

Cover Page



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CHAPTER 3

Impulsive sounds change European seabass swimming patterns: Influence of pulse repetition interval

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Abstract

Seismic shootings and offshore pile-driving are regularly performed, emitting significant amounts of noise that may negatively affect fish behaviour. The pulse repetition interval (PRI) of these impulsive sounds may vary considerably and influence the behavioural impact and recovery. Here, we tested the effect of four PRIs (0.5-4.0s) on European seabass swimming patterns in an outdoor basin. At the onset of the sound exposures, the fish swam faster and dived deeper in tighter shoals. PRI affected the immediate and delayed behavioural changes but not the recovery time. Our study highlights that (1) the behavioural changes of captive European seabass were consistent with previous indoor and outdoor studies; (2) PRI could influence behavioural impact differentially, which may have management implications; (3) some acoustic metrics, e.g. SEL_{cum} , may have limited predictive power to assess the strength of behavioural impacts of noise. Noise impact assessments need to consider the contribution of sound temporal structure.

Introduction

The ever increasing global energy demand has led to extensive exploitation of seas and oceans for both fossil and sustainable energy resources¹. Related human activities, such as seismic surveys and offshore constructions for wind farms and oil rigs, generate a substantial amount of noise in the underwater environment. This introduction of anthropogenic noise into the underwater acoustic scene may pose a threat to aquatic life, including fish, causing a range of negative effects, from physical injuries in close range, to behavioural changes further away from the sound sources²⁻⁴. To ensure the stability of marine ecosystems under increased pressure of ocean exploitation, it is important to understand whether and how underwater anthropogenic noise may affect fish behaviour, which in turn may have consequences on fish populations.

Whether behavioural changes will result in negative fitness consequences, depends partly on whether fish habituate to the noise exposures and recover from the changes. However, behavioural observations in previous noise impact studies generally did not last long enough to show recovery after initial behavioural changes^{5-8, but see 9}. Moreover, a recovery or a decrease in response does not necessarily denote habituation, where the animals hear selectively while filtering out repeated or irrelevant sound signals in the background¹⁰. A decrease in behavioural response could also be attributed to 1) sensory adaptation, i.e. the sensitivity of the hearing organs is reduced by loud exposures, leading to temporary threshold shift (TTS), or 2) motor fatigue, i.e. animals become unresponsive due to exhaustion¹¹. It is crucial to determine the mechanism of such behavioural recovery since the different mechanisms vary in their ecological implications.

Underwater noise impact assessments are complex also because anthropogenic noise shows a variety of amplitudinal, spectral and temporal variations. Of these, the temporal structure of sound is rarely studied, even though it may play a crucial role in triggering behavioural response in fish^{9,12}. For example, Neo *et al*⁹ showed that European seabass (*Dicentrarchus labrax*) recovered more slowly from impulsive sounds than from continuous sounds (despite the former having lower accumulated sound pressure level), after exhibiting consistent initial behavioural changes upon noise exposures. Considering that impulsive sounds differ in various temporal features, there is a need for systematic studies addressing other temporal parameters, such as pulse repetition interval, pulse repetition regularity, pulse duration and pulse shape (including rise time).

Among these temporal parameters, pulse repetition interval (PRI), which can also be expressed in pulse repetition rate (PRR, where $PRR = 1 / PRI$), is rather variable among the current practices in pile driving and seismic surveys. PRI generally varies between 1 – 4 s¹³ for pile driving and 5 – 15 s¹⁴ for seismic surveys. Different PRIs have been shown to influence the habituation rate to repeated sound stimuli in zebrafish and rats^{15,16}. However, it is unclear if PRI also contributes to fish habituation to impulsive anthropogenic sound exposures, such as pile driving and seismic shootings.

In this study, we used a similar setup as in Neo *et al*⁹ to answer two questions: 1) How do impulsive sounds of different PRIs (0.5 s, 1.0 s, 2.0 s, 4.0 s) affect the swimming patterns and behavioural recovery of European seabass? 2) Can the behavioural recovery be attributed to habituation? We expected larger PRIs to prolong the behavioural recovery and the recovery be attributed to habituation.

