound projector is that one has control over the signals. On could synthesize arbitrary signals or send out actually recorded sounds of piling strokes.

Excitation by an impact hammer

Alternatively, one could consider the use of an impact hammer to drive the fluid in the pipe. If the pipe would be driven via a rigid piston at one end, of 160 mm diameter, this would require a peak force of 643 N to generate a peak pressure level of 210 dB re 1 μPa^2 (i.e. 32 kPa). In a plane wave, the SEL corresponds with an acoustic energy $E = (A/\rho c)\cdot 10^{(SEL-120)/10}$, where A is the cross-sectional area of the fluid in the pipe and ρc is the characteristic impedance of the water in the pipe. Hence, a SEL of 190 dB re 1 μPa^2s in a PMMA pipe of 15 cm diameter filled with water equals about 0.55 J. The 20 dB lower level (for the pulse at 2 km from the pile) equals about 5.5 mJ.

Consider a rigid mass m with a velocity v_0 that impacts at time t=0 on the end plate of a semi-infinite fluid-filled pipe infinitely long elastic rod. The input mobility for the pipe wall, with area A_s, Young's modulus E_s and density ρ_s equals $Y_s = 1/A_s\sqrt{E_s\rho_s}$, the mobility of the fluid column within the pipe equals $Y_f = 1/A\rho c$. If the pipe is driven via a rigid end plate, the combined mobility equals $Y = 1/(1/Y_s + 1/Y_f)$. The driving force during impact depends on the details of the contact. The velocity of the end plate on impact follows from conservation of momentum. The required mass and impact velocity can be estimated for a fully elastic collision, estimating the momentum (I) of the pipe from the mass associated with a half wavelength in fluid and wall associated with the impact duration. The energy transmitted during the impact time t_I equals about $E \approx I^2 Y/\pi t_I$. The impact time depends on the contact area and contact stiffness. This can be influenced by the choice of 'hammer' shape and material.

This leads to the initial estimation that the required energy and peak pressure can be generated by dropping a mass[2] of 1 kg from a height of 0.5 m, provided that the impact time can be limited to 1 ms. This seems feasible.

[2] See the spreadsheet fishpipe.xls for details.

Using a hammer leads to a single short impulse that travels down the pipe. Although this impulse signal can fulfil the criteria that are described in §4, it deviates from actual offshore pile driving noise, which contains several compression and rarefaction peaks due to reflections in the pile and at water surface and bottom. In a 15 m long PMMA pipe (wave speed 355 m/s), the first reflections arrive after about 94 ms. More realistic times between reflections (in the order of ms) could be achieved in a much shorter pipe. But it will be very difficult to design that pipe and the pipe end in such a way that the reflections are representative for actual piling noise signals.

12 Option 3: Experiments in a compact chamber

A water volume that is small compared with the acoustic wavelength does not support acoustic waves, but behaves uniformly as a mass or stiffness, dependent on the boundary conditions. These uniform conditions are exploited in the 'fishabrator', see Figure 3.

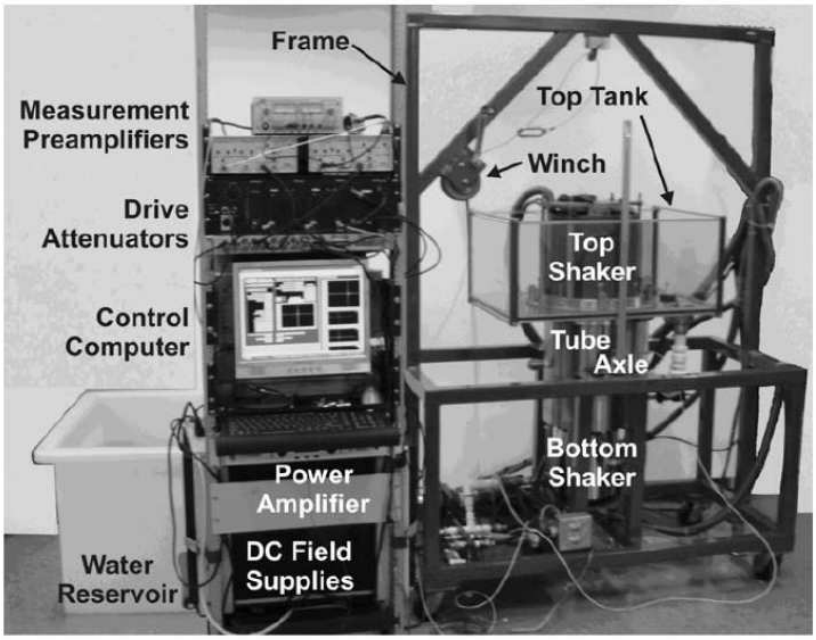

Figure 3 The 'fishabrator' at the George W. Woodruff School of Mechanical Engineering, Geogia Institute of Technology.

At a maximum frequency of 1 kHz, the acoustic wavelength in water is about 1.5 m, which means that a chamber with a maximum dimension smaller than 25 cm is smaller than $1/6^{th}$ of a wavelength and hence behaves uniformly.

In such a chamber, effects of pressure and particle velocity can be tested independently: By driving a rigidly enclosed chamber, the pressure is raised with negligible particle velocity, while by driving a semi-open chamber, the velocity is raised at negligible increase of pressure.

In the 'fishabrator', the control of the ratio between sound pressure and velocity is further enhanced by supplying two controlled exciters. For the purpose of the proposed study with fish larvae this is not necessary, which simplifies the design of the setup.

Figure 4 shows the geometry of the J11 projector[3]. It is proposed to design a cylindrical chamber that fits tight to the ring that surrounds the driving piston. The inner diameter of that chamber is then about 15 cm. With the height of the water column 10 cm, the water volume is about 1.8 litres, which should be large enough for the batches of larvae that we want to study.

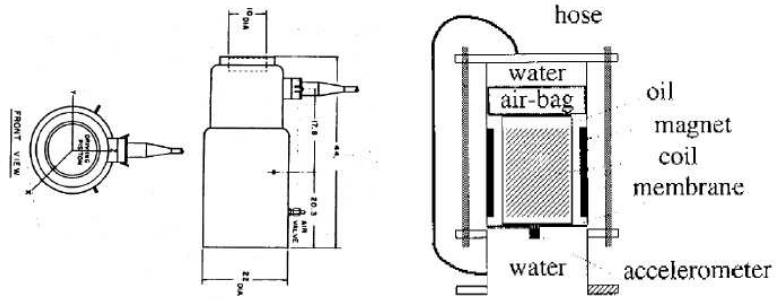

Figure 4 Sketch of the USRD J11 projector (left, dimensions in cm) and of a similar set-up (right) in which the J11 source was used to excite fluid pulsations in a pipe (from [12]).

Two configurations can be used:

1. **Pressure excitation**, with the chamber closed by a 'rigid' lid on top. Note that it is very important to avoid enclosing air bubbles in the chamber, because these have a large impact on the compressibility of the fluid. Only the larvae are allowed to influence that compressibility.
2. **Velocity excitation**, with the chamber open on top (or closed by a very flexible membrane, to keep the fluid and the larvae inside).

In configuration 1, chamber and lid should be tight and rigidly connected to the projector housing. The axial and circumferential stiffness of the chamber should be larger than the effective stiffness of the fluid volume. This can be achieved by a steel chamber with walls of (at least) 5 mm thickness.

The acoustic pressure can be measured by pressure transducers, mounted flush in the wall, half way the chamber. The particle velocity can be measure by a (watertight) accelerometer mounted on the surface of the piston of the projector.

Figure 5 shows the predicted response in the chamber for the two excitation types.

[3] As an alternative, TNO also can use the Actran LFPX projector, which can produce 10-12 dB higher levels than the J11, in the same frequency range.

For frequencies up to ca. 1.5 kHz, the calculated velocity response exhibits mass-behaviour ($v/p \propto 1/f$) with the lid open and stiffness-behaviour ($v/p \propto f$) with the lid closed. The set-up shows a ¼-wavelength fluid resonance near 3500 Hz. Note that these calculations assume a rigid connection between the chamber and the projector casing. This requires special attention, because any reduced stiffness in this connection may cause the resonance to shift down into the frequency range of interest (50 Hz-1 kHz).

With an open end, the acoustic velocity decreases with frequency, due to the inertia of the water mass. In order to obtain a realistic velocity pulse, the driving signal has to be equalized to correct for this effect. The required velocity level is -64 dB re 1 $(\text{nm/s/}\mu\text{Pa})^2$. The corresponding pressures are much lower than those for the closed chamber.

The predicted pressure response of the closed chamber is flat for frequencies up to ca. 1.5 kHz.

Note that the actual response of the projector and chamber will have to be determined experimentally. Additional resonances in the response may possibly be compensated by an appropriate matched filtering of the driving signal.

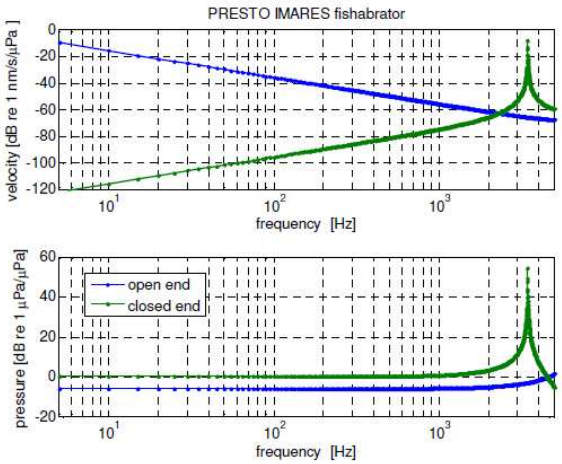

Figure 5 ***PRESTO*** **[12] simulation for the proposed exposure chamber (inner diameter 15 cm, wall thickness 1 cm, inner height 10 cm, lid thickness 1 cm, steel cylinder). The pulsations are generated by a 10 cm diameter piston at the lower end. The chamber wall is clamped at the lower end. Two configurations: with lid ('closed end', i.e. 'pressure excitation') and without lid ('open end', i.e. 'velocity excitation'). The calculated particle velocity (top) and pressure (bottom) at the mid plane of the chamber per unit pressure at the piston.**

13 Options 2 and 3 compared

In summary, both the pipe (option 2) and the chamber (option 3) can be used for the proposed exposure tests. Both can make use of an existing underwater loudspeaker to generate the required acoustic signals, by playing back actual recorded or synthesized sounds.

The chamber has the advantage of a compact and relatively simple set-up, which needs less space for mounting than the tube. Moreover, the sound exposure in the chamber is well defined: either uniform acoustic pressure or uniform acoustic velocity, that can be measured directly. It cannot represent the actual combination of pressure and velocity excitation that is experienced at sea during piling, but it can be argued that the physical effects that may lead to damage are more or less independent for larvae (which are much smaller than the acoustic wavelengths to which they are exposed): pressure fluctuations may lead to damage due to compression and decompression, while damage due to velocity fluctuations is mainly related with inertial effects (e.g. of the otolith organs).

The tube has the advantage that it provides a plane wave exposure, provided that the tube design avoids significant reflections at the end of the tube. However, the characteristic impedance in a PMMA tube differs by a factor of five from that in free water. In the tube, the particle velocity is measured indirectly: derived from measurements of the gradient of the acoustic pressure. Another disadvantage of the tube is that it is more difficult to design a facility for placing the larvae in the tube, without influencing the acoustic field with entrained air bubbles.

14 Conclusion

It seems possible to execute the pilot experiments in a laboratory setting. Three different options are worked out. The last option (the 'larvaebrator' exposure chamber) seems the most attractive. It provides a compact and simple setup with a possibility to test the response of the larvae to pressure and velocity signals independently. This independent testing requires a doubling of the amount of trials at a given level of exposure. This should be taken into account in the development of the test plans.

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Appendix B. TNO memo 2

TNO Science and Industry

Memorandum

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René Dekeling (Ministerie V&W)

From
Pieter van Beek

Copy to
Christ de Jong, Frank van den Berg, Dick Kaptein, Erwin Jansen, Frans Staats

Subject
The effect of piling noise on the survival of fish larvae - pilot experiments - memo-2: design of experimental test setup

Date
2 September 2010

Our reference
MON-MEM-2010-02151

Direct dialling
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1 Introduction

This is the second memorandum in the preparation of pilot experiments for determining the effect of underwater noise due to pile driving on the survival of fish larvae. It describes the practical design of the experimental test setup for testing the response of fish larvae to artificial piling noise. The concept of the setup is based on the first memo of this project [1], in which also already the final objective and background of the total project are described.
In [1] 3 options for a laboratory experiment setup were considered. It was concluded that option 3, the so-called 'larvaebrator', would be most promising: it is the most practical setup and it has the possibility to expose the larvae to pressure and velocity signals independently. During a progress meeting with IMARES on August the 3rd 2010 the recommendation for the concept of the 'larvaebrator' as experimental setup was adopted and approved by the project team [2].

The complete design phase of the experimental setup has been divided into the following items:
- Concept description,
- definition of requirement,
- technical/detailed design,
- fabrication/assembly,
- performance validation.

The latter 2 items will be covered in memo 3 of the project 'testing of the tube'.

2 Concept description

The general 'larvaebrator' design concept consists of a projector (underwater sound source) on which a compact chamber is placed. The chamber is filled with sea water and the larvae. The piston of the projector is also the bottom of the chamber and can directly excite the water with a given signal. Depending on the required boundary conditions, i.e. constant pressure or constant velocity, the top cover of the chamber can be closed (constant pressure) or released (constant velocity). Additionally, tests can be carried out with an increased pressure in the chamber, to simulate the effect of different depths in the water.

The constant velocity excitation will in that case be achieved with the cover closed, but with a small layer of pressurized air between the water surface and the cover.

3 Requirements

The experimental setup must fulfil a variety of requirements; from strict constraints to environmental conditions. Moreover there are 2 types of tests, i.e. with a pressure and with a velocity source signal, for which the setup requirements can differ.

Base requirement

The base requirement of the 'larvaebrator' is that it should be able to expose the larvae to a simulated piling noise signal, as representative as possible. Requirements regarding the pressure and velocity of the simulated noise signal itself are already given in [1]. Piling noise is a high pressure and/or high velocity transient pulse, which has consequences for the noise source i.e. projector, the housing i.e. larvae chamber and the sensors.

Projector

The projector should have enough power to reproduce the relevant characteristics of piling stroke noise as could be measured at distances between 100 and 2000 m from a pile in 20-25 m water depth at the North Sea, as explained [1]:

- It should be able to produce a broadband (50 – 1000 Hz) peak sound pressure level (SPL) between 190 – 210 dB re 1 μPa^2.
- The projector should also be able to produce a broadband (50 – 1000 Hz) peak particle velocity level between 127 – 147 dB re 1 $(nm/s)^2$.

In [1] it is demonstrated that 'broadband' in this case means that for a frequency range between 50 – 1000 Hz the waveform is hardly affected by the discarding lower and higher frequencies regarding both SEL and peak level. Therefore the requirements for the setup are limited to this frequency range.

