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Effects of anthropogenic noise on the behaviour of juvenile lobsters (*Homarus* gammarus)

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Contents

List of Figures

Summary

The German European lobster population of *Homarus gammarus* has been in decline since the 1960s and its recovery efforts with reared juveniles have yet to produce good results. Juveniles are one of the most vulnerable life stages as they depend on the availability of hard substrate that serves them as shelter, like rocks and boulders. Since the substrate in the German North Sea consists mostly of soft substrate like sand and mud, one viable habitat is the rocky island Helgoland. An increase of possible habitats could therefore increase the survivability of lobsters, wild and reared all together. Two of those new possible habitats are riffs constructed from the European oyster *Ostrea edulis*, with restoration attempts for rehabilitation in the North Sea already in progress. The other are the foundation of newly constructed Wind Farms, which often consists of rocks and boulders that envelope the foundation on the seabed. While this could lead to a surplus in new habitats due to the construction of several new Wind Farms over the next years, little is known about the possible effects' exposure to the low-frequency noise produced by the Wind Turbines operation. To understand habitat selection and anti-predator responses lobsters might face in these underwater structures, the present study aimed to test noise and predator effects on shelter preference and behavioural responses in a full factorial design. Juvenile lobsters (n = 104; mean and standard deviation total length of 12,29 ± 0,97 mm) were exposed to one of the four conditions (control, noise, predator, and noise + predator) for three hours during nighttime (when they are most active), and the time spend on predetermined behaviours was analysed from video records (20 min were analysed). Measured at the beginning and at the end of the exposure was the preferred shelter selection, lobsters selected the rocks over the oyster shells most of the time, which is supported by the literature. However, as oyster reefs represent much more complex habitats than the stacked shells setting used in the experiment, the use of oyster reefs as shelter cannot be discarded yet. The behavioural analysis highlighted that lobsters exposed to noise + predation were more active (general movement, shelter behaviour and exploration). The exploration behaviour was particularly predominant in comparison with the lobsters exposed to noise only. This higher activity made the lobster more visible to its predator, which possibly indicates that noise might distract the lobsters in their usual anti-predator responses. This study has therefore shown that noise exposure from Wind Farms could potentially affect the anti-predator behaviour of juvenile *H. gammarus* and possibly reducing its survival. In the view of the fishery industry interests in growing lobster in the Wind Farm foundations or in the view of the conservation actions of releasing reared juveniles in these, further research is needed to observe if such disturbance in the anti-predator responses occur in the wild. If so, the efforts of sustainable exploitation or conservation for the species might be in vain.

Zusammenfassung

Die deutsche Population des europäischen Hummers *Homarus gammarus* ist seit den 1960er Jahren zurückgegangen. Restaurationsvorhaben mit gezüchteten juvenilen Hummern haben bis jetzt wenige positive Resultate hervorgebracht. Juvenile sind die verwundbarste Lebensphase des Hummers, die sehr abhängig vom vorhandenem Hartsubstart, wie Felsen und Steine als Versteckmöglichkeit sind. Da die deutsche Nordsee allerdings zum Großteil nur aus Weichsubstrat, wie Sand und Schlamm bestehen, ist eine der einzigen möglichen Habitate die Felseninsel Helgoland. Ein Anstieg von möglichen Habitaten könnte deshalb die Überlebenschance von sowohl wilden als auch gezüchteten Hummer steigern. Zu möglichen Habitats Erweiterungen zählen Riffe, gebaut aus der europäischen Auster *Ostrea edulis*, für welche schon Rehabilitationsprozesse in der Nordsee durchgeführt werden und die Fundamente von neugebauten Wind Parks, die sehr häufig von Fels und Stein umgeben sind. Obwohl durch die Planung von mehreren neuen Wind Parks somit die Anzahl der Habitate steigern könnte, muss noch die möglichen Einflüsse von niedrigfrequentem Geräuschs Lärm, der erzeugt wird von den Motoren der Wind Turbine, erforscht werden. Diese Studie hat versucht, die möglichen Reaktionen der Hummer in ihrer Habitatwahl und ihrem Anti-Räuber Verhalten, in einem faktoriellem Experiment Design zu untersuchen. Die juvenilen Hummer (n = 104; Mittelwert und Standardabweichung der Gesamtpanzerlänge von 12,29 ± 0,97 mm) wurden zur Nachtzeit (Aktive Phase der Hummer) einer von vier Versuchsansätzen (Kontrolle, Lärm, Prädation und Lärm + Prädation) für drei Stunden ausgesetzt. Das Verhalten der Hummer, das vor dem Experiment festgelegt wurde ist auf Video festgehalten worden. Von dem Videomaterial wurden jeweils 20 Minuten analysiert, wobei die Zeit, die die Hummer für jedes Verhalten aufbrachten, festgehalten wurde. Zu Beginn und Ende des Durchlaufs wurde die Versteckwahl der Hummer geprüft. Die meiste Zeit bevorzugten sie die Steine gegenüber den Austern. Diese Beobachtung wird auch durch andere Literatur unterstützt, da allerdings ein natürliches Austernriff ein deutlich komplexeres Habitat im Vergleich zu locker gestapelten Austernschalen darstellt, kann die Auster als potenzielles Versteck noch nicht abgeschrieben werden. Die Verhaltensanalyse deutete darauf hin, dass juvenile Hummer, die sowohl Lärm als auch Räuberdruck ausgesetzt wurden, deutlich aktiver waren (generelle Regung, Versteckverhalten und Erkundung), mit dem Erkundungsverhalten deutlich höher wenn verglichen mit Hummer, die nur Lärm ausgesetzt waren. Durch die gesteigerte Aktivität waren die Hummer deutlich besser von ihrem Räuber zu entdecken. Daraus lässt sich schließen, dass Lärm das Anti-Rüber Verhalten verändert, indem es die begrenzte Aufmerksamkeitsspanne des Hummers beinträchtig und ihn somit ablenkt. Die Studie hat somit gezeigt, dass Hummer, welche dem Geräuschs Lärm von Wind Turbinen ausgesetzt wurden, ein reduziertes Anti-Räuber Verhalten aufweisen, was folglich eine verringerte Überlebenschance nach sich ziehen kann. Aus Sicht der Fischerei Industrie bezüglich der

Aufzucht von Hummern in Wind Parks und aus Sicht des Tier-/Meeresschutzes betreffend der Auswilderung von gezüchteten Larven in dem Bereich gilt, muss mehr Forschung betrieben werden um ausschließen zu können, das eine Störung der Anti-Rüber Reaktion der Tiere auch im Feld zu finden ist. Sollten neue Studien dies bestätigen könnte es der Nachhaltigen Erhaltung und Nutzung der Tiere entgegenwirken.

