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Short-term habituation of the longfin squid (*Doryteuthis pealeii*) to pile driving sound

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Offshore windfarms are a key renewable solution to help supply global energy needs. However, implementation has its challenges, including intense pile driving sound produced during constructions, which can affect marine life at the individual level, yet impacts at the group level remain poorly studied. Here, we exposed groups of longfin squid (*Doryteuthis pealeii*) in cages at multiple distances from consecutive pile driving events and sought to quantify responses at both individual and group levels. Pile driving induced short-term alarm responses at sound levels (in zero-peak) of 112–123 dB re 1 μm s^{-2} that were similar to those measured at kilometre scale from offshore windfarm constructions. The rate of individual alarm responses quickly decreased both within and across consecutive pile driving events, a result consistent with previous laboratory studies. Despite observing dramatic behavioural changes in response to initial pile driving sound, there were no significant differences in squid shoaling areas before and during exposure, showing no disruption of squid collective behaviours. Our results demonstrate rapid habituation of squid to pile driving sound, showing minimal effects on this ecologically and commercially key taxon. However, future work is now needed to assess responses of wild squid shoals in the vicinity of offshore windfarm constructions.

Keywords: collective behaviour, global change, machine learning, marine invertebrate, sound pollution.

Introduction

Anthropogenic sound is increasingly considered a major underwater pollutant of international concern that can affect sound-sensitive animals (Duarte *et al.*, 2021). Pile driving (PD) is associated with offshore windfarm (OSW) construction and generates repeated, high intensity impulsive sound that can propagate over tens of kilometres underwater, underscoring concerns for wide-ranging impacts (Bailey *et al.*, 2014; Dahl *et al.*, 2015; Mooney *et al.*, 2020). Several studies have described the various impacts of PD sound on marine mammals and fish, ranging from temporary changes in behaviour to mortality (Madsen *et al.*, 2006; Slabbekoorn *et al.*, 2010). However, little is known about the impact of PD activity on marine invertebrates despite their oft-central role in ecosystems and fisheries (Williams *et al.*, 2015; Popper *et al.*, 2022).

Cephalopods are sensitive to low-frequency sounds in the same bandwidth as PD sound (Packard *et al.*, 1990; Mooney *et al.*, 2010, 2020). Previous studies in tanks showed both artificial and PD sounds elicited short-term alarm responses in squid and cuttlefish, but these responses attenuated over time, suggesting a potential habituation to sound exposure (Samson *et al.*, 2014; Mooney *et al.*, 2016; Jones *et al.*, 2020). However, for many taxa, including marine invertebrates such as cephalopods, the translation of these laboratory results into the field and actual PD is not straightforward, especially when considering the different sound intensities as well as the spatial and temporal scales of potential impacts by anthropogenic activities (Popper *et al.*, 2022). An initial field-based study showed dose-dependent responses in individual squid (*Sepioteuthis australis*) to airgun sounds from seismic surveys, suggesting the severity of sound impact was related to the sound level and (often corresponding) distance from the sound source (Fewtrell and McCauley, 2012). Another field-based study used biologging tags and showed PD events disrupted individual squid fine-scale movements, but these impacts were transient, suggesting minimal energetic impacts over the entire exposure period (Cones *et al.*, 2022). However, all studies mentioned above have only examined individual-level responses. PD sound impacts on squid shoals are not yet known, a knowledge gap that is particularly striking because they live in groups (Hanlon and Messenger, 2018).

Intraspecific aggregations and collective movements are a widely conserved phenomenon across many distinct evolutionary trajectories (Allee, 1927). In many marine species, shoaling can decrease predation risk (Ioannou *et al.*, 2008), enable more efficient navigation through collective learning (Berdahl *et al.*, 2018), and decrease metabolic demands by leveraging beneficial flows from conspecifics (Marras *et al.*, 2015; Burford *et al.*, 2019). One laboratory study showed that PD sound disrupt the structure and dynamics of fish shoals (Herbert-Read *et al.*, 2017). In the field, free-ranging sprat and mackerel shoals exposed to PD sound increased dispersion (i.e. greater shoal area) and shoals moved to deeper water (Hawkins *et al.*, 2014). To date, there are no data of PD sound effects on shoal-level behaviours in marine invertebrates, leaving questions on how OSW development could impact the ecology of commercially important squid.

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Recent calls for future OSW constructions have been planned in nine US states (Musial *et al.*, 2019), encompassing the distribution area of many marine invertebrates, such as the longfin squid (*Doryteuthis pealeii*; Hanlon *et al.*, 2013). The occurrence rate and spatial range of PD exposure events are expected to affect this taxon and its vital habitat. Considering the economic importance of squid, which have contributed mean annual landings and value of 13000 mt and $26 million since 2000 (NMFS, 2019), conflicts between fishermen, policymakers, and the offshore wind industry are expected to increase dramatically (Lacroix and Pioch, 2011). New studies are now needed to develop effective management strategies and to design suitable mitigation methods (Popper *et al.*, 2022).

