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

Hubert, J.

### Citation

Hubert, J. (2021, September 9). *Sound investigation: effects of noise on marine animals across trophic levels*. Retrieved from <https://hdl.handle.net/1887/3209242>

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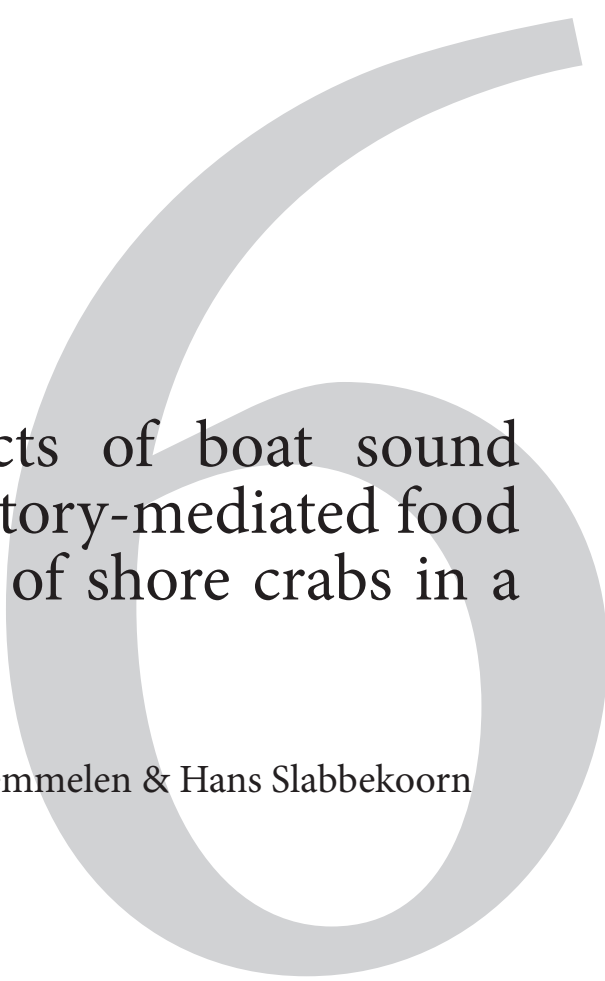


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**Author:** Hubert, J.

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**Issue Date:** 2021-09-09



# No negative effects of boat sound playbacks on olfactory-mediated food finding behaviour of shore crabs in a T-maze

Jeroen Hubert, Jostijn J. van Bemmelen & Hans Slabbekoorn

## Abstract

Anthropogenic noise underwater is increasingly recognized as a pollutant for marine ecology, as marine life often relies on sound for orientation and communication. However, noise may not only interfere with processes mediated through sound, but also have effects across sensory modalities. To understand the mechanisms of the impact of anthropogenic sound to its full extent, we also need to study cross-sensory interference. To study this, we examined the effect of boat sound playbacks on olfactory-mediated food finding behaviour of shore crabs (*Carcinus maenas*). We utilized opaque T-mazes with a consistent water flow from both ends towards the starting zone, while one end contained a dead food item. In this way, there were no visual or auditory cues and crabs could only find the food based on olfaction. We did not find an overall effect of boat sound on food finding success, foraging duration or walking distance. However, after excluding deviant data from one out of the six different boat stimuli, we found that crabs were faster to reach the food during boat sound playbacks. These results, with and without the deviant data, seem to contradict an earlier field study in which fewer crabs aggregated around a food source during elevated noise levels. We hypothesise that this difference could be explained by a difference in hunger level, with the current T-maze crabs being hungrier than the free-ranging crabs. Hunger level may affect the motivation to find food and the decision to avoid or take risks, but further research is needed to test this. In conclusion, we did not find unequivocal evidence for a negative impact of boat sound on the processing or use of olfactory cues. Nevertheless, the distinct pattern warrants follow up and calls for even larger replicate samples of acoustic stimuli for noise exposure experiments.

## Introduction

Over the last decades, increasing numbers of anthropogenic sound sources have been contributing to the marine soundscape (Andrew et al., 2002; Hildebrand, 2009). This has led to concerns about the effects of anthropogenic sound on the environment as many marine animals rely on sound for orientation and communication (Carroll et al., 2017; Slabbekoorn et al., 2010). However, noise may not only hinder processes mediated through sound, but may also have effects across sensory modalities (Halfwerk and Slabbekoorn, 2015). Such effects may be salient in a crab species that relies on chemical cues for food-finding. Relatively few sound impact studies have been done on effects of sound on crabs and other invertebrates (Williams et al., 2015). Yet, the abundance of invertebrates is critical for higher trophic levels as food or through ecosystem services (Morley et al., 2014; Solan et al., 2016).