1. Introduction

The European lobster *Homarus gammarus*, first described by Linnaeus in 1758, is a species of lobsters which can be found from Norway to the Mediterranean Sea, all along the northern European coast, the British Isles and Morocco coast. Beside its cultural and economic importance, *H. gammarus'* ecological role as predator/competitor species demonstrates its significant value for the local ecosystem (Boudreau and Worm, 2012).

The fishing of *H. gammarus* has been present for several centuries, especially around the Island Helgoland where the Lobster Fishery defined its local culture as well. Local fisheries peaked around the early 1900s until 1930s with landing number of up to 87 000 lobster in 1937. Following this peak, landing numbers decreased and continued to decrease after the second World War and nearly collapsed 1992, when only 102 lobsters were caught (Anger & Harms, 1994). Even though the precise causes for the decline are still unknown due to the lack of details in the fishery data, several speculations as to why still exist. After the end of the second world war, Helgoland was used by the British Royal Air Force as training grounds for Bomber targeting. This and the attempt to eradicate the island itself by blowing up 6 000 tons of ammunition could be responsible for destroying lobster habitat which might has included several important nursery grounds. Additional potential sources could also explain the decline such as harbour creation, oil spills (Walter, 2006), over-exploitation by the local fisheries or a combination of several of the aforementioned causes (Anger & Harms, 1994; Franke & Gutow, 2004).

In the present days, the European lobster is categorized as stable by The International Union for Conservation of Nature (Butler et al., 2009), reason being that *H. gammarus* can still be found in many areas and that its population decline was not globally severe and persistent over time (Dow, 1980). The population around the rock base of Helgoland is still weakened and has yet to recover completely. While the numbers of specimens declined around the island, the population of the edible crab *Cancer pagurus* drastically increased. The two species have always shared the same habitat, but this competition from the crab seems to have increased significantly enough to outperform *H. gammarus* in terms of food gathering and shelter use (Franke & Gutow, 2004).

In the year 2000, the Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung/ Biologische Anstalt Helgoland started a project to increase the understanding of the current state of the local lobster population, which included breeding of juvenile lobsters, tagging and release of 1-year old juveniles. Since 2018 this responsibility has fallen to Reefauna, a conservation company that specializes in the rearing of reef living animals.

Lobster females usually breed in the summer months from July to August immediately following moulting. Embryonic development of the eggs takes around 9 to 11 months and the new larvae hatch between May and August, when water temperatures reaches approximately 16 °C (Mehrtens ,2011). Newly hatched lobster larvae tend to immediately swim towards the surface (Dunn & Shelton, 1983), reacting to light, to disperse better (Schmalenbach & Buchholz, 2010). During the first three stages, larvae stay in the pelagic zone near the surface where they are mostly opportunity feeders, feeding on passing phyto- and zooplankton (Ennis, 1995). Once they reach the post-larval stage IV, larvae will slowly migrate to benthic habitats retaining their filter feeding capabilities and mostly hiding by burrowing themselves into the substrate or hiding in more complex shelters like rocks, seaweed and reefs (Lawton & Lavalli, 1995; Able et al., 1988). Early juveniles (stage V) are mostly still restricted to their shelter and its immediate surroundings, but are already seen to start foraging during nighttime (Lawton & Lavalli, 1995).

Throughout the larval and juvenile stages, the lobsters are most vulnerable to predation. Especially during the early pelagic life and during settling to lower benthic habitats probably due to insufficient hiding places (Ennis, 1995). Potential predators for the lobsters are visual oriented fish like the shorthorn sculpin (*Myxoxocephalus, scorpius*), the long-spined bulhead (*Taurulus bubalis*) and the rock cook wrasse (*Centrolabrus exoletus*) (Schmalenbach & Buchholz, 2013). Besides fish predation, mutual competition can also occur with the edible crab *C. pagurus* (Frank & Gutow, 2004) and the common shore crab *Carcinus maenas* (Van Der Meeren, 2005). Marine construction and mobile fishing gear like dredges and trawlers can disturb the lobster habitats and destroy potential shelters (Watling, 2005; Løkkeborg, 2005), which are paramount for lobster survival.

In order for the lobster populations to recover, it is indispensable that the successful recruitment of artificially or naturally reared lobster larvae and juvenile is not thwarted by anthropogenic influences. Considering the continued globalization of the ocean it becomes even more important to quantify the effect that human interference may have. A few of these effects that have been extensively researched are the ones associated to elevated pCO_2 on larval development (Agnalt et al., 2013, Small et al 2015/2016), reduction of seawater alkalinity during moults (Middlemiss et al., 2016) as well as increased water temperature (Schmalenbach and Franke, 2010).

Anthropogenic noise levels in the ocean are rising with human activities, such as fishing, marine trading routes, and offshore energy growing industry. Noise as a possible source of ocean pollution has been officially recognised by several legislations on the national and global scale (e.g. European Commission Marine Strategy Framework Directive and the US National Environment Policy Act). While the effects of noise on fish and marine mammals have been under the spotlight since decades, marine invertebrate research data remain relatively scarce, with an increasing interest only in the past few years. Research on noise effects on invertebrates can be roughly split into physiological effects and psychological/behavioural effects, with the latter being, again, scarcely researched at present.