In this field-based study, we assessed real-time behavioural responses of squid (*D. pealeii*) shoals exposed to real PD. Squid were placed in enclosures installed at different received sound levels and distances from the PD activity and subsequent responses were recorded using underwater cameras. We first characterized the effects of PD sound on individual behaviours and sought to assess potential habituation rates to repeated PD events. Next, we used video data to measure squid group cohesiveness by calculating the collective area of the shoals both prior to and while exposed to PD sound. Because different construction techniques are used in OSW constructions, we also examined the influences of the two main types of piling installation tools with different sound characteristics: the "impulsive" impact hammer, and "continuous" vibratory hammer (termed IH and VH, respectively). While IH is the most prevalent method, some OSWs have installed pile structures VH (OSPAR, 2014). Given that there is some interest in expanding this technique, we sought to compare sound effects from these multiple construction techniques in squid.

Material and methods

Animal collection and holding conditions

A total of 189 adult squid (dorsal mantle length = 17.9 ± 3.3 cm, mean ± standard deviation) were used for this study. Squid were collected from Vineyard Sound (41.22 N, 70.47 W) via trawling by the R/V Gemma of the Marine Biological Laboratory (Woods Hole, MA, USA). Squid were held in groups of four to seven individuals in cylindrical tanks (1.2 m diameter) constantly supplied with ambient seawater (temperature range: 21.1 to 22.5°C). Tanks rested on rubber gaskets and concrete blocks, both of which served to further isolate the squid from surrounding vibrations during their respective holding periods. The top of each tank was covered with plastic sheeting to create shaded zones. Squid were fed daily with mummichogs (*Fundulus heterclitus*) and grass shrimp (*Palaemonetes* spp.). Given that squid can be relatively fragile and healthy animals are vital to behavioural experiments, individuals were held <72 h before being used for experiments, and all animals incorporated had no visible skin damage and exhibited normal swimming behaviour (e.g. Jones *et al.*, 2023). All procedures regarding the use of animals in research followed local guidelines and were approved by the Woods Hole Oceanographic Institution's Institutional Animal Care and Use Committee (approval to TAM).

Location and PD characteristics

Ten days of PD experiments were conducted between the 14th and 29th of September 2021. Procedures took place at nearshore experimental testbed area off the Woods Hole Oceanographic Institution pier (41.52°N, 70.67°W; Figure 1a and b). It is a shallow water habitat with depth varying between 3 and 5 m depending on tide, the bottom is flat and consists of homogeneous sand and silt. PD incorporated a single 10 m long, 0.3 m diameter cylindrical steel monopile (wall thickness: 0.02 m). At the start of an experimental day, a crane (American 595) with a 20-m long boom moved a VH (weight: 212 kg, H&M model 135) into place to first secure the pile into the seabed. This VH, similar to a jack-hammer, would hit the pile at a rapid rate of 1150 blows per min. Once the piling was in position, squid were introduced into their respective cages (see details below). The VH was then removed and replaced with a steel IH (weight 1500 kg), the head of which was manually dropped onto the top of the pile from a height of 1.2 m at a rate of 10 strikes per min (~16 kJ per strike) for a duration of 15 min (constituting the IH exposure). After 15 min of IH, the piling was typically driven 5 m below the water sediment surface. The VH was then used to pull the pile out and reposition it in an adjacent location for another round of IH. This procedure started at 1330 each day to control for any potential circadian rhythm effects and was repeated five times within an afternoon. Hence, in total, each squid shoal was exposed to five IH and four VH sequences, always starting with the IH exposure. Control days were essentially mimics of this procedure (i.e. adding squid to cages shortly before 1330).

Experimental design

Prior to the first IH sound exposure, squid were quickly transferred from holding tanks to submerged 3.4 m^3 cubic cages (1.5 × 1.5 × 1.5 m) built with polyvinyl chloride (PVC) pipes and covered with 1.5-cm knotless polyester mesh netting (Figure 1c). Note that the shortest dimension of the cage (1.5 m) was always >5 body lengths even for the largest squid used, which allowed animals to naturally exhibit shoal behaviours throughout the experiments (see Supplementary materials). A door on the top of the cages permitted the squid transfer into the undersea cages; once all squid were introduced, the cage was lowered to 0.5 m from the seabed. Two cages were placed within 2–8 m and one cage was positioned at 50 m from the pile (Figure 1b). Squid were allowed 15 min of acclimatization in the cages to recover from handling before the first IH sound exposure started.

Within the cages, we sought to quantify squid shoaling behaviour, which was defined as three or more individuals swimming within one body length from each other (Oshima *et al.*, 2016). Each cage contained four to seven haphazardly chosen individual squid of mixed sexes. Hence, each day, two new squid shoals were studied at the near site (i.e. two replicants), while one new squid shoal was studied at the far site (Figure 1b). It is notable that this group size was certainly lower than that of many wild squid aggregations (often easily upwards of 100 individuals; see Shashar and Hanlon, 2013). Yet, this quantity provided a reasonable number of animals to coherently track and quantify shoaling behaviour (see below).