Auditory perception in crustaceans, including crabs, is still poorly understood, but is likely to be important for their survival and reproduction. They are thought to detect particle motion through mechanical stimulation of setae (hair-like) cells on the body-surface, chordotonal organs, and statocysts in contact with sensory hairs (Popper et al., 2001). Crustaceans lack gas-filled cavities such as a swim bladder and are assumed not to be sensitive to sound pressure, and they are therefore, most sensitive to low-frequency sound (Edmonds et al., 2016; Popper et al., 2001). Mud crabs (*Panopeus* spp.) have shown to be most sensitive to the lowest tested frequency (resp. 75 Hz) with decreasing sensitivity up to at least the highest tested frequency (1600 Hz; Hughes et al., 2014). There is evidence that natural sound is important to crabs: in their pelagic stages they were shown to use sound for orientation and navigation (Jeffs et al., 2003; Radford et al., 2007), and adult mud crabs changed foraging behaviour during the playback of predatory fish vocalisations (Hughes et al., 2014).

Elevated sound levels can have a range of effects on decapod crustaceans including crabs. Physiologically, sound exposures have been shown to increase oxygen consumption, exhibit changes in stress-related biochemistry, reduce growth and delay reproduction (Filiciotto et al., 2014; Filiciotto et al., 2016; Lagardère, 1982; Regnault and Lagardère, 1983; Wale et al., 2013a). Behaviourally, elevated sound levels have been shown to alter responses to a simulated predator, decrease resource assessment, change locomotor activity, decrease the amount of inter-individual encounters, change use of a shelter, disrupt feeding, and reduce aggregation at a food item (Chan et al., 2010a; Filiciotto et al., 2014; Filiciotto et al., 2016; Hubert et al., 2018; Wale et al., 2013b; Walsh et al., 2017). The underlying mechanism for most of the effects that have been found remain

however unknown.

Olfactory perception is considered to be an important sensory modality in decapod crustaceans (Krieger et al., 2012; Weissburg and Zimmer-Faust, 1994) and may be interfered by sound exposures. Crabs are shown to locate prey and conspecifics by sensing the direction of the water flow that contains olfactory cues of either prey or conspecifics (Weissburg and Zimmer-Faust, 1994; Zimmer-Faust, 1989). Even though there is no physical interference between the two modalities (e.g. sound and odour), simultaneous perception in an animal may hinder processing and interpretation of the stimuli through so-called cross-sensory interference (Halfwerk and Slabbekoorn, 2015). Studies across sensory modalities are therefore needed to truly understand the effects of noise pollution.

Cross-sensory interference has been found in a variety of species. The anti-predator response to a visual predator or a chemical alarm cue reduced during sound exposure in several fish species (Hasan et al., 2018; Simpson et al., 2014; Voellmy et al., 2014). Similarly, wild dwarf mongooses (*Helogale parvula*) responded less strong to predator faecal presentations during noise playbacks (Morris-Drake et al., 2016). Foraging behaviour has also shown to be affected across modalities. In great tits (*Parus major*), noise exposure increased approach and attack latencies of visually cryptic prey (Halfwerk and Van Oers, 2020). Several studies examined cross-sensory interference of sound exposures on hermit crabs, taxonomically more related to the current study species. Caribbean hermit crabs (*Coenobita clypeatus*) responded later to a silently approaching simulated predator during boat noise exposure (Chan et al., 2010b). Common hermit crabs (*Pagurus bernhardus*) invested less time in shell selection during white noise exposure (Walsh et al., 2017). Lastly, less Acadian hermit crabs (*Pagurus acadianus*) aggregated at the source of a chemical cue, indicative of new shells, during generation of impulsive sound (Roberts and Laidre, 2019). So, it may well be that the reduced aggregation at a food item by shore crabs during white noise exposures in our previous study (Hubert et al., 2018), can also be explained by cross-sensory interference due to the elevated noise levels.

Previous studies exploited the olfactory-mediated localization capability of crabs to assess their food and mate preferences using two-choice set-ups (Ekerholm, 2005; Hardege et al., 2011; Shelton and Mackie, 1971). A two-choice set-up, such as a T-maze, can be used to present a subject with two water flows with different chemical cues to assess a preference or capability to discriminate between cues. By visually blocking the source of the olfactory cues in the water flow, it can be assured that decision-making is based on olfactory perception alone (Zimmer-Faust, 1989). Such a set-up may prove to be useful in studying cross-sensory

interference of acoustic stressors on crustaceans.

In the current study, we explored the effect of experimental playback of boat sound on olfactory-mediated food finding behaviour of shore crabs. In the experiment, crabs were allowed to forage in a T-maze that contained a food item at the end of one of the arms. The food item was not visible from the intersection, but a water flow from the end of both arms towards the starting zone enabled the crabs to find food based on olfactory cues. We aimed at answering the following questions: (1) Is food finding success in crabs affected by the playback of boat sound? (2) Is food finding efficiency in crabs affected by the playback of boat sound?

## Materials and methods

### Study subjects

We used 239 wild-caught shore crabs (*Carcinus maenas*) with carapace widths ranging from 1.0 to 2.5 cm. The crabs were manually collected during low tides from rocks in the tidal area of the Jacobahaven, a cove in the Oosterschelde sea arm, the Netherlands. Before being used in the experiment, the crabs were group-housed in 1.7 L plastic boxes, stacked in a larger stock tank (1.2 x 1.0 x 0.6 m; L x W x H) that had a continuous inflow of fresh seawater and air supply. Each 1.7 L plastic box housed four crabs of similar size and also contained empty shells for shelter. Part of the walls of the plastic box was replaced by mesh to ensure fresh oxygen rich seawater to flow in. The crabs were housed in captivity for a maximum of 1.5 weeks and were fed with mussels (*Mytilus edulis*, without shell) on the day of catching and three days prior to the experiment. In this way, we standardized hunger-levels and made sure that each crab was familiar with the food source.

