Table 1. Attraction of worker bees to queen bees as a function of mandibular gland secretion.

Class of queens	Queens in class (No.)	Mean ranges of attracted workers*	Mean attraction of workers*
I. Laying queens with glands	5	36–75	48.5 ± 8.4†
II. Laying queens without glands	5	2–15	7.4 ± 2.7†
III. Aged virgins with glands	5	21–69	45.7 ± 8.7†
IV. Aged virgins without glands	4	10–23	16.4 ± 3.3†
V. Newly emerged virgins with glands	5	0–14	3.8 ± 4.1†

* Means result from four replicates, with same queens for each test (only three replicates for class V queens). † 95 percent confidence intervals.

Mandibular glands were removed (*3*) from class II one day before assay. Classes III and IV were sister queens. Class III queens were confined in cages within normal colonies from emergence until the attractiveness test 28 days later. Class IV queens were treated identically, except that the mandibular glands were extirpated within 24 hours after emergence. Class V queens were taken directly from an incubator within a few hours after emergence. All queens were tested for attractiveness simultaneously.

Queen attractiveness was assayed by confining the queens singly in new, 30- by 80- by 15-mm wooden cages (queen mailing cages) having approximately 10^3 mm^2 of wire screen (1.9-mm apertures) exposure on one side. The cages were randomized and distributed immediately on their sides over the top bars of an exposed colony, so that bees had easy access to the screened area of each cage. Then the hive was closed, the cover being supported well above the cages by an empty hive body. Thus the cages were enclosed in a dark chamber free of extraneous air currents. After an exposure period of 10 minutes, all cages with adhering workers were confined in separate cardboard containers. Bees in each container were anesthetized with carbon dioxide and counted.

Striking differences were found between the attractiveness of queens with and without mandibular glands (Table 1). Mated laying queens lost a mean of 85 percent of their attractiveness because of the loss of mandibular gland secretion. The results on attraction of aged virgin queens without glands are biased since 50 percent of the population from which these queens were selected died during the 28 days preceding the attractiveness test. The survival of these confined virgin queens depended upon their capacity to attract nurse bees to feed them. Hence the survivors which were assayed probably represent the most attractive half of that population.

No significant difference (Table 1) was found between the attractiveness of aged virgin queens and mated laying queens. It is therefore concluded that the secretion of attractive materials by the mandibular gland in the queen is a function of maturation rather than mating or egg laying, or both. This is an important consideration in biochemical studies of queen attractants since virgin queens, as opposed to mated queens, can be reared and aged conveniently in quantity.

The mechanism of attraction of workers to their queen serves an important function in the social organization of the colony. Recent research (*4*) has demonstrated that bioactive chemicals are produced by the queen and disseminated to worker bees. These chemicals, known as pheromones (*5*), are potent chemical messengers which mediate behavioral and physiological responses in the workers. One orally acting queen pheromone (9-oxodec-2-enoic acid) has been identified and synthesized (*6*). One or more olfactorily acting queen pheromones from the mandibular gland secretion therefore attract worker bees to the source of orally acting pheromones which are then passed from worker to worker through food-sharing activities (*4*, *7*). The combined effects of the queen pheromone complex ultimately contribute to the integration of thousands of bees into an organized insect social community (*8*).

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19 December 1960

Provisional Audiogram for the Shark, Carcharhinus leucas

Abstract. In an operant-conditioning study, a bull shark responded to signals at frequencies between 100 and 1500 cy/sec. In its band of greatest sensitivity (400 to 600 cy/sec), it discriminated, from high-level ambient noise, signals of amplitudes which the apparatus could not measure.

In an operant-conditioning study, which was prematurely terminated because of circumstances beyond our control, we obtained a provisional audiogram for the bull shark. To our knowledge, three precedents for the conditioning of sharks have been established. Vilstrup (*1*) conditioned spiny dogfish to a motor horn. Moulton (*2*) demonstrated the ability of the smooth dogfish, *Mustelus canis*, to associate an oscillator signal with an electric shock. Clark (*3*) showed that an instrumental (= operant) response could be elicited from large lemon sharks (*Negaprion brevirostris*).

Lowenstein and Roberts (*4*) reported an electrophysiological study of elasmobranch hearing, but expressed the belief that the frequency range derived therefrom had dubious physiological significance.

