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Sound investigation: effects of noise on marine animals across trophic levels

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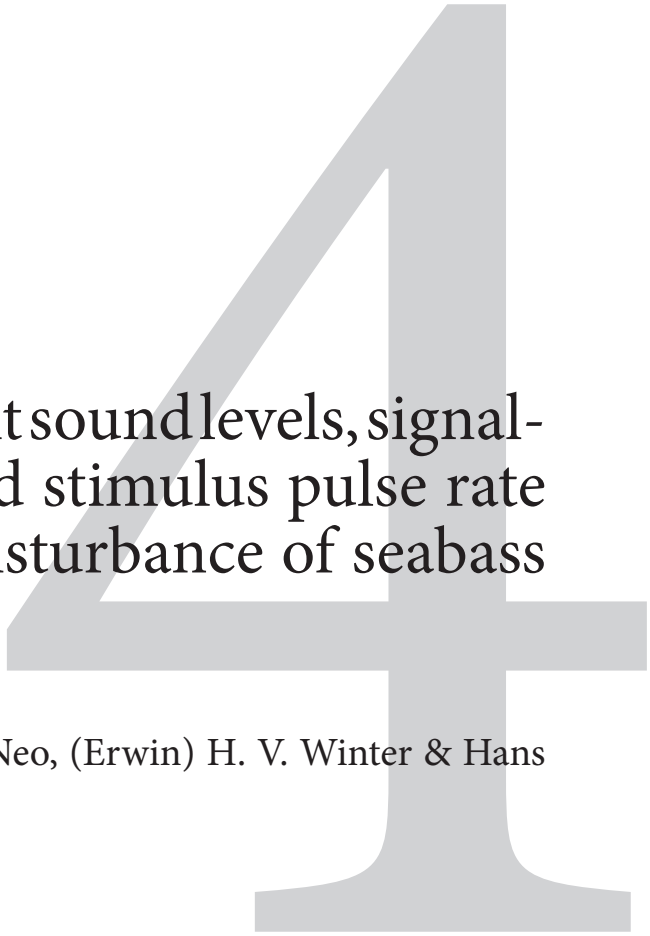


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The role of ambient sound levels, signal-to-noise ratio, and stimulus pulse rate on behavioural disturbance of seabass in a net pen

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Abstract

Anthropogenic sources increasingly contribute to the underwater soundscape and this may negatively impact aquatic life, including fish. Anthropogenic sound may mask relevant sound, alter behaviour, physiology, and may lead to physical injury. Behavioural effect studies are often seen as critical to evaluate individual and population-level impact. However, behavioural responsiveness likely depends on context and characteristics of sound stimuli. We pose that ambient sound levels, signal-to-noise ratio (SNR), and pulse rate interval (PRI), could affect the behavioural response of fish. To study this, we experimentally exposed groups of tagged European seabass (*Dicentrarchus labrax*) to different impulsive sound treatments that varied in pulse level, elevated background level, SNR, and PRI. Upon sound exposure, the seabass increased their swimming depth. The variation in the increase in swimming depth could not be attributed to pulse level, background level, SNR or PRI. It may be that the current range of sound levels or PRIs was too narrow to find such effects.

Introduction

The aquatic world is filled with a large variety of sounds, which may affect aquatic animals. A lot of these sounds originate from natural sources like water movements and animal activities (Hildebrand, 2009). However, over the last century, anthropogenic sounds have become much more prominent in this cacophony of underwater sounds (Andrew et al., 2002; Slabbekoorn et al., 2010). Anthropogenic sound sources include shipping, offshore constructions, sonar exploration, seismic surveys and underwater explosions. The increasing numbers and source levels of these sound sources have led to an increased interest in the impact of anthropogenic sound on fish and other aquatic animals (Carroll et al., 2016; Williams et al., 2015). Anthropogenic sound can potentially cause physical injury, increase hearing thresholds, mask relevant sounds and change physiology and behaviour in fish (Popper and Hastings, 2009; Slabbekoorn et al., 2010). Insights into the impact at individual and population level likely depend on revealing and understanding behavioural effects. This is, however, a complex task as there are many environmental factors and anthropogenic sound characteristics that may modulate their impacts on animal behaviour (Bejder et al., 2009; Slabbekoorn, 2016).

An environmental factor that may be especially relevant for assessing sound impact is the ambient noise level (Ellison et al., 2012). At sea, ambient noise mainly originates from water surface roughness as a function of weather conditions and boat noise (Carey and Browning, 1988; Wenz, 1962). The boat noise mentioned here does not refer to a single nearby ship whose sound can easily be discriminated from background noise, but to chronic omnipresent low-frequency noise produced by ships (Wright et al., 2007). This means that exposed waterbodies around busy shipping routes have relatively high ambient sound levels (Haver et al., 2018; Sertlek et al., 2016), whereas areas that are away from boat traffic and sheltered from wind and waves have relatively low ambient levels (e.g. Merchant et al., 2016).