### Experimental arena

The trials were performed using T-mazes with a continuous water flow from the ends of both arms to the starting zone. We created the two identical T-mazes from white acrylic sheets (0.2 cm thick) and submerged them partially (for 3.5 cm) in a tank that was identical to the stock tank. Holes at the ends of the arms allowed fresh seawater to flow in. Two tubes in the starting zone connected the water in the mazes to external boxes with a lower water level, allowing the water to flow out (fig. 1). The same principle applied to another pair of tubes connecting the water in the external boxes to a lower situated drain. We used the pumps in the external boxes to initialise the water flow. Once established, the water flow was maintained based on hydrostatic pressure, and the pumps

were turned off. The underwater speaker was placed between the two mazes on a 10 cm high frame at the tank floor. In this way, we maximized the distance between the speaker and the mazes to achieve a more homogeneous sound field within the mazes. We used a stationary hydrophone to record all trials to verify that sound exposure was played back correctly and no additional disturbance had taken place. All trials were recorded with a video camera (Everio GZ-R415, JVC, Japan) that was placed above the tank along with a LED tube. This set-up was covered up with black plastic sheets to ensure equal light conditions among trials and to prevent visual disturbance from the researcher or other objects in the direct vicinity.

## Experimental design

During the experiment, individual crabs were allowed to forage on an opened mussel (*Mytilus edulis*) of 3.0 to 4.5 cm. At the start of each trial, we placed a mussel at the end of one of the two arms of a maze. After this, we introduced a crab in a maze by gently pouring the crab in the starting zone and closing the zone with a transparent lid to avoid escapes. This was done within a 1 min period, during which ambient sound was played back and thereafter linearly crossfaded (10 s) into the 10 min playback of a boat or a different ambient track. The hatches in front of the starting zones were lifted 5 min after the crossfade, allowing the crab to emerge and find the mussel during the remaining 5 min. The hatches

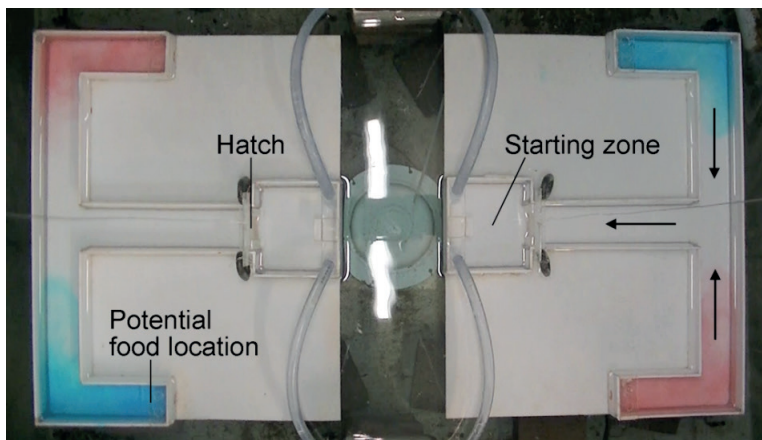


Fig. 1: A video still of the two T-mazes with the speaker in the middle. Crabs were introduced in the starting zone and after lifting the hatch, they could freely move through the T-maze. One of the ends contained a food item which could be found thanks to olfactory cues in the consistent water flow from both ends of the maze to the starting zone. The arrows indicate the direction of the water flow, we used colour dyes (blue and red) for demonstration purposes only (and not during the experiments with crabs). A movie of this can be found online (see Data accessibility). The dimensions of the mazes can be found in figure 3.



were opened from outside the plastic sheets around the experimental set-up by pulling wires. We tested two crabs simultaneously and each pair of trials was filmed for later analysis. After a pair of trials, we determined the crabs' sex and measured their carapace width to be able to examine potential differences sex and size differences in behaviour and a potential size-dependency in response to the sound. We also examined whether the crabs had a soft carapace due to recent moulting, these individuals were excluded from further analysis because of a potential difference in food-finding motivation. Each crab was used in a trial once. Between trials, we syphoned the mazes to clean them and placed new mussels in the mazes. We counterbalanced the order of the treatments and the position of the mussel.