Sensors

- Both pressure and velocity (or acceleration as measured in practice) have to be recorded during the measurements.
- The maximum acoustic peak pressure is about $3x10^4$ Pa.
- The maximum peak acceleration is about 140 m/s^2 at 1 kHz.
- For both velocity and pressure the required dynamic range is about 70 – 80 dB.
- The velocity sensor(s) and cable(s) have to be fully submergible for a longer period.
- The mass of the velocity sensor should be small compared to the mass of the projector piston.

Housing

- To avoid air bubbles sticking at the wall, the internal surface of the housing should be as smooth as possible as can be achieved with conventional mechanical (milling, turning, etc.) tooling: between 0.1 and 1 µm.
- The housing also has to contain an air bleeding system to release all the remaining air (bubbles).

- To check whether all the air is released and to observe the larvae during the experiments with the pressure excitation, the top cover has to be made from transparent material.
- The housing will be filled with salt sea water and therefore has to be made from rust-resistant (stainless) material(s). Stainless steel is allowed, whereas cupper/brass, etc. are not allowed.
- The 1st mechanical resonance frequency of each part of the housing has to be higher than the maximum frequency of interest, i.e. 1000 Hz.
- The volume of the housing should be between 1.5 and 2 L, with an extra requirement that the largest dimension of the volume should be less than 1/6th of the smallest acoustic wavelength in water, which is about 250 mm at 1000 Hz.
- The mechanical stiffness of the complete setup has to be at least 10 times higher than the equivalent 'stiffness' of the water volume/column inside.
- After each exposure the batch of larvae in the chamber has to be replaced. Therefore the top cover should be easy and quick to open and close.
- It should be possible to mount pressure sensors in the housing wall.
- The housing should contain a water and pressure tight cable transit for the velocity sensor i.e. accelerometer.
- It should be possible to pressurize the fluid in the chamberwith static pressures up to a maximum of 3 bar (representative of wate depths up to 30 m).

Other

- To avoid any influence on the experiments from the environment, the complete setup has to be acoustically decoupled from its surroundings, which means that the set-up will be installed on rubber mounts or on a rubber plate.
- For both the pressure and velocity experiment the larvae have to be exposed to a prescribed, simulated piling signal.
  The transfer function of the setup (projector, housing and water volume) will influence the signal. Therefore the source driving signal has to be corrected and filtered for this transfer function and other external disturbances, in order to retain the right piling signal in the water volume (covered in memo 3).
- The performance of the total assembled test setup has to be verified at TNO (covered in memo 3).

4 Technical design

Just like the requirements, the detailed, technical design will be split up in projector, sensor and housing part.

Projector

In [1] the USRD J11 projector is described as a possible noise source for the experiments. However, looking at the required peak pressure and velocity, it is doubted whether this projector will be able to fulfil these requirements. Therefore, another projector with more power is chosen: the USRD LFXP–4, which is also available at TNO. This projector can supply up to about 10 dB more acoustic power and should be able to fulfil the requirements. The global dimensions (in mm) of the projector are given in figure 1 and a photo is shown in figure 2. This projector can be driven by a Crown PSA-2 power amplifier, which is also available at TNO. This power amplifier can be fed with standard 230V mains voltage.

At TNO the LFXP–4 projector normally is operated in an underwater noise source set consisting of 4 equal projectors. Therefore no suitable connection cable is available and a new one has to be made.

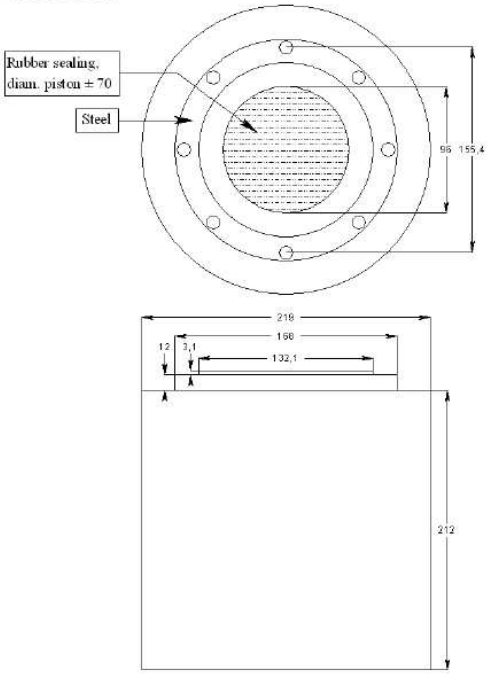

Figure 1 LFPX–4 Projector: top and side view, with global dimensions in mm.

Figure 2 LFPX–4 Projector.

Sensors

For the pressure measurements the PCB 116A02 high sensitivity, dynamic pressure transducers will be used. They have the following specifications:

- measurement range 1000 psi (≈ $7x10^6$ Pa),
- maximum pressure 5000 psi (≈ $3.5x10^7$ Pa),
- resolution 0.002 psi (≈ 14 Pa),
- nominal sensitivity 8 pC/psi (≈ $1x10^{-3}$ pC/Pa),
- rise time 5.0 µs
- resonant frequency 125 kHz

Via a charge amplifier the signal will be amplified and converted to voltage, which can be recorded with the B&K PULSE frequency analyzer.

To verify whether the pressure is about equal everywhere, 4 transducers will be installed equally spaced in the circumference of the housing wall. Via special adapters they can be 'flush mounted' in the side wall. The screw thread will be included in the housing design.

The velocity sensor, i.e. accelerometer in this case, will be rigidly glued on top of the rubber sealing of the moving projector piston. An Endevco 50 piezoelectric accelerometer will be used:

- Measurement range ± 40 g (≈ 400 m/s^2)
- resolution ≤ 0.001 g (rms)
- Nominal sensitivity 50 mV/g (≈ 5 $mV/(m/s^2)$)
- resonance frequency 10 kHz
- weight 3.8 gr

This transducer has an integrated amplifier that can be fed directly by the B&K PULSE analyzer. The sensor itself is hermetically sealed and thus water tight. It has a 3m long, already attached signal cable. However, the connection to the sensor itself was not water tight. It is made water tight with a special resin and the performance of the sensor is tested in a purpose-made experimental setup, as shown in the figure below. The accelerometer is placed in a cup on a shaker and the frequency response (FRF) was checked for 2 cases: the cup without water and filled with water.

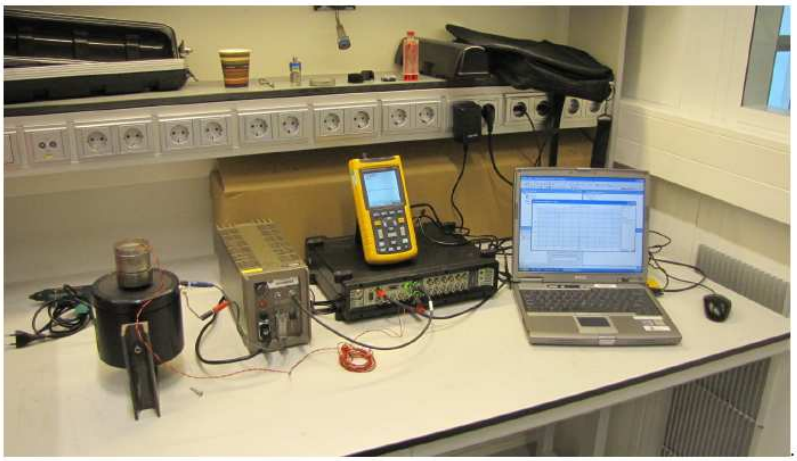

Figure 3 Experimental setup for testing water-resistant accelerometer.

In the larvae chamber/housing a 2.5 mm cable transit will be made. After installation of the signal cable, the remaining opening in the transit will be sealed with high resistive glue/resin. Finally a screwable connector will used to connect the transducer cable to the analyzer.

Housing

The housing consists of a cylindrical side wall and a top cover. As can be seen in figure 1 and 2 the projector has a 3mm thick steel ring that clamps the rubber sealing. This ring is used to centre the side wall above the projector piston. A 160 mm high and 28 mm thick tube, with a 110 mm inner diameter, will be placed on top of this ring; this will serve as the chamber side wall. The tube is made from stainless steel type 316, which is very suitable for salt water applications. The stiffness of the wall is in the order of magnitude of $1x10^{10}$ N/m in longitudinal direction and $1x10^{11}$ N/m in radial direction

The tube will be mounted on the projector with special studs (projector has UNC thread) and nuts, for which a cut-away (mounting hole) is made in the tube. In figure 1 the radius of the 8 holes in the projector was already given (155.4 mm). Special gasket material will placed underneath the tube for final sealing. A 3D sketch of the tube is given in figure 4, where also some specific positions for the transducers etc. are indicated.

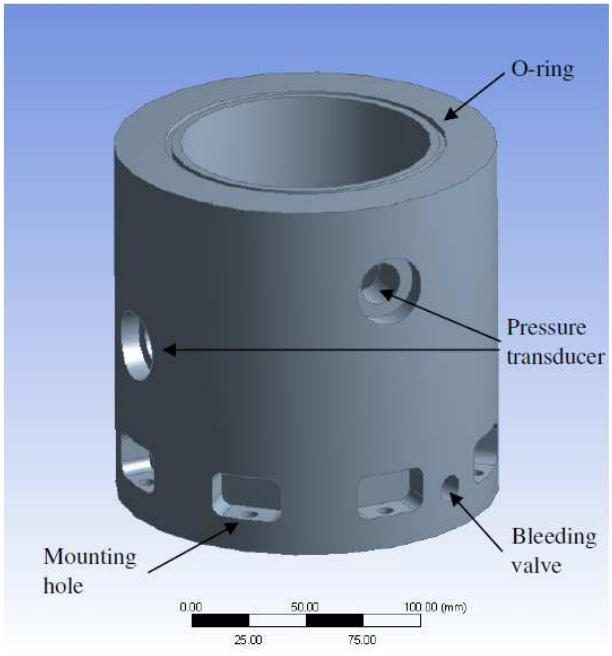

Figure 4 3D sketch of the housing side wall.

The central part of the top cover will be made from 35 mm thick circular plate made of transparent PMMA, also called Perspex. The outer part of the cover will be made from aluminium. This aluminium outer part makes the cover stiffer and screwable on the side wall of the setup. The bending stiffness of the top cover is about $1x10^9$ N/m. To be able to quickly mount the cover on the side wall, this will be a screwed connection, with female thread on the cover and male thread on the side wall. A rubber o-ring in a groove on top of the side wall has to ensure the final sealing between the 2 parts. A 3D sketch of the cover and total the assembled situation is shown in the figures below.

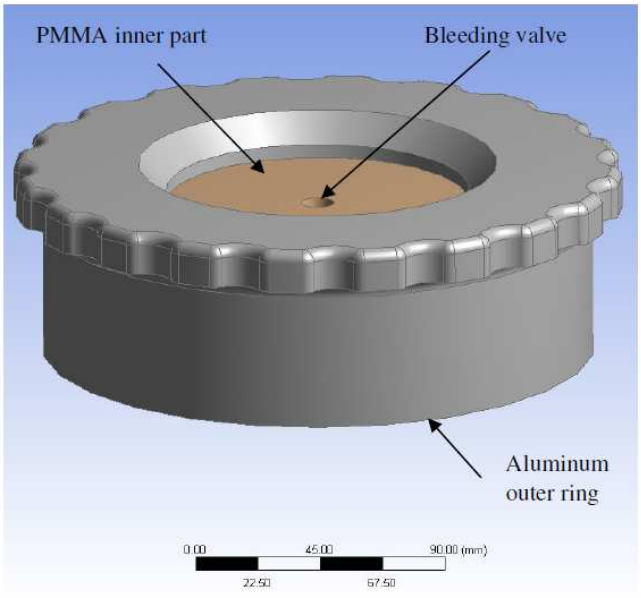

Figure 5 3D sketch of the top cover.

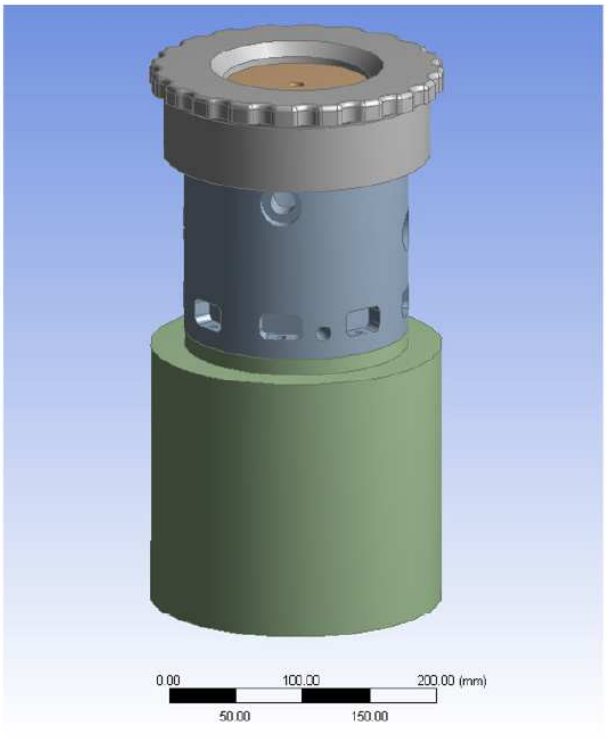

Figure 6 3D sketch of complete assembly, including projector.

The housing is designed in such a way that all the resonance frequencies are higher than 1000 Hz. The first 6 resonance frequencies of the housing (side wall plus cover) are given in the table below. Since the setup is not exactly axial symmetrical due to the mounting holes etc., the resonance frequencies of each set of 2 accompanying modes differ slightly. A few mode shape characteristic examples are given in figure 7 and 8.

Table 1 First 6 resonance frequencies experimental setup.

Mode no.	Frequency [Hz]	Mode type
1	1936	1st order axial bending
2	1975	1st order axial bending
3	3235	1st order torsional
4	4050	2nd order circumferential bending ('ovaling')
5	4063	2nd order circumferential bending ('ovaling')
6	4162	1st order bending PMMA part of cover

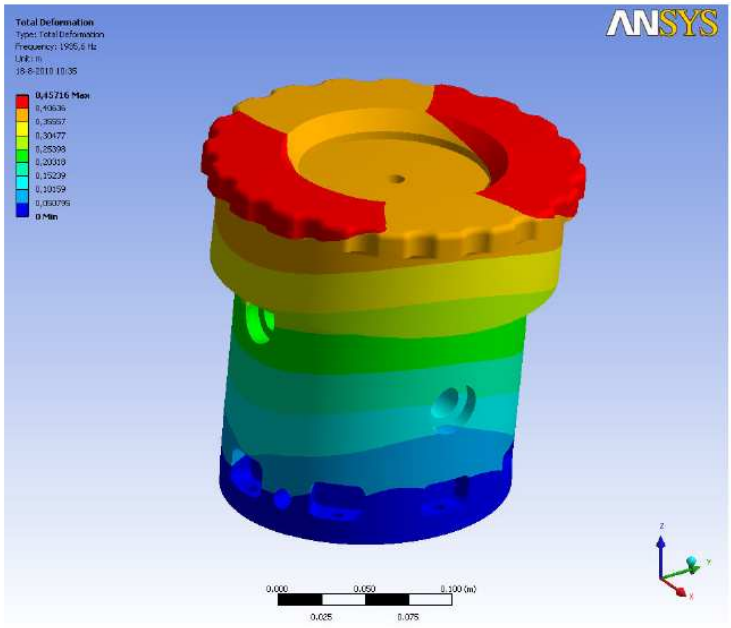

Figure 7 1st order axial bending mode of the assembled test setup at 1936 Hz.

