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To hum or not to hum: analyzing and provoking sound production in the American lobster (Homarus americanus)

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An Honors Project for the Department of Biology
By Renske Kerkhofs

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Abstract

American lobsters (*Homarus americanus*) produce humming sounds by vibrating their carapace. These sounds have a fundamental frequency on the order of 100 Hz, with multiple higher harmonics. Though I found no relationship between lobster carapace length and hum frequency, I observed sounds similarly structured to hums but with frequencies an order of magnitude higher, suggesting that lobsters may use a wider range of sounds than previously thought. Using laser vibrometry, I was able to pick up high frequencies of carapace vibration that were similar to those I observed on sound recordings. Lobsters seem to hum most readily when approached from above, but many studies have found it difficult to reliably find soniferous lobsters. To find a way to reliably evoke sound production in American lobsters without contributing to the sound environment, lobsters were exposed to overhead abstract visual stimuli on a screen, after which their behavioral reactions were recorded, as well as any sound production in response to the stimulus. Lobsters responded to the screen stimulus with the same types of behaviors with which they responded to general overhead physical stimuli. This study demonstrates that American lobsters may produce high-pitched sounds and that abstract visual cues can be used as a silent tool to elicit lobster behaviors, but not sound production.

Introduction

From insects to whales, and from mice to fish, many different animals make noise, and for many of them, this noise is a vital part of their daily communication (Fletcher, 1985). Some animals are more famous for their noises than others, however. Lobsters have been known to make noise since the end of the 19th century (Moulton, 1957), but this does not seem to be common knowledge, and the reason for the sounds they make remains largely unknown (Fish, 1966). My honors thesis explores the characteristics of the sounds of the American lobsters (*Homarus* *americanus*) and tests a novel approach of evoking sound production through abstract overhead screen stimuli.

What is a lobster?

In everyday language, we commonly refer to many different species within the order Decapoda as lobsters due to their relatively similar appearance. This causes an issue when discussing acoustics, however, since the different groups we refer to as lobsters do not produce sounds in the same way, or for the same reasons. Most recently, an updated phylogeny shows that the animals we refer to as lobsters belong to three infraorders of the order Decapoda (Fig. 1): Polychelidae, Achalata (which includes the Palinuridae and the Scyllaridea), and the Astacidea (which includes the Nephropidae, the Parastacidae, the Cambaroididae, the Astacidae, and the Cambaridae) (Wolfe et al., 2019). The members of the Astacidea infraorder look most like the common image of a lobster for people in the northeastern part of the United States; they have

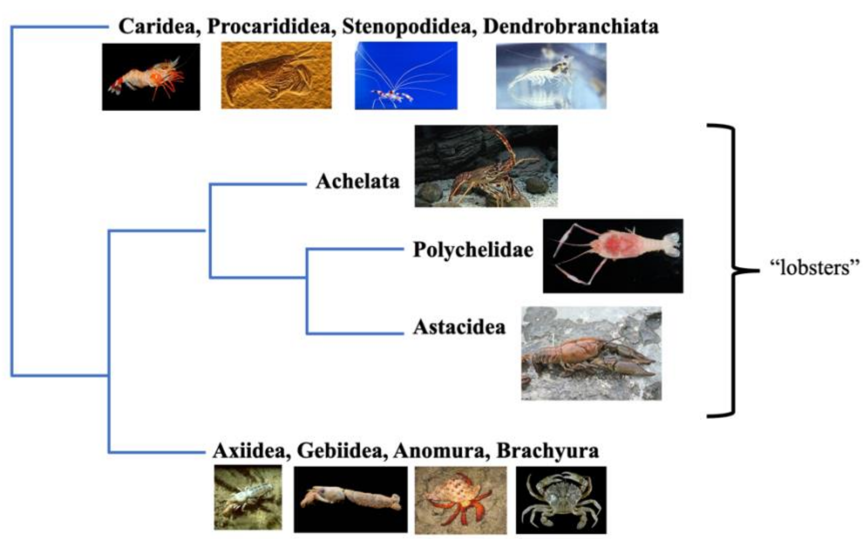


Fig. 1. Simplified figure based on Wolfe et al. (2019) showing the phylogeny of what is commonly referred to as lobsters, as well as some of their close relatives. Images from WikiMedia Commons.

large claws and thin, long antennae. The other two groups also look like lobsters at first glance, but they have some significant differences in morphology. The Polychelidae lobsters have claws that are much longer and thinner, whereas the Achelata lobsters lack claws altogether, and have much more pronounced, thick antennae. These morphological differences are important, as they inform differences in sound-producing mechanisms in different groups of lobsters.

Lobsters produce many types of sound

A variety of lobster sounds have been observed, as well as multiple mechanisms by which different types of lobsters produce those sounds. Moulton (1957) identified three different sounds in spiny lobsters (*Panulirus argus*), which he called rasps, rattles, and abdominal contractions. Two – the rasp and the rattle– are made by raising the antennae, which causes them to rub over a toothed ridge on the carapace. The toothed ridge Moulton (1957) described has since become known as the plectrum, which has been found to interact with a microscopically toothed file at the base the antennae (Meyer-Rochow and Penrose, 1976; Patek, 2002). The sticking and slipping of the smooth plectrum rubbing over the file as the antennae move causes a noise to be heard with each slip (Patek, 2001). The third type of sound, the carapace vibration, is produced by the contraction of two sets of muscles inside of the carapace, and sounds like a low buzz (Moulton, 1957). Additionally, Meyer-Rochow and Penrose (1976) found that western rock lobsters (*Panulirus cygnus*) grind their mandibles not only during feeding but also during moments of high stress, producing a crunching sound. In other words, though lobsters certainly make sound, they do so in different ways, based on their anatomy.

American lobsters ‘hum’ by vibrating their carapace

The carapace vibration is the only type of sound that American lobsters (*Homarus americanus*) are known to make, as they lack a plectrum and are therefore unable to rasp or rattle (Fish, 1966; Ward et al., 2011). The carapace vibration and its resulting noise are produced by the contraction of the antagonistic remotor and promotor muscles, located under the second antennae (Fig. 2). The remotor muscle appears to play a dominant role in noise production, as its incapacitation eliminates lobsters' ability to vibrate their carapace. Though sometimes lobsters use both the remotor and promotor muscles at the same time when producing sound, the promotor seems to play a secondary, unknown role (Henninger and Watson, 2005). In contrast, in spiny lobsters the promotor muscle is the main muscle used in sound production, as it is the muscle that moves the antennae to stick and slip and create a rasping noise (Patek, 2003). Since incapacitating the promotor muscle does not inhibit *H. americanus* from making sound, but these muscles are in fact engaged when producing carapace vibrations, Henninger and Watson (2005) hypothesized that the function of the promotor muscle might be to maintain waveform and intensity of the sounds by modulating the tension of the carapace.

