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

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Exploring effects of sound on the time budget of fishes: An experimental approach with captive cod

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Abstract

Energy intake and expenditure data are needed to estimate population level effects of anthropogenic sound on fish. We present an experimental design of a controlled behavioral experiment that allows to collect relatively long-term data (days) on Atlantic cod (*Gadus morhua*) during sound exposures. Data on the time spent foraging and swimming can be used as proxies for energy intake and expenditure. The wild-caught but captive Atlantic cod exhibited natural foraging behavior in the experimental basins and the design allowed for efficient scoring of the behavior throughout the 6-day trials. We conducted three pilot trials and share the experimental design to encourage other researchers to collect data on (proxies for) energy intake and expenditure to aid estimation of population level effects of sound exposures from noisy human activities such as seismic surveys.

Introduction

There is a growing interest in the effects of anthropogenic sound on marine life, including fish (Williams et al., 2015). While previous studies have found a range of effects on fish, one of the major challenges is to extrapolate results from controlled experiments to the population level (Carroll et al., 2016). For this purpose, population models that rely on Dynamic Energy Budgets (DEBs) can be used (Slabbekoorn et al., 2019). DEBs model the difference between acquired biomass and costs for maintenance, attributing the net energy to growth and reproduction (Leeuwen et al., 2013). To model the population level effects of anthropogenic sound, we need data on the effects of sound on energy intake and expenditure. Few studies have looked into the effects of sound on foraging behavior and most studies only examined short-term effects of a sound exposure, whereas long-term effects of repeated sound exposures are more relevant for models on population consequences.

Several tank studies, examining short-term effects (≤ 25 min) of sound exposure on foraging fishes, have found reduced feeding attempts and/or increased food handling errors (McLaughlin and Kunc, 2015; Purser and Radford, 2011; Shafiei Sabet et al., 2015a; Voellmy et al., 2014). Two studies, which did examine longer-term effects by exposing fish repeatedly (Magnhagen et al., 2017) and observing free-ranging fish (Bracciali et al., 2012), found reduced feeding events during sound exposure. However, these longer-term studies lack information on the energy expenditure during exposure. To our knowledge, only one sound impact study, on mulloways (*Argyrosomus japonicus*), examined both stomach content (food intake) and activity (a proxy for energy expenditure) of free-ranging fish. The authors found less stomach content on days with more boat traffic and activity levels were also lower on days with more boats (Payne et al., 2014).

Here, we present a design for a non-invasive, indoor mesocosm experiment which allows for high resolution quantification of both foraging (energy intake) and swimming behavior (expenditure) in a simulated shallow-water environment with acoustic disturbances at an ecologically realistic time-scale. Through personal observations, we noted that when provided with a sufficiently large basin and natural food items, Atlantic cod (*Gadus morhua*) exhibit discrete bouts of foraging behavior. This provided an opportunity to study the relatively long-term effects of seismic survey sound playback on foraging and swimming behavior of Atlantic cod in a closed system. Due to limited sample size and potential bias in the current study, we do not draw any conclusions from our results. Instead, our experimental design is presented as a promising approach to examine the effects of anthropogenic noise in a manner compatible with the parameterization of population level DEB models.

Materials and methods

Study subjects

We used three pairs of Atlantic cod of 30-50 cm body length in this study. The fish were caught from a charter fishing boat at shipwrecks in the Dutch North Sea on October 5th 2018. After catching, fish were kept in an indoor cylindrical holding tank (Ø 3.5 m, depth 1.1 m) at Stichting Zeeschelp (Kamperland, the Netherlands) for at least one week before being used in the experiment. The light-dark cycle followed the outdoor day-night cycle and the water in the holding tank was continuously filtered, cooled to 15 °C, and refreshed with seawater from the Oosterschelde, a sea inlet of the North Sea. The outlet of the cooling and filtering system caused a current against which the fish were swimming regularly. The bottom of the tanks was covered with sand and patches of live blue mussels (*Mytilus edulis*), Pacific oysters (*Magallana gigas*) and some rocks to provide shelter for the cod and small crabs. The cod were fed with dead European sprat (*Sprattus sprattus*) every other day and could feed ad-libitum on small live Asian shore crabs (*Hemigrapsus sanguineus* & *Hemigrapsus takanoi*) that were abundant in the tank. In the experimental tanks, we provided the cod with live shore crabs (*Carcinus maenas*) with a maximum carapace width of 1.5 cm. All live animals - other than the cod - were collected at the Jacobahaven, an adjacent cove of the Oosterschelde. Crustaceans, including crabs, form a dominant part of the natural diet of cod in the size class that we tested (Daan, 1973).

