Swimming bass under pounding bass : fish response to sound exposure

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CHAPTER 2

Temporal structure of sound affects behavioural recovery from noise impact in European seabass

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Abstract

Human activities in and around waters generate a substantial amount of underwater noise, which may negatively affect aquatic life including fish. In order to better predict and assess the consequences of the variety of anthropogenic sounds, it is essential to examine what sound features contribute to an impact. In this study, we tested if sounds with different temporal structure resulted in different behavioural changes in European seabass. Groups of four fish were exposed in an outdoor basin to a series of four sound treatments, which were either continuous or intermittent, with either consistent or fluctuating amplitude. The behavioural changes of the fish were analysed by a video-tracking system. All sound treatments elicited similar behavioural changes, including startle responses, increased swimming speed, increased group cohesion and bottom diving. However, with all other sound conditions being the same, intermittent exposure resulted in significantly slower behavioural recovery to pre-exposure levels compared to continuous exposure. Our findings imply that the temporal structure of sound is highly relevant in noise impact assessments: intermittent sounds, such as from pile driving, may have a stronger behavioural impact on fish than continuous sounds, such as from drilling, even though the latter may have higher total accumulated energy. This study urges regulatory authorities and developers to pay more attention to the influence of temporal structure when assessing noise impacts. However, more studies are needed to examine other sound parameters and to determine the generality of our observations in other species and in other outdoo water bodies.

Introduction

The underwater world is filled with a variety of biotic and abiotic sounds. In fact, these natural sounds are often so prominent that they have interfered with the underwater acoustic communication by the navy since the early 1900s[1]. However, as human exploitations of the marine environment increased over the years, a cacophony of anthropogenic sounds has also been introduced underwater through commercial shipping, offshore construction, sonar exploration, seismic surveys and underwater explosions. This change in the underwater acoustic scene may be posing a threat to marine life[2,3]. Consequently, underwater noise pollution has been listed in the European Union's Marine Strategy Framework Directive 2008/56/EC as one of the descriptors for achieving good environmental status, despite a considerable deficiency of empirical data.

In comparison to sea mammals, relatively few noise impact studies exist on fish, despite their high diversity, abundance, and economical importance[4,5]. All fish species studied to date can hear and many may use sound for habitat selection[6,7], conspecific communication[8,9] and predator-prey interactions[10,11]. Acoustic signals are especially effective over long distances or under low-visibility conditions. However, the biologically relevant sounds used by fish often overlap with anthropogenic noise, which typically also consists of relatively low frequencies[2,12]. This spectral overlap suggests that fish may be especially vulnerable to human-induced elevation of underwater noise levels.

Anthropogenic noise can be loud and localized or more moderate but widespread: both may affect fish differently. For example, several exposure experiments with high-intensity sounds, such as those resembling pile driving or explosions, have reported auditory tissue damage[13–17] or temporary hearing loss[18–22]. The exposure levels in these studies were usually very high, which in practice only happen when fish are in the immediate proximity of loud sound sources. In this regard, more moderate but widespread noise could be more critical to population and ecosystem stability as it covers wider areas affecting larger numbers of fish[2].

In particular, fish exposed to more moderate noise may also take an active role and alter their behaviour in response, which may alleviate some but induce other problems[23]. Anthropogenic sounds have, for example, been shown to disrupt spawning events[24], affect territorial dynamics[25] and reduce feeding efficacy[26]. Moreover, after seismic airgun shootings, fishing vessels have experienced significant catch reductions, suggesting active avoidance of the noise source by fish[27,28]. Many fish species also show startle responses[29] at the onset of noise exposure[26,30–33] and some dive to greater depth[34–38]. However, behavioural observations in these studies usually only lasted for several minutes and we still lack critical insights into the persistence of behavioural changes over longer periods[39], which may be related to long-term effects on growth and body condition e.g. [40,41].

The behavioural impact of anthropogenic sounds may not only be determined by their mere presence and level, but also by the frequency range, amplitude fluctuation and temporal structure of the sounds that arrive at a fish[2,42]. It is crucial to study these sound features explicitly because feature-dependent perceptual sensitivity may determine fish susceptibility to specific noise exposures. Among these sound features, little is known about the influence of temporal structure on noise impact but see [14,43], even though fish are known to be sensitive to the temporal characteristics of sounds, which may carry important information[44,45]. In terms of temporal structure, anthropogenic sounds vary with regard to intermittency (whether continuous or intermittent/impulsive), pulse duration, pulse repetition rate and pulse regularity. For example, seismic airgun and pile-driving noise are intermittent while wind turbine and ship noise are continuous. Moreover, sound amplitude may be fluctuating or consistent over time depending on the characteristics or movements of the sound sources. Hence, to assess the potential impact of anthropogenic noise, we need to understand what sound features actually contribute to the impacts.