## Sound exposure

During the trials, we exposed the crabs to a boat or ambient sound treatment. Boat sound is mostly produced by the on board machinery, water displacement, and cavitation (formation and collapse of bubbles) at propeller blades (Popper and Hawkins, 2019; Ross, 1976). Ambient sound in marine waters and coastal crab habitat mainly originates from water surface roughness as a function of weather conditions and distant shipping (Carey and Browning, 1988; Wenz, 1962). The playback tracks were constructed using seven ambient recordings and six recordings of different boats (3-~30 m long), all at different locations across the IJ (river) and Oosterscheld, the Netherlands. From these calibrated recordings, we selected windows of 35 to 50 s with relatively consistent amplitude and looped them into longer tracks using 3 s linear crossfades. We bandpassed the tracks with 100-1600 Hz filters to anticipate on low-frequency speaker limitations and based on expected high-frequency hearing limits of our study subjects, based on data from another crab species (Hughes et al., 2014). For the actual playbacks, each track started with 1 min of ambient sound and linearly crossfaded in 10 s to a 10 min boat sound or ambient sound from a different location than the first min of ambient sound. Sound treatments were played back with an underwater speaker (UW30, Lubell labs, US), from a recorder (PMD620, Marantz, Japan), through an amplifier (M033N, Kemo, Germany).

We used playback tracks that were constructed using seven ambient recordings and six boat recordings to increase external validity and to reduce possible confounding effects from a single recording. If a single boat sound would have been used, results would only be relevant for this single recording of this particular boat, while results from multiple stimuli better resemble the possible acoustic variation of boat sound in general. The use of more than six boats reduces potential issues with the well-known methodological flaw of pseudoreplication (Hurlbert, 1984; Slabbekoorn and Bouton, 2008). For this

study, it was explicitly not the goal to explore the effect of different boat types or boat sound characteristics. In do this, multiple boat stimuli would have been needed for each boat type or sound characteristic of interest. For linear correlations between sound characteristics and crab response levels, much more than six boat stimuli or highly controlled stimuli (e.g. Hubert et al., 2020) would have been needed. Boat recordings vary in numerous ways in frequency distribution, amplitude, and temporal pattern. With limited playback tracks, it is not possible to disentangle different characteristics from one another.

We recorded all playbacks in the experimental tank using a calibrated hydrophone (96-min, HTI, US) to gain insight into the sound levels and spectra for the exposure conditions of the crabs. Shore crabs are likely sensitive to the particle motion component of sound, rather than sound pressure. The acoustic conditions, in terms of the ratio between particle motion and acoustic pressure, and the directionality of sound velocity in small tanks is not comparable to the natural environment of aquatic animals. This is caused by the proximity of the water surface, tank walls, and bottom (Rogers et al., 2016). We just measured sound pressure levels because we did not have a particle motion sensor that is small enough to be expected to reliably measure particle motion in our setup. We did not measure the sound pressure levels at the position of the crabs (3.5 cm below the surface) but at a 15 cm depth because the water surface is a pressure release boundary. This means that sound pressure levels (SPL) decrease close to the water surface whereas particle motion levels increase. Therefore, SPL measurements at 15 cm depth are a better indication for the sound levels the crabs were exposed to than when we would have measured them closer to the surface. Importantly, these measurements do not provide absolute data on exposure conditions, but rather make exposure conditions repeatable and comparable. The geometric mean rms SPL (75-1600 Hz bandpassed) of the measurements of boat playbacks was 123.4 dB re 1  $\mu$ Pa and 103.9 dB re 1  $\mu$ Pa of the ambient playbacks (figure 2). We made additional recordings of a sweep tone (100 – 4000 Hz) at different locations in the tank and with and without the maze to gain insight into the sound propagation in the tank (supplementary material I).

## Behavioural observation

We analysed 239 trials: 117 trials with the ambient treatment, and 122 with the boat treatment. In blind scoring sessions (without audio), we manually scored whether the crab reached the food, the initial direction at the intersection, the time to emergence from the starting zone, and time from emergence till first physical contact with the mussel. Next, we used custom made video tracking software (depending on Python 3, FFmpeg and OpenCV 3.4.3; Bradski, 2000) to track the walking patterns of the crabs. We corrected the tracked positions

for camera angle, and only recognized new positions after a threshold of 2 cm was passed, in order to ignore movements of a single claw or leg (figure 3). We used positions from just after leaving the starting zone till the crab reached close

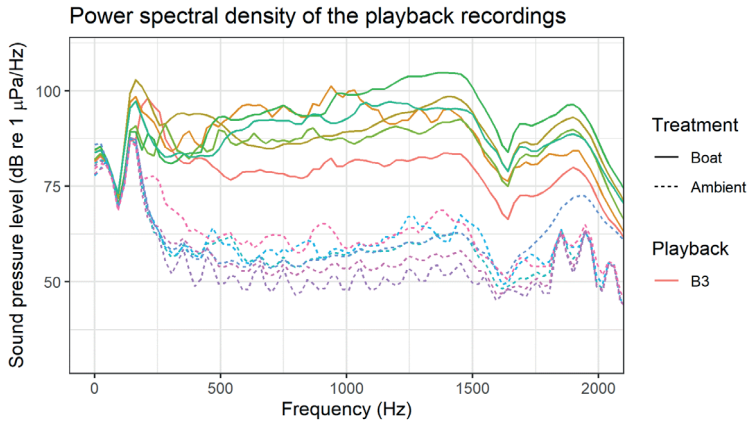


Fig. 2: Power spectral density (window length: 2048, window type: Hann) of all boat and ambient playbacks. The colours indicate the individual playbacks, the solid lines are the boat playbacks and the dotted lines are the ambient playbacks. We have highlighted boat playback B3 in the legend because this playback seemed to have yielded different results than the other boat playbacks.