Elevated ambient noise levels have been shown to negatively affect auditory detection and recognition thresholds due to masking in a variety of species, such as humans (Beattie et al., 1994), birds (Noirot et al., 2011), and fishes (Hawkins and Chapman, 1975; Ladich and Schulz-Mirbach, 2013; Wysocki and Ladich, 2005). However, masking can also be utilized for positive effects. In humans, masking effects have been exploited in open-plan offices to reduce intelligibility of background speech by playing sound (Hongisto et al., 2017; Schlittmeier et al., 2008). Similarly, fountain sound has been shown to reduce perceived loudness of traffic noise (Coensel et al., 2011; Nilsson et al., 2010). For non-human animals,

it has also been suggested to elevate ambient noise levels in zoo exhibits in order to decrease the signal-to-noise ratio (SNR) of potentially disturbing sounds from visitors (Wells, 2009). Similarly, a harbour porpoise (*Phocoena phocoena*) has been shown to reduce its behavioural response to a sound signal when ambient levels were artificially increased (Kastelein et al., 2011). While SNR may affect behavioural responsiveness of animals to sound exposures, it has not been systematically studied as a factor in underwater sound impact assessments.

Not only the environment, but also the anthropogenic sounds themselves vary in several acoustic characteristics that are likely to affect the behavioural responsiveness of fish. Although this is often neglected in sound impact assessments and legislation, fish are known to be sensitive to temporal patterns in sound exposures (Neo et al., 2014). Fish detect high temporal resolution in sound pulses which could mediate species and individual recognition (Marvit and Crawford, 2000; Wysocki and Ladich, 2002). Anthropogenic sounds vary considerably in temporal pattern; ship traffic and wind farms produce relatively continuous sound, whereas pile driving and seismic surveys produce impulsive sound. The pulse rate interval (PRI) is usually 1 to 4 s for pile driving (Hall, 2013; Matuschek and Betke, 2009) and 5 to 15 s for seismic surveys (McCauley et al., 2000). Such variation in temporal patterns in sounds has been shown to influence behavioural responses in fish (Neo et al., 2014; Neo et al., 2015a).

Most studies on temporal patterns have been conducted using European seabass (*Dicentrarchus labrax*). Seabass is a demersal fish that commonly inhabits shallow waters, where juveniles form schools and adults may shoal loosely with fewer individuals (Frimodt and Dore, 1995). They hear best up to 1000 Hz and are known to show behavioural and physiological responses to sound exposures (Bruintjes et al., 2016; Kastelein et al., 2008; Lovell, 2003). They increased their swimming depth (swam deeper) during both continuous and impulsive sound exposures, but during the latter, the seabass took twice as long to return to their baseline swimming depth (Neo et al., 2014). When comparing the effects of different PRIs, Neo et al. (2015a) found that smaller PRIs - faster pulse rates - increased group cohesion, and that PRI affected post-exposure swimming depth. These findings, together with a later outdoor study using the same species (Neo et al., 2016), suggest that sound pressure level (SPL) and cumulative sound exposure level (SEL_{cum}) alone are not sufficient in scaling acoustic conditions for assessing behavioural impact.

In the current study, we exposed hatchery-reared European seabass in a net pen to impulsive sound treatments and examined their behavioural changes. We aimed at gaining more insight into the limitations of SPL and SEL_{cum} as measures for

behavioural thresholds by exploring variation in responsiveness depending on ambient sound levels, signal-to-noise ratios, and stimulus pulse rate. We created artificial sound treatments that varied in pulse level and background noise level, causing variation in signal-to-noise ratio (SNR), and pulse rate interval (PRI). We assessed experimental sound exposure levels in both sound pressure and sound velocity levels. By comparing the behavioural response among the different sound treatments, we aimed to determine if SNR and PRI modulate behavioural responses.

Materials and methods

Study subjects

We used 16 groups of four European seabass (*Dicentrarchus labrax*) of 35 to 40 cm in body length. The fish were acquired from a hatchery (FRESH Völklingen GmbH, Germany) and kept in two indoor holding tanks (Ø 3.5 m, depth 1.1 m) at Stichting Zeeschelp (The Netherlands) in a light-dark cycle following the outdoor day-night cycle. The water in the holding tanks was continuously refreshed with seawater from the Oosterschelde, a sea inlet of the North Sea. The fish were fed commercial pellets (Aller Blue Organic EX 8 mm, AllerAqua, Denmark), whose amount was determined by the water temperature according to the description of the manufacturer.

Experimental arena

The experiment was conducted using a study island in the Jacobahaven, a man-made cove in the Oosterschelde (Fig. 1). 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 Oosterscheldekering and no external boat traffic is allowed in its proximity. We used a modular floating system (Candock, Canada) to assemble the study island. It consisted of a working platform for the equipment and researchers, and an octagonal walkway that supported a net pen as experimental arena (Ø 11.5-12.5 m, > 3 m deep). The two parts were separated by a 0.5 m distance aiming to reduce direct sound transmission from the working platform to the net pen. One end of the working platform held the underwater speaker at a distance of 7.8 m from the net to avoid unwanted near-field effects of the speaker. The set-up has been used for previous sound exposure studies and detailed measurements of the underwater soundscape revealed gradually decreasing sound levels with increasing distance from the speaker (Hubert et al., 2016; Neo et al., 2016; Neo et al., 2018). The study island was located in the middle of the Jacobahaven and anchored with dead weights in combination with chains and stretchable bungee ropes that kept the island in place throughout the tides.