In terms of physical impacts, effects range from change to early organism development to increased stress reactions and immune-system response. For example, de Soto et al. (2013) observed that scallop larvae (*Pecten novazelandiae*) that were exposed to long term intervals of airgun noise (mostly used in seismic surveys) caused malformations and a delay in development compared to their control group. An increase embryonic development failure could be observed in the sea hare *Stylocheilus striatus* following exposure to boat-noise, theorized to be due to stress or barotrauma (Nedelec et al., 2014). Boat noise was found to elicit an increased oxygen consumption due to stress in the shore crab *C. maenas* (Wale et al., 2013 a) and resulted in strong immune-system responses in the European spiny lobster *Palinurus elephas* (Celi et al., 2015).

On the other side of the spectrum, noise has shown to influence behavioural patterns of invertebrates, for example reducing foraging and anti-predator behaviour in *C. maenas* (Wale et al., 2013 b). Noise has the capability to mask and distract other sensorial cues which could lead organisms ignoring possible indications for the presence of predators such as in the experiment by Tidau and Briffa (2019). Their experiment showed that hermit crabs *Pagurus bernhadus* would ignore visual cues from a *C. maenas* model, representing a predator when choosing a new shell. Chemical cues detection capacity for new available shells were reduced in the hermit crab *Pagurus acadianus* under an impulsive noise source (Roberts and Laidre, 2019).

One of the possible sources of noise in the ocean that coincides closely with the theme of this thesis comes from Wind Turbines and Wind Parks. The expansion on the use of renewable energy sources is especially big in the North Sea where, in addition to the already constructed Wind Parks, several more are in planning or under construction (4C Offshore). While Wind Parks are a considerable source of noise in the ocean, Krone et al. (2017) believes that Wind parks are a potential habitat for benthic living organisms especially for those that prefer hard substrates. They found a distinct population of *C. pagurus* living at the base of several Wind Turbine Foundations, predicting that those foundations would serve as habitat the same way that shipwrecks do already. The European lobster would benefit especially from these new habitats since ship traffic is restricted within the zones of the Wind Park (SeeAnlV 1997; Berkenhagen et al., 2010). The remaining question is what kind of effect the noise produced from these new habitats could have on the newly settled organisms. Besides temporary noise exposure during construction, permanent exposure produced by the turbine itself should be considered.

In this thesis, it has been tested, if noise had an effect of the behaviour of juvenile lobsters during night-time and if the behaviour would change in the presence/absence of a predator. In addition, it has been investigated the effects of noise on the shelter preference by providing two choices of shelter: rocks and empty shells of the European oyster *Ostrea edulis*. Increased fishing that lead to overfishing, population destroying diseases and importation of foreign species had resulted in a near complete extinction in several regions (Héral & Deslous-Paoli, 1991). Their ability to create biogenic reefs by settling on top of each other, they could have potentially been used by juvenile *H. gammarus* as a shelter rich habitat (PROCEED, 2020). With the decline of the species the possibility as habitat but also other beneficial effects on the ecosystem have been lost. There have been multiple attempts at restoring the European oyster with minimal a success rate. A new project of AWI called PROCEED aim to successfully re-introduce *Ostrea edulis* into the German North Sea, thanks to the research done by a prior project: RESTORE. Early field-testing success in 2017 lead to the planning of a pilot reef creation in the Borkum Reef (RESTORE, 2020; PROCEED, 2020). Additionally, PROCEED is creating an oyster hatchery at AWI Helgoland to ensure a surplus of young oysters (PROCEED, 2020). A successful re-introduction of the European oyster could increase the availability of hard substrate for lobster in the future, creating more habitats and potential shelter and possibly helping in the restoration of the German lobster populations.

2. Methods

2.1 Experimental setup

Two rows of eight tanks were installed inside a temperature-controlled room set to 18° C. Each tank (56.6 x 36.0 x 42 [cm]) was arranged in the same way; 1.5 cm of sand at the bottom and filled with sea water (approximately 52 L). Two shelters made of rocks (6 - 14.5 cm) and oysters (9 - 12 cm) were arranged in the tanks, one in each corner diagonally opposite from each other. It is important to note that the area covered by the shelters was the same with sand strip in between to better observe the lobster behavior at the shelter edges (Fig. 1, right).

The tanks were divided into four different treatments: Control (C), Noise (N), Predator (P) and Noise + Predator (NP). Every day, two replicates of these treatments were assigned randomly to eight tanks using a software (miniwebtool.com/random-picker/). Since the experiment uses living predators, a small cage was made (12 x 5 x 6.5 [cm], mesh size: 5 mm) to hold the crab (*Carcinus maenas*) and not harm the lobsters which were borrowed from Reefauna, a lobster restauration company. In total, 104 lobsters (n = 26 per treatment; mean and standard deviation total length of 12,29 ± 0,97 mm) and 8 crabs (mean and standard deviation total length of 6.07 ± 0.38 mm) were used in the experiment over a timespan of thirteen days (from January 7 to 21th 2020). The contraption was composed of a waterproof diver egg attached to the cage and a tetrapod (179.8 g) that was fixed underneath (Fig. 1, left) to keep the cage and egg suspended in the middle of the tank (Fig. 2, right). In the N and NP treatments, a mobile phone vibration motor was enclosed in the diver egg to create a noise source of approximately 110 Hz in frequency (De Jong et al., 2017). In C and P treatments, the diver eggs were filled with rocks that had a weight similar to the motor. A crab was placed in the cage for the P and NP treatments, and the cage was left empty for C and N treatments.

At the top of the tanks, a makeshift scaffolding to hold small cameras (MQ8 Mini Camera, Shenzhen Qilesi Electronic Commerce Co, China) was placed approximately 30 cm above the tank to record the lobster behaviour during the experiment. On each tank row, four Mitras Lightbars (GHL) were used to illuminate the tanks during the experiment. They were hooked up to a Profilux light computer (GHL) that programmed the lights to red to avoid disturbing the lobster's natural clock, since the pigment of the lobster eye is not sensitive to red-light frequencies (Wald & Hubbard, 1957; Kennedy & Bruno, 1961).

