# AUDITORY CAPACITIES IN FISHES

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## *PURE TONE THRESHOLDS IN NINE SPECIES OF MARINE TELEOSTS*

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# INTRODUCTION

NOT UNTIL THE EARLY DECADES of the present century was it actually established that fishes could hear and respond to sound vibrations in the water. Parker's (1918) investigations conclusively proved that such capacity existed in a number of teleosts. Quantitative work on the frequency range of teleost hearing was undertaken mainly in Germany by von Frisch (1936) and his coworkers. The site of auditory reception in the labyrinth was located at the sacculus and lagena by Dijkgraaf (1949) and his colleagues in The Netherlands. Over the past 30 years, there have been numerous studies on the frequency range, auditory sensitivity, and discrimination in fishes. The literature has been thoroughly reviewed by Kleerekoper and Chagnon (1954).

The methods of most of the investigators consisted of conditioning the animals to a "feeding sound," and the positive response was the approach of the subject to the feeding area. Many workers also used an unconditioned reaction to sound as a sign of a positive response. In terms of the Pavlovian school of animal psychology, this would be called an "orientational reflex." Responses conditioned by a negative stimulus, such as an electric shock, have been used by relatively few investigators. Prominent among these have been Bull (1928), Froloff (1925), Maliukina (1960), Rough (1954) and, recently, Dijkgraaf (1963). In no case was instrumental conditioning (e.g., bar pressing) utilized.

The main problem with the feeding, orientational, and conditioned responses as reported heretofore has been that no clear-cut positive response was used as a criterion. Rather, the responses have been variously described as an approach, "fright reaction," increase in movement, or in similar nonspecific terms. Attempts to obtain a measurable activity change were made by Bull (1928), but in many reports the exact criteria for a positive response were not even described.

The use of such generalized responses, although adequate for qualitative work, is not sufficient for threshold determinations. It is well known, from the study of sensory thresholds, that the response of the subject becomes variable and erratic as the threshold is approached (Ash, 1951), which is to be expected, since a threshold is actually a statistically determined point and there is some probability of positive responses both above and below the calculated threshold point. What is needed, therefore, is a positive response on the part of the subject that is clear and unambiguous to the observer. The search for such responses led to the development of instrumental and avoidance conditioning techniques. On this basis we decided to use the avoidance response in a modified shuttle box as described by Horner, Longo, and Bitterman (1961), as a means of determining sound intensity and frequency thresholds in marine teleosts. Prior work by Behrend and Bitterman (1962) and Wodinsky, Behrend, and Bitterman (1962) demonstrated the feasibility of using this technique for a number of species.

In the majority of previous studies on teleostean auditory capacities, the objective was to determine the upper frequency limits to which the animals could respond. Only in a relatively few studies were absolute intensity thresholds attempted (Autrum and Poggendorf, 1951; Diesselhorst, 1938; Kritzler and Wood, 1961; Maliukina, 1960; Poggendorf, 1952; Stetter, 1929; von Boutteville, 1935), and, in these reports, only one or a few selected frequencies were actually tested. With the exception of the work of Griffin (1950), the intensity measurements were only approximations. Griffin's determinations were based on measurements taken with calibrated hydrophones, amplifiers, and decibel meters. For the present report, equipment was assembled that would give sound pressure measurements with considerable accuracy, i.e., within 1 decibel, an accuracy probably beyond that of the auditory apparatus of the fish.

Interest has always centered on those fishes that possess a Weberian apparatus, the Cypriniformes (Ostariophysi). Species such as the minnow (*Phoxinus*) and the catfish (*Ameiurus*)[1] have been studied most, since it was evident that the Weberian ossicles could act

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[1] The well-known generic name *Ameiurus* has been synonymized with *Ictalurus*, but many recent references still use the older name.

in a manner analogous to the mammalian middle ear bones and transmit sounds received by the swim bladder to the inner ear. It has been clearly shown by many workers that the Cypriniformes possess a higher frequency response and lower auditory threshold than most other forms. This group includes mostly fresh-water forms and some estuarine species. Truly marine forms and those that lack a Weberian apparatus have been investigated only sporadically as to hearing capacity. Most prominent of such studies is that of Maliukina (1960).

The nine species chosen for this report included some of the most common ones in the vicinity of Bimini Island, Bahamas. They can be considered as being representative of a large percentage of the shallow-water fauna of the Caribbean.

With the recent rise in interest in sound production among marine fishes (Fish, 1954; Tavolga, 1960; Schneider, 1961) it becomes of importance to determine what are the hearing capacities of the sound producers, and to what extent these sounds can be detected by other fishes. Also it would be desirable to know how much of the normal ambient water noise can be heard by fishes.

The purpose of this investigation, then, was to determine sound pressure thresholds at various frequencies and thereby enable the construction of an audiogram for several representative species of marine teleosts.

The technique and the apparatus used in the present study are described in detail in later sections of this report. The animals were trained and tested in an avoidance conditioning apparatus. This consisted of an aquarium tank, with two compartments separated by a barrier. The water level was adjusted so that the fish could swim across the barrier but would not remain there. The animal was exposed to a selected tone, produced by an under-water speaker concealed under the barrier, and this was followed in a short time (usually 10 seconds) by a series of intermittent electric shocks. During initial training, the fish learned to escape the shock by crossing the barrier, thus breaking a light beam to a photoelectric cell which terminated the trial. After a period of escape training, the animal began to cross the barrier after the onset of the sound (the conditioned stimulus) and before the onset of the shock (the unconditioned stimulus). This behavior, therefore, was an objective index of the fact that the fish responded to the sound. The sound was virtually a pure tone of a single frequency, and its intensity was measured, in terms of acoustic pressure, by a calibrated monitoring system. After the avoidance response was judged sufficiently reliable, the level of the sound was reduced in graded steps with each successive trial, until the subject failed to avoid, i.e., it crossed the barrier only after being shocked. The threshold for each given frequency was determined by the staircase or "up-and-down" method commonly used in psychophysical studies, and from a series of such determinations at various frequencies audiogram curves were constructed for each of the species tested.

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The illustrations were prepared by Mrs. Frances W. Zweifel.

# SPECIES TESTED

THE FOLLOWING NINE species of marine fishes were tested for this report:

Squirrelfish, *Holocentrus ascensionis* (Osbeck), Holocentridae
Dusky squirrelfish, *Holocentrus vexillarius* (Poey), Holocentridae
Cubbyu, *Equetus acuminatus* (Bloch and Schneider), Sciaenidae
Blue-striped grunt, *Haemulon sciurus* (Shaw), Pomadasyidae
Schoolmaster, *Lutjanus apodus* (Walbaum), Lutjanidae
Blue-head wrasse, *Thalassoma bifasciatum* (Bloch), Labridae
Beau-gregory, *Eupomacentrus leucostictus* (Müller and Troschel), Pomacentridae
Red hind, *Epinephelus guttatus* (Linnaeus), Serranidae
Slender sea robin, *Prionotus scitulus* (Jordan and Gilbert), Triglidae

These forms were chosen on the basis of several criteria. The species are representatives of different teleost families which are some of the major groups of shallow-water forms in the Bimini area. They are also representative of a number of very different configurations of swim-bladder structure. Included are species that are well known to be sound producers. All are quite common in the region.

The choice was restricted by the time available to train and test the animals, and in addition it was found that some species were not testable by the methods described here. Some preliminary attempts at avoidance conditioning were made with the goby, *Bathygobius soporator*, the toadfish, *Opsanus* sp., and numerous plectognaths such as puffers, triggerfish, and cowfish. The main reason for failure with these species appeared to be that their response to the electric shock was usually a freezing reaction, and a reliable escape response could not be elicited under the conditions used. Modifications of the apparatus or, possibly, the use of positive or reward conditioning will be required for adequate threshold determinations in such forms.

With the possible exception of the squirrelfish, all the species tested were strictly diurnal in habit. They were collected locally in traps or by hook and line. The squirrelfish and red hind were captured in the neighborhood of Turtle Rock, a rocky outcropping a few miles south of Bimini. The others were collected near the laboratory dock.

### SQUIRRELFISH (*Holocentrus ascensionis*)

The family Holocentridae is represented by only a few species in this area. The systematics of the western Atlantic forms was reviewed by Woods (1955), and the identification of this species, which is by far the most common in Bimini, was based on the descriptions given in that paper.

In captivity, squirrelfish are hardy but quite timid and usually seek out dark corners or shelters. As daylight wanes, they become more active and feed more readily. Their activity rhythm is distinctly crepuscular and possibly nocturnal (Moulton, 1958). The distinctive large eyes are obviously excellent light-gathering devices. In the field, they are usually to be found among rocks and corals. Territorial behavior has been reported by personal communications from many skin divers but has not yet been confirmed. Sex differences in behavior or external structure are not known, nor is there any information on reproductive behavior.

This species is of particular interest in auditory studies since it is well known to be a sound producer. In the Bimini area, *H. ascensionis* is probably one of the most regularly vociferous fishes. Moulton (1958) and Winn and Marshall (1960) have described the sound bursts that are emitted when the animals are threatened or sometimes merely approached by other squirrelfish or other species. The sonic mechanism has been described as consisting of a pair of muscles stretched across the dorsal surface of the swim bladder and attached to the anteriormost ribs (Winn and Marshall, 1963).

The probability that the squirrelfish has a good hearing apparatus is enhanced by the fact that the anterior end of the swim bladder in *H. ascensionis* is almost in contact with the otic region of the skull (Nelson, 1955), separated only by a tough band of connective tissue. If we assume that the swim bladder acts as a receiving transducer, then the direct or indirect physical contact with the skull should increase the efficiency of sound transfer to the inner ear in a manner analogous to that of the Weberian apparatus in the Cypriniformes.

A total of five *H. ascensionis* were used in threshold determinations, and several additional animals were used in preliminary tests. All specimens were mature and ranged in size from 17 to 20 cm. in standard length. This is a hardy species, and it submitted to handling without any damage. All the animals were kept in a large, concrete, holding tank (about 100 gallons) on the laboratory grounds. Running sea water was supplied, and the specimens were fed about every two days on pieces of conch (*Strombus*). The training and testing took place daily. Since the animals were kept in a single holding tank, each specimen was identified by fin clipping.

### DUSKY SQUIRRELFISH
### (*Holocentrus vexillarius*)

This is a smaller species, 5 to 8 cm. in standard length, usually found in rocky tide pools. Little is known about the behavior or ecology of *H. vexillarius*, and it is not known to be a sound producer. Dissections revealed that this species has the same type of swim-bladder structure as does *H. ascensionis*.

Three specimens of *H. vexillarius* were conditioned and tested. They were kept together in a 10-gallon aquarium and marked by fin clipping.

### CUBBYU (*Equetus acuminatus*)

This is probably the only representative of the family Sciaenidae that is found in the Bimini area. The species is not common but can be collected sporadically in the bay.

The swim-bladder structure in this species is very similar to that of the grunts and snappers. Schneider and Hasler (1960) reported the existence of "drumming" muscles in the lateral body wall of *E. lanceolatus*, but no sound-producing mechanism could be located in the specimens used here. *Equetus* has not been specifically reported as a sound producer.

There is no information on the auditory capacities of this species. Indeed, there has been no published study of hearing of any sciaenids, with one exception. The European genus *Corvina* was shown to have a high sensitivity to sound. Maliukina (1960), by the use of classical conditioning, demonstrated thresholds of −45 decibels (re 1 microbar) at 320 cps. and −50 decibels at 500–600 cps.

The data in the present report are based on determinations made on three specimens, 8 to 12 cm. in standard length.

### BLUE-STRIPED GRUNT (*Haemulon sciurus*)

This is certainly the most common member of the Pomadasyidae in the Bimini area. Small individuals are always present in the vicinity of the laboratory dock, and specimens can be collected in traps or by hook and line almost anywhere along the shore.

The species is not territorial in habit and is entirely diurnal. All the grunts are well known as sound producers. Burkenroad (1930) described the mechanism as being the pharyngeal teeth. The scraping of these patches of rasp-like denticles is presumably amplified by the swim bladder into the grunt-like sound heard when the fish is handled (Moulton, 1958). It is not known if these animals produce a sound under water during the course of their normal behavioral repertoire. Thus far the evidence appears to be negative.

There is no information on sound reception in any of the Pomadasyidae.

The swim bladder in *Haemulon* can be considered as typical in general form. It extends for almost the full length of the abdominal cavity. It protrudes into the perivisceral cavity and can be separated from the body wall easily, except just along the middorsal line. It is composed of tough, inelastic connective tissue and is rounded cephalad where it ends short of the occipital region of the skull.

Threshold determinations were based on four specimens. All were young, immature individuals of about 8 cm. in standard length. They were kept in individual 2-gallon aquaria and were trained and tested daily.

### SCHOOLMASTER (*Lutjanus apodus*)

This species was chosen as an easily available representative of the large snapper family (Lutjanidae). Young specimens of *L. apodus* were readily collected in traps around the laboratory dock.

The schoolmaster sometimes occurs in groups of up to a dozen individuals that remain in one general area for long periods of time. However, this species is not territorial, and the loose aggregations cannot be considered schools. None of the members of the Lutjanidae is known to be a sound producer, and there is no information on sound reception in this group.

The swim bladder in *Lutjanus* is essentially the same in general structure as that of *Haemulon.*

Threshold determinations were based on three specimens. All were young, immature individuals of about 10 cm. in standard length. They were kept in individual 2-gallon aquaria.

### BLUE-HEAD (*Thalassoma bifasciatum*)

The blue-head (*Thalassoma bifasciatum*) and the slippery dick (*Halochoeres bivittatus*) are the most abundant wrasses (family Labridae) in the shallow waters around Bimini. Curiously, *Halochoeres* turned out to be completely unsuitable for this type of conditioning in that individuals would not learn to escape the shock but remained in a corner of the tank and simply endured the shock until it almost killed them. *Thalassoma*, however, learned the escape and the avoidance problems readily. Specimens were captured in traps within 100 yards or so of the laboratory dock.

These wrasses are generally found in or around hiding places such as rock crevices, corals, and shells, but they are not territorial in habit. None of the labrids is known to be a sound producer. The only information on hearing in this group is the report of Bull (1928) who demonstrated the ability of *Crenilabrus melops* to respond to a sound of 128 cps. in frequency. However, the same species was not able to discriminate between the sound of a tuning fork and that of an electric buzzer (Bull, 1929).

The swim bladder in *Thalassoma* is small, ovoid, and in the posterior third of the abdominal cavity. It is less than one-fifth of the length of the cavity and is loosely attached to the dorsal body wall.

Threshold determinations were based on four specimens. These were all mature males, as judged by the predominant blue and black coloration of the head and body (Stoll, 1955), and they were about 5 to 7 cm. in standard length. The animals were kept in individual 2-gallon aquaria.

### BEAU-GREGORY (*Eupomacentrus leucostictus*)

This species is probably one of the most strongly territorial among the many reef-dwelling members of the Pomacentridae. It is quite common in the vicinity of the laboratory dock at Bimini, living in and around conch shells and pilings. Breder (1950, 1954) described some factors in their territorial behavior. Both males and females defend their selected shelters and areas vigorously. In captivity, it is usually necessary to keep the animals in separate aquaria, since they fight one another or other fishes until severe damage is inflicted.

Knudsen, Alford, and Emling (1948) reported the "damozel" as producing a "drumming, tapping" sound, presumably with its pharyngeal teeth. The specific identification is doubtful, but the animals were very likely to have been pomacentrids. *Eupomacentrus leucostictus* was definitely identified as a sound maker by Moulton (1958). Faint snapping sounds were produced by individuals in aquaria when one attacked another or when darting for cover (confirmed by observation, Tavolga, unpublished).