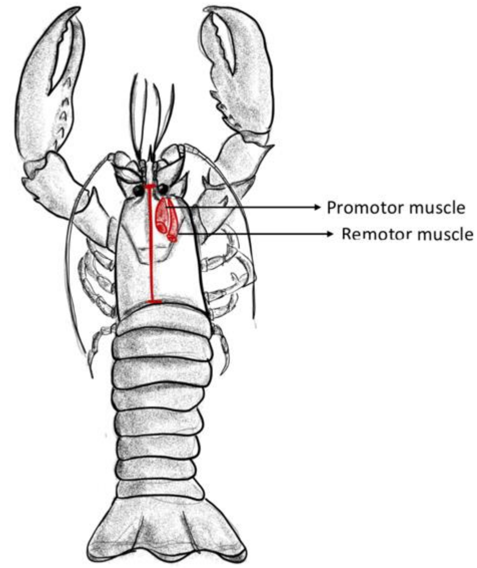


Fig. 2. Drawing of the dorsal view of an American lobster (*H. americanus*). The red line shows the carapace length, and the approximate location of the antagonistic promotor and remotor muscles under the carapace under the second antennae are marked in red and labelled. Only the promotor and remotor muscles on the right side of the body are shown, for clarity.

<u>American lobster hums have a harmonic, downward sloping structure</u>

Multiple studies have found the abdominal vibration of *H. americanus* to have a base frequency of about 180 Hz (Fish, 1966; Henninger and Watson, 2005; Ward et al., 2011), with no clear relationship between hum frequency and carapace size (Henninger and Watson, 2005). However, I believe that expressing the hum structure in terms of an average base frequency is not accurate to the actual structure of the sound. In fact, the hum itself has a distinct harmonic structure, meaning that there is a base frequency and several, evenly spaced harmonics above that frequency (Fig. 3). Furthermore, one of the hum's characteristics that is underappreciated in the literature is its overall downward slope, meaning each harmonic starts at a higher frequency than that at which it ends. I propose that the harmonic, downward-sloping structure of the hum characterizes it as a harmonic exponential down-chirp. In this term, the use of the word "harmonic" refers to the banded nature of the sound, and the "exponential down" refers to the fact that each harmonic or band starts at a higher frequency than it ends at (Fig. 3).

A.

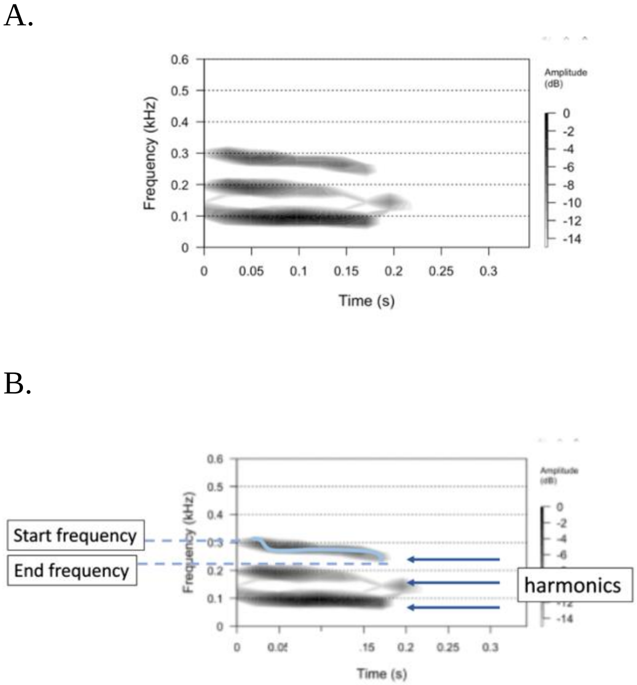


Fig. 3. A. Spectrogram generated in R of a hum I recorded using a GoPro Hero 11 showing clearly the harmonic structure of the hum. The important characteristics that make the hum a harmonic exponential down-chirp are indicated in B. This hum is very similar in structure to the hum depicted in figure 4B of Henninger and Watson's 2005 paper on sound production in *H. americanus*.

Lobsters make sound when threatened by predators

Lobsters appear to use sound most often when interacting with predators. In observed interactions between the Caribbean spiny lobster, *P. argus*, and the predator *Octopus briareus*, for example, *P. argus* stridulated (i.e. rasped with their antennae) throughout the entire physical encounter with the predator, and those who did were more likely to escape their attackers than those experimentally modified to not make noise (Bouwma and Herrnkind, 2009). Unlike spiny lobsters, American lobsters, *H. americanus*, do not need to be in direct contact with a predator to initiate their sound defense of buzzing their carapace. The average frequency of the hums falls within the range of frequencies that can be perceived by some predators that the lobsters are known to produce sound around, such as Atlantic cod (*Gadeus morhua*) (Offtutt, 1973). Along with the fact that potential predators such as cod and striped bass *(Morone saxatilis)* seemed to react behaviorally to the lobster sounds (Ward et al., 2011), this makes a compelling case for the theory that the lobster hum is a way to ward off predators.

Though evidence of the use of sound as a means of intraspecific communication is limited for lobsters, we know that American lobsters are capable of hearing. They do so not with statocysts, as was long thought to be the case, but with mechanoreceptors in structures on the outside of their body called hairfans. The frequency American lobsters hum at (~180 Hz) falls within the range of frequencies they are known to perceive (80-250 Hz) (Jezequel et al., 2021). Aside from frequencies they can perceive, some information is known about the loudness or amplitude (measured in decibels, dB) a stimulus needs to have for an American lobster to be able to perceive it. American lobsters’ threshold amplitude of sound they can perceive was found to range between 99 and 120 dB between different individuals, and this threshold amplitude also depended on the frequency of the sound presented to the lobsters. Knowing that *H. americanus* can both produce and hear sound indicates the possibility of noise production as a way of lobster intraspecific communication. European lobsters (*Homarus gammarus)*, for example, are known to repeatedly vibrate their carapace in antagonistic encounters with other European lobsters (Jezequel et al., 2020). Conversely, Ward et al. (2011) also found that *H. americanus* will occasionally vibrate when in the presence of other *H. americanus*. However, since they did not hum significantly more in the presence of conspecifics than when they are alone, the most popular hypothesis for the ecological purpose of the lobster hum remains that it is a reaction to predators.

Previous methods of provoking sound production in *H. americanus* are often noisy

Because *H. americanus* seem more likely to vibrate when disturbed or threatened by a predator than when left undisturbed, the general method of evoking noise production in the literature has long been direct grasping of the carapace by hand (Pye and Watson, 2004; Henninger and Watson, 2005; Ward et al., 2011). If not for the actual experiment itself, this method is commonly used to weed out soniferous lobsters from those that are not likely to make noise, as the carapace buzz can be felt directly when grabbing the lobster. Finding lobsters that are inclined to make noise this way is not as convenient as it may sound, however. Henninger and Watson (2005) tested 1723 *H. americanus* individuals, only 129 of which vibrated when grasped. They also found that size might influence *H. americanus* likelihood to vibrate, while sex did not. A reliable way to evoke noise production in lobsters that are likely to hum is to place them in the same tank as a potential predator. When doing so, Henninger and Watson (2005) observed an average of 15 vibration events in 30 minutes, as opposed to an average of 1.2 events per 30 minutes when soniferous *H. americanus* were alone. These methods introduce two big issues into the system. Firstly, it appears that though all *H. americanus* can make sound, those that readily will under laboratory conditions are rare. Secondly, current methods of inducing sound production are not soundless; grasping animals by hand introduces a lot of noise pollution from the handler, and using live fish is often not convenient.

