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Testing spatial avoidance and behavioural changes in European seabass in a floating pen in response to sounds from the FaunaGuard-Fish Module (FG-FM)

Research report 2

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Content

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Executive summary

Background

Aquatic animals live in an acoustic world and rely on sounds for survival and reproduction and can be negatively affected by noise pollution. GEMINI conducts construction activities in the Dutch North Sea, off the Dutch Wadden Coast, for building an offshore wind farm. The construction activities include pile driving activities that yield sound exposures at levels that are potentially harmful to nearby fishes. Van Oord Dredging and Marine Contractors has been exploring whether sound can also protect fishes and whether sound playback could be an effective mitigation measure against over-exposure. SEAMARCO and van Oord have therefore developed an Acoustic Deterrent Device (ADD), equipped with a sound sequence specifically for fish, labelled the FaunaGuard-Fish Module (FG-FM). The objective of the first part of the current study was to test the behavioural effect of exposure to the FG-FM sound series in natural sound field conditions with fish in an outdoor floating pen (Hubert et al. Research Report 1, 15-02-2017). Overall, the results of the first part were disappointing in terms of an overall lack of consistent responsiveness.

Objective and methods

The second part of the current project was already planned and conducted before the findings of part one had become clear. The design of this second part was, therefore, not adjusted based on the uncertainties resulting from the first part of the study and followed exactly the same protocol and procedures. The target of the second part was to explore kurtosis as an acoustic parameter that could objectively describe sound conditions in a single unit that would capture those features that determine acoustic responsiveness in aquatic animals. Kurtosis is a statistical measure that is used to describe a sample or data distribution and is a measure of how long and far the tails are relative to the rest of the distribution. In order to collect data on the potential validity of kurtosis as an acoustic responsiveness measure, we tested variability in response level to variation in sound energy distribution in terms of 1) pulse rate and 2) signal-to-noise level (expressed as crest factor) by exposing fish to pulses of brown noise spectra of three different levels against two levels of elevated ambient noise.

Results and interpretation

Fish from the same batch as used for experiment 1 responded consistently and thereby significantly to sound stimuli that were played at similar amplitude but were in terms of acoustic structure more like in previous studies (intermittent pulses of brown noise). This significant effect was found in one out of the four parameters: swimming depth. Pulse rate did not affect behavioural patterns during sound exposure in any of the four parameters, but a subtle but significant effect was found for pulse rate on a more detailed analysis for spatial avoidance. Detailed comparisons of behavioural response data from both experiment 1 and experiment 2 clearly reveal a distinct difference: only occasional cases of potential sound-induced changes in behaviour in response to FG-FM sounds and much more consistent changes in swimming depth in response to the intermittent pulse trains.

There was a non-significant trend for the crest factor to affect responsiveness. The effect may become significant with slightly more samples or slightly more contrast in this crest factor. The relevance of this is that not only how loud a pulse is will affect response tendencies in fishes, but also ambient levels matter. This means that at rough seas, when noise levels are higher, sound stimuli likely need to be louder to elicit deterrent responses. The apparent lack of any biologically relevant spatial response in the horizontal plane may be due to the fact that: the fishes are not able to localize the direction of the sound source; the natural response is just diving down, the pen size and the captive conditions in general restrict behavioural response freedom.

We used the potential value of kurtosis as a measure for impact assessment as our guideline for the experimental design in experiment 2. Traditional measures of impact just take amplitude in a particular frequency range into account (sound pressure or particle motion) or at best also the duration of exposure (sound exposure level, SEL). However, we found the intuitive match of the measure of kurtosis with the acoustic parameters that trigger responsiveness to be only partly valid and not very satisfying. Despite the fact that pulse rate and crest factor were two relevant parameters to explore in terms of behavioural responsiveness, we dismiss kurtosis as a very suitable measure to continue exploring.

Conclusions and recommendations

The FaunaGuard-Fish Module sound series did not elicit consistent behavioural responses in groups of four seabass swimming in a large outdoor floating pen. The same set-up, including species, group size, locality, sound levels, and playback procedure, but with different pulse-type sound series, did elicit significant changes, in particular in swimming depth, in previous studies and in the current study. These results confirm earlier studies that fishes in general and this species in particular respond to sound and shift down in the water column. The fact that other, longer and more tonal, sounds elicit less of a response at similar amplitude levels, indicates that acoustic structure matters and that the pulse-like structure, repetitive nature, and/or the bias to low-frequencies are critical for detection and/or decision making. This means that it is very likely possible to improve the deterrent capacity of the FG-FM sound series, although tests on multiple species and free-ranging conditions remain necessary. Furthermore, none of the response-triggering sounds played in earlier and the current experiments triggered a clear spatial avoidance response in the horizontal plane. As this is a critical aspect of the explicit target of an Acoustic Deterrent Device (ADD), more studies are needed to test and improve the ADD under field conditions. The currents state of the art in terms of the efficiency of ADDs in the application with fishes makes us come up with the following recommendations:

1. Improve responsiveness to FG-FM sounds directly by including more pulse train like sounds and explore the effect of higher pulse rates - tests in the floating pen at different sound levels would allow to compare pulse-rate dependent dose-response curves.
2. Investigate the effect of alternating or varying sounds in a sequence on response tendency and habituation – tests for adequate acoustic contrast should be included and are only feasible in a floating pen type set-up, with sufficient control and replication.
3. Assess the source level of the FG-FM and model spatial soundscape gradients for areas of application – this step is critical to match spatial information on fish position and response tendency with sound conditions.
4. Apply the FG-FM sound exposure at two distinct field sites (to get the minimal in replication and still a feasible challenge for fieldwork) with virtual source locations of anthropogenic acoustic danger and monitor free-ranging fish (multiple species – again probably best to select two ecologically distinct species) by telemetry – this step is critical for any final positive evaluation about ADD efficiency.

1. Introduction

GEMINI conducts construction activities in the Dutch North Sea, off the Dutch Wadden Coast, for building an offshore wind farm. The construction activities include pile driving activities that yield sound exposures at levels that are potentially harmful to nearby fishes and may have negative effects on local fish communities. It is in the context of this kind of activities and threats that Van Oord Dredging and Marine Contractors has been exploring whether sound can also protect fishes and whether sound playback could be an effective mitigation measure against over-exposure. SEAMARCO and van Oord have therefore developed an Acoustic Deterrent Device (ADD), equipped with a sound sequence specifically for fish, labelled the FaunaGuard-Fish Module (FG-FM). The FG-FM has been applied in the field in Sweden, Brasil and the Netherlands and anecdotal evidence suggests that it works well and could potentially save a lot of fish (Kastelein et al. 2011; Van der Meij et al. 2015).

1.1 Main findings of report 1

The FG-FM sound stimuli had only been tested in a fish tank context, in which the potential to trigger a startle response was used to improve the deterrent capacity of the 20 sounds that are put into a sequence with variable interval duration (Kastelein et al. 2011; 2012; reviewed in Hubert et al. Research Report 1, 15-02-2017). The objective of the first part of the current study was therefore to test the behavioural effect of exposure to the FG-FM sound series in natural sound field conditions with fish in an outdoor floating pen (c.f. Neo et al. 2016). A dose-response curve was the explicit target and we therefore tested behavioural responses of European seabass (*Dicentrarchus labrax*) to the maximum output level and a step-wise series of lower amplitude. We compared these to broad-band white noise sounds in the same temporal pattern as the FG-FM sounds at a slightly lower but similar amplitude level as a control.

Overall, the results of the first experiment were disappointing in terms of a lack of consistent responsiveness. There was no significant response to sound exposure in any of the four parameters: no decrease in swimming depth, no change in distance to the speaker, and also no change in swimming speed or group coherence. This was unexpected as typical response patterns such as diving down the water column, changing swimming speed and group coherence were reported in previous experiments, using the very same set-up, exposure tools and fish species (Neo et al. 2016; Neo 2016). We interpreted these results as that the FG-FM sounds (and the white noise control sounds) were less suitable in terms of temporal and spectral features, to elicit significant changes in behaviour than those sounds of previous experiments (Neo et al. 2016; 2016) and probably needed higher sound levels than tested to potentially trigger the same behavioural effects.

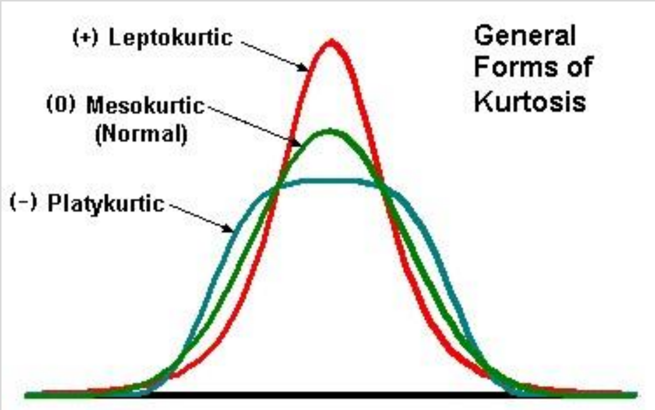
1.2 Remaining questions and current objectives

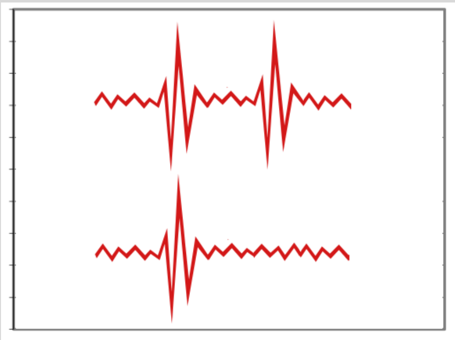
However, given the anecdotal evidence from efficacy of the FG-FM during field applications, it could be possible that free-ranging fish respond differently and stronger than captive fish in a net pen; and that fishes from other species or background than hatchery-reared seabass respond differently and stronger. Nevertheless, we also considered a number of alternative, but not mutually exclusive, explanations for the negative results that could serve as guidelines for follow-ups. 1) The batch of fish could have been less sensitive than previous batches, which were of the same species and of similar size, but came from another hatchery. 2) The temporal pattern of sound stimuli in earlier experiments (relatively brief pulses and brief intervals) were more potent in triggering a response. 3) The tones and spectral composition of FG-FM sounds and the white noise control sounds were spectrally less salient than previously used sounds (brown noise).

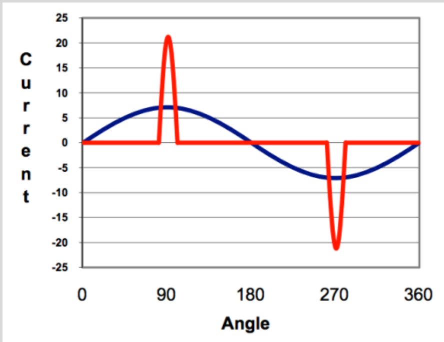
The second part of the current project was already planned and conducted before the findings of part one had become clear, due to time constraints and ending of the field season. The design of this second part was, therefore, not adjusted based on the uncertainties resulting from the first part of the study. The target of the second part was to explore kurtosis as an acoustic parameter that could objectively describe sound conditions in a single unit that would capture those features that determine acoustic responsiveness in aquatic animals. The background of this was that traditional measures just take amplitude in a particular frequency range into account (sound pressure or particle motion) or at best also the duration of exposure (sound exposure level, SEL). However, the distribution of sound energy in the spectrum (Estramil et al. 2010), but especially in the temporal range (Neo et al. 2014) can also affect response levels and is not represented in any current acoustic measure. Kurtosis is a statistical measure that is used to describe a sample or data distribution and is a measure of how long and far the tails are relative to the rest of the distribution (Box 1). The intuitive match of the measure of kurtosis with acoustic parameters that are known or expected to affect behavioural response levels is only partly valid as will be further addressed in the discussion.

In order to collect data on the potential validity of kurtosis as an acoustic responsiveness measure, we tested variability in response level to variation in sound energy distribution in terms of 1) pulse rate (c.f. Neo et al. 2015) and 2) signal-to-noise level (expressed as crest factor, see Box 1 for explanation) by exposing fish to pulses of brown noise spectra of three different levels against two levels of elevated ambient noise. These aims meant that the most urgent test target after the findings of report 1 was also accomplished: Test the same seabass batch in the same net pen to sounds of different temporal and spectral pattern that were more like those tested in Neo et al. (2016). This yielded critical insights into the negative data of the first experiment and revealed whether the fish tested in experiment 1 could be triggered by sound at all or if they were an exceptionally insensitive batch.

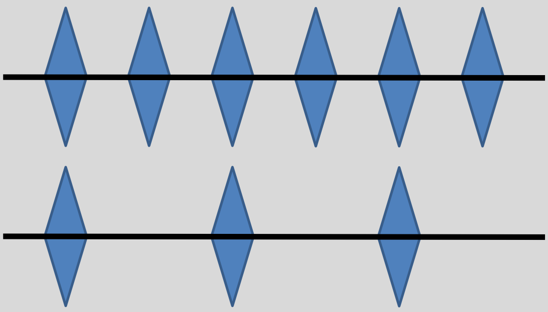
Box 1: Overview of the statistical concept of kurtosis, the phenomena of pulse rate and crest factor, and the acoustic parameters varied in our experimental design.

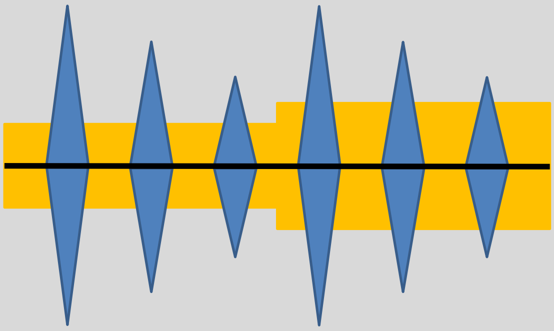






Top: Forms of *kurtosis*; variants in sample distributions that are all symmetrical but vary in shape of the tails on both ends. **Left:** *pulse rate* illustrated by two rates of a heart beat pulse signal. **Right:** *Crest factor* explained for a current: the red and blue waveforms both have an rms current of 5 A, but their crest factors are different. The sinusoidal current has a peak value of 7.07 A and a crest factor of 1.414 (7.07 A / 5 A). The non-sinusoidal current has a peak value of 21.21 A and a crest factor of 4.24 (21.21 A / 5 A).