Squid responses to PD sound were recorded using GoPro Hero 7 Black cameras. In each cage, one camera was positioned horizontally near the bottom against the net, while the second was mounted in the top corner and angled towards

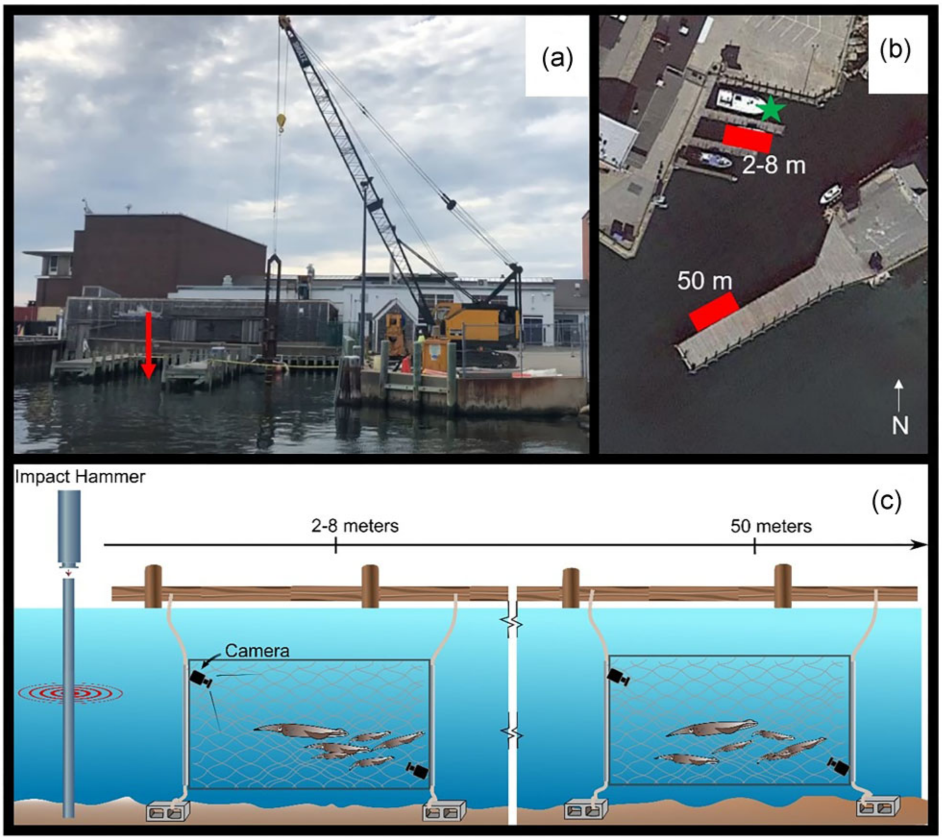

Figure 1. Experimental set-up used to investigate the behavioural responses of squid to repeated pile driving sound exposure. (a) Picture of the crane driving a pile into the seabed off a dock with the impact hammer. Red arrow shows the location of the cages placed within 2–8 m from the pile. (b) Map of the two near (2–8 m) and far (50 m) sites. The green star denotes the pile driving location, while the red regions are the positions of the squid cages. (c) Shoals of four to seven squid were placed in large cages and behavioural responses to repeated pile driving sound were monitored using two different cameras. Controls were performed using the same experimental design but without pile driving sound.

the cage centre (Figure 1c). The bottom camera was used to monitor individual squid responses, while the top camera was used for shoaling behaviour (see details in the section “Data analyses”).

Control experiments ($n = 7$ squid shoals) were conducted at both sites using the same experimental procedures, but without PD noise exposure. To compare metrics between the two experiment types, sound exposure time periods from experiment days were randomly assigned to control experiments.

Individual squid were used only once and released back into the marine environment at the end of each experiment.

Sound exposure

Squid detect low frequency (<1 kHz) underwater acoustic particle motion (Mooney *et al.*, 2010). Correspondingly, the sound field was measured and quantified in presence and absence of PD using a calibrated PCB triaxial accelerometer (model W356B11), and details of the particle acceleration levels (PALs) generated by the PD were presented in Cones *et al.* (2022). Briefly, the cages placed at the near site were exposed to “high” PALs (in 0-peak; $\mathrm{PAL_{0p}}$) from the impulsive IH that ranged between 112 and 123 dB re 1 μm s^{-2} at 8 and 1 m, respectively, while the cage at 50 m had lower $\mathrm{PAL_{0p}}$ (83 dB re 1 μm s^{-2}). The VH generated PALs (in rms; $\mathrm{PAL_{rms}}$) between 83 and 95 dB re 1 μm s^{-2} within 8 m, while $\mathrm{PAL_{rms}}$ decreased at 75 dB re 1 μm s^{-2} at 50 m. The PALs recorded at the near site were roughly equivalent to those measured at 1 km from OSW constructions (Sigray *et al.*, 2022). An example of underwater particle acceleration recordings from an entire PD experiment (including five IH and four VH sequences) is shown in Figure 2. Note that given the *in situ* and realistic nature of this actual PD, pulse amplitudes and inter-pulse time intervals did vary slightly within and across IH sequences as the pile was driven into the sediment (Figure 2). The variability in these metrics across impulses reflected that which a wild squid may experience near an offshore PD site (Amaral *et al.*, 2020). The near site was always characterized by higher PALs compared to the far site (∼30 dB difference). Here, we were interested in studying behavioural effects of PD sound at two distances from the pile, rather than studying responses dependent on these specific metrics.