Small tank acoustics complicate *H. americanus* hum recording and quantification

Though the carapace vibrations are easily felt by hand when they do occur, a way to quantify them is needed. Previous research has employed vibrometers, glued onto the carapace, to be able to link carapace vibration events to simultaneous sounds picked up by hydrophones. An alternative and possibly more precise way of detecting vibration was used by Taylor et al. (2019) when locating the sound-making mechanism in ghost crabs (*Ocypode quadrata*). Using doppler laser vibrometry, they were able to locate the place where the vibration was most intense - the gastric mill of the ghost crabs. Their work provides an alternative method of quantifying the vibrations of the American lobster carapace in a manner that does not involve gluing a vibrometer onto the lobster's carapace, possibly impeding or altering its movement. Laser vibrometry works under the principle that light reflected off a surface will have a change in frequency that is proportional to the change in velocity of the surface. Therefore, by shining a laser on the carapace, we can see the displacement of the carapace and the frequency (or speed) at which it vibrates at.

It is important to note that not all research was able to link each instance of *H. americanus* carapace vibration with an associated sound (Ward et al., 2011; Jezequel et al., 2020). When recording vibrations on the carapaces of male *H. gammarus* engaged in intraspecific agonistic encounters, Jezequel et al. (2020) were only able to link 15% of the buzzes found by the vibrometers to sounds picked up by the hydrophones, despite the hydrophones being hung directly above the animals. This could imply that not all carapace vibrations are sound producing, possibly depending on the intensity of the vibration or another unknown mechanism. However, Jezequel et al. (2020) offer an alternative explanation related to the acoustics in small tanks. They state that because of known issues of small tanks highly attenuating buzzing sounds, the hydrophones were unable to pick up these buzzing sounds, even though they were right above the source of the sound. Though this is certainly an issue in small tanks, it is one that can be mediated by proper characterization of the experimental tank. In general, one should pay attention to water depth, water temperature, and attenuation length of the tank used, as these will all influence the method needed to calibrate a hydrophone (Akamatsu et al., 2002; Okumura et al., 2002; Takahashi et al., 2018).

Unexpected high-frequency sounds

Although Henninger and Watson (2005) found no clear relationship between frequency and *H. americanus* carapace size, and found instead that the average frequency of the American lobster hum lies around 180 Hz. However, in my work with *H. americanus,* I observed noises that were much higher in frequency than the previously reported 180 Hz hum. After ruling out artefactual causes to the best of my ability, I paid close attention to noises in a register above what other research into *H. americanus* sound production has looked at, as well as reporting on some of the sounds I have observed that do not fit into the previous literature on *H. americanus* sound. Additionally, the use of laser vibrometry described above could prove to be useful when tackling such high frequencies, as the frequencies that are able to be resolved with laser vibrometry are higher than those able to be resolved with a vibrometer alone.

Project Goals

Given the above context, my project consists of two main parts. First, I used traditional overhead mechanical stimuli to evoke noise production and analyzed the characteristics of the sounds I found. I then investigated the possibility of using laser vibrometry to visualize carapace vibrations. Second, I looked for a reliable way to evoke a fear response and noise production in *H. americanus* under lab conditions. To avoid using physical human interference or the introduction of a live predator, I used a visual overhead stimulus in the form of a dark shape on a computer screen suspended above the experimental tank and looked for defensive behaviors and sound production in response to the stimulus. Lastly, to gain more insight into how *H. americanus* might use hums, I ran a play-back experiment that looked at lobsters' behavioral reactions to lobster hums.

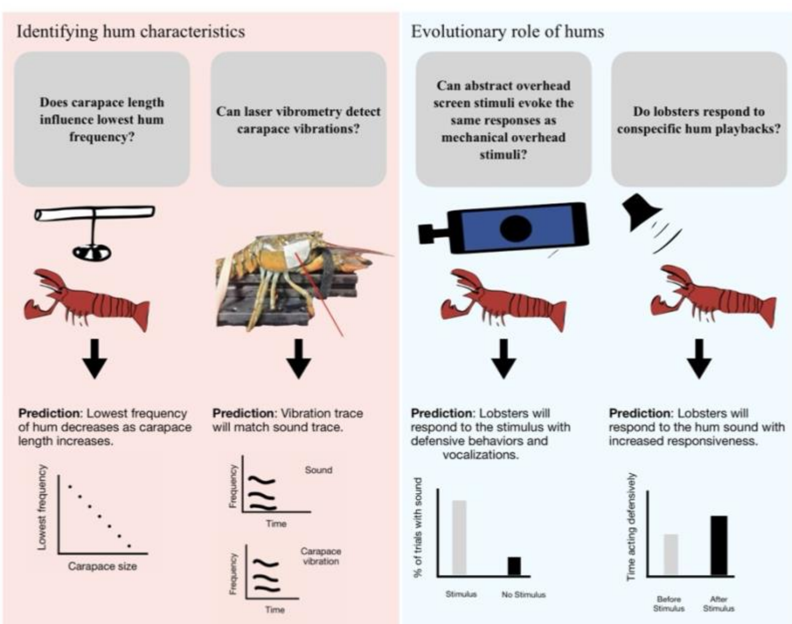


Fig. 4. Diagram walking through the two main parts of my project and the four experiments' methods and predictions.

Methods

<u>Animal collection and husbandry</u>

Lobsters were collected from Harpswell Sound, ME in October 2022, June 2023 and late October 2023, and returned to the sound after the conclusion of the experiments. For the first half of the experiments, lobsters were kept in separate baskets in the marine lab at the Bowdoin College Schiller Coastal Studies Center with flowing seawater from the Harpswell Sound. While at Schiller, lobsters were fed trout pellets and fresh mussels. During the 2023-2024 academic year, lobsters were housed in recirculating seawater tanks at Bowdoin College at 15.5° C in separate baskets and fed mussels and frozen shrimp ad libitum.

<u>Size experiment</u>

Preliminary testing

During preliminary tests, I placed individual lobsters in tanks of different materials and colors, and used several mechanical overhead methods to attempt to induce the lobsters to make sound. From my observations, lobsters seemed most responsive in white containers, and when being approached from above. These findings informed my final experimental design.