There is no information on the sound reception in this species or any other member of the Pomacentridae.

The swim bladder in *Eupomacentrus* is unlike that of any of the other species studied here. The bladder is essentially triangular in cross section, with the apex pointed dorsally. It is tightly wedged between the dorsolateral body walls, and on dissection of the perivisceral cavity, only the thin, flat ventral wall of the bladder is visible. The anterior end of the bladder does not abut the otic region as in *Holocentrus.* If the bladder acted as a transducer in sound reception, its coupling to the inner ear would be indirect, e.g., lateral musculature and ribs to vertebral column to skull.

Threshold determinations were based on four specimens. All were presumably mature individuals of about 7 to 8 cm. in standard length. They were kept in individual 2-gallon aquaria supplied with running sea water and fed about every two days on conch.

### RED-HIND (*Epinephelus guttatus*)

This species is considered representative of the family Serranidae, which includes the many species of sea basses and groupers. Specimens of *E. guttatus* were commonly captured on hook and line in the vicinity of Turtle Rock, south of Bimini. Nothing specific is known of the territorial habits of this form, but it is usually found in or around rock crevices, as are its close relatives, the rock hind (*Epinephelus adscensionis*) and Nassau grouper (*E. striatus*).

Sound production has been described for the latter species by Moulton (1958) as consisting of "vibrant grunts" when the animals were disturbed or approached by a foreign object. Tavolga (1960) obtained series of sound beats from the black grouper (*Mycteroperca bonaci*). These sounds were also elicited when the animal was disturbed or, in some cases, were produced when another fish swam close by. Electrical stimulation of the common sea bass (*Centropristis striatus*) resulted in drum-like thumps (Fish, 1954). Moulton (1958) believed that the sounds were produced by vibrations of the lateral body musculature, while Fish (1954) and Tavolga (1960) thought that the pounding of the operculum against the pectoral girdle was the mechanism involved. The swim bladder itself lacks drumming muscles. The quality of the sounds of these serranids is that of a low-pitched grunt or thump, with a fundamental frequency of 100 to 200 cps. Hazlett and Winn (1962) described the lateral body musculature involved in sound production in the Nassau grouper (*Epinephelus striatus*).

The swim bladder of the red hind, like that of all the serranids, is large and thin-walled and extends the full length of the abdominal cavity. It is composed of tough, inelastic, connective tissue and, anteriorly, there is no direct connection with the occipital region of the skull. Essentially, it is similar to that of *Haemulon* and *Lutjanus*.

Although a number of specimens were used, the threshold determinations given here are based on the responses of a single animal. This one was immature, about 27 cm. in standard length.

### SLENDER SEA ROBIN (*Prionotus scitulus*)

The sea robins, as are most of the Triglidae, are well known as sound producers, and references to this ability go back hundreds of years. The sonic mechanism was described by Tower (1908) as consisting of a pair of muscles attached to the lateral walls of the swim bladder. The muscles are intrinsic, i.e., completely detached from the lateral body wall. The studies of Moulton (1956) showed that sound production in *Prionotus* may be involved in some form of communication.

The auditory capacity of a sea robin (*Prionotus evolans*) was investigated by Griffin (1950). In a preliminary study, he showed that this species can respond to a sound of 100 cps. at a pressure level of about 17 decibels re 1 microbar, but such response was not construed to be a threshold value.

Although this species is normally not common in the Bimini area, three specimens of 15 to 20 cm. in standard length were collected, and threshold determinations were made.

# EQUIPMENT

Briefly stated, the equipment used consited of five systems: (1) the experimental aquarium tank in which the animals were trained and tested, (2) the test sound-producing system, (3) the sound monitoring and measuring apparatus, (4) the electric shock system for the unconditioned stimulus, and (5) the control apparatus interconnecting all of the above.

## The Experimental Aquarium

A standard glass aquarium tank was lined on the inside with a 2-inch layer of rubberized hair on the sides and floor. The rubberized hair was the type commonly used as padding within packages containing fragile items. The insulating material was found, after prolonged tests, to be inert as far as any deleterious effects on fish were concerned. It consists of a mixture of curled horse and hog hair impregnated with latex. As an under-water sound insulator, it decreased the background noise level in the tank by about 20 decibels, and, as a sound baffle, it virtually eliminated sound reflections and standing waves. One important property of this material was that, when water was introduced into the tank, it penetrated through the insulation without significant trapping of air bubbles. Air bubbles are known to be excellent sound reflectors, and these could be eliminated by slowly filling the tank with its interior insulation. This material possesses some of the sound-absorbing properties of various materials of fiber type (Tamm, 1957) and has been widely used as an acoustical curtain in under-water sonic studies. To eliminate further the effects of background noise, the tanks were set on 2-inch cushions of foam rubber at the corners. Most low-frequency noise was eliminated in this way.

Two experimental tanks were constructed. One was a 20-gallon aquarium, and the other a 5-gallon size. The larger one was used for testing squirrelfish (*Holocentrus ascensionis*) and red hind (*Epinephelus*), while the smaller one was used for the other, smaller species.

Transversely, across the center of each experimental tank, a partial barrier was constructed of rubberized hair (solid, flat surfaces were avoided to prevent sound reflections and possible standing waves). The tank, therefore, was divided into two equal compartments, with the floor slanted up toward the center divider. The water level was adjusted so that the center barrier was covered by an inch or so. The fish was able to swim from one compartment into the other, but in doing so was forced to cross the barrier on its side or with a large part of its dorsal surface protruding out of water.

The water level over the center barrier was found to be a critical dimension. It had to be high enough to permit the fish to swim over, although with some difficulty, yet low enough to inhibit the animal from remaining in this center area. In training, the water level was generally higher, but, as intertrial crossings of the barrier increased, it was gradually dropped to a level optimal for each species. A light beam to a photoelectric cell was placed so that the crossing of the barrier could be detected.

The under-water speaker was concealed under the center barrier. The shocking electrodes were inserted into the inner insulator walls on the sides of each compartment. The hydrophone was placed in the tank usually at one of the ends farthest from the sound source, but it was normally not left in the tank during the course of a training or testing run. A mirror was clamped in a position above the tank, so the center barrier could be observed.

Figure 1 is a diagrammatic section of the small experimental tank. The large tank and its dimensions are shown in figure 2.

The water in the small tank was changed completely after each series of trials, and the large tank was supplied with running sea water which was turned off temporarily during threshold determinations to lower the level of ambient noise.

## Sound-producing System (the Conditioned Stimulus)

In all this work, single frequency sine waves were used as the conditioned stimulus. The system was arranged so that the conditioned stimulus was virtually a pure tone (as determined from the sound monitoring system). The sine wave was generated by an

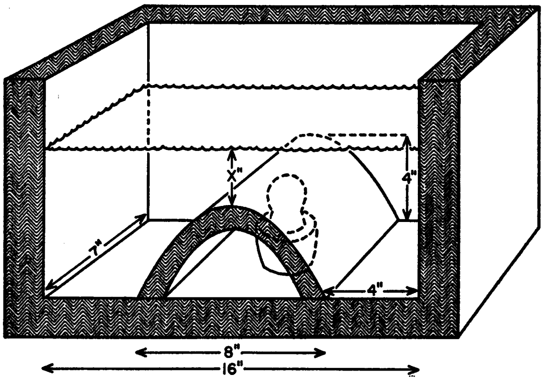

FIG. 1. Diagrammatic longitudinal section of the small experimental aquarium used in the testing of auditory capacities in seven species of marine fishes. The dimensions are in inches. The insulating material was 2 inches thick, and the entire structure was set inside a glass aquarium. The height of the water above the central barrier (X) was varied with the species used. The under-water speaker was within the central barrier, as shown.

audiogenerator (Heath Model AG-1A) that possessed an output meter, which permitted constant control of the output level. After appropriate calibration by means of the sound-monitoring system, the intensity of the output from the speaker could be controlled with this meter alone.

The power amplifier was a 14-watt unit (Heath Model EA-3), the gain and tone controls of which were set to produce the output of least distortion consonant with the generator output and the frequency and intensity required for the conditioned stimulus. The switching of the signal took place, by means of the control system, between the generator and the amplifier. The snapping noise or transient click of the switch was not detectable by the monitoring system and was probably far below background noise level in intensity. Some blank trials were included in random fashion in the testing and training series in which the gain control of the generator was turned down to the minimum level and the shock system was disconnected. The possible conditioning of the animals to the transients of switching noise was largely eliminated.

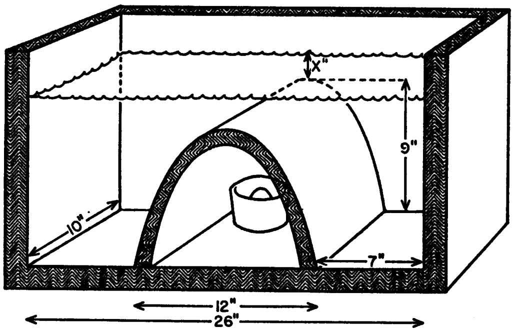

FIG. 2. Diagrammatic longitudinal section of the large experimental aquarium used in the testing of two of the species (*Holocentrus ascensionis* and *Epinephelus guttatus*). Except in size, this tank was essentially like that shown in figure 1.

The under-water loud-speaker in the small tank was constructed of a public address driver unit (University Model SA-HF) with a stiff rubber bulb attached over the horn end. The entire unit was waterproofed with tar, tape, and rubber. The rubber bulb served as a good coupling device for transmitting the sound into the water, and there was enough air inside so that the excursions of the voice coil were not inhibited. Distortion-free sine waves were obtainable from this unit in the 200- to 5000-cps. range at pressure levels of up to 50 decibels above 1 microbar. At lower frequencies, however, harmonic distortion and clipping took place at levels above 30 to 35 decibels. The acoustic pressure was measured with the monitoring system at distances of 6 to 8 inches from the sound source, and distortions were observed through the monitoring system on an oscilloscope screen.

The large tank was equipped with an under-water speaker designed by University (Model MM-2), with a plastic expansion bulb as the driving surface. Under the conditions of the present investigation, the frequency response and distortion level of this unit were slightly superior to those of the one described above.

In the process of testing the frequency response of the speakers, it was found that the rubberized hair insulation eliminated standing waves in the tanks, at least at the frequencies and acoustic pressures that were used.

### SOUND-MONITORING SYSTEM

The hydrophone used as the pick-up unit was a disc-shaped barium titanate unit (Model SB-154C, Chesapeake Instrument Co.)

approximately 5 cm. in diameter and 1.5 cm. in thickness. The output was given by the manufacturer as −89 decibels (re 1 volt per microbar of sound pressure) and a frequency response essentially flat from 50 cps. When the output was monitored from the speaker, the hydrophone was always placed in the same position in the tank, i.e., suspended at the wall farthest from the sound source.

The pre-amplifier was a transistor unit designed by Robert Laupheimer of the Courant Institute of Mathematical Sciences, New York. Its frequency response was flat from 50 cps., and it had a calibrated gain control with settings at 40, 60, and 80 decibels. Its internal noise level was below 1 microvolt at the output. It possessed a cut-off switch at 5, 10, and 20 kilocycles per second.

The sound levels were measured on an audio volt meter (Heath Model AV-3) the calibration of which was checked against a standard root-mean-square decibel meter. In all, the accuracy of the sound pressure determinations at the frequencies and intensities used was in the order of ±0.5 decibel.

All the decibel measurements given here refer to acoustic pressure levels rather than acoustic intensity *per se*. Albers (1960) makes the distinction between these two values clearly. "Acoustic intensity" is measured in ergs or watts per square centimeter and is proportional to the square of the pressure. "Acoustic pressure" is given in terms of dynes per square centimeter and, for arithmetic convenience, is usually expressed in decibels in reference to some standard value such as 1 microbar (=1 dyne per square centimeter). The following formulas for acoustic power, intensity, and pressure will facilitate the conversion of our data into other units.

Acoustic power: $P = p^2A/\rho c$ (in ergs per second)

Acoustic intensity: $I = p^2/\rho c$ (in ergs per square centimeter), or $I = (10^{-7}p^2)/\rho c$ (in watts per square centimeter)

Acoustic pressure:

$$p = \sqrt{\frac{10^7 P\rho c}{A}}$$

(in dynes per square centimeter), or $p = 20 \log_{10} (p/p_0)$ (in decibels)

In the above equations for plane waves, $A$ is the area in square centimeters through which the acoustic energy must flow. The factor $\rho c$ is the acoustic resistance of the medium (the acoustic ohm) and is the density of the water times the velocity of sound in this medium. The value of this factor for water is usually taken as 150,000 grams per square centimeter per second, and for air it is 42 grams per square centimeter per second. Temperature and salinity, of course, affect both the density and velocity figures. In the last equation, $p_0$ is the reference value of acoustic pressure. All measurements given in this report are in reference to 1 microbar (which equals 1 dyne per square centimeter), rather than the idealized and commonly used 0.0002-microbar threshold of human hearing at 1000 cps. In under-water acoustics, the human threshold actually has little real meaning and the 1-microbar reference level is becoming more widely used (Horton, 1959). Most of the earlier reports use the 0.0002 level as 0 decibel, which is equivalent to −74 decibels re 1 microbar, so that conversion is a simple arithmetic matter.

Although the distance of the hydrophone from the sound source was constant in all determinations, the distance of the fish from the sound source varied. The transmission loss of sound energy over the length of the experimental tank had to be considered. Based on the data and equations given by Albers (1960) and Horton (1959), the attenuation of the sound pressure because of scattering, absorption, and air-bubble effects is negligible over the distances and frequencies involved in these experiments. The loss of energy from spreading or divergence, however, follows the inverse square law. Since intensity varies as the square of the pressure, the sound pressure should vary in inverse proportion to the distance from the sound source. Assuming a point source of sound in our experimental tanks and measuring the range of distances from the sound source where the experimental animal was most likely to position itself at the start of a trial, we calculated that the transmission loss was about 2 decibels in the small tank and 3 decibels in the large tank. By placing the hydrophone in different positions in the experimental tank, we found that the actual transmission loss was somewhat more than the theoretical value. In the large tank, there

TABLE 1

AMBIENT NOISE LEVELS IN EXPERIMENTAL TANKS

| Band Width in Cycles per Second | Sound Pressures in Decibels (re 1 Microbar) | |
|---|---|---|
| | Large Tank | Small Tank |
| 37.5– 75 | <−50 | −43 |
| 75– 150 | <−50 | <−50 |
| 150– 300 | −50 | <−50 |
| 300– 600 | −46 | −43 |
| 600–1200 | −43 | −39 |
| 1200–2400 | −39 | −34 |
| 2400–4800 | −35 | −29 |
| 4800–9600 | −20 | −20 |

was a 5-decibel decrease in sound pressure from the base of the central barrier to the wall farthest from the speaker, a distance of about 6 inches. The corresponding loss in the small tank was 3 decibels. Height above the bottom made no perceptible difference, but, if the hydrophone was in direct contact with the insulating material of the central barrier which covered the speaker, the increase in sound pressure was about 2 decibels. Thus, if the starting points for the fish were randomly distributed within its compartment, the accuracy of the sound determination with respect to the actual pressure received by the fish was ±3.5 decibels in the large tank and ±2.5 decibels in the small tank. Actually, the threshold determinations made were more accurate, since the fish generally made a habit of assuming the same position during the intertrial period. For most individuals, this starting position was close to the far wall of the compartment of each fish, although there were many exceptions. This question is discussed in greater detail in the section on Results, below.