Bottom left: Schematic illustration of *Pulse rate variation* as applied to artificially generated sound files for four different test conditions: 0.5, 1.0, 2.0 and 4.0 pulses per second. **Bottom right:** *Crest factor variation* as applied (not in a single series) by artificially generated sound files for pulse signals of three different amplitude levels (in blue) and background noise at two different amplitude levels (in orange), yielding six different crest factor test conditions.

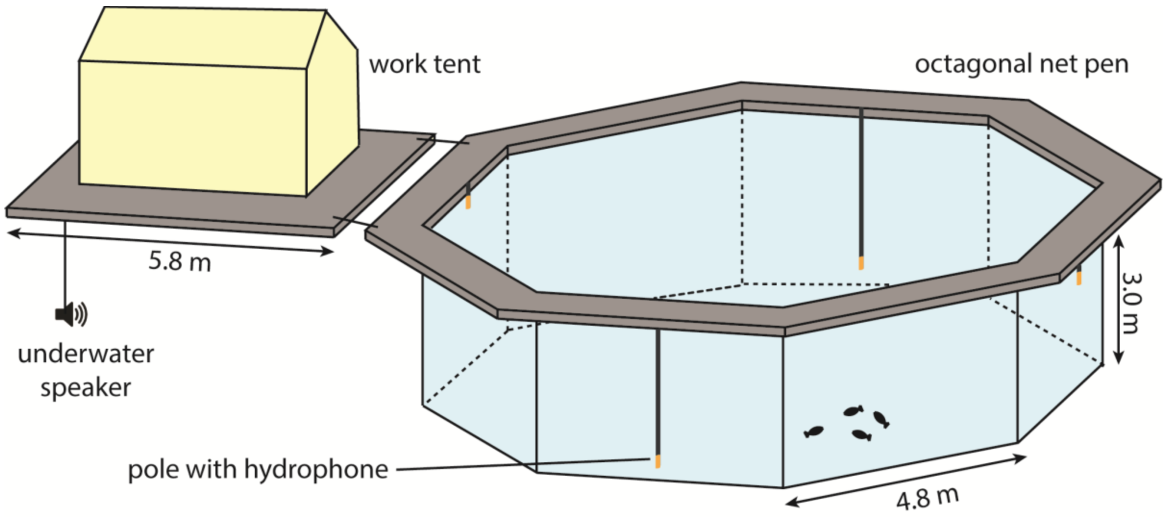
1.3 Research questions

1. Do fish from the same batch used for experiment 1 respond to sound stimuli that are played at similar amplitude but in terms of acoustic structure more like in previous studies (brown noise and intermittent)?

2. Does pulse rate affect behavioural patterns during sound exposure for a replicated set of four seabass (n = 16) swimming in an outdoor floating pen?

3. Do brown noise pulse amplitude and ambient background level (both affecting signal-to-noise ratio), affect behavioural responsiveness?

4. If any of these questions would get a positive answer, we would follow-up with a question based on one of the objectives for the FG-FM-sounds: Do fish show spatial avoidance in the horizontal plane during exposure in a natural sound field?



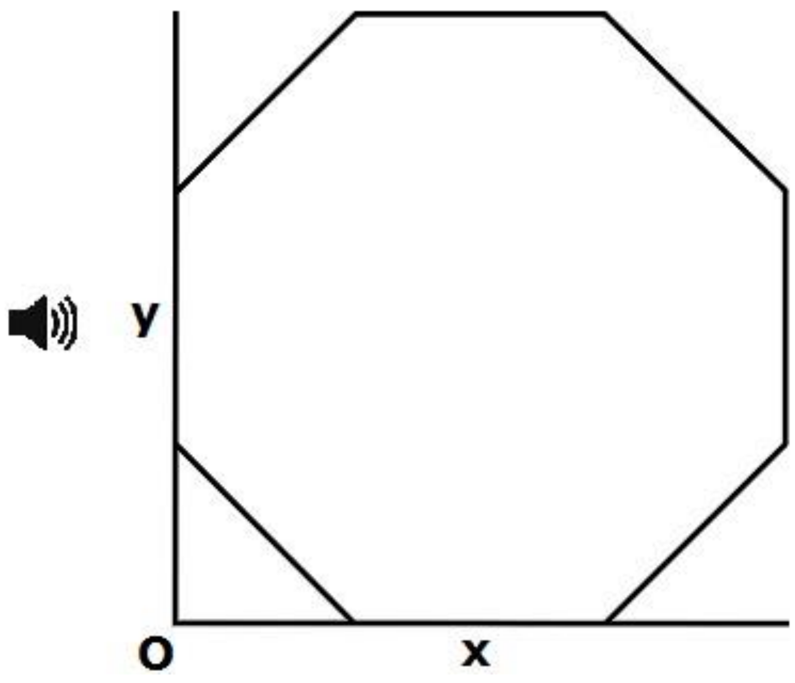


Figure 1: Schematic representation of the floating pen, with work tent on adjacent platform and speaker and hydrophone positions (top) and the axes derived from this set-up for the fish' positions in the horizontal plane relative to the speaker side. x = 0 means at the side closest to the speaker, x = 11.5 means at the side furthest from the speaker. The x-values are expressed in meters.

2. Materials and methods

2.1 Subjects

We used 16 groups of four European seabass (*Dicentrarchus labrax*) of 35 to 40 cm in body length from the same batch as used for the first experiment of this study. The fish were acquired from a hatchery (FRESH, Völklingen, Germany) and kept in two indoor holding tanks (Ø 3.5 m, depth 1.2 m) at Stichting Zeeschelp (Kamperland, The Netherlands) in a dark-light cycle similar to the outside day-night cycle. The water in the holding tanks was continuously refreshed with seawater from the Estern Scheldt (Oosterschelde), an estuary of the Dutch North Sea. The fish were fed with commercial pellets (Aller Blue Organic EX 8 mm, AllerAqua), for which the amount was determined by the temperature of the water. All experiments were conducted in accordance with the Dutch Experiments on Animals Act and approved by the Dutch Central Commission Animal Experiments (CCD) under no. AV D106002016610.

2.2 Experimental arena

The experiment was conducted using a study island in the Jacobahaven, a man-made cove in the Eastern Scheldt. The Jacobahaven is about 200 m wide, 300 m long and depending on the tides 2-5 m deep. The Jacobahaven is situated near the Eastern Scheldt storm surge barrier and no external boat traffic is allowed within 1 km of the site. We assembled the study island using a modular floating system (Candock, Canada), consisting of a working platform for the equipment and researchers and an octagonal net pen (Ø 11.5-12.5 m, >3 m deep) as arena for the fish (Fig. 1). The two platforms were separated with a 0.5 m distance to avoid noise transmission from the working platform to the net pen. The working platform supported the speaker, in this way the distance from the speaker to the net was 7.8 m and unwanted near-field effects of the speaker were avoided. The set-up has been used for previous sound exposure studies (Neo et al. 2016; Neo 2016) and detailed measurements of the underwater soundscape revealed a clear sound level gradient across the net pen (see Hubert et al. Research Report 1, 15-02-2017).

2.3 Tagging fish

We tracked the swimming patterns of the four fish in the net pen using acoustic tags (Model 795-LG, HTI, US) that emitted 307 kHz pings at an ~ 1s pulse rate interval (PRI). Fish could be identified and tracked individually because of subtle differences in the programmed PRI of the different tags. At the net pen, the pings of the tags were received by four hydrophones (Model 590-series, HTI, US), two close to the surface and two close to the bottom, and stored on a laptop via a specialized oscilloscope (Model 291, HTI, US). The spatial resolution is determined by the potential of data on 60 fish locations per minute (for each of the four fish) and the amount of missing values for one or more hydrophones at any particular time point, due to signal collision or problematic signal-to-noise ratios. The percentage of missing value is typically around 20-30%, yielding spatial resolution of about 40 samples per minute. Before tagging, the fish were anaesthetised in a bath with 2-phenylethanol (0.5ml/l seawater) similar to the tagging procedure conducted for the first experiment (see Hubert et al. Research Report 1, 15-02-2017).

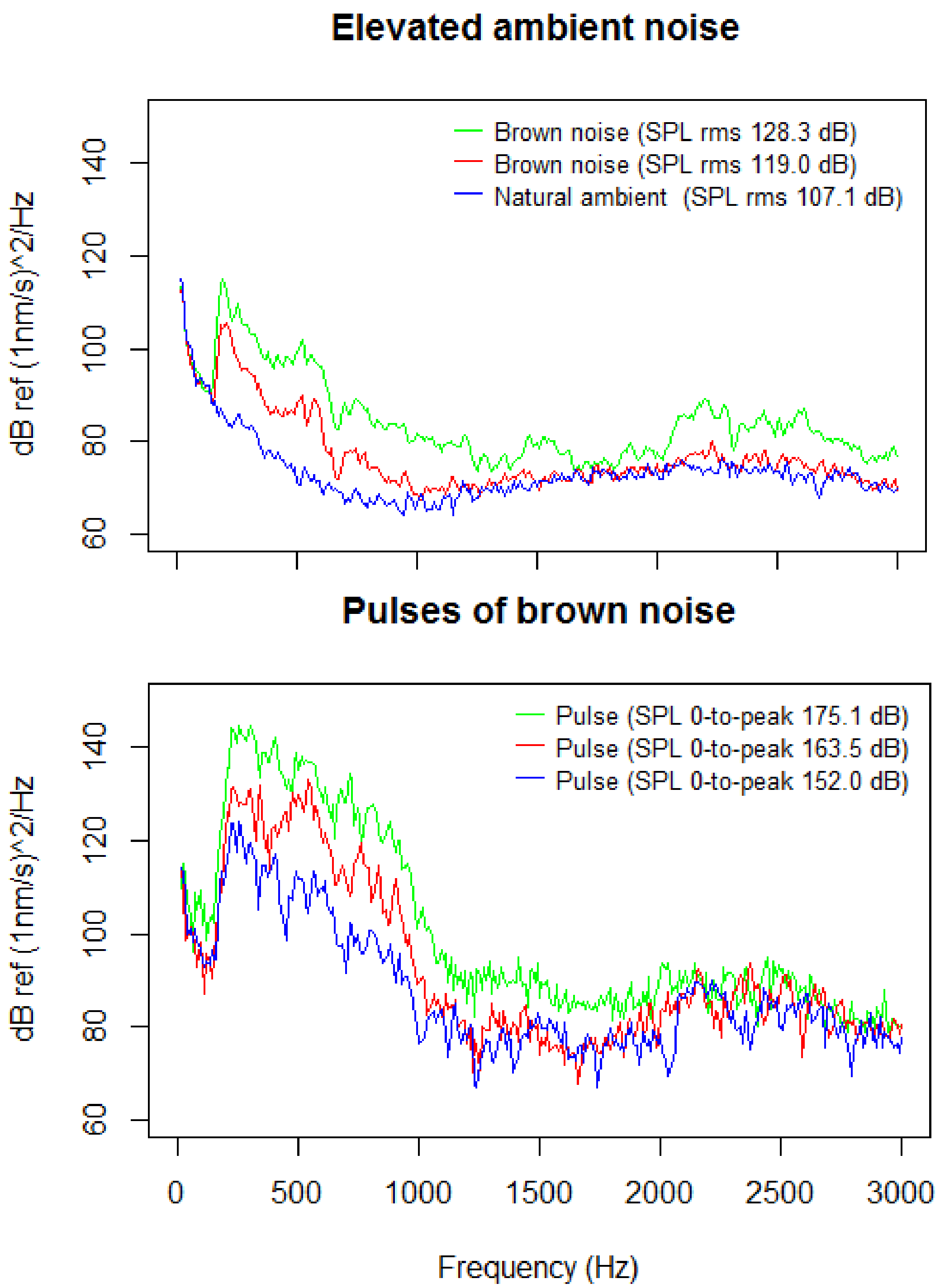


Fig. 2. Power spectral density plots of the ambient noise spectrum and the two levels of elevated ambient background conditions (top) and the brown noise pulse spectra (bottom).

2.4 Treatment series

We exposed all groups of fish to six sound treatments consisting of 0.1 s pulses and elevated ambient noise. The treatments differed in pulse rate interval (PRI), sound level of the pulses, sound level of the elevated ambient noise and thereby also in signal to noise ratio (SNR, see 2.5). The sound treatments were created in Audacity 2.0.5. For the pulses, we generated a track of brown noise and applied a high-pass filter of 200 Hz and a low-pass filter of 1000 Hz (Fig. 2). The actual pulses were created by making silences in the track, such to obtain a fixed PRI of 0.5, 1.0, 2.0 or 4.0 s. For the elevated ambient noise, we generated another track of brown noise with a fade-in of 5 minutes to smoothen the transition from the natural ambient noise to the elevated ambient noise. The elevated ambient noise started 20 minutes before the first pulse and ended 5 minutes after the last pulse, the impulsive sound took 30 minutes, so the total playback lasted for 55 minutes. The 0-to-peak sound pressure levels (SPL) of the pulses were 152, 164, and 175 dB ref 1uPa (resp. 94, 106, and 116 dB ref 1nm/s) (Fig. 3), rms SPL of the elevated ambient was 119 or 128 dB ref 1uPa (resp. 61 or 70 dB ref 1nm/s). The stimuli used in experiment 1 (Fig. 4) and in the current experiment 2 (Fig. 5) were played at similar amplitude but differed strongly in spectrum and temporal pattern.