In this study, we investigated whether intermittency and amplitude fluctuation of noise exposure contribute to behavioural changes and recovery in the European seabass (*Dicentrarchus labrax*), an important commercial fish species. The fish were exposed to artificially generated sounds resembling man-made noise, and their swimming patterns were analyzed with movement-tracking software.

Materials and methods

STUDY SPECIES

The European seabass is a demersal species that is commonly found in shallow waters in the North Sea and the Mediterranean Sea. It is an oceanodromous species and can tolerate a wide range of temperature and salinity[46]. The juveniles form schools but the adults are less gregarious and may shoal loosely with fewer individuals[46]. The species is known to hear best below 700 Hz[31] and has no accessory hearing organs besides the otoliths and the swim bladder.

ANIMAL MAINTENANCE

The European seabass used in this study came from a commercial hatchery (Ecloserie Marine, Gravelines, France) and were about 35 cm in total body length and 350 g in weight. The fish were kept in round polyester holding tanks (2.2m in diameter, 1m deep) 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 estuary. The fish were fed Neo Grower Extra Marin pellets (Le Gouessant Aquaculture, Lamballe, France) every other day based on the temperature-dependent prescription by the manufacturer. Water temperature varied from 9 to 16 °C throughout the one-and-a-half-month experimental period (May-June 2012). All experiments were performed in accordance with the Dutch Experiments on Animals Act (DEC approval no: 12026) which serves as the implementation of the Directive 86/609/EEC by the Council of the European Communities regarding the treatment of animals used for scientific purposes.

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 [see 31 for details]. Next to the basin, there was a research cabin containing sound generating and monitoring equipment and video recording and monitoring equipment. During the exposure trials, fish were placed in a white nylon net enclosure (1.6 x 1.6 x 2.0 m) in the basin to ensure full coverage by two video cameras (Lanmda, China) for observation (Fig. 1). White tarps were positioned at the bottom and the background to ensure sufficient contrast in video images, without causing abnormal swimming behaviour in the fish.

TREATMENT SERIES

The fish were subjected to a series of four sound treatments: continuous consistent (CC), continuous fluctuating (CF), intermittent

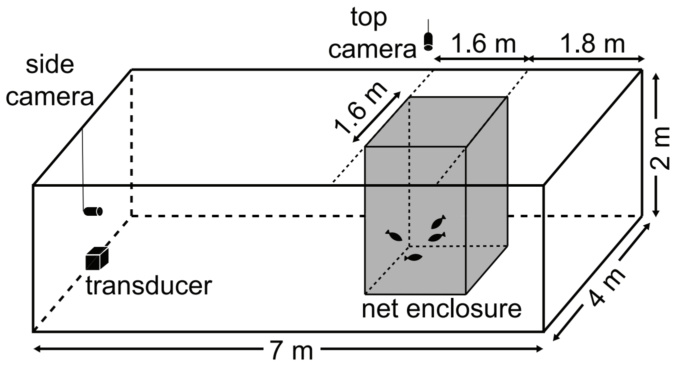


Fig. 1 Experimental arena in the outdoor basin at SEAMARCO. The transducer for playback is indicated on the left near the bottom and the net enclosure with the restricted swimming space for the four fish is indicated in grey.