Figure 1. **Left:** The contraption used for the experiment was composed of a waterproof diver egg attached to a cage and a tetrapod that was fixed underneath to keep the cage (12 x 5 x 6.5 [cm], mesh size: 5 mm) and egg suspended in the middle of the tank. In the noise and noise + predator treatments, a mobile phone vibration motor was enclosed in the diver egg to create a noise source of approximately 110 Hz in frequency (De Jong et al., 2017). In control and predator treatments, the diver eggs were filled with rocks that had a weight similar to the motor. A crab (*Carcinus maenas*) was placed in the cage for the predator and noise + predator treatments, and the cage was left empty for control and treatments. **Right:** Tank Layout (56.6 x 36.0 x 42 [cm]), oyster shelters and similar sized rock shelters, shelters are divided by sand strips to better observe the lobster behavior at the shelter edges*.*

2.2 Preparations and procedures

Each day, eight tanks were used to conduct the experiment. Since the seawater that was used to fill the tanks was cold, the tanks were filled the day before and left overnight to acclimate to the temperature of the TK room. Every day, before running the experiment, salinity (Cond 3110 SET 1 [WTW]), pH (pH 315i/SET [WTW]) and temperature (Pocket-DigiTemp Insertion thermometer (short version) [DOSTMANN electronic GmbH], Germany) were measured. Temperature was measured one hour before each day’s run to make sure that the ideal temperature was reached. The four crabs used in the experiment were collected 45 minutes before conducting the experiment to avoid keeping them in the small cage for longer than necessary. Similar to the crabs, the juvenile lobsters were collected in the dark ten minutes before the start to avoid day-night-cycle disturbances.

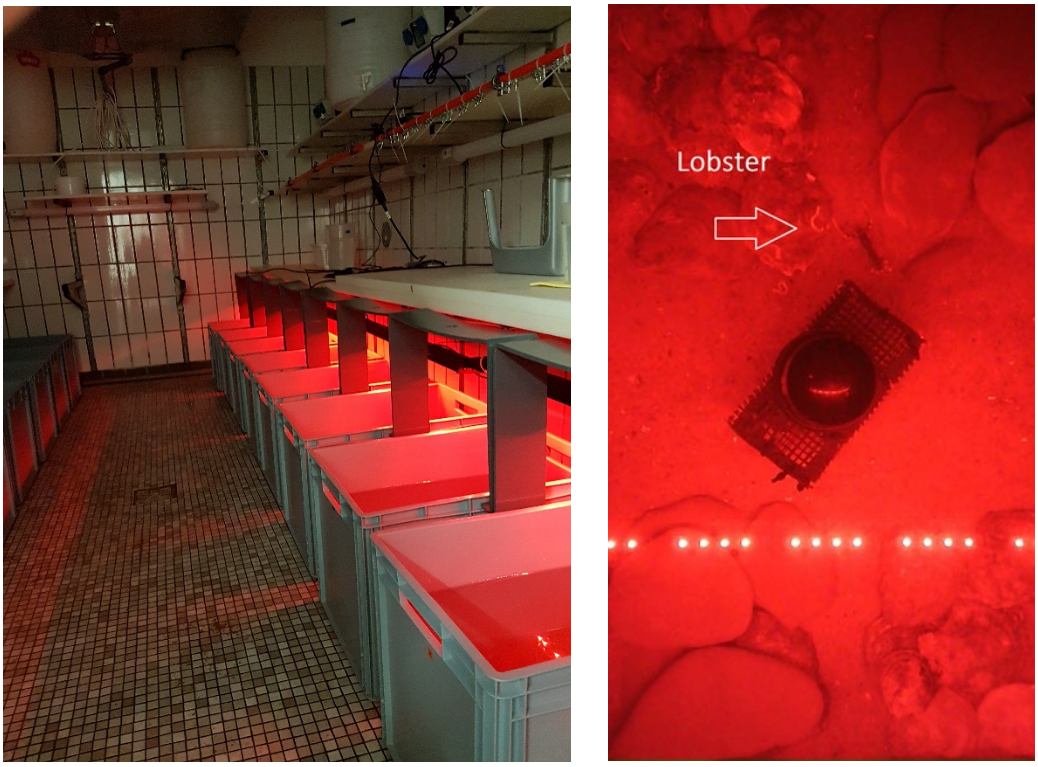

Figure 2. Left: General view of the *experimental setup and the illumination.* ***Right:*** *Tank Layout during the Experiment at night, contained in the cage underneath the noise egg is the predator. The lobster can be seen moving around the box. Picture depicts noise and predator treatment.*

The experiment started every day at 18:00 CET. The lobsters were placed inside the tanks and the cameras placed onto the scaffolding and setup to record for approximately two hours. The lobsters were kept in the tanks for three hours until 21:00 CET. At the end of the trial, the shelter where the lobster was found was registered. The eight lobsters were measured with a Vernier and returned to Reefauna tanks, while the four crabs used as predators brought back to their tank. A new set of eight lobsters was used every day, but the two sets of 4 crabs alternated every other day. After returning the animals, two calibrated SM3 hydrophones (sensitivity of -165 dB re: 1 V/uPa, Wildlife Acoustics Song Meter Automated Audio Recorder SongMeter, Wildlife Acoustics Inc., USA) were installed at 10 cm from the noise source to measure the level of noise inside the tanks and to ensure that the noise tanks (N or NP) would not affect the tanks next to it (C or P). The noise egg produces sound at a frequency of 110 Hz, in order to detect this sound only 220 Hz are necessary. The sample rate was set to 44100 (44.1 kHz) samples per second. When using 44.1 kHz instead the creates a frequency power spectrum with a higher resolution. The hydrophones were left in the tank overnight and recorded from 0:00 CET to 4:00 CET.