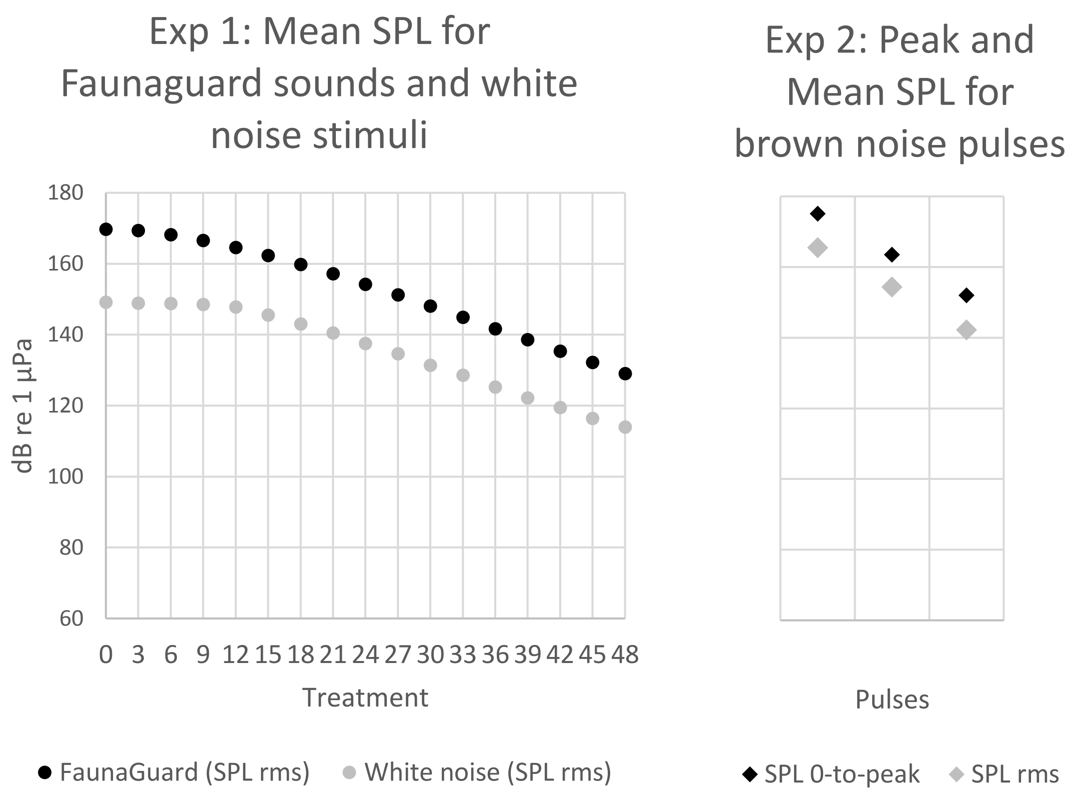


Fig. 3. Sound pressure levels of the different treatments in experiment 1 of this study (Exp 1, left) as reported before (see Hubert et al. Research Report 1, 15-02-2017) and the sound pressure levels of the brown noise pulses used in the current experiment 2 (Exp 2, right). Note that the black and grey dots on the left should be compared only to the grey diamonds on the right (all rms-values). 4 out of 17 treatment levels in Exp 1 were played at a louder rms-value than any of the 3 pulse levels in Exp 2. Also 4 out of the 17 treatments in experiment 1 were less loud than any pulse level in Exp 2. The black diamonds are peak measures of the 100 ms pulses used in the second experiment, not measured for the long-drawn sound stimuli used in the first experiment.

The sounds were played back with an underwater transducer (LL-1424HP, Lubell Labs, Columbus, US) using a laptop, a power amplifier (DIGIT 3K6, SynQ) and a transformer (AC1424HP, Lubell Labs). To examine the actual sound levels present in the net pen, we measured sound pressure levels (SPL) and sound velocity levels (SVL) twice at six distances from the speaker (every 2.1 m, from 8.3 to 18.8 m from the speaker). The measurements were done using the M20 particle velocity sensor (GeoSpectrum Technologies, Canada), this sensor measures sound pressure using an omnidirectional hydrophone and 3D particle velocity using three accelerometers. Calibration of the sensor was provided by the manufacturer. The signals were stored on a laptop at 40 kHz via a current-to-voltage convertor box (GeoSpectrum Technologies Inc., Canada) and a differential oscilloscope (PicoScope 3425, Pico Technologies, UK). The recordings were later processed with the Matlab application paPAM (c.f. Nedelec et al., 2016) using a 200-1000 Hz band-pass filter (all procedures and processing were the same as for the first experiment: see Hubert et al. Research Report 1, 15-02-2017).

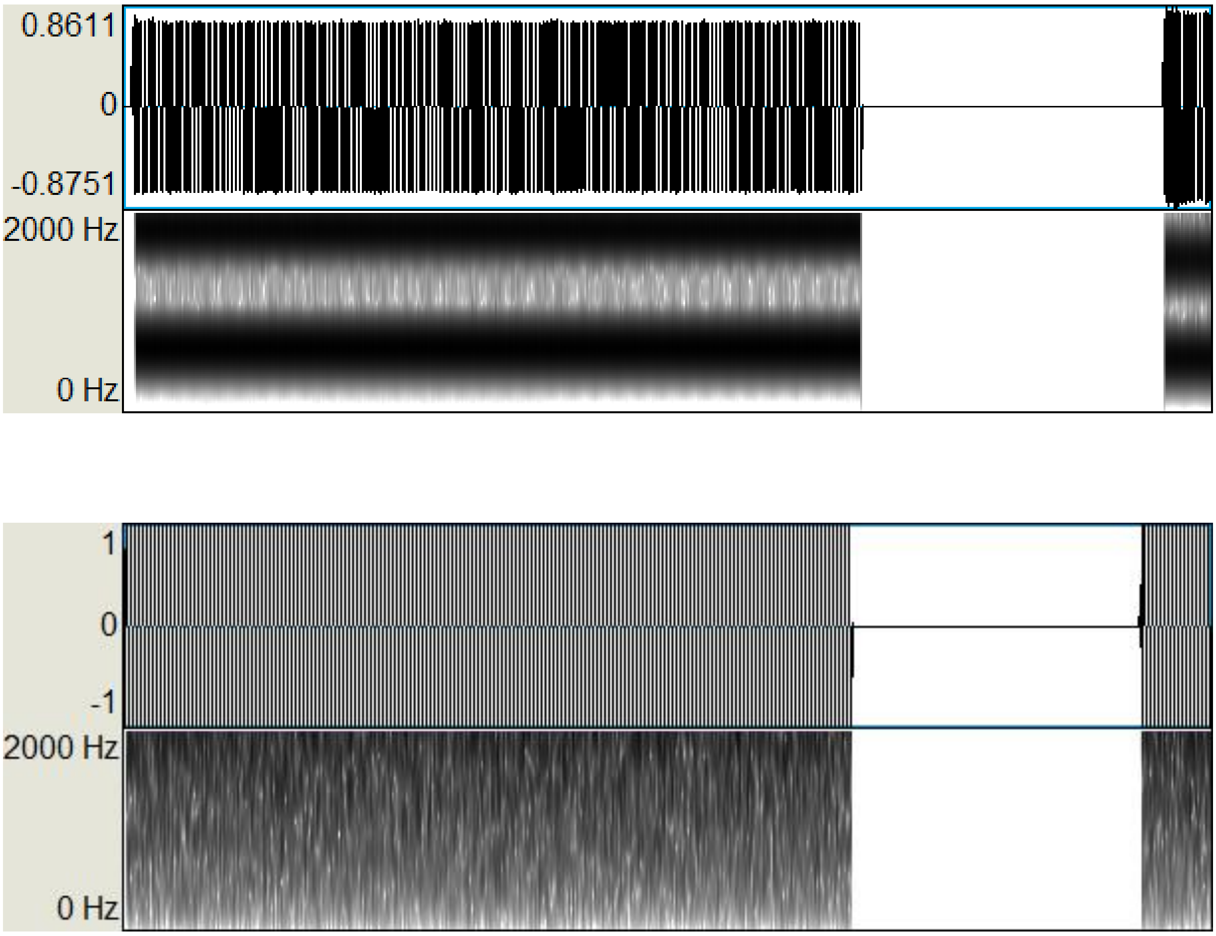


Figure 4: First 15 seconds of the FaunaGuard sound series (top) and the white noise sound treatment as used in experiment 1 and reported before (see Hubert et al. Research Report 1, 15-02-2017). Each sound is depicted with a time domain waveform (upper part) and a spectrogram (bottom part) for comparison with the sounds of experiment 2 (Fig. 4).

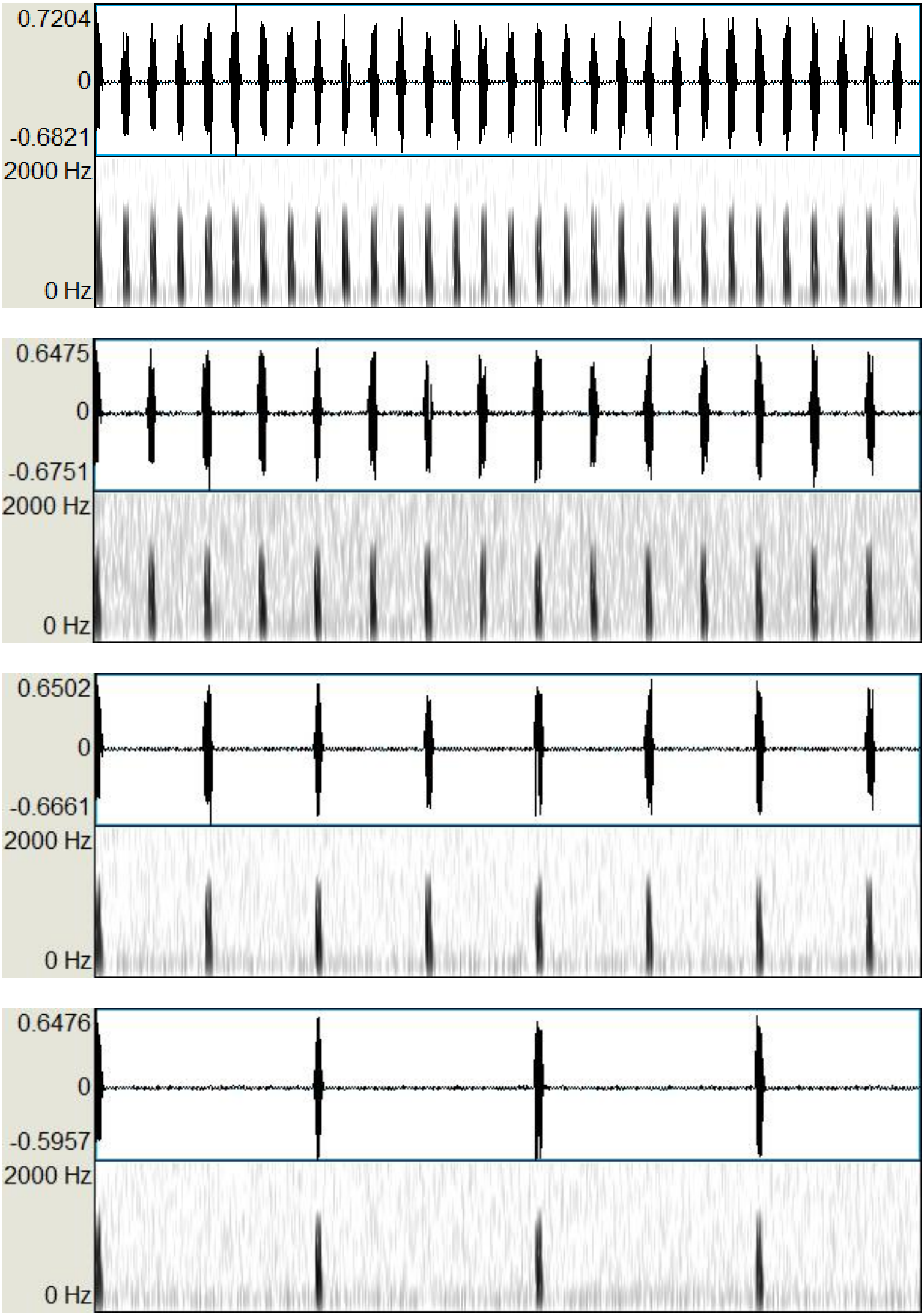


Figure 5: First 15 seconds of the impulsive sound series of brown noise pulses used for the current experiment: from top to bottom: PRI = 0.5 s, 1.0 s, 2.0 s, and 4.0 s, respectively (wave form and spectrogram for each).

2.5 Experimental design

We exposed each group of four fish to six different sound treatments. The order of the treatments followed a complete counterbalanced design, each group was exposed to all PRI's at least once, all pulse sound levels twice and both ambient levels thrice. Each group of fish was tagged at least two days (>40 h) before transfer to the net pen. Transfer took place in a plastic container (60L) and the fish could acclimatize overnight, for at least 8 h. A group was exposed to three treatments per day, for two days (Fig. 6). We conducted one trial at flood tide (starting 2:45 h before absolute high tide), one at high tide (starting 0:20 h before absolute high tide) and one at ebb tide (ending 2:45 h after absolute high tide). This schedule was chosen to ensure that the water level in the Jacobahaven was deep enough (>3 m) during the trials to maintain a fixed difference in depth between the tag-receiver hydrophones.

The researchers arrived at the platform about 25 min before the start of the playback of the sound treatment. Upon arrival, all equipment was switched on. 15 minutes after the start of the sound treatment, the software to track the positions of the fish was started and ran until the end of the sound treatment. In this way, we tracked the fish 5 minutes before the start of the impulsive sound, during the 30 minutes of impulsive sound and 5 minutes after the impulsive sound. After a group was exposed to six treatments, it was caught and sacrificed (all procedures and processing were the same as for the first experiment: see Hubert et al. Research Report 1, 15-02-2017).

2.6 Statistics

The received tag signals were processed on a computer using MarkTags v6.1 & Acoustic Tag v6.0 (HTI, US). This led to the x, y, z coordinates of the 3D swimming patterns of all fish. These coordinates were used to calculate swimming depth, distance from the speaker, swimming speed and average inter-individual distance (group cohesion). To test for behavioural responses, we used 5-minute-bin-averages of these parameters from before the impulsive sound ('before'), after the start of the impulsive sound ('during1'), before the end of the impulsive sound ('during2') and after the end of the impulsive sound ('after') (cf. Neo et al. 2014). To capture the transient speed change we used 10-s-bin-averages for the parameter swimming speed.

All statistics were done using R-Studio and the packages nlme and multcomp. We used linear mixed effects models, followed by Tukey-HSD post-hoc tests to statistically compare the bin-averages of the different behavioural parameters. We used group as a random effect and the parameter(s) of interest as fixed effects. After this we used linear mixed effects models to correlate changes in behavioural parameters to the acoustical parameters that varied between exposures. We selected the best model based on AIC. One of the acoustical parameters was the signal-to-noise ratio, which we calculated as follows:

$$[1] \qquad SNR_{dB} = \frac{P_{signal,dB\,(0-to-peak)}}{P_{noise,dB\,(rms)}}$$

In the results section, we also show the results of the previous experiment in which we compared the effect of the sounds of the FaunaGuard-Fish module with white noise. No new statistics have been applied on these results and we did not include these results into our control for multiple comparisons.

