Sound exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure

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Abstract

Underwater sound from human activities may affect fish behaviour negatively and threaten the stability of fish stocks. However, some fundamental understanding is still lacking for adequate impact assessments and potential mitigation strategies. For example, little is known about the potential contribution of the temporal features of sound, the efficacy of ramp-up procedures, and the generalisability of results from indoor studies to the outdoors. Using a semi-natural set-up, we exposed European seabass in an outdoor pen to four treatments: 1) continuous sound, 2) intermittent sound with a regular repetition interval, 3) irregular repetition intervals and 4) a regular repetition interval with amplitude ‘ramp-up’. Upon sound exposure, the fish increased swimming speed and depth, and swam away from the sound source. The behavioural readouts were generally consistent with earlier indoor experiments, but the changes and recovery were more variable and were not significantly influenced by sound intermittency and interval regularity. In addition, the ‘ramp-up’ procedure elicited immediate diving response, similar to the onset of treatment without a ‘ramp-up’, but the fish did not swim away from the sound source as expected. Our findings suggest that while sound impact studies outdoors increase ecological and behavioural validity, the inherently higher variability also reduces resolution that may be counteracted by increasing sample size or looking into different individual coping styles. Our results also question the efficacy of ‘ramp-up’ in deterring marine animals, which warrants more investigation.

Keywords: anthropogenic noise, *Dicentrarchus labrax*, fish behaviour, field study, sound characteristics, stress response

Introduction

The rise of underwater noise pollution resulting from human activities at seas may threaten the health and stability of fish populations (Hawkins et al., 2014a, Normandeau Associates, 2012, Popper and Hastings, 2009a, Popper and Hastings, 2009b, Radford et al., 2014, Slabbekoorn et al., 2010). This concern needs to be corroborated by understanding how critical fish behaviours change in response to the exposure of man-made noise (Hawkins et al., 2014a, Slabbekoorn et al., 2010). For example, man-made noise has been shown to affect fish by changing their swimming patterns (Hawkins et al., 2014b, Neo et al., 2014, Neo et al., 2015a; Robertis, 2013, Sarà et al., 2007), territorial dynamics (Sebastianutto et al., 2011), antipredator vigilance (Simpson et al., 2014, Voellmy et al., 2014a), foraging efficacy (McLaughlin and Kunc, 2015, Payne et al., 2015, Purser and Radford, 2011, Shafiei Sabet et al., 2015, Voellmy et al., 2014b) and other fitness-related activities (Boussard, 1981, Picciulin et al., 2010). These studies were conducted using different sound sources, which reflected the diversity of man-made noise sources in reality, and varied in their spectral, amplitudinal and temporal characteristics (Slabbekoorn et al., 2010). Different acoustic features likely differ in their relative importance in exerting behavioural effects, but such findings cannot be properly interpreted without deeper fundamental understanding (Hawkins et al., 2014a, Normandeau Associates, 2012).

It was only recently that the temporal characteristics of sound were shown to affect the on-set and recovery of behavioural changes for fish (Neo et al., 2014, Neo et al., 2015a). For example, the behavioural recovery of captive European seabass (Dicentrarchus labrax) in a large basin was faster when exposed to continuous sound than to impulsive sound (Neo et al., 2014). In addition, impulsive sound exposure induced initial and delayed behavioural changes that were influenced by the pulse repetition interval (PRI) (Neo et al., 2015a). Moreover, amplitude fluctuations were shown to affect shoaling behaviour of the seabass

(Neo et al., 2014). The latter effect is interesting as amplitude fade-in, usually called 'ramp-up' or 'soft-start', is widely recognised and has been applied as a mitigation strategy (Hawkins et al., 2014a, JNCC, 2010, Normandeau Associates, 2012, Weilgart, 2007). A gradual rise in sound level, before a pile-driving or seismic shooting operation at full power, is assumed to drive away marine mammals and fish, in order to prevent injuries caused by intense sound exposure close to the sound source. However, the efficacy of the procedure still needs to be demonstrated (Cato et al., 2013).

Behavioural studies often carry implications that are difficult to ascertain because of interpretation discrepancies and generalisation uncertainties inherent to different experimental approaches. For example, tank-based and laboratory studies examining the behavioural impact of sound on captive fish have methodological advantages but also apparent extrapolation limitations (Calisi and Bentley, 2009, Hawkins et al., 2014a, Popper et al., 2014, Slabbekoorn, 2014). Such confined set-ups have high internal validity but lack ecological validity, wherein the acoustic fields likely differ from natural waters in a complex and unpredictable manner (Parvulescu, 1967), and the fish behaviour different and more constrained than in the wild (Hawkins et al., 2014a, Radford et al., 2014). However, this concern has not been substantiated with empirical evidence showing in what ways these limitations result in different behavioural observations between tank-based and open-water studies. Comparisons of behavioural responses to the same stimuli in the same social setting in both tank-based and open-water conditions could improve the external validity of test results and may provide additional insights into the underlying mechanisms (Brewer, 2000, Campbell, 1957).

Field studies on free-ranging animals have the highest ecological validity, but conducting well-replicated and well-controlled sound exposure studies at sea is exceedingly costly and logistically challenging. Moreover, discrepancies between contradictory results from different field studies can often not be sufficiently explained (see Hawkins et al., 2014b), due to unknown and potentially confounding or modulating factors. Consequently, a semi-natural approach with semi-controlled setting and a size-appropriate enclosure in the fish natural environment may sometimes be an optimal compromise (Calisi and Bentley, 2009, Slabbekoorn, 2014).

In this study, we used European seabass in a large floating pen in a man-made cove within a tidal marine inlet, to test the impacts of sound exposure with different temporal structures. We tested four sound treatments varying in intermittency (continuous vs impulsive), repetition interval regularity and the presence of ‘ramp-up’ to test the following hypotheses: 1) Upon sound exposure, fish change their swimming speed, swimming depth, group cohesion and swim further away from the sound source; 2) the behavioural changes are affected by the different temporal structures, including intermittency, repetition interval regularity and the presence of ‘ramp-up’; 3) the behavioural changes are in agreement with previous indoor studies which had the same experimental design (Neo et al., 2014, Neo et al., 2015a).

Materials and methods

ANIMAL MAINTENANCE

Mixed-sex European seabass from a hatchery (Ecloserie Marine de Gravelines, France) with a total body length of about 30 cm were used in this study (Neo et al., 2014, Neo et al., 2015a). Before and after the experiment, the fish were kept in two cylindrical holding tanks (Ø 3.5 m, depth 1.2 m) in an 8:16 dark-light cycle at Stichting Zeeschelp research institute in Zeeland, the Netherlands. The water in the holding tanks was continuously refreshed with water from the nearby Oosterschelde marine inlet and the water temperature varied from 17 to 22 °C throughout the experimental period (June–August 2014). The fish were fed pellets (Le Gouessant Aquaculture, France) every other day based on a temperature-dependent prescription. All experiments were in accordance with the Dutch Experiments on Animals Act and approved by the Animal Experiments Committee at Leiden University (DEC approval no: 14047).

EXPERIMENTAL ARENA

The experiment was conducted in the Jacobahaven, a man-made cove in the Oosterschelde. The cove is about 200 m wide, 300 m long and 2–5 m deep depending on tides. It has a level and muddy bottom. The water is relatively calm in the summer and is home to wild European seabass. No external boat traffic is allowed within about 2 km of the cove, making it quiet and ideal for noise impact studies.