Materials and methods

ANIMAL MAINTENANCE

The European seabass (mixed sex; 20-25 cm in total body length) came from a commercial hatchery (Ecloserie Marine, Gravelines, France) and were kept in four round holding tanks (diameter: 2.2 m; depth: 1 m) before and after the test trials at the Sea Mammal Research Company (SEAMARCO) in Wilhelminadorp, The Netherlands. Water was refreshed continuously with a recirculating system connected to the nearby Oosterschelde marine inlet and the water temperature varied from 4 to 12 °C throughout the experimental period (May-June 2013). Fish were fed pellets (Le Gouessant Aquaculture, Lamballe, France) every other day based on a temperature-dependent prescription. All experiments were performed in accordance with the Dutch Experiments on Animals Act and approved by the Animal Experiments Committee at Leiden University (DEC no: 13023).

EXPERIMENTAL ARENA

The experiment was conducted in a large outdoor rectangular basin (7 x 4 x 2 m) equipped with a water recirculating system at SEAMARCO. During the exposure trials, fish were put in a white nylon net enclosure (4 x 1.6 x 2 m) to ensure full coverage by an underwater video camera for observation (Fig. 1). A white tarp was placed at the bottom and in the background to ensure sufficient contrast in video images, without disrupting the normal swimming behaviour of the fish. Beside the basin, there was a research cabin containing sound generating and video monitoring equipment.

TREATMENT SERIES

We exposed the fish to a series of four regularly repeated impulsive sound treatments differing in PRI: 0.5 s, 1.0 s, 2.0 s and 4.0 s (Table 1). The pulse duration of all the treatments was the same, which was around 0.15 s (Fig. 2a).

The pulses were created in Adobe Audition 3.0 using filtered brown noise (bandpassed: 200-1000 Hz; matching the hearing range of European seabass) and played back with an underwater transducer (LL-1424HP, Lubell Labs, Columbus, US) from a laptop through a power amplifier (Macrotech 5000 VZ, Crown Audio, Elkhart, US). The whole experimental arena had a very homogenous sound pressure field during the playback of broadband sounds⁹. The average root-mean-square sound pressure level (SPL_{rms}) before the exposure (ambient) in the experimental basin was 104 dB re 1 μPa, which was comparable to the ambient noise levels of our measurements in the Oosterschelde marine inlet. To quantify the amplitude level of the impulsive sound treatments, single-strike sound exposure level (SEL_{ss}) and zero-to-peak sound pressure level (SPL_{z-p}) were measured (Table 1). The amplitude levels were chosen to represent received level of pile driving at a range of around 50-100 km according to ideal cylindrical spreading. Spectral investigation confirmed that most of the sound energy of the pulses was concentrated between 200 and 1000 Hz (Fig. 2b).

Particle motion may be perceptually dominant in European seabass hearing¹⁷, but we were unable to measure this. However, we believe that the lack of this information is not a concern in this study, since our aim was not to assess absolute threshold levels that can be extrapolated to outside conditions. Our main interest was to compare the effects of PRI on behavioural response while keeping other acoustic parameters constant.

EXPERIMENTAL SET-UP

We tested twelve groups of four fish, where each group was exposed to all four treatments (N = 12, 48 fish). The order of the treatments per fish group followed an incomplete counterbalanced design (12 of 24 possible orders), to minimise the potential 'carry-

Table 1. Relevant acoustic parameters of the four sound treatments: pulse repetition interval (PRI), pulse repetition rate (PRR), exposure duration, average zero-to-peak sound pressure level (SPL_{z-p}), average single-strike sound exposure level (SEL_{ss}), number of pulse and average cumulative sound exposure level (SEL_{cum}).