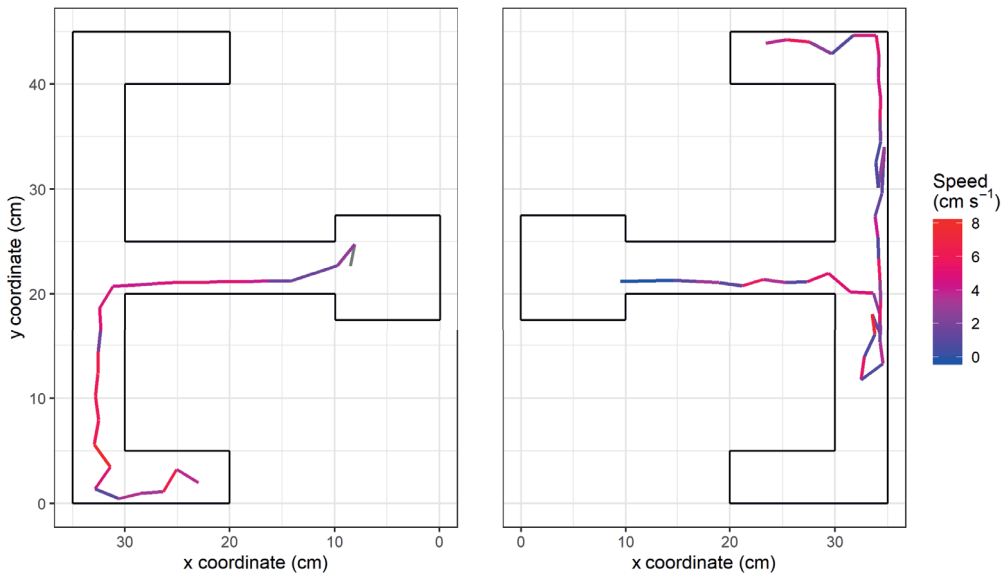


Fig. 3: Walking tracks of individuals in two simultaneous trials, from the opening of the hatches till the individuals reached the food. A new position was only recognized after a threshold of a 2 cm distance relative to the previous recognized position was passed. So, the speed – indicated by the colour – is the average speed over the previous ~ 2 cm.

proximity to the food (both with a 2.0 cm margin). Using the tracked walking paths, we determined the total walking distance.

## Statistics

We used the observations from the videos as response variables in generalized linear models (GLMs) in R (R Core Team, 2016). For the response variables ‘reached food’ (Y/N) and ‘Initial direction’ (Towards food/Away from food/None), we used a binomial error distribution, and for ‘Emergence time’, ‘Foraging time’ and ‘Walking distance’, we used a negative binomial error distribution. We used ‘Crab sex’, ‘Crab size’, ‘Treatment’ (Ambient/Boat), and the interaction between the latter two as covariates in the full model. The best model was selected based on lowest AICc score (using the function `Dredge`, R package `MuMIn`; Barton, 2016), if ‘Treatment’ was not part of the best model, we kept it in the final model anyway because this was our variable of interest. To determine the effect and significance of the covariates, we ran the final models. Lastly, we determined the 95% confidence intervals of the intercept and slope (using the function `confint`, R package `MASS`; Venables and Ripley, 2002).

## Results

225 of 239 crabs emerged from the starting zone and reached the food item, 5 did not emerge from the starting zone and 9 did emerge, but did not reach the food (fig. 4). There was no significant effect of sound treatment on the number of individuals that reached the food (Intercept: 2.75, CI: 2.07 – 3.62; Boat treatment: 0.04, CI: -1.06 – 1.15; p-value: 0.94; fig. 4a) or their initial direction at the intersection (Intercept: 2.75, CI: 2.07 – 3.62; Boat treatment: 0.21, CI: -0.92 – 1.37; p-value: 0.72; fig. 4b).

The size of the crabs was significantly negatively correlated with both emergence time (Intercept: 4.24, CI: 3.59 – 4.90; Crab size slope: -0.62, CI: -1.02 – -0.21; p-value: < 0.01; fig. 6a) and foraging time (Intercept: 4.31, CI: 3.90 – 4.73; Crab size slope: -0.37, CI: -0.62 – -0.11; p-value: < 0.01; fig. 6b). This means that larger crabs were faster than smaller crabs in emerging and reaching the food. However, there was no significant effect of the sound treatment on emergence time (Intercept: 4.24, CI: 3.59 – 4.90; Boat treatment: 0.18, CI: -0.08 – 0.43; p-value: 0.17; fig. 5a), foraging time (Intercept: 4.31, CI: 3.90 – 4.73; Boat treatment: -0.11, CI: -0.27 – 0.04; p-value: 0.15; fig. 5b), and walking distance (Intercept: 4.15, CI: 4.07 – 4.22; Boat treatment: -0.05, CI: -0.16 – 0.05; p-value: 0.33; fig. 5c).