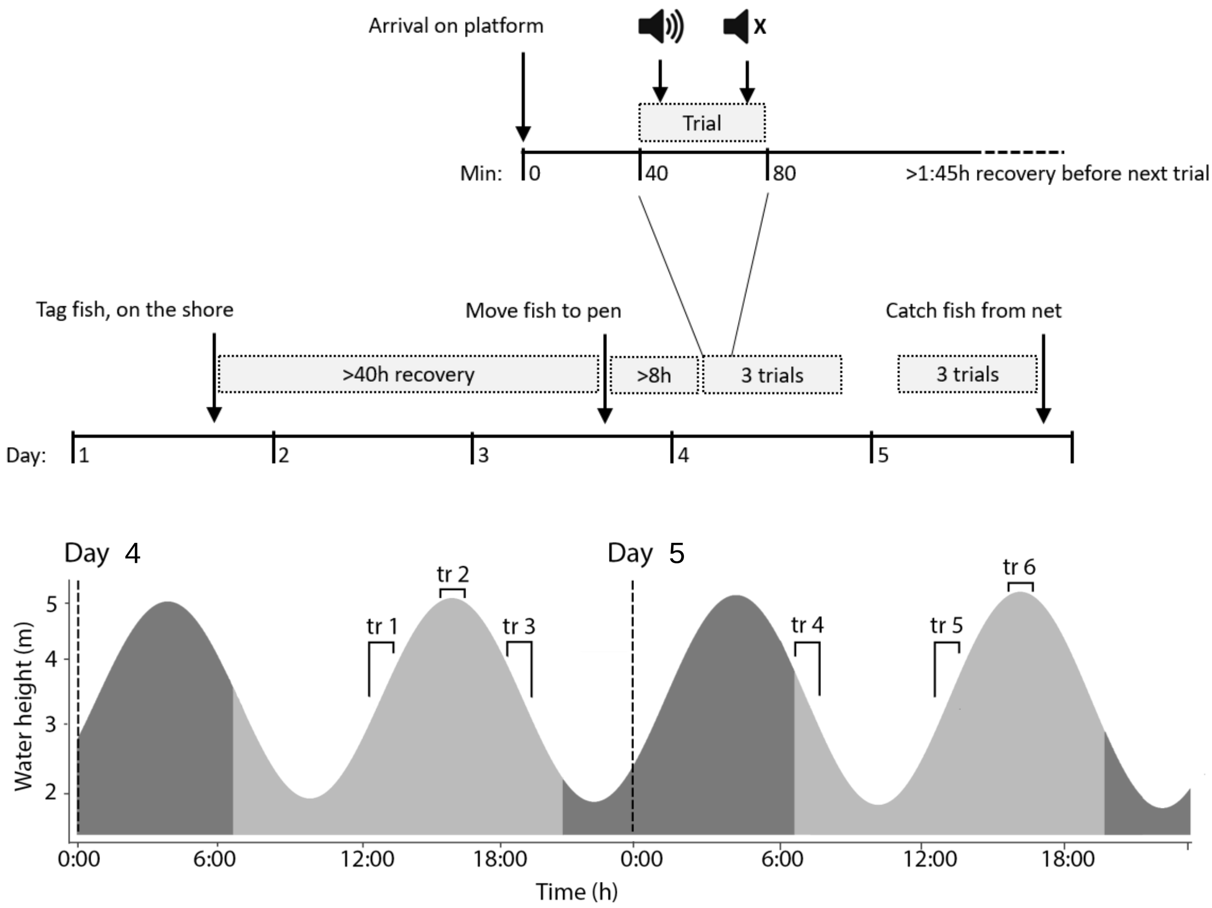


Figure 6: Timetable for the five-day experimental processing of a single group of four fish (top panel). Tagging was done on day 1, transition to the floating pen on day 3, and six half-hour sound exposure trials took place on day 4 and 5. We conducted three trials per day, before, during and after absolute high tide (bottom panel).

3. Results

Using the x, y, z coordinates of the fish positioning, we calculated the group averages for swimming depth, distance from speaker, swimming speed and group cohesion over the course of each trial. As an example, we provide plots of the progression of each of these behavioural parameters during all six trials of group 7 (Fig. 7a-f).

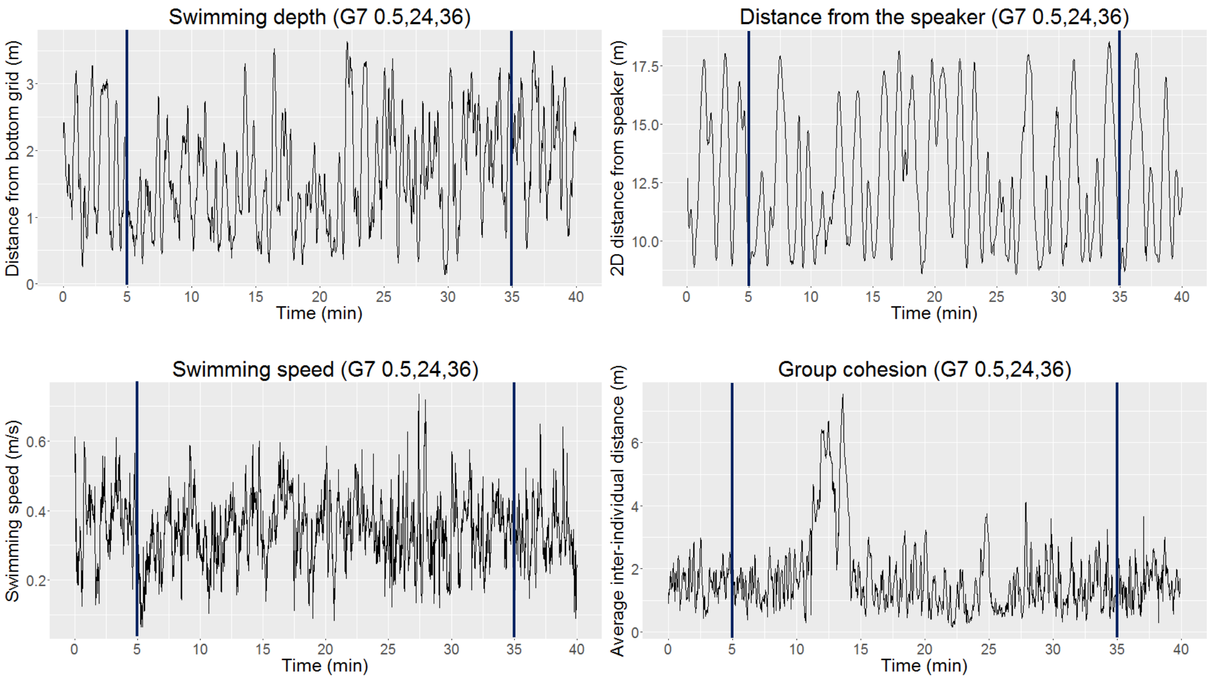


Figure 7a: Behavioural parameters of group 7 during the first trial. The vertical lines at 5 and 35 min delimit on-set and off-set of sound exposure. The fish typically swam up and down and around in the pen, along the net, but show a brief drop in depth and speed right after sound on-set. They changed depth continuously and tended to maintain a reasonably constant speed. The group split up for a few minutes, several minutes into the exposure period. PRI 0.5 s; 0-to-peak SPL 152 dB; Ambient SPL rms 128 dB (settings 24 and 36, respectively).

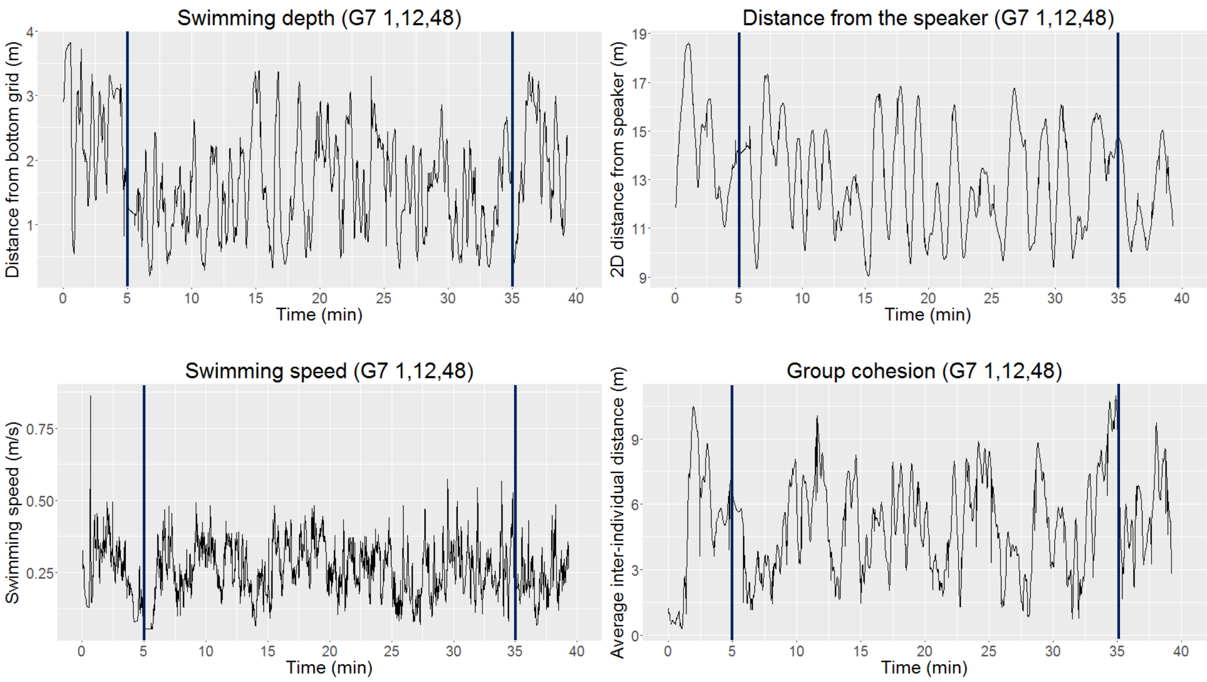


Figure 7b: Behavioural parameters of group 7 during the second trial. The vertical lines at 5 and 35 min delimit on-set and off-set of sound exposure. There is a clear drop in swimming depth visible right after sound on-set. The other parameters fluctuate, but not obviously related to the exposure period. PRI 1.0 s; 0-to-peak SPL 163.5 dB; Ambient SPL rms 119 dB (settings 12 and 48, respectively).

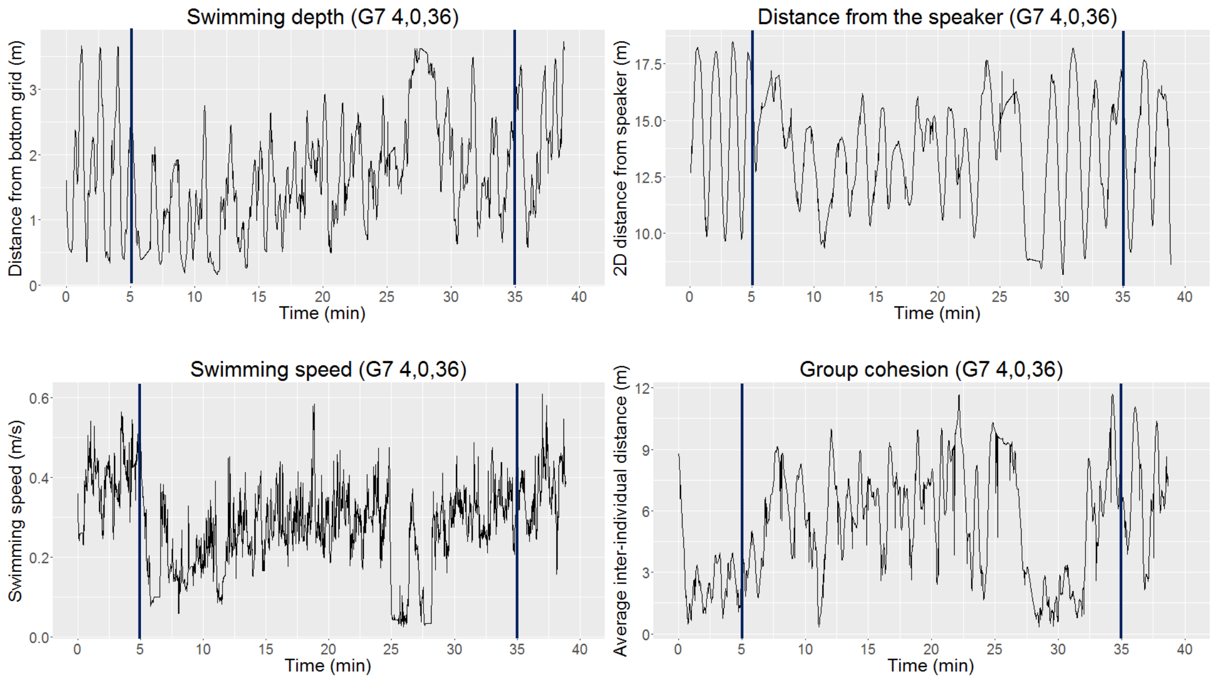


Figure 7c: Behavioural parameters of group 7 during the third trial. The vertical lines at 5 and 35 min delimit on-set and off-set of sound exposure. There is a clear drop in swimming depth and speed visible right after sound on-set, which only slowly returned to baseline. Also the stable patterns of distance to speaker and group coherence are distorted after sound on-set. PRI 4.0 s; 0-to-peak SPL 175 dB; Ambient SPL rms 128 dB (settings 0 and 36, respectively).

