The Effects of Noise on Aquatic Life IV
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Insights of a revised dose-response methodology to investigate behavioural responses of juvenile seabass exposed to offshore wind turbine noise

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Abstract

Anthropogenic noise has become a widespread pollutant in the world's oceans, having an impact on the biology and ecology of various marine species. However, due to the challenges of reproducing realistic conditions in experimental setups, behavioural effects of continuous anthropogenic noise remain poorly studied. The aim of this study was to develop a laboratory-based experimental protocol to evaluate the behavioural responses of juvenile European seabass (*Dicentrarchus labrax*) to representative and realistic playback of offshore wind turbine noise. The acoustic exposure simulated the gradual approach of fish swimming toward an operating turbine, using field recordings collected *in situ* sea conditions. Acoustic calibrations were conducted prior to testing to ensure the spectral and spatial realism of the sound within the experimental environment.

Keywords

Anthropogenic noise; fish behaviour; laboratory experiments; acoustic playback; sound calibration; dose-response

State of the art

The expansion of marine renewable energy projects, particularly the proliferation of offshore wind farms, is contributing to increase anthropogenic noise emissions in the ocean. Marine soundscapes are initially altered during the construction phase of wind farms, when large working vessels are deployed and turbine foundations installed. Indeed, these vessels produce broadband noise, and the installation of wind turbine foundations often requires pile driving - a process known to generate impulsive noise (Benhemma-Le Gall et al. 2021). By generating low-frequency noise, generally below 1 kHz, the operational phase of wind farms will then contribute to changing the surrounding marine soundscapes (Tougaard et al. 2020).

To understand the impact of these environmental disturbances on marine fauna, research has primarily focused on determining acoustic thresholds that cause physical damage, especially in response to impulsive noise. More indirect effects, such as behavioural responses that are first-line defences to adverse environmental change (Schreck et al. 1997) have largely been overlooked.

Dose–response approaches are commonly used in ecotoxicology and sensory ecology to assess the relationship between the intensity of a stimulus, often chemical, and the resulting biological response. In the context of underwater noise pollution, dose–response methods could be applied to identify behavioural thresholds. However, such approaches usually involve sudden or fixed exposure levels. These levels do not accurately reflect how marine animals experience noise *in situ*, where exposure often changes gradually as the animals move toward or away the source of the noise.

Teleost (*i.e.*, bony fish) species occupy a wide range of ecological niches, notably within marine food webs. They range from primary consumers to top predators playing an important role in maintaining ecosystem balance (see Shekhar 2025). Due to the similarity in density between their body tissues and the surrounding water, fish are considered acoustically transparent (Popper and Hawkins 2019). This property makes them particularly sensitive to variations in sound pressure, increasing their susceptibility to be disturbed by anthropogenic underwater noise. Therefore, given their ecological importance and acoustic sensitivity, teleosts are relevant subjects for investigating the effects of noise pollution and the potential consequences on marine ecosystems.

In practice, studying fish reactions under realistic acoustic conditions is technically difficult. This can, for instance, involve *in situ* distribution monitoring using technologically advanced approaches, which may be particularly difficult to implement (*e.g.,* telemetry, echosounder). To accurately observe and monitor behavioural reactions, experiments can also be conducted in a controlled environment. In this case, the challenge is to realistically reproduce the sound environment, considering the various acoustic constraints, such as tank resonance and/or sound field heterogeneity, associated with an enclosed environment (Campbell 2019; Jones et al. 2019). These constraints can alter the spectral and spatial characteristics of the playback signal, potentially leading to a wrong interpretation of behavioural responses. Addressing these issues is therefore essential to ensure the acoustic realism of the experimental conditions and the accuracy of the results.

This chapter describes the experimental setup and methodology developed to expose juvenile European seabass (*Dicentrarchus labrax*) to operational wind turbine noise under controlled laboratory conditions. This approach aimed to reproduce wind turbine noise as realistically as possible by addressing technical considerations, such as the selection of the equipment and the acoustic properties and constraints of the experimental environment. The methodology also incorporated a revised dose–response approach, with a gradual increase in noise exposure being simulated, reflecting the natural movement of fish towards the noise source.

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From field to laboratory: creating a realistic exposure