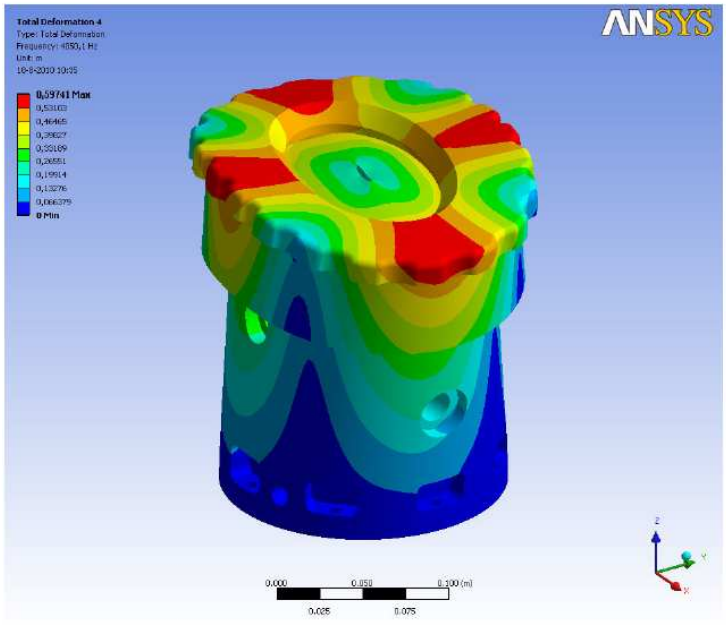

Figure 8 2nd order circumferential bending ('ovaling') mode of the assembled test setup at 4050 Hz.

Finally a 1.52 L cylindrical volume is obtained. The 1st resonance frequency of this water filled volume occurs at about 11 kHz, which is also high above the frequency range of interest. The equivalent 'stiffness' of the water column is approximately $1.3x10^8$ N/m, which is considerably lower than the stiffness of all the housing parts.

Air bleeding system
All the parts will be fabricated in such a way that they will fit close to each other, without any remaining hollow spaces, etc. The side wall and PMMA part of the cover will be polished, so as little as possible air bubbles will 'stick' to them.
At the bottom of the side wall and in the middle of the cover a valve will placed. Via these valves the remaining air can be removed, after the cover has been closed. The underside of the cover will be turned off slantwise (2 mm) to the middle cover, so the valve is always located at the highest point of the chamber.
High quality Stainless steel Festo valves and quick push-in couplings will be used, which have the advantage that the cover can be screwed on to the housing side wall without winding the connection hose. They are also suited to withstand a water pressure up to 10 bar if needed.

A bin filled with about 2 L of sea water and placed at an adjustable stand higher than the rest of the setup, will provide the small amount of overpressure that is needed to release the remaining air underneath the cover valve. A 3D sketch of the installed bleeding system is shown in figure 9, which also shows the total setup on its supports. Underneath the supports adjustable feet with vibration isolators are mounted.

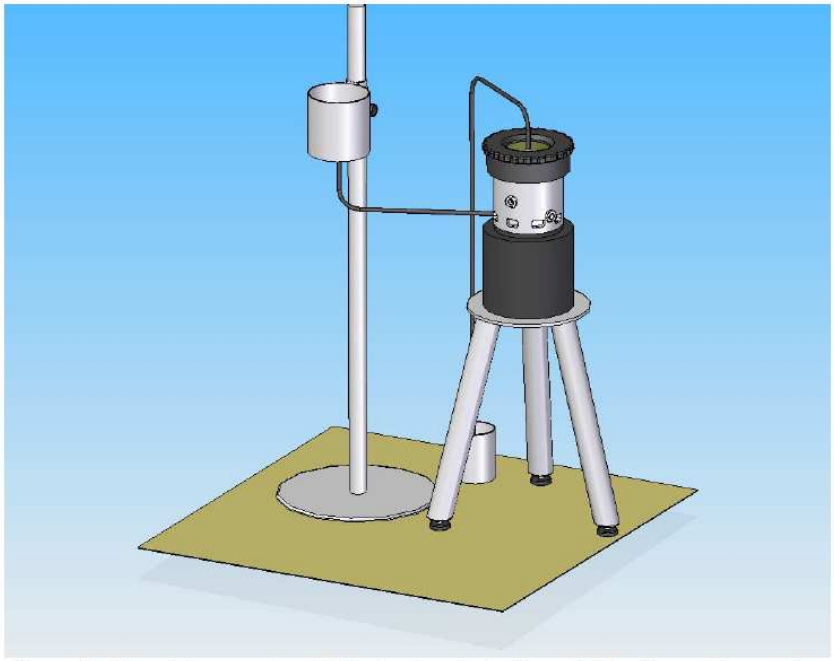

Figure 9 Complete experimental test setup, including air bleeding system and supports.

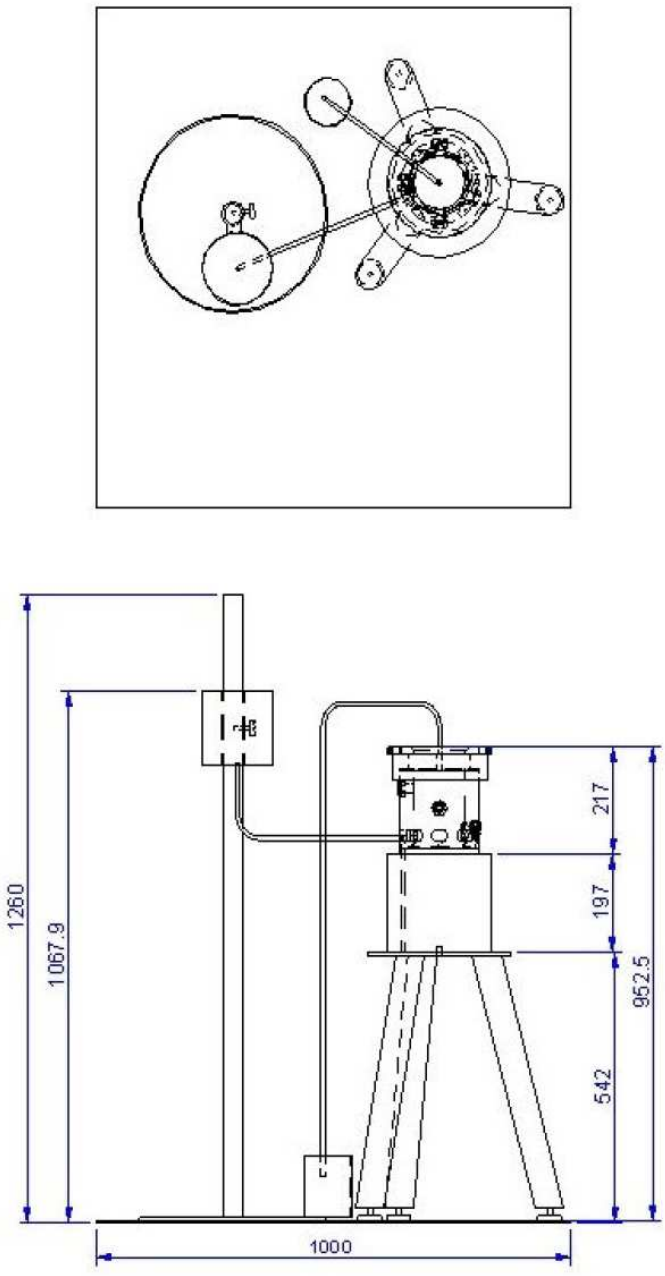

Figure 10 Possible global dimensions of the complete experimental test setup.

Appendix C. TNO memo 3

TNO Science and Industry

Memorandum

Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek / Netherlands Organisation for Applied Scientific Research

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To
Loes Bolle e.a. (IMARES)

From
P.J.G. van Beek

Copy to
Christ de Jong, Frank van den Berg, Dick Kaptein, Erwin Jansen

Subject
The effect of piling noise on the survival of fish larvae - pilot experiments - memo-3: Testing of experimental setup

Date
20 december 2010

Our reference
MON-MEM-2010-03087

Direct dialling
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1 Introduction

This is the third memorandum in the preparation of pilot experiments for determining the effect of underwater noise due to pile driving on the survival of fish larvae. It describes the performance validation test of the experimental test setup for testing the response of fish larvae to artificial piling noise. In the 2nd memo a practical design was made, which was based on the 'larvaebrator' concept of the 1st memo of the project 'testing of the tube'.
In memo 2 the design phase of the experimental setup was divided into different items, from which the following 2 are covered in the current memo:

- fabrication/assembly,
- performance validation.

2 Fabrication & assembly

First of all it has to be noticed that after the preparation of memo 2, an extra specification has been added to the design requirements of the test setup: for both the velocity and pressure source test conditions, it should be possible to introduce a static overpressure inside chamber, varying between about 0.2 and a maximum of 3 bar. This overpressure should better simulate the variety of underwater conditions at the range of depths at which the larvae are situated. It is obvious that this has some consequences for the original design.

For the velocity source test now the top cover also is installed and via a precise pressure regulator a static overpressure can be introduced. For this case the lower bleeding valve is already closed at the beginning of the test. When the required pressure is achieved, the upper bleeding valve also is closed.
At the same time exactly the same pressure also is applied to the compensation chamber on the backside of the projector. This ensures that the piston of the projector remains at its original position in case of no excitation signal. To achieve this, both chambers are connected to the same static pressure source (air compressor in this case) via a T-joint in the tube between the chambers and the source. Then the pressure is automatically levelled. The compensation chamber of the projector is equipped with a so-called Schrader valve, which is also used for vehicle tyres. Note that this is a one-way valve that automatically holds to the maximal applied pressure. This means that

when the test condition is returned to a lower static pressure, first the compressed air in the compensation chamber has to be released by hand. This can be done easily with special tool that opens the valve in the other direction. After that the required static pressure can be applied. A schematic overview of the connections, valves, etc. is given in figure 1.

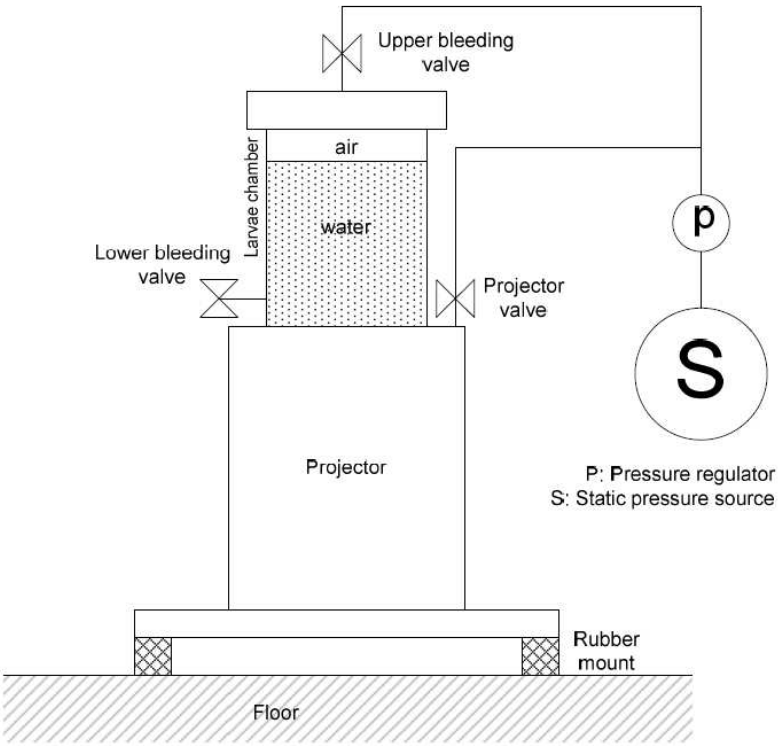

Figure 1 Schematic overview static pressure regulation in case of velocity excitation signal.

In case of the dynamic pressure signal test condition in principal the application of the overpressure is the same as in the velocity test condition. However, in this case the test chamber must be fully filled with water. Therefore the static air pressure is applied to another reservoir that is partially filled with water. This reservoir is connected to the lower bleeding valve of the test chamber. In this case first a small overpressure is applied to the reservoir. When all the remaining air has left the larvae test chamber via a separate small tube that is connected to the upper bleeding valve, this valve is closed. After that, when the required static pressure is achieved, the lower bleeding valve is also closed. A schematic overview of the static pressure regulation is given in figure 2. Finally the actual testing can be started.

After memo 2 it was also decided that it is better to place the projector plus larvae chamber directly on decoupling mounts on the floor, in stead of the using the tripodsupports. These relatively 'flexible' supports might cause extra, unwanted vibrations i.e. resonances in the measured response signals. Therefore the projector is mounted on a 30 mm thick steel plate of 400 x 400 mm. Underneath the plate on each corner a rubber mount is placed with a Shore-A hardness of 50, which results in a resonance frequency of the complete setup of about 15 Hz. A 3D impression of the updated design is given in figure 3, together with a side view in figure 4.

The complete manufactured and installed laboratory setup is shown in figure 5

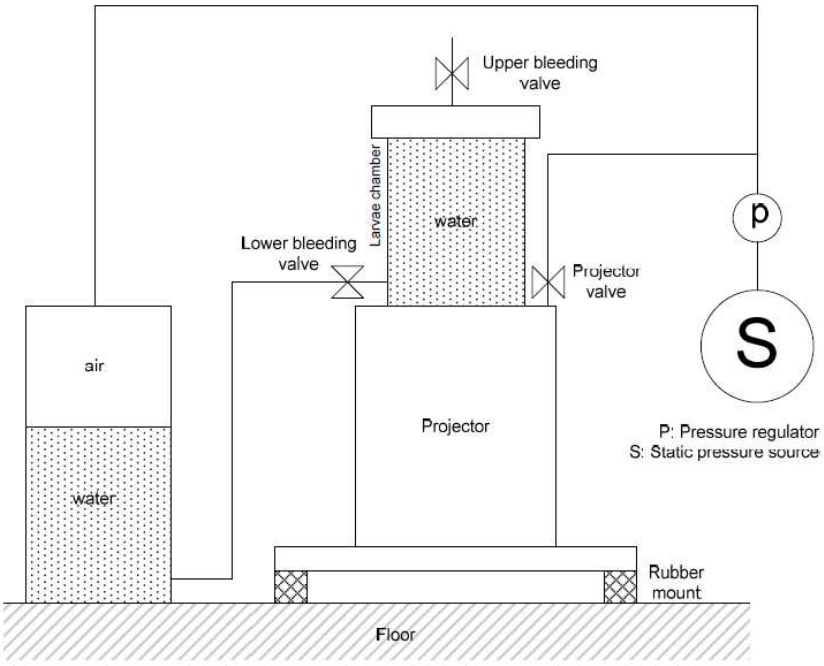

Figure 2 Schematic overview static pressure regulation in case of pressure excitation signal.