The PALs of ambient sound in the holding tanks and underwater in absence of PD sound were below the self-noise floor of the accelerometer, which was evidenced by flat power spectral densities of these recordings at 30 dB re 1 (μm s^{-2})^2 Hz^{-1} (Figure 2; Cones *et al.*, 2022). These relatively quiet environments enabled us to isolate and assess potential effects of PD sound on squid while minimizing extraneous stimuli that are typically found in natural field environments.

Data analyses

Individual behaviour

Behavioural responses of individual squid were assessed using bottom-mounted camera videos. Manual annotations started

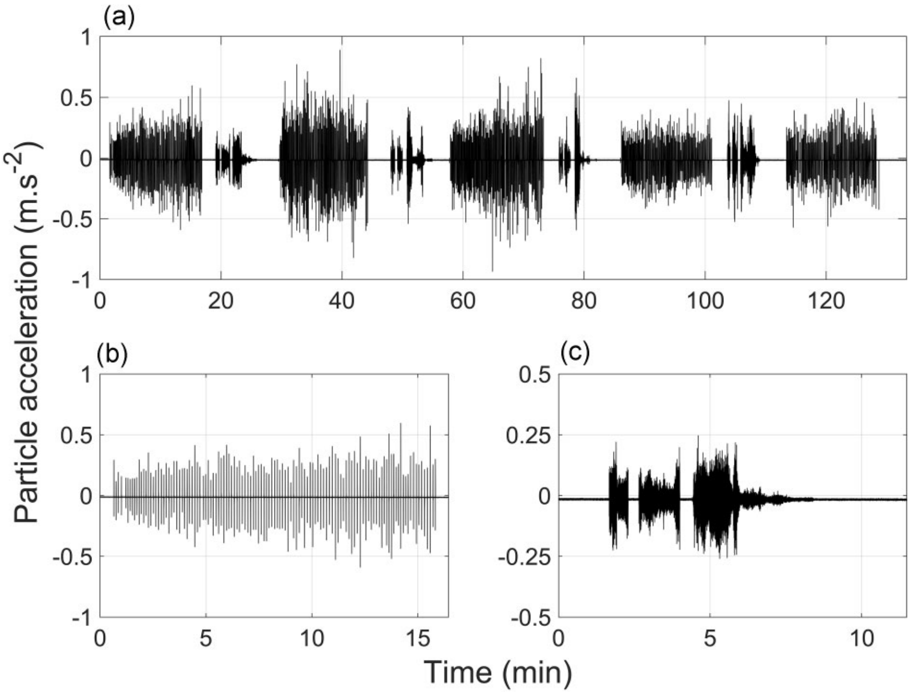

Figure 2. Example of underwater particle acceleration (*x*-axis) from a field-based *in situ* pile driving experiment recorded at 8 m from the pile (a). Sound exposures consisted of five 15-min long IH sequences (transient pulses, b) that were separated by VH sequences (continuous vibrations, c). There were 5 min "silent" sequences between each IH and VH sequence due to the crane switching hammers. Each experiment started daily at 1330 when squid shoals were first exposed to the IH pile driving.

5 min after squid were transferred inside the cages. All visible squid were observed and behaviours were annotated using tools in BORIS (v7.12.2; Friard and Gamba, 2016). Videos were first viewed by a trained observer at half-speed without sound (i.e. blind to the sound treatment) to quantify occurrences of four alarm responses (inking, jetting, startle, and body pattern change) following descriptions from previous studies (Hanlon *et al.*, 1999; Mooney *et al.*, 2016; Jones *et al.*, 2020). Because the GoPro cameras also recorded sounds from PD at both sites, a second annotation was then performed by listening to the audio, which permitted us to synchronize particular behaviours to either IH or VH sequences. Annotations were then compared with another independent observer (see Cones *et al.*, 2022) who manually annotated one third of the video footages, and comparisons showed 100% agreement. Body pattern changes were defined as alterations in chromatic components of at least half of the squid bodies (Hanlon *et al.*, 1999). Squid raising their arms as postural component was termed startle. Jetting was characterized by a rapid jet-propelled escape, which was sometimes followed by inking. Alarm responses related to agonistic encounters were not taken into account in this study. Because squid were not tagged with a specific mark, it was not possible to assess behavioural responses by specific squid throughout an entire experiment. However, most squid were present in the video recordings majority of the time. Hence, we reported the proportion of individual squid responding to PD sound per number of squid visible on the video. Using the sound recordings on the camera to synchronize both video and acoustic data, we were able to associate observed responses to each strike from the IH events.