Visual inspection of the foraging times per playback track indicated that the effect of a single boat playback track deviated from the other boat tracks (fig. 7).

We performed a parallel analysis, excluding the data from this particular boat track, and found that crabs exposed to the other five boat stimuli were affected by the sound and had a significantly lower foraging time (Intercept: 4.22, CI: 3.80 – 4.64; Boat treatment: -0.18, CI: -0.34 – -0.02; p-value: 0.03). The noisy conditions appeared not to interfere, but made the crabs reach the food more quickly. Excluding this boat stimulus did not yield different results for the walking distance of the crabs (Intercept: 4.13, CI: 4.06 – 4.21; Boat treatment: -0.07, CI: -0.18 – 0.04; p-value: 0.20).

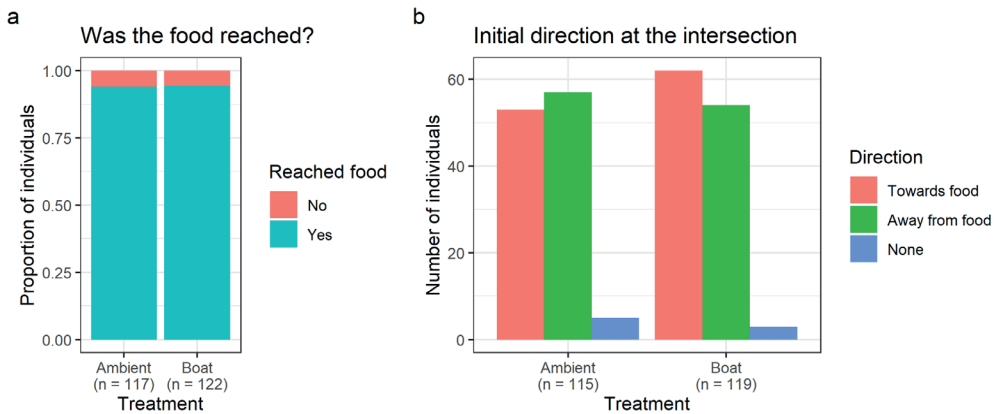


Fig. 4: (a) The proportion of individuals that reached the food item before the end of the trial, in both ambient as boat playback conditions. (b) The number of emerged individuals that initially moved towards, away from the food at the intersection, and did not reach the intersection.

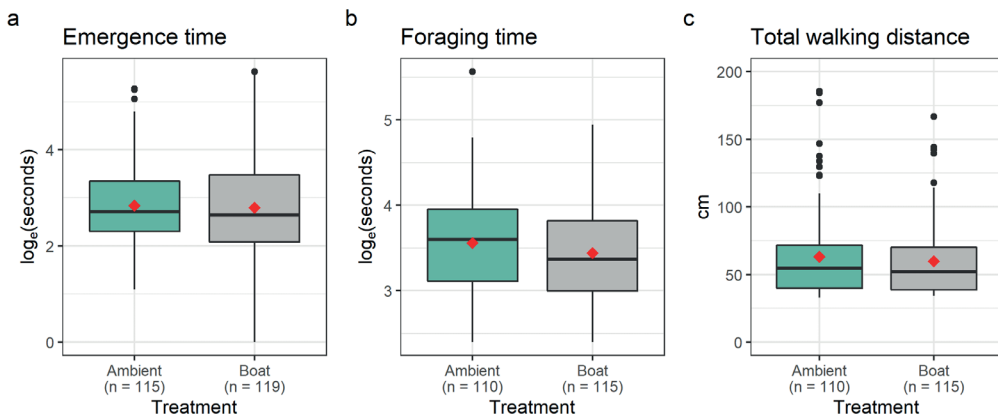


Fig. 5: (a) Emergence time, which is the time individuals took, from the opening of the hatch, to leave the starting zone. The red diamonds indicate the means. (b) Foraging time, which is the time the individuals took, from leaving the starting zone, to reach the food. (c) Total walking distance of the individuals, from leaving the starting zone till reaching the food.

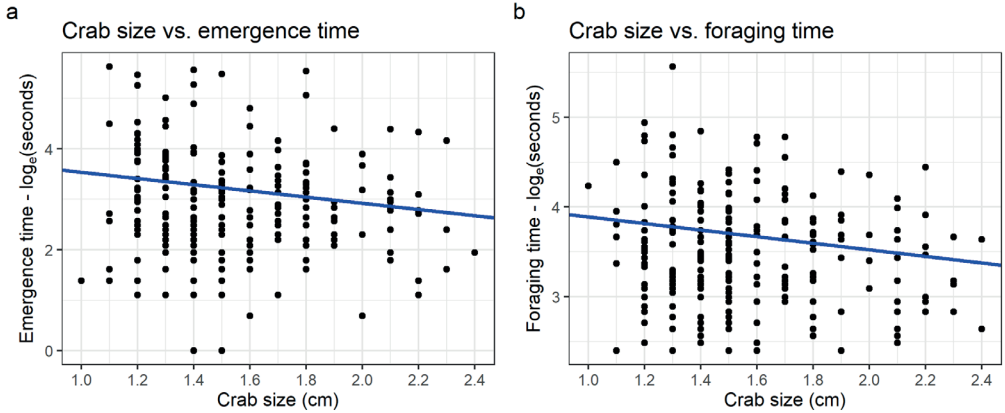


Fig. 6: (a) Correlation between crab size and emergence time. (b) Correlation between crab size and foraging time. The points indicate single individuals, and the blue trendline indicates the significant negative correlations between crab size and (a) emergence time and (b) foraging time. Note that the y-axes of both plots are not identical.