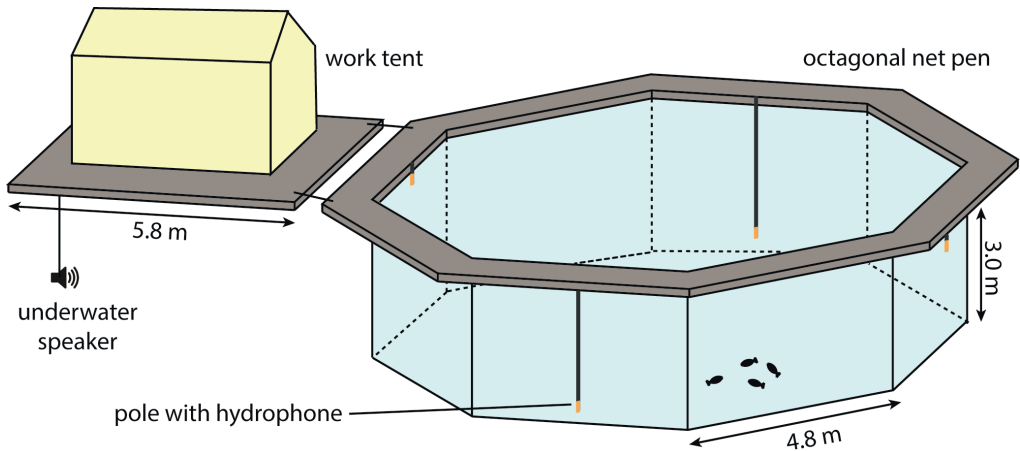


Fig. 1: A schematic overview of the net pen and research platform (figure from Neo et al., 2016). The square working platform was connected to the octagonal walkway used ropes, leaving a gap of approximately 0.5 m. The far end of the working platform held the underwater speaker at a depth of 2.2 m and a distance of 7.8 meter to the net. The four hydrophones recorded the acoustic signals from the tags. The distance between adjacent hydrophones was 8.7 m.

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 0.5 ms 307 kHz signals at a fixed ~ 1 s interval. Fish could be identified and tracked individually because of small differences in the programmed signal interval of the different tags. At the net pen, the acoustic signals of the tags were received by four hydrophones (Model 590-series, HTI, US); two close to the surface and two close to the bottom. The received signals were logged on a laptop via a tag receiver (Model 291, HTI, US).

Before tagging, each fish was anaesthetised in a bath with 2-phenoxyethanol (0.5 ml/l seawater). Once anaesthetised, the fish was placed on its back in a v-shaped cradle to keep the fish's abdominal wall above water and its head submerged in seawater with half the amount of 2-phenoxyethanol (0.25 ml/l) to maintain anaesthesia. We then made an 1.5-cm incision in the abdominal wall, implanted a tag (volume: 1.4 cm³; in air weight: 4.6 g) in the intraperitoneal cavity and sutured the opening. After the tagging, the fish could recover in a rectangular tank (1.20 x 1.00 x 0.65 m) with continuously refreshed seawater for at least two days (> 40 h).

Treatment series

Using Audacity 2.0.5, we generated 24 different sound treatments, all consisting of 0.1 s pulses and elevated background noise. We used impulsive sound because

this elicited the strongest behavioural response in a previous study on European seabass (Neo et al., 2014) and continuous sound as elevated background noise because continuous sound has the most potential to mask other sound. All sound treatments followed the same structure and only differed in sound level of the pulses, sound level of the elevated background noise, and pulse rate intervals (PRI) (Fig. 2). To make 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. This frequency range was selected because it was not possible to playback sound below 200 Hz due to speaker limitations and physical limitations for propagation of low frequencies in shallow water and because seabass hear best up to 1000 Hz. The actual pulses were created by making silences in the track to obtain a fixed PRI of 0.5, 1.0, 2.0 or 4.0 s and we created tracks of three different sound levels for all PRIs. For the elevated background 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 background noise. We created two different sound levels of this