Shoaling behaviour

We used top camera videos to assess the potential impacts of PD sound exposure on the cohesion of squid, quantified as the variance in the area covered by the shoal using a trained neural network. Analyses were conducted for the near site only (within 8 m from the pile) because there were no behavioural reactions of squid found at the far site (50 m form the pile). To compute the shoal area, we first tracked each squid individually in the video by anchoring and tracking three virtual point markers along the length of their body (arms, centre, and mantle tip). Then, we computed the convex hull of the set of all points from all squid visible in a frame to find the smallest bounding convex polygon, and then computed its 2D area in pixel space. To train the squid tracker, we used 260 randomly selected frames and annotated them by marking the arms, centre, and mantle tip of each squid. Then, we used the DeepLabCut (Lauer *et al.*, 2022) algorithm to train the squid tracker (Figure 3), and then apply it to rest of the data to track the squid in all frames of all the videos. We analysed 6 out of 18 squid shoals when the water turbidity was low enough for all squid within a shoal to be detectable by our algorithm throughout the entire video recordings.

False positives and negatives in the squid detections can result in extremely noisy estimates of the shoal area. Instead of using the standard outlier elimination approach based on running averages, we found the Hampel Filter to be far more robust to the noisy detection (Davies and Gather, 1993). Any value that was considered as an outlier by the filter was replaced by the median of the filter's running window. We set the window size to be the same as the number of frames per second for that particular video.

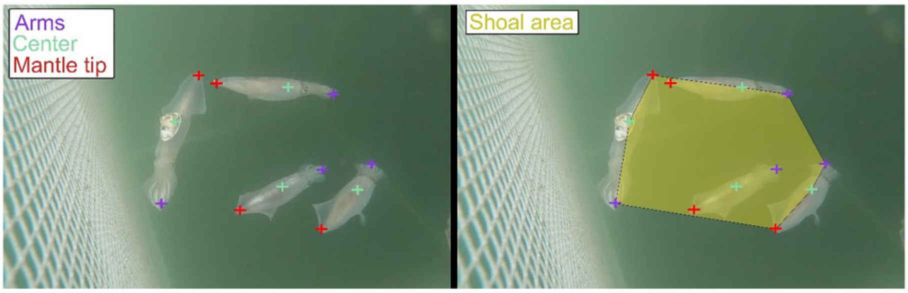

Figure 3. Example of a manually annotated frame (a) and associated polygon (b) created by the machine learning model using DeepLabCut software (Lauer *et al.*, 2022) to estimate the area of the squid shoal during pile driving sound exposure.

Statistical analyses

Statistical analyses were performed using R v4.1.3 (http://www.R-project.org/). Behaviours from individual squid ($n = 103$) were measured as the proportion of visible squid that responded to PD exposure at the near site. No statistics were conducted for the far site samples since squid did not respond to either IH or VH sequences at 50 m (see the section "Results"). We focused our analyses on IH events because most alarm responses occurred during IH compared to VH events (see the section "Results"). We also focused on the first 30 strikes of each IH event to compare our results with previous laboratory and bioenergetic studies (Samson *et al.*, 2014; Mooney *et al.*, 2016; Jones *et al.*, 2020; Cones *et al.*, 2022). Generalized linear models (GLMs) were used for regression analysis to describe the occurrence of each behaviour over the first 30 strikes. The strike numbers and IH sequences were used as explanatory variables, and ordinary Poisson (log-link) GLMs were performed. Since two different squid shoals were exposed daily at the near site, making them non-independent samples, we included "day" as a nested factor in the model.

Finally, we used one-way repeated measures analyses of variance (ANOVAs) to determine the effects of PD treatment on the squid shoaling area at 8 m, as no alarm responses were found at 50 m (see the section "Results").

Results

Individual behaviour

A total of 30 different squid shoals (near site: $n = 18$; far site: $n = 12$) were experimented upon during 10 days of PD sound exposure. Another seven shoals were monitored as controls without PD. Individual alarm responses only occurred at the near site. No alarm responses besides natural conspecific interactions were found at the far site (50 m form the pile) during PD exposure and during controls.

At the near site, a total of 305 alarm responses were detected from individual squid in response to PD sound (Figure 4, Table 1). Among these responses, 89% occurred during the IH exposure, while only 11% occurred during VH exposure. While all types of alarm responses were exhibited by squid exposed to the IH (i.e. inking, jetting, colour change, and startle), only colour change patterns and startle responses occurred during VH exposure (Table 1). During IH exposure, the most common alarm response was jetting (65%) and startling (21%); inking only occurred four times and was associated with jetting (Table 1).

During IH sequences at the near site, 80% of alarm responses were seen during the first two IH sequences of the day and squid showed significantly more responses to the first IH sequence compared to subsequent IH sequences (LMM: $X^2 = 764$, df $= 2095$, $p < 0.001$; Figure 5). In addition, 84% of alarm responses were seen within the first five strikes, which corresponded to the first 30 s of each IH sequence (Figure 5). Hammer strike number was a significant predictor of the alarm response rate over the first 30 strikes for all IH sequences (Poisson GLM, $p < 0.001$; Figure 5). There were also individual variations in alarm responses among squid shoals, with <40% of individual squid within a shoal reacting to PD sound (Figures 4 and 5). Finally, for two squid shoals, no alarm responses were exhibited by any squid over the entire PD sound exposure experiment at the near site.