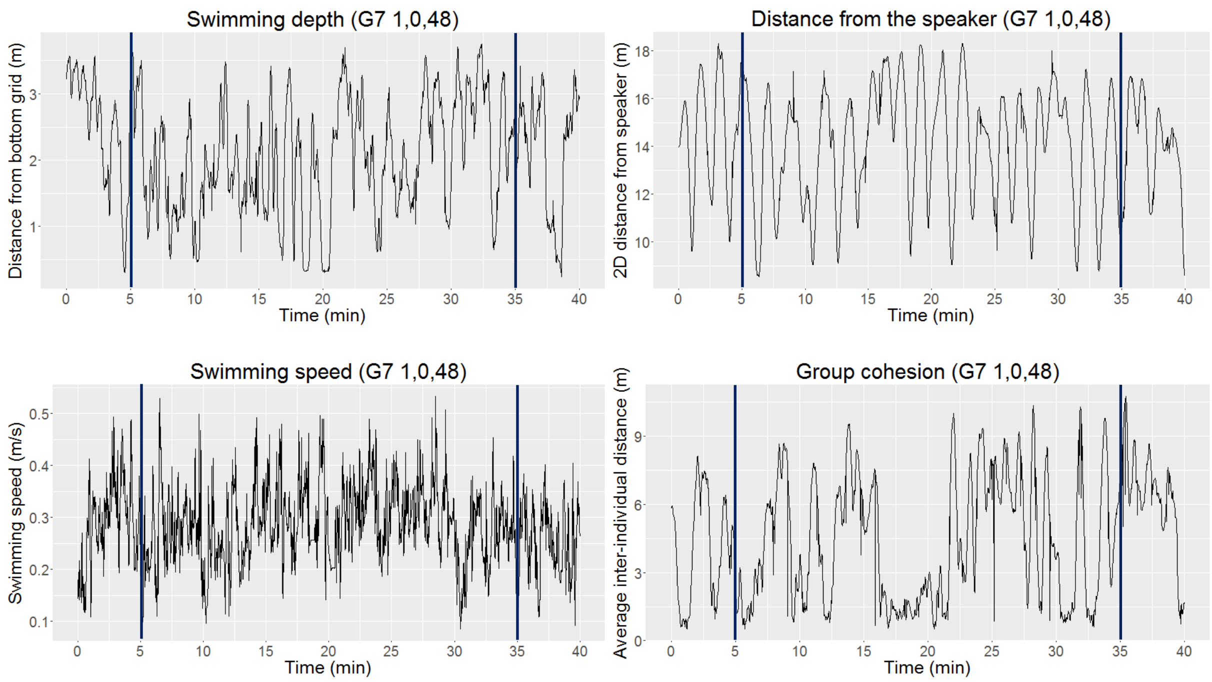


Figure 7d: Behavioural parameters of group 7 during the fourth trial. There is something of a drop in swimming depth and speed after sound on-set, but less clear patterns in distance to speaker and group coherence. PRI 1.0 s; 0-to-peak SPL 175 dB; Ambient SPL rms 119 dB (settings 0 and 48, respectively).

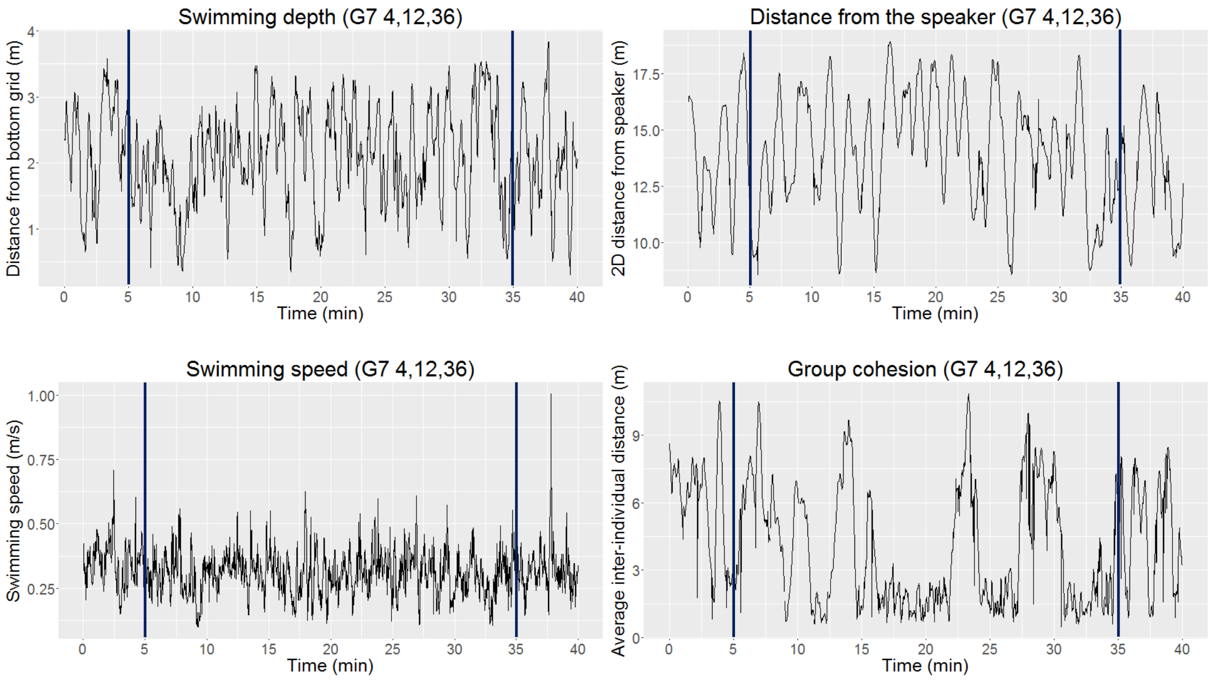


Figure 7e: Behavioural parameters of group 7 during the fifth trial. The vertical lines at 5 and 35 min delimit on-set and off-set of sound exposure. There are again somewhat lower values of swimming depth and speed visible after sound on-set. No clear patterns for distance to speaker and group coherence. PRI 4.0 s; 0-to-peak SPL 164 dB; Ambient SPL rms 128 dB (settings 12 and 36, respectively).

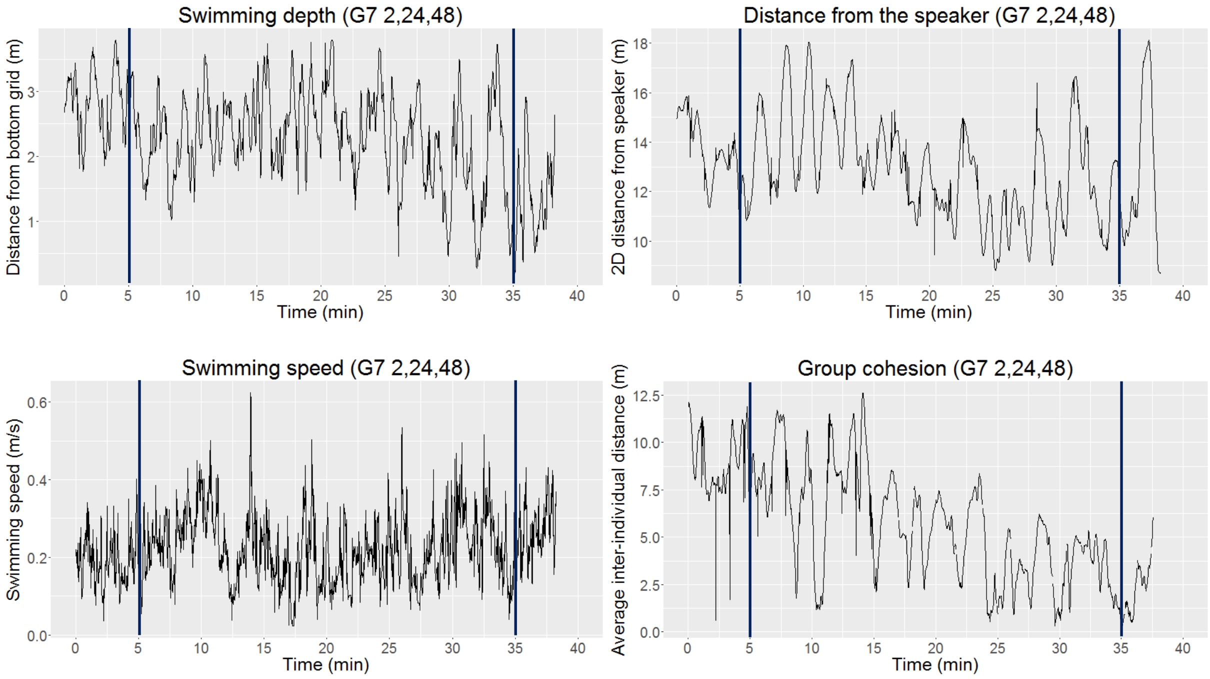


Figure 7f: Behavioural parameters of group 7 during the sixth trial. The vertical lines at 5 and 35 min delimit on-set and off-set of sound exposure. There is a clear drop in swimming depth, but a gradual rise in speed after sound on-set. No clear patterns for distance to speaker and group coherence. PRI 2.0 s; 0-to-peak SPL 152 dB; Ambient SPL rms 119 dB (settings 24 and 48, respectively).

It should be stressed that the patterns of a single group of fish (Fig. 7a-f) just serve as an example and we only attribute meaning to consistent patterns across the 16 groups, for which we combined the data in the following part. To assess the effects of the sound exposures on the groups of fish we used 5-min-bin-averages. There was a significant decrease in swimming depth after the start of the sound exposure (QUESTION 1, Fig. 8a, right). There were no significant changes in any of the other parameters (7b-d). The boxplots depict four time periods: 'Before' concerns the 5 minute bin group averages just before sound on-set, 'During1' the 5 minute bin just after on-set, 'During2' the 5 minute bin just before the sound is turned off and 'After' concerns the 5 minute bin without sound just after sound exposure has ended.

To increase our understanding of the progression of swimming depth after the start of the sound treatments we made line graphs in which we show average swimming depth of all trials of each group and again compared the results for the first and second experiment directly (Fig. 9). This showed most clearly how the overall response of the fish from the same batch differed in consistency between experiment 1 and the current experiment 2 (which was a key test target for this second experiment after the negative results of the first experiment).

After this we tested whether pulse rate affected response strength and whether response strength faded over the trials due to habituation and whether the baseline swimming depth affected changes in swimming depth. We also explored whether the change in swimming depth was correlated to the ambient or pulse sound level and tested whether the signal-to-noise level affected swimming depth (Fig. 10a-e). There was no significant effect of pulse rate (PRI) on swimming depth (QUESTION 2, Fig. 10a), but there was a non-significant trend ($p < 0.1$) for a correlation between signal-to-noise ratio and the change in swimming depth (QUESTION 3, Fig. 10b).

Finally, we explored the spatial response behaviour of the fish in the horizontal plane, using the x-coordinate (position relative to the sound source) of all individual fish. If we plot all fish before sound exposure and check a 10 seconds interval, we see that the typical circle-swimming yields a significant negative correlation (Fig. 11a). If fish are close to the speaker side they tend to swim away and when they are far from the speaker side they tend to come closer. This significant pattern is also present after on-set of exposure (Fig. 11b), and a linear mixed-effects model revealed a significant interaction ($p < 0.01$), which may indicate a change in swimming speed but not necessarily a directional change or avoidance response (QUESTION 4).

Interestingly, an exploration of pulse rate indicates no significant correlation just before, but significant correlation after sound on-set (Fig12a-b). Although the effect size appears subtle, this pattern suggests a differential effect related to pulse rate in that there is a stronger effect of the pulsed sound stimuli at higher pulse rates (QUESTION 4 & 2).

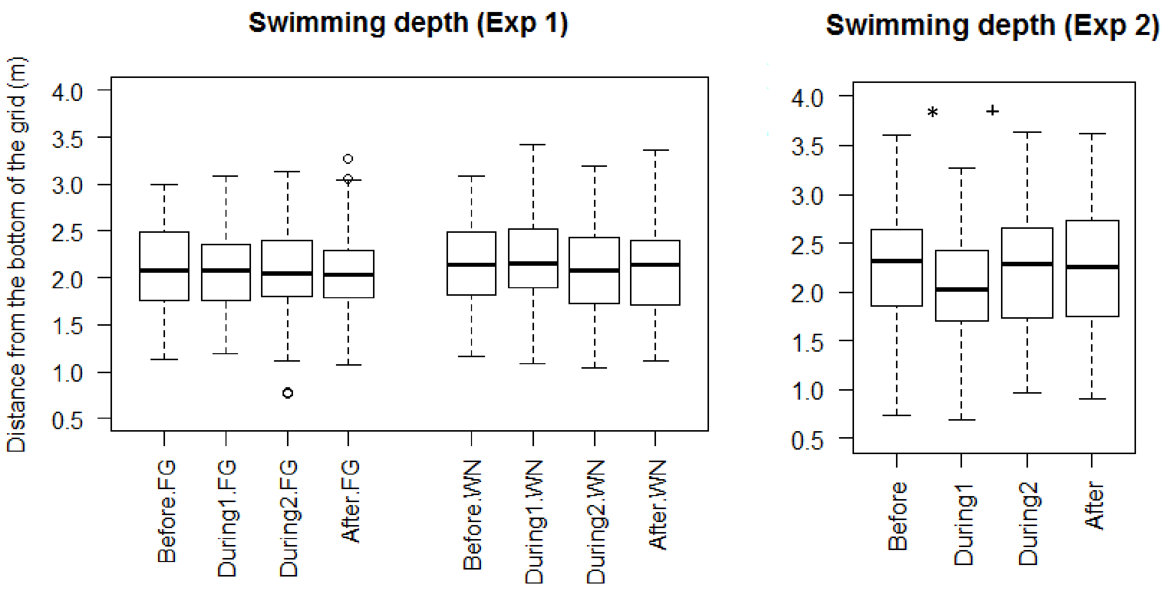


Figure 8a: Swimming depth during the trials of experiment 1 (Exp 1, left) as reported in the first report (see Hubert et al. Research Report 1, 15-02-2017) and the current experiment 2 (Exp 2, right). The results of the first experiment are divided into trials with FaunaGuard (FG) exposures and white noise (WN) exposures. Both experiments have been conducted using 16 groups of four fish. During experiment 1, they have been exposed thrice to a FG treatment and thrice to white noise. During experiment 2, they have been exposed to a treatment with impulsive sound six times. Here, we did see a significant drop in swimming depth after the start of the impulsive sound (before vs. during1; $p = 0.005$) and an indication for habituation during the exposure period in a non-significant trend (during1 vs. during2; $p < 0.1$)