In the middle of the Jacobahaven, a floating island consisting of two platforms (Fig. 1) was constructed from a modular floating system (Candock, Canada) and anchored to the sea bottom with dead weights, chains and stretchable bungee ropes that kept the island in place at all tides. The octagonal platform (Ø 11.5–12.5 m) supported a custom-made octagonal net (volume 334 m3), in which test fish were held during sound exposures; the square working platform supported a work tent (4 × 5 m), which protected all equipment from the weather and served as a working space during the experiment. The two platforms were kept at 0.5 m distance from each other to minimise unwanted noise transmission from the working platform to the octagonal platform during sound exposure. The working platform was detachable from the octagonal platform, and for every quarter of the total trials, it was repositioned at another orthogonal arm of the octagonal platform. The use of four different positions facing the four cardinal directions was intended to minimise the influence of extraneous factors (e.g. seabed topography, tide flows) on fish swimming patterns.

Figure 1

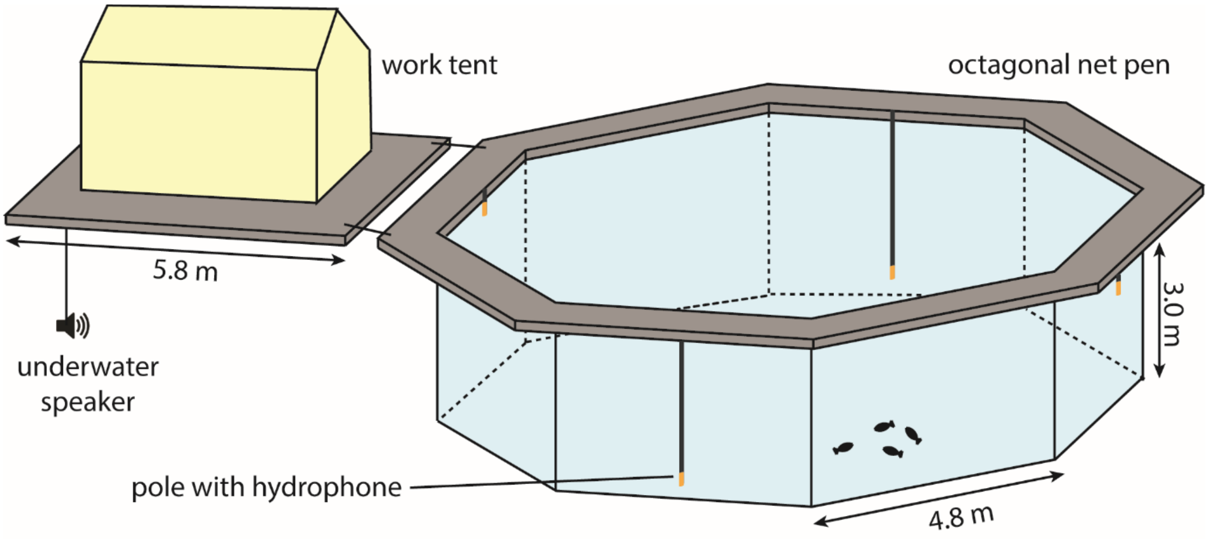


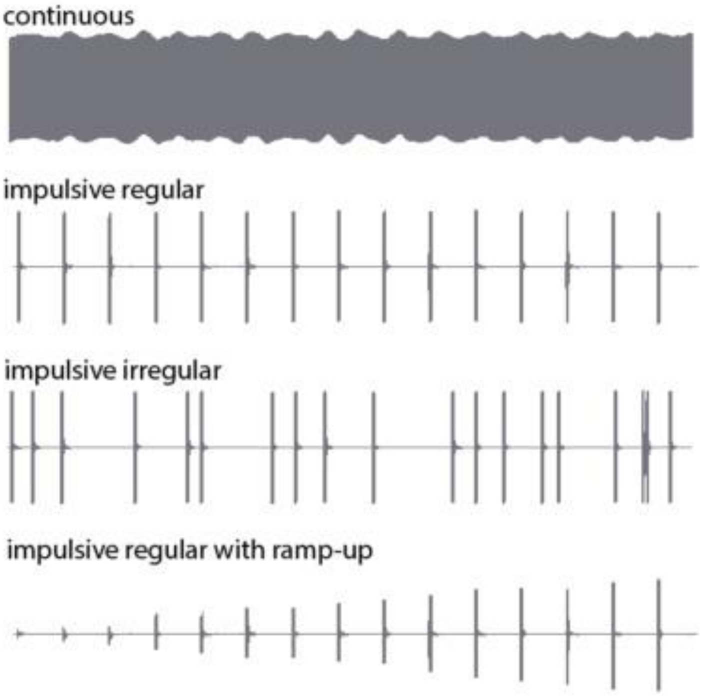
Fig. 1. Floating island where experiment was conducted. The square working platform is connected to the octagonal platform by two ropes, leaving a gap of 0.5 m between the two platforms. On the working platform, there is a work tent (5 × 4 m). The underwater speaker hangs on the far end of the working platform at a depth of 2.2 m. The distance of the underwater speaker and the closest side of the net is 7.8 m. The four poles with hydrophones are responsible for tracking the four test fish via acoustic telemetry. Two of the hydrophones are placed near the surface and the other two near the bottom. The distance between adjacent hydrophones is 8.7 m.

TREATMENT SERIES

We exposed the fish to a series of four sound treatments: continuous, impulsive regular, impulsive irregular and impulsive regular with ‘ramp-up’ (Fig. 2a). In order to vary only the temporal parameters of interest in the treatments while keeping all other sound parameters constant, the sound treatments were created in Audition 3.0 (Adobe, San Jose, US) using filtered brown noise (band-passed: 200–1000 Hz). The continuous treatment consisted of uninterrupted sound elevation with constant amplitude. The other three impulsive treatments consisted of a pulse train with 0.1 s pulses, repeated at either a regular PRI (pulse repetition interval) of 2 s, or an irregular PRI of 0.2–3.8 s (random; average 2 s). The ‘ramp-up’ treatment consisted of 20 min of fade-in from ambient level to the same amplitude as the other treatments. All sound samples were created in Adobe Audition 3.0 using filtered brown noise (band-passed: 200–1000 Hz; matching the hearing range of European seabass (Kastelein et al., 2008, Lovell, 2003)) and played back with an underwater transducer (LL-1424HP, Lubell Labs, Columbus, US) from a laptop through to a power amplifier (DIGIT 3K6, SynQ) and a transformer (AC1424HP, Lubell Labs).