Shoaling behaviour

We studied the area change of squid shoals during PD sound exposure at the near site and during the first strikes of each PD sequences (i.e. where most individual alarm responses occurred). Our computer vision-based model successfully computed the areas of six different squid shoals. While alarm responses occurred in response to IH strikes, squid quickly (within few seconds) returned to the shoal (Figure 4). This was further confirmed by comparing shoal areas at different time scales prior to sound exposure, after the first IH strike (5 s) and after 10 IH strikes (60 s). Indeed, despite these short behavioural disruptions, the squid shoal areas were not significantly affected by PD sound after the first IH strike (one-way ANOVA, $p = 0.299$) nor after the last 10 IH strikes of a sequence (one-way ANOVA, $p = 0.345$; Figure 6).

Discussion

This field-based study is the first to quantify both shoaling behaviour and individual alarm responses within squid shoal exposed to actual PD. Behavioural changes were transient and occurred mostly at the onset (<1 min) of the PD sound exposure, and response rates decreased after consecutive PD sequences. Despite these short-term responses, PD sound did not disrupt overall squid shoaling behaviour. Our results suggest potential quick habituation of wild squid to PD sound during OSW constructions.

The alarm responses observed during PD sound exposure were typical squid behaviours to perceived predatory threats (Figure 4; Hanlon and Messenger, 2018). These results are consistent with previous tank studies on the same species as well as cuttlefish in response to both artificial and PD sounds (Samson *et al.*, 2014; Mooney *et al.*, 2016; Jones *et al.*, 2020). This striking similarity highlights the valuable asset of sound

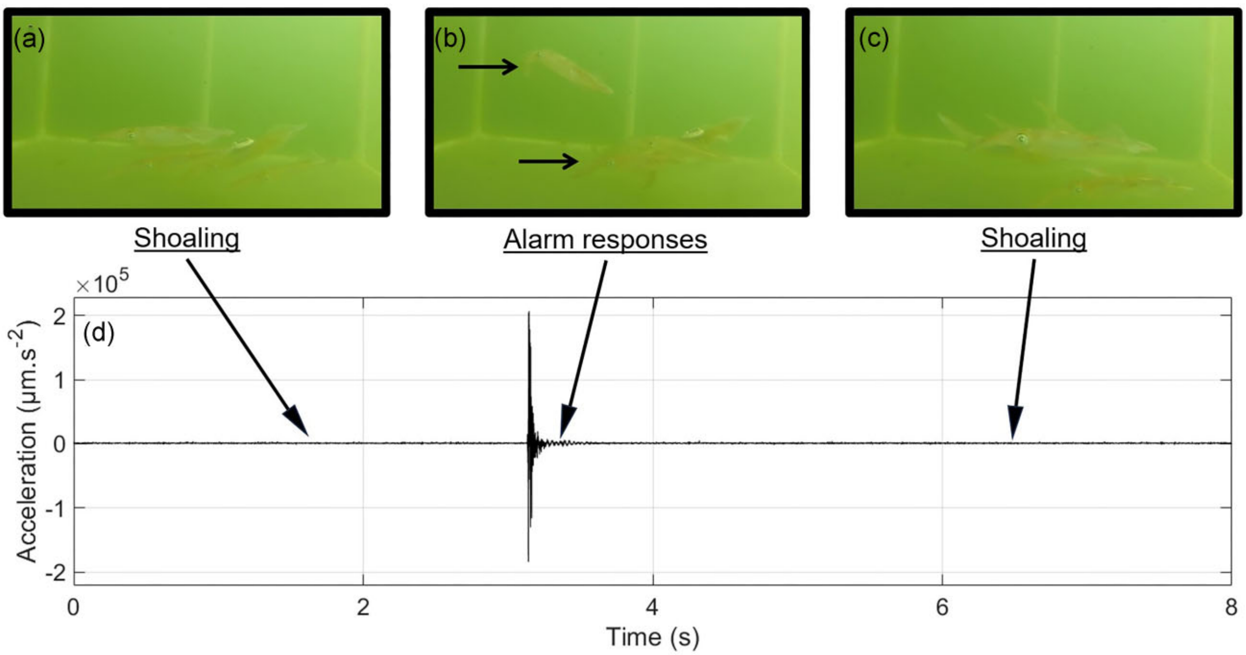

Figure 4. Examples of squid alarm responses observed before (a), during (b), and after (c) exposure to the first strike of an IH sequence (d). Arrows indicate the time when the captions occurred during sound recordings in (d). Amongst the five squid present in the shoal, two individuals reacted by jetting and startling in response to the strike (b, horizontal black arrows), yet quickly returned to the shoaling behaviour (c).

Table 1. Summary of individual alarm responses annotated during pile driving sound exposure at the near (*n* = 103) and far (*n* = 56) sites and during control (*n* = 30). Note that squid exhibited alarm response only at the near site.