Experimental tanks

We used two experimental tanks that were identical to the holding tank. To film the behavior of the fish, five GoPro cameras were equally spaced along the tank wall, just below the water surface and pointed downwards at an angle of about 45°. The cameras were connected to a power grid to enable them to film for seven hours continuously. Several screens of white netting were attached along the tank wall to increase the visibility of the fish (Fig. 1a). The basins were covered with black plastic sheets and three TL light bars (36 W/840 lm) provided constant light conditions to ensure consistent video quality and to keep investigators out of sight from the fish (Fig. 1b). We used an amplifier (M033N, Kemo) and underwater speaker (UW30, Lubell), attached in the middle of the tank and middle of the water column, for the experimental sound exposures. We also placed three PVC pipes, vertically along the tank wall, to be able to release crabs into the experimental tank, while minimizing the chance they would be preyed upon already while on their way to the bottom of the basin.

Fig. 1: Pictures of a cylindrical experimental tank. (a) The tank before being filled with water, but with sand on the bottom and patches of mussels, oysters, and some rocks for shelter. Also visible are the frame holding the TL light bars and underwater speaker and the white netting to increase the visibility of the fish and the PVC pipes to release the crabs. (b) The same tank now filled with water and covered with black plastic sheet.

Experimental design

The evening before the start of a trial, a pair of individuals was introduced into an experimental tank. Paired individuals were selected to differ in body length, so they could be easily discriminated on video. Each trial consisted of six days; three consecutive days with two sound exposures of one hour each day, and three days without exposure. Trial 1 started with three days without exposure, followed by three with exposure. Trial 2 and 3 started with three days with sound exposures, followed by three days without. We made daily video recordings from approximately 10:00 to 17:00 CET. The two one-hour sound treatments started at 11:00 and 14:00. During the period without sound playback, we played back a silent track. Each day, just before the start of the video recordings, six small shore crabs were released in the tank as cod prey (Fig. 2). The crabs typically sought

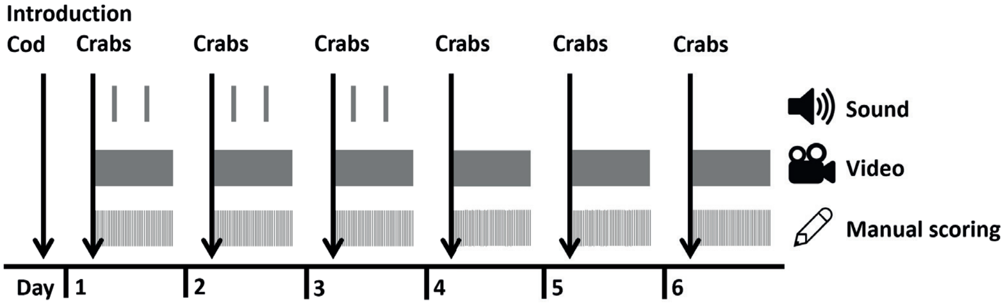

Fig. 2: Schematic of a single six-day trial: introduction events of the two cod and daily groups of six crabs are indicated by arrows, while the presence of grey bars reflect the time in which we played back sound (six traces on top) and in which we made video recordings (middle), which were later used for manual scoring of behavior (bottom).

refuge between or under the mussels and oysters. The cod were observed to scout the patches of mussels and oysters, lifting some shells and, when successful in finding a crab, gulping their target.

