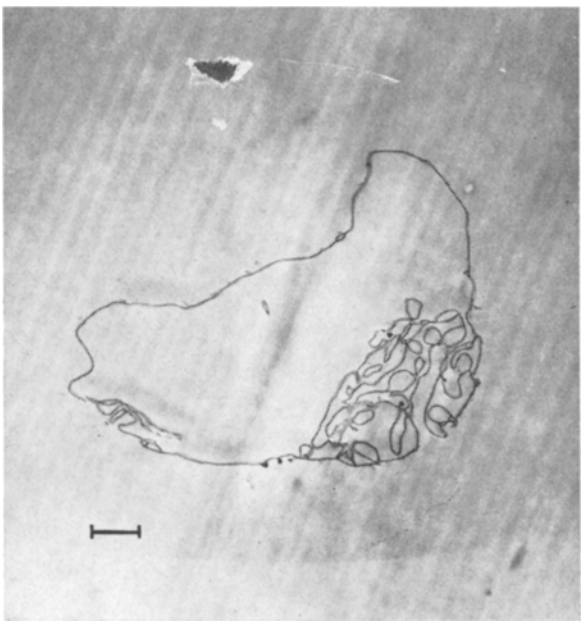

Fig. 3. Balloon-formation of salt chloroplasts in distilled water. The grana discs are so strongly distorted that individual discs are seldom recognizable and it is seen just like the unknitted configuration. × 6500.

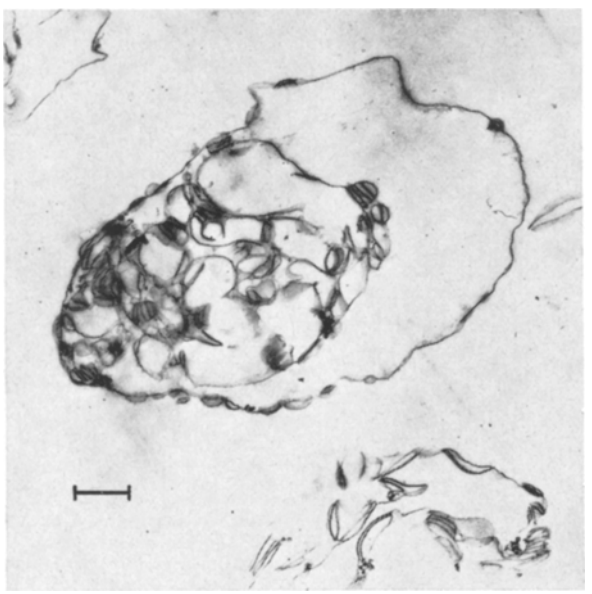

Fig. 4. Balloon-formation of sugar chloroplasts in 0.2 m*M* $MgCl_2$. The grana discs and vesicles are observed inside the balloon. × 7600.

observed (Figure 2). These observations clearly show that the balloon-formation results from the extension of stroma lamellae of chloroplasts. The tangled structure (Figure 3), which is represented the unknitted configuration, may be formed from the further swelling of the grana discs adherring to the balloon surface.

The explanation of the formation described above is based on the general model of lamellae structure that grana discs are stacked on the stroma lamellae. On the basis of this model, however, it cannot be explained from the balloon-formation how vesicles or grana discs are frequently observed inside the balloon (Figure 4). MENKE[11] reported that thylakoid stack may be formed by invagination, and WEHRMEYER[12] showed that at the edge of a granum, a lamellae may bifurcate or fold back on itself, thus contributing 2 discs to the same granum. Furthermore, WEIER et al.[13] showed the three-dimensional model of a single fret connected with several adjacent loculi. From the model of lamellae system as described above, it is not to be understood how the vesicles or grana discs observed inside the balloon are formed.