2.3 Analysis of Behaviour Footage

Before starting the analysis of the recorded footage, certain behavioural patterns were established that would serve as a focus and would simplify the process. Some cameras had technical issues, and some of the lobsters were thus not considered for the behaviour analysis. The total number of footage available is 86 (control n = 23, noise n = 19, noise + predator n = 22, predator n = 22).

These behavioural patterns were:

- Peeking: the lobster would, most of the time slowly and cautiously, move its upper body out from the shelter scanning its surroundings, usually with its claws vertically parallel to each other (Wickins et al., 1996).
- Shelter: combination of burrowing and pushing sand to fortify its shelter (focus on sand moving while analysing) (Botero & Atema, 1982).
- Exploration: observed when the lobster would leave its shelter with its whole body, completely exposing itself (Cooper & Uzmann, 1980; Mehrtens et al., 2005).
- Escape: defined by extremely quick backwards movements after being frightened, for example when getting too close to a crab (Lang et al., 1977).
- Movement/Moving: this pattern is quite broad and encompasses movement seen through the holes or the edges of the shelter and behaviour too short or undecipherable to assign to any other pattern (developed on my own criteria).
- Hidden: no behaviour recorded. Lobster is assumed to be hiding when not seen in the footage.

The behaviours that occurred in the first five minutes of footage were ignored to account for acclimation in the conditions of the experimental unit. Only the chosen shelter and the time it took to hide was noted during this acclimation time. Then, the 20 following minutes of footage was analysed and the active time doing each behaviour was recorded (in seconds). Only behaviours that lasted at least one second were analysed.

2.4 Data analysis

For the statistical analysis, RStudio (R Core Team, 2020) was used. To assess the power spectral density of each treatment, the package 'psd' (Barbour &Parker*,* 2014) and 'tuneR' (Ligges *et al.,* 2018) were used. The package 'dplyr' (Wickham et al. 2020) was used for data management, while data mean and standard deviation were calculated using 'doBy' (Højsgaard & Halekoh, 2020). Data were tested for normality and homogeneity and were transformed if necessary, to meet parametric assumptions. Since normality and homogeneity could not be achieved with data transformation, Kruskal-Wallis (KW) was used to test for significant variations for each behaviour instead of a one-way analysis of variance (ANOVA). For this case, post-*hoc* non-parametric multiple comparison test was done using 'pgirmess' (Giraudoux, 2018). For all the statistical tests, significant difference was set at $p < 0.05$. The package 'ggplot2' (Wickham, 2016) was used for data visualization.

3. Results

3.1 Hydrophone measurements

To prove that there was actual noise in the respective treatments and that this noise did not influence the other treatments, two tanks, one with and one without noise were tested for noise using hydrophones. The experimental tanks set with the "noise egg" (Fig. 3 B, D) showed several frequency bands (harmonics) compared to the tanks without the vibration motors (Fig. 3 A, C).

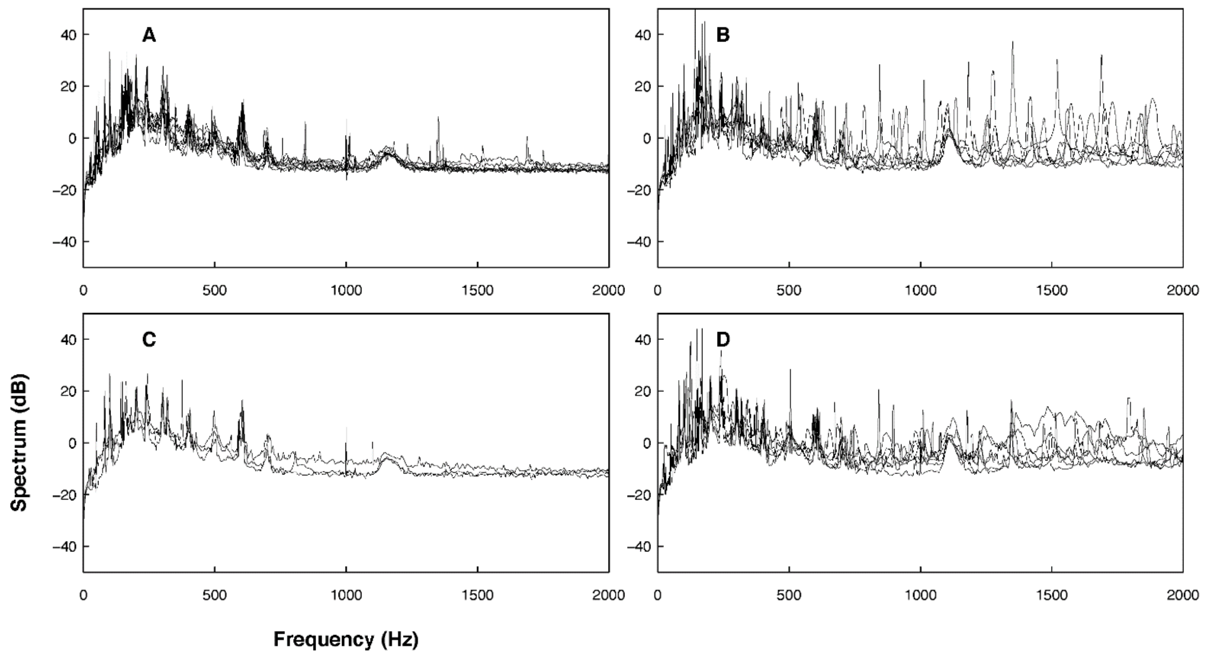

Figure 3. Noise power spectrum density for each treatment, A = control, B = noise, C = predator, D = noise + predator

3.2 Shelter preference and side bias

For the shelter preference, the initial choice (first choice of the lobster during the 5 min acclimation) and the final choice (the shelter where the lobster was found after 3 h) were compared for each treatment (Fig. 4). Oyster were equally chosen by juvenile lobsters as shelter compared to rocks only for the control treatment in the initial choice. Rocks were the more preferred shelter for the three other treatments, both at the beginning and at the end of the trial.