Experimental design

A white cooler without lid with dimensions 74 x 41 x 47 cm was used as an experimental tank. Water in the tank came directly from the Harpswell Sound and was changed out between lobsters to avoid the water warming up over time and to avoid any effects of scent cues left over by other lobsters on sound production. I measured the carapace length of all lobsters using a caliper before the start of the experiment (measured from the tip of the rostrum to the lower edge of the carapace, Fig. 2). Over the course of the experiment, lobsters (N = 25) were tested for their sound production on three different materials: the plastic of the cooler itself, white foam, and white felt. In three different sets of trials, the cooler was lined with each of these materials on the bottom and walls to dampen the noise of lobster carapace on plastic. For each of these materials, the same procedure was followed. The lobsters were put into the tank with a GoPro Hero 8 or Hero 11 video camera (30 frames per second) already recording, and the tank was then covered with a white cloth. Lobsters were allowed to acclimate in the tank for 10 minutes. After 10 minutes, I removed the cloth and repeatedly lowered a Secchi disk (20 cm in diameter with alternating black and white quadrants) suspended from a plastic frame over the lobster in the tank. When the lobster had habituated to the disk (i.e. no longer responded), the lobster was left to sit for 5 more minutes before being removed from the tank by hand. I did not turn the GoPro off until after the lobsters were removed from the tank, in case they made noise in the process of getting picked up. Of 25 lobsters, 11 were tested for noise production on all three materials. The remaining 14 were only tested on foam.

Control of lobster legs scraping on different materials

As a control, I obtained frozen lobster legs from the Dickinson lab at Bowdoin College. After thawing at room temperature, I manually rubbed both a cheliped (walking leg with claw) and a normal pereiopod leg on the three materials (foam, felt, and plastic) for 15 minutes each underwater to see if I was able to produce noises. I also analyzed how likely lobsters were to make noise during 15-minute trials on each respective material.

Software

Data were compiled in Excel (v16.67). Video files were converted to audio files using iMovie (v10.3.5), and I used Cornell’s free Raven Lite software (v2.0.4) to analyze audio. Statistical analyses were carried out using RStudio (v4.2.0; R Core Team 2022).

Statistical analyses

I analyzed the binary sound (1) – no sound (0) data using a binomial logistic regression to determine whether carapace size affected likelihood of making sound. I then ran a linear regression to see if carapace size influenced lowest frequency of sound produced. The presence-absence sound data from the control experiment of manual rubbing on different materials was analyzed using a Cochran Q test from the R package RVAideMemoire (Hervé, 2022).

<u>Detection of carapace vibrations using laser vibrometry</u>

Preliminary testing

The Polytec laser vibrometer (Polytec Inc., Hudson MA) was tested by playing lobster hums through an underwater speaker to ensure that the laser was able to pick up the vibrations on the surface of the speaker. After this was confirmed, I used a vibrating underwater Hexbug (a children's toy that moves through an internal motor that causes rapid vibration) to mechanically vibrate the lobster carapace, ensuring that these vibrations could be picked up as well.

Experimental design

To better characterize the nature of carapace vibration and confirm high-frequency sounds I observed, I used a laser vibrometer loaned from Polytec to quantify carapace vibration, inspired by the methods by Taylor et al. (2019). Lobsters were strapped down to limit vibrations due to other movements (Fig. 5). Lobsters were stimulated by approaching by hand from above. Reflective tape was placed on the carapace with underwater glue to aid in focusing the laser.

Fig. 5. American lobster strapped to a weight with reflective tape on the carapace for laser vibrometry.

Software and data analysis

Data were extracted using Polytec's complimentary software VibSoft and visualized in Raven Lite (v2.0.4).

<u>Response to overhead screen stimuli</u>

Preliminary testing

Lobsters were shown varying abstract shapes moving at different speeds on an overhead monitor to determine which visual was most effective at evoking a defensive response. I qualitatively determined the most effective stimulus to be a rapidly enlarging black circle (Fig. 6).

Experimental design

Lobsters (N = 17) were placed alone in the experimental tank of 45.7 x 30.4 x 25.4 cm with a hydrophone behind a grate and a computer monitor overhead. Lobsters acclimated to the tank for five minutes with the screen overhead set to a blue background. Then, lobsters were shown a rapidly enlarging black dot animation created in PowerPoint 2021, meant to mimic the shadow of a predator approaching overhead (Fig. 6) three times in a row, with 60-second intervals. After three stimuli, the lobsters were left to rest for five minutes, after which the procedure was repeated two more times. In total, each lobster was exposed to the stimulus nine times. For each 180 second trial with three stimuli, I recorded sound using a hydrophone and visualized the sound as a spectrogram using Spike (v2.9). For each stimulus, I evaluated the lobster's visual reaction (Table 1).

Fig. 6. Overhead stimulus shown to lobsters. The dot increased in size 3000% (from the left to the right image) in 0.5 seconds to imitate the experience of a predator rapidly approaching overhead.

Software

Data were extracted from Spike (v2.9), compiled using Excel 2021, and analyzed using and Prism (v10.2.2) and the Seewave package in RStudio 2022 (Sueur et al., 2008).

Statistical analyses

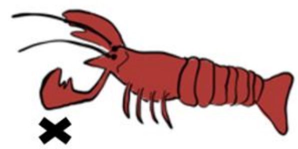
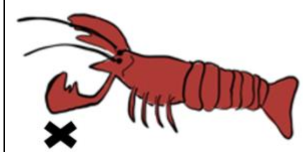
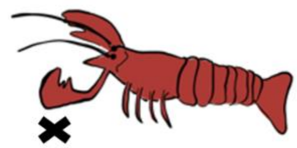
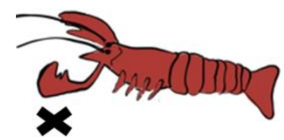
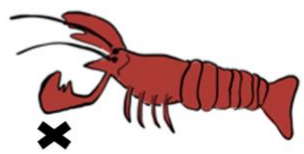
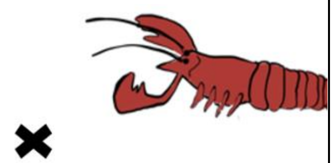
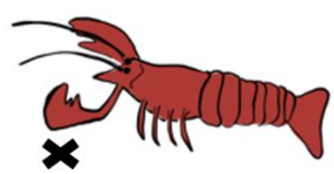
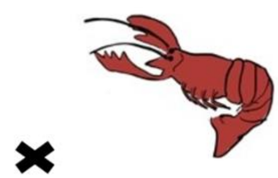
I performed a repeated measures ANOVA to test for effect of individual lobster and the effect of treatment (stimulus/ no stimulus) on likelihood to show defensive/evasive behaviors (Table 1). I used a linear regression to characterize the pattern of average reaction intensity over trials and a repeated measures ANOVA to see the effect of individual lobsters on this trend. The effect of treatment on likelihood to produce sound was tested using a chi-squared test.

<u>Behavioral response to auditory cues</u>

Experimental design

During the summer of 2023, I exposed seven *H. americanus* caught from the Harpswell Sound to silence, white noise, and a lobster hum recording, and evaluated their behavior following the stimulus to determine whether the hums elicited different reactions than other sounds. Lobsters were placed in an experimental tank (dimensions 101.6 x 241.3 x 88.9 cm) with aquarium sand as substratum and an Olympus TG-6 underwater camera recording (30 frames per second). Each

Table 1. Behavioral scale used to evaluate *H. americanus* responses to an abstract overhead screen stimulus.