Treatment no	PRI (s)	PRR (s^{-1})	Duration (min)	Avg SPL_{z-p}	Avg SEL_{ss}	Pulse no	Avg SEL_{cum}
1	0.5	2.00	60	158	140	7200	179
2	1.0	1.00	60	158	140	3600	176
3	2.0	0.50	60	158	140	1800	173
4	4.0	0.25	60	158	140	900	170

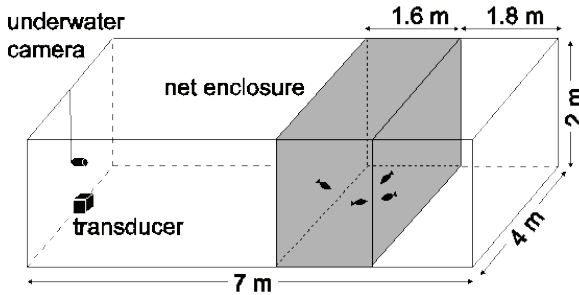
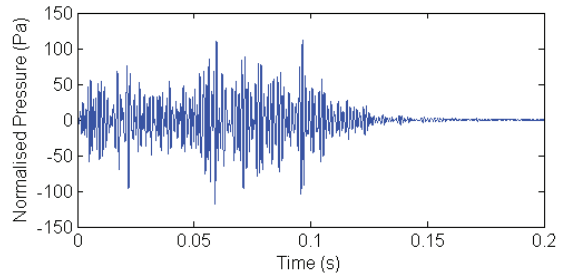


Fig. 1 Experimental basin at SEAMARCO. Shaded area is the net enclosure with restricted swimming space for four fish.

(a) Time domain waveform



(b) Power density spectra

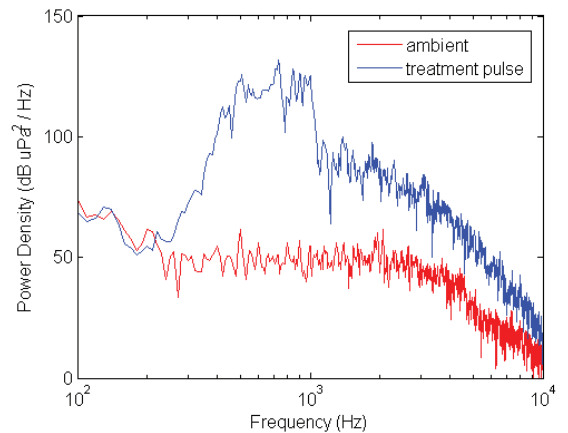


Fig. 2 (a) Time domain waveform of a single pulse used in the treatments and (b) power density spectra of the same pulse and the ambient noise before the playback of the pulse.

over' effect due to sequential exposures. At least 17 h prior to the trials, each fish group was transferred to the experimental basin to allow acclimatisation. 30 min before each trial, the transducer and the lights above the experimental basin were turned on. We conducted two trials per day: one in the morning and one in the afternoon, with a break of at least three hours in between. There was no external anthropogenic noise or disturbance near the study area during the trials. The trials consisted of 10 min of pre-exposure silence and 60 min of sound exposure. Based on pilot and previous studies⁹, we expected the fish behaviour to recover within 60 min of sound exposure. Right after the playback of the 60-min treatment sound, a 2-second 600Hz tone ($SEL_{ss} = 156$ dB re $1\mu Pa$, $SPL_{z-p} = 157$ dB re $1\mu Pa$) was played back to test for stimulus specificity of the behavioural recovery, which was to demonstrate that the recovery was due to habituation and not sensory adaptation or motor fatigue¹⁰. The fish were expected to show startle response again upon presentation of the tone, if they had only habituated to the repeated impulsive treatment sound and could still hear and react to a novel sound stimulus.

BEHAVIOURAL OBSERVATION & ANALYSIS

The fish were video-recorded during the entire exposure sessions (10 min before, 60 min during and 10 s after sound exposure). The full video recordings were analysed with tracking software, Logger Pro 3.8.5.1 (Vernier Software & Technology, Beaverton, US), with manual placement of all fish coordinates every second. The coordinates were then used to calculate the swimming speed, the swimming depth and the group cohesion (average inter-individual distance).