The total ambient noise levels in the experimental tanks ranged from −10 to −15 decibels (re 1 microbar). The actual background sound was mostly a combination of high-frequency hissing produced by the running sea-water system in other aquaria in the laboratory and some low-frequency ground vibration. Both noises were considerably reduced by the rubberized hair insulation and the foam rubber padding beneath the tanks. The noise, as measured by the sound monitoring system, also included electrically induced and intrinsic noise, i.e., 60-cps. hum and transistor and tube thermal noise. The noise spectrum was determined by the insertion of an Allison band filter into the system. Table 1 shows the band widths and background sound pressures (the system was not accurate below the −50-decibel level).

From table 1 it can be seen that in the range used in this work (50 to 3000 cps.), the noise level was quite low and at least 10 decibels below the lowest auditory threshold obtained for any of the species tested.

### ELECTRIC SHOCK SYSTEM (THE UNCONDITIONED STIMULUS)

The source for the unconditioned stimulus was the output from a variable autotransformer (Fisher Powerstat). In some of the preliminary work, a direct-current source, i.e., battery, was used, but the autotransformer output was more easily variable and the electrodes were not polarized. The voltages, as measured at the output of the transformer, ranged from 5 to 30 volts. By trial and error, the optimal voltages for each species were determined, and it was found that some forms could not tolerate more than about 7 volts, while others showed no response until 25 or 30 volts. The optimal voltages are given in the Results section for each species tested.

The shocking electrodes had to be of some material that could withstand the corrosive effect of sea water and be highly conductive and yet yield no toxic by-products. Short pieces of silver solder (a type of brazing material) were found to be satisfactory. These were twisted into circles, a pair for each compartment of the experimental tank, and attached to the side walls by our inserting them into the stiff insulating hair. During a shock pulse, the electrical field was strong enough to affect the fish at any point in the experimental tank.

The shock was applied intermittently by the control system in pulses of about one-tenth of a second in duration and at a repetition rate of approximately 40 pulses per minute.

Because of the high conductivity of sea water, it is assumed that the amount of current passed between the electrodes was high, but no means were available for actual measurement of this factor. All that can be said is that the shock level was adjusted so that a clear response could be obtained from the fish without damaging it. The fact that some animals were tested daily for up to two months with no deleterious effects shows that such a level was empirically achieved.

The range of shock intensities was affected by several variables: species differences in tolerance; development of tolerance with training; changes in effective shock intensities as a result of corrosion of the electrodes; changes in the position of the fish; and line-voltage fluctuations. The shock intensities used for each species are given in the Results section.

In cases in which the shock level was too high but still not lethal, the effect on the behavior of the fish was immediately apparent. Both avoidance and escape responses ceased, even in fish that had been well trained previously. On the basis of a number of such observations, we concluded that, if the shock level were too high, both avoidance and escape behavior were disrupted, and that in the majority of our tests the shock was below the disruptive levels. Short of this extreme, we are not in a position to evaluate the effects of variations in shock intensities on the threshold determinations.

### CONTROL SYSTEM

The apparatus was essentially the same as that used in shuttle-box avoidance conditioning by Horner, Longo, and Bitterman (1961), Behrend and Bitterman (1962), and Wodinsky, Behrend, and Bitterman (1962).

To begin a trial, the "start" button was pressed, which turned on the switch between the audiogenerator and the power amplifier, i.e., the conditioned stimulus was on. Simultaneously, an electric clock (graduated to one one-hundredth of a second) was started. A standard interval between the onset of the sound and the shock of 10 seconds was used. If the animal crossed the barrier from one compartment into the other during this period, it broke a light beam to a photoelectric cell. This stopped the sound and the clock.

If the animal did not "avoid" during the 10-second period, the shock was applied with a pulse repetition rate of about 40 per second. The sound continued. When the animal escaped by crossing the barrier, the shock, sound, and clock were stopped. The time for an animal either to avoid or to escape was shown on the clock. After an appropriate intertrial interval, the trial was repeated, with the animal crossing the barrier in the opposite direction.

Wodinsky, Behrend, and Bitterman (1962), using some of the same species but with light as the conditioned stimulus, found that 10 seconds was an optimal interval between the conditioned and unconditioned stimuli for the response to take place. Generally, if the animal did not avoid within that time, it did not do so if given more time. In addition, a longer interval led to a confusion between an avoidance response and an intertrial response. If the time before onset of the shock were too long, the association (contiguity learning) between the two stimuli was not developed. If the interval between the stimuli were too short, the slow-reacting species or individuals lacked enough time to respond.

The intertrial interval had to be varied for the species used, i.e., ranging from one-half of a minute to five minutes. In addition, for each series of trials, the intertrial interval was varied in a random fashion to prevent the animal from anticipating the onset of a trial. For example, if an intertrial interval of two minutes were chosen for a particular series, the actual intervals were one minute, two minutes, and three minutes, varied at random, i.e., averaging two minutes. The specific intervals are given below in the sections under the various species.

# METHODS

## Training Techniques

Initially, the acoustic stimulus level was set at a point some 20 or 30 decibels above the estimated threshold level. In the early experiments, no estimates were available, and in some cases this initial training level turned out to be as much as 50 decibels above or 10 decibels below the actual threshold. The frequency of the training tone was varied with different animals, but often we began at 400 or 440 cps. Some fish were trained at other frequencies to check points on the audiograms.

The shock level was also estimated. After some trial and error, including the death of some specimens, the optimal shock levels were determined for each species.

The first series of trials can be termed "escape training." The test animal was placed in one of the compartments in the test tank, the start button on the control apparatus was pressed, and the trial was begun. The interval between the onset of the sound and the shock was uniformly 10 seconds (with one exception, i.e., *Prionotus*).

At the onset of the intermittent shocks the animal reacted with visible, violent twitches to each shock and an increase in general activity. The shock levels during these early trials were kept as low as possible, so as not to produce any damage to the fish. The water level over the barrier was usually at least twice the optimal value (see below under Results), so that the fish had no difficulty in crossing the barrier.

In the majority of cases, no crossing took place in the first few trials, and, in order to prevent the animals from receiving an excessive number of shocks, the fish was guided or pushed across the barrier, after the fish had received not more than 20 or 30 shocks.

The escape response consisted of the fish's swimming across the barrier after receiving one or more shocks. Once the animal escaped a number of times, the technique of guiding the fish was discontinued, unless there was no response for about 20 seconds in the duration of shocks. In the majority of cases, the escape training was achieved in the first day of trials. Twenty-five trials per day were used as a standard during the training period, but was varied from 10 to 50 on a few occasions. The latency of each response was measured on an electric clock which was part of the control apparatus.

The intertrial period was varied in random fashion so that the animal should not become conditioned to the time intervals and to control for the coincidence of intertrial crossings with the onset of the sound stimulus. The average intertrial periods for each species are reported in the Results section.

Once escape training was on its way, the water level across the barrier was lowered to such a point that the crossing of the fish was not prevented but intertrial crossings were inhibited. Often the lowering was not done until later testing trials, since intertrial crossings rarely took place during the training period.

The intensity of the shock usually had to be raised as escape training progressed. The animals seemed to develop a tolerance for the shock, and the level for each species, as given in the Results section, was that used during the test trials.

During training, the subject had to learn two things. First, it had to learn to cross the barrier in response to the shock, which we call "escape training." Second, it had to associate the sound with the shock and learn to cross over as soon as the sound came on, which we call "avoidance training."

With each day's trials, the number of avoidances increased. Often, in a series of avoidance responses, the response time gradually increased from one or two seconds to a maximum of 10 seconds. This was followed by one or more escape responses; then the animal began to avoid again. The number of training days for an adequate number of avoidances to be achieved varied, ranging from three days for *Equetus* up to 12 days for *Lutjanus*, at 25 trials per day.

Since the rate of learning to avoid was not the primary concern of this investigation, only rough indications of this rate can be given. In some cases, the intensity of the conditioned stimulus had to be raised during the course of initial training, because the starting levels were too close to threshold values, and avoidance learning was abnormally slow. The number of trials per day was not constant during the training period. Also, if an animal did not escape during the first day's trials, it was guided across the barrier with a plastic paddle, to reduce the total number of electric shocks received by the subject. For these reasons, then, the conditions during early training were not constant, and the records do not provide a reliable index of the rate of learning to avoid. With an average of 25 trials per day, most of the animals reached a criterion of 90 per cent avoidance within five or six days. This compares favorably with the results reported by Wodinsky, Behrend, and Bitterman (1962) who used light as the conditioned stimulus.

A behavioral feature common to most of the animals tested was their slow response at the beginning of any given day's series of trials. Even after the animals were well conditioned and had been tested a number of times, they rarely avoided on the first few trials, even though the sound level was initially 20 decibels above threshold. It was as though the animals required a short "refresher course." These "warm-up" trials at the beginning of each series were characteristic of nearly all the animals tested. The criterion, therefore, for successful avoidance training was set at a minimum of 18 avoidances out of 20 trials, with the first five trials in a day's sequence discarded as "warm-up" trials.

Figure 3 presents the entire training sequence of one animal, a squirrelfish (*Holocentrus ascensionis*). The response times (in seconds) are plotted on the ordinate. P on the graph represents cases in which the animal was guided or prodded across the barrier in order to prevent its receiving too many shocks. Note that this had to be done in 20 out of 25 trials during the first day but only sporadically on other trial days. The second and third days of the trials showed good escape learning and a few avoidances. On the fourth day there were eight avoidances in 25 trials, and on the fifth day the criterion of avoidance learning was attained with 22 avoidances in 25 trials.

Figure 4 shows a threshold determination made for the same animal, the training protocol of which is illustrated in figure 3. This threshold was taken immediately following the twenty-fifth trial on the fifth training day. After each avoidance response, the conditioned stimulus level was reduced by 5 decibels at the subsequent trial. If there was no avoidance, the animal usually escaped promptly, and at the next trial the condi-

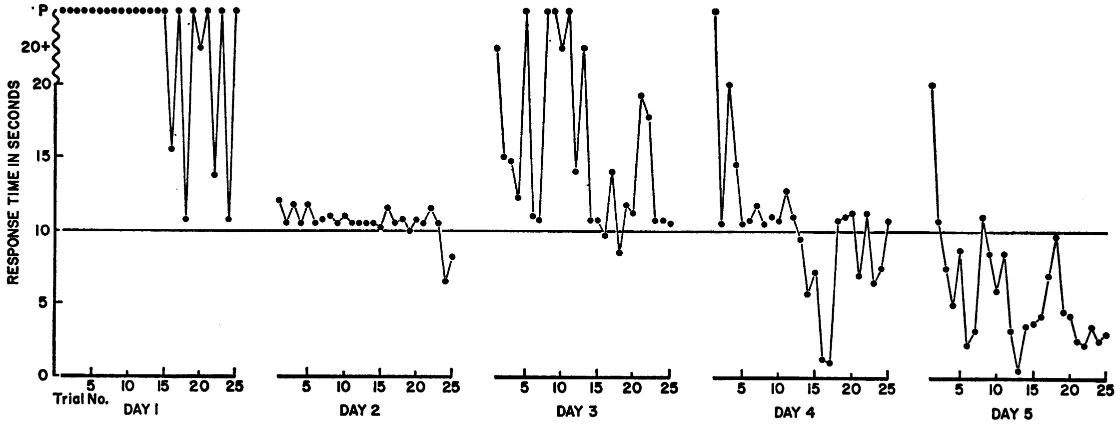

FIG. 3. Example of a five-day training sequence of a squirrelfish (*Holocentrus ascensionis*, no. DF-2), with the use of 25 trials per day and an interval of 10 seconds between the onset of the sound and the onset of the shock. The conditioned stimulus in this case was a tone of which the frequency was 1600 cps. at a pressure level of 30 decibels (re 1 microbar). In the first 15 trials, the responses were forced, i.e., after about 30 seconds from the start of the trial, the animal was pushed across the barrier with a plastic paddle. These forced responses are indicated by P on the ordinate. Response times of over 20 seconds are all grouped together, indicated by 20+ on the ordinate. All responses under 10 seconds were avoidances.

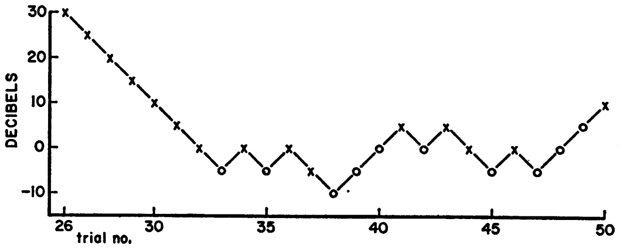

Fig. 4. An example of a threshold determination made for the same animal the training protocol of which is illustrated in figure 3. After each avoidance response (indicated by X), the sound pressure was reduced by 5 decibels. After each escape response (indicated by O), the sound pressure was increased by 5 decibels. The calculated threshold for this series of trials was −1.25 decibels re 1 microbar.

tioned stimulus level was raised by 5 decibels.

## Testing Techniques

Once the animal achieved a high enough score of avoidances, it was clear that the acoustic stimulus was perceived and that we could begin to test for the threshold level for that particular training tone.

The acoustic pressure of the test tone was generally 20 to 30 decibels above the estimated threshold, and, with each avoidance response, the stimulus level was lowered for the subsequent trial. For most of the species, the stimulus level was lowered in steps of 2 decibels. In two cases (*Holocentrus ascensionis* and *Epinephelus guttatus*), 5-decibel steps were used, because it was found that these animals stopped responding after more than 40 or 50 trials, and coarser steps had to be used.

When the stimulus level was dropped to a point near or below the range of the threshold, the animal did not avoid during the 10-second interval and received one or more shocks. In these cases, the escape was usually a prompt one, often occurring after a single shock. In the next trial, the stimulus level was raised the appropriate 2- or 5-decibel step. In short, each time the animal avoided, the stimulus was lowered, and each time there was no avoidance (i.e., the subject escaped after being shocked) the stimulus was raised in intensity.

In this manner, the threshold range could be bracketed. The technique is a modification of the method of minimal changes, often called the "staircase" or "up-and-down" method (Guilford, 1954).

With increased practice, the fish became more efficient in crossing the barrier, and the number of intertrial crossings increased to a point at which a test trial response could not be distinguished from an intertrial crossing. The animals also may have learned to respond on a time basis. In such situations, the water level was lowered to inhibit these intertrial responses, and the intertrial interval was increased (sometimes up to 15 minutes) to give the animal a chance to settle down. General activity was always higher immediately after a trial, and the subject often crossed the barrier back and forth a few times after which it became quiescent in its typical position in the compartment.

Since it was evident that intertrial crossings increased in frequency as the threshold range was approached, such responses are properly to be considered as false positives or "false alarms." In our study, we attempted not to count these responses but rather, by adjusting the water level at the barrier, to reduce their occurrence.

The fish learned rapidly that the place of the light beam and the top of the barrier were of critical importance. Some of the subjects learned to remain in the beam and partially across the barrier, which, of course, made a trial impossible, since the sound was turned off as soon as the trial was begun, and the fish did not make the required response. The solution, again, was to lower the water level to a point that would inhibit any attempt on the part of the subject to remain in the beam, but not low enough to prevent avoidance or escape responses.

A similar situation was one in which, at the onset of the sound, the fish swam up to the light beam and broke it, but did not cross over to the other compartment, by nosing the beam or breaking it with a fin movement. These partial or accidental responses were considered a true avoidance response for two reasons. If it be assumed that the fish had learned the relevance of breaking the beam or approaching the barrier to the occurrence of the sound, these partial responses are just as objective and meaningful as is the complete avoidance response, and they should be treated as such. If the breaking of the beam were a chance occurrence, it would fall in the same category as an apparent avoidance response that was due to a chance intertrial crossing by the fish. Selected sampling of intertrial crossings indicated that the obtained threshold data cannot be accounted for by the frequency of their occurrence. A chance response or "false alarm," therefore, was counted as a positive response.