Experimental playbacks

We generated the exposure playbacks by randomly selecting airgun shots from recordings from a real seismic survey (Thompson et al., 2013). For each trial, we selected random shots that were recorded at either 10, 15 or 20 km from the survey. The recordings of the shots were band-passed between 150-1000 Hz and normalized with respect to peak amplitude. We determined the sound pressure levels (SPL) of the playback and ambient levels with an HTI-96-min hydrophone. The geometric mean zero-to-peak sound pressure level ($\text{SPL}_{z\text{-}p}$) of the shots was 150 dB re 1 μPa in the middle of the water column at 80 cm from the speaker. The geometric mean ambient SPL in the tank was 88 dB re 1 μPa (100 - 1000 Hz) (Fig. 3a).

To characterize spatial variation in the sound field across the basin during sound exposure, we recorded pure tones, that swept from 150 to 1000 Hz. We sampled at 20 cm radial distance steps ranging from 35 cm to 135 cm from the speaker (Fig. 3b). Up to approximately 600 Hz - the upper limit of the cod hearing (Chapman and Hawkins, 1973) - there is a monotonic decrease in power spectral density with increasing distance from the speaker, spanning a maximum of ~25 dB.

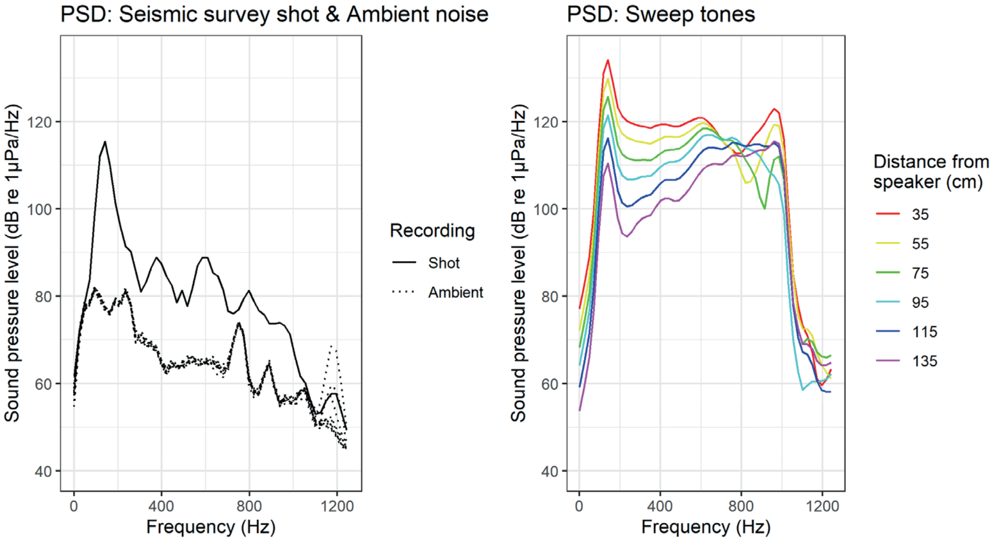

Fig. 3: (left) Power spectral density plot of a seismic airgun shot and ambient recordings in the basin. (right) Power spectral density plot of recordings from a sweep (150 to 1000 Hz) at various distances from the speaker (window length: 2048, window type: Hann).

Data scoring and analysis

We sampled the behavior of both individuals in each pair by scoring the most dominant behavior observed during 20 s video clips. We extracted video clips for observation at 5 min incremental steps throughout the daily seven-hour recording period. The behavioral states were: 'foraging', 'swimming' and 'stationary'. 'Foraging' indicated that an individual was swimming near the bottom, while turning frequently and occasionally lifting rocks or bivalves with its nose. 'Swimming' indicated swimming and turning infrequently, typically around the perimeter of the basin and in the mid or upper part of the water column. 'Stationary' indicated that the fish was not moving while being on the bottom of the tank. A custom-made Python script (utilizing FFmpeg) was used to extract and concatenate synchronized video clips from all cameras into one video (Fig. 4) to facilitate efficient scoring.