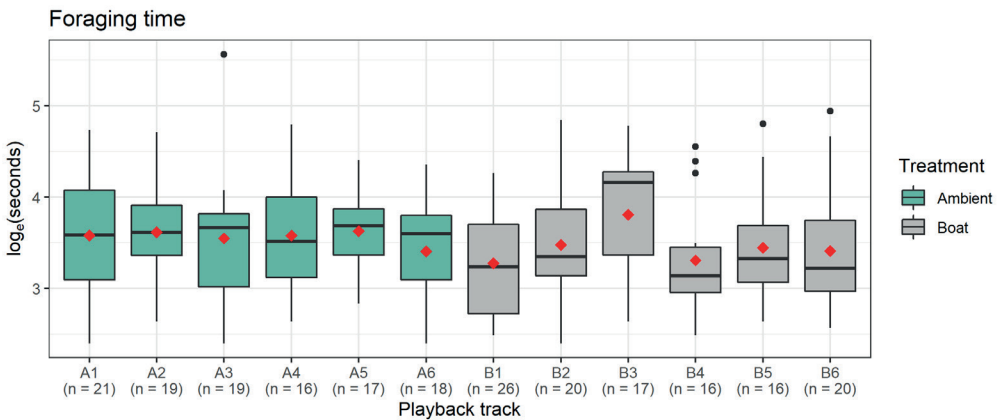


Fig. 7: Foraging duration for each of the playback tracks in box-and-whisker plots, indicating the median, first and third quartile, min and max excluding outliers, and outliers. The red diamonds indicate the means. There was no significant overall effect of sound treatment, although median values for all six ambient stimuli were higher than the median values of all but one boat sound stimulus. When excluding the data from the deviating boat sound stimulus B3, crabs under noisy boat conditions were not slower, but reached the food more quickly than under ambient conditions.

## Discussion

In the current study, we allowed individual shore crabs in a T-maze to forage on an opened mussel, while exposing the crabs to a boat sound playback or an

ambient control. Our results demonstrated that food finding success and foraging efficiency of crabs in a T-maze was not affected by the boat sound playback. However, when we excluded deviant data from one out of the six boat playbacks, we found that crabs were faster to arrive at the food item during boat playbacks than crabs exposed to ambient playbacks. In any case, boat playbacks did not lead to increased foraging duration, which suggests that olfactory mediated food finding was not negatively affected by boat noise playback in this study. Additionally, we found that larger crabs were faster to emerge and to reach the food, but we found no size dependent response to sound.

## Foraging behaviour during sound exposures

Overall, boat sound exposures did not affect food finding success, foraging duration, and walking distance during foraging. However, after exclusion of the results for the boat stimulus B3, the foraging duration of crabs that were exposed to boat sound appeared to be shorter. Two earlier studies examined the effect of sound exposures on the foraging behaviour of shore crabs. Wale et al. (2013b) also found no effect on food finding success and foraging time in a tank. Food finding might have been relatively easy in that study because the experimental tank was relatively small (30 x 30 cm; L x W), with the food item in the centre, and there were no visual or physical blockages between the crab and food. In the current study, the crabs had to walk at least ~ 45 cm and could only find the food based on olfactory cues.

However, the results of both studies (Wale et al., 2013b and the current study) are not in line with the results of Hubert et al. (2018), where we found reduced aggregation at a food item in the field. Differences between field and tank studies may be explained by the sound field, from which the particle motion component is typically not mapped. Crabs are thought to be sensitive to particle motion (Popper et al., 2001), for which levels and direction in tanks are influenced by the proximity of the water surface, tank walls, and bottom, which yields different conditions than in the natural environment (Rogers et al., 2016). For these foraging studies specifically, differences between the field and tank studies may also be explained by the hunger level of the study subjects. Although we do not know the hunger level of the free-ranging crabs, in both captive studies (Wale et al 2013b and our current study), crabs were food-deprived for three days before a trial, which may change the motivation to find food and take risks.

Various studies have shown that food-deprivation influences behaviour including foraging. Food-deprived individual three-spined sticklebacks (*Gasterosteus aculeatus*) were more likely to initiate predator inspection visits and had higher feeding rates than well-fed shoal mates (Godin and Crossman, 1994). Hungry



fifteen-spined sticklebacks (*Spinachia spinachia*) were more likely than partially satiated individuals to feed at a food source associated with predator threat (so hungry sticklebacks were shown to be less risk-averse) (Croy and Hughes, 1991). Well-fed crayfish (*Procambarus clarkii*) close to a food odour, were more likely to respond to a moving shadow by a tail-flip escape response, whereas food-deprived individual were more likely to freeze, potentially a riskier response (Schadegg and Herberholz, 2017). For the current study species, it has been shown that increased hunger levels can lead to decreased prey selectivity (Jubb et al., 1983) resulting in greater variation in prey size, including suboptimal sizes (Morris, 2008). So, it may well be that the lack of an overall negative effect of sound exposure on food finding success and foraging time in the current study and in Wale et al (2013b), in contrast to Hubert et al (2018), can be explained by the increased hunger levels, but future research has to confirm this hypothesis.