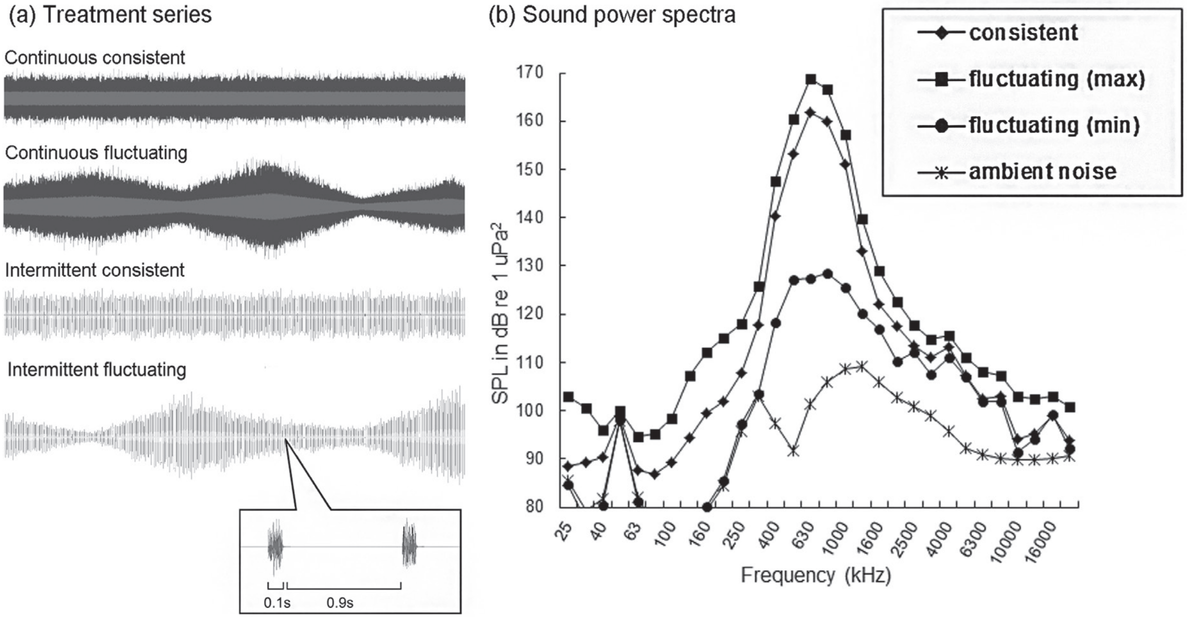


Fig. 2 (a) Time-domain waveforms showing 5 min of the four treatments exposed to each group of fish and a 2-second magnification of the intermittent treatments. (b) Frequency-domain power spectra in 1/3 octave bands of the four conditions measured for their SPL. The SPLs of consistent treatment, the maximum and minimum amplitude of fluctuating treatment and ambient noise are 165, 172, 134 and 115 dB re 1µPa respectively.

consistent (IC) and intermittent fluctuating (IF) (Fig. 2a). The treatments vary only in terms of the two temporal parameters of interest, i.e. intermittency and amplitude fluctuation, and have all other sound parameters (e.g. frequency bandwidth, start amplitude, average amplitude and exposure duration) kept the same. The continuous treatments consisted of elevated noise levels without interruption, while the intermittent treatments consisted of 0.1-second noise pulses interspersed with 0.9-second silent intervals, with a regular repetition rate of 1 pulse per second, comparable to the range of pile-driving sounds. The continuous and intermittent treatments were played back at either a consistent (CC & IC) or a fluctuating amplitude level (CF & IF). In order to vary only the temporal parameters of interest in the treatments while keeping all other sound parameters constant, the sound samples were created in Audition 3.0 (Adobe, San Jose, US) using filtered brown noise (bandpassed: 300-1000 Hz) and played back with an underwater transducer (LL-1424HP, Lubell Labs, Columbus, US) from a laptop connected to an attenuator (AS-2008-2, SEAMARCO, Harderwijk, Netherlands), a pre-amplifier (SEAMARCO, Harderwijk, Netherlands), a power amplifier (Macrotech 5000 VZ, Crown Audio, Elkhart, US) and a bandwidth filter (KrohnHite Corporation, Brockton, US).

Prior to the exposure trials, the sound pressure level (SPL) of the treatments was determined in the experimental basin with a calibrated system consisting of a broad-band hydrophone (8101, Brüel & Kjær, Denmark; frequency range 0–100 kHz), a voltage amplifier system (TPD, TNO, Netherlands; frequency range 0–300 kHz) and a personal computer with spectral analysis software (Cool Edit Pro, Syntrillium Software Corp, US; sample frequency 11–96 kHz, frequency range 0–48 kHz, df = 15–115 Hz). The SPL was calculated as the root mean square of sound pressure over 10 s. Four conditions were measured: the ambient noise level without playback, the CC treatment, the maximum amplitude of the CF treatment and the minimum amplitude of the CF treatment (same level as when no sound was played but with the transducer switched on). Since the intermittent treatments were created from the continuous treatments in the computer program, their equivalent SPL was assumed to be the same as their corresponding continuous treatments.

Measurements made at 56 points in the basin (in a 7 x 4 x 2 grid) showed that the sound pressure field was rather homogenous in the whole basin (± 0.8 dB). The ambient SPL in the experimental basin was 115 dB re 1µPa. When the transducer was switched on, the SPL increased to 134 dB re 1µPa, even though no sound was played, due to a low humming sound made by the transducer. However, this increase in SPL did not trigger any fish response during the trials. The SPL of the consistent amplitude treatments was 165 dB re 1µPa and the SPL of fluctuating amplitude treatments changed gradually every minute between random levels ranging from 134 to 172 dB re 1µPa.