Numerical value	**Response**	**Description**	**Schematic**	
			Before Stimulus	**After stimulus**
0	No response	No visual movement		
1	Flinch	Carapace moved down without relocation of body		
2	Retreat	Walking backward, either with or without flinching the body down		
3	Tail flip	Rapid backward movement characterized by flipping the tail		

lobster was placed into the tank in a flower pot they had gotten accustomed to in their enclosure. After 10 minutes, lobsters were exposed to one type of stimulus at a time (white noise, a lobster hum, or silence) and left to sit for another five minutes. The stimuli were each 200 ms in duration, based on my observations of the average length of a lobster hum. Each lobster (N = 7) underwent three trials total, one for each stimulus. Lobsters were only exposed to one treatment per day, and the order of the trials was randomized.

Software and analysis

I analyzed lobster behavior visually for every five seconds of the minute leading up to and following the stimulus according to Table 2 and noted their behavior as either reacting (1) or not reacting (0). Data were compiled in Excel 2021 and analyzed in RStudio 2022. I performed a two-way repeated measures ANOVA to determine the effect of stimulus type on time spent engaging in defensive behaviors before and after the stimulus.

Table 2. Binary notation used to score lobster reactions during playback experiments.

Notation	Description
0	**No movement**
1	**Fleeing**: out of shelter and actively retreating away from sound. **Avoidance -** remained in shelter but retreated further into shelter than before. **Initiation 1:** in shelter, but moved forward out of shelter without tail fully leaving shelter **Initiation 2:** out of shelter, closer to sound source than to flowerpot. **Threat display 1:** in shelter, but showing other signs of defensiveness or aggression (like raising claws in meral spread, high on legs, antenna point, claw forward) **Threat display 2:** out of shelter, moved towards the sound source with other signs of defensiveness (like raising claws in meral spread, high on legs, antenna point, claw forward). **Physical contact:** out of shelter, making physical contact with the fence protecting the sound source. (adapted from Atema & Karavanich, 1998)

Results

<u>Analysis of hum characteristics</u>

Carapace size and hum frequency comparison

Carapace length did not have a significant effect on how likely a lobster was to make sound (Binomial logistic regression, $p = 0.6$). Furthermore, carapace size did not have a significant effect on the lowest frequency of noise made by soniferous lobsters (Linear regression, R-squared: 0.013, $p = 0.3$) (Fig. 7). The frequency characteristics of the sounds observed will be explored in more depth later with sound data of better quality.

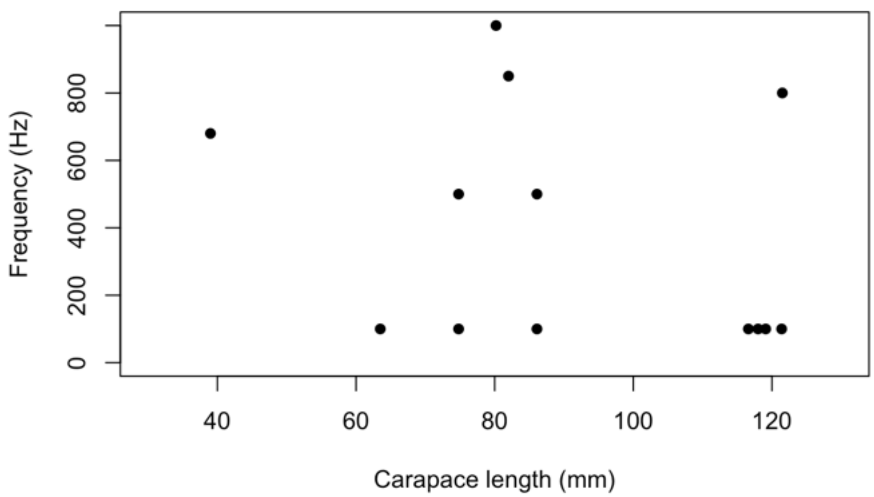
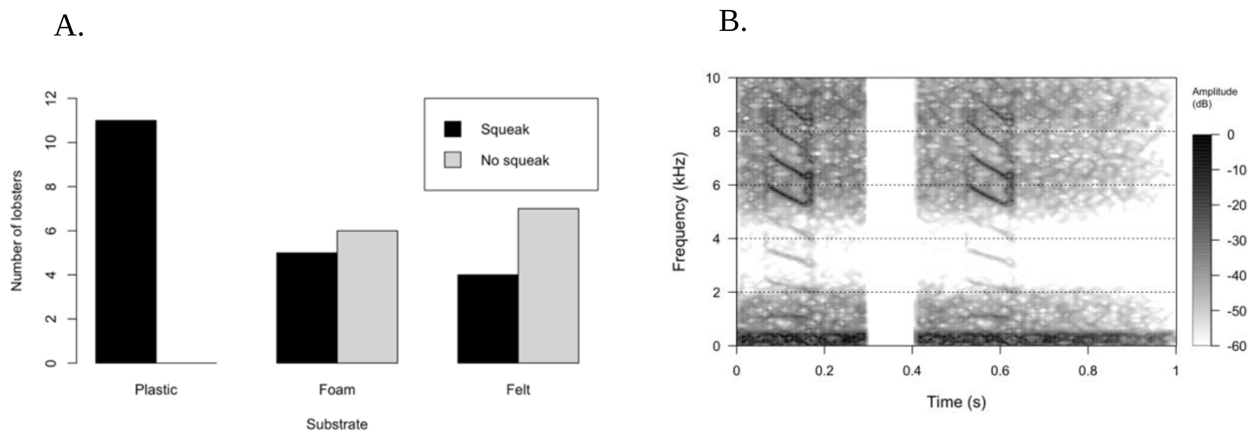


Fig. 7. Lowest frequency of sounds produced by lobsters of different carapace lengths (mm). There was no significant relationship between carapace length and lowest frequency of noise produced (Linear regression, R-squared: 0.013, p = 0.3).

Unexpected sounds

While conducting the size experiments and the playback experiments, I observed sounds with harmonics at much higher frequencies than previously reported (hereafter referred to as 'squeaks') (Fig. 8). To rule out artefactual causes, I tested lobsters on several substrates (plastic, foam, and felt) and manually rubbed pieces of lobster carapace on those substrata as well. Manual rubbing of lobster legs on foam and felt produced no high-pitched sounds in 15 minutes each, whereas the rubbing of the same legs on plastic produced over 100 squeaks in that same 15-minute period. The use of plastic substrata was thereafter discontinued. To ensure that there was no other, non-lobster related source of the sounds (such as birds outside or creaking of the tank), I recorded 13 trials of lobsters sitting alone in an experimental tank on aquarium sand substratum for 15 minutes, and recorded that same tank empty for 15 minutes for 13 trials as well. None of the empty trial recordings showed high-pitched noises, whereas 3 out of 13 of the trials with lobsters did.