STATISTICS

To test for the change in swimming depth and group cohesion during the trials, we conducted two-way repeated measures

ANOVAs comparing three 5-minute bins of exposure sequence from our data set: 5 min right before sound exposure ('before'), the first 5 min of exposure ('start') and the last 5 min of exposure ('end'). The bin length was chosen based on a previous study with comparable setup⁹. Both exposure sequence and treatment were treated as within-subject (repeated) factors. If sphericity cannot be assumed, Huynh-Feldt correction was used. To test for the change in swimming speed, we used the same procedure but the bins were 10 s instead of 5 min, in order to capture the transient nature of speed change. The order effect was also tested in the model as a covariate but was subsequently excluded when it showed no correlation.

To understand the interaction of the two factors in the previous test and find out if the behavioural changes varied systematically across treatments of different PRIs, we performed repeated measures linear contrast analyses to compare the difference before and at the start of exposure, as well as before and at the end of exposure for the three parameters above. This statistical test has a higher power than omnibus ANOVA tests, given that we had a priori expectation that our variables correlated in a linear manner. We also performed one-sample *t*-tests to see if each difference mentioned was significantly larger than 0.

We also analysed the recovery time of the behavioural changes, which was defined as the time that the fish took to revert back to the pre-exposure swimming depth and group cohesion. The 5-minute average of swimming depth and group cohesion before exposure was used as a baseline to compare with the 5-minute moving averages (shifting forward every second) during exposure, to see when the baseline was reached again. If the baseline was not reached by the end of the trial, the recovery time was counted