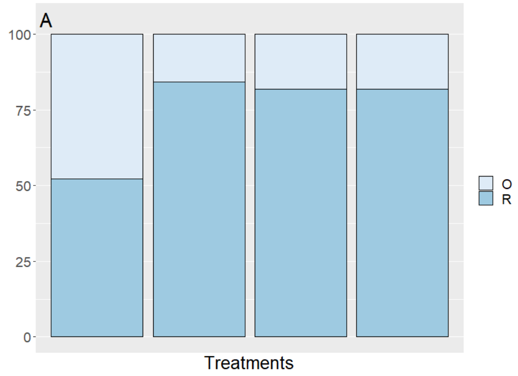

Figure 4. Shelter preference during the first 5 min acclimation (A) and at the end (B) of the experiment (3h after) for each treatment. O = Oyster shelter, R = Rock shelter,

Since the lighting over each tank was not evenly distributed and therefore more prominent on one side than on the other, testing for a distinct bias was necessary. For this a rank was assigned for every time a shelter on the top side or the bottom side of the tank was chosen. Important to note is that the top side is where the lights was stationed and were illumination was most prominent. The same method as for the shelter preference was used, to see if the choice changed over the course of the experiment (Fig. 5). Whereas at the beginning the bottom seems to be more preferred, the distribution was relatively balanced at the end of the trial. No strong bias relatively to the light exposure was thus considered for this experimental design.

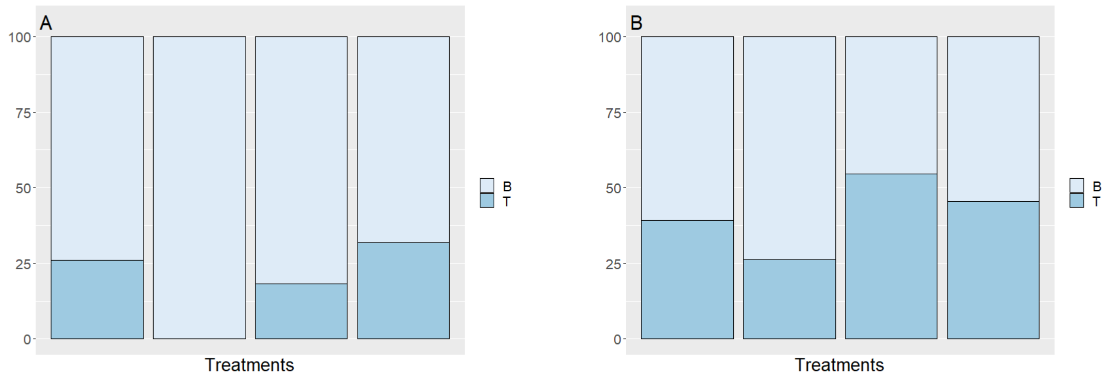

Figure 5. Side preference during the first 5 min acclimation (A) and at the end (B) (3h after) for each treatment. B = Bottom side (darker side), T = Top side (brighter side)

3.3 Behavioural analysis

The primary focus during analysis were the different behavioural patterns established in the methods section and the active time doing each behaviour or remaining hidden.

The lobsters spend 78 % of the time analysed completely hidden inside their shelter. The remaining 22 %, were split between the observed behaviours. Looking at the total active time on each behaviour for every treatment, shelter (mean and standard deviation for all treatments: **control** 85.43 ± 130.58, **noise** 68.16 ± 83.02, **noise + predator** 138.77 ± 174.85, **predator** 54.41 ± 53.47) and exploration (mean and standard deviation for all treatments: **control** 50.65 ± 56.31, **noise** 38.53 ± 61.94, **noise + predator** 119.23 ± 82.78, **predator** 66.50 ± 98.17) were the dominant ones (Fig. 6).