To test whether time budgets of cod behavioral states were different on days with two one-hour sound exposures, we determined daily proportions of video clips in which cod were in the foraging, swimming or stationary state. These proportions were fit to Generalized Linear Mixed-effect Models (GLMMs; R-package lme4 by Bates et al., 2015) with a binomial error distribution, 'day number' and 'treatment' as covariates, and 'fish ID' as random effect. We calculated the marginal (R2m) and conditional (R2c) R2 values of the models to show the proportion of variance of the response variable explained by the fixed effects (R2m) and the entire model (R2c) (Nakagawa and Schielzeth, 2013). For each model, a parametric bootstrap procedure (10,000 resamples) was performed, where the random effect intercepts from the fitted model were

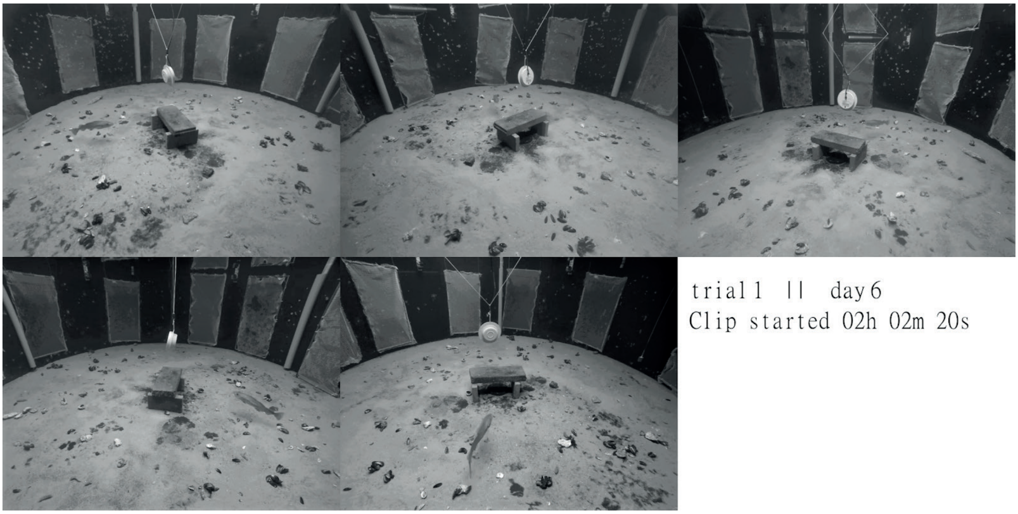

Fig. 4: A screenshot from a 20 s video clip with synchronized footage of all five cameras. The dominant behavior was scored for both individuals by an observer.

not resampled and where we used the fitted estimates instead. The bootstrap was used to determine the 95% confidence interval of the 'treatment' covariate estimate. If this confidence interval did not overlap with 0, we considered the sound exposure to have significantly changed the time expenditure in this behavioral state.

Results

Based on the behavioral scores from the video clips, we determined the daily proportion of time during which an individual was foraging, swimming, or remaining stationary (Fig. 5). Based on daily proportions of time spent in each behavioral state of all six individuals, we found that the subjects did not change the amount of time foraging (Intercept: -3.21, Sound Treatment 95% CI: -0.03 – 0.36, R2m: 0.00, R2c: 0.44). However, the subjects did increase the time swimming (Intercept: -1.07, Sound Treatment 95% CI: 0.06 – 0.40, R2m: 0.01, R2c: 0.33) and decreased the time being stationary (Intercept: 1.37, Sound Treatment 95% CI: -1.64 – -0.92, R2m: 0.07, R2c: 0.66).