Although there have been electron microscopic investigations[6–8] on structural changes of isolated chloroplasts which were suspended in distilled water or hypotonic solutions, they have not observed the balloon as shown in our experiments, but separate small vesicles, separately swollen grana discs, swollen grana-fretwork system, irregular stroma lamellae lacking the grana discs, or swollen chloroplasts. In our observations, we never saw swollen grana-fretwork system[7] and irregular stroma lamellae lacking the grana discs[6,8]. The swollen grana-fretwork system shown with photomicroscope[13] is probably identical with the blebs formed in 0.05–0.1 *M* sucrose as reported by SPENCER and WILDMAN[3].

Zusammenfassung. Die Ballonbildung isolierter Spinatchloroplasten wurde elektronenoptisch untersucht.

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Acoustic Stimulus Perception by the American Lobster *Homarus americanus* (Decapoda)

Crustaceans have been used extensively for physiological studies of vision and the nervous system[1], less so for the auditory system. LAVERACK[2] studied *Homarus* and determined the physiological sensitivity of hair-fan organs to various low frequency water vibrations. Drops of water and a moving diaphragm in the end of the test tank were two of the stimuli used. Recordings were made from nerves leading from hair-fan organs on the chelipeds and carapace. COHEN[3] found that one type of receptor in the statocyst of *H. americanus* responded to substratum vibration but not to air- or water-borne vibrations. A frequency response curve for substratum vibrations has been determined for the crab *Uca* with an unconditional response[4]. There have been a few reports of responses to sound stimuli by crustaceans[5].

In these studies there have been few adequate behavioral measures of what stimuli crustacea are able to perceive. It is hoped that the present technique of con-

ditioning the heart rate may provide a behavioral measure of sensory ability.

Lobsters (carapace length 6.6–7.2 cm) were obtained by otter trawl from Narragansett Bay. While being tested, the lobsters were held in a nylon mesh net suspended in a plastic tank. The tank was placed in a small reverberation chamber with a 16″ speaker mounted in its wall. The apparatus and test equipment were the same as was used for the tautog and codfish[6]. Measurements of the stimulus field were made for both pressure and particle velocity. Through the region where the animal was held, the field had a variability of 3 db or less except at 18.7 and 37.5 Hz (6 and 4 db, pressure) and 75 Hz (9 db, velocity). Ambient noise was below the internal hydrophone noise (pressure, — 21 db per octave) and therefore masking was not a factor. All pressure measurements are given as db re: 1 μ bar (dyne/cm²) and particle velocity measurements as db re: 1 μ var (6.7×10^{-6} cm/sec)[7]. A temperature range of 16.4° to 18.9 °C was observed during testing.

Heart rates were obtained with electrodes made of No. 24 silver coated copper wire with about 1 cm of the teflon insulation removed. The exposed wire was inserted into the paricardial cavity through a small hole in the carapace. The area on the carapace around the wire was then covered with cranial cement. After the cement

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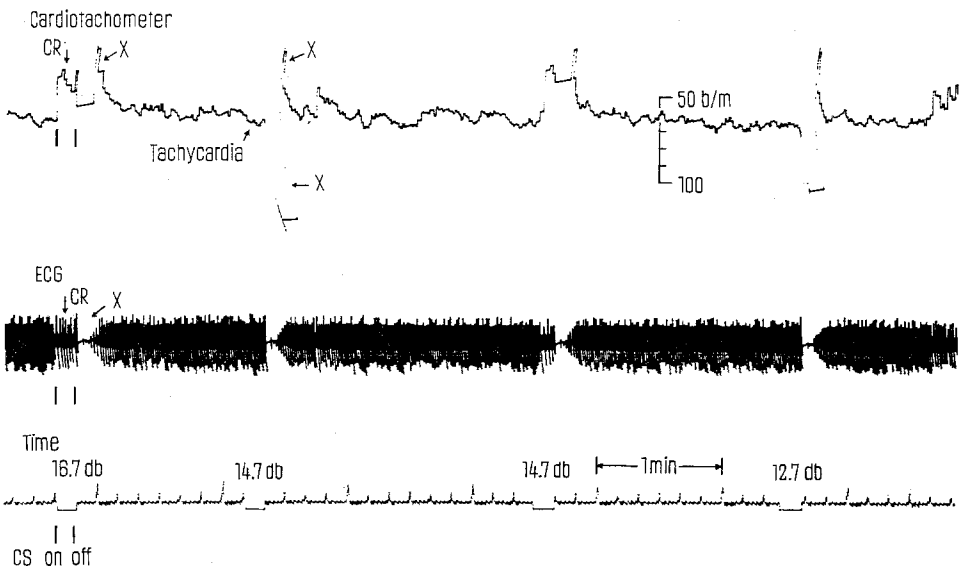