Wind turbine and *in situ* acoustic recordings

Floatgen is a floating wind turbine deployed at the SEM-REV offshore test site (managed by OPEN-C foundation), located 22 km off the coast of Le Croisic in the Atlantic Ocean (Pays-de-la-Loire, France, Figure 1). Between June 2020 and March 2021, the acoustic environment and noise emissions generated by Floatgen during electricity production were characterised. First, to estimate the site-specific acoustic properties, large-band acoustic signals were emitted from various locations using a sound emission system provided by AudioSud (France), comprising a digital player (TAG, France) and an amplifier (Lubell Labs Inc., Ohio, USA) connected to a loudspeaker (Lubell LL916C, frequency response: 200 Hz – 20 kHz, Lubell Labs Inc., Ohio, USA). The corresponding sound pressure levels were recorded with a receiving chain composed of a recorder (Tascam, sampling frequency: 192 kHz, 24-bit resolution; TEAC, Wiesbaden, Germany) and a calibrated hydrophone (Brüel & Kjær, bandwidth: 10 Hz – 100 kHz, sensitivity: -174,1 dB re 1V/µPa, Hottinger Brüel & Kjaer GmbH, Germany) deployed at a fixed location. The distance–sound pressure pairs were then used to model acoustic propagation losses in the environment – that is $15\log(d)$, where d is the distance between the source and the measurement point. Then, audio recordings were conducted at multiple locations to determine both the ambient soundscape and the acoustic radiation pattern of Floatgen turbine under operational conditions. Additionally, a long-term audio monitoring campaign was conducted over one month with the hydrophone located at 214 meters from the turbine base, at a depth of 33 meters. Audio files obtained from these field campaigns were then used to isolate both sea ambient noise of the site without any anthropogenic noise and the operational acoustic signature of Floatgen. Sea ambient noise was extracted from a recording made under wind force 4 on the Beaufort scale (BF4) and sea state 3 (SS3). BF4 represents the most frequent wind condition at the site over a year, and SS3 was the most common sea state observed under BF4 conditions in the available recordings.

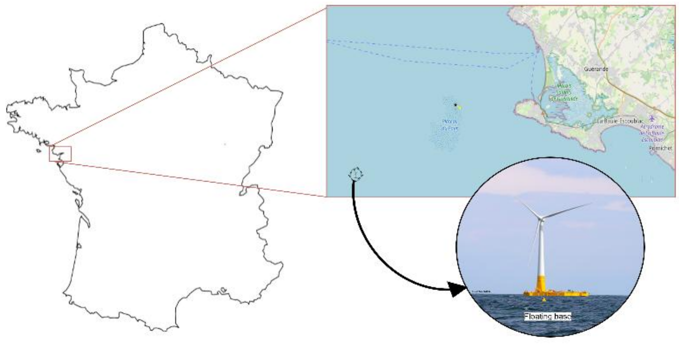

Figure 1: location and photography of the experimental floating wind turbine Floatgen

Experimental environment

Experiments were conducted at the LIENSs laboratory facilities (La Rochelle Université/CNRS, France) in a 11,000-litre indoor cylindrical seawater pool (Ø 3.66 m, water level: 1.05 m) equipped with three experimental arenas (Figure 2a). Each arena was built with a white rigid expanded PVC bottom and a cylindrical lateral enclosure made with oyster mesh, allowing water flow while containing fish. The enclosure was acoustically transparent, ensuring propagation of the sound. With a diameter of 80 cm and a water level of 20 cm, the arenas provided sufficient space for representative behavioural monitoring of the experimental fish groups (Figure 2b).

The pool was installed on several layers of insulating material, including two types of foam panels and a cork underlay, to minimise noise disturbances from outside. Using the same equipment as in the field, acoustic characterisation of the pool was achieved by sending and recording white noise. White noise was recorded at various locations and depths within both the main pool and the experimental arenas to enable an accurate modelling of acoustic propagation and losses, and to verify that reverberation remained minimal in the experimental pool.