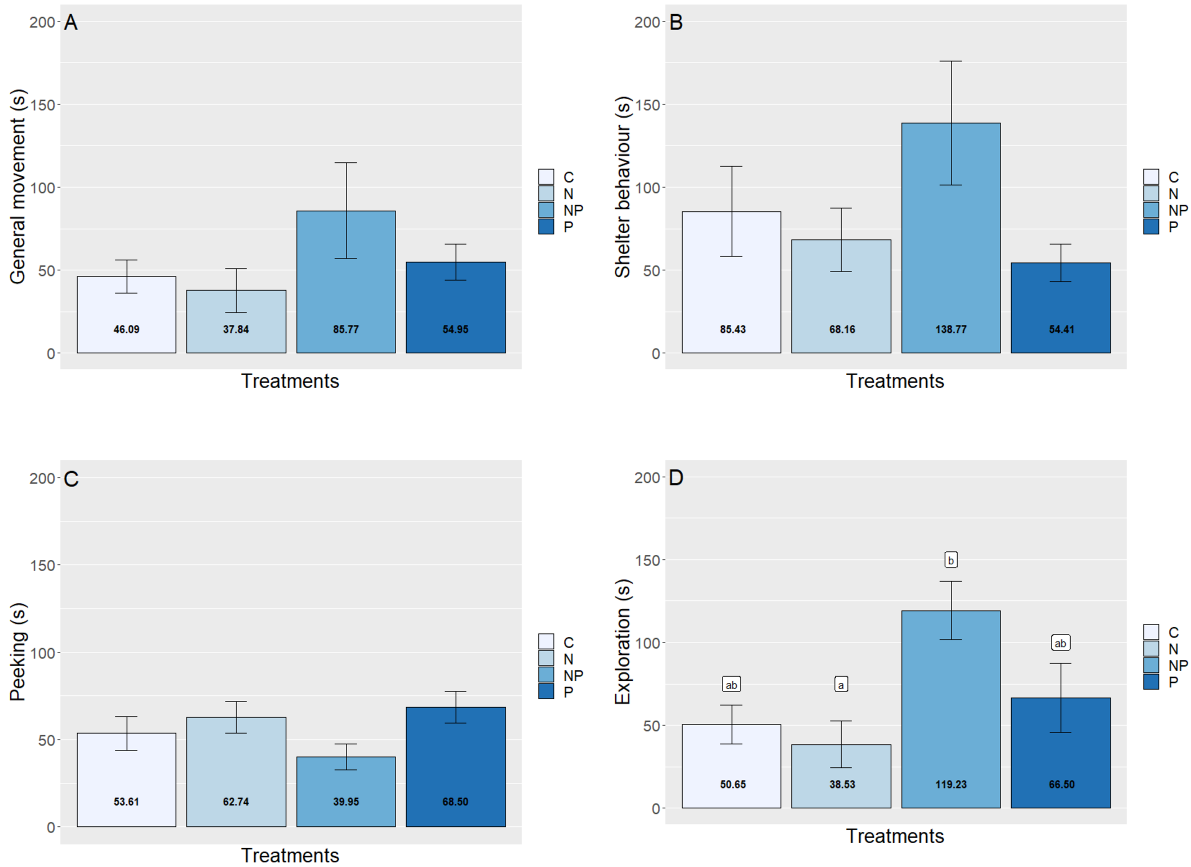

Figure 6. Mean and standard error of the active time of each behavior in (s), for every treatment: Control [C], Noise [N], Noise + Predator [NP] and Predator [P]. **A:** general movement, **B:** shelter behavior, **C:** peeking, **D:** exploration. Lower-case letters indicate significant differences ($p < .05$) among treatments.

A significant difference (KW, chi-squared = 12.588, df = 3, p-value = 0.005619) was recorded on lobsters doing the exploration behaviour (Fig. 6 D). The multiple comparison test denoted that juvenile lobsters under noise + predator condition were exploring more than the ones under noise treatment (observed difference = 25.7, critical difference = 20.6). This data is reinforced by the amount of lobsters that changed shelter during the experiment, mimicking the trend seen in the exploration behaviour (Fig. 7) No other significant differences were detected for the other behaviours, but similar higher trends were observed in the noise + predator condition concerning general movement and shelter behaviours (Fig. 6 A, B). A lower trend in the same treatment was observed for the peeking behaviour (Fig. 6C).

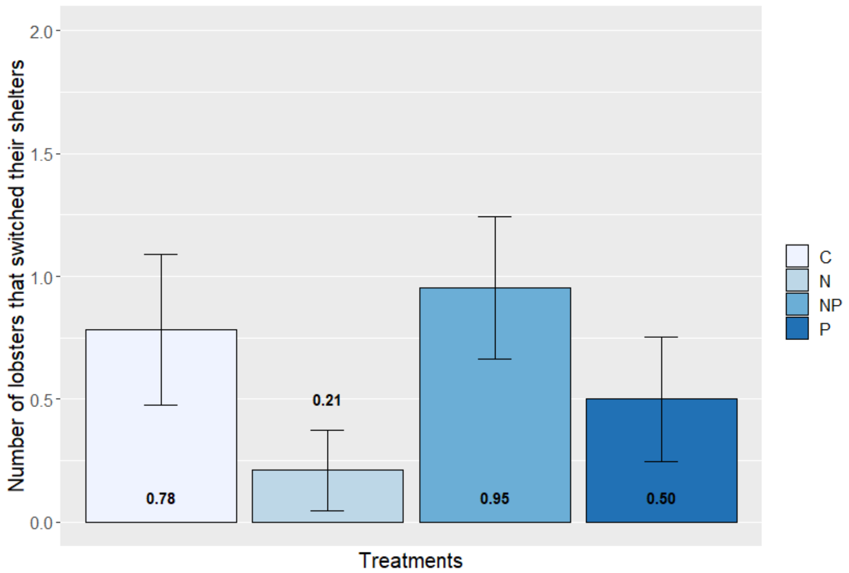

Figure 7. Mean and standard error of the number of individuals that changed their shelter during the experiment for every treatment, C = control, N = noise, NP = noise + predator, P = predator.

4. Discussion

When it came to shelter preference, the lobster preferred rocks over the oysters in all treatments, some of them having switched shelter during the experiment. The behaviour analysis shown that noise seems to have no effect on its own, with the noise treatment data closely resembling that of the control treatment for every behaviour. Same for the predator treatment, but when noise and predator treatments were combined, the behaviour starts to differ. In this combined condition, the lobster increases their activity in exploration, shelter behaviour, and general movement in comparison to the other treatments.

4.1 Shelter Preference

The results from the experiment show clearly that the lobster preferred the rocks in all treatments over 70 % of the time. Even though at the beginning in our control unit nearly half of the lobster chose a shelter consisting of oyster shells, over the timespan of the experiment most of them switched to one of the two shelters made of rocks. Those lobsters that choose oysters at the beginning of the trial corresponded to the individuals that switched shelter during the 20 video minutes analysed of the control treatment. Tests done on *Homarus americanus* confirm this notion, that the lobster prefer rocks and boulders over other provided shelter when they get the possibility to choose (Wahle & Steneck, 1991; Botero & Atema, 1982). Even if lobsters prefer a rockier substrate as shelter, fact of the matter is that the German Bight and the North Sea consist of mostly soft substrate with a few hard substrates around Helgoland and a marginal number of glacial boulder reefs (Figge, 1981; reviewed in Krone et al., 2017 & BAW, 2013). Soft substrates like sand and mud do not provide sufficient shelter against predators (Lavali & Barshaw, 1986; Barshaw & Lavali, 1988). The results could stem from the arrangement of the oyster shells, noise as a source was excluded since there was no noticeable difference between the noise and control treatments. In the experimental setting, the oyster shells were loosely stacked besides and on top of each other creating a feeble covering that would shift even in response to movement from the lobsters, making it less effective against bigger predators. In reality, a reef made of *Ostrea edulis* shells should create more complex and sturdy shelters. This is definitely something worth looking into more details since every possible addition of hard substrate to lobster habitats could potentially lead to an increase in survival.