Fig. 1. A copy of the original data for the lobster showing the cardiotachometer record (giving the beat-by-beat rate), the ECG and the time. The 4 trials are indicated by the depressed time base line. The acoustic pressure for each trial is shown. There was a CR (conditional response) on the first and third trials. At the end of each trial the US caused an artifact (indicated by X) to appear in the ECG and in the cardiotachometer record. Tachycardia was observed on the second trial.

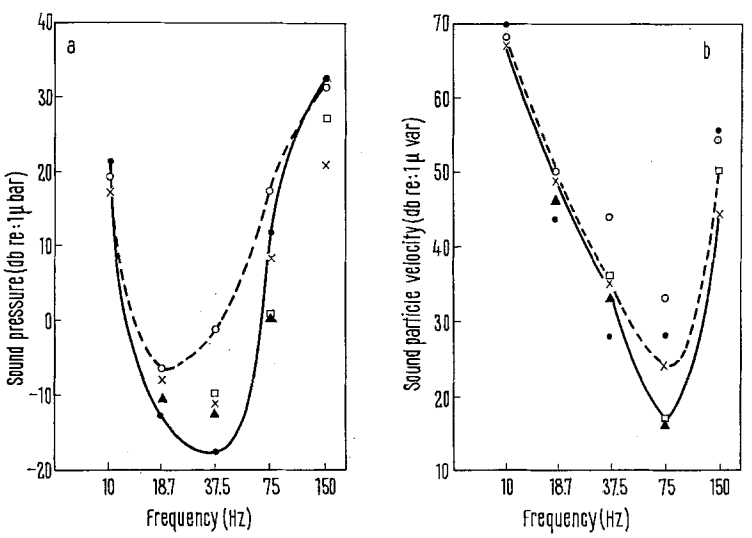

Fig. 2. The response thresholds for 5 lobsters (a different symbol used for each animal) relative to the 2 measured components of the acoustic stimulus: a) sound pressure; b) sound particle velocity. Curves are drawn visually through the data for selected animals.

hardened, a more permanent attachment to the carapace was made with Eastman 910 adhesive. An indifferent electrode was placed in the water.

When first placed in the test tank, the lobster's heart rate was always high (100–125 beats per minute, b/m). They were given 20 min to an hour to reach a steady state before testing was begun. Most testing was done with heart rates of 40 to 70 b/m although a few thresholds were determined using lower or higher rates. In 2 animals the audiograms were not completed, one because the rate became too slow and irregular and the other because a low ECG signal was masked by noise.

LARIMER[8], investigating heart rate responses in crayfish, showed that many external stimuli caused the animal to exhibit bradycardia (slowing of the heart rate). In my experiments the lobsters were shielded from most of these stimuli although occasional noises resulted in bradycardia. These were not common and within a short time (usually less than 30 sec) a more regular heart rate would be reached. Testing on any day was often terminated due to the heart rate becoming irregular.

Training was usually started with the conditional stimulus (CS) set at 37.5 Hz with pressure between 20 and 29 db. The CS lasted 10–11 sec. For the last 0.14 sec of the CS the unconditional stimulus (US) was applied. The US consisted of an AC current regulated by a variable transformer and administered through clips attached to the last pair of walking legs. Levels of US between 10 and 55 volts were used but 15–20 volts (about 0.15 to 0.2 amps) were found to be most effective.