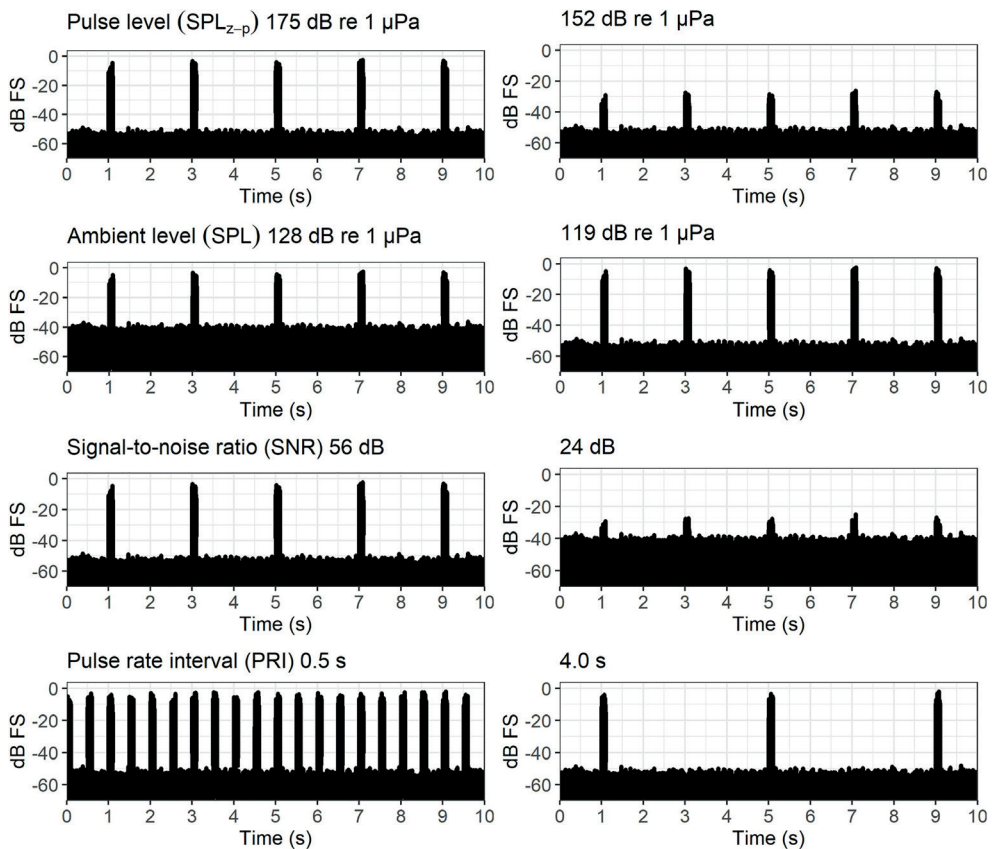


Fig. 2: Waveform in decibels relative to full scale (dB FS) to display the maximum variation in pulse level, ambient level, SNR, and PRI across the 24 sound treatments.

track. The elevated background noise started 20 minutes before the first pulse and ended 5 minutes after the last pulse. The impulsive sound series took 30 minutes, so the total playback lasted for 55 minutes. Altogether this resulted in 24 different sound treatments (four PRIs, three pulse levels and two background levels). The sounds were played back with an underwater transducer (LL-1424HP, Lubell Labs, US) using a laptop, a power amplifier (DIGIT 3K6, SynQ, Belgium) and a transformer (AC1424HP, Lubell Labs, US).

To examine the actual sound levels in the net pen, we measured sound pressure levels (SPL) and sound velocity levels (SVL) twice during flow, high and ebb tide and with both elevated background levels at 2 m deep at six distances from the speaker (every 2.1 m, from 8.3 to 18.8 m from the speaker). The measurements were conducted using the M20 particle velocity sensor (GeoSpectrum Technologies, Canada), which measures sound pressure using an omnidirectional hydrophone and 3D particle velocity using three orthogonal 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, Canada) and a differential oscilloscope (PicoScope 3425, Pico Technologies,

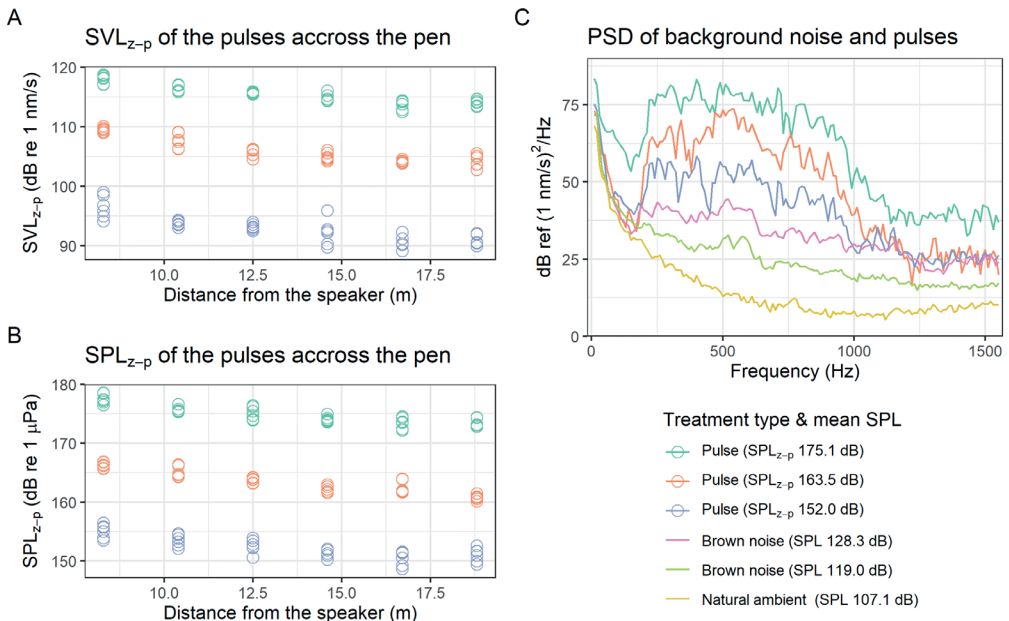


Fig. 3: (A) Zero to peak sound velocity levels (SVL) and (B) sound pressure levels (SPL) of the impulsive sound, across the net pen. This shows the variation of sound levels the fish have been exposed to. (C) Power spectral density (PSD) plots of the sound velocity of the elevated background noise and the pulses, both measured at 12.5 m from the speaker in the middle of the water column. The mean sound pressure levels are depicted in the legends.

UK). The recordings were later processed with Matlab application paPAM (c.f. Nedelec et al., 2016) using a 200-1000 Hz bandpass filter. Across measurement locations and replicate recordings, the mean zero to peak sound pressure levels (SPL_{z-p}) of the pulses in the 200-1000 Hz bandwidth range of the different playback levels were 175.1, 163.5 or 152.0 dB re 1 μ Pa (resp. 115.6, 106.3 or 93.3 dB re 1 nm/s; Fig. 3). The mean rms SPLs of the elevated background noise were 128.3 or 119.0 dB re 1 μ Pa (resp. 69.6 or 61.1 dB re 1 nm/s).