An additional behavioral observation in a few cases was that, for reasons unknown to us, the fish developed an asymmetry in the avoidance response. That is, a given fish avoided from one compartment and ceased to avoid from the other compartment. This asymmetrical response occurred at intensities that were sufficiently high to eliminate all possibility that it was the result of an unequal sound level in the two compartments. There were also no differences in the two compartments as measured by our monitoring system. This one-sided avoidance took one of two forms. First was the simple form of one-sided avoidance. This problem was handled by our presenting each sound level twice, so that at least one stimulus was presented to the fish in the compartment from which he was avoiding. In the second form, not only was there one-sided avoidance, but the fish also showed a preference for remaining in a given compartment during the intertrial interval. That is, if the fish crossed from compartment 1 to compartment 2 as a result of a trial (escape or avoidance), it then returned to compartment 1 shortly thereafter, during the intertrial interval. The result was that the fish was always presented with the conditioned stimulus in only one compartment. Such a one-sided avoidance presented no problem, providing that the preferred compartment was the one from which the fish avoided. The problem would become almost insoluble if the fish simultaneously developed one-sided avoidance and a preference for the compartment from which it did not avoid. Fortunately, this behavior pattern was manifested infrequently among the subjects, and, when it did appear in an individual fish, it lasted for only a few days. An attempt was made to break it by shocking the fish whenever it entered the non-avoiding compartment. At times this was successful, but the total amount of shock that the subject received on that day made the threshold determination somewhat questionable, and these data were excluded from the results presented here.

## CRITERIA OF THRESHOLD

A graph of a threshold determination (fig. 4) appears as a series of vertical zigzags. The range between the peaks and valleys of this graph includes the threshold. This is essentially the "up-and-down" or "staircase" technique used by Dixon and Mood (1948), Dixon and Massey (1951), Blough (1958), and others in their method of minimal changes for determining visual thresholds in birds and other animals. The well-known audiometer paper of von Békésy (1947) described this technique for human auditory studies.

One problem in this method is to select the point in the graph at which to begin the calculation. In all of our studies, we started the test trials with a suprathreshold stimulus and gradually worked the animal down to the threshold range, but the slope of this initial part of the graph was often not smooth. The question, then, was: At what point in the graph can it be said that the asymptote was reached? No statistical manipulation is known to us that gives a satisfactory answer to this problem. As shown in several of the figures in this report, the point from which the calculation was made was chosen on the basis of its apparent relation to the beginning of an asymptote. All the points of inflection from there on were used for the calculation of the threshold. A recent description of this staircase technique was given by Cornsweet (1962).

Each segment of the broken zigzag line represents a range that includes the threshold value. We assume, therefore, that the threshold is on a midpoint between each peak and valley of the graph. The mean value of all these midpoints for the entire graph, then, becomes the calculated threshold value. This represents a theoretical point at which the signal is received and responded to 50 per cent of the time and is essentially the same as the calculations used for the method of limits (Guilford, 1954). The calculations were also checked by the frequency analysis method of Dixon and Massey (1951), and the results were virtually identical.

The curves for the several audiograms shown in the following figures were fitted to the data by the method of orthogonal polynomials (Pearson and Hartley, 1956) except in cases of large gaps, in which the lines were drawn in by visual inspection and approximation.

The total number of trials for a test series varied. In training, 25 trials per day were used as a standard, and the range in a threshold determination was from 25 to 150 trials per fish per day. Often a series of a given day was terminated by the animal. In such instances, it simply stopped all avoidances and sometimes even ceased escape behavior. In many cases, the trials for the day were stopped when it appeared that the threshold data obtained were sufficient for a reliable calculation. In general, the criterion was considered reached after at least 10 consecutive reversals of response from avoidance to escape to avoidance, and so on. In some cases, more than 40 such reversals were achieved in a single test series.

Once a threshold for a particular frequency in a given specimen was determined, on the following day the frequency of the test tone was changed and the animal was retested on the new frequency. Eventually some of the animals were retested at the same frequencies, and in most cases the thresholds for different frequencies were replicated at least once by other specimens of the same species.

The number of cases on which an individual threshold is determined is not necessarily the only or the best indication of the reliability of that determination. If an individual threshold falls in line with the slope of an audiogram curve and is consistent with the thresholds obtained with higher and lower frequencies around it, its validity is increased regardless of the number of cases on which it is based. Thus, there is a great deal of confidence in the current audiograms for the higher frequencies. On the other hand, among the low frequencies, where there seem to be at least two threshold functions, not only is a large sample size necessary, but great care must be invoked in fitting any obtained threshold into an appropriate function.

The previous amount of training and the previous number of testing sessions may influence the obtained threshold. A large body of data (Adams, 1957; Teichner, 1954) suggests that the thresholds change as a function of practice. An attempt has been made in the present research to control for this factor by testing some of the animals of a given species in ascending frequencies, and some in descending frequencies. After thresholds were determined for various fish at different frequencies, some new fish were then trained at selected frequencies for the purpose of adding additional subjects, and for checking the obtained values, as well as checking the effect or previous training on the threshold.

In addition to the appearance of the graph of avoidances and escapes, the adequate number of points of inflection as the asymptote is reached, and the consistency of the results with other threshold determinations, another criterion for a reliable threshold was the range of variability in the test series. A maximum acceptable variability was set at 10 decibels, but the range between the maximum and minimum inflection points was usually less. All results reported present the arithmetic means and standard deviations based on the points of change between avoidance and escape. Most of the standard deviations varied from ±2 to ±3.

# RESULTS

## PART 1

THE THRESHOLD DETERMINATIONS on the three species described in the present part (*Holocentrus ascensionis*, *H. vexillarius*, and *Equetus acuminatus*) were consistent at all the frequencies tested. Variation among different individuals within a species was small. The tabular data are arranged on the basis of the frequencies tested.

### AUDITORY THRESHOLD DETERMINATIONS IN *Holocentrus ascensionis*

During the preliminary escape training, the optimum shock levels for this species were determined to be 15 to 20 volts. After the avoidance response became well established and the specimens received fewer actual shocks, the shock level was raised to 25 volts. The intertrial intervals were varied on a random basis, but it was found that the minimum was three minutes. Shorter intertrial intervals resulted in a general increase in activity and numerous intertrial crossings. The interval was varied randomly from three to five minutes, with an average of four minutes. Longer intervals were occasionally introduced if intertrial crossings took place at frequencies greater than one per minute. Intervals up to 10 or 15 minutes usually gave the animal a chance to settle down in a corner.

The water level over the barrier was a critical variable. The squirrelfish, unlike the other species tested, rarely turned on its side to cross over, even if the water level was very low. Levels of 5 cm. or more were readily traversed, even though the dorsal fin and a large part of the dorsum protruded from water during the crossing. At a water level of 2.5 cm., crossings were greatly inhibited, and the animal often went part way over and then dropped back. After initial training, a level of from 3.5 to 4.0 cm. was found to be optimal, i.e., spontaneous crossings were not very frequent and escape or avoidance crossings were not inhibited.

Once the training parameters were established, i.e., the intertrial interval, the shock level, water level, and so on, it took about four to six days for a squirrelfish to reach a reliable criterion of avoidance. The animal was given 25 trials per day. On the first day, it had to be helped across the barrier by a plastic paddle in the first five to 10 trials. Help was given to speed up the escape learning, because, for purposes of this work, the learning time was not important. On the second and third days, escape from the shock was regular and rapid, usually after the first one or two stimuli, and there were sporadic avoidances. By the fifth day, the number of avoidances was usually over 50 per cent (it was 90 per cent in one instance). By the sixth or seventh day, the animal was avoiding in 80 to 90 per cent of the trials. Most avoidances took place within five seconds of the onset of the conditioned stimulus. In almost every day's series of trials, however, there was a "warm-up" period in which, for the first five or 10 trials, there were no avoidances, and thereafter the response was close to 100 per cent. This warm-up period persisted even

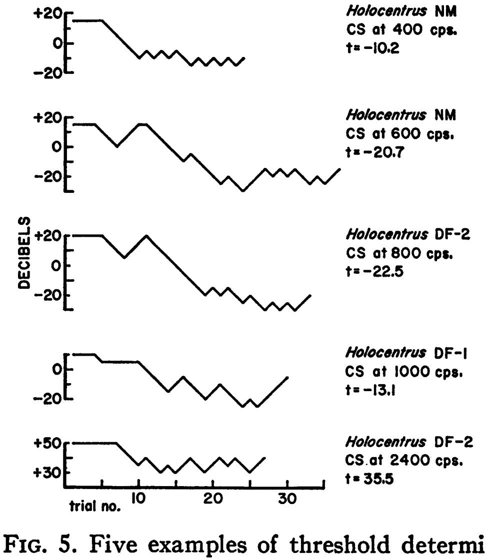

FIG. 5. Five examples of threshold determinations made on the squirrelfish (*Holocentrus ascensionis*). The initial horizontal line represents the "warm-up" trials; the downward slopes represent avoidance responses, and the upward slopes are escape responses. The sound frequencies (conditioned stimulus = CS) and the calculated threshold values (t), in decibels, are given for each protocol.

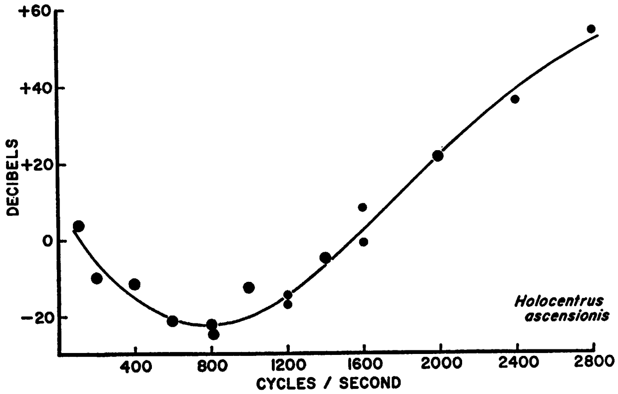

FIG. 6. Summary of threshold determinations made for five specimens of the squirrelfish (*Holocentrus ascensionis*). The data are given in table 2. The larger spots indicate two or more values at almost the same point.

after more than 30 days of testing in some individuals.

When an individual fish was tested, it was found that after 40 or 50 trials the avoidance responses became sporadic and often ceased entirely. In many instances even escape responses became slow and irregular. It was as if the animal tired or the response became extinguished. To keep the animals from getting shocked too frequently, the trials were terminated for the day when the avoidance responses became obviously slow and unreliable.

Once avoidance responses became stabilized and a threshold determination was in progress, the animals consistently took up the same position in the compartment during the intertrial interval. This position faced into a corner or against the far wall of the compartment, i.e., about where the hydrophone was placed for sound measurements. It is fair to say, therefore, that the accuracy of the threshold measurements for *Holocentrus* was probably in the order of ±2 decibels, if equipment variability and small changes in the initial position of the animal at the onset of the conditioned stimulus are allowed for.

Figure 5 shows five sample records obtained with *Holocentrus* auditory threshold determinations.

Figure 6 and table 2 summarize all the data on threshold determinations in this species. A total of five animals were used. The larger spots on the graph indicate two or more threshold values of almost the same value.

This species was one of the most sensitive tested. Its lowest threshold was at 800 cps. at an acoustic pressure level of below −20 decibels. The squirrelfish also possessed the broadest frequency-response spectrum, exhibiting reliable thresholds at 2400 and 2800 cps.

## AUDITORY THRESHOLD DETERMINATIONS IN *Holocentrus vexillarius*

The general behavior of this smaller species of squirrelfish was remarkably similar to that of *H. ascensionis.*

The optimum shock levels were 15 to 20 volts, and the level was maintained at 20 volts in most of the threshold determinations. The intertrial periods were varied from one to three minutes, with occasional periods of up to 10 minutes when intertrial crossings became too frequent. The water level at the barrier was extremely critical, with 2 cm. as the optimal value. Seven to eight days of training were usually required, with 25 trials per day, until the 90 per cent avoidance criterion was reached. The interval between the onset of the sound and the delivery of the shock was 10 seconds. This species was tested at sound-pressure changes of 2 decibels, i.e., after each avoidance the sound level was reduced 2 decibels or raised 2 decibels after an escape. Like *H. ascensionis*, these animals also regularly took up a position against the far wall of the compartment between trials.

Figure 7 and table 3 present the data on threshold determinations in *H. vexillarius*. Three animals were used. There were some clear differences between this species and *ascensionis*. The lowest threshold values were at 600 cps. instead of 800 cps., and these were higher by more than 10 decibels over the lowest values in *ascensionis*. The audiogram of *H. vexillarius* showed a steeper rise both above and below the point of highest sensitivity. Although attempts were made to test these animals at frequencies above 1200 cps., no reliable threshold determinations could be

TABLE 2

AUDITORY THRESHOLD DETERMINATIONS IN THE SQUIRRELFISH, *Holocentrus ascensionis*

| Fish No. | Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | $\sigma$ |
|---|---|---|---|---|---|---|
| NM | 7/10 | 100 | 15 | 18 | 4.1 | 2.3 |
| LP | 7/16 | 100 | 10 | 21 | 3.8 | 2.1 |
| LP | 8/2 | 100 | 12 | 15 | 3.6 | 1.7 |
| LP | 7/15 | 200 | 11 | 23 | −10.0 | 4.3 |
| LP | 8/3 | 200 | 17 | 21 | −9.8 | 2.1 |
| NM | 7/9 | 200 | 10 | 12 | −10.2 | 1.5 |
| NM | 7/11 | 400 | 14 | 16 | −10.2 | 2.5 |
| LP | 8/5 | 400 | 15 | 22 | −13.5 | 1.4 |
| NM | 7/14 | 400 | 10 | 12 | −12.8 | 1.2 |
| NM | 7/12 | 600 | 11 | 16 | −20.7 | 2.8 |
| DF-1 | 7/9 | 600 | 13 | 14 | −21.0 | 3.1 |
| DF-1 | 7/11 | 800 | 13 | 17 | −24.6 | 2.2 |
| NM | 7/13 | 800 | 9 | 11 | −23.2 | 1.1 |
| UC | 7/11 | 800 | 12 | 18 | −24.1 | 3.5 |
| DF-2 | 8/5 | 800 | 11 | 15 | −22.5 | 4.6 |
| DF | 7/12 | 1000 | 8 | 19 | −13.1 | 6.5 |
| UC | 7/9 | 1000 | 12 | 22 | −12.7 | 5.4 |
| DF | 7/13 | 1200 | 16 | 24 | −17.0 | 3.1 |
| UC | 7/13 | 1200 | 15 | 20 | −15.2 | 2.9 |
| DF-1 | 7/15 | 1400 | 13 | 17 | −5.2 | 2.8 |
| UC | 7/15 | 1400 | 9 | 12 | −4.3 | 4.5 |
| UC | 7/21 | 1600 | 12 | 19 | 8.3 | 3.4 |
| DF-2 | 7/31 | 1600 | 12 | 21 | −1.3 | 2.5 |
| UC | 7/22 | 2000 | 7 | 9 | 20.7 | 2.4 |
| DF-2 | 8/2 | 2000 | 5 | 9 | 21.5 | 1.3 |
| DF-2 | 8/3 | 2400 | 11 | 17 | 35.5 | 1.9 |

[a] Number of points of inflection in the threshold determination curve.

[b] Number of trials on which the threshold determination was made.

made because of extremely erratic avoidance behavior.