After exclusion of the data from boat stimulus B3, we found a significantly lower foraging duration during boat sound playbacks, but not a lower walking distance during foraging. This suggests that the crabs walked faster during boat sound, rather than navigating more efficiently. Crabs may have walked faster during boat sound playbacks to reduce predation risk or due to elevated stress levels, but they nevertheless had enough motivation to find the food, both in line with the previously stated hypothesis. Humans have also been shown to walk faster under traffic noise conditions, an effect that is possibly related to stress (Franěk et al., 2018). However, based on the current study, we cannot draw strong conclusions on the subjects' stress levels, anti-predator response, or a combination of both.

## Cross-sensory interference

One of the major goals of this study was to examine whether boat sound playbacks interfered with olfactory-mediated food finding. This would be an indication of cross-sensory interference; meaning that increased capacity of the nervous system to process the acoustic stimulus interfered with the detection and information use of other sensory inputs (Halfwerk and Van Oers, 2020). If cross-sensory interference would have taken place, we expected a lower food finding success and/or a longer food-finding duration. Our results show that food finding success was not negatively affected by boat sound, in our set-up. Depending on including or excluding data from deviant response patterns to one of the six boat recording stimuli used, crabs were respectively either equally fast or even faster in reaching the food than crabs that were exposed to an ambient control. Thus, there is no evidence that cross-modal interference took place in the current study. Several other studies found cross-sensory interference, including effects on foraging behaviour in great tits (Halfwerk and Van Oers, 2020) and anti-predator behaviour, and aggregation and selection of a potential



new shell in hermit crabs (Chan et al., 2010b; Roberts and Laidre, 2019; Walsh et al., 2017). Not having found evidence for cross-sensory interference in shore crabs in the current study, does not exclude that this process may play a role in shore crabs under other conditions.

## Boat stimuli

We provided the results of two parallel analyses: one for all our trials and one for a subset in which we excluded the trials of boat stimulus B3. The foraging times of the crabs that had been exposed to this particular boat seemed to deviate from the foraging times of the other boats. The analysis with all boats did not reveal an effect of boat playbacks on foraging duration, whereas the analysis without this particular boat showed a lower foraging duration for individuals that were exposed to boat sound. This provided some statistical support for the visually observed difference between B3 and the other boats.

We here speculate about some possible explanations for the deviant response patterns to the boat stimulus B3. The spectral profile of this playback was distinct from the other boats in being equally high in the low-frequency range, but the least loud above 500 Hz. Additionally, unlike the others, this boat was regularly used in the Jacobahaven, where all study subjects were caught. Future studies are needed to examine whether crabs respond differently to boat sounds of different spectral profiles or to familiar and unfamiliar boat sounds. However, the current results indicate that one out of six stimuli can still influence the results substantially. We therefore recommend the use of even more different playback stimuli for future noise impact studies, beyond our current set of six recordings, to further reduce problems of pseudoreplication (Hurlbert, 1984; Slabbekoorn and Bouton, 2008).

## Conclusions

Our study examined the effect of boat sound playbacks on olfactory-mediated foraging behaviour in shore crabs. Our results do not provide evidence for a negative effect of boat sound playbacks on food finding success and foraging duration. However, after exclusion of the deviant data for one of the boat stimuli, crabs that were exposed to boat sound appeared to have reached the food faster than crabs that were exposed to ambient playbacks. These results add to what seems an emerging picture of anthropogenic noise exposure potentially affecting behaviour, but with contrasting results from captive and field studies on crabs (Hubert et al., 2018; Wale et al., 2013b; and the current study) We hypothesise that this contrast can be explained by a potential difference in hunger level, as crabs in both lab studies were food-deprived for three days, but future research

has to confirm this. Lastly, we call for sufficient replication of playback stimuli in sound exposure experiments, to reduce pseudoreplication and large single-stimulus impact (Hurlbert, 1984; Slabbekoorn and Bouton, 2008).

## Ethical statement

We adhered to the Guidelines for the treatment of animals in behavioural research and teaching (ASAB, 2018). There are no legal requirements for studies involving crabs and bivalves in the Netherlands.

## Supplementary information

Supplementary material associated with this article is available from the publication DOI:10.1016/j.envpol.2020.116184.

## Data accessibility

All data used for the analyses reported in this article and some videos are available from the Zenodo Repository, DOI:10.5281/zenodo.4135946.

## Acknowledgements

We thank James A. Campbell for his help with the experimental design and making video tracking software. We thank the personnel from Stichting Zeeschelp for making use of their facilities. JH was funded by the E&P Sound & Marine Life Joint Industry Programme during data collection by JvB.

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