The subjects of the present experiments were a healthy male *Carcharhinus leucas* which had been captured in the spring of 1959, and a female which was introduced into the pens in the early spring of 1960. All experiments reported here were conducted in one of the 12- by 24-m shark pens at the Lerner Marine Laboratory, Bimini, Bahamas, described by Gilbert and Kritzler (*5*).

Originally, our subjects were trained to manipulate a bar near the surface of the water as the conditioned response to a motor horn signal (peak intensity at 610 cy/sec). In the course of these training exercises, and after the female of the pair had achieved a high level of performance, we became aware of the fortuitous appearance of an operant response in which both sharks demonstrated perception of the sound stimulus by abruptly terminating their normal random movements and swimming, with accelerated velocity, directly to the location of the sound source, about 6 m from the reinforcement area. Subsequent experiments, in which the sound source was moved at random with respect to the reinforcement area, confirmed that the location of the former, rather than of the latter, governed the orientation of the subjects.

The data reported here were secured by exploiting the turning and acceleration components of this orientation response; only the male was used. Attempts to capture a replacement for the female, which died in late June 1960, were unsuccessful.

The experimental design was as follows. At the north end of the pen (immediately beneath the observers' platform) there was centered an underwater loudspeaker, about 6 m south of which was suspended a hydrophone. The stimulus, consisting of successive 2-second pure tones at the frequency and amplitude being tested, was sounded when the shark, moving *away* from the speaker, was at a distance about equal to that between the speaker and the hydrophone. Thus the signal received by the observers through the hydrophone was assumed to be equivalent to that arriving at the subject. The response was considered acceptable only when the stimulus elicited an instantaneous and vigorous 180° turn and an accelerated movement back toward the sound source. Reinforcement (consisting of a 50-g portion of fish) was given when the subject had arrived within a meter of the speaker.

The acoustic apparatus was composed of separate emitting and receiving systems. The emitting system operated satisfactorily with the exception that when, in order to compensate for rising thresholds at the limits of the shark's frequency range, it became necessary to increase greatly the output power of the system, the loudspeaker's normal sine-wave signal became severely distorted. Limitations imposed by the receiving system were more serious. Two factors precluded expressing thresholds for intensity in terms of a standard reference. First, the ambient noise in the pen was consistently at a high level. When measured on one occasion with a visitor's AN/PQM-1A noise measuring set, it averaged about 60 db above a reference of 0.0002 dy/cm^2. Thus, despite the use of an efficient filter (*6*), the subject, at frequencies within its optimal sensitivity band (400 to 600 cy/sec), discriminated signals which were below the indicated filtered noise level and were therefore not measurable. Second, we were unable to procure instruments which would calibrate the receiving system within the sensitivity range of the hydrophone or of the shark. The only reference level available, therefore, was the mean of the filtered ambient noise. This was of nearly equal intensity throughout the shark's range, during the trials reported here, and was adopted as a zero line in plotting the audiogram (Fig. 1*a*).

Each time the subject responded to a stimulus of selected frequency and amplitude, the intensity was reduced until the subject no longer responded. The points on the audiogram represent half the difference, in decibels, between the lowest amplitude at which the stimulus evoked an acceptable response and the next lower at which it did not.

The audiogram is based upon 105 trials which took place on 2 days in August 1960. The broken lines in the right and left ends of the curve denote those points at which the shark failed to respond to stimuli of such high intensity that further increases resulted in the signal distortion noted above. At the center of the curve the sensitivity of the subject exceeded that of our instruments; our ignorance of the curve's shape here is shown by the broken line. The number in parentheses besides each frequency point refers to the total trials upon which the point is based.