	Near site		Far site		Control (no sound)	
Behaviour	IH	VH	IH	VH	IH	VH
Startle	57	20	0	0	0	0
Body pattern change	34	14	0	0	0	0
Jet	176	0	0	0	0	0
Ink	4	0	0	0	0	0

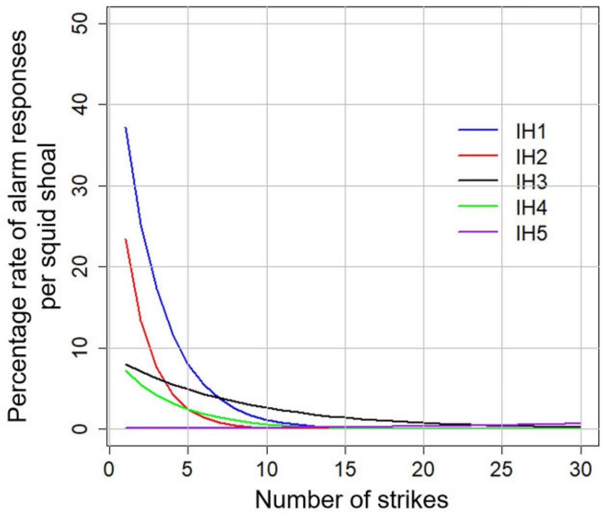

Figure 5. Proportion of alarm responses (percentage) across daily consecutive IH sequences (from one to five) for squid located at the near site within 8 m from the pile driving (*n* = 103). IH1 to IH5 represent the five sequences within a single day of pile driving exposure. Lines represent Poisson GLMs performed on the first 30 strikes of each IH sequence.

exposure experiments in tanks when the sound field is accurately calibrated (Popper and Hawkins, 2018). Using the same field set-up, our previous study used movement sensors to demonstrate that while alarm responses were mostly high acceleration jetting responses, these behavioural changes were transient and persisted for <15 s (Cones *et al.*, 2022). Additionally, there was no evidence of activity changes on longer time scales, suggesting minimal effects on squid energetics (Cones *et al.*, 2022). However, our results are different from another study where PD exposure repeatedly affected scallop behaviours (Jézéquel *et al.*, 2022), showing the importance of studying anthropogenic sound impacts across different taxa.

The logarithmic decrease in alarm responses within and across PD events is similar to previous tank studies that reported rapid habituation of squid and cuttlefish to sound (Figure 5; Samson *et al.*, 2014; Mooney *et al.*, 2016; Jones *et al.*, 2020). This reduction in alarm responses over several sound impulses indicates increased tolerance over time to PD sound, and suggests these squid may have behaviourally habituated. Interestingly, habituation to repeated sound exposures has also been noted in some fish (e.g. Nedelec *et al.*, 2016; Neo *et al.*, 2018; Currie *et al.*, 2020). However, using the same experimental set-up as in this study, Jézéquel *et al.* (2022) found that scallops did not habituate to repeated PD exposure, suggesting inter-specific differences for marine invertebrates in response to PD activity. Behavioural habituation can be defined as a learned, persistent reduction of an individual's response to a stimulus repeated over time, as individuals learn the stimulus has neither adverse nor beneficial consequences

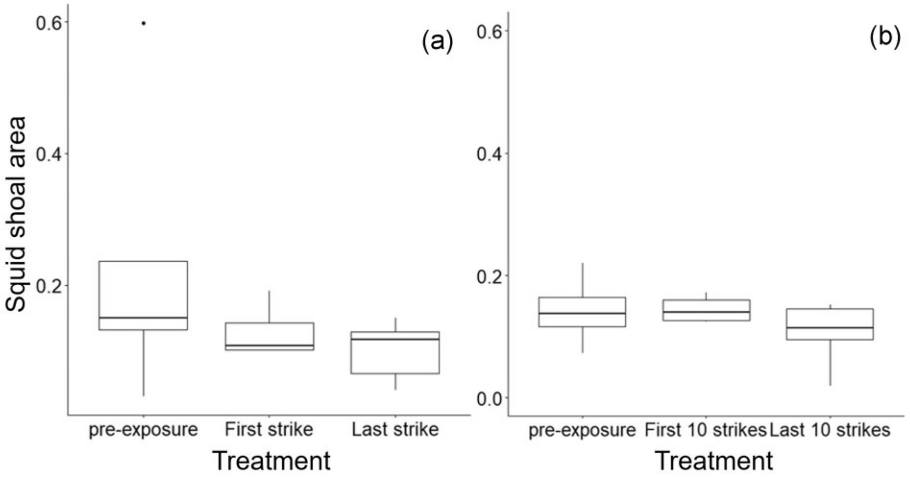

Figure 6. Areas computed on six squid shoals exposed to PD sound at the near site. Two different temporal resolutions are shown: during the first strike and the last strike (i.e. 5 s scale; a) and the first 10 first strikes and last 10 strikes (i.e. 60 s scale; b) of IH sequences. No significant differences were found in both treatments.