3 of 7 animals showed bradycardia at the first CS presentation. One animal habituated to this response (no US given) in 8 trials. In all animals strong bradycardia or conditional response (CR) appeared within 4 reinforced trials. At high intensities the CR was as great as 20–30 b/m. However, this response was graded and near threshold the amount of change usually became less. Figure 1 is a polygraph record of 4 tests made near threshold and lasting about 7 min. 2 tests resulted in very distinct CR's. On some trials close to the threshold level tachycardia (increased rate) was observed. This was not considered as a CR, however if it was very strong (i.e. greater than about 7 b/m), the test level was repeated. The second trial in Figure 1 shows an example of this type of response.

Training and testing were similar to that used for fish[6]. The response threshold was determined using an up-and-down procedure with 2 db changes of the stimulus. At least 9 crossings or reversals of the CS amplitude were used for each threshold determination. Threshold values obtained early in training were always found to be higher than during later tests. Additional training, particularly at a second frequency, resulted in a lowered threshold (plateau effect). This was often observed during one testing session as a precipitous drop of about 10 db to a lower threshold level.

Frequencies tested were 10 Hz and at octave intervals from 18.7 to 150 Hz. Figures 2, a and b show the lowest thresholds for 5 animals relative to pressure and particle velocity respectively. Removal of the second antennae and chelipeds in one animal did not change its response level. There is reasonable agreement among 4 of the lobsters but the fifth one had a higher threshold in the more sensitive region for particle velocity. This animal was being retested when it molted. It may have had a higher threshold during premolt or it may have been less responsive to conditioning.

The 4 other animals showed the lowest threshold at 37.5 Hz for pressure (mean -13 db) and at 75 Hz for particle velocity (mean 19 db). It is not known which sound component acts as the stimulus, but it is believed that hair cells on the surface of fish are responsive to particle velocity or displacement (both vector quantities)[9].

Hair-fan organs, observed on various parts of the body and appendages, can probably respond to an adequate acoustic stimulus. Using a falling drop of water, LAVERACK[2] calculated the pressure to be 0.40 dyne/cm^2 (e.g. -8 db) at the physiological threshold of the hair-fan organ. This value is remarkably close to the threshold of pressure found in the present study (e.g. -13 db at 37.5 Hz). LAVERACK did not measure particle velocity.

COHEN[3], using tuning forks of 128–320 Hz, found that water-borne vibrations were ineffective. These frequencies are above the sensitive region found in this study and may account for the lack of response. The statocyst and other hair cells, as on the antenna, may also be responsive to acoustic stimuli. It may be that the perception of acoustic stimuli is not a simple process and multiple receptors may be the basis of the observed plateau effect as has been shown for fish[6].

Résumé. Pour le stimulant acoustique entre 10 et 150 Hz les seuils de réponses ont été obtenus sur 5 homards (*Homarus americanus*). La plus grande sensibilité était de 37,5 Hz pour une pression de -13 db re: 1 bar, ou bien de 75 Hz pour une rapidité de particules de 19 db re: 1 var, conditions classiques réalisées pour obtenir en bradycardie un si haut seuil tonal de stimulation.

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[10] My thanks to J. COBB for his encouragement, to P. CAHN and W. SILER for lending and demonstrating the particle velocity transducer, and to H. E. WINN who critically reviewed the manuscript. Instrumentation was provided under USPHS grant No. NB 06397-05. Support was through NIH traineeships to the Graduate School of Oceanography (No. 5 T1-ES-41-03) and to the URI Institute of Environmental Biology (No. PHS 1 T01 ES00104).

Fungistatic Action of the Pigment Secreted by the Fungus *Epicoccum nigrum* Link

It was discovered by chance that when *Epicoccum purpurascens* and *Helminthosporum sativum* were grown side by side on agar media, the former fungus elaborated a diffusible substance which inhibited the hypal growth of the latter fungus. BAMFORD et al.[1] confirmed the production of an anti-fungal anti-biotic by *E. purpurascens* and by *E. andropogonis*.

The present investigation was carried out to determine whether the pigment secreted by *E. nigrum* (Strain 5-1-3) possessed any such anti-fungal effect.