As noted above, the greatest single

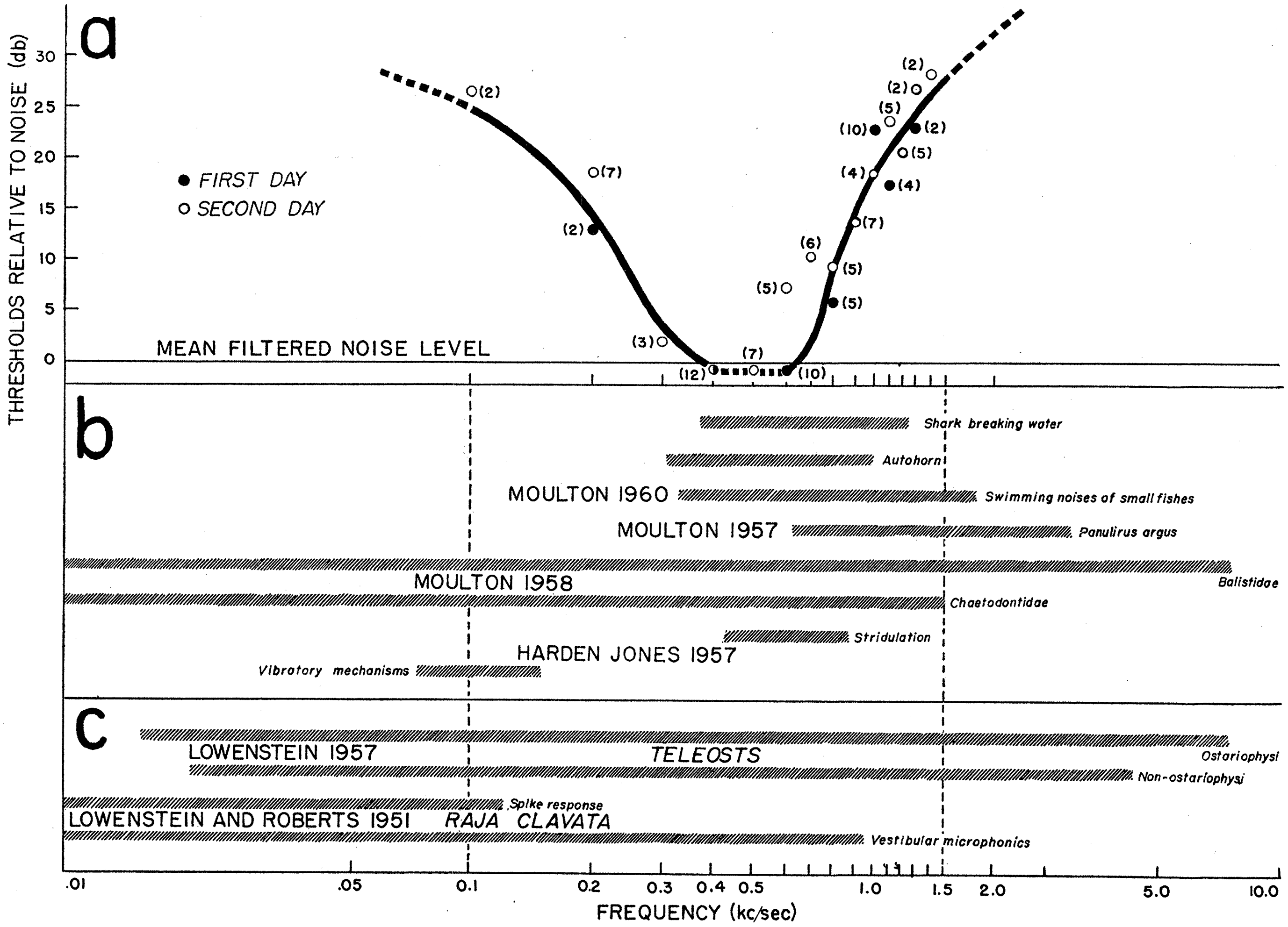

Fig. 1. (*a*) Relative auditory thresholds and frequency range of *Carcharhinus leucas*. (*b*) Spectra of sounds of the natural environment of *C. leucas*. (*c*) Auditory ranges of teleosts and one other elasmobranch.

difficulty in the present investigation was the persistence of high-level noise in the acoustic environment of the pens. Quantitative studies (*7*) of teleost audition suggest that their intensity thresholds differ little from those of man, though their optimal frequencies — like those reported here — are in a lower part of the spectrum. With an assumed noise level of about 60 db, the shark was faced with a problem in discrimination rather than the simple detection of low-intensity sounds in a sea of silence. That it was able to discriminate in the 400- to 600-cy/sec band, when the signal-to-noise ratio indicated by our equipment was *less* than unity, suggests that, if these experiments had been done in a silent environment, the shark's thresholds, at each frequency in its range, would conceivably have been as much as 60 db lower. This would modify the shark's audiogram to be more nearly approximate to those of teleosts and, hence, to that of man.