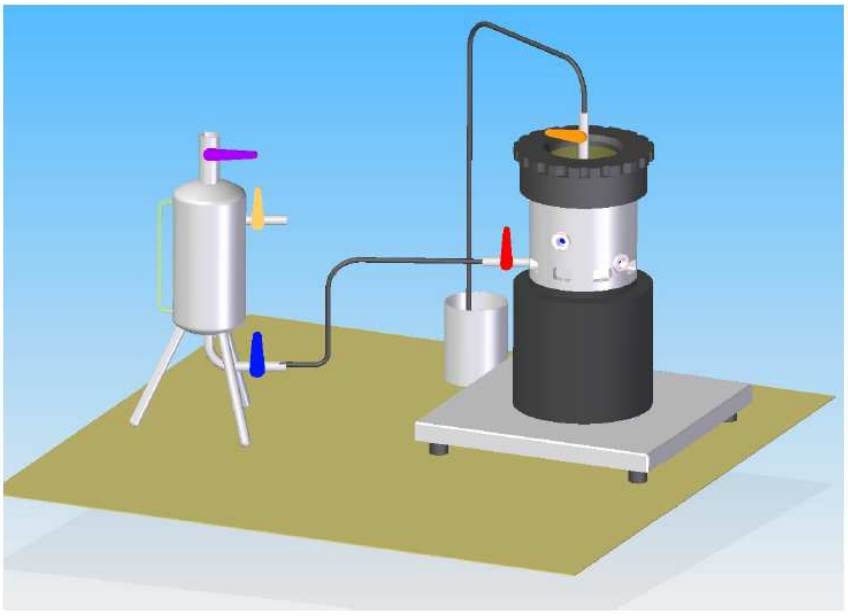

Figure 3 A 3D impression of the updated experimental test setup.

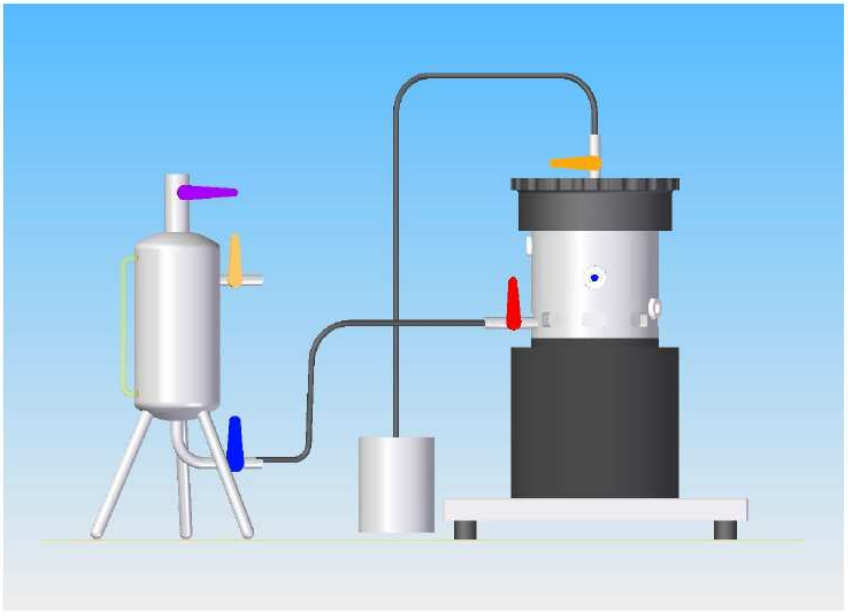

Figure 4 Side view of the updated experimental test setup.

Figure 5 Overview of the laboratory test setup, including measurement equipment.

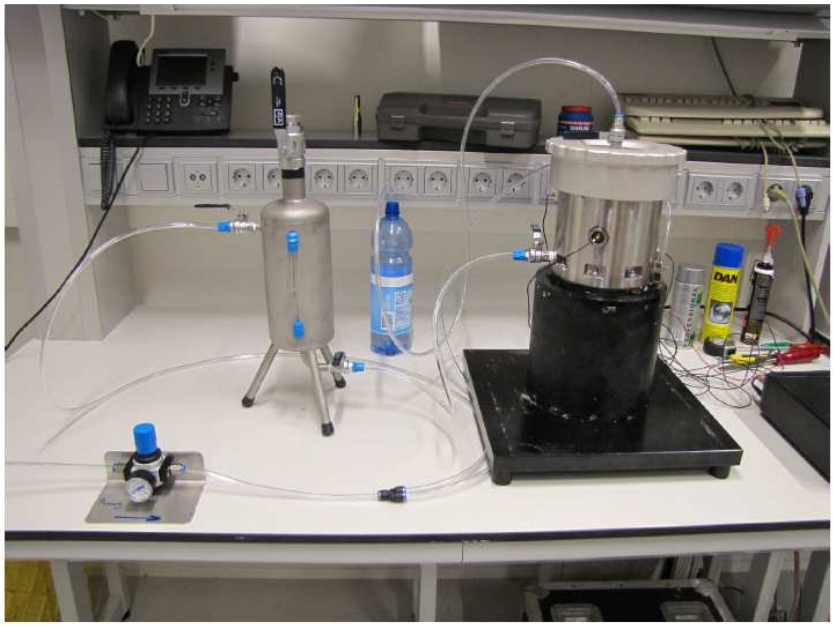

Figure 6 Laboratory test setup: projector and larvae chamber (right), reservoir (middle), pressure regulator (left)

3 Performance validation

In memo 1 already an example of a piling pressure signal was shown. A similar signal is used for both pressure and velocity excitation. A real, measured piling pressure signal (measured at 100 m from a pile at the OWEZ wind farm) is filtered between about 20 and 3000 Hz with a 3rd order Butterworth filter and tapered to zero around the signal with a Tukey window. Finally it is normalized to a signal with a maximum of 1 and converted to a 16 bit .wav file, which can be used as an output signal for the generator and thus as input signal for the projector amplifier. The B&K Pulse LanXI analyzer has an integrated signal generator, so the output signal and measured velocity and pressure signals are always synchronized. An example of a normalized excitation signal is given in figure 7. The signal can be repeated as many times as required. Initially for the performance validation the same signal is used for both pressure and velocity excitation.

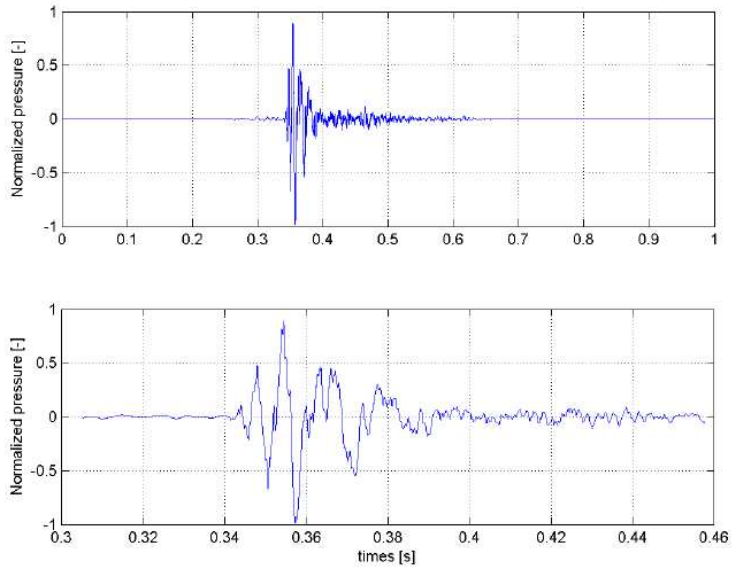

Figure 7 Normalized excitation: complete signal (upper) and zoomed (lower).

4 Piling noise signals

As explained in the first memo, the main characteristics of the piling noise pulses in connection with the potential effects are the peak level and the integrated exposure level of the sound pressure and acoustic particle velocity. In the selection of the signals for the controlled exposure, care is taken that the peak and integrated exposure levels have the correct ratio, so that the signals have the correct 'impulsiveness'. It is unknown which other properties of the signals might have an effect. Therefore it is decided to use actually recorded piling noise instead of synthetic signals. However, recorded pulses are not available for all the specific distances that were selected for this exposure study. Therefore it was decided to use recorded signals that are scaled to

the correct peak and/or exposure levels at the required distance. Hence, the signals to which the larvae will be exposed are characteristic for piling underwater noise, with the correct peak and integrated exposure level, instead of actually recorded signals at the various distances. In real life, the actual wave shape of piling noise will vary a lot, due to variations in pile, hammer and environment, but the characteristic parameters will be similar to the ones chosen for this study.

Two measured signals are selected, one at 100 m and one at 800 m from a pile at the OWEZ wind farm. The amplitude will adapted to the various distances according to a $15\log_{10}$(distance) scaling (i.e. 4.5 dB decrease for each doubling of the distance), according to the following table.

Table 1

distance	peak pressure	SEL	peak velocity	integrated velocity	wav-file
m	dB re 1 μPa^2	dB re 1 μPa^{2}s	dB re 1 (nm/s)^2	dB re 1 (nm/s)^{2}s	
100	210	188	147	124	pressure_100m_filter.wav
200	205	183	142	119	pressure_100m_filter.wav
400	201	179	138	115	pressure_100m_filter.wav
800	196	174	133	110	pressure_800m_filter.wav
1600	192	170	129	106	pressure_800m_filter.wav
3200	187	165	124	101	pressure_800m_filter.wav

Note that the applied distance scaling is not generally applicable for all piling locations, because this will depend on the actual local propagation conditions. However, the main aim of the proposed controlled exposure study is to obtain dose-response relationships, where the dose is quantified according to the four parameters in the above table.

5 Test results

The experimental set-up (filled with clean tap water) was tested at the maximum achievable acoustic level in four different configurations:

a Velocity excitation at 0 bar overpressure
b Velocity excitation at 2 bar overpressure
c Pressure excitation at 0 bar overpressure
d Pressure excitation at 2 bar overpressure

In each configuration the modified wav-file of the 100 m recording was sent to the projector at a level close to the maximum allowable level for the projector. The resulting acoustic signals in the chamber were measured by the accelerometer on the piston and by the four pressure transducers in the wall of the chamber.

The results are shown in the following two figures. Note that the pressure sensors are numbered from bottom (close to the piston) to top.

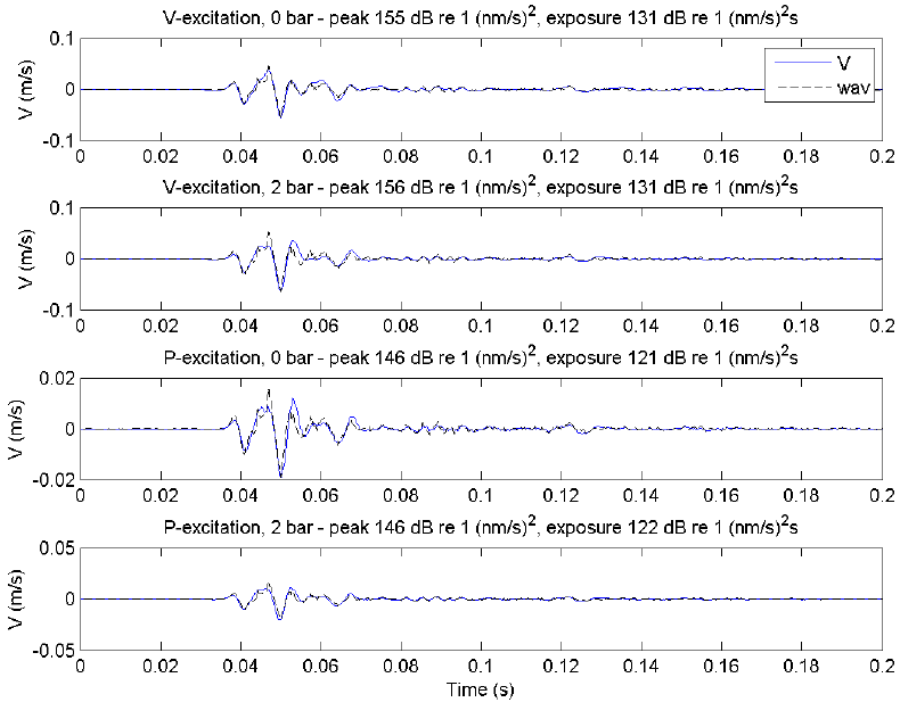

Figure 8 The velocity of the piston of the projector for the four different excitation configurations. The black dashed line is the waveform of the wav-file, scaled to match the peak level of the measured velocity (blue line). The header gives the peak and integrated particle velocity levels that were obtained in these tests. These may be considered the maximum achievable levels.

It can be seen that the piston reproduces the original recorded wav-files quite accurately. In the configurations with pressure excitation, the velocity levels are substantially lower than for velocity excitation.

The maximum achievable velocity levels for velocity excitation are about 8 dB higher than required for this study (see table 1).

In case of pressure excitation, the piston velocity level is relatively high, probably due to remaining flexibility (air/membrane) in the chamber. The observed pressure to velocity ratio is actually close to the ratio in a plane wave in unbound water. So the present set-up does not produce a pure pressure excitation.