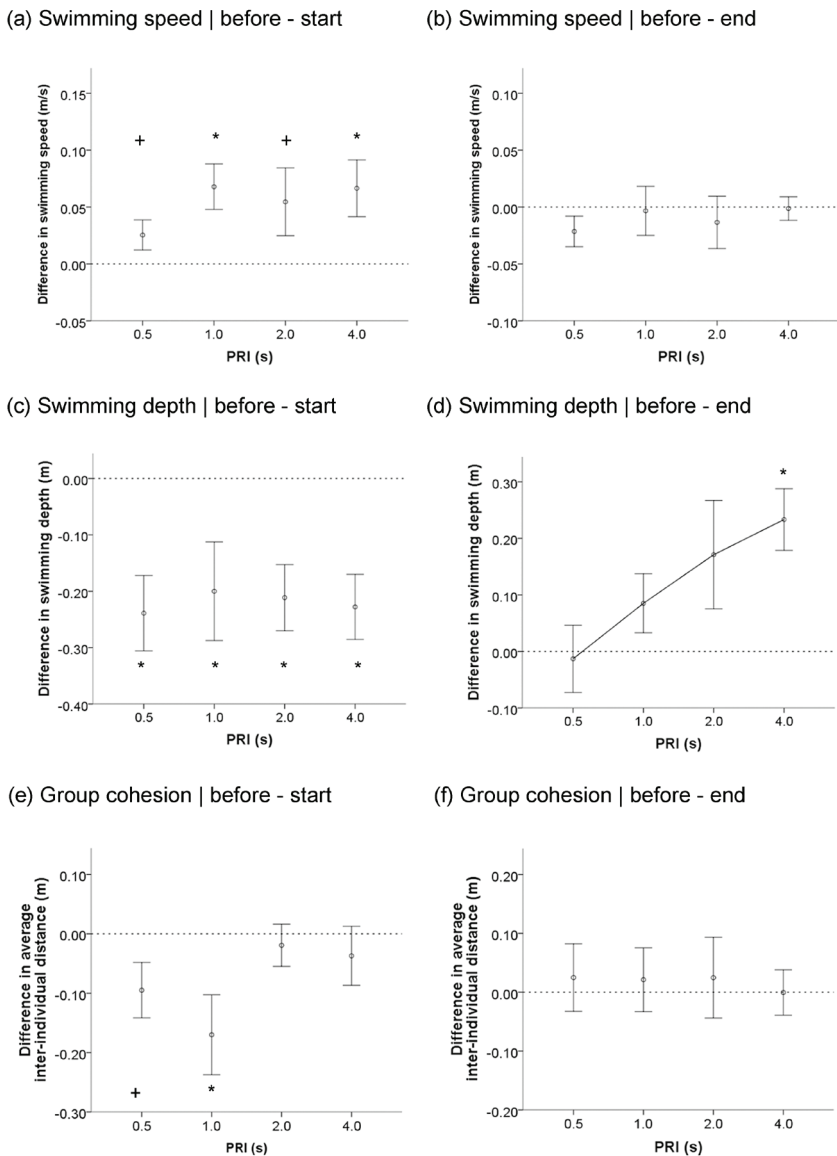


Fig. 3 The difference in swimming speed, swimming depth and group cohesion (mean \pm SE) before and at the start of the exposure, and before and at the end of the exposure, for the four different pulse repetition interval (PRI) treatments. (a) Swimming speed increases in all the treatments at the start of the exposure, (b) and resumes back to baseline level at the end of the exposure. (c) The change in swimming depth is the same for all treatments at the start of the exposure, (d) but the difference in swimming depth between before and the end of the exposure is positively correlated with the PRI (linear contrast analysis: $F_{1,11} = 9.754$, $P = 0.01$). (e) The difference in group cohesion before and at the start of the exposure is significant only for PRI 1.0 s, (f) while there is no significant difference before and at the end of the exposure for all four treatments. An asterisk (*) denotes a significant difference from 0 ($P \leq 0.05$) and a plus (+) denotes a non-significant trend ($0.05 < P \leq 0.1$).

as 60 min (occurrence frequency: 2/48 for swimming depth and 2/48 for group cohesion). The recovery time of swimming speed was analysed in the same manner but with 10-second averages. To compare the effect of different treatments on recovery time, we performed non-parametric Friedman test, which accounted for the repeated nature of the treatments. To demonstrate that the behavioural recovery observed was due to habituation and not sensory adaptation or motor fatigue, we compared 10-second bins of swimming speed before and after the start of sound treatments, and before and after the playback of a 600-Hz tone right after the sound treatments.

Results

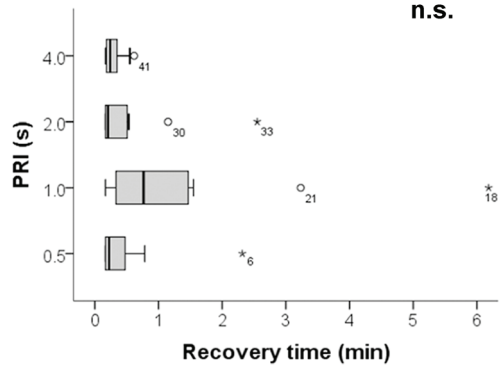
The fish significantly increased in swimming speed (two-way rANOVA: $F_{2, 22} = 12.108$, $P < 0.001$; Bonferroni-Holm *post-hoc*: 'before' vs 'start', $P = 0.008$) and swam to significantly greater depth at the start of the exposure (two-way rANOVA: $F_{2, 22} = 28.121$, $P < 0.001$; Bonferroni-Holm *post-hoc*: 'before' vs 'start', $P = 0.005$) in significantly tighter shoals (two-way rANOVA: $F_{2, 22} = 6.886$, $P = 0.005$; Bonferroni-Holm *post-hoc*: 'before' vs 'start', $P < 0.05$). By the end of the exposure, swimming speed and group cohesion recovered to pre-exposure level (Bonferroni-Holm *post-hoc*: 'before' vs 'end', $P = 1.000$ & $P = 0.454$ respectively) but swimming depth became even shallower than pre-exposure level (Bonferroni-Holm *post-hoc*: 'before' vs 'end', $P < 0.01$). The analysis also showed that for swimming depth, there was a non-significant trend on the interaction between PRI and the 5-min exposure sequence (Huynh-Feldt corrected two-way rANOVA: $F_{4.7, 51.8} = 2.136$, $P = 0.079$).