Spectral investigation confirmed that most of the sound energy during experimental exposure was concentrated between 250 and 1600 Hz (Fig. 2b), around the best hearing sensitivity for seabass [31,47]. Due to technical limitations, measurements were only conducted for SPL and not for particle motion. Both sound components are probably perceived by European seabass, but particle motion may be perceptually more dominant[48], although there is still a general lack of insight about the relative contributions of both sound components to hearing in most species[49,50]. The ratio of sound pressure to particle motion in our basin is probably not constant like in the far-field at open sea[51]. Nonetheless, we believe this potential acoustic heterogeneity is not a concern for our bio-assay, as we use broadband sound treatments that differed only in the temporal structure. We expect that the temporal variation in SPL that we measured reflect the temporal variation in particle motion, which makes our set-up valid for comparing the effects of sound temporal structure and relative amplitude variation.

EXPERIMENTAL SET-UP

We tested twelve groups of four fish, where each group was exposed to all four treatments (48 fish, N = 12). The order of the treatments per fish group followed an incomplete counterbalanced design. At least 17 h prior to the trials, each fish group was transferred to the experimental basin to allow acclimatization. The fish acclimatized quickly to the conditions in the experimental arena after being introduced in groups of four from their holding tanks. Within one or two hours, they swam at a relaxed speed throughout the experimental arena. 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. The trials consisted of 30 min of silence before and after 30 min of sound exposure. 30 min before each trial, the transducer and the lights above the experimental basin were turned on (to ensure good video quality), while the oxygen pump and the water recirculating system were turned off (to minimise ambient noise). Water temperature (9-16 °C), light intensity (320-1280 lux), wind speed (13-35 kmh^{-1}) and weather condition (sunny, cloudy or rainy) were recorded before the start of the trials, to be used as covariates in the data analysis. During the trials, no external anthropogenic noise or disturbance was allowed near the study area.

BEHAVIOURAL OBSERVATION & ANALYSIS

Fish behaviour was video-recorded during the entire exposure sessions (30 min before, 30 min during and 30 min after sound exposure). The recordings from the top camera were used for the analysis of startle responses, defined as sudden changes in swimming direction accompanied by acceleration. Four 5-minute fragments were cut from the recordings: right before sound exposure ('before'), at the start of sound exposure ('during 1'), right before the end of sound exposure ('during 2') and right after sound exposure ('after'). The number of all startle responses exhibited by the four fish was then scored manually, blind to the observer (J.S.) for the treatment type and exposure period. For the analysis of other behavioural parameters, the full recordings from the side camera were analysed with tracking software, Logger Pro 3.8.5.1 (Vernier Software & Technology, Beaverton, US), which assigned a pair of coordinates to each fish in a trial every second by on-screen visual tracking and manual cursor placement. The coordinates were then used to calculate swimming depth, group cohesion (the average distance between pairs of individuals) and swimming speed.

STATISTICS

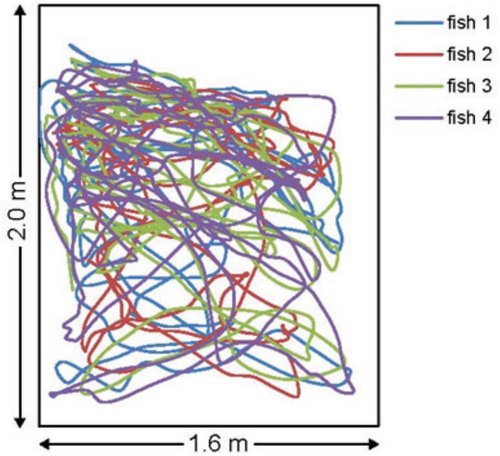
Startle responses were only observed at the onset of sound exposure and the effect of treatments on the number of startle responses was analysed with the Friedman test. To test for the magnitude of change in swimming depth and group cohesion during the trials, we compared four 5-minute period bins from our data set: 'before', 'during 1', 'during 2' and 'after' (like the analysis of startle responses). Two-way repeated measures ANOVAs were then performed, with treatment and period as the withinsubject factors. For swimming speed, the analysis was the same, but the period bins used were 10 s instead of 5 min due to the transient nature of the increase in speed. We also analysed the recovery time of 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 5minute moving averages (shifting forward every second) during and after exposure, to see when the baseline was reached again. If the baseline was not reached after 60 min (30 min after sound exposure), the recovery time was counted as 60 min (occurred in 4 out of 48 trials for swimming depth and 2 out of 48 trials for group cohesion). For swimming speed, the analysis was the same, but the averages used were 10 s instead of 5 min. The effects of temporal parameters on the recovery time were then analysed with twoway repeated measures ANOVAs, with intermittency and amplitude fluctuation as the withinsubject factors. The data of the recovery time were log-transformed to fulfil the assumptions of normality and homoscedasticity. When sphericity could not be assumed in a repeated-measures ANOVA, Huynh-Feldt correction was used. For ANOVAs with a significant outcome, Bonferroni-corrected posthoc tests were conducted.