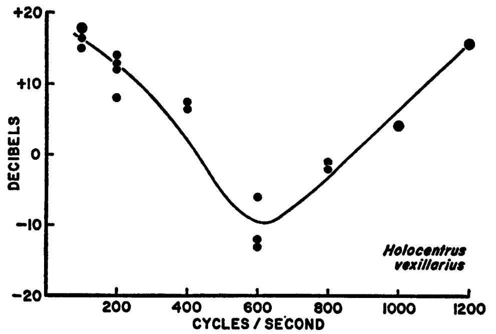

FIG. 7. Summary of threshold determinations made for three specimens of the dusky squirrelfish (*Holocentrus vexillarius*). The data are given in table 3. The larger spots indicate two or more values at almost the same point.

### AUDITORY THRESHOLD DETERMINATIONS IN *Equetus acuminatus*

The optimum shock voltage was 12 to 15 volts. The intertrial intervals averaged two minutes. The water level above the barrier was varied from 2.5 to 3.0 cm. The interval between sound and shock was 10 seconds, and the sound level was varied in steps of 2 decibels during the threshold determinations. Three specimens were used.

This species learned the avoidance problem by far the most rapidly. For example, initial training of specimen A at 600 cps. was begun

TABLE 3

AUDITORY THRESHOLD DETERMINATIONS IN THE DUSKY SQUIRRELFISH, *Holocentrus vexillarius*

| Fish No. | Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | σ |
|---|---|---|---|---|---|---|
| A | 7/13 | 100 | 9 | 21 | 15.0 | 2.2 |
| B | 7/10 | 100 | 12 | 32 | 17.3 | 3.2 |
| C | 7/13 | 100 | 14 | 27 | 16.4 | 3.0 |
| B | 7/16 | 100 | 15 | 30 | 17.8 | 2.1 |
| A | 7/13 | 200 | 10 | 27 | 8.2 | 1.1 |
| B | 7/12 | 200 | 16 | 31 | 14.1 | 1.8 |
| C | 7/12 | 200 | 19 | 23 | 12.2 | 1.8 |
| B | 7/17 | 200 | 23 | 47 | 12.5 | 2.1 |
| A | 7/12 | 400 | 18 | 44 | 6.4 | 1.9 |
| B | 7/13 | 400 | 20 | 43 | 7.3 | 3.2 |
| A | 7/10 | 600 | 9 | 26 | −6.0 | 2.7 |
| B | 7/11 | 600 | 13 | 28 | −13.2 | 1.8 |
| A | 7/11 | 600 | 15 | 27 | −12.4 | 1.2 |
| A | 7/14 | 800 | 18 | 40 | −1.0 | 2.3 |
| C | 7/14 | 800 | 20 | 38 | −2.2 | 2.5 |
| A | 7/14 | 1000 | 19 | 42 | 3.8 | 2.0 |
| C | 7/14 | 1000 | 12 | 26 | 4.2 | 2.1 |
| A | 7/15 | 1200 | 15 | 36 | 16.1 | 1.8 |
| C | 7/15 | 1200 | 21 | 39 | 15.5 | 2.4 |

[a] Number of points of inflection in the threshold determination curve.

[b] Number of trials on which the threshold determination was made.

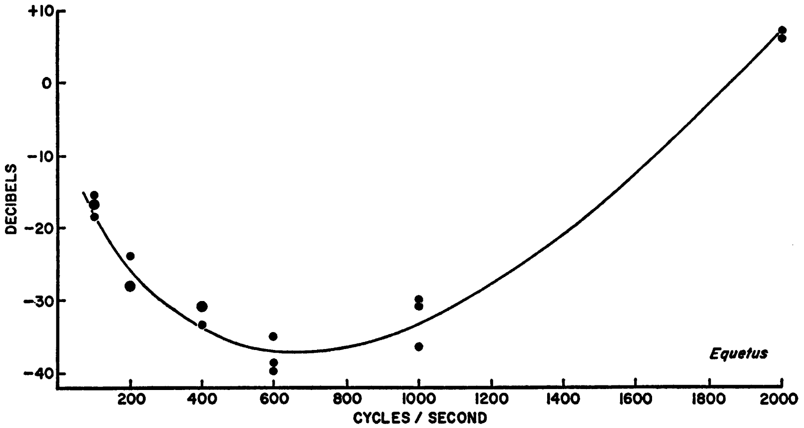

FIG. 8. Summary of threshold determinations for three specimens of the cubbyu (*Equetus acuminatus*). The data are given in table 4. The larger spots represent two or more congruent points.

TABLE 4

AUDITORY THRESHOLD DETERMINATIONS IN THE CUBBYU, *Equetus acuminatus*

| Fish No. | Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | $\sigma$ |
|---|---|---|---|---|---|---|
| A | 6/27 | 100 | 14 | 34 | −8.3 | 2.7 |
| B | 6/28 | 100 | 23 | 36 | −5.4 | 1.2 |
| C | 6/29 | 100 | 20 | 34 | −6.2 | 2.2 |
| C | 6/30 | 100 | 18 | 25 | −7.1 | 1.8 |
| C | 7/1 | 100 | 16 | 24 | −6.5 | 2.4 |
| C | 7/2 | 100 | 19 | 28 | −6.8 | 1.0 |
| B | 6/27 | 200 | 8 | 16 | −24.0 | 1.2 |
| A | 6/28 | 200 | 13 | 29 | −28.1 | 2.4 |
| C | 6/27 | 200 | 17 | 30 | −27.4 | 2.3 |
| C | 7/3 | 200 | 22 | 38 | −27.7 | 3.1 |
| A | 6/26 | 400 | 11 | 25 | −31.3 | 2.8 |
| B | 6/26 | 400 | 12 | 26 | −33.3 | 1.0 |
| C | 6/26 | 400 | 15 | 34 | −30.5 | 3.2 |
| A | 6/24 | 600 | 8 | 18 | −35.0 | 1.2 |
| B | 6/25 | 600 | 9 | 22 | −39.8 | 1.3 |
| C | 6/25 | 600 | 14 | 24 | −38.8 | 1.9 |
| A | 6/29 | 1000 | 18 | 34 | −36.3 | 1.7 |
| B | 6/30 | 1000 | 25 | 38 | −30.1 | 2.2 |
| B | 7/1 | 1000 | 27 | 37 | −30.7 | 1.9 |
| A | 6/30 | 2000 | 19 | 30 | 6.2 | 3.4 |
| A | 7/1 | 2000 | 10 | 22 | 7.3 | 2.9 |

[a] Number of points of inflection in the threshold determination curve.

[b] Number of trials on which the threshold determination was made.

on June 21. In 25 trials, the first two had to be forced escapes (i.e., the animal was prodded across the barrier), but in the remaining trials of this first day, the subject avoided on the tenth trial and escaped rapidly in all the others. On the second day, this animal avoided in 12 out of 25 trials. On the third day, it avoided in 48 out of 50 trials. On the fourth day, June 24, its threshold at 600 cps. was determined.

During preliminary trials, intertrial crossings were virtually absent, but, as in all other species tested, as the threshold was approached, the animals continued to cross the barrier every five to 10 seconds shortly after a trial, but within one to two minutes they settled down to a stable position. This position was usually close to the center of the compartment, facing the barrier. Avoidances were usually rapid, usually about two seconds after the onset of the sound.

Coincident with the rapid learning of the avoidance problem, this species exhibited the lowest threshold values of any of the forms tested. Table 4 and figure 8 show the threshold determination results for *Equetus*. Thresholds as low as almost −40 decibels at 600 cps. were determined. There was little variability among the three animals tested.

## PART 2

This group consists of four species: *Haemulon sciurus*, *Lutjanus apodus*, *Thalassoma bifasciatum*, and *Eupomacentrus leucostictus*.

At frequencies above 400 cps., all the members of these species gave threshold values that showed only small variations among different individuals of the same species, but at the low frequencies a high degree of variability began to appear as the animals were retested and the points on the audiograms were replicated. These variabilities took two forms. One was an abrupt change in the threshold determinations as compared to values obtained in early tests, and the other was the appearance of a temporary threshold during a given series of test trials.

### SECONDARY LOW-FREQUENCY THRESHOLDS

A feature exhibited by several of the animals tested was the presence of what appeared to be two very different thresholds for the same frequency. This was evident only at frequencies below 500 cps. During the replicating of the low-frequency threshold determinations, it was found that some of the subjects showed a drop in threshold values of as much as 20 decibels below the previously determined levels. This occurred only after the animals had been tested over a period of several days or weeks. As an example, in grunt no. 3 (*Haemulon sciurus*) the threshold values for 100 cps. and 200 cps. were determined to be +11.3 and +12.1 decibels when the fish was tested on June 20 and June 21, respectively. After additional testing at these and other, higher frequencies, the thresholds at 100 cps. and 200 cps. became −17.3 and −16.4 decibels on August 20 and August 15, respectively. The data on these low frequencies, therefore, had to be divided into two groups—early and late determinations. It

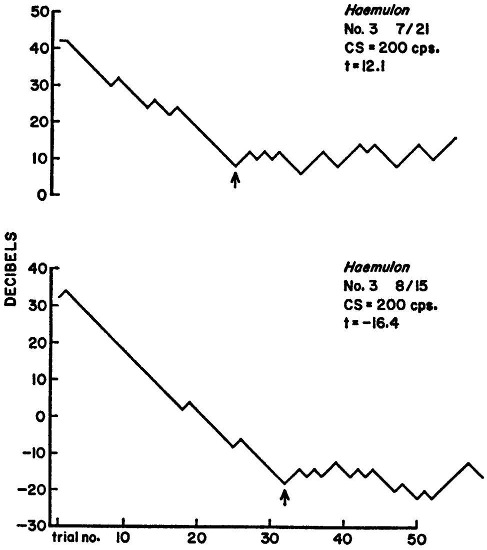

FIG. 9 Graph of two representative threshold determinations made on a specimen of grunt (*Haemulon sciurus*, no. 3). The arrow indicates the point at which the calculation of the threshold was begun. CS is the frequency of the conditioned stimulus. Note that the threshold values (t) of the two determinations are 28.5 decibels apart. The lower value was obtained after the animal had had considerable testing experience (see table 5).

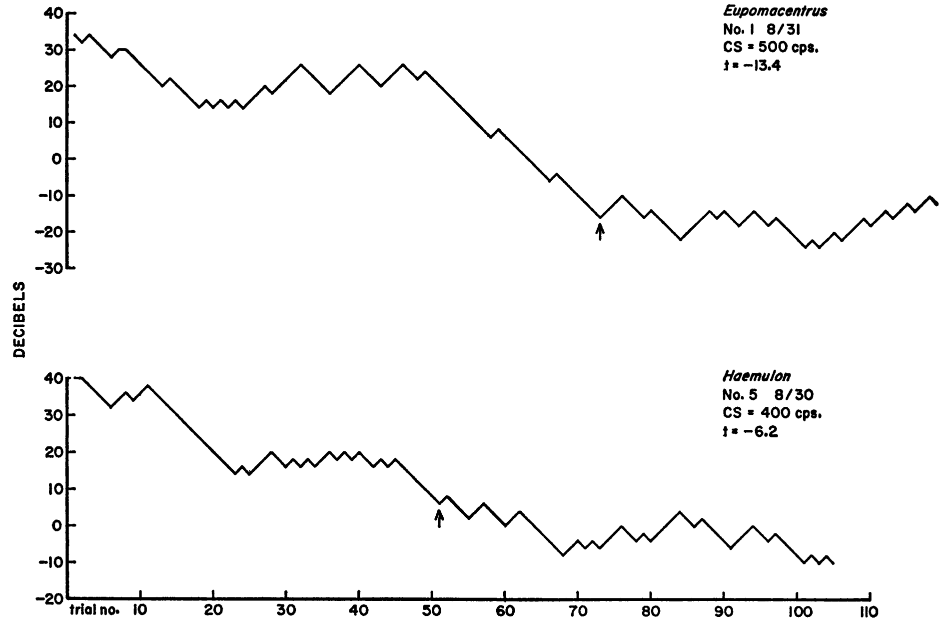

FIG. 10. Two graphs of threshold determinations that illustrate the plateau effect. The arrow indicates the point at which the calculation of the threshold was begun. The upper graph is for a beau-gregory (*Eupomacentrus*, no. 1), and the lower is for a grunt (*Haemulon*, no. 5).

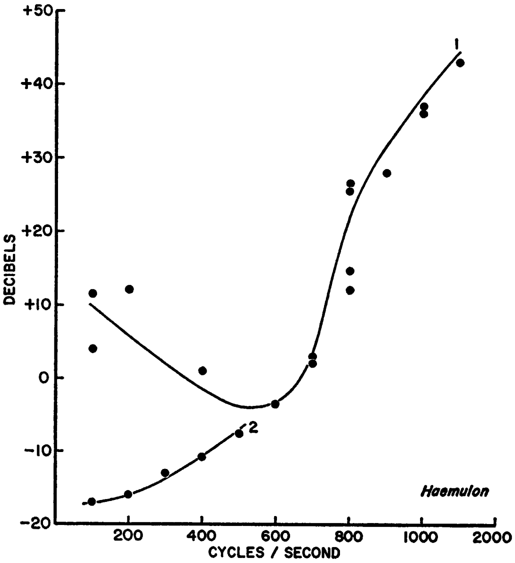

FIG. 11. Summary of threshold determinations for the grunt (*Haemulon sciurus*, no. 3). The values are listed in table 5. Curve 1 represents the primary threshold determinations made during the period July 13 to August 14. Curve 2 is the secondary low-frequency series of determinations made August 15 to 27.

was evident that the testing procedure itself constituted additional training and that an improvement in the performance would not be surprising. What was surprising, however, was the abrupt and major change in the threshold values. In species in which this change occurred, we grouped the threshold data into two separate curves. The first determinations (both low and high frequencies) we call the "primary threshold curve." The later low-frequency determinations comprise what we call the "secondary low-frequency threshold curve." These are described in detail in the sections under each species and are shown in the tables and graphs.

The data in the tables for these species are presented in chronological order for each individual subject, so that the threshold changes with time can be seen.

## PLATEAU EFFECT

During the course of many of the threshold determinations at frequencies below 500 cps., a temporary threshold effect was obtained as the sound level was lowered or raised in the step method described above. As trials were

TABLE 5

AUDITORY THRESHOLD DETERMINATIONS IN A SINGLE SPECIMEN (NO. 3) OF THE BLUE-STRIPED GRUNT, *Haemulon sciurus*

| Date | Frequency, in Cycles per Second | Threshold, in Decibels |
|---|---|---|
| 7/10 | 700 | 1.3 |
| 7/13 | 400 | 0.5 |
| 7/20 | 100 | 11.3 |
| 7/21 | 200 | 12.1 |
| 7/23 | 100 | 3.7 |
| 7/24 | 600 | −3.9 |
| 7/25 | 800 | 25.2 |
| 7/26 | 800 | 26.4 |
| 7/28 | 700 | 2.7 |
| 7/31 | 800 | 11.9 |
| 8/3 | 900 | 27.5 |
| 8/9 | 1000 | 35.8 |
| 8/11 | 1100 | 42.7 |
| 8/12 | 1000 | 36.7 |
| 8/14 | 800 | 14.4 |
| 8/15 | 200 | −16.4[a] |
| 8/20 | 100 | −17.3[a] |
| 8/22 | 300 | −13.4[a] |
| 8/25 | 400 | −11.1[a] |
| 8/27 | 500 | −7.7[a] |

[a] Secondary low-frequency thresholds.

continued, the record of avoidances showed a sharp drop and an eventual stabilization at a lower sound pressure. We call this temporary threshold the "plateau effect."

The plateau was usually of brief duration, usually involving fewer than 10 points of inflection in the test series, but on a few occasions as many as 20 such points were included in a plateau. After the appearance of a plateau, the threshold curve sometimes dropped as much as 20 or 30 decibels before becoming asymptotic (fig. 9).