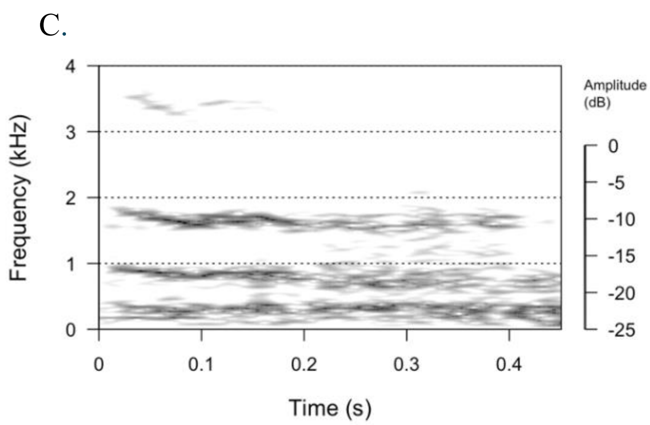


Fig 8. A. Number of lobsters out of 11 whose trials showed squeaks (black bars) or did not show squeaks (grey bars) on three different materials. Material did not significantly affect the probability of a ‘squeak’ being produced (Cochran Q, $p = 0.14$). Still, plastic substratum was no longer used after these findings, since I was able to reproduce squeaking sounds on plastic with severed lobster legs. B. and C. show spectrograms of the high-pitched sounds (squeaks) that I observed in trials with lobsters present.

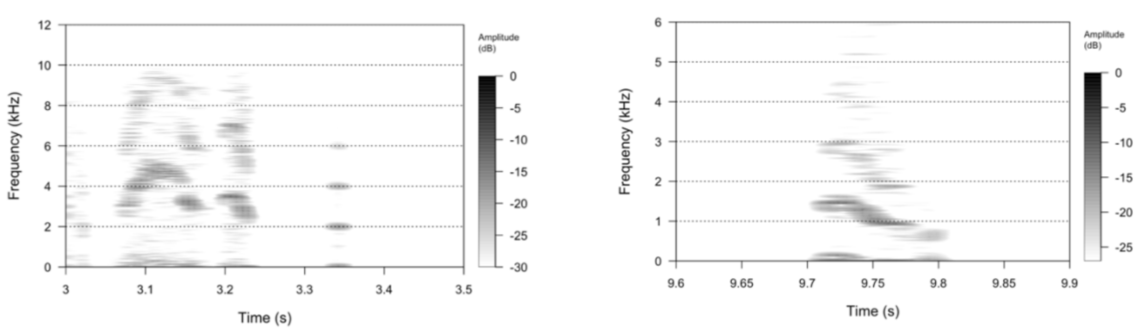


Fig. 9. Spectrograms showing vibration of a lobster’s carapace detected by the laser vibrometer. Those areas where the plot is darker show a stronger signal of that particular frequency. Vibrations have the same sloping structure of a harmonic, exponential down-chirp as those shown in the spectrograms based on sound recordings of the hums (Fig. 8). Note that all frequencies are reported in kHz, meaning that they lie an order of magnitude higher than previously reported for hums (Fig. 3), but are similar in frequency to the high-frequency sounds.

Detecting carapace vibration using laser vibrometry

Using a laser vibrometer, I was able to record vibrations of the surface of the lobster carapace that were similar in structure to the previously recorded hums. In particular, the downward-sloping structure and harmonics were preserved (Fig. 9).

Response to overhead screen stimuli

Behavioral responses

H. americanus did not show behaviors as described in Table 1 when sitting in a tank with the screen illuminated without a stimulus on the screen. In contrast, they showed a reaction in 100 out of 153 trials with the stimulus. The presence of the overhead screen stimulus had asignificant effect on the likelihood of reaction, and there was a significant difference in the likelihood to react to the stimulus between lobsters (repeated measures ANOVA, $p < 0.001$ and $p = 0.001$) (Fig. 10A). Average reaction intensity (as in Table 1) decreased over trials (linear regression, R

A. B.

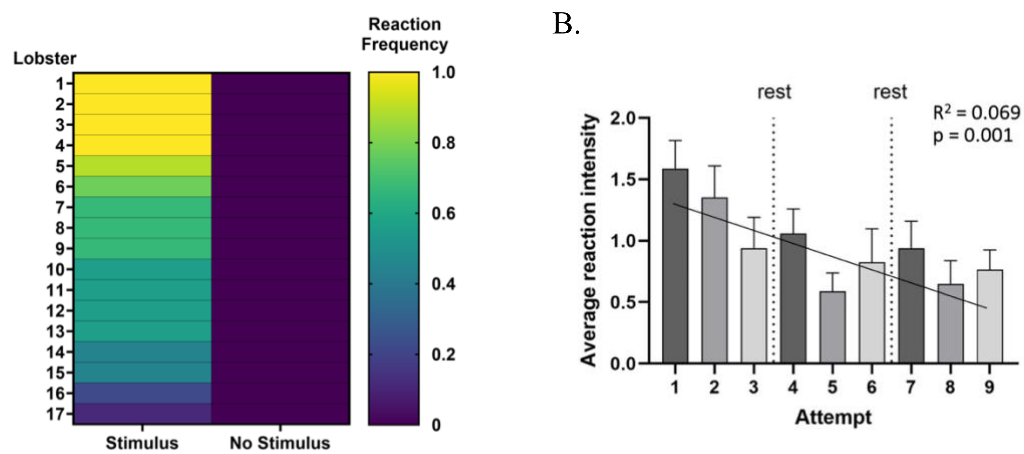


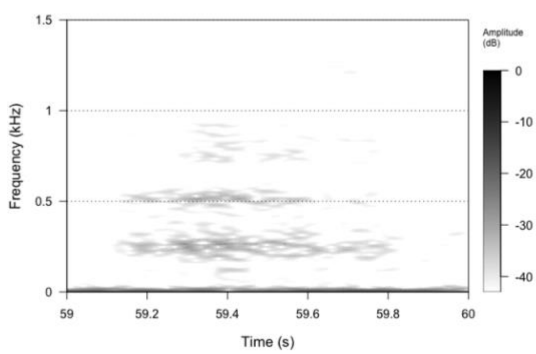
Fig. 10. A. The presence of the overhead screen stimulus had a significant effect on the likelihood of reaction, and there was a significant difference in the likelihood to react to the stimulus between lobsters (repeated measures ANOVA, $p < 0.001$ and $p = 0.001$). B. Average reaction intensity (as in Table 1) decreased over trials (linear regression, R squared = 0.069, p = 0.001).

squared = 0.07, p = 0.001) (Fig. 10B). As with the likelihood to react, there was also a significant difference between lobsters in reaction intensity across attempts (repeated measures ANOVA, $p < 0.05$).