Results

Startle responses were seen in 41 of the 48 trials and typical only in the first 10 s of sound exposure (all within period 'during 1', except in 2 of the 48 trials where it happened also in period 'after'). The number of startle responses in the first 5 min of exposure did not differ among treatments of different temporal structure (Friedman's test: $X^2_3 = 1.991$, $P = 0.574$). Fish typically dived to the bottom during the first 5 min of exposure (Fig. 3), which is supported by a significant drop in swimming depth of 32.19 ± 19.62 (SD) cm (repeated-measures ANOVA: $F_{3,27} = 25.457$, $P < 0.001$; Bonferroni-corrected *post-hoc*: 'during 1' compared to three other periods, all Ps < 0.005). However, there were no significant effects of treatment and treatment-period interaction ($F_{3,27} = 1.094$, $P = 0.368$; $F_{5.7,51.4} = 1.132$, $P = 0.357$ respectively). The distance between individuals also became 7.01 ± 8.00 (SD) cm closer during the exposure, leading to a significantly tighter group cohesion (repeated-measures ANOVA: $F_{3,27} = 11.078$, $P < 0.001$; Bonferroni-corrected *post-hoc*: 'during 1' compared to three other periods, all Ps < 0.05). Again, no significant effects of treatment and treatment-period interaction were found ($F_{3,27} = 0.441$, $P = 0.726$; $F_{9,81} = 0.643$, $P = 0.757$ respectively). Besides, group cohesion did not correlate with swimming depth ($R^2 = 0.06$). Furthermore, a significant increase in swimming speed of 53.99 ± 42.84 (SD) cms^{-1} was also observed at the onset of sound exposure (repeated-measures ANOVA: $F_{1.60,17.65} = 35.330$, $P < 0.001$; Bonferroni-corrected *post-hoc*: 'during 1' compared to three other periods, all Ps < 0.05).

Besides analysing the magnitude of the behavioural changes, we also examined the recovery of these behavioural patterns (Fig. 4). We found that the recovery time (log-transformed) of swimming depth was affected by sound intermittency (Fig. 5), with intermittent treatments showing 19.74 ± 20.70 (SD) min longer recovery time than continuous treatments (repeated-measures ANOVA: $F_{1,11} = 23.569$, $P = 0.001$). However, it was not influenced by amplitude fluctuation (repeated-measures ANOVA: $F_{1,11} = 0.046$, $P = 0.835$). In contrast, the recovery time (log-transformed) of group cohesion was not significantly influenced by sound intermittency (repeated-measures ANOVA: $F_{1,11} = 0.001$, $P = 0.978$) and amplitude fluctuation (repeated-measures ANOVA: $F_{1,11} = 4.264$, $P = 0.063$), although the latter was a non-significant trend with a difference of 9.01 ± 21.93 (SD) min. The recovery time (log-transformed) of swimming speed was also not significantly influenced by sound intermittency (repeated-measures ANOVA: $F_{1,11} = 0.107$, $P = 0.749$) and amplitude fluctuation (repeated-measures ANOVA: $F_{1,11} = 1.353$, $P = 0.269$).