Experimental design

We exposed each of the 16 groups of four fish to six of the 24 sound treatments. The order of the treatments followed a counterbalanced design; each group was exposed to all PRIs at least once, all pulse sound levels twice and both background levels thrice. Each group of fish was tagged at least two days (> 40 h) before being transferred to the net pen (Fig. 4). The fish could acclimatize overnight, for at least 8 h. Each group was exposed to three sound treatments per day, for two days. We conducted one trial at flood tide (starting 2:45 h before

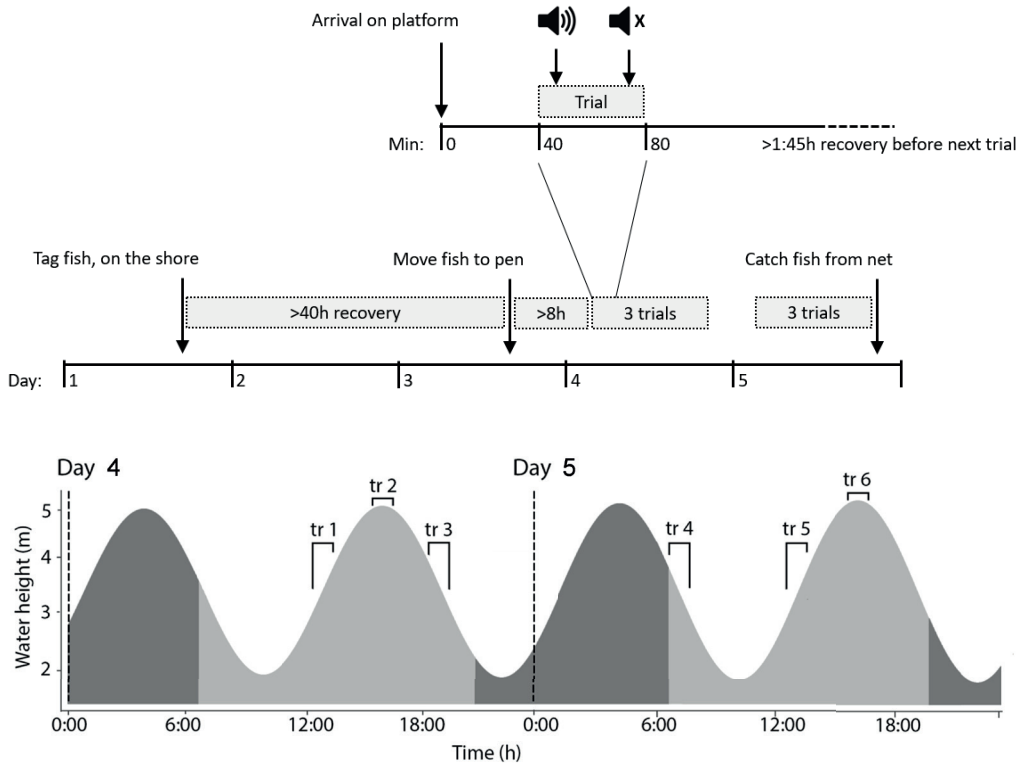


Fig. 4: Timetable for the five-day experimental processing of a single group of four fish (top panel). Tagging was done on day 1; moving to the net pen on day 3; and the six half-hour sound exposure trials took place on day 4 and 5. We conducted three trials per day, before, during and after absolute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

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 constant difference in depth between the tag receiving hydrophones and to allow most of the energy of the sound treatment to propagate (cut-off frequency <250 Hz).

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 till 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 each group was exposed to six treatments over two days, the fish were caught and a new group was released into the pen.

Statistics

The received tag signals were processed on a computer using MarkTags v6.1 & AcousticTag v6.0 (HTI, US), generating 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 to the impulsive sound, we used 5-minute-bin-group-averages of these parameters from before the impulsive sound ('before'), after the start of the impulsive sound ('during') (cf. Neo et al., 2014). To capture the transient speed change, we used 10-s-bin-averages for the parameter swimming speed. These four parameters were used as response variables in four Linear Mixed-effect models with Period ('before' or 'during') as a fixed effect and Group ID as a random effect. We compared these models with their corresponding null models using Akaike information criterion for small sample sizes (AICc). If the best model contained Period, we performed a parametric bootstrap procedure (10,000 resamples) where the random effect intercepts from the fitted model were not resampled and instead the fitted estimates were used. The bootstrap was used to determine the 95% confidence interval of the covariate estimate. If this confidence interval did not overlap with 0, we considered the sound exposure to have significantly changed this behavioural parameter.

The behavioural parameters that significantly changed during the sound exposure, were used to explore the effects of our acoustical parameters of interest: PRI, SPL_{z-p} of the pulses, rms SPL of the elevated background noise and the ratio between the latter two (signal-to-noise ratio; SNR), which was calculated as

follows (formula I):

$$\text{SNR}_{\text{dB}} = P_{\text{signal,dB (z-p)}} - P_{\text{noise,dB (rms)}} \quad (\text{I})$$

We used these acoustical parameters, together with other factors that may affect the change in swimming depth (tide, trial order, depth before the start of the sound), as fixed effects in a Linear Mixed-effect model to explain the variation in the change in a behavioural parameter. The best model was chosen by AICc using dredge model selection (package MuMIn) and a bootstrap procedure was used to determine significance of the covariates from the best model.