The magnitude of change from before to the start of exposure did not vary systematically across the four PRIs for swimming speed, swimming depth and group cohesion (linear contrast analysis: $F_{1, 11} = 1.232$, $P = 0.291$, $F_{1, 11} = 0.015$, $P = 0.904$; $F_{1, 11} = 0.063$, $P = 0.128$ respectively) (Fig. 3). When testing for PRI-specific effects in the difference of swimming speed, the difference was significantly larger than 0 for PRI 1.0 s and 4.0 s (one-sample *t*-test: $t_{11} = 3.388$, $P = 0.006$; $t_{11} = 2.666$, $P = 0.022$ respectively) but a non-significant trend for PRI 0.5 s and 2.0 s (one-sample *t*-test: $t_{11} = 1.921$, $P = 0.081$; $t_{11} = 1.832$, $P = 0.094$ respectively). For swimming depth, the difference was significant for all the treatments (one-sample *t*-test: $-3.944 \leq t_{11} \leq -2.285$, all P s < 0.05). For group cohesion, only PRI 1.0 s was significantly larger than 0 (one-sample *t*-test: $t_{11} = -2.523$, $P = 0.028$), while PRI 0.5 s was a non-significant trend (one-sample *t*-test: $t_{11} = -2.035$, $P = 0.067$).

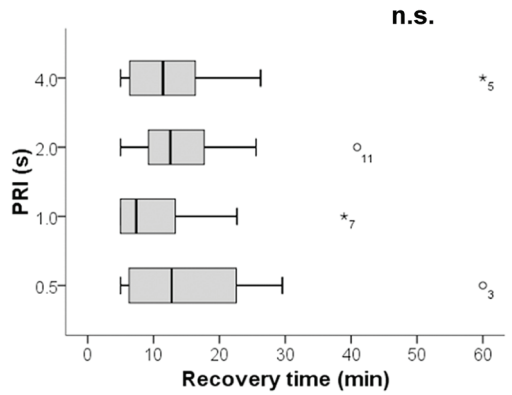
For the difference between before and the end of the exposure, swimming speed and group cohesion was the same across all treatments (linear contrast analysis: $F_{1, 11} = 1.021$, $P = 0.334$; $F_{1, 11} = 0.133$, $P = 0.722$ respectively), but swimming depth showed a significant positive linear effect (linear contrast analysis: $F_{1, 11} = 9.754$, $P = 0.01$). The difference with baseline ranged from no significant difference for PRI 0.5 s (one-sample *t*-test: $t_{11} = -0.218$, $P = 0.831$), 1.0 s (one-sample *t*-test: $t_{11} = -0.218$, $P = 0.831$) and 2.0 s (one-sample *t*-test: $t_{11} = -1.630$, $P = 0.131$), to a significant difference of 0.23 ± 0.19 (SD) m for PRI 4.0 s (one-sample *t*-test: $t_{11} = 4.285$, $P = 0.001$).

We found that the fish habituated to the treatment sounds within an hour (Fig. 4), but the habituation time did not vary significantly among all treatments, for swimming speed (Friedman test: $X^2_3 = 5.625$, $P = 0.131$), swimming depth (Friedman test: $X^2_3 = 2.806$, $P = 0.423$) and group cohesion (Friedman test: $X^2_3 = 1.216$, $P = 0.749$). When comparing

(a) Recovery of swimming speed



(b) Recovery of swimming depth



(c) Recovery of group cohesion

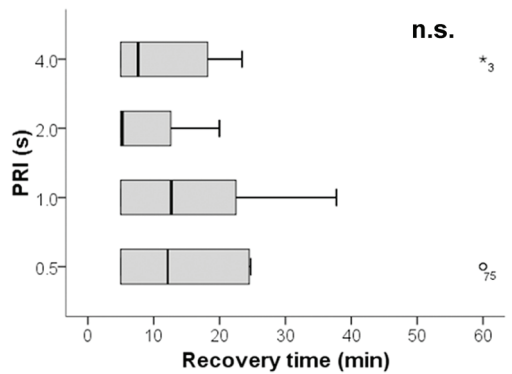


Fig. 4 Boxplots showing the recovery time of (a) swimming speed, (b) swimming depth and (c) group cohesion for the four pulse repetition interval (PRI) treatments. The fish habituate to the sound exposure within the same time for the four treatments.