(a) Before sound exposure



(b) During sound exposure

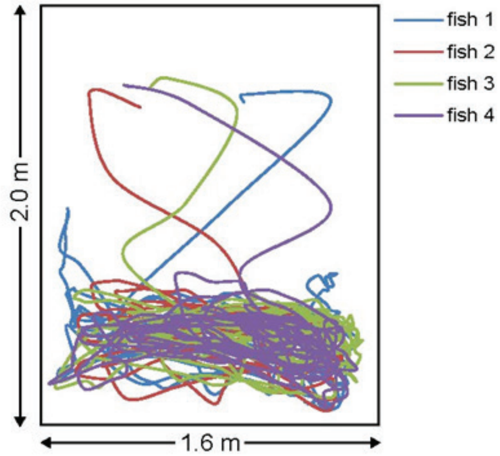
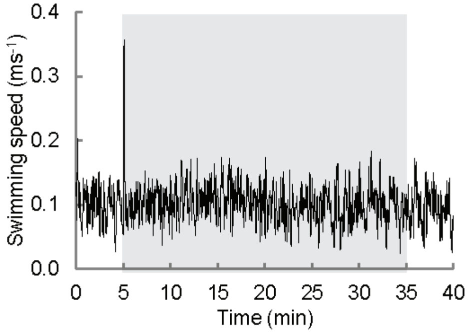
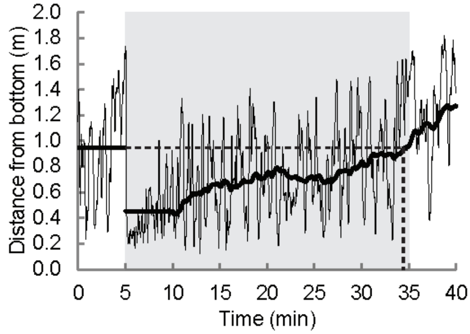


Fig. 3 Typical swimming track images of four fish within the net enclosure viewed from the side camera (a) 5 min before and (b) 5 min at the start of sound exposure (example of an IF treatment trial). The fish occupy the whole study arena before sound exposure but dive to the bottom at the onset of sound exposure and stay there for the next 5 min.

(a) Swimming speed



(b) Swimming depth



(c) Group cohesion

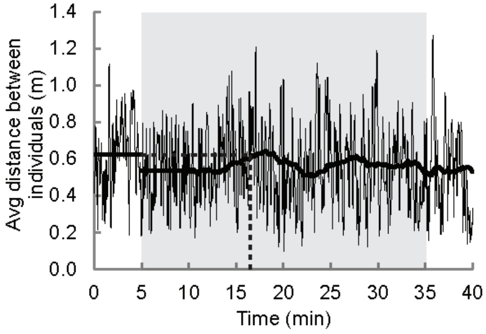
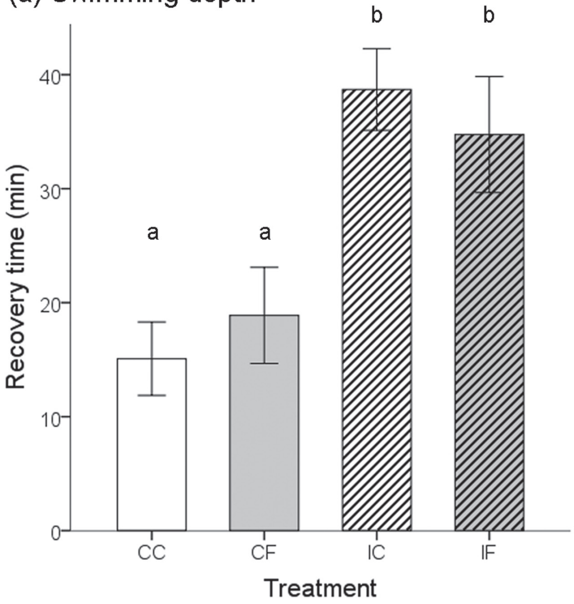


Fig. 4 Time series showing typical (a) swimming speed, (b) swimming depth and (c) group cohesion of fish 5 min before, 30 min during (shaded) and 5 min after sound exposure (example of an IC treatment trial). In (b) & (c), the thick line in the first 5 min is the average level before sound exposure; the thick line thereafter is the 5-minute moving averages shifting forward every second; the dashed line is the trace of how recovery time is determined.