Figure 2

(a) Time-domain waveform



(b) Power spectral density

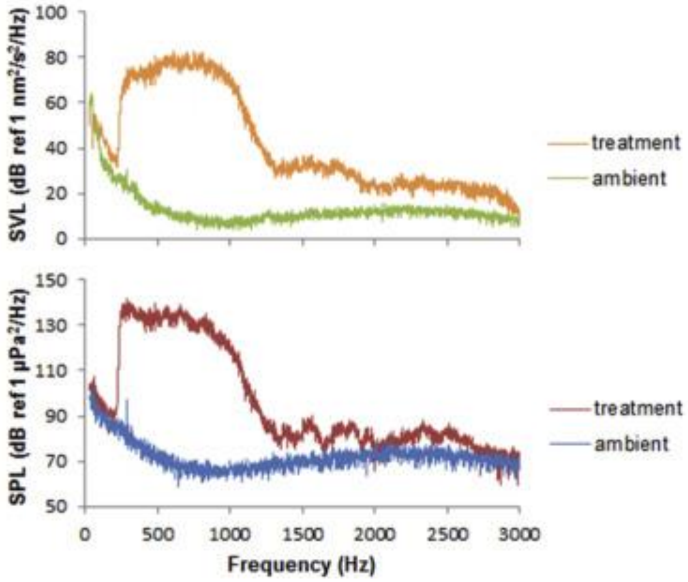


Fig. 2. (a) Time-domain waveforms showing 30 s of the four treatments exposed to each group of fish. Note that the rate of amplitude change for impulsive regular with ‘ramp-up’ is adjusted for illustration purposes; the original exposure consists of 20 min of ‘ramp-up’ followed by 50 min of exposure at the same amplitude as the other treatments. (b) Power spectral density plots of measurements in the middle of the octagonal net showing SPL and SVL of 1 s continuous noise and their respective ambient levels. Most energy concentrates between 200 and 1000 Hz as intended, overlapping with the hearing range of European seabass. The original spectra contained a dent between 500 and 1000 Hz, which was caused by the splitting of the original sound files for the avoidance of overload during measurements. The dent was removed in post-processing to reflect the actual acoustic conditions during the exposure trials.

To examine the soundscape of the whole experimental arena, we measured both sound pressure level (SPL) and sound velocity level (SVL). These metrics relate to sound pressure and particle motion, which are both involved in fish hearing, although their relative importance are not always clear. The rms SPL and SVL of the continuous treatment were measured over 1 s at 360 points - three depths (0.5, 1.5 & 2.5 m) x 120 points - within the octagonal net (Fig. 3a). These measurements were performed during both ebb and flow tides for all four positions of the working platform, totalling eight sets of 360 measurements. The measurements were made using the M20 particle motion sensor (GeoSpectrum Technologies, Canada), whose pre-amplifier was powered by a 12 V car battery. The sensor was connected to a current-to-voltage convertor (GeoSpectrum Technologies, Canada) that gave an output of four channels: three for the 3D particle velocity directions (u, v & w) and one for sound pressure. These channels were connected to a laptop via an oscilloscope (PicoScope 3425, Pico Technologies, UK) and data were logged at 40 kHz using a script in Microsoft Access and subsequently analysed with MATLAB.

Figure 3

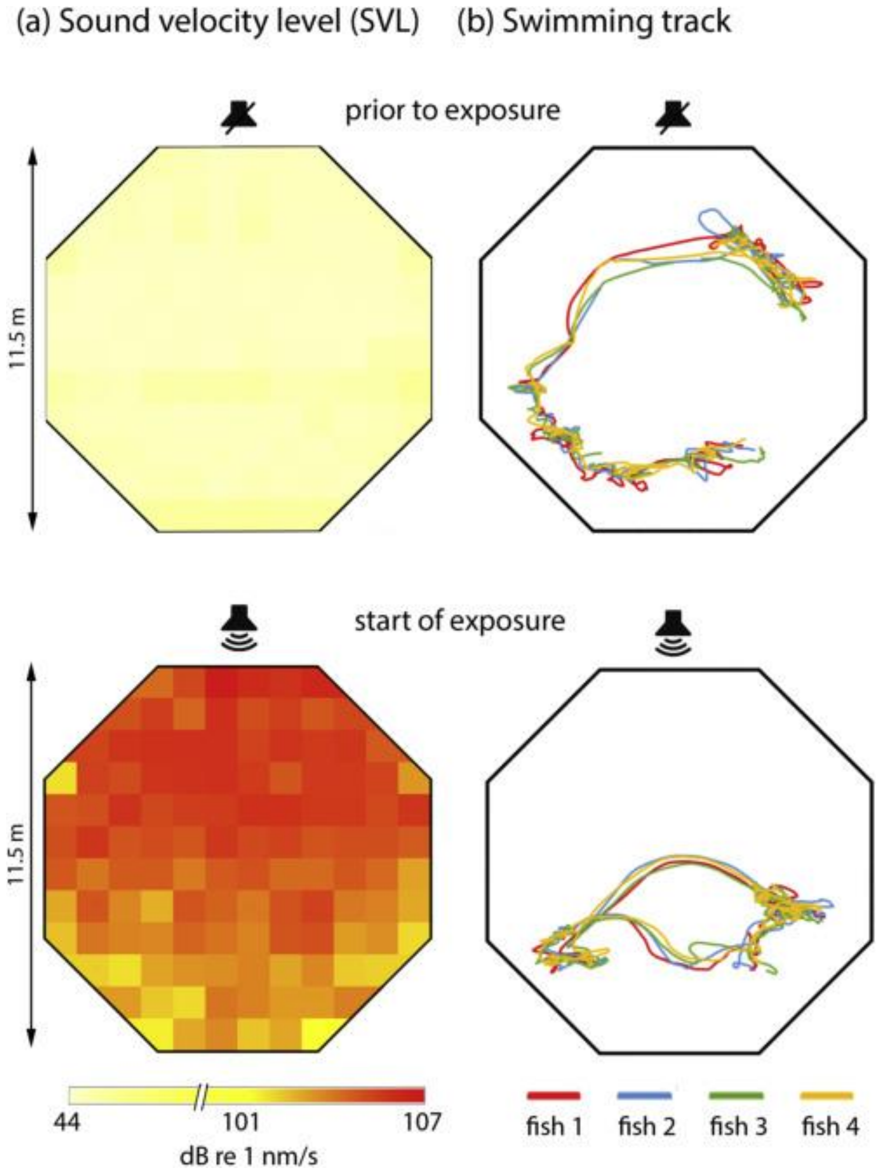


Fig. 3. (a) 2D soundscape maps in sound velocity level (SVL) prior (ambient) and at the start of sound exposure, measured at 1.5 m water depth. The speaker is 7.8 m away from the experimental arena, making the experimental arena outside the postulated acoustic nearfield <7.5 m (sound treatments had minimum frequency 200 Hz). There is a clear amplitude gradient, also in sound pressure level (not shown). (b) Aerial view of swimming tracks of four fish 10 min prior and 10 min at the start of sound exposure. The fish swim around the periphery of the whole study arena before sound exposure but swim away from the speaker at the onset of sound exposure.

Measurements closer to the speaker caused signal overload. To avoid signal overload, we split the original sound file into two files of 200–560 Hz and 560–1000 Hz bandwidth, and redid the measurements. The readouts of the two files were merged during post-processing (Fig. 2b). The splitting caused a dent in the power spectra around the splitting frequency, leading to a slight underestimation of amplitude level (consistent throughout all measurements and not reflecting the actual exposure conditions). The mean rms SPL and SVL of the ambient noise were 108 dB re 1 μPa and 47 dB re 1 nm/s respectively. The mean rms SPL and SVL for the continuous treatment were 163–169 dB re 1 μPa and 101–105 dB re 1 nm/s respectively (the range indicates values from the furthest to the nearest points from the speaker within the experimental arena). For the impulsive treatments, the mean zero-to-peak SPL ($SPL_{z\text{-}p}$) and SVL ($SVL_{z\text{-}p}$) were 180–192 dB re 1 μPa and 124–125 dB re 1 nm/s respectively; the mean single-strike sound exposure level (SEL_{ss}) and velocity exposure level (VEL_{ss}) were 156–167 dB re 1 μPa2 s and 99–100 dB re 1 nm2/s respectively.