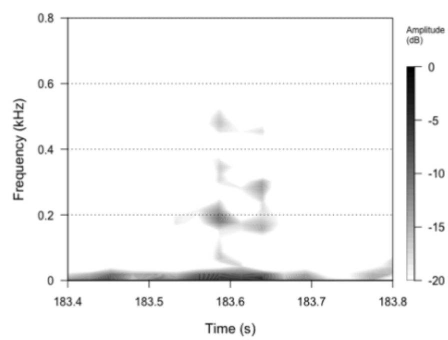
Sound responses

Hum-like sounds were observed in 13 out of 153 stimulus trials, and 6 out of 153 control trials, and presence of stimulus did not significantly affect the likelihood of observing a hum (X^2 (1, $N = 306$, $p = 0.1$) (Fig. 11).

A.



B.



C.

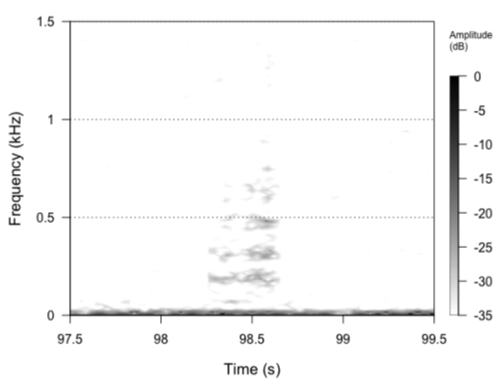


Fig. 11. Examples of sounds lobsters produced while being shown the stimulus (A and B), and without being shown the stimulus (C). The sounds are harmonic in structure.

<u>Behavioral response to auditory cues</u>

I performed an outlier analysis and removed data from lobster A2, who was an extreme outlier as determined by the boxplot method. After removing data for A2, the only treatment that significantly impacted the fraction of time spent reacting before and after the stimulus was the white noise treatment (Repeated measures two-way ANOVA, $p < 0.05$). Lobsters did not change the amount of time they were engaging in defensive behaviors (Table 2) after hearing a conspecific hum.

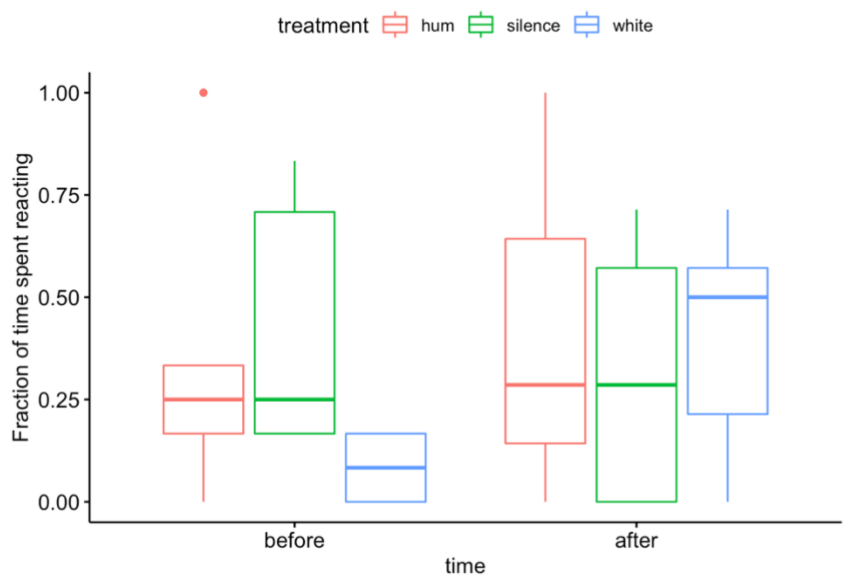


Fig. 12. Fraction of time that lobsters spent engaging in defensive behaviors (Table 2) before and after hearing silence, white noise, or a conspecific hum. Only white noise had a significant interaction with time (Repeated measures two-way ANOVA, $p < 0.05$), and lobsters did not show more defensive behaviors after hearing a hum than before. The red dotrepresents the outlier that was removed for data analysis.

Discussion

<u>Hum characteristics</u>

Carapace length does not influence hum frequency, and unexpected high-frequency sounds were not due to identifiable artefactual causes.

Like Henninger and Watson (2005), I found no relationship between carapace length and lowest frequency of hum produced (Fig. 7). This is counterintuitive, as one would expect the frequency of sound produced by vibrating a material to increase as the length of the material decreases. However, another property of the material that can influence the frequency of the sound produced is stiffness. Henninger and Watson (2005) hypothesized that the promotor muscle – whose current function is unknown – could be used to modulate frequency by adjusting the tension, and therefore firmness, of the carapace. This could explain the lack of correlation between carapace length and the lowest frequency of hum produced.

An essential component of the project was to rule out artefactual sounds. I was able to rule out several artefactual causes of the high-frequency ‘squeaks’ I observed, such as the scraping of lobster carapace on tank material by manual controls and outside sources of the sound by recording tanks while empty. After discontinuing the use of the plastic substratum because of its tendency to make squeaking noises when rubbed on by lobster carapace, I continued to hear the high-frequency sounds even on those materials that I was unable to reproduce squeaks on by manually rubbing severed lobster legs. Furthermore, none of the empty tank experiments showed similar squeaks, and therefore it is likely that the lobsters were the source of these unexpected sounds (Fig. 8B-C).

High-frequency sounds appear to be modulated hums.

The range of frequencies I observed in some lobster sounds lay an order of magnitude higher than the frequencies previously reported (1000 – 15000 Hz compared to the previously reported 100 - 600 Hz), but the characteristics of the previously reported hums are clearly visible in the higher-frequency sounds as well, specifically the harmonic nature and downward-sloping structure of the hums (Fig. 8B-C). The high-frequency exponential down-chirps could therefore be modified hums. One hypothesis is that the promotor muscle is responsible for the modulation in frequency of the buzzes, as proposed by Henninger and Watson (2005). Though severing of the promotor muscle did not change the frequency at which lobsters buzz or even inhibit the lobsters from buzzing at all, both the remotor and the promotor muscles showed activity during buzzes, implying that the promotor muscle has at least some purpose here (Henninger and Watson, 2005). This hypothesis is supported by laser vibrometer data, which shows vibrations of the same structure as the hums, but at higher frequencies (Fig. 9). Still, further research is needed to confirm the exact mechanism behind these high-frequency carapace vibrations. Though it is known that the remotor muscle can contract repeatedly at frequencies that account for the previously accepted hum sound baseline of around 180 Hz (Mendelson, 1969), it is unclear if they are able to contract this muscle or the promotor muscle at frequencies adequate to explain the harmonics of the squeaks I observed, which spanned up to almost 16 kHz. I believe that laser vibrometry will eventually prove to be useful in resolving these high-frequency vibrations of the carapace surface.

Lobster reactions to overhead stimuli

Abstract overhead screen stimuli evoked behavioral responses similar to physical overhead stimuli.