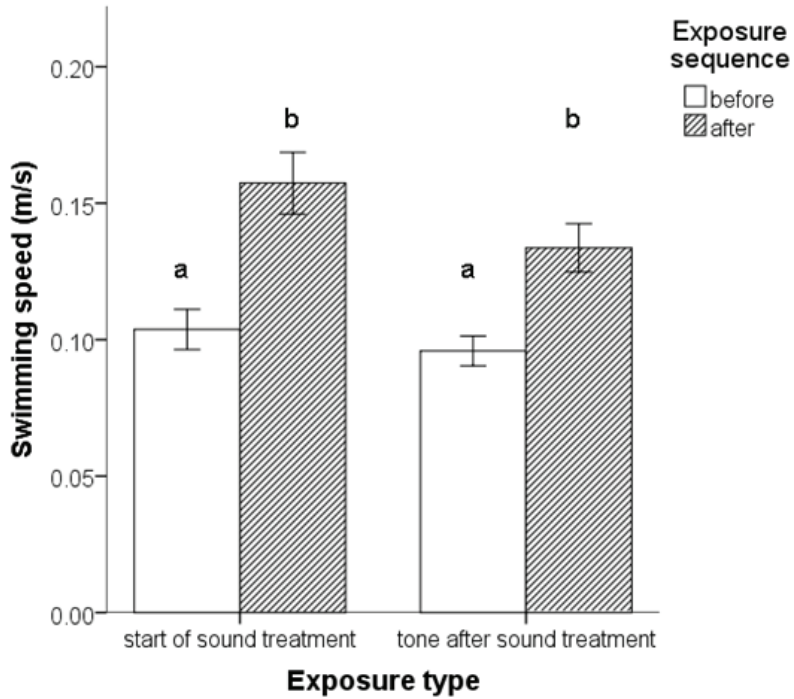


Fig. 5 Average swimming speed (\pm SE) before and after the exposure of two different acoustic stimuli. The swimming speed changes in the same way during the playback of 600 Hz tone compared to the playback of sound treatments, indicating that the fish could still hear and react to a novel acoustic stimulus after having habituated to the treatment exposure.

the swimming speeds before and after the first pulse of sound treatments, and before and after the playback of a 600-Hz tone right after the sound treatments, both exposure types resulted in a rapid increase in swimming speed (startle response) (two-way rANOVA: $F_{1,46} = 32.377, P < 0.001$ for exposure sequence; $F_{1,46} = 1.997, P = 0.164$ for exposure type; $F_{1,46} = 0.669, P = 0.418$ for the interaction of the two factors), indicating that at the end of the trials, the fish could still hear the tone and react to it to the same degree as to the treatment sound at the start of the trials (Fig. 5). The PRI of the treatment did not influence the speed change caused by the 2-second tone (two-way rANOVA: $F_{1,44} = 16.192, P < 0.001$ for exposure sequence; $F_{1,44} = 0.147, P = 0.931$ for the interaction of exposure sequence and PRI).

Discussion

The European seabass in this study swam to the bottom of the basin and formed tighter shoal upon exposure to impulsive sounds. All treatments seemed to be equally effective in eliciting an initial response of increased swimming speed and bottom diving, independent of the PRI, except for group cohesion, which was less affected by the slower than the faster pulse rates. Over time, they recovered by swimming higher up in the water column and shoaling less tightly again. Recovery occurred at the same rate regardless of the PRI of the sound treatments. While swimming speed and group cohesion reverted back to the baseline level and remained there till the end of the exposure, swimming depth went beyond baseline level by the end of the exposure depending on the PRI of the sound treatments: the longer the PRI, the shallower the fish swam. We also demonstrated that the behavioural recovery could be attributed to habituation to the sound treatments.

IMMEDIATE BEHAVIOURAL CHANGES

The startle response, bottom diving and shoaling behaviour observed at the start of sound exposure in this study mirror a previous study using larger European seabass (35 cm) in a smaller experimental enclosure⁹, although the magnitude of changes was smaller in the current study. The observed behavioural changes were also consistent with several other studies conducted on other captive or wild species using various sound sources^{5-7,18,19}. These behaviours have typically been associated with fright and anxiety in neurophysiological studies²⁰⁻²², and each of them is likely related to specific stress-related contexts⁹. These behavioural changes may incur short-term energetic costs to the fish, but it is still unknown if they will lead to disruptions of other important behaviours and have long-term consequences on fish populations.