(a) Swimming depth

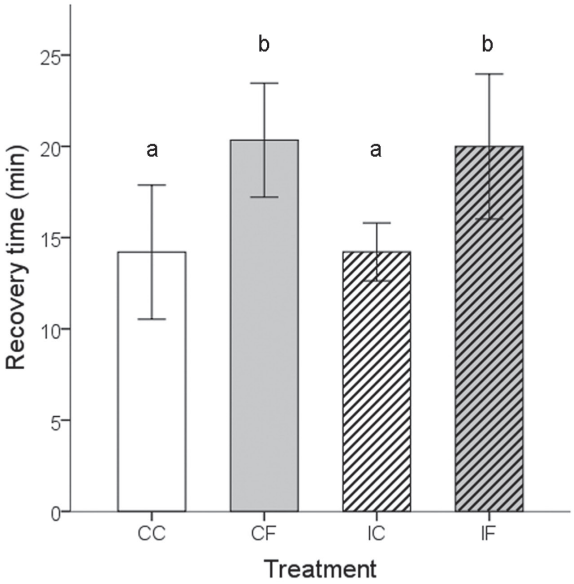

(b) Group cohesion

Fig. 5 Recovery time (± SE) of (a) swimming depth and (b) group cohesion of fish. The recovery time of swimming depth is significantly longer for intermittent treatments than for continuous treatments (a's and b's reflect statistically different groups, $P = 0.001$). For the recovery time of group cohesion, there is a trend for the recovery time of amplitude fluctuation to be longer than amplitude consistency (a's and b's reflect statistical trend for being different groups, $P = 0.063$). Note that the graphs are not log-transformed but do show the same patterns after transformation.

Discussion

Our results revealed that all the different noise treatments initially resulted in a similarly strong response with behavioural changes that are typically associated with fright and anxiety[52–54]. However, intermittent exposure prolonged the recovery of swimming depth significantly compared to continuous exposure.

BEHAVIOURAL RESPONSES RELATED TO ANXIETY?

Caution is necessary when making direct comparisons between studies in captivity and in the wild, as there are important behavioural and acoustic differences[55]. However, the nature of the behavioural changes observed in our study on captive European seabass mirrors earlier observations on other captive, semi-wild, or wild species. For example, several studies have shown that fish dive deeper upon noise exposure[34–38]. Considering that our experimental basin had a rather homogenous sound pressure field independent of depth, we believe that the response of our seabass is not an active avoidance of the sound source but a typical anxiety-related reaction. Many fish species have been reported to dive to greater depth when subjected to various stressors such as predation cues[56,57], novel environments[58–60], chemical pollution[61] and anxiogenic drugs[53,58]. Moreover, two field studies have shown that vessel noise induced more immediate and prominent fish movements in the vertical axis down the water column than in the horizontal axis away from the vessel[34,37]. Therefore, diving behaviour may be a good indicator, in both indoor and outdoor settings, of a primary stress response, by which pelagic and demersal fish species may try to escape danger and minimize potential risks of predation.

Changes in group cohesion as a result of anthropogenic noise exposure have also been documented in previous studies[35,62]. Some species may form tighter shoals[35] while others may decrease school coordination[62]. Both directions of change may reflect an anxiety-driven response related to the perception of potential danger[63]. The difference in response may be related to the variation in test conditions, group sizes[64] or species-specific escape strategies that may be different for shoaling and highly coordinated schooling aggregations[65].

We also observed startle responses and sudden accelerations of fish at the onset of exposures, which have been reported by many other researchers[26,30–33,35]. These erratic movements in the form of rapid flight reflexes are mediated by Mauthner cells in the hindbrain and were an anti-predation strategy that have been associated with anxiety[29,58]. They have also been shown to co-occur with an increase in lactate and haematocrit levels in our study species, the European seabass, when exposed to artificial sound[66]. Although we did not take any physiological measurements, the behavioural changes observed in our study could have been accompanied by an increase in anxiety-related hormones (e.g. cortisol) upon noise exposure, as reported in several other studies[20,67–69]. However, the correlation between behaviour and stress physiology is not always clear[70].

BEHAVIOURAL RECOVERY FROM NOISE EXPOSURE

Our experimental noise exposure resulted in a highly consistent initial behavioural response among all four treatments. However, the recovery time varied significantly among treatments, allowing the investigation into the relative impact of the temporal variation in sound exposure. Three mechanisms may explain the behavioural recovery observed in our study: 1) habituation, 2) sensory adaptation and 3) motor fatigue[71]. Habituation happens in the central nervous system, filtering out irrelevant stimuli and allowing animals to focus selectively on biologically important stimuli; sensory adaptation happens in sensory organs as a result of decreased sensitivity induced by acute stimulations, e.g. temporary threshold shift (TTS); motor fatigue happens in muscles due to exhaustion.

We believe that the first explanation, habituation, is most likely the dominant determinant for the behavioural recovery during the noise exposure in our study. One of the characteristics of habituation is that higher stimulation rates result in faster habituation[72,73]. The faster recovery rate for continuous noise (constant stimulation, thus high stimulation rate) compared to intermittent noise (relatively low stimulation rate) in our study, implicitly suggest that the recovery was possibly due to habituation. Furthermore, we observed fast inter-trial recovery, where a successive noise exposure after 3 h induced the same level of behavioural changes as the previous exposure. This renders sensory adaptation a less likely explanation for the behavioural recovery, as TTS would usually take days to weeks to recovery[20,21,74]. Even if TTS did occur, it was probably not strong enough to hinder fish hearing of excessive sounds. Motor fatigue may have been responsible for the rapid recovery (< 20 s) of swimming speed[75], but less likely for swimming depth and cohesion, as these parameters are generally not associated with extensive muscle use. However, further investigations are necessary before we can be more conclusive about the mechanism underlying the behavioural recovery in our assay[72].