We are not able to state with any certainty whether these plateaus are caused by high sound or shock intensity, stage of practice, or a shift in sensory modality. One difficulty that is introduced by the presence of these plateaus is the uncertainty as to whether or not the asymptote of any individual threshold has been reached. Most of the thresholds reported here are based on 10 to 20 points of inflection. The fact that plateaus of comparable duration can occur invites caution, to assure that the reported values are true thresholds and not merely plateaus and thus preludes to lower thresholds.

The plateau effect and the secondary low-frequency thresholds seem to be related in that they occur only at frequencies below 500 cps. The repeated trials, either in a single day's series or over many days of testing, add to the experience of the subject and lead to the expression of either the plateau effect or the secondary threshold.

### AUDITORY THRESHOLD DETERMINATIONS IN *Haemulon sciurus*

The optimum shock levels were 7 to 10 volts, and the intertrial intervals averaged two minutes. The water level at the barrier ranged from 0.4 to 1.2 cm. At 25 trials per day, the animals learned to avoid regularly in four or five days. The interval between the onset of the sound and the shock was 10 seconds, and the staircase method of testing used steps of 2 decibels. Intertrial crossings (i.e., "false alarms") were infrequent and occurred only occasionally when the sound

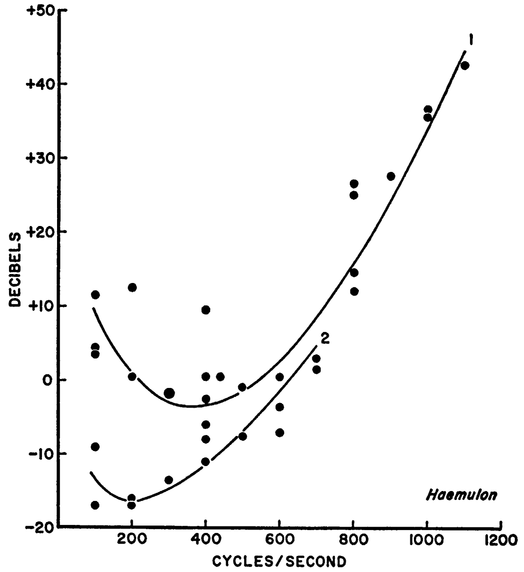

FIG. 12. Summary of threshold determinations made for four specimens of the blue-striped grunt (*Haemulon sciurus*). The data are given in table 6. Curve 1 represents the primary thresholds, and curve 2 is the secondary low-frequency series of determinations. The larger spots indicate two or more values at almost the same point.

TABLE 6

AUDITORY THRESHOLD DETERMINATIONS IN THE BLUE-STRIPED GRUNT, *Haemulon sciurus*

| Fish No. | Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | $\sigma$ |
|---|---|---|---|---|---|---|
| 1 | 8/4 | 100 | 11 | 25 | 4.0 | 2.4 |
| 3 | 7/20 | 100 | 16 | 31 | 11.3 | 3.2 |
| 3 | 7/23 | 100 | 20 | 38 | 3.7 | 2.6 |
| 3 | 8/20 | 100 | 14 | 18 | −17.3[c] | 1.5 |
| 5 | 9/5 | 100 | 20 | 44 | −9.1[c] | 3.2 |
| 1 | 7/31 | 200 | 11 | 20 | 0.3 | 1.8 |
| 3 | 7/21 | 200 | 16 | 32 | 12.1 | 1.9 |
| 3 | 8/15 | 200 | 17 | 29 | −16.4[c] | 2.6 |
| 5 | 9/3 | 200 | 15 | 31 | −16.6[c] | 1.7 |
| 1 | 7/26 | 300 | 27 | 46 | −2.2 | 3.9 |
| 3 | 8/22 | 300 | 15 | 22 | −13.4[c] | 1.5 |
| 5 | 9/1 | 300 | 21 | 48 | −1.8 | 2.5 |
| 1 | 7/17 | 400 | 21 | 48 | −2.9 | 4.0 |
| 1 | 7/25 | 400 | 20 | 30 | 9.2 | 2.1 |
| 3 | 7/13 | 400 | 15 | 28 | 0.5 | 2.9 |
| 3 | 8/25 | 400 | 20 | 36 | −11.1[c] | 2.2 |
| 4 | 7/18 | 400 | 14 | 29 | −7.7[c] | 2.7 |
| 5 | 8/30 | 400 | 21 | 39 | −6.2[c] | 3.5 |
| 4 | 7/11 | 440 | 9 | 18 | 0.4 | 2.1 |
| 4 | 7/14 | 440 | 19 | 35 | −11.8[c] | 2.0 |
| 3 | 8/27 | 500 | 25 | 44 | −7.7[c] | 2.4 |
| 5 | 8/29 | 500 | 11 | 19 | −0.9 | 3.2 |
| 1 | 8/6 | 600 | 11 | 25 | −7.3 | 1.5 |
| 3 | 7/24 | 600 | 34 | 57 | −3.9 | 2.3 |
| 4 | 7/20 | 600 | 23 | 36 | 0.6 | 2.5 |
| 3 | 7/10 | 700 | 14 | 32 | 1.3 | 4.6 |
| 3 | 7/28 | 700 | 28 | 61 | 2.7 | 3.6 |
| 3 | 7/25 | 800 | 41 | 73 | 25.2 | 4.1 |
| 3 | 7/26 | 800 | 13 | 25 | 26.4 | 2.9 |
| 3 | 7/31 | 800 | 21 | 40 | 11.9 | 3.6 |
| 3 | 8/14 | 800 | 17 | 28 | 14.4 | 2.7 |
| 3 | 8/3 | 900 | 29 | 59 | 27.5 | 4.3 |
| 3 | 8/9 | 1000 | 39 | 93 | 35.8 | 5.8 |
| 3 | 8/12 | 1000 | 19 | 36 | 36.7 | 6.6 |
| 3 | 8/11 | 1100 | 29 | 61 | 42.7 | 3.6 |

[a] Number of points of inflection in the threshold determination curve.

[b] Number of trials on which the threshold determination was made.

[c] Secondary low-frequency thresholds.

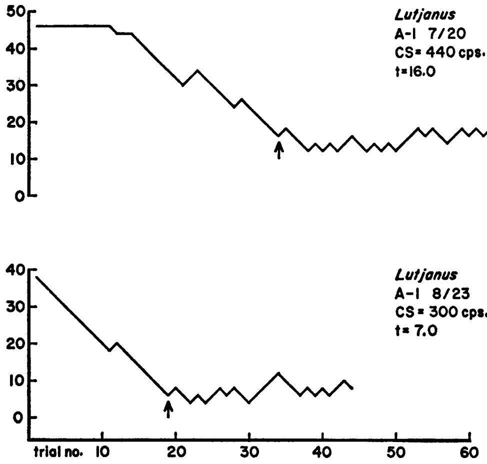

FIG. 13. Two graphs of threshold determinations made on a specimen of schoolmaster (*Lutjanus*, No. A-1). The arrow indicates the point at which the calculation of the threshold was begun. The upper graph is an example of a long "warm-up" period, while the lower graph shows no "warm-up."

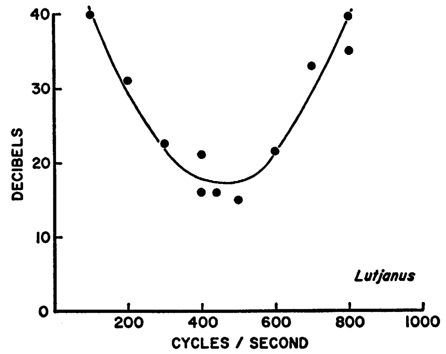

FIG. 14. Summary of the first 11 threshold determinations (see table 7) made for a single specimen of schoolmaster (*Lutjanus*, no. A-1) during the period July 16 to 30. This represents the primary threshold curve for the specimen.

level was close to threshold. The usual position of the animal just prior to a test was against the far wall of the compartment, but sometimes it remained in the center of the compartment with its snout pointing toward the barrier. Four specimens were used in these determinations.

Figure 9 shows two representative threshold determinations of *Haemulon* no. 3 at 200 cps. The arrow in the graph indicates the point from which the threshold value was calculated. Note that the threshold values of these two determinations are 28.5 decibels apart. The upper graph was a determination made during the early stages of testing on July 21. Prior to this test, this animal had three previous threshold tests at 100, 400, and 700 cps. The lower graph was a threshold measurement taken on August 15 after this animal had had 15 threshold determinations at frequencies of from 100 to 1100 cps. This latter value is considered here as representing a secondary low-frequency threshold.

Figure 10, lower, is an exceptionally long record consisting of more than 100 trials. In contrast to records as exemplified in figure 9, this showed a plateau effect from the twentieth to the forty-fifth trial. Such plateaus were generally evident in tests of frequencies of 400 cps. or below and also, in most cases, occurred during the later periods of testing, i.e., when the secondary low-frequency thresholds began to appear.

Table 5 lists the threshold data in chronological order for a single animal (no. 3), and figure 11 shows these data in graph form. The curve labeled "1" represents the primary thresholds (as defined above), which were determined during the period July 13 to August 14. Replications of the low-frequency thresholds at later dates (August 15 to 27) showed an abrupt change in value. These determinations, labeled "2" on the graph,

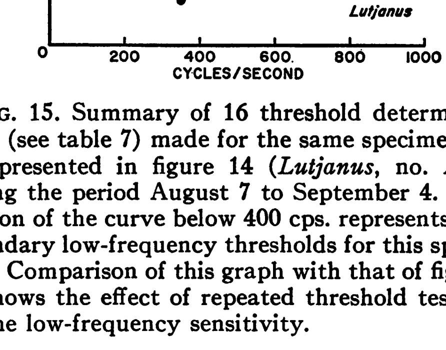

FIG. 15. Summary of 16 threshold determinations (see table 7) made for the same specimen as is represented in figure 14 (*Lutjanus*, no. A-1) during the period August 7 to September 4. The portion of the curve below 400 cps. represents the secondary low-frequency thresholds for this specimen. Comparison of this graph with that of figure 14 shows the effect of repeated threshold testing on the low-frequency sensitivity.

TABLE 7

Auditory Threshold Determinations for a Single Specimen (No. A-1) of the Schoolmaster, *Lutjanus apodus*

| Date | Frequency, in Cycles per Second | Threshold, in Decibels |
|---|---|---|
| 7/16 | 400 | 15.9 |
| 7/20 | 440 | 16.0 |
| 7/22 | 400 | 21.2 |
| 7/23 | 600 | 21.2 |
| 7/24 | 800 | 35.1 |
| 7/25 | 800 | 39.1 |
| 7/26 | 700 | 32.8 |
| 7/27 | 300 | 22.6 |
| 7/28 | 200 | 30.9 |
| 7/29 | 100 | 39.7 |
| 7/30 | 500 | 14.7 |
| 8/7 | 600 | 27.4 |
| 8/9 | 700 | 30.4 |
| 8/11 | 800 | 31.5 |
| 8/13 | 800 | 30.4 |
| 8/14 | 1000 | 39.6 |
| 8/16 | 200 | 12.3[a] |
| 8/19 | 200 | 11.9[a] |
| 8/21 | 100 | 19.3[a] |
| 8/23 | 300 | 7.0[a] |
| 8/24 | 400 | 21.3 |
| 8/26 | 350 | 5.5[a] |
| 8/28 | 380 | 15.1 |
| 8/30 | 390 | 19.6 |
| 8/31 | 470 | 18.0 |
| 9/2 | 360 | 15.8 |
| 9/4 | 350 | 12.6 |

[a] Secondary low-frequency thresholds.

represent the secondary low-frequency thresholds by virtue of a drop of more than 10 decibels from the earlier determinations. The determination at 500 cps. is considered as part of the secondary curve only because it was obtained after the subject had considerable experience in the testing situation.

Figure 12 is a composite graph for all four subjects of all the determinations listed in table 6. From 700 cps., the audiogram curve resembles that of *Holocentrus* and that of *Equetus* in general shape and consistency. Below 400 cps., the data break into two distinct groups, with the lower values being the later determinations of subjects nos. 3 and 5. The 400- to 600-cps. region shows a high degree of variability. This is evidently where the primary and secondary curves overlap. The curves as drawn in figure 12 are only approximations and interpretations.

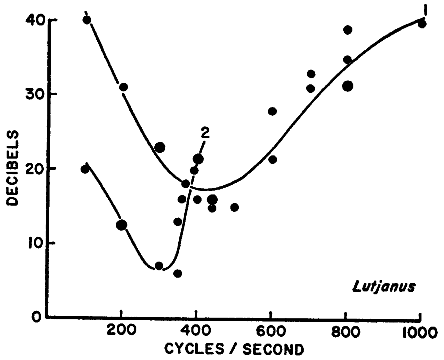

Fig. 16. Summary of threshold determinations for three specimens of the schoolmaster (*Lutjanus apodus*). The data are given in table 8. Curve 1 represents the primary thresholds, and curve 2 is the secondary low-frequency series. The larger spots indicate two or more congruent values.

## Auditory Threshold Determinations in *Lutjanus apodus*

The optimum shock level was 7 volts; this species was extremely sensitive to higher voltages. The intertrial intervals averaged two minutes. The water level at the barrier ranged from 0.6 to 1.3 cm. This species was one of the slowest to learn the avoidance problem. As many as 12 days were required before avoidances became regular enough for threshold determinations. The interval between sound and shock was 10 seconds, and the sound level was varied in steps of 2 decibels during testing. Intertrial crossings were very infrequent. There was some tendency to establish a "preferred" side, and, after an avoidance or escape, the animal immediately crossed the barrier to return to the original compartment. The positions of the animals prior to a test varied widely but usually showed some orientation with respect to the barrier which indicated a readiness to respond. One common position was with the snout leaning against the top of the barrier in such a way that a single flip of the tail sent the fish across the barrier. Three specimens were used.

Figure 13 shows two sample threshold determinations in *Lutjanus*. The upper graph

TABLE 8

AUDITORY THRESHOLD DETERMINATIONS IN THE SCHOOLMASTER, *Lutjanus apodus*

| Fish No. | Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | $\sigma$ |
|---|---|---|---|---|---|---|
| A-1 | 7/29 | 100 | 33 | 47 | 39.7 | 4.7 |
| A-1 | 8/21 | 100 | 31 | 42 | 19.3[c] | 4.3 |
| A-1 | 7/28 | 200 | 31 | 42 | 30.9 | 2.2 |
| A-1 | 8/16 | 200 | 23 | 34 | 12.3[c] | 3.2 |
| A-1 | 8/19 | 200 | 19 | 28 | 11.9[c] | 2.2 |
| A-1 | 7/27 | 300 | 21 | 30 | 22.6 | 3.7 |
| A-1 | 8/23 | 300 | 17 | 27 | 7.0[c] | 1.5 |
| N | 9/9 | 300 | 24 | 41 | 23.3 | 2.3 |
| A-1 | 8/26 | 350 | 22 | 47 | 5.5[c] | 5.1 |
| A-1 | 9/4 | 350 | 21 | 46 | 12.6 | 2.9 |
| A-1 | 9/2 | 360 | 13 | 48 | 15.8 | 4.4 |
| A-1 | 8/31 | 370 | 11 | 38 | 18.0 | 2.7 |
| A-1 | 8/28 | 380 | 24 | 40 | 15.1 | 2.5 |
| A-1 | 8/30 | 390 | 18 | 30 | 19.6 | 1.6 |
| A-1 | 7/16 | 400 | 7 | 18 | 15.9 | 2.3 |
| A-1 | 7/22 | 400 | 25 | 46 | 21.2 | 2.0 |
| A-1 | 8/24 | 400 | 23 | 33 | 21.3 | 2.1 |
| P-4 | 7/11 | 440 | 17 | 29 | 15.5 | 2.6 |
| P-4 | 7/13 | 440 | 20 | 42 | 14.6 | 3.1 |
| A-1 | 7/20 | 440 | 24 | 34 | 16.0 | 1.8 |
| A-1 | 7/30 | 500 | 11 | 51 | 14.7 | 3.3 |
| A-1 | 7/23 | 600 | 33 | 44 | 21.2 | 2.3 |
| A-1 | 8/7 | 600 | 21 | 81 | 27.4 | 3.1 |
| A-1 | 7/26 | 700 | 29 | 39 | 32.8 | 1.9 |
| A-1 | 8/9 | 700 | 10 | 35 | 30.4 | 2.2 |
| A-1 | 7/24 | 800 | 19 | 35 | 35.1 | 5.2 |
| A-1 | 7/25 | 800 | 33 | 42 | 39.1 | 2.2 |
| A-1 | 8/11 | 800 | 17 | 26 | 31.5 | 1.3 |
| A-1 | 8/13 | 800 | 11 | 17 | 30.4 | 1.2 |
| A-1 | 8/14 | 1000 | 17 | 41 | 39.6 | 3.1 |

[a] Number of points of inflection in the threshold determination curve.