EXPERIMENTAL DESIGN

Sixteen groups of four fish (64 fish, N = 16) were used and each group was exposed to all four sound treatments sequentially in two days; the exposure order followed an incomplete counterbalanced design (16 of 24 possible orders), to minimise potentially confounding effect of the treatment orders. Each group of fish was transferred to the floating pen in a black plastic container (56 × 39 × 28 cm) enriched with oxygen (OxyTabs, JBL, Germany) and allowed to acclimatise for at least 8 h. At least 30 min before the start of each trial, researchers arrived on the floating island by a small motorised rubber boat. The arrival triggered slight behavioural changes but the fish recovered within 30 min, before the start of the trial. We conducted two trials per day, one during ebb tide (starting 1.5 h after the high tide) and one during flow tide (ending 1.5 before the high tide), ensuring that the water depth was always between 3 and 4 m during the trials. Each trial lasted for 1.5 h and comprised 60 min of sound exposure and 15 min of silence before and after, except for trials with ‘ramp-up’, where the exposure consisted of 20 min of ‘ramp-up’ plus 50 min of standard sound exposure (overall energy equalled 60 min standard exposure). During the exposure trials, the researchers stayed in the work tent and did not set foot on the octagonal platform. Light intensity, weather condition and water temperature were recorded during each trial and subsequently used as covariates in the statistical analyses. After each group of fish went through four trials, they were transferred back to the onshore holding tank.

ACOUSTIC TELEMETRY

The swimming patterns of the fish were studied with a 3D telemetry system using acoustic tags (Model 795-LG, HTI, US). The tags were programmed with a programmer (Model 490-LP, HTI, US) to emit 307 kHz pings (inaudible to the fish) of 0.5 ms at four different PRIs (985, 995, 1005 and 1015 ms), in order to identify the four fish in a group. The fish were tagged externally, directly under the first and second dorsal fin (cf. FISHBIO, 2013). After tagging, the four fish were kept in a rectangular recovery tank (1.20 × 1.00 × 0.65 m), which was continuously refreshed with filtered water from the Oosterschelde. The fish stayed in the recovery tank for at least two days before being transferred to the outdoor pen. The pings emitted from the fish were received by four hydrophones (Model 590-series, HTI, US) attached to the octagonal platform (Fig. 1). The signals were then digitised by an acoustic tag receiver (Model 291, HTI, US) connected to a laptop. The digital data were subsequently processed by computer programs MarkTags v6.1 & AcousticTag v6.0 (HTI, US) into 3D coordinates (x, y, z), with a temporal resolution of one position every second for all four fish (position accuracy = ±0.5 m). The 3D coordinates were used to calculate four behavioural parameters: swimming speed, swimming depth, average inter-individual distance (group cohesion) and distance from the speaker.

STATISTICS

To test for the changes in the behavioural parameters during the trials, we used linear mixed models to compare four 5-min bins in the exposure sequence from our data set: the 5 min right before sound exposure (‘before’), the first (‘start’) and the last 5 min of exposure (‘end’), and the 5 min right after exposure (‘after’) (cf. Neo et al., 2014). Both exposure sequence and treatment were treated as repeated variables, with covariance structure defined as compound symmetry. We used the same procedure for swimming speed but the bins were 1 min instead of 5 min in order to capture the transient speed change. To understand the impact differences between the treatments, we subsequently ran the same test for each treatment separately, treating exposure sequence as a repeated variable. In addition, we compared the difference of the behaviours before and at the start of exposure between impulsive regular and the other three treatments separately, treating treatment as a repeated variable. We subsequently performed one-sample t-tests to see if the calculated differences were significantly larger than 0. In all tests, tide (ebb/flow), water temperature, light level and trial order were fitted as covariates. To select for the best model, irrelevant variables were omitted from the model through backward stepwise selection based on Akaike information criteria. All post-hoc tests and multiple comparisons were corrected using the Holm-Bonferroni method.

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 level. The ‘before’ bin was used as a baseline to compare with 5-min moving averages during exposure shifting forward every second, 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 as 60 min (occurrence frequency: 3/64 for swimming depth, 9/64 for average inter-individual distance and 8/64 for distance from speaker). To compare the difference in recovery time between impulsive regular and the other three treatments separately, we used linear mixed models like above on ranked data, since the original data were not normally distributed.

Results

BEHAVIOURAL CHANGES

We were able to generate high-resolution swimming tracks of four fish for all trials (see Fig. 3b). At the start of the exposure, the fish increased the swimming speed (linear mixed model: $F_{3,237} = 4.978$, $P = 0.002$; Holm-Bonferroni post-hoc: 'start' vs 'before', 'end' & 'after' $P = 0.026$, 0.007 & 0.007 respectively) and swimming depth (linear mixed model: $F_{3,240} = 3.913$, $P = 0.009$; Holm-Bonferroni post-hoc: 'start' vs 'before', 'end', 'after', all $Ps < 0.001$), and swam further from the speaker (linear mixed model: $F_{3,240} = 2.654$, $P = 0.049$; Holm-Bonferroni post-hoc: 'start' vs 'before', 'end' & 'after' $P = 0.021$, 0.068 & 0.099 respectively), without changing the group cohesion. All the interaction terms between exposure sequence and treatment were not significant and were removed from the final models.

When the treatments were analysed separately, swimming speed did not increase significantly for all treatments, except for the continuous treatment, although the change was not significant in the post-hoc test after correcting for multiple testing (linear mixed model: $F_{3,48} = 4.910$, $P = 0.005$; Holm-Bonferroni post-hoc $Ps > 0.1$). Swimming depth increased consistently across all treatments (linear mixed model: $F_{3,48} = 3.144$, $P = 0.034$ for continuous; $F_{3,48} = 5.141$, $P = 0.004$ for impulsive regular; $F_{3,49} = 4.277$, $P = 0.009$ for impulsive irregular; $F_{3,48} = 5.702$, $P = 0.002$ for impulsive regular with ramp-up; all Holm-Bonferroni post-hoc: 'start' vs 'before', 'end', 'after', all $Ps < 0.05$) but distance from speaker did not increase significantly for any of the treatments. The group cohesion increased significantly for impulsive regular (linear mixed model: $F_{3,43} = 3.916$, $P = 0.015$; Holm-Bonferroni post-hoc: ‘start’ vs ‘before’, ‘end’ & ‘after’ $P = 0.026$, 0.032 & 0.134) and continuous treatment, although post-hoc test did not reveal a significant change for the continuous treatment (linear mixed model: $F_{3,47} = 4.639$, $P = 0.006$; Holm-Bonferroni post-hoc Ps > 0.1). The increase in group cohesion was not significant for impulsive irregular treatment and impulsive regular with ramp-up.