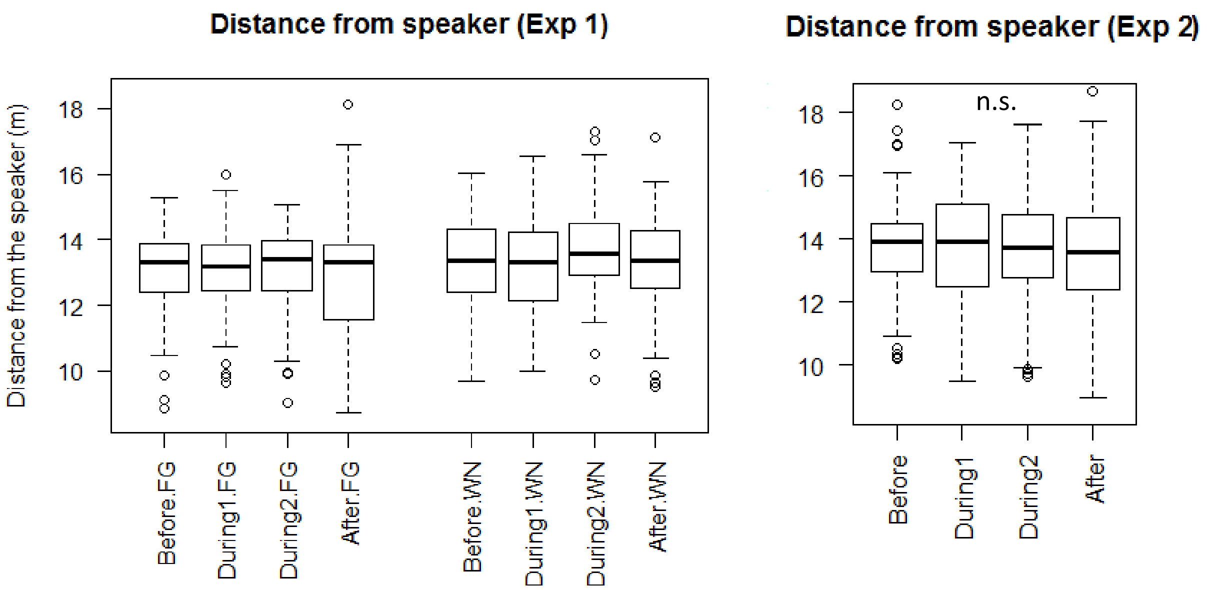


Figure 8b: Distance from the speaker during the trials of experiment 1 (Exp 1, left) as reported in the first report (see Hubert et al. Research Report 1, 15-02-2017) and the current experiment 2 (Exp 2, right). For experiment 1, we calculated the 3d distance from the speaker whereas we now calculated the 2d distance for experiment 2. No significant differences were found for either method.

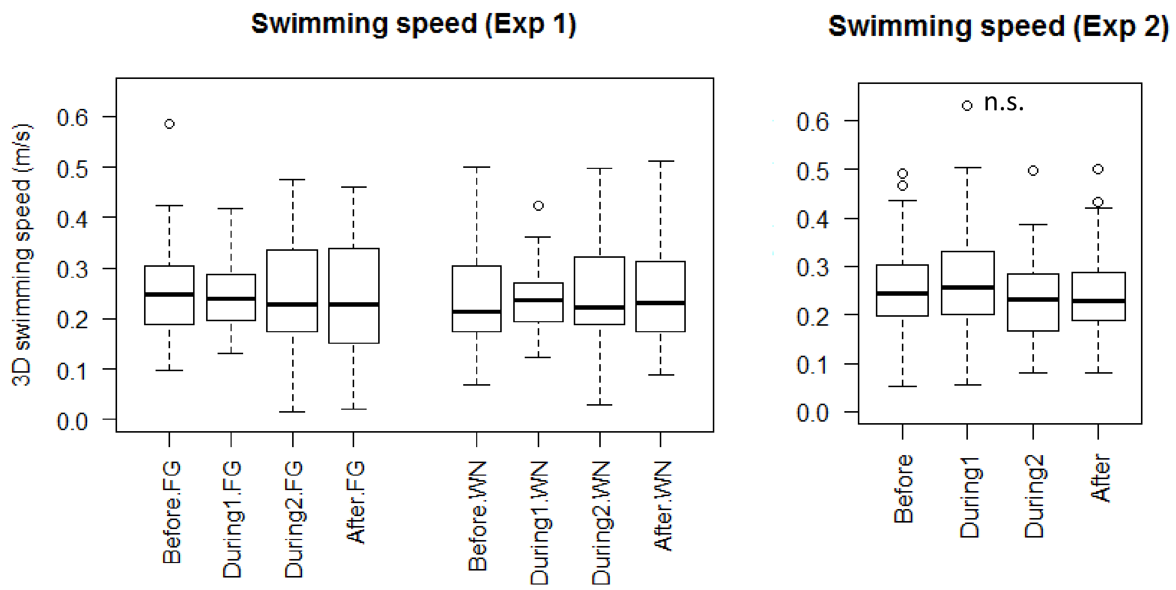


Figure 8c: Swimming speed during the trials of experiment 1 (Exp 1, left) as reported in the first report (see Hubert et al. Research Report 1, 15-02-2017) and the current experiment 2 (Exp 2, right). For experiment 1, we used 1 minute bins whereas we used 10 s bins for experiment 2. No significant differences were found for either method.

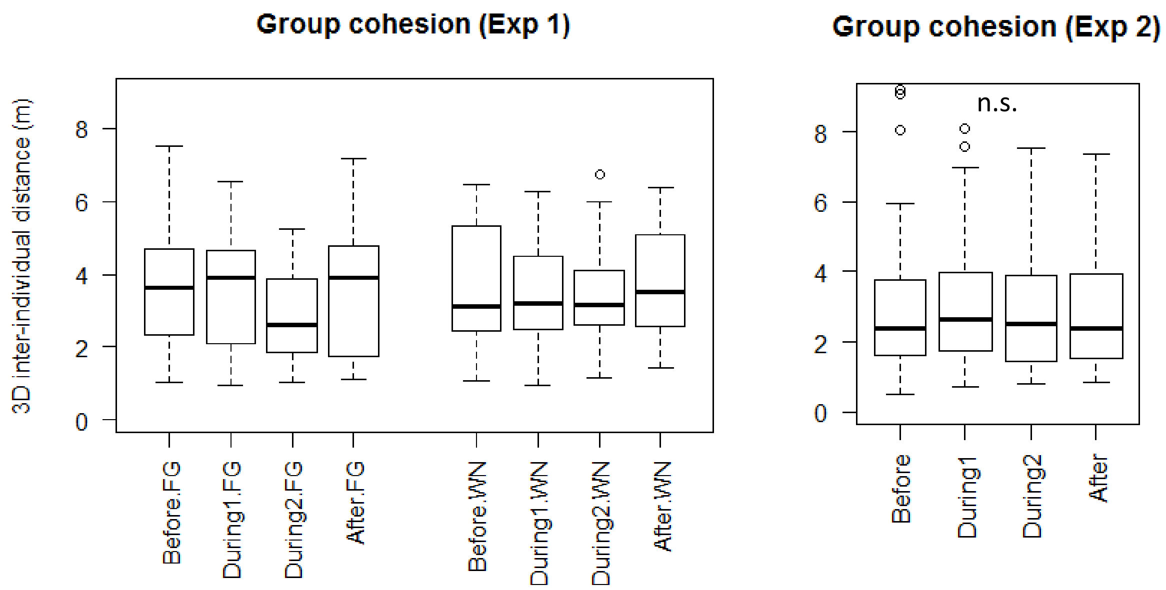


Figure 8d: Group cohesion during the trials of experiment 1 (Exp 1, left) as reported in the first report (see Hubert et al. Research Report 1, 15-02-2017) and the current experiment 2 (Exp 2, right). No significant differences were found.

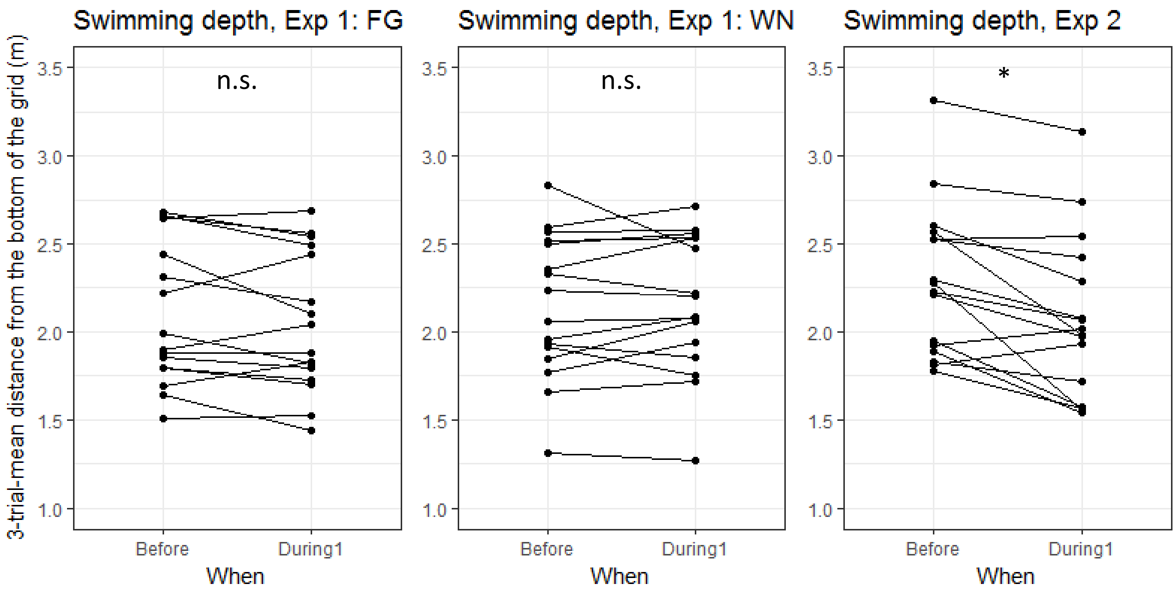
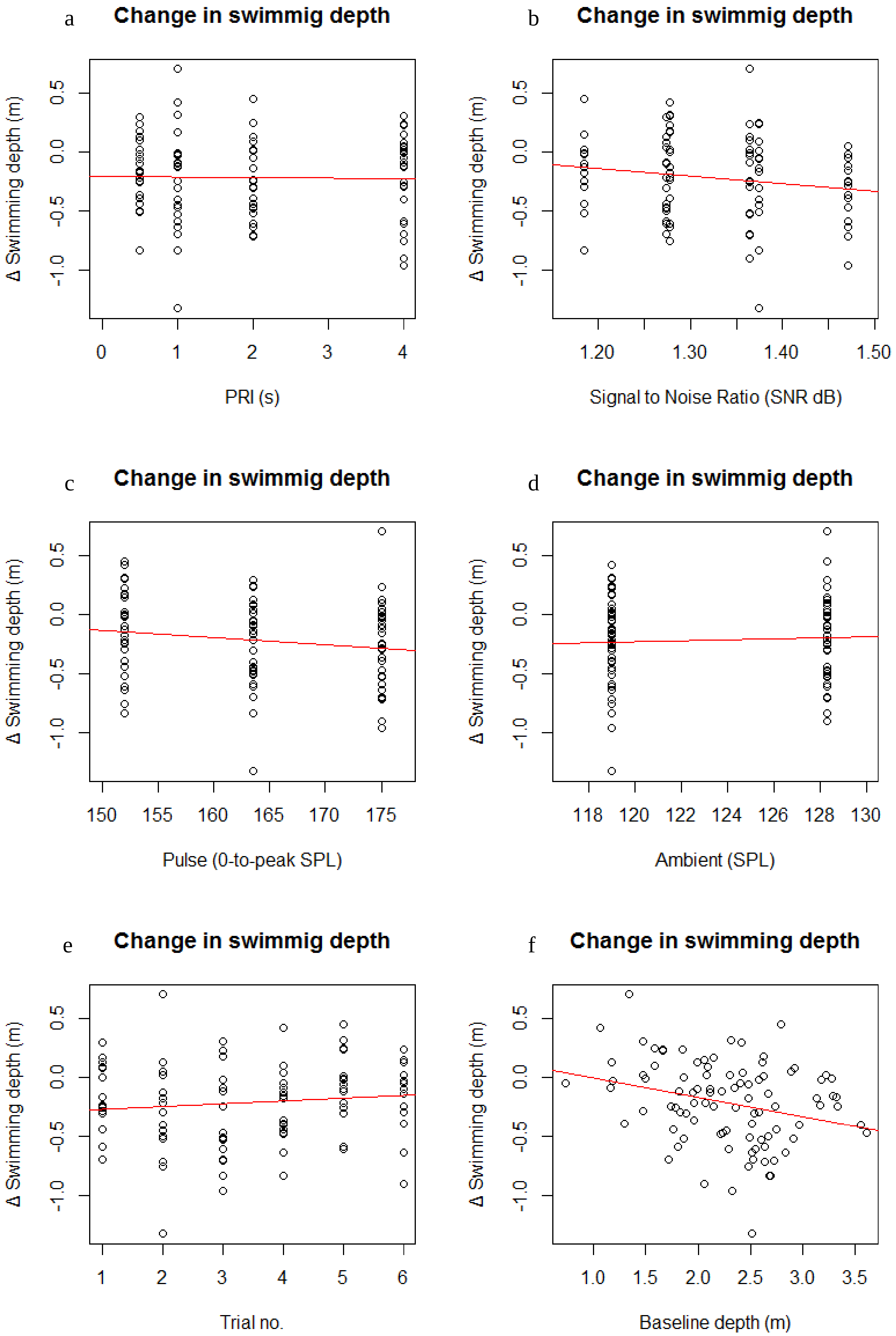


Figure 9: Average swimming depth during three trials in experiment 1 (Exp 1: FG & WN) as reported in the first report (see Hubert et al. Research Report 1, 15-02-2017) or six trials in the current experiment 2 (Exp 2). During experiment 2, there was a significant drop in swimming depth after the start of the exposure (same data as in Fig. 7a, right). There is a clear contrast in consistency between both experiments.