[b] Number of trials on which the threshold determination was made.

[c] Secondary low-frequency thresholds.

TABLE 9

AUDITORY THRESHOLD DETERMINATIONS IN THE BLUE-HEAD, *Thalassoma bifasciatum*

| Fish No. | Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | σ |
|---|---|---|---|---|---|---|
| 1 | 8/18 | 100 | 21 | 41 | 27.1 | 2.8 |
| 1 | 8/22 | 100 | 25 | 35 | 20.4 | 2.2 |
| 1 | 8/6 | 200 | 15 | 28 | 6.0 | 1.9 |
| 2 | 8/6 | 200 | 13 | 22 | 5.5 | 1.6 |
| 2 | 8/18 | 200 | 21 | 35 | 9.4 | 2.8 |
| 4 | 9/10 | 200 | 12 | 31 | 22.3 | 4.7 |
| 1 | 8/3 | 300 | 14 | 25 | 11.9 | 2.4 |
| 1 | 8/25 | 300 | 15 | 24 | 1.5 | 1.4 |
| 2 | 8/4 | 300 | 14 | 23 | 14.4 | 1.6 |
| 3 | 9/9 | 300 | 11 | 17 | 17.4 | 1.4 |
| 4 | 8/4 | 300 | 15 | 42 | 17.2 | 4.3 |
| 1 | 8/15 | 400 | 19 | 39 | 9.9 | 3.1 |
| 2 | 8/15 | 400 | 17 | 37 | 10.7 | 3.5 |
| 3 | 9/3 | 500 | 22 | 51 | 4.8 | 2.8 |
| 1 | 8/8 | 600 | 15 | 27 | 9.0 | 1.9 |
| 2 | 8/8 | 600 | 19 | 30 | 10.1 | 2.9 |
| 1 | 8/10 | 800 | 21 | 33 | 19.3 | 1.8 |
| 2 | 8/10 | 800 | 25 | 39 | 23.6 | 2.0 |
| 1 | 8/11 | 900 | 15 | 30 | 22.9 | 3.2 |
| 2 | 8/14 | 900 | 17 | 26 | 29.4 | 1.8 |
| 1 | 8/13 | 1000 | 11 | 16 | 26.1 | 1.0 |
| 2 | 8/12 | 1000 | 21 | 37 | 31.3 | 2.6 |
| 1 | 8/14 | 1200 | 13 | 25 | 34.8 | 2.2 |

[a] Number of points of inflection in the threshold determination curve.
[b] Number of trials on which the threshold determination was made.

is an example of a long "warm-up" period. Here the first 10 trials were not avoidances, and the sound level was not changed. After the eleventh trial, the sound pressure was reduced 2 decibels with each avoidance or increased 2 decibels after each escape. In contrast, the lower graph shows a case in which there were no "warm-up" trials, and the sound level was reduced regularly until the threshold range was reached.

As in *Haemulon*, a secondary low-frequency threshold curve was present in *Lutjanus*. Figures 14 and 15 show this. Figure 14 is a graph of the first 11 threshold determinations made for specimen no. A-1, during the period July 16 to 30. This graph represents the primary threshold curve. Figure 15 is a graph of 16 threshold determinations made for the same specimen during the period August 7 to September 4. The upper portions of these curves, from 500 cps., are similar. At 100 and 200 cps., however, the later determinations (fig. 15) show as much as a 20-decibel drop in the thresholds, and a 15-decibel drop at 300

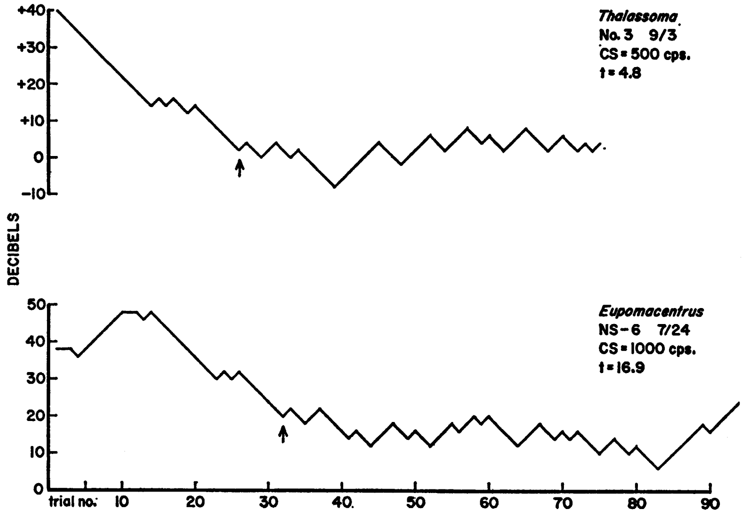

FIG. 17. Two graphs of threshold determinations. The arrow indicates the point at which the calculation of the threshold was begun. The upper graph, for a blue-head wrasse (*Thalassoma*, no. 3), illustrates the absence of a "warm-up" period and a trace of the plateau effect. The lower graph, for a beau-gregory (*Eupomacentrus*, no. NS-6), illustrates a prolonged "warm-up" period.

cps. The region of overlap at 400 to 500 cps. shows a high variability but no clear threshold drop.

Table 7 lists, in chronological order, all the determinations made on the above specimen, A-1. The results at 800 and 600 cps. show a high degree of consistency between tests made in July and those made more than a week later in August, whereas, when the animal was retested at 100 and 200 cps., there was a significant drop in the threshold. The last eight determinations were all made in the frequency band where the primary and secondary curves appear to overlap, and the table shows a variability range of more than 10 decibels in this region. The 5.5-decibel threshold obtained at 350 cps. on August 26 is assumed to be on the secondary curve, while the higher value (12.6) for the same frequency nine days later is thought to represent a return to the primary curve.

Table 8 and figure 16 present all the data on threshold determinations in this species. Most of the data are based on a single subject, but two additional animals were used to check specific points. Specimen N, for example, was both trained and tested at 300 cps., and gave a value within 1 decibel of that obtained as a primary threshold for specimen A-1.

## AUDITORY THRESHOLD DETERMINATIONS IN *Thalassoma bifasciatum*

The optimum shock levels were 10 to 15 volts. The intertrial intervals varied from two to 15 minutes because of frequent intertrial crossings. Often, after a trial, the animal continued to cross back and forth every few seconds for several minutes. The observer, then, had to wait until these crossings slowed down to fewer than one per minute before another trial could be begun. The water level had to be very low, usually 0.6 cm. or less. These fish regularly assumed a position with the nose at the barrier, and, if the water level was too high, the light beam along the top of the barrier remained cut. The interval between sound and shock was 10 seconds, and the sound level was varied in 2-decibel steps. Four animals were tested and reached the criterion of avoidance training in three to four days.

Figure 17, upper, is a representative threshold record for a specimen of *Thalassoma*. In this instance, no "warm-up" trials were required, but there was a trace of a plateau

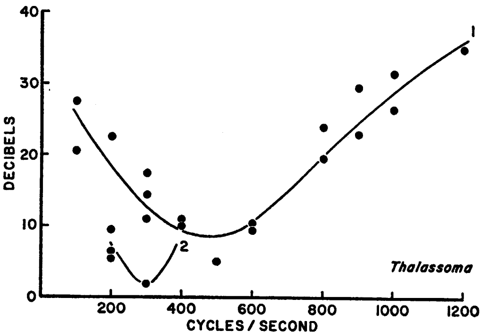

FIG. 18. Summary of threshold determinations for four specimens of the blue-head wrasse (*Thalassoma bifasciatum*). The data are given in table 9. Curve no. 1 represents the primary thresholds and curve no. 2 is the secondary low-frequency series.

effect about 10 decibels above the threshold level.

Table 9 and figure 18 summarize all the threshold determinations in this species. The secondary low-frequency curve is not so clear in this species as in *Haemulon* or *Lutjanus*. It is possible, for example, that the lower point at 100 cps. should be part of this secondary curve, but there are not sufficient data to establish this 7-decibel difference as significant. Furthermore, at 200 cps., it is not clear whether the threshold point at 9.4 decibels really belongs with the secondary curve or not, but we have assumed that it does because it occurred after this animal (no. 2) had been

TABLE 10

AUDITORY THRESHOLD DETERMINATIONS IN A SINGLE SPECIMEN (NO. NS-5) OF THE BEAU-GREGORY, *Eupomacentrus leucostictus*

| Date | Frequency, in Cycles per Second | Threshold, in Decibels |
|---|---|---|
| 7/10 | 440 | 14.2 |
| 7/15 | 400 | 4.0 |
| 7/19 | 400 | 6.8 |
| 7/21 | 200 | 11.8 |
| 7/22 | 100 | 23.3 |
| 7/24 | 100 | 23.6 |
| 7/26 | 100 | 4.6[a] |
| 7/27 | 200 | −4.5[a] |
| 7/29 | 600 | 21.3 |
| 7/31 | 600 | 18.9 |
| 8/3 | 600 | −10.4 |
| 8/5 | 800 | −2.0 |
| 8/6 | 1000 | 13.0 |
| 8/8 | 1100 | 22.7 |
| 8/10 | 1200 | 36.5 |
| 8/12 | 900 | 6.8 |

[a] Secondary low-frequency thresholds.

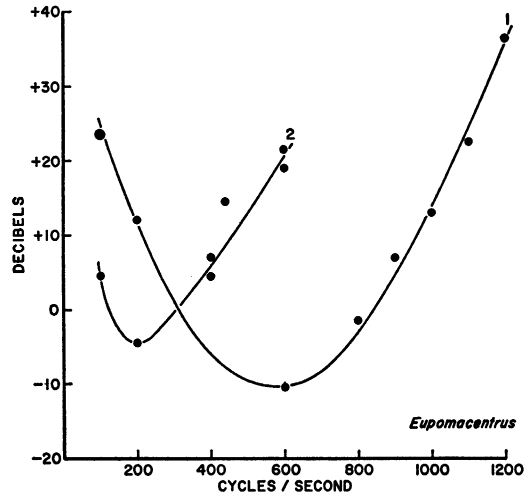

FIG. 19. Summary of threshold determinations made for a single specimen of the beau-gregory (*Eupomacentrus*, no. NS-5). The values are listed in table 10. Curve 1 is the primary threshold series, and curve 2 is the secondary low-frequency curve.

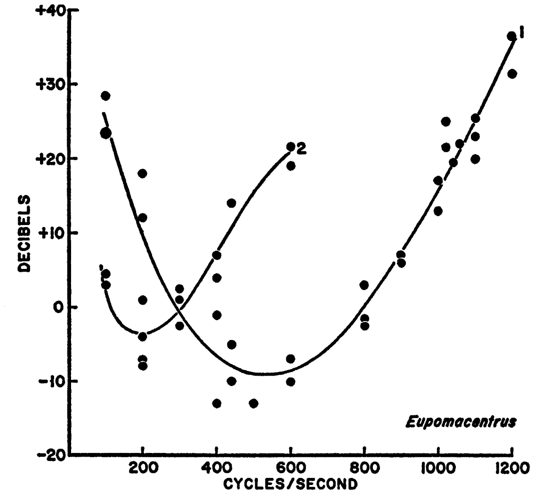

FIG. 20. Summary of threshold determinations for four specimens of the beau-gregory, *Eupomacentrus leucostictus*. The data are given in table 11. Curve 1 is the primary threshold series, and curve 2 is the secondary low-frequency curve.

TABLE 11

Auditory Threshold Determinations in the Beau-gregory, *Eupomacentrus leucostictus*

| Fish No. | Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | σ |
|---|---|---|---|---|---|---|
| NS-5 | 7/22 | 100 | 23 | 48 | 23.3 | 4.6 |
| NS-5 | 7/24 | 100 | 17 | 26 | 23.6 | 2.0 |
| NS-5 | 7/26 | 100 | 20 | 31 | 4.6[c] | 1.7 |
| No-3 | 9/10 | 100 | 22 | 48 | 23.6 | 3.8 |
| No-3 | 9/11 | 100 | 24 | 53 | 28.2 | 3.5 |
| No-1 | 9/9 | 100 | 20 | 37 | 3.0[c] | 1.7 |
| NS-5 | 7/21 | 200 | 35 | 73 | 11.8 | 5.2 |
| NS-5 | 7/27 | 200 | 20 | 33 | −4.5[c] | 2.6 |
| NS-6 | 8/17 | 200 | 11 | 18 | 19.0 | 1.9 |
| NS-6 | 8/23 | 200 | 15 | 22 | −7.3[c] | 1.4 |
| No-3 | 9/4 | 200 | 19 | 49 | 0.6[c] | 5.1 |
| No-1 | 8/7 | 200 | 17 | 24 | −7.6[c] | 1.7 |
| NS-6 | 8/15 | 300 | 21 | 41 | 2.4 | 3.6 |
| No-3 | 9/2 | 300 | 22 | 37 | −2.6 | 1.9 |
| No-1 | 9/4 | 300 | 26 | 43 | 1.1 | 3.2 |
| NS-5 | 7/15 | 400 | 19 | 30 | 4.0 | 12.1 |
| NS-5 | 7/19 | 400 | 33 | 57 | 6.8 | 2.1 |
| NS-6 | 7/19 | 400 | 23 | 36 | −1.4 | 2.0 |
| No-1 | 8/2 | 400 | 20 | 37 | −13.6 | 2.4 |
| NS-5 | 7/10 | 440 | 25 | 41 | 14.2 | 3.7 |
| NS-6 | 7/10 | 440 | 9 | 16 | −9.9 | 1.3 |
| NS-6 | 7/12 | 440 | 10 | 21 | −5.2 | 2.0 |
| No-1 | 8/31 | 500 | 25 | 48 | −13.4 | 3.6 |
| NS-5 | 7/19 | 600 | 19 | 37 | 21.3 | 3.1 |
| NS-5 | 7/31 | 600 | 23 | 62 | 18.9 | 5.6 |
| NS-5 | 8/3 | 600 | 5 | 13 | −10.4 | 2.1 |
| NS-6 | 7/20 | 600 | 23 | 35 | −7.6 | 2.8 |
| NS-5 | 8/5 | 800 | 9 | 34 | −2.0 | 2.7 |
| NS-6 | 7/22 | 800 | 17 | 37 | −2.2 | 4.2 |
| NS-6 | 8/14 | 800 | 13 | 24 | 2.8 | 1.5 |
| NS-5 | 8/12 | 900 | 15 | 21 | 6.8 | 1.2 |
| NS-6 | 8/12 | 900 | 10 | 27 | 6.2 | 2.8 |
| NS-5 | 8/6 | 1000 | 17 | 32 | 13.0 | 2.2 |
| NS-6 | 7/24 | 1000 | 30 | 65 | 16.9 | 3.5 |
| NS-6 | 7/26 | 1020 | 11 | 25 | 21.6 | 3.1 |
| NS-6 | 7/30 | 1020 | 19 | 38 | 24.9 | 1.5 |
| NS-6 | 7/31 | 1040 | 19 | 44 | 19.2 | 4.0 |
| NS-6 | 8/2 | 1060 | 19 | 35 | 22.1 | 3.8 |

[a] Number of points of inflection in the threshold determination curve.