For all Linear Mixed-effect models, we calculated the marginal (R^2_{m}) and conditional (R^2_{c}) R^2 values to show the proportion of variance in the response variable that is explained by the fixed effects (R^2_{m}) and the entire model (R^2_{c}) (Nakagawa and Schielzeth, 2013). All statistics were done using RStudio (R Core Team, 2016) and the packages lme4 (Bates et al., 2015), MuMIn (Barton, 2016) and piecewiseSEM (Lefcheck, 2016).

Results

We first examined the overall effects of the sound exposures on the swimming patterns of the groups of seabass. The fish significantly increased their swimming depth (swam deeper) upon sound exposure according to model selection (Table 1b; Intercept: 2.28, During: -0.21; Fig. 5B) and bootstrap procedure (95% CI During: -0.34 to -0.08). Model selection of the other behavioural parameters did not show any change related to the sound exposure (Table 1a,c,d; Fig. 5A,C,D). Secondly, we examined the parameters that could explain the variation in the change in swimming depth. Model selection showed that this variation was best explained by the depth before the start of the impulsive sound ($df = 4$, $R^2_{\text{m}} = 0.13$, $R^2_{\text{c}} = 0.44$, Table 1e). Running this model showed that fish that were higher in the water column before the start of the sound, showed a larger increase in depth (Intercept: 0.29, slope Depth before: -0.22; Fig. 6E), the bootstrap procedure showed that this was a significant correlation (95% CI slope Depth before: -0.31 to -0.09). The model selection also showed that none of the treatment manipulations appeared to have an effect on the change in swimming depth (Fig. 6).

Discussion

In the current study, we experimentally exposed European seabass in a net pen to impulsive sound treatments, while varying the pulse rate intervals (PRI),

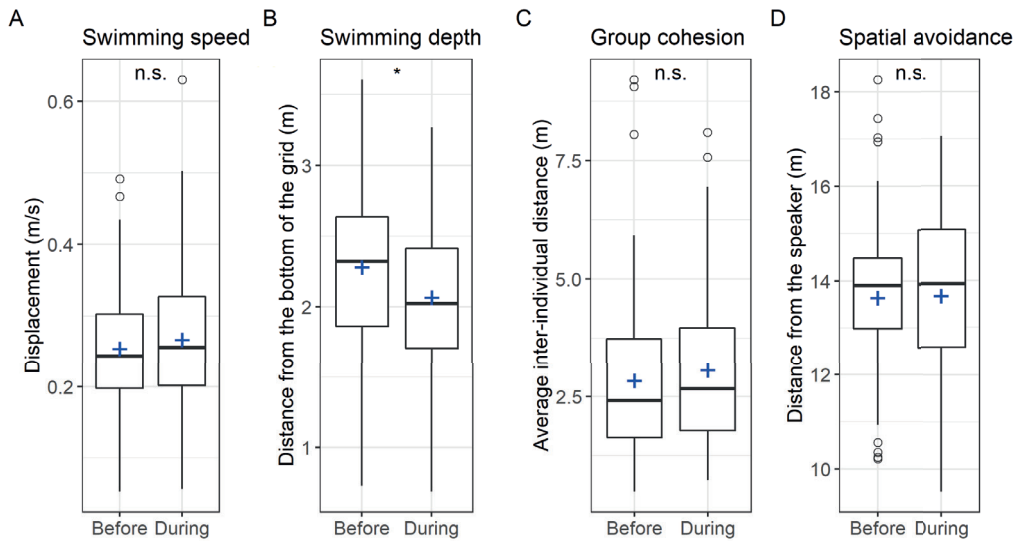


Fig. 5: Four behavioural parameters 5 minutes before and 5 minutes after the start of the impulsive sound, except for swimming speed, where we used 10-s-bins. A blue + indicates the mean and an * indicates a significant difference between the two time bins.

pulse levels, artificial background noise levels, and thereby also the signal-to-noise ratio (SNR). Our results demonstrated that seabass in outdoor conditions responded to the impulsive sound by increasing their swimming depth, but they did not change their swimming speed, group cohesion or distance from the speaker. Pulse level, elevated background level, SNR, or PRI were not significantly correlated to the increase in swimming depth. However, the baseline swimming depth was significantly correlated to the change in swimming depth.

Level dependent response

We did not find a significant effect of pulse level, ambient level or signal-to-noise ratio on the increase in swimming depth of the seabass. Several studies have found level-dependent reactions to sound exposures in a variety of species (e.g. Dunlop et al., 2018; Hawkins et al., 2014; Kok et al., 2018). Also in the current study species, a positive relationship has been found between pile driving pulse level and percentage of groups of four fish that showed a startle response (Kastelein et al., 2017). This contrast of the latter with the current study may be explained by the narrower range of sound levels in the current study. The different findings may also be explained by the type of behaviour that was analysed; startle responses are transient reflexes whereas the current analysis used 5-min-bins of swimming depth. These two measures were also shown not to correlate in a sound exposure study on zebrafish (*Danio rerio*) (Shafiei Sabet et al., 2016).

Table 1: Results of model selection (ranked by ΔAICc) for all four response variables (in front of ~). The marginal R^2 (R^2_m) shows the proportion of variance explained by the fixed effects, the conditional R^2 (R^2_c) shows the proportion of variance explained by the entire model and w_i is the Akaike weight of the model. $\Delta\text{AICc} \geq 2$ indicates a significant difference between the models. An * indicates the best model.