EFFECTS OF PULSE REPETITION INTERVAL

Contrary to our expectation, PRI of sound exposure had no influence on the habituation rate of the fish. This may be either because the range of PRIs tested in this study was not large enough to provide sufficient resolution to yield an observable difference in habituation rate, or because our setup did not allow inter-pulse spontaneous recovery, like in other studies that tested different PRIs on sound habituation^{15,16}. Nevertheless, our fish appeared to perceive the difference of the four PRIs tested, since they changed their group cohesion at the start of the sound exposure depending on the PRI and showed a PRI-dependant swimming depth difference at the end of the sound exposure. The correlation of PRI and 'post-habituation' swimming depth suggests that PRI of impulsive sound exposure can cause fish swimming behaviour to deviate from baseline level, although the underlying mechanisms are still unknown.

One possible explanation is that the fish swam higher up in the water column due to reduced general wariness as a result of attentional shift caused by the constant input of the habituated sounds, despite immediate stress-related response at the onset of exposure^{20,21,23}. Although the fish might be in a lower anxiety state, there could be other negative consequences during noise exposure, such as masking of important acoustic cues and distraction from potential predators and prey^{23,24}. Another explanation is that the observation was in fact habituation that proceeded beyond the baseline and reached a different asymptotic level^{10,25,26}. This implies that the treatments with larger PRIs in our study resulted in longer habituation time, which is in agreement with the literature^{10,27}. However, it is unclear if the elevated swimming depth would eventually revert back to the baseline, as this potential recovery could not be determined in our experimental setup, where a 600 Hz tone was played right after the treatment trials and the fish readily dived to the bottom regardless of the PRIs.

IMPLICATIONS FOR NOISE IMPACT ASSESSMENTS

Our findings suggest the potential contribution of PRI of impulsive sound exposure in changing the swimming patterns and perhaps the state of wariness of fish during sound exposures. This effect was seen at the start of the exposure for group cohesion and the end of the exposure for swimming depth, but not for the habituation rate to the sound exposure. However, the mechanisms of the observed effects are unclear, which renders the interpretation problematic. Therefore, caution is necessary when translating this finding to management strategies.

Nevertheless, our results suggested that the treatment with 0.5 s PRI had the least impact on fish swimming depth compared to other treatments with larger PRIs, even though its

SEL_{cum} and number of strikes were the highest. This implies that some standard metrics such as SEL_{cum} and the number of pulse strikes may have limited predictive power for assessing potential behavioural impacts of impulsive sounds²⁸. This is in agreement with the results of Neo *et al*⁹, where impulsive sound treatments prolonged the behavioural recovery of European Seabass for twice as long compared to continuous sound treatments (with about double the SEL_{cum}). In view of this, our results agreed with Halvorsen *et al*²⁹, who investigated the thresholds for impulsive sounds to cause barotrauma injuries in Chinook salmon (*Oncorhynchus tshawytscha*), in rejecting the 'Equal Energy Hypothesis', which states that the same amount of acoustic energy will cause the same level of impact severity, regardless of how the energy is distributed in time^{30,31}.

In regard to relevant metrics for noise assessment on behavioural impacts, we believe that the qualitative characteristics of sound, such as various temporal structure parameters, including PRI, may be very useful⁹. Therefore, more temporal parameters, such as pulse repetition regularity, pulse duration and pulse shape, still need to be studied, so that some well-grounded quantification metrics and assessment methods can eventually be developed, which will provide practical information to inform management decisions regarding anthropogenic noise impacts.

CONCLUSION

Our study suggests that different PRIs of impulsive sounds may affect the immediate or delayed behavioural changes of fish differentially, without influencing the rate of the behavioural recovery. It is unclear whether these differences in behavioural changes would matter when it comes to the fitness consequences of the fish, especially in the wild. Nevertheless, despite the uncertainties, our findings provide insights into the relative impact strength of PRI, and highlight the

importance of future studies conducted in the field, examining the temporal variations of sound exposures in assessing impact severity.

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