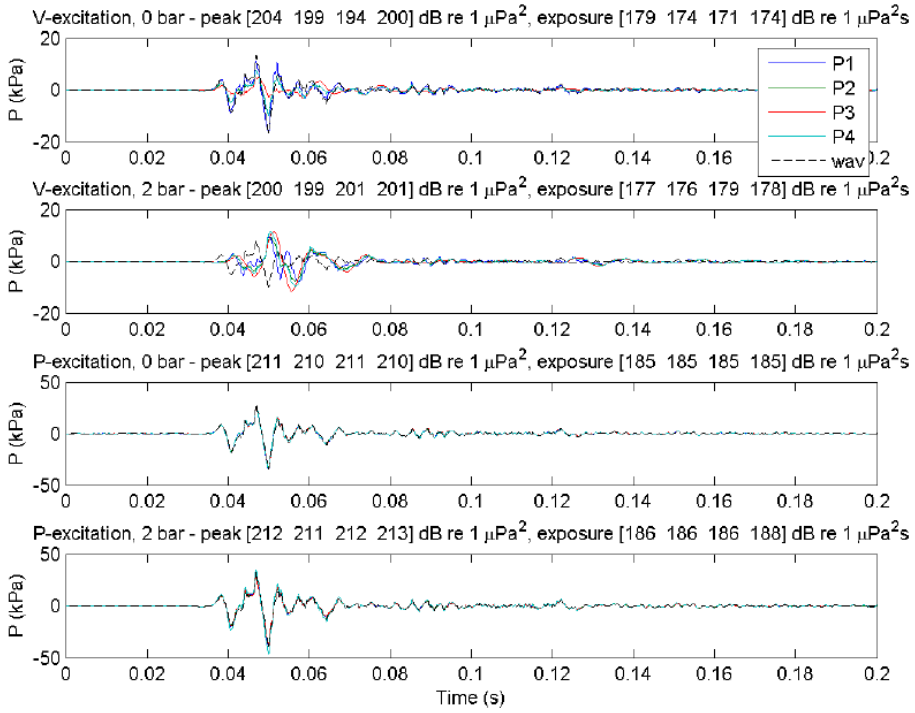

Figure 9 The sound pressure at the four sensors in the larvaebrator chamber for the four different excitation configurations. The black dashed line is the waveform of the wav-file, scaled to match the peak level of the measured pressure at sensor 2. The header gives the peak and integrated pressure levels that were obtained in these tests. These may be considered the maximum achievable levels

It can be seen that the sound field reproduces the original recorded wav-files quite accurately in case of pressure excitation (the two lower figures). The pressure distribution in the chamber is very homogeneous in that configuration.

The maximum achievable pressure levels for pressure excitation are about 1-2 dB higher than required for this study (see table 1).

In case of maximum velocity excitation, the pressure levels are 8-13 dB lower than in case of pressure excitation. Because the required velocity levels are about 8 dB lower

than the maximum velocity levels, it follows that the pressure levels in case of velocity excitation are negligibly small, compared to the levels for pressure excitation.

So the two different excitation types create two very different exposures:

a *Predominant velocity excitation*

b *Pressure and velocity excitation at a ratio in the same order of magnitude as the ratio in acoustic waves in unbound water*

Figures 10 and 11 show that the main characteristics of the frequency spectra of pressure and velocity are reproduced to an acceptable level.

We conclude that the set-up is ready for the larvae exposure tests.

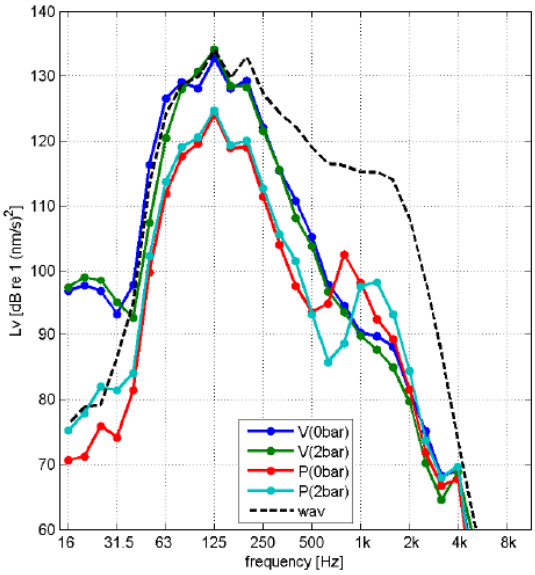

Figure 10 velocity spectrum (1/3-octave bands) for the four configuration, compared with the spectrum of the wav-file, scaled to match the peak level of the measured velocity

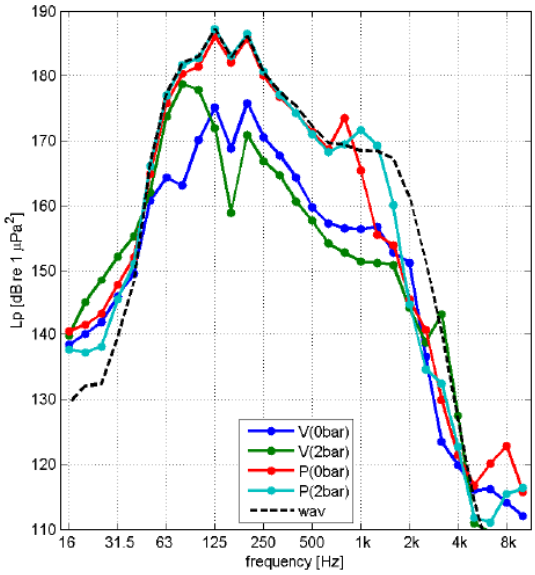

Figure 11 pressure spectrum of sensor 2 (1/3-octave bands) for the four configuration, compared with the spectrum of the wav-file, scaled to match the peak level of the measured pressure

Appendix D. IMARES memo 1

IMARES
WAGENINGEN UR

IMARES

memo

TO

Christ de Jong, Dick Kaptein, Pieter van Beek, Frank van den Berg (TNO)
René Dekeling (Ministerie V&W)

FROM

Loes Bolle, Olvin van Keeken, Erwin Winter, Dick de Haan, Cindy van Damme, Jan van der Heul, Victor Simoncelli

EMAIL

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DATE

17 September 2010

SUBJECT

The effect of piling noise on the survival of fish larvae – pilot experiments.
IMARES memo 1: Phase 1

Introduction

The aim of this project is to examine the effect of piling noise on the survival of fish larvae by means of laboratory experiments. This approach is novel and requires considerable preparations and testing before the actual exposure experiments can be carried out. These preparations were carried out during the first phase of the project and are documented in 4 memo's (3 TNO memo's and this memo).

An overview of the activities carried out during the first phase:

- Definition of the acoustic signals including a discussion on how representative of pile driving noise these signals can be made (TNO memo 1)
- Design of the experimental set-up (TNO memo 1 & 2)
- Construction of the experimental set-up (TNO memo 3)
- Acoustic testing of the experimental set-up (TNO memo 3)
- Obtaining fish larvae (this memo)
- Obtaining approval by the DEC (Animal Experiments Commission) (this memo)
- Preparation laboratory facilities (this memo)
- Development protocol for handling larvae, maintaining larvae and scoring survival (this memo)
- Estimation larval mortality (this memo)
- Development experimental design (this memo)

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The first phase will be completed with a go/no-go decision before the second phase (i.e. the actual exposure experiments) is started. The decision will be based on an evaluation of the feasibility of:

- Generating loud impulse sounds in an experimental setup without distortion due to reflections.
- Generating artificially sound which is representative of the noise from a typical offshore piling installation for a steel mono-pile wind turbine foundation, at distances of 100-2000m.
- Experiments with fish larvae without high mortality due to the handling of larvae during the experiment.

Fish larvae

In principle, larvae can be obtained from 3 sources: catching live larvae, rearing larvae in the laboratory and commercial hatcheries.

Catching larvae is not a realistic option, because it is impossible to catch large amounts of larvae in a healthy state during ichthyoplankton surveys.

Larvae can be reared in the laboratory; this has been done successfully for a number of species. Laboratory rearing requires a major effort: ripe adults are caught in the spawning season, eggs and sperm are collected from these ripe adults, eggs are fertilised *in vitro* and then reared to the larval stage. Laboratory rearing is not considered to be a realistic option for this pilot study in view of the costs involved and the limited time frame in which larvae can be made available this way.

Larvae can be obtained in large numbers and at relatively low costs through commercial hatcheries. For this pilot study we choose sole (*Solea solea*) larvae obtained from a hatchery in IJmuiden (SOLEA BV), because of the high frequency of spawning episodes in this hatchery and for practical reasons (quick and easy delivery of larvae due to close connections with IMARES). The multiple spawning episodes (approximately once in 6-8 weeks) increases the time-frame in which experiments can be carried out. However, the duration of the pelagic larval stages is short and larvae of a certain stage are only available in restricted periods (few days to 1 week). Furthermore, the SOLEA spawning episodes are not planned on a regular basis, but in response to the demand by commercial customers, which complicates planning of experiments. The onset of a spawning episode is usually planned a few weeks in advance, but the precise date when larvae of a certain age are available depends on several factors including temperature regime, condition of the adult stock and feeding success.

Hatchery reared larvae can also be obtained for other species (e.g. sea bass), but this pilot study will be limited to one species, i.e. sole. Consequently, conclusions on inter-specific differences in the impact of piling noise can not be given. For adult fish it has been shown that the impact of sound depends on fish species and fish size (Hastings & Popper 2005). We expect that inter-specific differences will be smaller in the larval stage as physiological differentiation between species is less in the larval stage.

DEC

Approval by the DEC (Animal Experiments Commission) has been granted for the use of 1500 larvae. This number was based on the original plan in the tender of this project. In the meanwhile the project team has decided to reduce the period of effect measurements in a large proportion of the experiments to enable more experiments. Hence more larvae will be required. Although formally an approval is only required in the case of experiments with larvae after the yolk-sac phase, the DEC has been informed about our intention to increase the number experiments.

Laboratory facilities

Laboratory facilities (such as aquaria, climate chambers and clean/filtered sea water), which are required for maintaining fish larvae and maintaining the copepod cultures used to feed fish larvae, are installed at IMARES.

The larvae per delivery (spawning event) will initially be kept in 1 container to avoid different environmental circumstances. The temperature in the hatchery is lower than the ambient temperature in the IMARES laboratory, so an acclimatisation period is required (at least 1 day). Batches of larvae will be prepared prior to the experiments, to minimize handling time during the experiment. Originally we intended to separate the batches by small floating pens placed in one large holding tank, but the experience gained during the test trials showed that contact with netting should be avoided to minimise mortality. The batches will therefore be kept in small containers.

Test trials

Several test trials, i.e. experiments without exposure to noise, have been carried out prior to the actual exposure experiments.

During the first test trial the procedures for handling larvae were optimised to ensure minimal mortality due the experiment itself. Furthermore a protocol for scoring survival was developed.

Mortality due to handling can be minimized if any contact with the larvae is avoided, i.e. all transferring of larvae should be done within water and all movements of water containing larvae should be done very slowly and carefully. If these handling techniques are applied then the mortality of yolk-sac larvae on the short-term (3 days) is low (<5%). These findings led to a change in the plans on how to insert larvae in the test chamber of the experimental set-up: the larvae will be put directly into the water-filled test chamber in stead of inserting a floating pen or a plastic bag with water and larvae. This approach facilitates the removal of air bubbles from the test chamber, but care has to be taken that the water temperature in the test chamber remains equal to that in the larvae containers.

During the first test trial we discovered that the transition from yolk-sac to feeding is a critical phase, irrespective of any handling. High mortality rates occurred, probably due to poor timing of feeding the larvae. Maintaining larvae after the yolk-sac stage proves to be time-consuming (food items have to be provided and removed once a day). Based on these experiences we have reconsidered the experimental design: we will limit the number of larvae batches maintained for a longer period after the experiment, which enables us to do more experiments on short-term effects.

Survival of larvae will be scored by visual inspection. Originally we envisaged image-analyses of the mobility of larvae to determine if a larva is dead or alive, but it turns out that mobility is not a good criterion as live larvae can be quite immobile. Most dead larvae can easily be recognized by sight and in case of doubt the heart-beat or respiratory activity is checked using a microscope or magnifying glass.

During the second test trial the potential accumulation of larvae at the surface was examined, as this may have consequences for the experimental set-up. The results showed that accumulation of larvae at the surface is limited and can further be limited by the selection of larvae. Hence, potential bias due to heterogeneous distribution and the risk of losing larvae through the top valve of the set-up is limited.

Short-term mortality was estimated more precisely during the second test trial. Three batches of larvae were treated in the same way, i.e. simulating the handling which will be required during the experiments. Each batch consisted of 25 larvae, i.e. the sample size which will be used for the first trial with exposures. The age of the larvae at onset of the experiment was 2-3 days (yolk-sac stage). The larvae were not fed. Mortality was scored after 5 days. Two larvae died in 1 of the 3 batches, no mortality occurred in the other 2 batches. This gives an average mortality of 3% with a standard deviation of 5%.

During the third test trial we focussed on optimising the procedures for maintaining larvae after the yolk-sac stage. The optimised protocol is described in the following section.

Short-term and long term mortality were estimated during the third test trial. Three batches of larvae (batch size=25, age at onset experiment=3 days) were maintained under *ad libitum* food conditions until approximately 50% of the larvae had reached metamorphosis, which was after 16 days. These 3 batches were not exact replicates because the way of providing and removing food differed between the batches. Average mortality was 4% (sd=4%) after 5 days and 11% (sd=12%) after 16 days. These mortality rates are considered to be low, i.e. much lower than natural mortality in the field. Although average mortality was low, variability between batches was high with no apparent explication. The low mortality rate is encouraging for the actual sound exposure experiments, but the high variability between (almost) identical treatments may indicate that we need more replicates in the final exposure trials.

Until now we had not considered introducing overpressure (water pressure) in the experimental set-up of laboratory experiments. Water pressure may be an important factor in the effect of sound on fish (larvae) and overpressure can be incorporated in the experimental set-up. However, applying overpressure quickly may affect the survival of larvae; larvae may need an acclimatisation period for changes in water pressure as they do for changes in temperature. Before introducing overpressure as a factor in the actual exposure experiments, a test trial is required to examine the effect of changes in pressure. A separate test chamber has been developed for this purpose as the experimental set-up is not yet available. The pressure test trial will be carried out as soon as larvae are available.

The next spawning episode is expected to take place in the week of 20-24 September. This raises the question whether the first sound exposure trial should be postponed until after the 4th test trial or whether the first sound exposure should be carried out without or with low overpressure.

Protocol

For experiments with yolk-sac larvae, recently hatched larvae (one day old) are selected at SOLEA and taken to IMARES. For post yolk-sac experiments, larvae are selected 2 days before the experiments. The larvae are kept for at least 1 day in the transportation container, to acclimatise them to the ambient temperature in the IMARES laboratory.

The larvae are taken from the transportation container with a glass measuring cup. From this cup the larvae are selected and divided into batches. Each batch is placed into a small container with 500ml water. As larvae are vulnerable to mechanic damage, no aeration will be used in the batch-containers to reduce the chance of larvae hitting the walls.

When selecting the larvae from the glass measuring cup, larvae are taken from the water column and not from the surface. Larvae floating at the surface tend to remain at the surface (test trial results). Selection of surface larvae would increase the risk of an heterogeneous distribution of larvae in the test chamber and loss of larvae through the top valve of the experimental set-up.

Larvae are transported to and from different water bodies using a plastic pipette, from which the front part is cut off to increase the size of the opening. This method minimises mortality due to handling. A cut-off pipette is used to transport the larvae from the glass measuring cup to the batch-containers and from the batch-containers to the test chamber. To retrieve the larvae from the test chamber an extended pipette is required.

The duration of the yolk-sac stage (at ambient temperature in the IMARES laboratory) is 3-4 days. The larvae clearly feed at an age of 4 days (after hatching). In principle, food is provided from 3 days after hatching onwards. But in the case of experiments with yolk-sac larvae, in which only the short-term effect of exposure

is monitored (i.e. the age of the larvae at the end of the monitoring period ≤5 days), the larvae will not be fed.