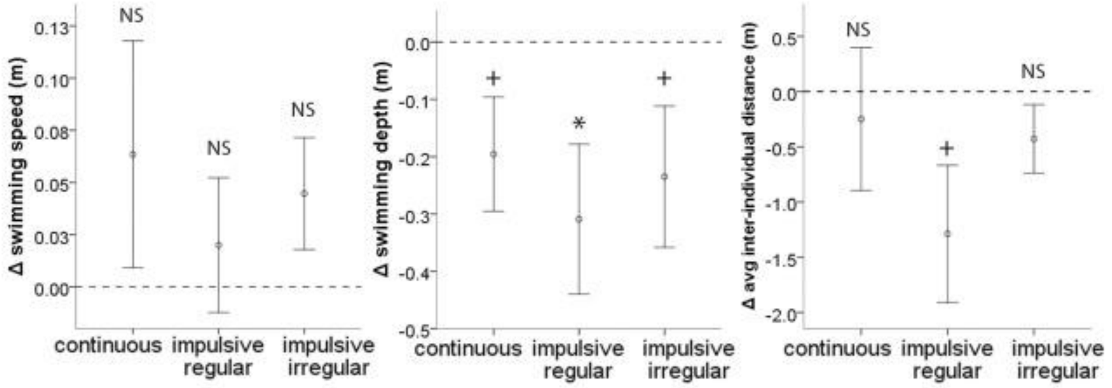
TREATMENT EFFECTS

INTERMITTENCY

Comparing between continuous and impulsive regular treatment, the increase in swimming speed, swimming depth and group cohesion did not differ significantly from each other, although the increase in swimming depth and group cohesion seemed larger in impulsive sound (Fig. 4). This was suggested by one-sample t-tests, where for swimming depth, there was a significant difference from zero for impulsive treatment and a non-significant trend for continuous treatment ($t15 = -2.362$, $P = 0.032$; $t15 = -1.773$, $P = 0.096$ respectively); and for group cohesion, there was a non-significant trend for impulsive treatment and no significant difference for continuous treatment ($t14 = -1.815$, $P = 0.091$; $t14 = -0.114$, $P = 0.911$ respectively). Contrary to the previous study, the recovery time of continuous and impulsive treatment did not differ significantly for swimming depth or group cohesion.

Figure 4

(a) Behavioural changes



(b) Recovery time

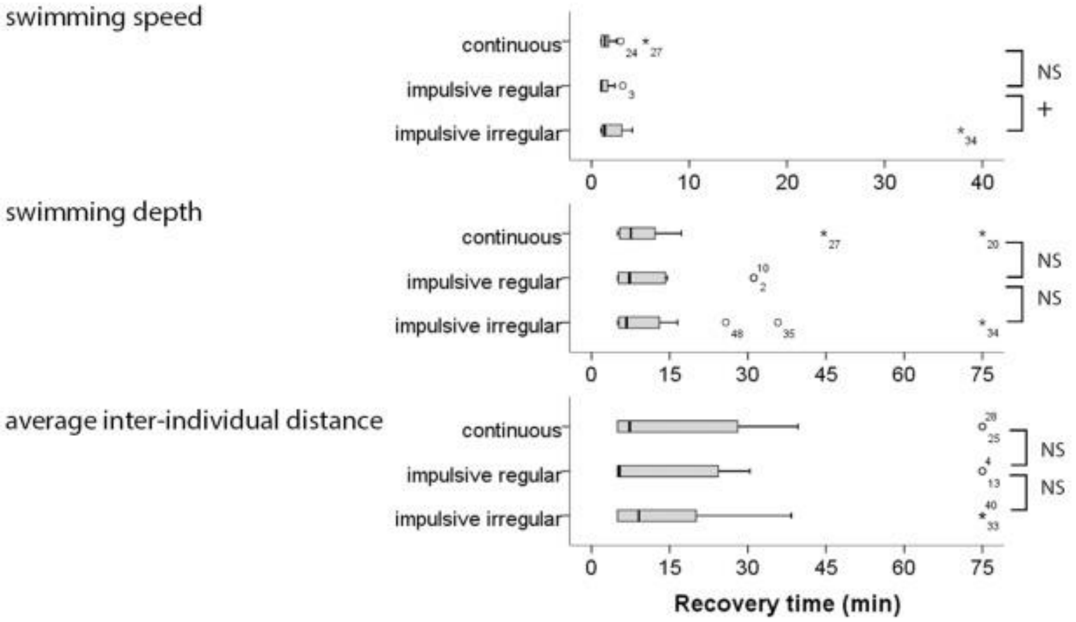


Fig. 4. (a) Changes in swimming speed, swimming depth (from net bottom) and average inter-individual distance (mean ± SE) from before to start of exposure for continuous and impulsive regular treatments. (b) Recovery time of swimming speed, swimming depth and average inter-individual distance for continuous and impulsive regular treatments. An asterisk (*) denotes a significant difference from 0 ($P \leq 0.05$), a plus (+) denotes a non-significant trend ($0.05 < P \leq 0.1$) and NS denotes non-significance ($P > 0.1$).

INTERVAL REGULARITY

Behavioural changes caused by the impulsive irregular treatment did not significantly differ from the regular treatment. Subsequent one-sample t-tests for irregular treatment yielded a non-significant trend in swimming depth ($t_{15} = -1.905$, $P = 0.076$) and no significant difference in group cohesion ($t_{14} = -1.378$, $P = 0.191$), which contrasted with the regular treatment in the same way as continuous treatment (see previous paragraph). However, for distance from speaker, the increase was significantly larger than zero for the irregular treatment, but not for the regular treatment (one-sample t-test: $t_{15} = 2.595$, $P = 0.020$; $t_{15} = 1.744$, $P = 0.102$ respectively). Furthermore, the irregularity of PRI did not significantly affect the recovery of swimming depth, group cohesion and distance from speaker, although there was a non-significant trend that the recovery of swimming speed was prolonged (linear mixed model: $F_{1,15} = 6.346$, $P = 0.071$).

‘RAMP-UP’ PROCEDURE

‘Ramp-up’ caused diving behaviour already within the first 5 min, similar to the treatment without it (Fig. 5). Within this period, the amplitude levels were still much lower than the full standard levels. Exposures at these sound levels triggered behavioural changes not significantly different from the treatment without the ‘ramp-up’, which had constant sound levels from the exposure on-set that were at least 16 dB (up to 31 dB) higher. The ‘ramp-up’ procedure also did not affect the recovery of the behavioural changes.