#	Model	df	R^2_m	R^2_c	AICc	ΔAICc	w_i
A: Swimming speed ~ ...							
null*	(1 Group)	3		0.11	-380.5	-	0.98
1	Period + (1 Group)	4	0.01	0.12	-372.6	7.96	0.02
B: Swimming depth ~ ...							
1*	Period + (1 Group)	4	0.03	0.48	278.5	-	0.91
null	(1 Group)	3		0.45	283.2	4.74	0.09
C: Group cohesion ~ ...							
null*	(1 Group)	3		0.29	582.8	-	0.75
1	Period + (1 Group)	4	0.00	0.30	585.0	2.24	0.25
D: Distance from the speaker ~ ...							
null*	(1 Group)	3		0.13	723.9	-	0.83
1	Period + (1 Group)	4	0.00	0.13	727.1	3.19	0.17
E: Change in swimming depth ~ ...							
1*	Depth before + (1 Group)	4	0.13	0.44	61.6	-	0.83
null	(1 Group)	3		0.29	67.7	6.07	0.04
3	Depth before + Tide + (1 Group)	6	0.14	0.44	68.2	6.61	0.03
4	Depth before + Pulse SPL + (1 Group)	5	0.16	0.47	68.9	7.33	0.02
5	Depth before + PRI + (1 Group)	5	0.13	0.44	69.0	7.41	0.02

Signal-to-noise ratio has received limited attention in sound impact studies. Kastelein et al (2011) showed that a harbour porpoise increased its number of surfacings in response to signal sweeps, but less strongly during artificially elevated background levels. Similarly, several studies have shown that animals respond less strongly to experimental sounds in high disturbance areas (Bejder et al., 2006; Harding et al., 2018). This may be partly explained by SNR, but can only be shown by experiments – like the current – in which both background noise and signal levels are varied. The current study found no evidence for SNR as a covariate for behavioural response. However, given that auditory detection and

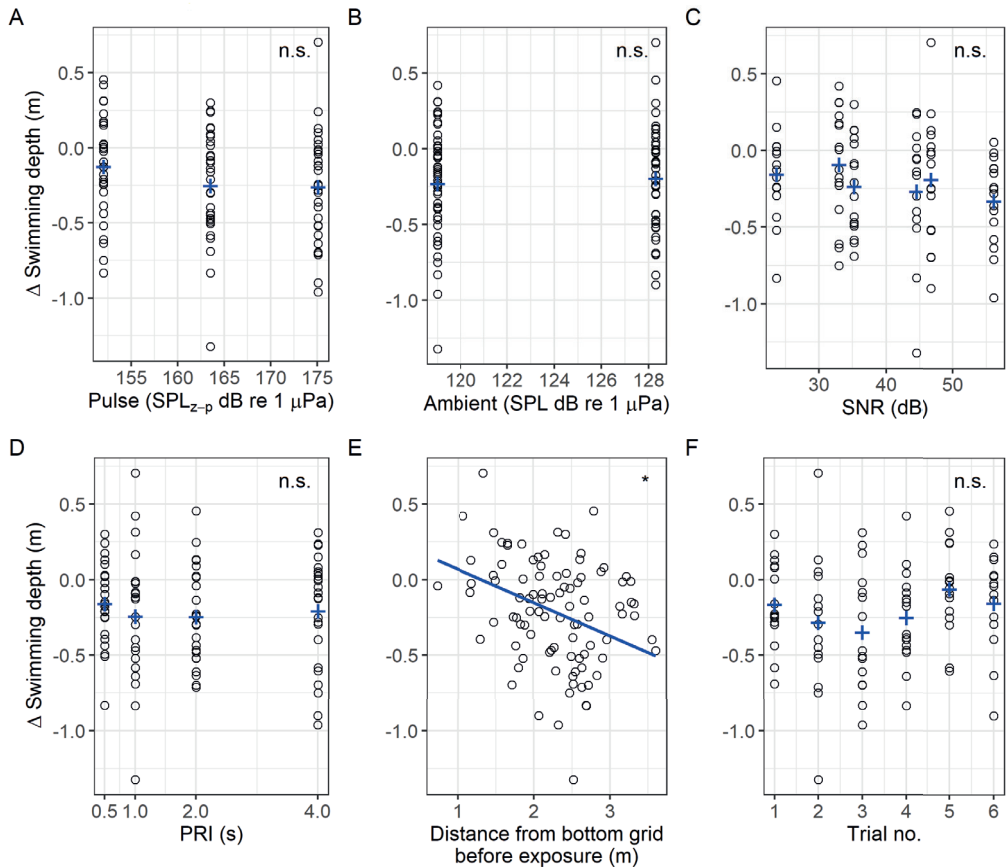


Fig. 6: The effects of pulse level, ambient level, SNR, PRI, depth before exposure and trial number on the change in swimming depth after the start of the impulsive sound. An * in the top-right corner of a plot indicates a significant correlation.

recognition thresholds in fish increase under higher background levels (Hawkins and Chapman, 1975; Wysocki and Ladich, 2005) and perceived loudness of traffic noise reduced with the playback of fountain sound in humans (Coensel et al., 2011; Nilsson et al., 2010), further studies to SNR using larger ranges of signal levels and SNRs are warranted. It may be most fruitful to compare the explanatory value of signal level and SNR in an experimental design and with a study species that has already revealed level dependent responses. Anyway, we strongly recommend to always report both ambient and signal levels in sound impact studies. For field studies that are conducted on different days or different locations, variation in ambient levels may be expected and may explain part of the variation in behavioural responses to sound stimuli.