Though clawed lobsters such as *H. americanus* appear well-armed, they have been found to suffer higher mortality during predation events than slipper lobsters and spiny lobsters (Barshaw et al., 2003). Due to their vulnerability to predation events, American lobsters and many other crustaceans have developed a peculiar escape response, sometimes called a ‘tailflip’ or ‘escape swimming’, in which they flick their tail forward violently, effectively propelling themselves backward (Neil & Ansell, 1995). American lobsters tailflip more when they feel more vulnerable (e.g. while in post-molt state), and therefore the tailflip response and the behaviors often preceding a tailflip (such as flinching and retreating) are good indicators of a lobster that is feeling threatened (Cromarty et al., 1991). I observed all of these behaviors when conducting the size experiments and while handling lobsters, during which I was physically approaching the lobsters from above with either my hand or an object. With their eyes positioned atop their head, American lobsters are thought to be sensitive to detecting small changes in light under low light intensity conditions to hunt for live prey and look out for swimming predators (fish, rays, etc.) (Atema and Voigt, 1995). It was this fact that inspired me to attempt to evoke escape responses, and potentially the sounds accompanying them, using a less invasive, abstract overhead screen stimulus. The high instance of defensive reactions to the stimulus (in 65.4% of trials) confirms that it is possible to move away from mechanical, often noisy, overhead stimuli to evoke defensive and escape behaviors in American lobsters. Since American lobsters have no true color vision, it is likely that the defensive reactions I recorded in response to my overhead screen stimulus were due to its mimicking of a shadow approaching rapidly (Atema and Voigt, 1995). Though I opted for a blue background for my stimulus, the stimulus could probably be set up using any color, as long as the light levels change sufficiently throughout the stimulus. Lobsters did acclimate slightly to the stimulus over the nine times they were shown it, which implies that the exposure to the stimulus should be limited to ensure its effectiveness (Fig. 10B).

Presence of the overhead abstract screen stimulus did not affect the likelihood of sound production.

Lobster hums are a relatively rare phenomenon. Out of the 1723 lobsters Henninger and Watson (2005) picked up by hand, only 129 hummed (7.5%). It is therefore not surprising that in my 306 trials with 17 lobsters, I only detected hums in 19 (6.2% of trials). Because it is known that manual grasping of the lobster carapace or introduction of a fish predator are effective in evoking sound production in soniferous individuals (Henninger & Watson, 2005; Ward et al., 2011), I hoped that the overhead screen stimulus could provide a similar effect without the interference of an observer or fish. However, presence of the stimulus did not significantly affect likelihood of hearing a hum in any given trial. It is possible that this result would change with a bigger sample size. To date, the most effective way of provoking soniferous lobsters to produce sound has been the introduction of a live predator (Ward et al., 2011). Ward et al. (2011) reported that lobsters hummed an average of 30 times in 15 minutes when in a tank with a predatory fish. There are several reasons the fish approach is that much more effective than my abstract overhead stimulus. Firstly, it is possible that although lobsters can and do use changes in light level to look for threats, the way they assess threats is multimodal, meaning sight alone is not enough (Weissburg et al., 2014). In the case of Ward et al. (2011), therefore, the lobsters might have been reacting to both the sight and scent of the cod, and in both the case of a fish predator and a manual approach, the physical water displacement due to the threat might be vital in determining the lobsters' responses as well. To investigate the exact type of stimuli that trigger hum production, it would be interesting to repeat this experiment with predator-scented water.

Sound production of American lobsters: response to predators or intraspecific communication?

Both the above discussion and these experiments in general assume that the hum sound is produced in response to a threat, as is the most common hypothesis. An alternative hypothesis is that the hum is used for intraspecific communication between lobsters. Although the amplitude of the sound cannot be determined from my data, the sounds are likely only audible at very close range, and could therefore be used as communication between lobsters, especially because the base frequency *H. americanus* hum at (~180 Hz) falls within the range of frequencies they are known to perceive (80-250 Hz) (Jezequel et al., 2021). When exposing lobsters to lobster hums, white noise, and silence, however, I found no significant change in lobster activity before or after any stimulus but the white noise (Fig. 12). This is contrary to what I had expected, based on observations by Jezequel et al. (2020) that European lobsters (*Homarus gammarus*) hum when engaged in agonistic encounters. It was hard to control for amplitude of the different stimuli, however, so it is likely that the stimuli were not played loudly enough. The experiment should be repeated with a better understanding of the amplitude of the stimuli. Alternatively, like in the case of the overhead threat, one part of the signal (audio) might have not been enough to evoke a response.

Overall, there seem to be some contradicting lines of evidence that make it hard to determine what the role of the hum sound is in lobster ecology. This is further complicated by the possible existence of the high-pitched sounds I observed. If American lobsters are modulating their pitch, why and how are they doing it? It is of note that Ward et al. (2011) did not link every occurrence of a carapace vibration with a sound. Similarly, Jezequel et al. (2020) could only link 15% of the carapace vibrations they recorded with a vibrometer to a sound picked up by a hydrophone, which they ascribed to small tank effects. Though my recordings also suffered from small tank effects (Fig. 13), the attenuation was not nearly strong enough to mask the hums entirely. I propose instead that it is possible that the hum sound is merely a by-product of vibrating the carapace. In that case, we should not be focusing on what the function of the sound may be or how to evoke the sound itself, but what the function of the carapace vibrations and the variation within these vibrations might be.

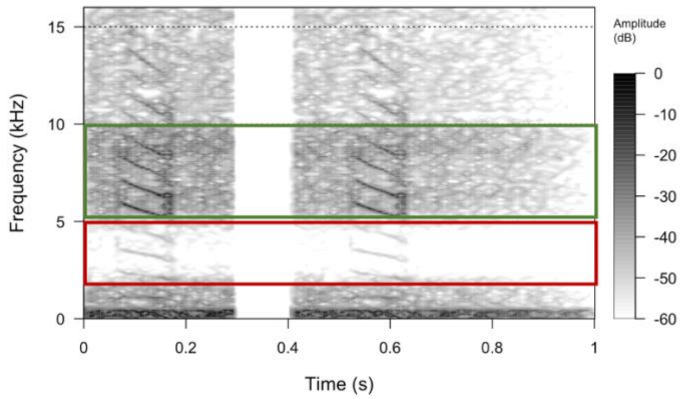


Fig. 13. Spectrogram showing a compilation of two hums with a harmonic, exponential down-chirp structure. Higher frequencies are overrepresented (green box) due to their short wavelength, and low frequencies are underrepresented (red box) due to their long wavelength compared to the small experimental tank length.

Conclusion

H. americanus hums seem to vary more in frequency than has been previously reported, and the frequency produced does not depend on the carapace length of the animal. Furthermore, I determined that laser vibrometry is a viable method for investigating American lobster carapace vibrations and to pick up the higher frequency harmonics I noticed in audio recordings. Though I was not successful at finding a reliable way to evoke sound production in the American lobster, I did find that abstract overhead screen-stimuli that mimic a shadow are effective at inducing defensive and escape responses in American lobsters.

Future directions

From this study, several more questions arise about the role of the promotor muscle, the relationship between carapace vibrations and sound, and the ecological use of the hum sound in American lobsters. Future studies should assess the concurrence of sound with carapace vibration events, and look further into the existence of and mechanisms behind high-frequency carapace vibrations.

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