Figure 5

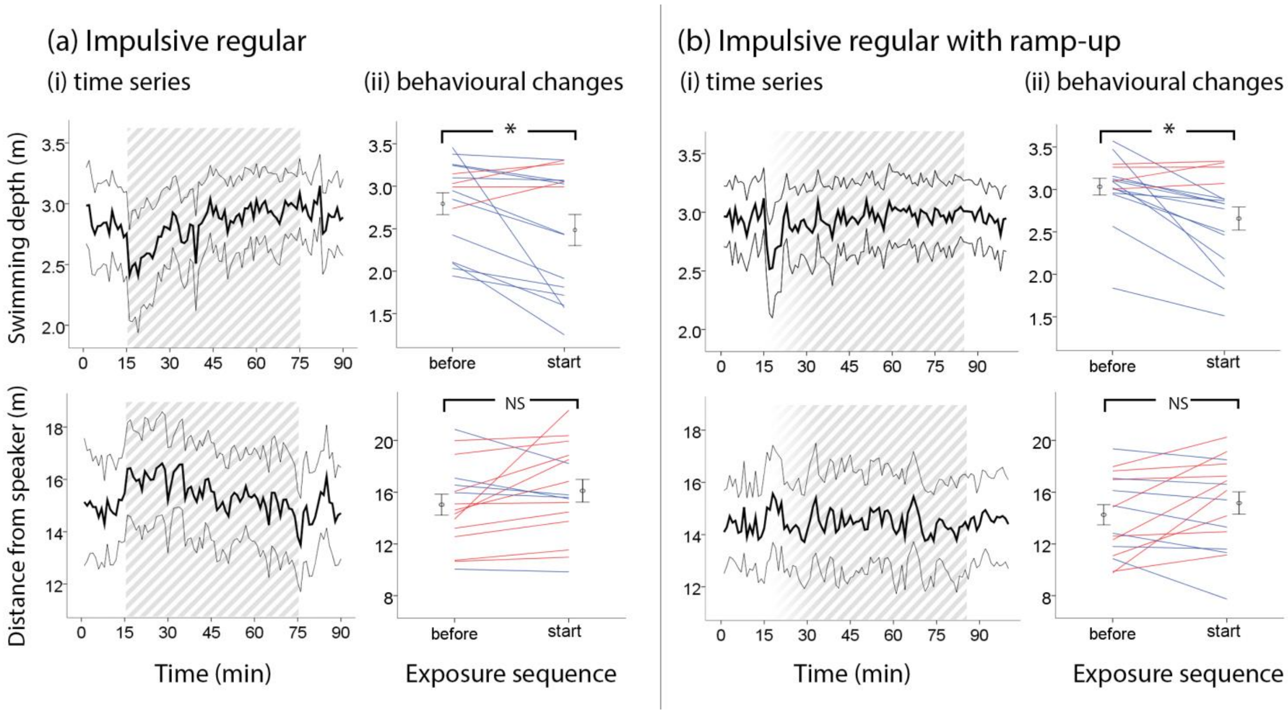


Fig. 5. Comparisons between (a) the absence and (b) presence of ‘ramp-up’, showing (i) the time series plots of the whole exposure period (with 95% confidence interval) and (ii) the changes in swimming depth (from net bottom) and distance from speaker (mean ± SE) for all 16 fish groups. In the time series plots, the shaded area indicates noise exposure; in the behavioural changes plots, red lines indicate increases while blue lines indicate decreases for the different groups. An asterisk (*) denotes significance ($P \leq 0.05$) and NS denotes non-significance ($P > 0.1$).

Discussion

In the current study, we could observe detailed swimming patterns of fish in a large floating pen in outdoor conditions. Upon sound exposure, the fish swam faster, deeper, and further away from the speaker. Within 30 min, most fish returned to their baseline behaviour. Despite some noticeable patterns, sound intermittency and interval regularity did not significantly influence fish response and recovery. In addition, the ‘ramp-up’ procedure triggered a behavioural response as immediate as when the procedure was absent, but did not make fish move away from the speaker. Some fish even seemed to approach the sound source, at least in the beginning. In general, the fish response was qualitatively similar to earlier indoor experiments, but the behavioural changes and recovery in the current study were more variable. We also found horizontal avoidance behaviour that was absent in the previous studies.

NO INFLUENCE OF TEMPORAL STRUCTURES

In contrast to our expectations, the impulsive treatment did not prolong the recovery of swimming depth as in previous indoor study (Neo et al., 2014). One explanation may be that the current set-up allowed the fish to swim away from the speaker to quieter areas. As a result, the fish had some control over the sound exposure levels they experienced, and increased the variability of their swimming depth such that this differential impact on recovery was invisible. It should be noted that the absence of a significant difference does not necessarily mean the absence of an effect, because individuals may respond to sound using different coping strategies (Koolhaas et al., 2011, Silva et al., 2010), e.g. freeze versus flight, and the causal relationship between sound exposure and behavioural changes may be moderated by some unknown environmental factors (Brewer, 2000). These context-dependent effects of noise exposure can only be answered with more well-controlled studies (Radford et al., 2014, Slabbekoorn, 2014).

Consistent with a previous study conducted on groups of five zebrafish in aquaria (Neo et al., 2015b), we found no significant effects of repetition interval regularity. Irregular pulses could be less predictable, potentially resulting in higher anxiety response as well as slower habituation (Koolhaas et al., 2011, Rankin et al., 2009). However, evidence for these effects were so far only found in an indoor study on individual zebrafish, where the speed change was higher upon exposure to irregular sounds (Shafiei Sabet et al., 2015). The influence of pulse repetition regularity may be too subtle to show in groups of fish where behavioural responses are strongly influenced by group dynamics. This implies that pulse repetition regularity might be less important in inducing behavioural impacts, at least within the temporal resolutions used in the current study (random PRI range: 0.2–3.8 s) and the previous study (random PRI range: 1–17 s) (Neo et al., 2015b).

EFFICACY OF ‘RAMP-UP’

To our knowledge, the inclusion of ‘ramp-up’ procedure allowed us to test for its efficacy on fish for the first time. The ‘ramp-up’ procedure caused fish to dive deeper without delay, which implies that the fish were sensitive to the presence of impulsive sound already at relatively low sound levels. However, when it comes to avoidance of the sound source, the effect was not very clear. When all treatments were analysed together in the statistical model, the avoidance effect was significant; but when analysed separately, the effect was not significant for any treatment. It can be seen in Fig. 5(a, ii) & (b, ii) that not every group of fish responded to the noise exposure by swimming away from it. A smaller but considerable proportion of the groups even initially approached the speaker, possibly due to sound-independent swimming pattern where the fish continuously circled the pen periphery, or due to a phonotactic response, potentially driven by curiosity for novel sounds (Nelson and Johnson, 1972, Weilgart, 2007). Therefore, contrary to our expectation, ‘ramp-up’ may not necessarily drive fish away from ensuing intense noise exposure and some fish may actually stay where they are or even swim closer to the noise source.

Furthermore, the gradual increase in sound level of the ‘ramp-up’ procedure may allow the fish to habituate to the sound exposure more easily (Groves and Thompson, 1970, Rankin et al., 2009) and stay within the exposure area without avoidance behaviour. This suggests that ‘ramp-up’ procedure may actually reduce the effect of horizontal displacement of fish, instead of inducing deterrence as intended. In view of this, ramp-up procedures may actually be used to prevent distribution changes of fish if an exposed site is critical for foraging or breeding. However, fish may consequently experience other negative effects resulting from the ensuing noise exposure, such as physiological stress (Buscaino et al., 2010, Celi et al., 2015), auditory masking (Codarin et al., 2009) and attentional shifts (Bell et al., 2012). The effect of ‘ramp-up’ has also been suggested to be species-dependent, as some species are more mobile or more ready to swim from one area to another (Von Benda-Beckmann et al., 2014). However, empirical data on more species is still unavailable. Therefore, extrapolating our findings to other species or other ‘ramp-up’ procedures is unadvisable at this stage. More studies are needed to test the efficacy of different ‘ramp-up’ procedures, which should not only consider a gradual rise in amplitude, but also a start with slower pulse repetition rates or ‘ramp-up’ in other relevant temporal characteristics.

FROM INDOOR TO OUTDOOR

One of the explicit aims of the current study was to compare findings from this study with a previous indoor study (Neo et al., 2014). The indoor study was conducted within a net enclosure (1.6 × 1.6 × 2 m) in a large basin (7 × 4 × 2 m), using a very similar experimental design and exposure scheme on captive European seabass of similar sizes. Two of the four sound treatments used were similar to the current study: continuous versus impulsive regular. The indoor study reported increased swimming speed, swimming depth and group cohesion upon sound exposures, irrespective of the treatment types. However, the impulsive treatment caused swimming depth to recover twice as slowly compared with the continuous treatment. The study successfully highlighted the relatively stronger impact of impulsive sound, but had extrapolation limitations because 1) the natural swimming behaviour of fish might be constrained by the small experimental enclosure and 2) the acoustic characteristics in the basin were quite different from natural waters, such as the lack of natural acoustic gradient due to near-field effects and reverberation, and the potentially complex sound pressure and particle motion ratios in the basin.