Our results also seem to suggest qualitatively different influence of the two temporal parameters tested: intermittent treatments affected the recovery time of swimming depth, while fluctuating treatments did not, but they tended to affect the recovery time of group cohesion. This finding suggests that different stress responses exhibited by fish may be determined by specific acoustic characteristics of a sound stressor. For example, compared to the continuous treatments, the fluctuating treatments had an unpredictable rise and fall in amplitude that may resemble more natural and biologically relevant sounds, such as an approaching predator. These fluctuations may have been perceived as potentially more dangerous, which made the fish stay close together for longer. When Speedie & Gerlai[76] exposed zebrafish (*Danio rerio*) to an alarm pheromone, the fish also showed tighter group cohesion without spending more time close to the tank bottom like when exposed to other stressors[56,58]. However, more studies are needed in order to shed light on the potential relationship between qualitatively different behavioural responses and stressor types.

MANAGEMENT IMPLICATIONS

Our findings call for a reconsideration of current practices in noise impact assessments, which typically only consider the standard metrics, such as SPL, sound exposure level (SEL) and exposure duration, but not the temporal patterns of noise exposure. Although these metrics may be important in determining the impacts of high-intensity exposure in terms of auditory tissue injury and temporary hearing loss[16,17,21], here we show that they are not always sufficient when assessing the behavioural impact of exposure to more moderate levels. For example, while having comparable SPL, our intermittent treatments with a 10% duty cycle would only accumulate ~10% of the energy of the continuous treatments, hence in principle had about 10 dB lower cumulative SEL (SEL_{cum}) than the continuous treatments. Yet, our results revealed that the intermittent treatments prolonged the behavioural recovery for twice as long as the continuous treatments, suggesting that noise treatments with a lower

SEL_{cum} may sometimes lead to a stronger behavioural impact. Since the behavioural impact of anthropogenic noise is more widespread than physical harm[2], temporal structure of sound should be considered more prominently in noise impact assessments and taken into account when devising mitigating measures.

Our findings suggest that noise impact on fish may be alleviated by modifying the temporal pattern of exposure schemes. For example, offshore piling may become more fish-friendly by using more continuous drilling techniques instead of the conventional impulsive pile-driving methods. Drilling may reduce not only the risk of physical impact for fish near the construction site due to lower SPL and SEL[77], but also the extent of behavioural impact for fish in a much larger area through faster habituation and recovery of natural activities. Because of the different impact strengths related to the temporal structure of sound exposure, the thresholds of detrimental effects on fish behaviour are possibly also lower for intermittent sounds compared to continuous sounds, although it is still not easy to suggest any quantitative adjustments.

However, at this early stage of research, considerable care should be exercised when assessing management implications[78]. On the one hand, even though all the behavioural changes observed in our study point to anxiety and may involve stressful physiological changes, little is known about whether these behavioural changes will result in fitness consequences through reduced survival, growth and reproduction. On the other hand, even in the absence of direct behavioural reactions, subtle aspects of behaviour such as the time spent on nest guarding, may still be affected[39], potentially leading to significant consequences for life-time reproductive success.

We also need to be cautious with the extrapolation of data from captive to wild animals[55,79]. Wild fish have, for example, the potential to swim away from the sound source, which could result in a different kind of behavioural response. Furthermore, wild fish have been shown to be more vulnerable to some stressors than captive ones[80,81], suggesting that behavioural impact of noise exposure may be stronger for wild fish. Absolute threshold levels assessed under captive conditions are therefore likely to have limited value in the wild and we emphasize that the value of our findings is in the relative impact of the different treatments varying in the temporal patterns of noise exposure.

CONCLUSIONS

Our study provides clear evidence of the contribution of sound temporal pattern to the behavioural recovery of fish after noise disturbance. Many fundamental questions remain, but we argue that the insights from our study provide important implications for underwater noise impact assessments. We believe that temporal variations are critical when assessing or predicting the severity of anthropogenic noise impact on fish behaviour and may have applied value for devising mitigating measures.

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