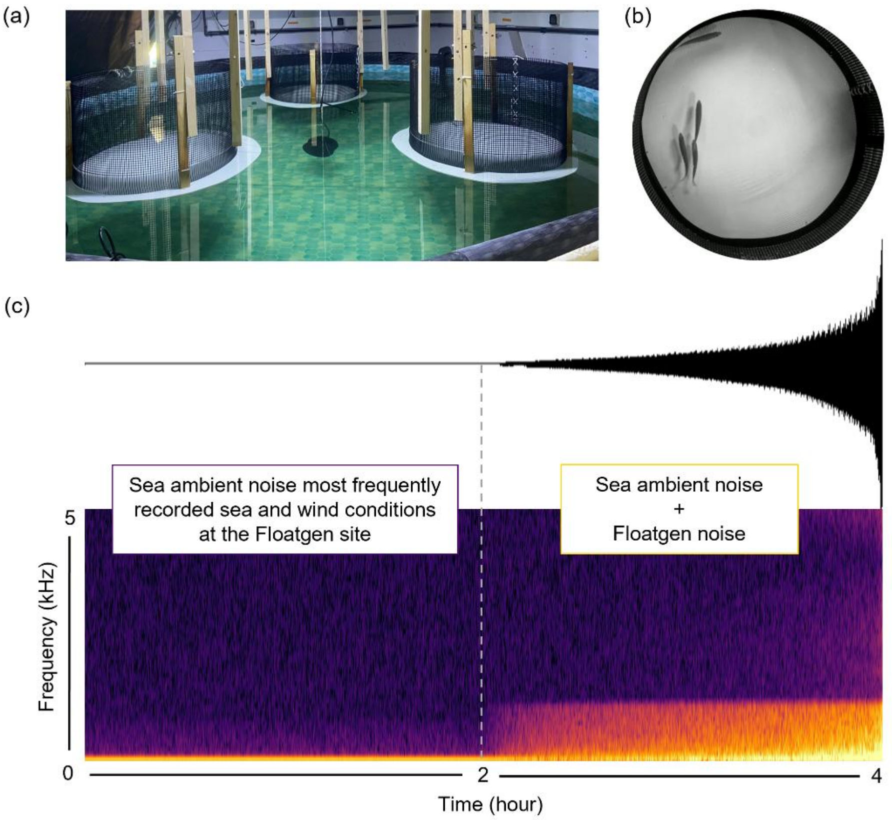

Figure 2: Illustration of the setup and the sound track used in the study. (a) the experimental pool with the three experimental arenas; (b) view from above of an experimental group of juvenile seabass within an arena, and (c) the oscillogram and spectrogram of the four-hour sound track.

Sound track

The playback design was developed to simulate the natural movement of fish from an area located outside the influence of Floatgen noise (approximately 4 km away) toward the base of the turbine. Based on an average optimal swimming speed of 0.54 cm.s^{-1} assessed in 16°C acclimated juveniles of European seabass (Claireaux et al. 2006), fish would require around two hours to cover the 4 km distance. Accordingly, the soundtrack used in the experiment was four hours long. The first two hours consisted of a control phase with only the natural sea ambient noise from the site. During the subsequent two-hour exposure phase, the Floatgen noise was superimposed on the sea ambient noise. In line with the simulation of seabass moving towards Floatgen, the intensity gradually increasing from 0 to 145 dB re 1 μPa. This increase in intensity was adjusted according to the propagation loss formula [15log(*d*)] derived from the acoustic field measurements and modelling conducted at the Floatgen site (Figure 2c).

The four-hour soundtrack was broadcast through an emitting chain in the middle of the pool. This chain was composed of a Clark Synthesis loudspeaker (Diluvio™, frequency response: 20 Hz – 17 kHz, Inc., Colorado, USA) connected to the sound emitting device gathered by AudioSud (France). The sound received within the three experimental arenas was recorded using the same calibrated recording chain as in the field. By analysing these recordings and comparing the sea ambient noise recorded *in situ* with the sound measured inside the arenas, differences in frequency levels were identified. Levels of certain frequencies were then adjusted on the soundtrack to ensure that the acoustic envelope within the arenas closely matched the conditions recorded at sea.

Experimental design

A total of six groups, each consisting of four juvenile European seabass, were tested within a week in May 2025. As the experimental pool was only equipped with three arenas, all the groups were tested across two sessions. Experimental groups of fish were formed four days prior to be exposed to the soundtrack. Once established, all groups followed the same protocol and were immediately acclimatised to the experimental conditions, including the arena environment and water parameters such as salinity, temperature, and oxygen levels. Trials were conducted at a water temperature of 16±1°C, with dissolved oxygen levels maintained above 80% and salinity at 31±1 PSU. To standardise metabolic conditions, fish were fasted for 48 hours before the beginning of the exposure.

Each arena was monitored using a camera (ZowieCAM - 4K NDI POV, 5-50 mm Mount Zoom Lens, Zowietek Electronics, Ltd, China) filming continuously from above to record fish behavioural responses throughout the four-hour exposure. Experiments began with the activation of an autonomous recorder (OSEAN, sampling frequency: 400 kHz, 24-bit resolution; OSEAN technology, France), capturing sound via a hydrophone (Neptune D140, bandwidth: 7 Hz – 200 kHz, sensitivity: -207 dB re 1V/μPa, Neptune Sonar Ltd, UK) positioned between two arenas. Video recordings were then initiated from the control room adjacent to the pool via a Python interface specifically developed for this study (Tourte 2025). Finally, acoustic playback was also remotely broadcast from the control room through the Clark Synthesis loudspeaker using the AudioSud sound emission system.

Experimental reliability and perspectives

To ensure the realism of the playback under laboratory conditions, the methodology presented in this chapter combines acoustic calibration, environmental context, and species-specific biological considerations. The design of the playback first considered site-specific acoustic properties and the most frequently recorded sea and wind conditions at the Floatgen site. The acoustic constraints of the experimental enclosure, as well as the emission characteristics of the underwater loudspeaker, were also carefully addressed to optimise sound propagation within the pool. Furthermore, the playback signal was designed to simulate the gradual acoustic exposure experienced by a fish naturally approaching the operating wind turbine. By incorporating these multiple realistic dimensions into the protocol, this methodology aims to reduce the risk of artefactual behavioural responses and to support a more accurate interpretation of the results. While developed in the context of juvenile European seabass (*Dicentrarchus labrax*) exposed to operational wind turbine noise, this methodology can be adapted to other species and noise sources.

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