Young larvae are fed with 1-day-old copepods. Older larvae are fed with 2-day-old 'enriched' copepods; these copepods have been fed for 1 day with algae to increase the nutritional value and size of the copepods. This diet is sustained until metamorphosis. The food items are provided *ad libitum*.

After feeding has started the water in the containers has to be refreshed each day, partly due to the fact that the containers are not aerated, but mainly due to the necessity of removing old food items. The quickest and most effective way of doing this is by transferring the larvae to a new batch-container holding water of ambient temperature with fresh copepods.

In all experiments, the larvae are examined right after the experiment and after 1-3 days to assess mortality. In a limited number of experiments the larvae are monitored for an extended period.

Survival of larvae is scored by visual inspection. Dead larvae can easily be recognized by sight. Within a day (probably within a few hours) after death, the shape of the larvae clearly indicates that the larvae is dead (Figure 1). When in doubt, a larvae is viewed using a microscope or a magnifying glass to examine the heart-beat and/or respiratory activity.

Figure 1. Left: dead larvae, right: live larvae

The dead larvae and (part of) the live larvae at the end of the monitoring period will be preserved to enable future examination of physiological damage. The larvae will be preserved in 3 ways (to enable both histological and SEM analyses):

- 3.6% formaldehyde solution
- glutaraldehyde-formaldehyde solution
- supercold alcohol (-70ºC)

Experimental design

The experiments will be performed at IMARES. The experiments will be supported by TNO in operating the experimental set-up and measuring and analysing the evoked sound levels.

Three trials will be carried out. Each trial consists of ±20 experiments to be carried out in 1 day. During the 1st trial, the sensitivity range of larvae to various sound parameters will be examined crudely. The results of

the 1st trial will be used to focus on relevant sound parameters during the 2nd trial. If all goes well then the 3rd trial can be used to examine the role of biological (age of larvae) and environmental (water depth) factors in the effect of sound on fish larvae. As the results of the previous trial will form the basis for the next trial, 3 spawning events are required to be able to carry out the 3 trials (see paragraph 'fish larvae'). We aim at examining the effect of the variables (at the levels) listed in Table 1.

Table 1. Variables and levels of variables examined during the SMW pilot experiments.

Variables	Levels	Values					
Sound level at distance (m)	6	100	200	400	800	1600	3200
Strokes (no.)	3	1	10	100			
velocity or pressure	2	V	P				
Water pressure (bar)	2	0.5	2				
Age larvae at T=0 (days after hatching)	2	2-3	±8-9				

The sound level is expressed in distance to the pile driving site. Each distance corresponds with a certain value for the sound exposure level (SEL) and peak exposure level (see memo 1). The maximum sound level that can be generated by the experimental set-up corresponds to a distance of 100m. Sound measurements within 100m of a pile driving site are not yet available. The larvae will be exposed to single or multiple blasts: 1, 10 or 100 strokes. We choose 3 values for this variable rather than calculating the number of strokes for each distance based on the average current, to be able to test this variable independently. The effect of sound pressure and particle velocity will also be measured independently (see memo 1 and 2).

Two values for the factor age larvae are chosen corresponding to larval stages 1 and 3 (according to Al-Maghazachi & Gibson 1984). Larval stage 1 is the yolk-sac phase. These larvae do not require feeding and are therefore easily maintained and kept alive. Furthermore, the yolk-sac itself may be an organ sensitive to sound pressure or velocity. Larval stage 3 is selected because in this stage the swim bladder is fully inflated (Al-Maghazachi & Gibson 1984). The swim bladder diminishes in stage 4 and completely disappears in stage 5. Metamorphosis is completed by the end of stage 5 (Ryland 1966, Al-Maghazachi & Gibson 1984). The duration of larval stages 1 to 3 is estimated to be approximately 9 days at ambient temperature in the IMARES laboratory, based on a review of temperature dependent development rates presented in Bolle et al. (2005). Development is however also dependent on feeding success.

Two values for the factor water pressure are chosen based on the geographical and vertical distribution of sole larvae. No studies on the vertical distribution of sole larvae have been carried out in the southern North Sea. A North Sea & Irish Sea study on the planktonic stages other fish species shows that, overall, larvae occur in the entire water column with higher concentrations in the top water layers (<25m), but this study also shows inter-specific differences (Conway et al. 1997). Vertical distribution has been examined in other sole populations, but most of these studies focused on the transition from pelagic to demersal life style and only discriminated between the bottom water layer (1-1.5m above seabed) and the rest of the water column (e.g. Lagardère et al. 1999, Grioche et al. 2000). Only 1 study, carried out in the Bay of Biscay (published in Koutsikopoulos et al. 1991 and Champalbert & Koutsikopoulos 1995), presented data on the distribution of sole larvae in the entire water column. This study showed that the early larval stages (stage 1-2) mainly occur in the bottom half of the water column, whereas the later stages (stage 3-4) occur in the whole water column. A diel vertical migration pattern is observed in which the larvae move up in the water column at night and down during daytime. This pattern was clearly observed in larval stages 3 and 4, but was less evident for the stages 1 and 2. By stage 5, sole larvae disappeared from pelagic catches and were only observed close to the seabed. Sole spawning grounds are further offshore in the Bay of Biscay compared to the North Sea (Arbault et al. 1986, Koutsikopoulos & Lacroix 1992). In the North Sea, sole spawn within the 50m depth contour (Houghton & Riley 1981, Riley et al. 1986, van der Land 1991) and mayor spawning activity is observed at a water depth of 10-25m (Bolle et al. in prep). Taking into account both the vertical

distribution pattern observed in the Bay of Biscay and the geographical distribution of sole spawning in the North Sea, we concluded that sole larvae will certainly occur at a depth of 5m and 20m (i.e. 0.5 and 2 bar overpressure). Furthermore, these 2 values differ sufficiently to test the effect and water depth and they are also realistic for larvae of other fish species.

The response variable that will be measured is mortality. In all experiments, mortality will be measured directly after the experiment and after 1-3 days (T=0 - 3 days). In a limited number of experiments mortality will be monitored for an extended period (T=0 - ±10 days).

During the first trial all experiments, including a control experiment without sound exposure, will be carried in duplo. The sample size for the experiments in the first trial will be set at 25. This number is reduced compared to the original plans in the tender of this project because of the low mortality observed in the test trials.

The levels of the variables will be limited in the first trial compared to Table 1. The age of the larvae will be set at 2 or 3 (yolk-sac stage). Water pressure will be set at 2 bar, if the results of the 4th test trial are available and show that it is possible to increase pressure quickly (i.e. within a few minutes) without affecting the larvae. Otherwise the first trial will be carried out using a low overpressure (0.5 bar). The number of strokes will only be varied between 1 or 100 strokes.

An iterative approach has been chosen for the 1st trial; the results of the first experiment determine the choice of the next exposure. This approach is the most effect way to find the critical sound exposure levels, but it strongly depends on immediate visibility of effects of sound exposure.

We expect to be able to carry out 26 experiments during the first trial (1 day): 1 control experiment in duplo, 6 exposures to sound pressure in duplo, and 6 exposures to particle velocity in duplo. An exposure refers to the combination of the sound level at a certain distance and the number of strokes (1 or 100). A test-scheme has been developed for the first trial (Table 2). This scheme consists of 14 series of exposures. The first exposure in all scenario's is 1 stroke at a sound level corresponding to a distance of 800m. If this has no direct effect then the next exposure is 100m / 1 stroke; if this has no effect then the next exposure is 100m / 100 strokes; etc. This scheme is carried out for both sound pressure as well as particle velocity, as the critical exposure values may be different for pressure and velocity. The test scheme has been worked out for 9 exposures, to be prepared if more than 6 exposures are possible during the first trial.

The left part of Table 2 presents the exposures necessary to determine the critical exposure values for instantaneous effects. The right part of Table 2 lists additional experiments focused on non-instantaneous effects. The emphasis of the latter experiments is on multiple strokes as it is expected that non-instantaneous effects will mainly be determined by the number of strokes. If all relevant combinations of sound level and number of strokes have been tested then the remaining capacity can be used to fine-tune the dose-effect relationship, or in the case of an effect at a distance of 3200m, further reduce the sound level (scenario's 11-14).

The iterative approach adopted for the first trial depends on the assumption that effects are immediately detectable. If this assumption proves to be untrue then the first trial will consist of the exposures listed in scenario 1.

Table 2. Scenario's for first trial with sound exposures. See text for further explanation.

Scenario	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	*Exp7?*	*Exp8?*	Exp4	Exp5	Exp6	*Exp7?*	*Exp8?*	*Exp9?*
1	800/1	100/1	100/100						400/100	1600/100	3200/100	200/1	400/1	1600/1
2a	800/1	100/1	100/100	800/100	400/100	200/100			-	-	-	1600/100	3200/100	200/1
2b	800/1	100/1	100/100	800/100	400/100	200/100			-	-	-	1600/100	3200/100	200/1
3	800/1	100/1	100/100	800/100	400/100				-	-	1600/100	3200/100	200/1	400/1
4a	800/1	100/1	100/100	800/100	3200/100	1600/100			-	-	-	200/1	400/1	1600/1
4b	800/1	100/1	100/100	800/100	3200/100	1600/100			-	-	-	200/1	400/1	1600/1
5	800/1	100/1	100/100	800/100	3200/100				-	-	200/1	400/1	1600/1	3200/1
6	800/1	100/1	400/1	400/100	200/100				-	-	800/100	1600/100	3200/100	200/1
7a	800/1	100/1	400/1	400/100	200/100	200/1			-	-	-	800/100	1600/100	3200/100
7b	800/1	100/1	400/1	400/100	200/100	200/1			-	-	-	800/100	1600/100	3200/100
8a	800/1	100/1	400/1	400/100	800/100	200/1			-	-	-	1600/100	3200/100	1600/1
8b	800/1	100/1	400/1	400/100	800/100	200/1			-	-	-	1600/100	3200/100	1600/1
9a	800/1	100/1	400/1	400/100	800/100	3200/100	1600/100	200/1	-	-	-	-	-	1600/1
9b	800/1	100/1	400/1	400/100	800/100	3200/100	1600/100	200/1	-	-	-	-	-	1600/1
9c	800/1	100/1	400/1	400/100	800/100	3200/100	1600/100	200/1	-	-	-	-	-	1600/1
9d	800/1	100/1	400/1	400/100	800/100	3200/100	1600/100	200/1	-	-	-	-	-	1600/1
10a	800/1	100/1	400/1	400/100	800/100	3200/100	200/1		-	-	-	-	1600/1	3200/1
10b	800/1	100/1	400/1	400/100	800/100	3200/100	200/1		-	-	-	-	1600/1	3200/1
11a	800/1	100/1	400/1	1600/100	800/100				-	-	3200/100	1600/1	3200/1	*
11b	800/1	100/1	400/1	1600/100	800/100				-	-	3200/100	1600/1	3200/1	*
12a	800/1	100/1	400/1	1600/100	3200/100				-	-	1600/1	3200/1	*	*
12b	800/1	100/1	400/1	1600/100	3200/100				-	-	1600/1	3200/1	**	**
13a	800/1	1600/1	1600/100						3200/100	3200/1	*	*	*	*
13b	800/1	1600/1	1600/100	3200/100					-	3200/1	*	*	*	*
13c	800/1	1600/1	1600/100	3200/100					-	3200/1	**	**	**	**
14a	800/1	1600/1	3200/1	3200/100					-	*	*	*	*	*
14b	800/1	1600/1	3200/1	3200/100					-	**	**	**	**	**
14c	800/1	1600/1	3200/1						3200/100	**	**	**	**	**

no direct effect
direct effect

* fine-tune dose-effect relationship
** further reduce SEL until no direct effect, then fine tune dose effect relationship

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Appendix E. TNO memo 4

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Memorandum

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To
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René Dekeling (Ministerie V&W)

From
P.J.G. van Beek

Copy to
Christ de Jong, Frank van den Berg, Dick Kaptein

Subject
The effect of piling noise on the survival of fish larvae - pilot experiments – memo-4: first trail at Imares

Date
3 November 2010

Our reference
MON-MEM-2010-02684A

Direct dialling
+31 88 866 63 66

1 Introduction

This is the fourth memorandum of the pilot experiments for determining the effect of underwater noise due to pile driving on the survival of fish larvae. At October 6th the first trail of experiments with real fish larvae was carried out at IMARES in IJmuiden. In total 12 valid measurements with piling excitation were performed, see table 1.

Table 1 Overview of the first day pilot experiments trail with real larvae.

Measurement no.	Excitation type	Larvae container no.	Simulated distance	Number of strokes
2	velocity	4	800	1
3	velocity	5	800	1
4	velocity	6	100	1
5	velocity	7	100	1
6	velocity	8	100	100
7	velocity	9	100	100
8	pressure	12	800	1
9	pressure	13	800	1
10	pressure	14	100	1
11	pressure	15	100	1
12	pressure	16	100	50
13	pressure	17	100	50

As can be seen in table 1, each experiment was carried out twice. Each container consisted of 25 larvae (batch). Furthermore first two reference batches (container no. 1 and 2) of larvae were exposed only to the static pressure of 0.5 bar. During the day, actually in between the velocity and the pressure excitation, the static test was repeated with two other batches (container no. 10 and 11). This 0.5 bar static pressure is used for all the measurements during trail 1. Note that in practice the pressure excitation in fact is a combined pressure and velocity excitation.

2 Acoustic results Trail 1 experiments

The time signal of the (normalized) piling excitation is given in figure 1. The time signals of the resulting, measured velocity at the piston are given in figure 2 and 3. Figure 2 shows the measured velocities due to velocity excitation a respectively 800m and 100m (latter with 1 stroke and 100 strokes). For the tests with 100 strokes one representative stroke is used for further analysis (almost no difference between individual strokes). In figure 3 the measured velocities due to pressure excitation at respectively 800m and 100m (latter with 1 and 50 strokes) are given. The peak and SEL levels are also shown in the figures. The accompanying measured pressures are given in figure 4 and 5. The spectra are given in figure 6 and 7.
All results correspond very well to the requested levels from table 1 in memo 3, which is repeated here [1]:

Table 2 Expected velocity and pressure levels at different distances due to piling excitation.

distance	peak pressure	SEL	peak velocity	integrated velocity
m	dB re 1 μPa^2	dB re 1 $\mu Pa^2 s$	dB re 1 $(nm/s)^2$	dB re 1 $(nm/s)^2 s$
100	210	188	147	124
200	205	183	142	119
400	201	179	138	115
800	196	174	133	110
1600	192	170	129	106
3200	187	165	124	101

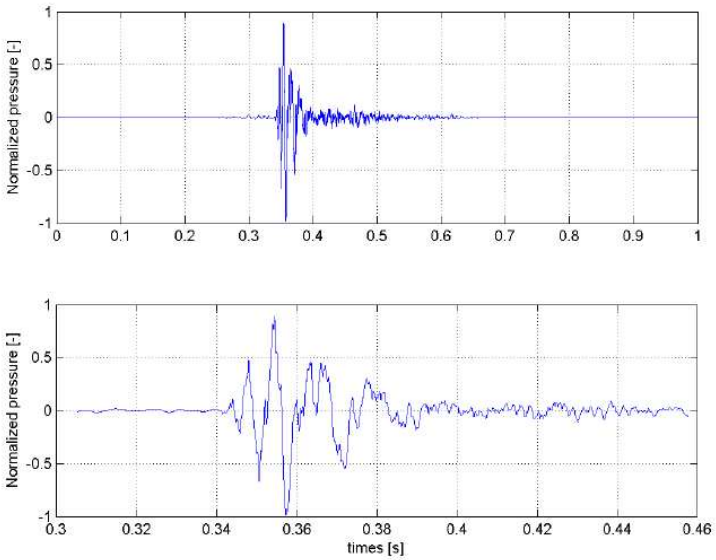

Figure 1 Normalized piling excitation at 100m: complete signal (upper) and zoomed (lower).