Pulse rate interval

We did not find an effect of PRI on the change in depth of the seabass. Previous studies have found effects of PRI, however, across studies no clear patterns have emerged. In zebrafish, a significant increase in group cohesion and swimming speed in response to 1 s pulses with 1 s intervals has been found, but not to the same pulses with 9 s intervals (Neo et al., 2015b). However, another study on zebrafish found no differences in immediate response to 1 s pulses with either 1 or 4 s intervals (Shafiei Sabet et al., 2015). In a previous European seabass study with the same PRIs as the current study, faster PRIs were found to increase group cohesion whereas slower rates did not. Additionally, the difference between post-exposure swimming depth and baseline swimming depth was positively correlated with PRI (Neo et al., 2015a). Humans and rats (*Rattus norvegicus*) showed a faster decrease in startle-like responses to pulse trains with a lower PRI (humans: 20 vs. 100 s; rats: 2 vs. 16 s) (Davis, 1970; Gatchel, 1975), the tested range of PRI is however bigger than those described in the fish studies. Thus, it may be that the range of PRI in the current study was too small to find an effect, although, a bigger range would be beyond the range in which pile driving occurs. The PRI used for seismic surveys is usually 5 to 15 s (McCauley et al., 2000) and this range has not been tested for fish yet.

Due to the different PRIs, the sound treatments in the current study differed in cumulative sound exposure level (SEL_{cum}). The absence of an effect of SEL_{cum} on fish behavioural response is in line with other studies that compared the behavioural effect of different sound treatments (Neo et al., 2014; Neo et al., 2015a; Neo et al., 2015b; Neo et al., 2016; Shafiei Sabet et al., 2015). SEL_{cum} was originally proposed – along with zero-to-peak sound pressure level (SPL_{z-p}) – to assess the risk of physical injury to fish by pile driving (Stadler and Woodbury, 2009; Woodbury and Stadler, 2008). For physical injury, SEL_{cum} has been shown to be useful, but not as a single metric (Halvorsen et al., 2012). For behavioural impact assessments, new metrics are needed, and we may also require a combination of metrics. Kurtosis has been suggested as a good metric for continuous sounds (Hastings, 2008; Popper and Hawkins, 2019). However, behavioural response studies scaled to kurtosis are lacking and it is unsuitable as a metric for intermittent sounds, as pulse rate would dominate the metric.

Previous studies with European seabass

The current study followed a similar design as four earlier sound exposure studies on European seabass, this enabled us to qualitatively examine the consistency of seabass reaction to sound across years, seasons, experimental arenas, fish batches and sizes. The seabass in the current study swam to greater

depth upon the start of the sound exposure. Such a response to sound exposures has been shown before in the same species in very similar experimental designs (Neo et al., 2014; Neo et al., 2015a; Neo et al., 2016; Neo et al., 2018). However, we found no effects on group cohesion, swimming speed and spatial avoidance, while they have been found in some of the aforementioned studies (Table 2). In general, fewer behavioural changes have been found in the outdoor studies. In the outdoor studies, the fish were situated in a bigger experimental arena and experienced acoustic conditions that were more similar to those experienced in natural waters. These factors may offer fish more control over their environment (Koolhaas et al., 2011), as they may move around more freely and may perceive sound directionality better, which may reduce their stress levels caused by the sound exposure.

This comparison among well-replicated studies with different testing conditions nicely shows the value of captive studies and their complementary role to field studies. The overview above indicates that indoor studies with high resolution of measurements may be best to explore mechanistic processes and make progress in conceptual understanding. Once we have consensus about an optimal acoustic metric to scale responsiveness to anthropogenic sound of variable features and in variable conditions, we would need to follow up with tests in the field. Insight

Table 2: Overview of sound impact studies on European seabass with a similar design and testing the same behavioural parameters: depth (swimming depth), cohesion (inter-individual-distance), speed (swimming speed) and avoidance (distance from the speaker). The table provides an overview of the significant effects of the sound exposure, ignoring trends. Additional information on these experiments can be found in Supplementary material I.

Study	Arena	Changes in behavioural parameters, before vs. during sound exposure			
		Depth	Cohesion	Speed	Avoidance
Neo et al., 2014	Indoor	Deeper	Tighter	Faster	Not tested
Neo et al., 2015a	Indoor	Deeper	Tighter	Faster	Not tested
Neo et al., 2016	Outdoor	Deeper	No effect	Faster	Further
Neo et al., 2018	Outdoor	Deeper, only at night	No effect	Faster, only at night	No effect
Current study	Outdoor	Deeper	No effect	No effect	No effect

about absolute response thresholds that would be applicable to free-ranging fish in a natural context will not come from studies in captivity and will always require behavioural response studies in that natural context, replicated at the context of interest (i.e. with respect to habitat, species, season, etc.).

Conclusions

In the current study, we provided conceptual background to the potential importance of SNR and PRI in predicting the behavioural effects of sound exposures on animals and experimentally tested this in European seabass. The seabass increased their swimming depth upon sound exposure, however the variation in this increase could not be explained by any of the acoustic parameters of interest; PRI, pulse level, (elevated) background level or SNR. Several earlier studies are indicative of potential explanatory value of SNR, further testing with wider ranges of signal and background levels may yield different results.

Ethical statement

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. AVD106002016610.

Supplementary information

Additional information on the studies from the overview in Table 2 is available from the publication DOI: 10.1016/j.beproc.2019.103992.

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