Discussion

The current experimental design allowed us to expose Atlantic cod to repeated playback of seismic survey recordings over multiple days in a simulated shallow-

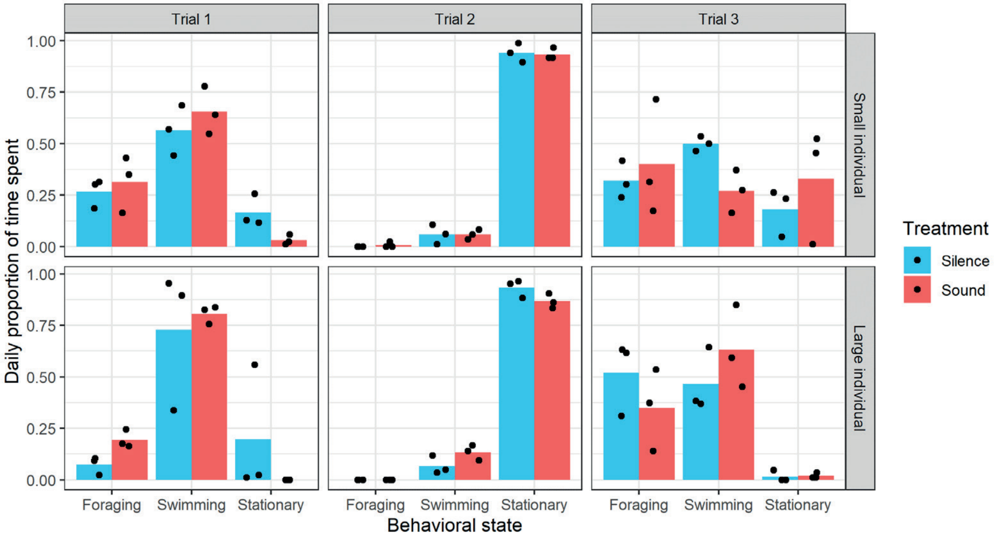

Fig. 5: Distribution of behavioral states as daily proportion of time spent Foraging, Swimming, and remaining Stationary. The black points indicate the proportions of individual days and the bars indicate the mean proportion over three sequential days during Silence playback or Sound exposure. The three facet columns depict the three trials, and the facet rows show the data for the smaller (above) and larger individual (below) of this trial.

water habitat. By scoring short video clips throughout six days, we were able to quantify time expenditure in the discrete behavioral states: foraging, swimming, and remaining stationary during days with sound exposures and days with silent controls. The current experiment was ceased after three trials due to concerns about the health of the study subjects, as their livers were discolored. This discoloration did likely not emerge during their short time in captivity as the discoloration was also reported in other non-experimental individuals that were caught and killed on the same day. Nevertheless, the fish in trial 1 and 3 regularly scouted the patches of mussels, oysters, and rocks; they sometimes lifted or pushed the patches with their nose; aiming to catch crabs that were hiding. Paired individuals were also observed to synchronize their foraging behavior. When one individual initiated foraging behavior, the other often immediately joined and they examined the same crab refuge patches together. These foraging patterns, including the social context, are in line with reports on free-ranging Atlantic cod (Rose, 2019).

The current study subjects, in the conditions of our experimental set-up, did increase the time swimming and decreased the time remaining stationary on days with sound exposures, but did not change the time spent foraging. Due to the limited sample size and the possibly biased sample because of the discolored livers, we do not make inferences to a healthy wild population from our results. However, we argue that this type of data, with adequate sample size and certainty about individual health status, can serve as proxies for energy intake and expenditure and could thereby be useful to parametrize DEB models (Slabbekoorn et al., 2019; Soudijn et al., 2020). In contrast to earlier studies (e.g. McLaughlin and Kunc, 2015; Shafiei Sabet et al., 2015b), we here examined both foraging and swimming activity at a relatively large time scale of days, which may be more relevant than minutes or an hour, as input to energy budget models that aim to investigate population effects (Soudijn et al., 2020).