Despite apparent differences between the experimental arenas, the current study found comparable immediate behavioural changes. The increase in swimming depth was especially clear, implying that it is a robust indicator for behavioural impact, while other read-outs, such as group cohesion, may only become informative with the high resolution of indoor studies. Social effects that explain group cohesion are possibly lessened in the outdoor conditions due to the large experimental arena and the inherently reduced mutual visibility. In addition, we also showed that the fish avoided the sound source by swimming away from it (Fig. 3). The absence of this behaviour in the previous indoor study confirmed that either the artificial sound fields or the spatial restrictions in tanks could prevent some response patterns from emerging (Oldfield, 2011). In view of this, the behavioural validity of any studies conducted in a confined or unnatural setting (especially the absence of effects), needs to be assessed critically before implications for noise impact assessments can be drawn. This can be achieved by comparing studies across different contexts from laboratory to field, to find out what behavioural parameters are generalisable, and whether they depend on specific contexts. For example, diving behaviour occurs in indoor studies conducted in reverberant enclosures without acoustic gradient (Fewtrell and McCauley, 2012, Neo et al., 2014, Neo et al., 2015a) and has typically been associated with anxiety across contexts (Cachat et al., 2010, Israeli-Weinstein and Kimmel, 1998, Kuwada et al., 2000, Luca and Gerlai, 2012, Skilbrei and Holst, 2009, Wilson and Dill, 2002). By knowing this, diving behaviour in outdoor studies (Gerlotto and Fréon, 1992, Handegard et al., 2003, Slotte et al., 2004) can be interpreted, at least partly, as related to anxiety instead of acoustic avoidance by vertical displacement. In any case, researchers, as well as regulators, should never take the findings of a single study at face value, but advice management decisions based on studies over a variety of contexts and approaches.

Performing indoor studies using robust behavioural parameters can increase their external validity, so that researchers can take advantage of the high controllability and practicality of tank-based set-ups to support outdoor experiments, which are typically more challenging to perform (Radford et al., 2014, Slabbekoorn, 2014). Currently, most studies so far were conducted on captive fish from a hatchery, which may be less or differently affected by environmental stressors than wild fish (Benhaïm et al., 2012, Lepage et al., 2000), making the observed response levels potentially less strong than in the wild. Moreover, there is still a need in determining which behavioural parameters may lead to long-term consequences on fish populations.

CONCLUSION

The findings from our semi-natural set-up successfully breached the extrapolation gap between laboratory and field studies. We showed that certain behavioural changes were qualitatively consistent with previous indoor studies, while horizontal avoidance behaviour only occurred outdoors. In addition, the outdoor conditions increased the variability of the behavioural response and did not reveal the effects of different temporal structures. Furthermore, we showed that a ‘ramp-up’ procedure had unexpected results, where fish startled already at the start of the ‘ramp-up’, without swimming away from the speaker. This observation implies that the ‘ramp-up’ procedure may affect fish behaviour, but not necessarily have the mitigation effect that is generally assumed.

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References

Bell, R., Röer, J.P., Dentale, S., Buchner, A., 2012. Habituation of the irrelevant sound effect: evidence for an attentional theory of short-term memory disruption. J. Exp. Psychol. Learn. Mem. Cogn. 38, 1542-1557.

Benhaïm, D., Péan, S., Lucas, G., Blanc, N., Chatain, B., Bégout, M.-L., 2012. Early life behavioural differences in wild caught and domesticated sea bass (*Dicentrarchus labrax*). Appl. Anim. Behav. Sci. 141, 79-90.

Boussard, A., 1981. The reactions of roach (Rutilus rutilus) and rudd (Scardinius erythrophthalmus) to noises produced by high speed boating. In: O'Hara, K., Barr, C.D. (Eds.), Proceedings of 2nd British Freshwater Fisheries Conference. University of Liverpool, Liverpool, pp. 188-388.

Brewer, M., 2000. Research design and issues of validity. In: Reis, H., Judd, C. (Eds.), Handbook of Research Methods in Social and Personality Psychology. Cambridge University Press, Cambridge, pp. 3-16.

Buscaino, G., Filiciotto, F., Buffa, G., Bellante, A., Di Stefano, V., Assenza, A., Fazio, F., Caola, G., Mazzola, S., 2010. Impact of an acoustic stimulus on the motility and blood parameters of European sea bass (*Dicentrarchus labrax L.*) and gilthead sea bream (*Sparus aurata L.*). Mar. Environ. Res. 69, 136-142.

Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Chung, K.M., Wu, N., Wong, K., Roy, S., Suciu, C., et al., 2010. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat. Protoc. 5, 1786-1799.

Calisi, R.M., Bentley, G.E., 2009. Lab and field experiments: are they the same animal? Horm. Behav. 56, 1-10.

Campbell, D.T., 1957. Factors relevant to the validity of experiments in social settings. Psychol. Bull. 54, 297-312.

Cato, D.H., Noad, M.J., Dunlop, R. a., McCauley, R.D., Gales, N.J., Kent, C.P.S., Kniest, H., Paton, D., Jenner, K.C.S., Noad, J., et al., 2013. A study of the behavioural response of whales to the noise of seismic air guns: design, methods and progress. Acoust. Aust. 41, 88-97.

Celi, M., Filiciotto, F., Maricchiolo, G., Genovese, L., Quinci, E.M., Maccarrone, V., Mazzola, S., Vazzana, M., Buscaino, G., 2015. Vessel noise pollution as a human threat to fish: assessment of the stress response in gilthead sea bream (*Sparus aurata*, Linnaeus 1758). Fish. Physiol. Biochem. 42, 1-11.

Codarin, A., Wysocki, L.E., Ladich, F., Picciulin, M., 2009. Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). Mar. Pollut. Bull. 58, 1880-1887.

Fewtrell, J.L., McCauley, R.D., 2012. Impact of air gun noise on the behaviour of marine fish and squid. Mar. Pollut. Bull. 64, 984-993.

FISHBIO, 2013. Predation Study Report Don Pedro Project FERC No. 2299.

Gerlotto, F., Fréon, P., 1992. Some elements on vertical avoidance of fish schools to a vessel during acoustic surveys. Fish. Res. 14, 251e259.

Groves, P., Thompson, R., 1970. Habituation: a dual-process theory. Psychol. Rev. 77, 419-450.

Handegard, N.O., Michalsen, K., Tjøstheim, D., 2003. Avoidance behaviour in cod (*Gadus morhua*) to a bottom-trawling vessel. Aquat. Living Resour. 16, 265-270.

Hawkins, A.D., Pembroke, A.E., Popper, A.N., 2014a. Information gaps in understanding the effects of noise on fishes and invertebrates. Rev. Fish. Biol. Fish. 25, 39-64.

Hawkins, A.D., Roberts, L., Cheesman, S., 2014b. Responses of free-living coastal pelagic fish to impulsive sounds. J. Acoust. Soc. Am. 135, 3101-3116.

Israeli-Weinstein, D., Kimmel, E., 1998. Behavioral response of carp (*Cyprinus carpio*) to ammonia stress. Aquaculture 81-93.

JNCC, 2010. JNCC guidelines for minimising the risk of injury and disturbance to marine mammals from seismic surveys, 16.

Kastelein, R.A., Van Der Heul, S., Verboom, W.C., Jennings, N., Van Der Veen, J., de Haan, D., 2008. Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. Mar. Environ. Res. 65, 369-377.

Koolhaas, J.M., Bartolomucci, a, Buwalda, B., de Boer, S.F., Flügge, G., Korte, S.M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., et al., 2011. Stress revisited: a critical evaluation of the stress concept. Neurosci. Biobehav. Rev. 35, 1291-1301.