While the question of sensitivity is of basic importance, so also are two other functional dimensions of hearing. We have already mentioned our observations of these subjects' ability to localize accurately the source of sound in the water. The third consideration is the extent to which this shark's provisional frequency range is useful in the perception of its natural sonic environment. If we compare this range to the spectra of sounds in the sea which may be significant to such a predatory animal (Fig. 1*b*), we find that components of these sounds, in every case we know of (*8*), fall within the suggested range of *Carcharhinus leucas*. In Fig. 1*c*, we have compared it to those reported for other fishes (*7*). Any apparent discrepancies between our results and those of Lowenstein and Roberts may be attributable to the normal differences which might be expected between results from whole, living animals and isolated preparations and, of course, from two different though related species (*9*).

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Perspective on Function of Free Space in Ion Uptake by Roots

Abstract. An observed effect of longitudinal flow rate through corn roots on phosphate transport is shown to be inconsistent with diffusion theory. R. C. Smith's results confirm rather the existence of a diffusion barrier between the xylem and the free space external to the central cylinder of the root.

Whether apparent free space in roots (the partial volume of the roots into which components of a solution appear to diffuse freely) extends to the xylem elements or is interrupted by a differentially permeable cell barrier (the endodermis) has been much disputed. As Russell and Barber (*1*) point out, the endodermis is regarded as a free-space barrier mainly on the basis of its structure, but there is no direct proof of such function. Since recent findings (*2*) would appear to assign free space to cell walls and intercellular space only (and not to the cytoplasm), the suberized Casparian strips of the endodermal cell walls could be an effective barrier to free diffusion and therefore could delimit the radial extent of free space into the root.

The increased solute absorption associated under some conditions with increased transpiration has been a major argument in favor of transport of solute by way of free space directly into the vascular tissues of the plant axis (*3*). Some 20 percent of the total water absorbed is believed to enter through free space together with the solutes it contains. An alternative explanation for the transpiration effect has been advanced (*4*). Solutes may accumulate in free space as water is differentially absorbed by cells bordering a free-space system that reaches uninterruptedly only as far as the endodermis. Absorption of solute from such higher concentrations in free space may, according to this theory, account for the transpiration effect.

Smith (*5*) has presented evidence for an effect of water movement through the vascular tissue on solute uptake which he believes occurred by simple diffusion through free space into the xylem. Young corn-root segments about 3 cm long were so mounted that nutrient solution lacking phosphate could be forced into one end of the root while nutrient solution containing isotopically labeled phosphate (1 mmole/lit.) bathed the outer surface of the root. The efflux was collected at the other end of the root, and its phosphate content was determined. Curves *A* and *B* (Fig. 1) show the relationships of phosphate concentration and total phosphate collected in the efflux to the rate of flow through the root. Contrary to Smith's conclusion, analysis of these data shows that the phosphate could not have moved directly into the stream flowing through the root by simple diffusion through free space. The equation for diffusion into a hollow cylinder is (*6*):

$$Q = \frac{2\pi D(C_o - C_i)}{\ln \frac{r_o}{r_i}} \qquad (1)$$

where Q equals quantity diffused per second per centimeter of length, D is the diffusion coefficient in $\text{cm}^2\ \text{sec}^{-1}$, and C_o and C_i the concentrations outside the cylinder and inside it at some radius r_i respectively; r_o is the radius of the root, and r_i is the average radial distance of a system of sinks, the conducting elements of the xylem. For a given system such as a corn root, Q becomes simply proportional to $C_o - C_i$ and the remaining components can be lumped into a single constant k. Thus:

$$Q = k(C_o - C_i) \qquad (2)$$

It is immediately apparent that the amount of phosphate recovered from the root was not proportional to the quantity $C_o - C_i$, because the concentration C_i decreased hyperbolically with increasing rate of flow through the root, but the phosphate collected increased linearly. This relationship does not conform to Eq. 2, which would require the amount collected to be a hyperbolic function of rate of flow just as the concentration of phosphate was. (The rate of flow has no direct bearing on Q. It affects it indirectly through its effect on C_i only.) Second, with 1 μmole/ml in the external solution and only 0.02 to 0.08 μmole/ml in the solution flowing out of the root, the value of $C_o - C_i$ would hardly be affected. No such large increase in the amount of phosphate collected could result from the very small changes in gradient. These qualitative conclusions may be assessed quantitatively as follows.

If the values are taken at the lowest flow rate, a C_i which rose to 0.076