It remains important to stress that fish behavior in captivity, and sound propagation in tanks, is different from outdoor conditions (Calisi and Bentley, 2009; Rogers et al., 2016). So, absolute response levels of fish to sound from captive and/or indoor experiments likely do not represent absolute response levels in the field (and also in the field, response levels will vary with life phase, season, experience, and weather, and with local water depth and propagation conditions). Ultimately, all experiments on the effects of sound are more realistic when conducted in situ, however, outdoor experiments on free-ranging animals are challenging and some research questions are currently impossible to address due to technical limitations (e.g. high-resolution telemetry to assess data on behavioral states at a resolution comparable to video observations). For this reason, optimal progress in studies on response levels is likely achieved by complementary indoor and in situ observations and experiments.

Sound propagation in the experimental tanks is expected to differ from propagation in outdoor settings, largely due to the close proximity of tank walls, water surface and tank floor. Especially particle motion levels and directions are expected to differ from natural habitat (Rogers et al., 2016). In the current experimental tanks, we found a monotonic decrease in sound pressure levels with increasing distance from the speaker that was relatively consistent across frequencies up to 600 Hz. At ~750 Hz and above, complex acoustic artefacts were observed: The PSD was no longer monotonically decreasing with distance from the speaker at certain frequencies, indicative of a standing wave marking the shallow-water cut-off frequency (Akamatsu et al., 2002). In the current study particle motion measurements were unavailable. However, with detailed pressure measurements, it is feasible to make rough estimates of particle velocity in a geometrically simple basin setup or estimate particle motion by measuring pressure gradients with hydrophone pairs (Gray et al., 2016).

For the current dataset, we scored the behavioral state in short video segments, equally spaced throughout the daily seven-hour video recordings. This proved to be a quick and efficient method. For more detailed behavior measurements, the current camera set-up could facilitate the processing of videos with 3d tracking. Automated tracking would provide high resolution data on swimming behavior (energy expenditure) without requiring subsampling of videos. Additionally, swim tracks are suitable input for Hidden Markov Models (HMMs) for animal movement, allowing for automated quantification of behavioral states. While HMMs have been used before to study the behavior of large mammals over time and space at large scales by statistical ecologists (Langrock et al., 2012), recent advancements in the accessibility of these models to non-statisticians facilitate their use in broader applications (McClintock and Michelot, 2018). HMMs expect multivariate and temporally auto-correlated input and provide direct estimates of an animals' latent behavioral state, making them suitable for the analysis of swimming tracks. In addition, a fitted HMM can act as a foundation for simulating animal responses in the presence/absence of acoustic stressors, allowing one to extrapolate a fitted HMM to population level consequences (DeRuiter et al., 2017).

Conclusions

We here reported on an experimental design, that allowed us to collect data on foraging and swimming behavior of a demersal fish species during scaled seismic survey sound exposures and a silent control in captive conditions. The current design allowed for efficient scoring of the behavioral states throughout the daily seven-hour video recordings. We found a shift in time spent swimming and remaining stationary, but not in the time spent foraging. However, the sample size was limited and possibly biased, so more data is required to confirm these results. These behavioral states from wild-caught but captive individuals can serve as proxies for energy intake and expenditure, and have high potential as complementary data to field studies on the same species. The proxies for energy intake (time spent foraging) and expenditure (time spent swimming and stationary) can be further specified by investigating catch success rates and prey energy content and swimming speed correlates to oxygen consumption from swim tunnel experiments. We believe these are feasible steps towards parametrizing Dynamic Energy Budget models which will allow estimates and predictions on population levels effects of acoustic disturbances such as during seismic surveys.

Ethical statement

This experiment was conducted in accordance with the Dutch Experiments on Animals Act and approved by the Dutch Central Commission Animal Experiments (CCD) under no. AVD1060020171085.

Data accessibility

All data used for the analyses reported in this article and some example 20 s video clips are available from the Zenodo Repository, DOI:10.5281/zenodo.3595208.

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