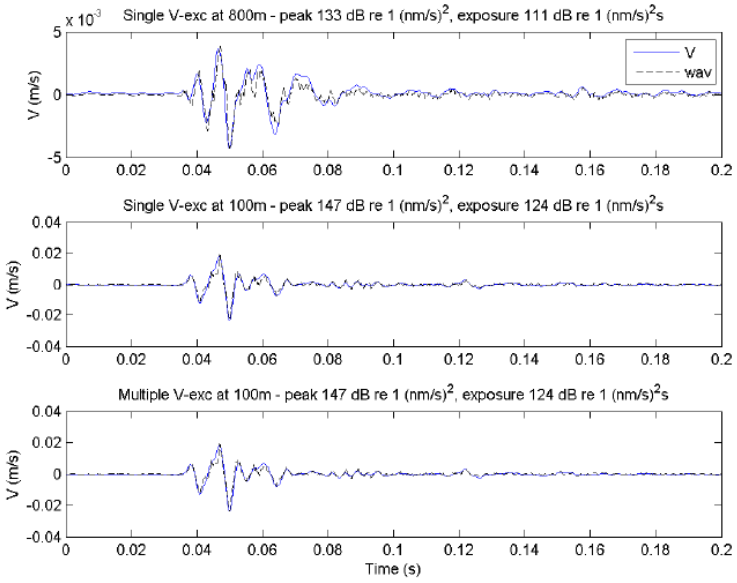

Figure 2 Measured velocity at 800m with one stroke (upper), 100m with one stroke (middle) and 100m with 100 strokes (lower), due to velocity excitation.

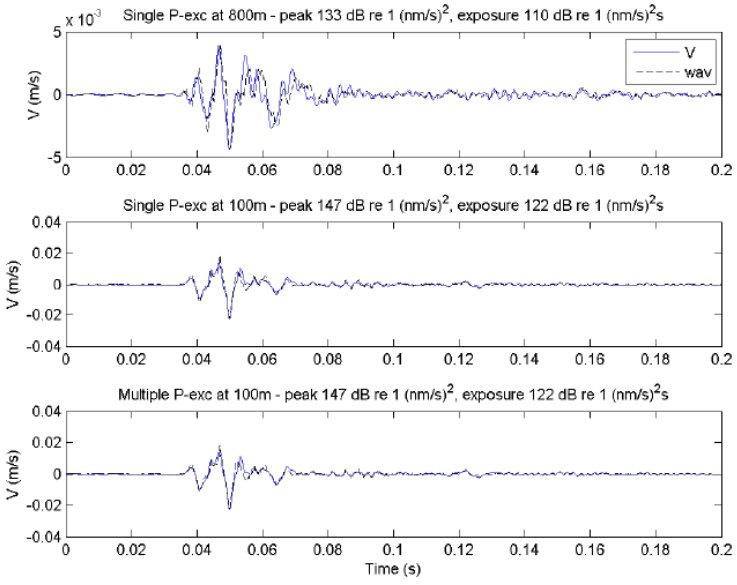

Figure 3 Measured velocity at 800m with one stroke (upper), 100m with one stroke (middle) and 100m with 50 strokes (lower), due to pressure excitation.

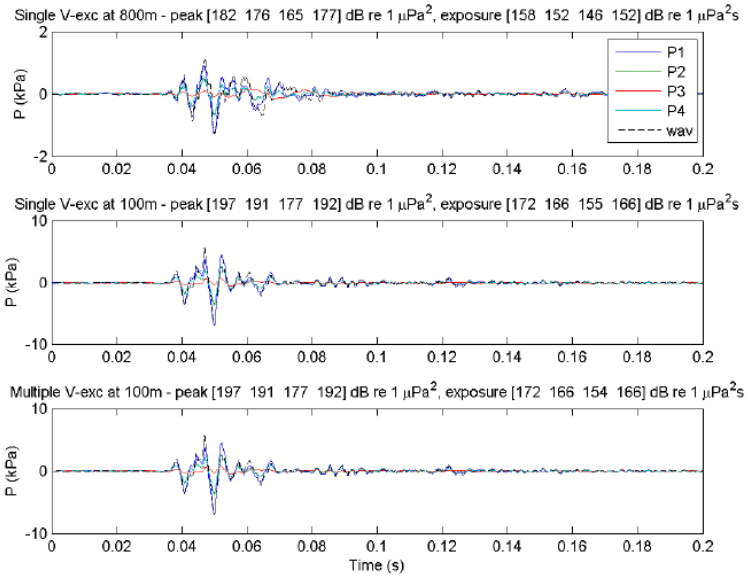

Figure 4 Measured pressures at 800m with one stroke (upper), 100m with one stroke (middle) and 100m with 100 strokes (lower), due to velocity excitation.

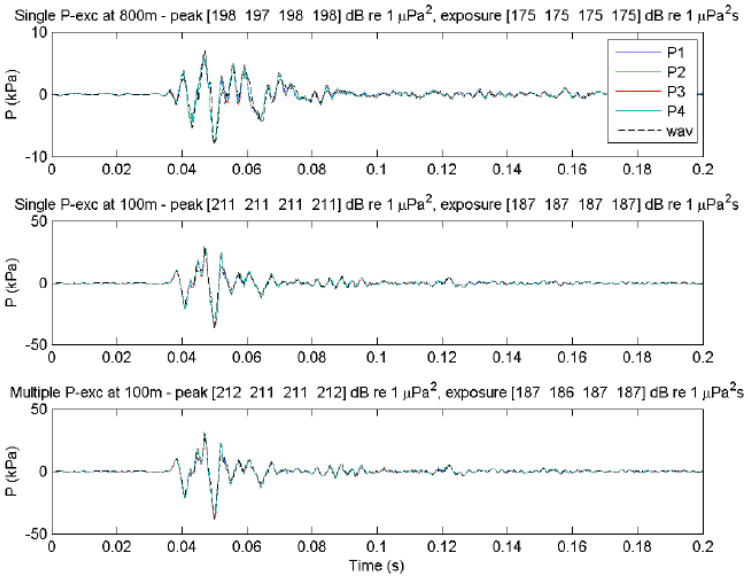

Figure 5 Measured pressures at 800m with one stroke (upper), 100m with one stroke (middle) and 100m with 50 strokes (lower), due to pressure excitation.

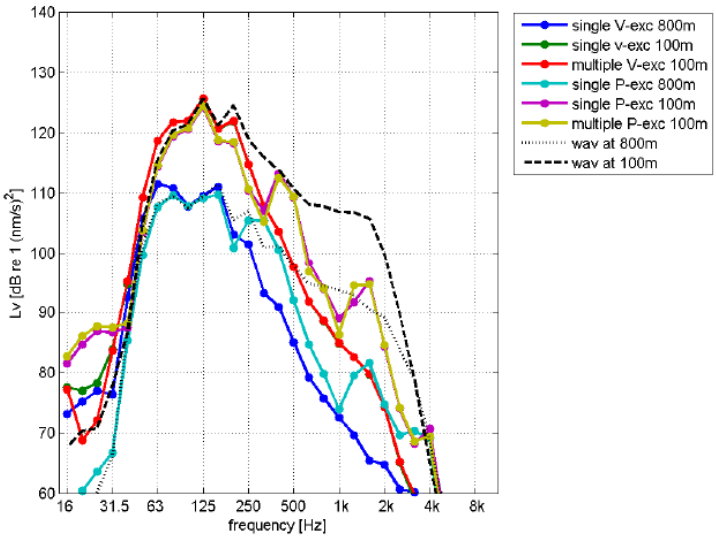

Figure 6 Velocity spectra of the time signals as given in figure 2 and 3, including the excitation wav signal at 100m and 800m (reference).

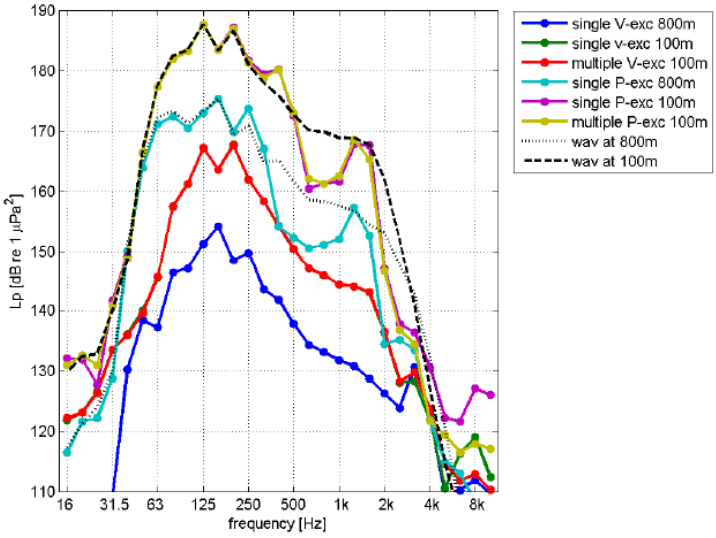

Figure 7 Pressure spectra of the time signals as given in figure 4 and 5, including the excitation wav signal at 100m and 800m (reference).

Appendix F. IMARES memo 2

IMARES
WAGENINGEN UR

IMARES

memo

TO
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Olvin van Keeken, Cindy van Damme, Erwin Winter, Dick de Haan (IMARES)
René Dekeling (Ministerie V&W)

FROM
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EMAIL
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DATE
16 December 2010

SUBJECT
The effect of piling noise on the survival of fish larvae – pilot experiments.
IMARES memo 2: Preliminary results phase 2

Introduction

The aim of this project is to examine the effect of piling noise on the survival of fish larvae by means of laboratory experiments. This approach is novel and requires considerable preparations and testing before the actual exposure experiments can be carried out. These preparations were carried out during the first phase of the project and are documented in 4 memo's (TNO memo's 1-3 and IMARES memo 1).

The "go" decision for the 2nd phase of the project was taken on 20 September 2010. Since then 3 trials and 1 additional test trial have been carried out. The preliminary results of these trials are presented in the this memo. Furthermore, an update of the procedures for handling larvae, maintaining larvae and scoring mortality, based on experiences obtained during the 2nd phase of the project, is presented.

Procedures for handling larvae, maintaining larvae and scoring mortality (updated)

Batches of eggs are obtained from an hatchery. The eggs and larvae are reared to the required developmental stage in large cultivation chambers at the IMARES laboratory. The temperature is slowly raised to the ambient temperature in the IMARES laboratory. Advantage of rearing the larvae at IMARES, rather than obtaining larvae shortly before the experiment, is that the developmental stage can be manipulated by temperature adjustments.

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Ample larvae are carefully collected from the cultivation chamber using a small container. The required number of larvae are selected from this container and inserted into the test chamber of the experimental set-up. After the treatment, the larvae are transferred to a small container and examined for instantaneous effects. The water in the test chamber is refreshed before the next experiment is carried out.

The larvae are transferred to and from different water bodies using a plastic pipette, from which the front part is cut off to increase the size of the opening. This method minimises mortality due to handling.

After the experiments, the batches of larvae are held separately in small containers for a period of 10-12 days. Larvae are vulnerable to mechanic damage, therefore no aeration is used in these small batch-containers. The water in the containers is refreshed each day, because the containers are not aerated, and because of the necessity of removing old food items. The quickest and most effective way of doing this is by transferring the larvae to a new batch-container. Whilst doing this the mortality rate is scored.

The duration of the yolk-sac stage (at ambient temperature in the IMARES laboratory) is 3-4 days; the larvae clearly feed at an age of 4 days (after hatching). Food is provided each day from 3-4 days after hatching onwards. Young larvae are fed with 1-day-old copepods. Older larvae are fed with 2-day-old 'enriched' copepods; these copepods have been fed for 1 day with algae to increase the nutritional value and size of the copepods. This diet is sustained until metamorphosis. The food items are provided *ad libitum*.

Survival of larvae is scored by visual inspection. Dead larvae can easily be recognized by sight. Within a day (probably within a few hours) after death, the shape of the larvae clearly indicates that the larvae is dead (Figure 1). When in doubt, a larvae is viewed using a microscope or a magnifying glass to examine the heart-beat and/or respiratory activity.

Figure 1. Left: dead larvae, right: live larvae

The live larvae at the end of the monitoring period are preserved to enable future examination of physiological damage. The larvae are preserved in 3.6% formaldehyde solution for histology or in a glutaraldehyde-formaldehyde solution. This preservation method allows both light microscopy and SEM analyses.

Test trial 4

Initially we had not considered introducing overpressure (water pressure) in the experimental set-up, but recent work (presented at an international conference in August 2010) showed that the effect of sound may be proportional to water pressure. The design of the experimental set-up was changed to enable overpressure. However, applying overpressure rapidly may affect the survival of larvae. The larvae may need an acclimatisation period for changes in water pressure as they do for changes in temperature. Before introducing overpressure as a factor in the actual exposure experiments, a test trial is required to examine the effect of changing overpressure. A test chamber has been developed by IMARES to test this.

Two larval stages were exposed to several treatments to examine the effect of overpressure (Table 1). The larvae exposed to overpressure showed a higher mortality rate than the control group (Figure 1). The difference with the control group is considered to be small enough to risk this source of additional mortality in the actual sound exposure experiments.

Table 1. Treatments in test trial 4.