(Bejder *et al.*, 2009). Another hypothesis to the decrease of alarm responses over time could be the occurrence of temporary thresholds shifts due to sound exposure in squid. Some studies highlighted that artificial sound can induce anatomical damages on statocysts (André *et al.*, 2011), the sensory organ responsible for sound detection in squid (Mooney *et al.*, 2010). While we did not analyse potential squid statocyst damages after sound exposure, acoustic trauma could have been responsible for the decrease of squid sensitivity to sound.

We found that only a portion of squid in each shoal responded to PD sound, with overall <40% of squid showing alarm responses (Figures 4 and 5). These results suggest inter-individual differences in squid sensitivity, which has been highlighted in fish (Harding *et al.*, 2019). This result is vastly different from previous tank studies where >90% of squid showed alarm responses to PD sound (Jones *et al.*, 2020). This difference can be attributed to the fact that the previous studies tested solitary squid, which may be more sensitive to predator cues than squid in a shoal (Hanlon and Messenger, 2018). This also highlights the importance of studying anthropogenic sound impacts on shoals and realistic groupings, rather than individuals for social species (Popper *et al.*, 2022). Another hypothesis is that sound exposure levels were not high enough to induce alarm responses in all squid. Our measured sound levels corresponded to roughly 1 km from actual windfarm constructions (Sigray *et al.*, 2022). Hence, it is possible that more squid would react at closer ranges where sound levels are much higher.

Despite the occurrence of short-term alarm responses, PD exposure did not disrupt squid shoaling areas (Figures 4 and 6). This result is consistent with the findings of Ginnaw *et al.* (2020) where fish shoal collective motion was not affected by pure low-frequency tones in tanks, but differs from Herbert-Read *et al.* (2017) who found that PD sound exposure (5 min duration) in tanks disrupts collective dynamics of fish shoals. First, it is possible that the cages used in our study, while relatively large and comparable to other studies (e.g. Dahl *et al.*, 2020), could have influenced squid shoal movements, preventing any horizontal dispersion. Despite these physical constraints, squid exhibited normal shoaling behaviours without any collisions with the cage netting (see Supplementary materials). For example, wild shoals of fish can disperse and even change depth when exposed to PD sound (Hawkins *et al.*, 2014). Second, shoaling is a vital behaviour for squid to reduce predatory threats and for reproduction, thus there is a likely high motivation for individuals to return quickly to shoaling after behavioural disruption (Figure 3; Hanlon and Messenger, 2018). While our study was performed using four to seven squid per shoal, wild *Doryteuthis* spp. shoals can be composed of 100 s of individuals (Shashar and Hanlon, 2013), and dynamics could be different for larger groups. Thus, while our data reflect behavioural responses and interactions within small (but more manageable) shoals, there is still a need to address how PD sound may influence larger, wild mating squid. Finally, while the PD sound levels at our near site were roughly equivalent to 1 km from OSW constructions (Sigray *et al.*, 2022), they might not have been high enough to induce any shoal disruption (Cones *et al.*, 2022). Further studies should assess potential horizontal dispersion of wild squid shoals in the vicinity of OSW constructions, which have much higher intensity PD activity (Sigray *et al.*, 2022).

The present study focused on behavioural responses of both squid individuals and shoals to repeated PD sound exposure. Here, we did not study dose-dependent responses that could have occurred in individuals located at the near site where PAL_{0p} varied between 112 and 123 dB re 1 μm s^{-2}, depending upon the distance pile-cages (Cones *et al.*, 2022). Dose-dependence behavioural responses were previously described in *D. pealeii* to pure tones in tanks (Mooney *et al.*, 2016), as well as in *S. australis* exposed to seismic air gun sound (Fewtrell and McCauley, 2012). Squid elicited a higher proportion of alarm behaviours with increasing sound levels, implying the severity of sound impact on squid is related to the distance from the source. Considering the higher sound levels and propagation distances arising from OSW constructions (Sigray *et al.*, 2022), further studies are now needed to assess dose-dependent responses, as well as minimum acoustic thresholds that induce alarm behaviours in *D. pealeii* in the marine environment.

To conclude, our results highight two main potential mitigation procedures that could be used by OSW developers to reduce behavioural impacts on squid. First, far fewer behavioural reactions were found in squid exposed to the VH continuous and low-amplitude signals, which is consistent with our previous study in scallops (Jézéquel *et al.*, 2022). This suggests that the VH can be used as a mitigation technique to drasticaly reduce behavioural impacts on squid during OSW constructions. Second, the fact that squid mostly responded at the onset of the IH events highlights that the first IH blows have the most impacts on squid. Here, ramp-up could also be used as a mitigation technique. This soft-start procedure with slowly increasing sound levels could provide animals with a warning signal before sound exposure levels rise to the full power (Robinson *et al.*, 2007; Bailey *et al.*, 2014). The gradual increase in sound level of the ramp-up procedure may allow the squid to habituate to the sound exposure faster and stay within the exposure area without avoidance behaviour.

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Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

YJ, SC, and TAM designed research; YJ, SC, SF, and NA, performed research; YJ, PJ, and SC analysed the data; YJ, PJ, SC, YG, and TAM wrote the paper. TAM acquired funding. All authors read and approved the last version of the manuscript.

Data availability

All data used for the analyses are available on request to the corresponding author.

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