Figure 10a-f (next page): Regressions of the change in swimming depth after the start of the impulsive sound series. A linear mixed effects model showed no significant effect of pulse rate (NS) and a non-significant trend for a correlation between SNR and change in depth ($p = 0.054$). We also explored pulse amplitude and ambient level independently, but these were not significantly affecting swimming depth (both NS). The slope of fading response strength with repeated exposure was also not significant (NS), but we found a significant correlation between depth before the sound and the change in depth after the start of the sound ($p < 0.001$).


a
Change in swimmig depth
Δ Swimming depth (m)
PRI (s)
b
Change in swimmig depth
Δ Swimming depth (m)
Signal to Noise Ratio (SNR dB)
c
Change in swimmig depth
Δ Swimming depth (m)
Pulse (0-to-peak SPL)
d
Change in swimmig depth
Δ Swimming depth (m)
Ambient (SPL)
e
Change in swimmig depth
Δ Swimming depth (m)
Trial no.
f
Change in swimming depth
Δ Swimming depth (m)
Baseline depth (m)

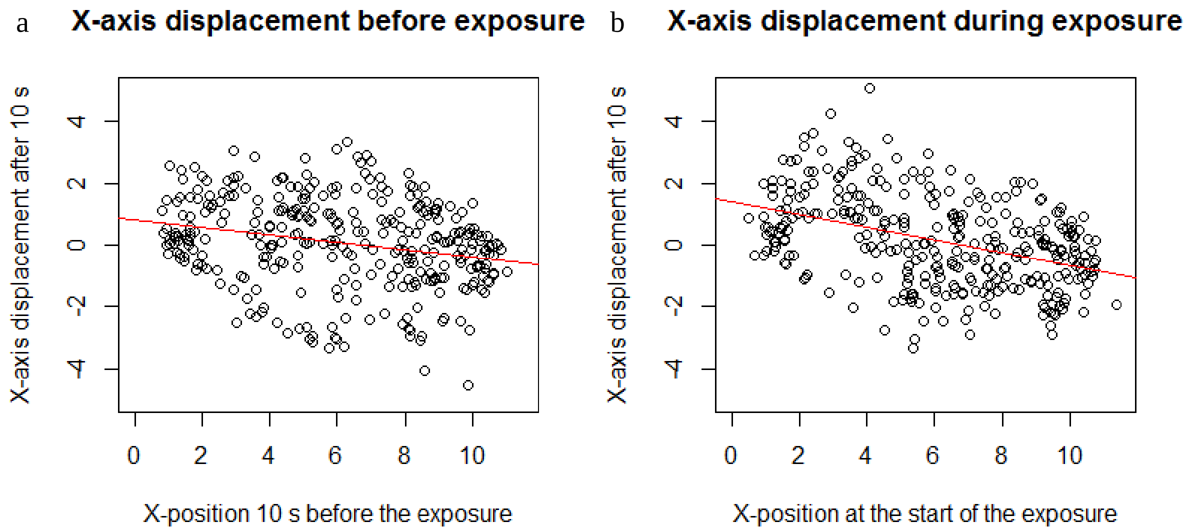


Figure 11a-b: Here we plotted the x-coordinate at one point (see x-axes of the plots) to the change in x-coordinate 10 s later. We did this right before the start of the impulsive sound (a, left) and right after the start of the impulsive sound (b, right). Both plots show a significant linear relation (both $p < 0.001$). Taking together data of both regressions in a linear mixed-effects model revealed that they are not shifted relative to each other, but that there was a significant interaction ($p < 0.001$), indicating that the slopes are different.

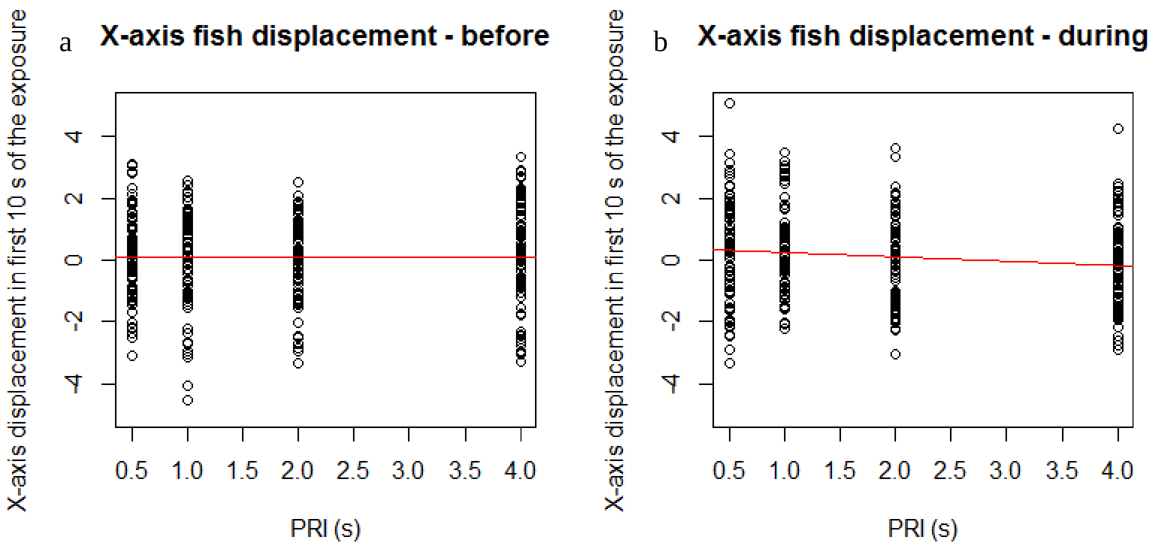


Figure 12a-b: Correlation of the pulse rate interval (PRI) with the x-displacement (change in x-coordinate) in the last 10s before and first 10 s during the impulsive sound. There is no linear relation between PRI and x-displacement before the impulsive sound ($p = 0.996$), but after the start of the sound there is ($p = 0.023$).

4. Discussion

4.1 Main results and interpretation

In this second experiment, we have shown that fish from the same batch as used for experiment 1 responded consistently and thereby significantly to sound stimuli that are more like in previous studies (brown noise and intermittent, c.f. Neo et al. 2016; Neo 2016). This significant effect was found in one out of the four parameters: swimming depth. Pulse rate did not affect behavioural patterns during sound exposure in any of the four parameters that we tested before: swimming depth, distance from speaker, swimming speed, and group cohesion. In an additional and detailed exploration of distance changes in the horizontal plane relative to the speaker, it appeared that higher pulse rate had a slightly stronger effect in displacing fish away from the speaker. There was a non-significant trend for the signal-to noise ratio of the brown noise pulse amplitude and ambient background level and no significant effects for either of them independently. Spatial avoidance in the horizontal plane during exposure in a natural sound field was non-existing or very subtle.

These results confirm earlier studies that fishes in general and this species in particular respond to sound and shift down in the water column. This behaviour can be interpreted as anxiety-related or the result of an increase in the perceived risk of predation or other dangerous event. Short pulses of 100 ms of broad –band (brown) noise within the audible range of the fish (200-1000 Hz), with variable intervals (0.5-4.0 s) appear reliable acoustic triggers. The fact that other, longer and more tonal, sounds elicit less of a response at similar amplitude levels, indicates that acoustic structure matters and that the pulse-like structure, repetitive nature, and/or the bias to low-frequencies are critical for detection and/or decision making. It may be that these sounds are more like natural biotic or abiotic sounds that fish more often react to and that may indicate some danger (predator attack) or other relevant event or location (object drop, rocky current, rain- or waterfall).

Pulse rate had little impact and may not be meaningful to fish at all or just not at the temporal scales tested. There was a slightly stronger impact for higher pulse rates to be more effective in eliciting a spatial response in the horizontal plane. This may suggest that pulses with even shorter intervals may be worthwhile to explore for their response-eliciting potential c.f. Dunning & Ross 2010; Gurshin et al. 2014). The signal-to-noise ratio, expressed as the crest factor (or how much the pulse peak stood out against the average ambient noise level) revealed a non-significant trend effect. This indicates an effect that may become significant with slightly more samples or slightly more contrast in this crest factor. The relevance of this is that not only how loud a pulse is will affect response tendencies in fishes, but also ambient levels matter. This means that at rough seas, when noise levels are higher, sound stimuli likely need to be louder to elicit deterrent responses.

The apparent lack of any biologically relevant spatial response in the horizontal plane may be due to the fact that the fishes are not able to localize the direction of the sound source (Shafiei Sabet et al. 2016). It may also mean that the natural response is just diving down, which may be species specific and depend on location. Finally, it may also be due to the restrictions in pen size and the captive conditions in general, although the area is quite spacious for the size of the fish and there is potential for over 10 m directional escape sprints. Many studies indeed report vertical response patterns (Sarà et al. 2007; De Robertis & Handegard 2013; Hawkins et al. 2014; Neo et al. 2014; 2015; 2016), but reports on horizontal moves do exist (Sand et al. 2000; Vetter et al. 2015) and just more studies are needed, preferentially on free-ranging fish or with comparative insights for various captive conditions (Neo 2016).

4.2 Kurtosis explored and dismissed

We used the potential value of kurtosis as a measure for impact assessment as our guideline for the experimental design in experiment 2. Traditional measures of impact just take amplitude in a particular frequency range into account (sound pressure or particle motion) or at best also the duration of exposure (sound exposure level, SEL). However, the distribution of sound energy in the spectrum (Estramil et al. 2010), but especially in the temporal range (Neo et al. 2014), can also affect response levels and is not represented in any current acoustic measure. Kurtosis is a statistical measure that is used to describe a sample or data distribution (Box 1). The potential validity of kurtosis as an acoustic responsiveness measure depends on how well it objectively describes sound conditions in a single unit that would capture those features that determine acoustic responsiveness in aquatic animals. We tested and confirmed to some extent variability in response level to variation in sound energy distribution in pulse rate and crest factor (see Box 1 for explanation). However, we found the intuitive match of the measure of kurtosis with these acoustic parameters to be only partly valid and not very satisfying.

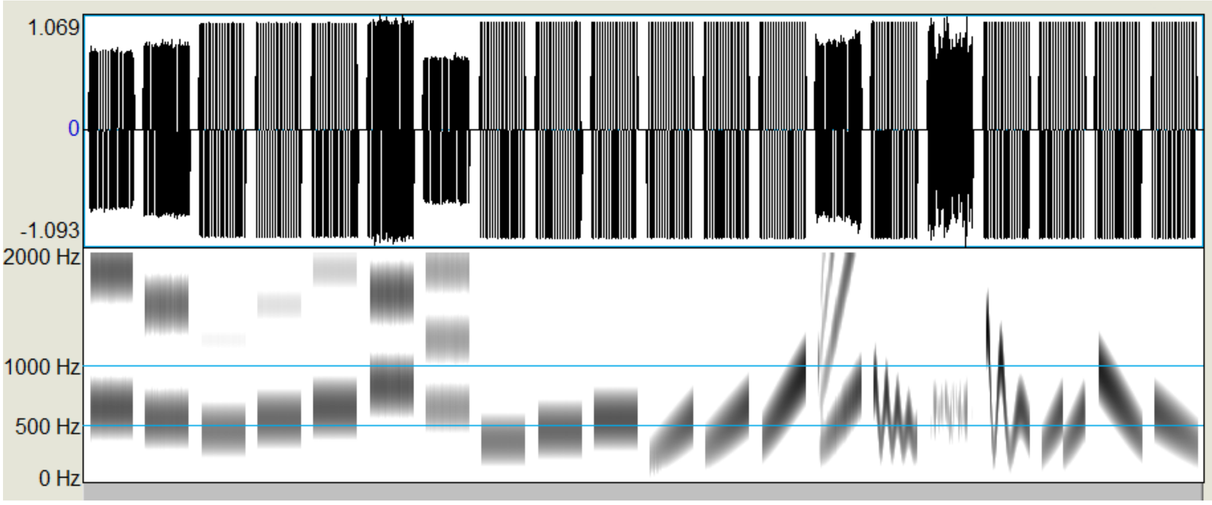
The main problems lay in the time factor. First of all, a higher pulse rate leads to more pulses in the same amount of time and is thereby affecting the level of kurtosis. However, a critical and arbitrary protocol aspect would be the time frame for analysis, as the duration of that will also affect kurtosis. Furthermore, the spread in time of regularity of inter-pulse intervals within the analysis period will not affect the kurtosis measure, while it is likely to affect the behavioural responsiveness (Shafiei Sabet et al. 2015). Variation in crest factor may be a closer match at first sight, as it also provides some measure reflecting peak height relative to ambient background tails. Nevertheless, also here the time frame of analyses is critical and arbitrary, changing kurtosis measure for the same sound conditions. Any variation in energy distribution below the peak value is also not taken into account, while amplitude fluctuations above the ambient and below the peak may also affect behavioural or physiological responsiveness (Wysocki et al. 2007). Furthermore, initial exploration yielded the insight that the pulse rate completely overruled the crest factor in impact on the kurtosis measure. We know now that both parameters may have some effect, but certainly not as heavily skewed as this bias in the kurtosis measure. Anyway, we concluded that, despite the fact that pulse rate and crest factor were two relevant parameters to explore in terms of behavioural responsiveness, kurtosis seems not a very suitable measure to continue exploring. Some sort of index, in which relevant parameters can be represented according to their weight on impact, seems a more promising pathway for future exploration.

4.3 Lack of response to the FaunaGuard-Fish Module

Given that previous experiments with different sound stimuli have triggered consistent response patterns at high, but also at very low levels (Neo et al. 2016; Neo 2016), we argued that the FG-FM sounds (and the white noise control sounds) in experiment 1 were not loud enough to elicit significant changes in behaviour in the current test conditions and set-up (see Hubert et al. Research Report 1, 15-02-2017). The findings of the second experiment, reported here, confirm however that the fish of this batch were responsive to other sounds at the amplitude level used in experiment 1 and strongly suggest that the sound structure of the sounds of the FaunGuard-Fish Module (FG-FM) are not optimal for eliciting a behavioural change in seabass in our floating pen. It may be relevant to once more address the detailed structure of the FG-FM and discuss whether we can understand the apparent variation in response triggering potential related to acoustic structure.