Kuwada, H., Masuda, R., Shiozawa, S., Kogane, T., Imaizumi, K., Tsukamoto, K., 2000. Effect of fish size, handling stresses and training procedure on the swimming behavior of hatchery-reared striped jack: implications for stock enhancement. Aquaculture 185, 245-256.

Lepage, O., Øverli, Ø., Petersson, E., Järvi, T., Winberg, S., 2000. Differential stress coping in wild and domesticated sea trout. Brain. Behav. Evol. 56, 259-268.

Lovell, J.M., 2003. The Hearing Abilities of the Bass, Dicentrarchus labrax. Technical Report Commissioned by ARIA Marine Ltd. For the European Commission Fifth Framework Programme. Project Reference: Q5AW-CT-2001-01896.

Luca, R.M., Gerlai, R., 2012. In search of optimal fear inducing stimuli: differential behavioral responses to computer animated images in zebrafish. Behav. Brain Res. 226, 66-76.

McLaughlin, K.E., Kunc, H.P., 2015. Changes in the acoustic environment alter the foraging and sheltering behaviour of the cichlid Amititlania nigrofasciata. Behav. Process. 116, 75-79.

Nelson, D., Johnson, R., 1972. Acoustic attraction of Pacific reef sharks: effect of pulse intermittency and variability. Comp. Biochem. Physiol. Part A Physiol. 42, 85-95.

Neo, Y.Y., Seitz, J., Kastelein, R.A., Winter, H.V., ten Cate, C., Slabbekoorn, H., 2014. Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biol. Conserv. 178, 65-73.

Neo, Y., Ufkes, E., Kastelein, R., Winter, H., ten Cate, C., Slabbekoorn, H., 2015a. Impulsive sounds change European seabass swimming patterns: influence of pulse repetition interval. Mar. Pollut. Bull. 97, 111-117.

Neo, Y.Y., Parie, L., Bakker, F., Snelderwaard, P., Tudorache, C., Schaaf, M., Slabbekoorn, H., 2015b. Behavioral changes in response to sound exposure and no spatial avoidance of noisy conditions in captive zebrafish. Front. Behav. Neurosci. 9, 1-11.

Normandeau Associates, 2012. Effects of Noise on Fish, Fisheries, and Invertebrates in the U.S. Atlantic and Arctic from energy industry sound-generating activities.

Oldfield, R.G., 2011. Aggression and welfare in a common aquarium fish, the midas cichlid. J. Appl. Anim. Welf. Sci. 14, 340-360.

Parvulescu, A., 1967. The acoustics of small tanks. In: Tavolga, W.N. (Ed.), Marine Bio-Acoustics, vol. 2. Pergamon Press, London, pp. 7-13.

Payne, N.L., van der Meulen, D.E., Suthers, I.M., Gray, C. a, Taylor, M.D., 2015. Foraging intensity of wild mulloway *Argyrosomus japonicus* decreases with increasing anthropogenic disturbance. Mar. Biol. 162, 539-546.

Picciulin, M., Sebastianutto, L., Codarin, A., Farina, A., Ferrero, E. a, 2010. In situ behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacentridae) living in a marine protected area. J. Exp. Mar. Biol. Ecol. 386, 125-132.

Popper, A.N., Hastings, M.C., 2009a. The effects of human-generated sound on fish. Integr. Zool. 4, 43-52.

Popper, A.N., Hastings, M.C., 2009b. The effects of anthropogenic sources of sound on fishes. J. Fish. Biol. 75, 455-489.

Popper, A.N., Hawkins, A.D., Fay, R.R., Mann, D.A., Bartol, S., Carlson, T.J., Coombs, S., Ellison, W.T., Gentry, R.L., Halvorsen, M.B., et al., 2014. Sound Exposure Guidelines for Fishes and Sea Turtles: a Technical Report Prepared by ANSI-accredited Standards Committee S3/SC1 and Registered with ANSI.

Purser, J., Radford, A.N., 2011. Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (Gasterosteus aculeatus). PLoS One 6, e17478.

Radford, A.N., Kerridge, E., Simpson, S.D., 2014. Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Behav. Ecol. 25, 1022-1030.

Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J., Coppola, G., Geyer, M. a, Glanzman, D.L., Marsland, S., et al., 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol. Learn. Mem. 92, 135-138.

De Robertis, A., Handegard, N.O., 2013. Fish avoidance of research vessels and the efficacy of noise-reduced vessels: a review. ICES J. Mar. Sci. 70, 34-45.

Sarà, G., Dean, J., D'Amato, D., Buscaino, G., Oliveri, A., Genovese, S., Ferro, S., Buffa, G., Martire, M., Mazzola, S., 2007. Effect of boat noise on the behaviour of bluefin tuna *Thunnus thynnus* in the Mediterranean Sea. Mar. Ecol. Prog. Ser. 331, 243-253.

Sebastianutto, L., Picciulin, M., Costantini, M., Ferrero, E.A., 2011. How boat noise affects an ecologically crucial behaviour: the case of territoriality in *Gobius cruentatus* (Gobiidae). Environ. Biol. Fishes 92, 207-215.

Shafiei Sabet, S., Neo, Y.Y., Slabbekoorn, H., 2015. The effect of temporal variation in experimental noise exposure on swimming and foraging behaviour of captive zebrafish. Anim. Behav. 49-60.

Silva, P.I.M., Martins, C.I.M., Engrola, S., Marino, G., Øverli, Ø., Conceicao, L.E.C., 2010. Individual differences in cortisol levels and behaviour of Senegalese sole (*Solea senegalensis*) juveniles: evidence for coping styles. Appl. Anim. Behav. Sci. 124, 75-81.

Simpson, S.D., Purser, J., Radford, A.N., 2014. Anthropogenic noise compromises antipredator behaviour in European eels. Glob. Chang. Biol. 586-593.

Skilbrei, O., Holst, J., 2009. Vertical movements of “escaped” farmed Atlantic salmon (*Salmo salar L.*)-a simulation study in a western Norwegian fjord. ICES J. Mar. Sci. 66, 278-288.

Slabbekoorn, H., 2014. Aiming for progress in understanding underwater noise impact on fish: complementary need for indoor and outdoor studies. In: Popper, A.N., Hawkins, A.D. (Eds.), The Effects of Noise on Aquatic Life, Budapest Conference Proceedings. Elseviers.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., Popper, A.N., 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419-427.

Slotte, A., Hansen, K., Dalen, J., Ona, E., 2004. Acoustic mapping of pelagic fish distribution and abundance in relation to a seismic shooting area off the Norwegian west coast. Fish. Res. 67, 143-150.

Von Benda-Beckmann, A.M., Wensveen, P.J., Kvadsheim, P.H., Lam, F. P. a, Miller, P.J.O., Tyack, P.L., Ainslie, M. a, 2014. Modeling effectiveness of gradual increases in source level to mitigate effects of sonar on marine mammals. Conserv. Biol. 28, 119-128.

Voellmy, I.K., Purser, J., Simpson, S.D., Radford, A.N., 2014a. Increased noise levels have different impacts on the anti-predator behaviour of two sympatric fish species. PLoS One 9, e102946.

Voellmy, I.K., Purser, J., Flynn, D., Kennedy, P., Simpson, S.D., Radford, A.N., 2014b. Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Anim. Behav. 89, 191-198.

Weilgart, L.S., 2007. The impacts of anthropogenic ocean noise on cetaceans and implications for management. Can. J. Zool. 85, 1091-1116.

Wilson, B., Dill, L.M., 2002. Pacific herring respond to simulated odontocete echolocation sounds. Can. J. Fish. Aquat. Sci. 59, 542-553.