Stage larvae	Treatment	Overpressure (bar)	Duration pressure (min)	No. of replicates
1	control	0	21	2
1	stepwise 0.5 bar	2	21	2
1	instantaneous	2	21	2
1	instantaneous	3	21	2
2	control	0	21	2
2	instantaneous	3	21	2

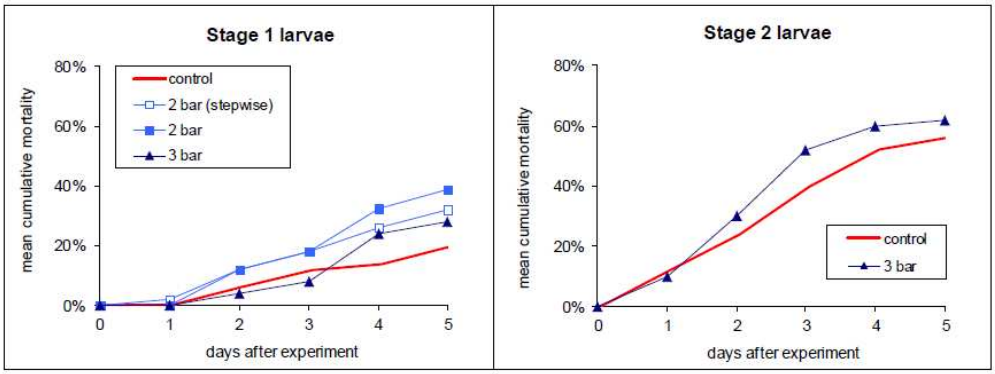

Figure 2. Mean cumulative mortality rates for each treatment 0-5 days after the experiment.

Trial 1

As little is known about the critical values for sound parameters with regard to larval survival, the aim of the first trial was to examine the sensitivity range. Hence we choose to maximise the number of exposures and minimise the number of replicates. A test scheme was designed in which each exposure depended on the results of the previous exposure (IMARES memo 1). This iterative approach is the most effective way to find critical sound exposure levels, but it depends on immediate visibility of the effects of sound exposure.

It was not possible to carry out the 4th test trial before the first sound exposure trial, due to the availability of fish larvae and the duration of the project. The first trial was therefore carried out with a small overpressure (0.5 bar). Young fish larvae (stage 1, yolk-sac stage) were used in the first trial. All experiments were carried out in duplo. The batch size for each experiment was 25 (± 2) larvae.

No immediately visible effects were observed, therefore scenario 1 of the test scheme (IMARES memo 1) was followed. The pressure excitation exposure representing 100m and 100 strokes was replaced by an exposure representing 100m and 50 strokes because of the risk of overheating the set-up. The number of experiments possible in 1 day was lower than anticipated; 6 exposure and 2 control experiments were

carried out in duplo (Table 2). The first control group received exactly the same treatment as the exposure groups. The second control group was not inserted in the test chamber but otherwise received the same treatment. The sound parameters for each distance and number of strokes (Table 2) are estimated based on a 'typical' North Sea piling event (Q7 characteristics: 4m diameter pile, sandy bottom, 20m depth; TNO memo 1).

Table 2. Treatments in trial 1.

Treatment	Over-pressure	Velocity or pressure excitation	Distance	No. of strokes	Peak pressure	SEL	Peak velocity	Integrated velocity	No. of replicates
	bar		m		dB re 1 uPa^2	dB re 1 uPa^2s	dB re 1 $(nm/s)^2$	dB re 1 $(nm/s)^2s$	
control 1	0				0	0	0	0	2
control 2	0.5				0	0	0	0	2
sound exposure	0.5	P	800	1	197	173	133	108	2
sound exposure	0.5	P	100	1	211	187	147	122	2
sound exposure	0.5	P	100	50	211	204	147	139	2
sound exposure	0.5	V	800	1	183	158	133	110	2
sound exposure	0.5	V	100	1	197	172	147	124	2
sound exposure	0.5	V	100	100	197	192	147	144	2

No instantaneous effects were observed. Mortality rates were scored daily until 12 days after the experiment. All experiments (instead of a selection, i.e. contrary to the plans presented in IMARES memo 1) were monitored for a period of 12 days. No clear differences were observed between the different treatments during this period (Figure 3, left panels) Variability between batches with the same treatment was high (illustrated by the error bars in the right panels of Figure 3). Preliminary statistical analyses (ANOVA) show no significant differences between the treatments after 12 days. Final statistical analyses (mixed modelling) have not been carried out yet.

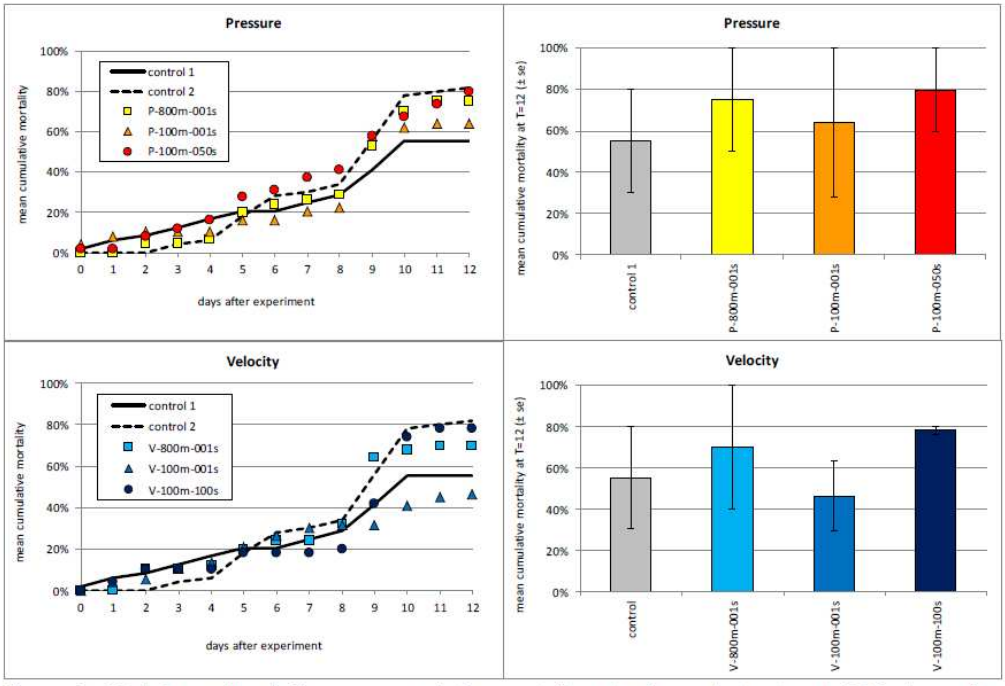

Figure 3. Trial 1 results. Left: mean cumulative mortality rate for each treatment 0-12 days after experiment. Right: mean cumulative mortality rates (± se) for each treatment 12 days after the experiment (95% confidence limit = 12.7*se at n=2).

Trial 2

High 'batch variability' (variability between batches with the same treatment) was observed in the previous trials. The number of replicates required to statistically assess a certain difference between treatments increases with an increase in batch variability. Therefore the number of replicates for each treatment was increased in the 2nd trial, at the expense of the number of exposures. The iterative approach was reduced to 1 exposure representing 100m and 1 stroke and 2 follow-up scenario's. Each scenario consisted of 4 replicates of 6 treatments (4 exposures and 2 controls) in randomised sequence. The randomisation was applied to avoid bias due to potential serial effects in batch variability. A 5th exposure was defined in both scenario's in case time allowed additional experiments.

The intention was to carry out all experiments employing 2 bar overpressure, however, due to technical problems we had to change the overpressure to 0 bar. Stage 2 larvae were used in the second trial. The batch size for each experiment was 25 (±2).

No immediately visible effects were observed, therefore the scenario consisting of high sound exposures was followed (Table 3). The number of experiments which could be carried out in 1 day was higher than during the previous trial (because of experience gained, increased size of larvae and omission of static pressure). Four exposure and 2 control experiments were carried out in 4-fold and randomised sequence. A 5th exposure (100m, 100 strokes) was carried out in 4-fold at the end of the day. The sequence of monitoring was randomised over all treatments. The first control group received exactly the same treatment as the exposure groups. The second control group was not inserted in the test chamber but otherwise received the same treatment. The sound parameters for each distance and number of strokes (Table 3) are estimated based on a 'typical' North Sea piling event (Q7 characteristics: 4m diameter pile, sandy bottom, 20m depth; TNO memo 1).

Table 3. Treatments in trial 2.

Treatment	Over-pressure	Velocity or pressure excitation	Distance	No. of strokes	Peak pressure	SEL	Peak velocity	Integrated velocity	No. of replicates
	bar		m		dB re 1 uPa^2	dB re 1 uPa^2s	dB re 1 $(nm/s)^2$	dB re 1 $(nm/s)^2s$	
control 1	0				0	0	0	0	4
control 2	0				0	0	0	0	5
sound exposure	0	P	200	200	206	205	142	140	4
sound exposure	0	P	100	50	211	204	147	139	4
sound exposure	0	P	100	100	211	207	147	142	4
sound exposure	0	V	200	200	192	190	142	142	4
sound exposure	0	V	100	100	197	192	147	144	4

No instantaneous effects were observed. Mortality rates were scored daily until 10 days after the experiment, for all experiments. The only exposure which appeared to have an effect on mortality after 5-10 days was the highest pressure exposure, corresponding to a distance of 100m and 100 strokes (Figure 4, left panels). After 10 days, a cumulative mortality rate of 80% was observed for this exposure compared to 60% in the control group (i.e. 50% of the larvae which survive 'natural mortality' are killed due to noise). Preliminary statistical analyses indicate that the difference is not significant (ANOVA, P=0.14). Final statistical analyses (mixed modelling) have not been carried out yet. A larger number of replicates is necessary to be able to assess the statistical significance of a difference of this magnitude, given the high variability between batches with the same treatment.

If significant, the results indicate a sharp threshold at 207 dB (cumulative SEL), which is 24 dB above the threshold suggested by the US Caltrans Fisheries Hydro-acoustic Working Group for fish <2 gram (Oestman et al. 2009). If significant, the hypothesis of 100% mortality up to a distance of 1000m from the pile driving site (as assumed in the Appropriate Assessment Dutch offshore wind farms, Prins et al. 2009) can be rejected.

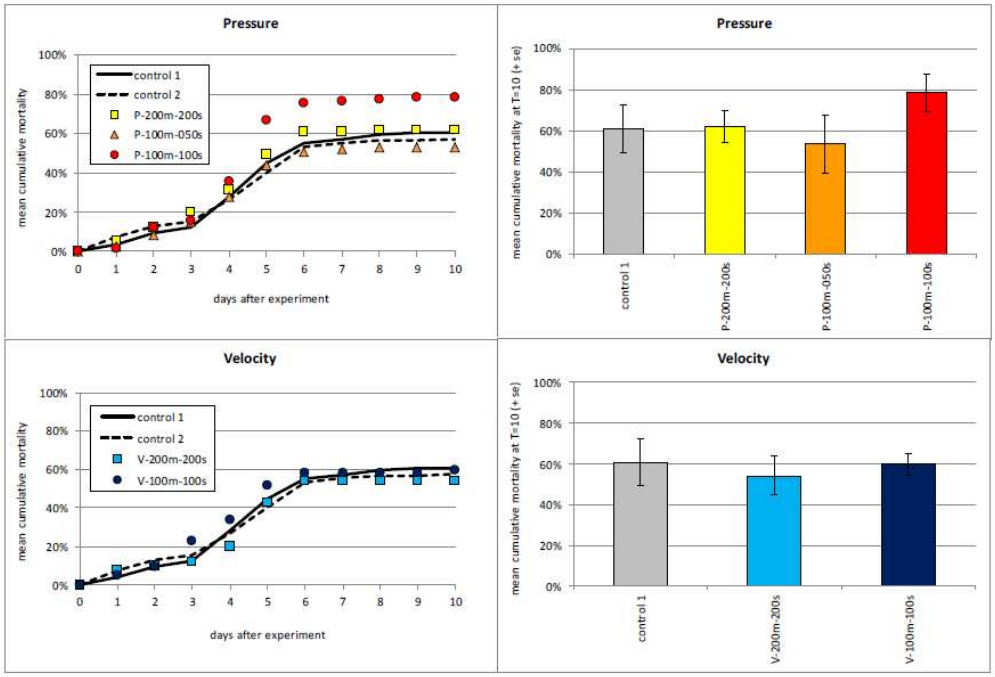

Figure 4. Trial 2 results. Left: mean cumulative mortality rate for each treatment 0-10 days after experiment. Right: mean cumulative mortality rates (± se) for each treatment 10 days after the experiment (95% confidence limit = 3.2*se at n=4)

Trial 3

The same approach was chosen for trial 3 as for trial 2, that is 1 exposure representing 100m and 1 stroke and 2 follow-up scenario's. The number of replicates for each treatment was further increased to 5 (95% confidence limit = 2.8*se at n=5). Like in trial 2, 4 exposures and 2 controls were carried out in randomised sequence, and a 5th exposure was defined in case time allowed additional experiments.

Stage 3 larvae were used in the third trial. In this larval stage the swim bladder is maximally inflated and hence a higher sensitivity to sound waves is expected compared to the larval stages used in the previous trials. The 2nd trial actually already aimed at stage 3 larvae but the development rates proved to be lower than expected based on a literature review (IMARES memo 1). A sample of larvae was examined on the day of the experiments and the stages ranged from 3a to 4a with the majority of larvae in stage 3b (stages according to Al-Maghazachi & Gibson 1984).

The batch size for each experiment was 28 (±2). All experiments were carried out with no overpressure to be consistent with the previous trials.

No immediately visible effects were observed, therefore the scenario consisting of high sound exposures was followed (Table 4). Four exposure and 2 control experiments were carried out in 5-fold and randomised sequence. A 5th exposure (100m, 10 strokes) was carried out in 4-fold at the end of the day. The sequence of monitoring was randomised over all treatments. The first control group received exactly the same treatment as the exposure groups. The second control group was not inserted in the test chamber but

otherwise received the same treatment. The sound parameters for each distance and number of strokes (Table 4) are estimated based on a 'typical' North Sea piling event (Q7 characteristics: 4m diameter pile, sandy bottom, 20m depth; TNO memo 1). The first 4 exposures consisted of 2 pressure excitation exposures with different peak pressure levels and the same cumulative SEL, and 2 velocity excitation exposures with different peak velocity and the same integrated velocity. The additional exposure was a pressure excitation exposure in which the cumulative SEL was reduced by 10 dB compared to the previous pressure excitation exposures.

Table 4. Treatments in trial 3.

Treatment	Over-pressure	Velocity or pressure excitation	Distance	No. of pulses	Peak pressure	SEL	Peak velocity	Integrated velocity	No. of replicates
	bar		m		dB re 1 uPa^2	dB re 1 uPa^2s	dB re 1 $(nm/s)^2$	dB re 1 $(nm/s)^2s$	
control 1	0				0	0	0	0	5
control 2	0				0	0	0	0	5
sound exposure	0	P	100	10	211	197	147	132	4
sound exposure	0	P	200	300	206	207	142	142	5
sound exposure	0	P	100	100	211	207	147	142	5
sound exposure	0	V	200	300	192	192	142	144	5
sound exposure	0	V	100	100	197	192	147	144	5

No instantaneous effects were observed. Mortality rates will be scored daily until 10-12 days after the experiment, for all experiments. These results are not yet available.

References

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Oestman R, Buehler D, Reyff JA, Rodkin R (2009) Sacramento: California Department of Transportation. 'CALTRANS Technical Guidance for Assessment and Mitigation of the Hydroacoustic Effects of Pile Driving on Fish'

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