4.2 Behavioural analysis

When noise and predator were combined, certain lobster behaviours increase in activity. In particular, exploratory behaviour of these lobster was significantly increased compared to the lobsters exposed to noise only. This promotes the idea that noise by itself does not influences the lobster's behaviour but that only the combination of the two stimuli of noise pollution and predation do in fact create a response. It was therefore theorized that noise pollution could influence a lobster's anti-predator behaviour. This reaction has been observed in numerous other taxa in both aquatic and terrestrial habitats. An experiment done on seabass (*Dicentrarchus labrax*) tested its response to two different kinds of noise pollution, a steadily increasing noise in form of drilling noise and a rapidly increasing noise in form of piling (Spiga et al., 2017). The seabass reacted with startle responses to piling noise and an overall increased ventilation rate indicating a higher stress level under noise exposure in general. In addition, the noise was affecting the seabass' anti-predator behaviour when exposed to noise and the simulated predator, responding differently to both of the different noise pollutants. While under normal circumstances the fish would decide to stay immobile when confronted with a predator, exposure to piling noise lead to increased turning behaviour and less time spent in the safe zone (zone of the tank furthest from the predator). Drilling noise in comparison lead to the fish spending most of the time inside the safe zone. While the fish appeared to exhibit increased vigilance, they also reduced their predator inspection behaviour (Spiga et al., 2017). A different experiment done on the response of the two sympatric fish species *Gasterosteus aculeatus* and *Phoxinus phoxinus* to boat noise resulted in *G. aculeatus* responding quicker to predator stimuli, but in turn spend less time on foraging and reduced foraging success, while *P. phoxinus* did not react to the noise pollution at all (Voellmy et al., 2014).

The anti-predator behaviour of juvenile *Homarus* spp. revolve around their ability to find a suitable shelter. While additional behaviour exists, ranging from tail flipping (Lang, 1977) to freezing and slowly retreating to the next shelter (Johns & Mann, 1987), survival is unfavourable without a shelter (Wahle & Steneck, 1992). To summarize, lobster anti-predator behaviour consists of predator avoidance strategies with an important emphasis to hide inside sturdy shelters. In my experiment, lobster's activity increased under noise + predator exposure, which contradicts normal anti-predator behaviour. Whale (1992) was the first to provide evidence that *H. americanu*s can detect predators simply by olfactory cues beside visual stimuli. Considering this fact, if the lobsters in the present study were for some reason not able to visually confirm the presence of the predator that was held in a cage above the noise source, they should have been able to sense the crab chemically.

In conclusion, noise pollution can interfere with the lobster's anti-predator behaviour, specifically by distracting it. Animals are shown to possess a finite amount of attention that they have to divide to different tasks, with stress and other stimuli able to affect this and subsequently ensure a distraction (Mendl, 1999). This is similar as with the aforementioned experiment including the European seabass, were they also concluded that the behaviour changes were a result of distraction via noise pollution. While there is the possibility that the observed behaviour is not a result of distraction but masking, this is usually connected with the noise overshadowing other similar auditory cues. For example, Jung et al. (2020) showed that traffic noise impacted the behaviour of two American songbirds, titmice

(*Baeolophus bicolor*) and nuthatches (*Sitta carolinensis*), to respond to predator calls with alarm calls of their own. Despite that *H. americanus* was observed to have the necessary receptor organs to theoretically receive sound signals (Budelmann, 1992), there is to date no research suggesting that they are able to use these to detect predators. Therefore, the possibility of the noise masking specific stimuli was excluded.

In case that some juvenile lobsters decide to migrate to some of the Wind Parks in the German Bight in search for a new habitat of hard substrate, the results of this study could impose several problems that would offset the increased survivability gained from a potentially more secure habitat. The success for survival at the Wind Turbines consists of a multitude of variables that could be either beneficial or disadvantageous all together. While the results show that noise influences the anti-predator behaviour of *H. gammarus*, it is not yet clear if this will indeed have an adverse effect on the lobster's survival. Thus, more studies need to be carried out. There is always the possibility that not only the prey but also the predator is affected by the noise. Indeed, studies on *Carcinus maenas* showed reduced foraging behaviour during noise exposure, increased stress reactions and reduced anti-predator behaviour (Wale et al., 2013a, Wale et al. 2013b). Considering that lobsters also use olfactory cues to detect food (Mackie, 1973), European lobsters could also show reduced foraging behaviour partly explained by the distraction effect shown in this study.

Also, still unknown are the effects of long-term noise exposure on lobster behaviour and the potential of sensitization or habituation and possible long-term damage that could persist even after exposure ended. As well as the possibility of noise produced particle motion to create soil vibrations that could also influence invertebrate and lobster behaviour respectively (Roberts & Laidre, 2019). While the results show that noise influences the anti-predator behaviour of *H. gammarus*, the fact that the experiment was done under laboratory conditions should not be disregarded. In addition, the noise source is supposed to simulate as closely as possible the effects of a Wind Turbine, but is unable to correctly emulate the *in situ* equivalent. The constraints of in-tank testing could also result in reverberating particle motions, bouncing of the tank walls and extrapolating the noise exposure in the test animals (Nedelec et al., 2016). The sound source used in the present experiment is a cheap and easy to replicate source for noise that can increase the amount of data currently available on effects of noise pollution by making research more viable, and cost efficient (De Jong, 2017). Further, the egg can be used in the field, which open the door for an infinity of future studies without tank boundaries (De Jong, 2017).

In summary, the results of this study still showed the potential impact the effect of noise on anti-predator behaviour in *H. gammarus* could have. Further research has to be conducted to uncover the unknown variable that still play a role in the effect of noise pollution on the entire benthic community, some of which have been suggested above.

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Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig verfasst und gelieferte Datensätze, Zeichnungen, Skizzen und graphische Darstellungen selbstständig erstellt habe. Ich habe keine anderen Quellen als die angegebenen benutzt und habe die Stellen der Arbeit, die anderen Werken entnommen sind – einschl. verwendeter Tabellen und Abbildungen – in jedem einzelnen Fall unter Angabe der Quelle als Entlehnung kenntlich gemacht.

Bielefeld, den 17.08.20

Sön Scholz

Unterschrift