The FaunaGuard-Fish Module (FG-FM) played a series of twenty 10-second sounds with variable intervals. The first ten were each continuous sounds at a single frequency, but each at a slightly different frequency (ranging between 200-800 Hz) and of a different wave type (sine, triangle, square, sawtooth wave). The second ten sounds were a variable set of upsweeps, downsweeps and more complex frequency-modulated sound elements and a noise band. All these sounds were carefully selected based on distinct, visually determined startle responses in fish tank conditions (Kastelein et al. 2011; 2012). However, much of the acoustic energy of the sounds is above 500 Hz (Fig. 13), which is above the frequency of best hearing of most marine fish species. Furthermore, an effect of wave type on response tendency has not been reported and is also not expected as wave type is determined by harmonic components that are certainly beyond the hearing range of the fish (Hubert et al. Research Report 1, 15-02-2017).

The rationale behind having a variable series of sounds, with each subsequent one being acoustically distinct, was to counteract habituation and is reasonable in itself (c.f. Teilmann et al. 2006; Rankin et al. 2009; Neo et al. 2015; Radford et al. 2016). However, the nature of the relatively long duration of the sounds and the relatively subtle frequency differences, together with an overall moderate to low expectation about audibility and a complete lack of insight with respect to perceptual contrast for the fish, make the efficiency in counteracting habituation at least questionable. A thorough investigation into perceptual contrast and mode of variation (e.g. number of serial repeats, number of distinct sound stimulus variants, interval duration and stereotypy) is required for a better understanding of the nature of habituation to sound exposure in fish in general and to counteract the phenomenon through stimulus design in acoustic fish deterrent devices in particular.



Frequency (Hz) Time (10 sec)

Figure 13: Amplitude wave form and sonogram of a cut of each of the 20 FG-FM sounds in the order of application during experiment 1. The blue line at 500 Hz indicates the upper boundary of the spectral range of best hearing for European seabass and the blue line at 1000 Hz indicates the upper boundary of hearing for this species. Note that all harmonics (many are beyond the scale) are beyond the hearing range of the model species and most marine fish species (and there is therefore no reason to assume that they will affect responsiveness). Also note that the original temporal pattern is not reflected in this figure as each sound is in reality 10 s by itself (for a better comparison of the temporal structure of sounds used in both experiment 1 and 3, see 15 s cuts of the original sound series in Fig. 4 and 5). The second series of ten sounds has a more complex structure of modulations that may be perceived by fish as more intermittent than the first ten sounds.

4.4 Application of the FaunaGuard-Fish Module in open water

The current results are in contrast with the anecdotal evidence from previous applications in open water in Sweden, Brasil and the Netherlands, which suggested that the FaunaGuard-Fish Module worked well and could potentially save a lot of fish (Kastelein et al. 2011; Van der Meij et al. 2015). Obviously, we need independent research and a better and objective quantification to make strong statements about such observations on deterrent efficiency. However, it may well be that different species, free-ranging individuals, or particular field conditions may make fishes more responsive. It may also be that the FG-FM sounds were played back at a higher amplitude level, which may mean it may have reached zones of response triggering potential in the field that were not accomplished in the floating pen (Fig. 14). However, as far as we know, there were no sound level measurements taken in those field sites and we therefore do not know whether this has been the case. Furthermore, sound propagation will inevitably lead to attenuation over distance and we have to assume that different zones of triggering potential have been present (Fig. 15), including those with no spatial responsiveness and maybe even zones in which fishes were attracted to the source. However, although attenuation with distance is a certainty, there are no studies that have tested the existence of these theoretical zones of behavioural impact in the context of fish deterrent devices and such studies are badly needed for future applications.

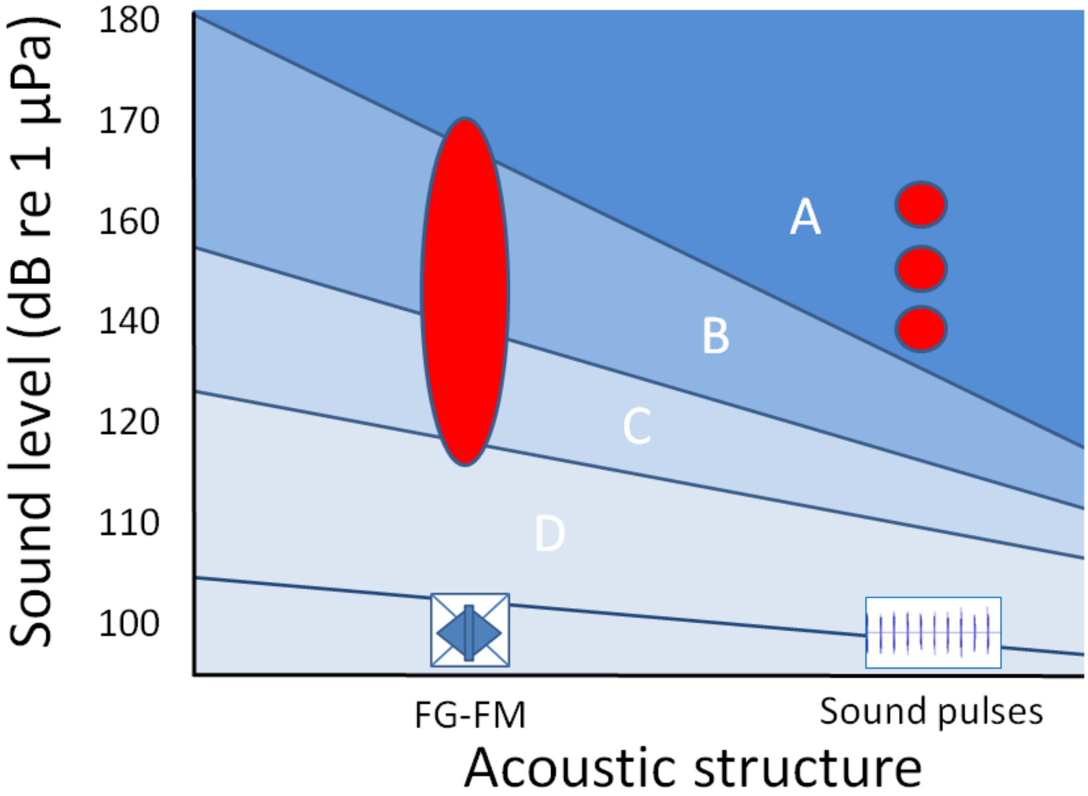


Fig. 14: Schematic illustration of the discrepancy between the lack of significant behavioural effects to the 10.0 s FaunaGuard-Fish Module (FG-FM) sounds in experiment 1 and the significant changes in behaviour to the series of 0.1 s sound pulses in the current experiment 2. The axes represent variation in acoustic structure on the x-axis and sound level of exposure on the y-axis. The blue zones correspond with theoretical effect zones (Fig. 15): Zone A is where there is a significant target response; Zone B is where fish groups may be affected but do not move away or towards the ADD; Zone C is where fish exhibit phonotaxis; and Zone D is where the sounds are still audible, but do not elicit any spatial response. The large red oval represents the range of rms sound levels at which the FG-FM sounds were tested in experiment 1, while the three small red circles represent the rms sound levels of experiment 2 (see Fig. 3). The behavioural response tendency is determined by a combination of acoustic structure and sound level; moving the FG-FM up in sound level may improve performance, but pulse-type sound stimuli have similar response triggering potential at lower sound levels.

The current insights from both experiments together indicate that it is very likely possible to improve the FaunaGuard-Fish Module by modification of the sound stimulus series. More brief (duration e.g. 0.1-0.5 sec) and broad-band (frequency range e.g. 200-1000Hz) sounds, repeated at relatively high rates (interval e.g. 0.1-2.0 sec) are likely more effective in triggering a behavioural response. Tones have been reported to be suitable for conditioning, but to be much less efficient in eliciting a spatial avoidance response than complex, broad-band sounds (Sloan et al. 2013; Vetter et al. 2015), which are often used in acoustic fish guiding efforts especially in the context of riverine passage problems for migratory fish (Popper & Carlson 1998; Schilt 2007; Noatch & Suski 2012). There are several examples of acoustic deterrence studies with sounds that cover the audible range of a wide variety of fishes (Maes et al. 2004; Perry et al. 2014; Ruebush et al. 2012). If reported, the sound stimuli used often concern relatively fast repetitions of brief sound bursts (Sand et al. 2000; Gibson & Meyers 2002; Maes et al. 2004; Gurshin et al. 2014). In addition, we know still very little about the potential effect of using multiple sound variants in a sequence, but this seems also a fruitful alley for future research and potential tool to counter-act habituation.

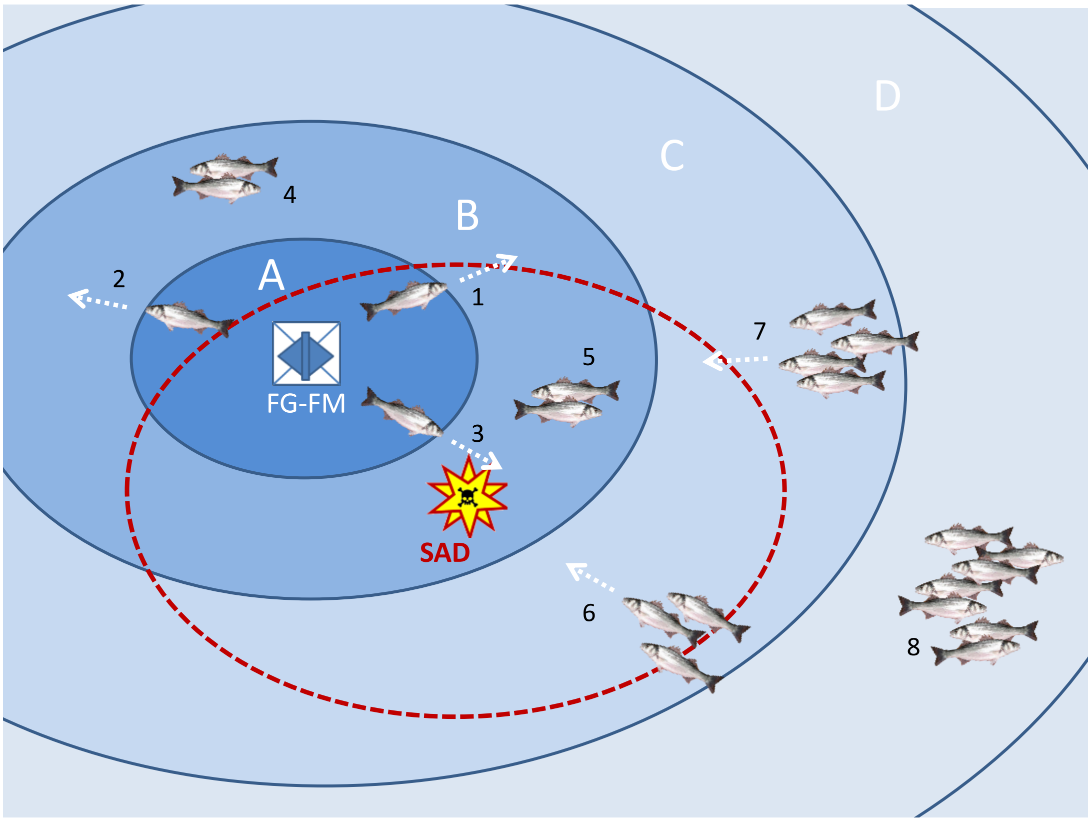


Figure 15: Schematic illustration of hypothetical zones around an Acoustic Deterrent Device (ADD), such as the FG-FM, placed at a certain distance from an anthropogenic Source of Acoustic Danger (SAD). The target is to deter fish away from and outside the area that may cause harm (red dashed oval), which is achieved for fish 1 and 2 that are in zone A in this example, but not for fish 3. Inherent to sound level decline with distance, beyond deterrent zone A, there may be a zone B, in which fish may startle or respond otherwise, but do not make a spatial shift, which yields no problem for fish group 4, but renders the ADD ineffective in saving fish group 5. Beyond zone B, there may be a zone C in which fish even exhibit phonotactic behaviour, approaching the ADD, inducing trouble for fish groups 6 and 7 (note that wider circles have a probability to involve a larger number of fish). In zone D, the ADD may still be audible but not having a spatial effect. The presence and size of each zone may depend on species and environmental conditions and will be determined by ADD sound features, source level and sound propagation (affected by e.g. depth, bathymetry, temperature and currents).

5. Conclusions and recommendations

The FaunaGuard-Fish Module sound series did not elicit consistent behavioural responses in groups of four seabass swimming in a large outdoor floating pen. The same set-up, including species, group size, locality, sound levels, and playback procedure, but with different pulse-type sound series, did elicit significant changes, in particular in swimming depth, in previous studies (Neo et al. 2016; 2016) and in the current study. This is in line with data from the literature that also suggest that repeated series of short, broad-band, sound bursts are more potent than long-drawn tonal sounds in triggering behavioural responses in fishes (reviewed in Hubert et al., Research Report 1, 15-02-2017). This means that it is very likely possible to improve the deterrent capacity of the FG-FM sound series, although tests on multiple species and free-ranging conditions remain necessary.

None of the response-triggering sounds played in earlier experiments (Neo et al. 2016; 2016) or in the current experiment triggered a clear spatial avoidance response in the horizontal plane. As this is a critical aspect of the explicit target of an Acoustic Deterrent Device (ADD), more studies are needed to find out whether this is due to the specific species tested, the captive conditions, the locality, or the specific sound patterns played. The currents state of the art in terms of the efficiency of ADDs in the application with fishes makes us come up with the following recommendations:

1. Improve responsiveness to FG-FM sounds directly by including more pulse train like sounds and explore the effect of higher pulse rates - tests in the floating pen at different sound levels would allow to compare pulse-rate dependent dose-response curves.
2. Investigate the effect of alternating or varying sounds in a sequence on response tendency and habituation – tests for adequate acoustic contrast should be included and are only feasible in a floating pen type set-up, with sufficient control and replication.
3. Assess the source level of the FG-FM and model spatial soundscape gradients for areas of application – this step is critical to match spatial information on fish position and response tendency with sound conditions.
4. Apply the FG-FM sound exposure at two distinct field sites (to get the minimal in replication and still a feasible challenge for fieldwork) with virtual source locations of anthropogenic acoustic danger and monitor free-ranging fish (multiple species – again probably best to select two ecologically distinct species) by telemetry – this step is critical for any final positive evaluation about ADD efficiency.

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