[b] Number of trials on which the threshold determination was made.

[c] Secondary low-frequency thresholds.

TABLE 11—(*Continued*)

| Fish No. | Date | Frequency, in Cycles per Second | N | T | Mean, in Decibels | σ |
|---|---|---|---|---|---|---|
| NS-5 | 8/8 | 1100 | 17 | 53 | 22.7 | 5.0 |
| NS-6 | 8/6 | 1100 | 19 | 43 | 25.4 | 4.5 |
| NS-6 | 8/8 | 1100 | 11 | 22 | 19.6 | 1.7 |
| NS-5 | 8/10 | 1200 | 21 | 36 | 36.5 | 1.7 |
| NS-6 | 8/10 | 1200 | 19 | 30 | 31.0 | 2.3 |

tested five previous times. Were it not for the strong indications of this secondary curve in other species, the lower threshold points at 200 and 300 cps. might have been overlooked as simply representing a high degree of variability.

## AUDITORY THRESHOLD DETERMINATIONS IN *Eupomacentrus leucostictus*

This species was resistant to electric shock, and the optimum shock levels were varied from 10 to 35 volts. Usually the higher voltages had to be used during the threshold tests, because the animal developed a tolerance for the shock. The water level at the barrier was about 1.2 cm. Intertrial crossings were frequent, and the intertrial intervals had to be varied from two to five minutes. The positions of the animals in the test compartment before a trial were extremely variable, and, unlike the other species tested, there appeared to be no habitual position for any individual. The interval between sound and shock was 10 seconds, and the sound level was varied in steps of 2 decibels. Four animals were tested, and these reached the criterion of avoidance training in about four days.

Figure 17, lower, shows a sample threshold record for specimen NS-6 of *Eupomacentrus.* This record is an example of a long "warm-up" effect. Figure 10, upper, is an example of the plateau effect. Note that the plateau represents a temporary threshold value of about 20 decibels, i.e., more than 33 decibels above the actual threshold determination.

Above 600 cps., the threshold data for this species are consistent and show only a small degree of variability. The determinations from 600 cps. down show extreme variability. Were it not for the secondary threshold data in *Haemulon* and *Lutjanus*, these data at low frequencies might be interpreted as representing individual differences, intrinsic variability, or as the result of some inadequacy in the testing methods.

Table 10 is a list of threshold determinations, in chronological order, made on a single specimen (NS-5). Figure 19 is a graph of these same determinations. This specimen was initially trained at 440 cps., and the test frequencies were subsequently lowered to 400, 200, and 100 cps., in that order. After two determinations at 100 cps. on July 22 and 24, the threshold at the same frequency on July 26 dropped almost 20 decibels, and the next determination at 200 cps. (July 27) showed a 16-decibel drop from the previous one at that frequency. On this basis, therefore, these lower values are assumed to be secondary thresholds. The next series of determinations at 600 cps. gave high threshold values on July 29 and 31, and a 29-decibel drop on August 3. Subsequent tests at higher frequencies showed that the lower value at 600 cps. was clearly part of the primary curve. The assumption was made, therefore, that the two high points at 600 cps. were part of the secondary curve.

Specimen NS-6 was also trained at 440 cps., but subsequent testing was done at higher frequencies, and these values fell in line with those for NS-5. When NS-6 was tested at 300 and 200 cps., there was a drop of 25 decibels from the primary to the secondary curve at 200 cps. The small number of tests on the remaining two specimens did not elicit a clear difference between primary and secondary thresholds.

Plateau effects were commonly noticed when these animals were tested at frequencies below 800 cps. The record shown in figure 10, upper, is typical of the results obtained. In this example, the plateau is at a level that would place it close to curve 2 of figure 19. The high thresholds obtained at 600 cps. may actually represent such plateaus, and, had it been possible to continue the trials, these same determinations might have shown values some 20 or 30 decibels lower.

Figure 20 and table 11 are composites of the data on all the specimens of this species that were tested. The separation of the data into the primary and secondary low-frequency threshold curves follows the distinction between determinations made early and late in the study, as described above for figure 19.

## PART 3

The data on two species (*Epinephelus guttatus* and *Prionotus scitulus*) are incomplete and must be treated as preliminary. It is not possible to place these species in either of the other groups, since the data are not sufficient for the presence or absence of secondary low-frequency thresholds to be demonstrated.

### Auditory Threshold Determinations in *Epinephelus guttatus*

Threshold determinations were made on only a single individual, although additional animals were trained and tested.

This species was difficult to test. Training to the avoidance problem was slow. After seven days of daily training (20 trials per day), the animal began to avoid the shock sporadically, and it was not until 10 days later that the first threshold determination could be made. The critical period in a series of trials came when the threshold was approached. The fish began to cross the barrier regularly about every five to 10 seconds. The intertrial intervals were increased to 15 minutes, in some instances, before a significant trial could be run. The water level at the barrier was extremely critical. If it was increased to 10 cm., the crossings went on regularly for periods of up to 30 minutes before they slowed down sufficiently to permit the starting of a trial. At a level of 7.5 cm., the animal had great difficulty in crossing, and often both avoidances and escapes ceased. The optimum level was found to be 9 cm., and a variation of 1 cm. either permitted numerous intertrial crossings or inhibited avoidances.

The shock levels had to be varied during the course of training and testing. In initial training, 25 volts was adequate to produce escapes and, eventually, avoidances, but as avoidances became more regular, the shock level was increased to 30 volts or the avoidances soon became irregular. When a threshold was tested for, however, shock levels had to be reduced to 15 or 20 volts, since it was found that the higher levels not only inhibited avoidances but also inhibited escapes, and the animal often attempted to escape in the wrong direction or burrow down between the layers of rubberized hair insulation.

The animal exhibited a number of irregularities in performance which became especially pronounced as the threshold was approached. The stimulus intensity was reduced in 5-decibel steps after each avoidance, and,

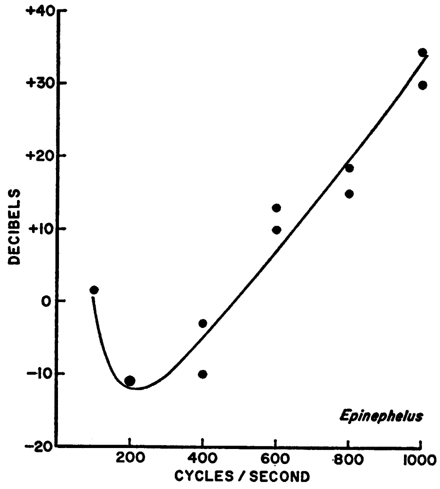

FIG. 21. Summary of threshold determinations for one specimen of the red hind, (*Epinephelus guttatus*). The data are given in table 12.

TABLE 12

AUDITORY THRESHOLD DETERMINATIONS IN THE RED HIND, *Epinephelus guttatus*

| Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | σ |
|---|---|---|---|---|---|
| 8/6 | 100 | 9 | 16 | 1.4 | 3.3 |
| 7/12 | 200 | 7 | 13 | −10.7 | 3.5 |
| 7/14 | 200 | 8 | 15 | −11.2 | 3.8 |
| 7/29 | 400 | 10 | 18 | −10.2 | 4.1 |
| 7/31 | 400 | 6 | 20 | −3.3 | 5.1 |
| 8/1 | 600 | 11 | 21 | 12.5 | 3.1 |
| 8/4 | 600 | 13 | 23 | 9.8 | 4.2 |
| 8/2 | 800 | 5 | 12 | 14.6 | 6.8 |
| 8/5 | 800 | 10 | 17 | 18.5 | 4.8 |
| 8/3 | 1000 | 8 | 15 | 34.4 | 4.7 |
| 8/6 | 1000 | 9 | 14 | 29.8 | 3.5 |

[a] Number of points of inflection in the threshold determination curve.

[b] Number of trials on which the threshold determination was made.

after the first negative response in which the animal received a shock, intertrial crossing began. As mentioned above, excessive intertrial crossings usually could be controlled by an increase in the intertrial interval, but on some occasions, the trials had to be terminated. On a number of trial sessions, the fish showed a preference for one side of the test tank, and it persisted in avoiding from one side and only escaping from the other. If this behavior remained consistent, then the trials were doubled, i.e., two trials at the same intensity. Unfortunately, after about 10 or 15 trials, the animal changed its preferred side. The initial position of the fish at the onset of the sound varied and was important to the response. If, in the initial position, the animal faced away from the central barrier, with its nose in a corner, the response was quite slow, and a full 10 seconds of the sound-shock interval elapsed before the fish turned around and crossed. Often the response was a partial one in that the fish just turned around but did not cross. Partial responses were not rewarded by termination of the trial, because it was feared that the full response would be weakened thereby. Usually, the observer waited until the animal turned with its nose toward the barrier before beginning a trial. The threshold records that were considered valid were only those in which these irregularities were virtually absent, which meant that a number of partial records had to be discarded.

Despite the fact that the threshold curve given here is based on only a single individual, it is still considered a valid series of determinations.

Table 12 and figure 21 present the available threshold data on this specimen. No evidence of a secondary threshold curve was present.

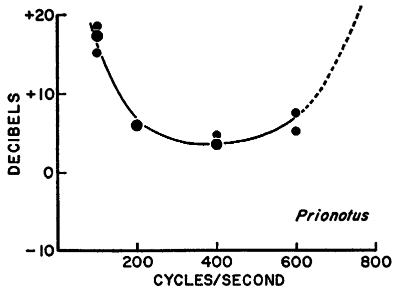

FIG. 22. Summary of threshold determinations for three specimens of the slender sea robin (*Prionotus scitulus*). The data are given in table 13.

TABLE 13

AUDITORY THRESHOLD DETERMINATIONS IN THE SEA ROBIN, *Prionotus scitulus*

| Fish No. | Date | Frequency, in Cycles per Second | N[a] | T[b] | Mean, in Decibels | $\sigma$ |
|---|---|---|---|---|---|---|
| A | 7/9 | 100 | 27 | 42 | 15.3 | 2.4 |
| B | 7/9 | 100 | 30 | 67 | 18.5 | 3.1 |
| B | 7/10 | 100 | 26 | 50 | 17.4 | 3.1 |
| B | 7/11 | 100 | 23 | 39 | 17.1 | 2.8 |
| B | 7/12 | 100 | 24 | 38 | 17.8 | 2.5 |
| A | 7/7 | 200 | 17 | 33 | 6.5 | 1.5 |
| B | 7/8 | 200 | 19 | 34 | 5.8 | 2.1 |
| B | 7/13 | 200 | 24 | 44 | 5.9 | 2.9 |
| A | 7/6 | 400 | 10 | 28 | 4.8 | 2.6 |
| B | 7/7 | 400 | 16 | 33 | 3.5 | 2.1 |
| C | 7/9 | 400 | 28 | 45 | 3.9 | 2.9 |
| C | 7/10 | 600 | 30 | 67 | 5.1 | 3.1 |
| A | 7/11 | 600 | 28 | 49 | 7.4 | 2.0 |

[a] Number of points of inflection in the threshold determination curve.

[b] Number of trials on which the threshold determination was made.

It is not known, therefore, if this audiogram represents only a single curve or if it is a combination of the two that could be detected in other species tested.

## AUDITORY THRESHOLD DETERMINATIONS IN *Prionotus scitulus*

The optimal shock voltage was 12 volts. The intertrial intervals averaged three minutes. The water level was 1 to 2 cm., and the animals crossed the barrier by almost leaping out of the water. Early in the training period, it was found that an interval of 10 seconds between the onset of the sound and the onset of the shock was insufficient for this species. The usual behavior of the animal was that it started to move toward the barrier slowly after the sound had been on for about five to eight seconds, hesitate at the barrier for as long as 10 seconds, and then abruptly hurtle across. A water level that was higher than 2 cm. enabled the animal to remain partially across the barrier. An interval of 20 seconds between the onset of the sound and the shock was found to be optimal, and the sound level was varied in 2-decibel steps during the threshold determinations.

Crossings during the intertrial interval were virtually absent. The animals normally assumed a quiescent position against the far wall of the compartment immediately after a trial. Three specimens were used in this study, but, unfortunately, all three died before additional data could be gathered. This species is uncommon at Bimini, so that the likelihood of obtaining more animals was small, and the data, although incomplete, are presented here.

Table 13 and figure 22 present the threshold data on *Prionotus*. Despite replications at the low frequencies, there was no evidence of a secondary curve. The dotted line in figure 22 is an extrapolation of the audiogram above 600 cps. The lowest threshold values were at 400 cps. at about 4 decibels.

Some preliminary tests indicated that the thresholds for frequencies above 600 cps. rose sharply, and the extrapolation of the curve in figure 22 is based on these partial records.

# DISCUSSION

## Generalizations and Comparisons with Other Reports

For the nine species tested, a number of features in common can be seen from the data. The lowest thresholds were in the 300–500-cps. range, clearly so in *Eupomacentrus*, *Thalassoma*, *Lutjanus*, *Epinephelus*, and *Prionotus*, and, aside from the secondary low-frequency thresholds, also essentially true for *Haemulon*. The lowest thresholds in *Holocentrus* and *Equetus* were almost an octave higher (600–800 cps.).

The upper frequency limits were about 1000–1200 cps. in all except *Holocentrus ascensionis*, for which the upper limit was 2800 cps. It is important to qualify conclusions about upper frequency limits with a statement of the sound pressures at which the animals were tested. A pressure of 45–50 decibels (re 1 microbar) was the limit here, primarily because above this level considerable harmonic distortion and clipping of the output signal occurred. It is also a fact that these pressures are well above the sound levels normally present in the area (see below).

The equipment also limited the tests of low-frequency limits, in that 100 cps. was the lowest undistorted frequency that could be reliably achieved with the speakers. The range of thresholds for the six species was from −15 to +35 decibels. Here it becomes important to separate the data into primary and secondary low-frequency thresholds. As here defined, the primary thresholds were those determinations that were made during the early phases of training of the subjects. After replication and additional training, the threshold values dropped abruptly in four of the species. These are defined as the secondary low-frequency thresholds. In summary, the highest primary threshold at 100 cps. was that of *Lutjanus* (36 decibels). *Eupomacentrus* and *Thalassoma* were next (about 25 decibels), followed by *Holocentrus* and *Haemulon* (5 to 10 decibels) and *Epinephelus* (about 0 decibel). The secondary low-frequency thresholds in *Lutjanus*, *Eupomacentrus*, *Thalassoma*, and *Haemulon* were lower by 15 to 20 decibels. *Holocentrus* and *Equetus* did not show a secondary curve even after extensive training, and the data on *Epinephelus* and *Prionotus* were probably insufficient for this phenomenon to be detected.

From the data presented, it was evident that the squirrelfish (*H. ascensionis*) had the broadest frequency spectrum as far as sound reception in general is concerned. The highest sensitivity was at 800 cps., with a threshold of −24 decibels, and positive responses went up to 2800 cps., at an intensity of +53 decibels. The other, smaller species of squirrel fish (*H. vexillarius*) gave threshold values 10 decibels or more above those for *H. ascensionis*.

The highest sensitivity of all species tested here was shown by the cubbyu (*Equetus*), with a threshold of almost −40 decibels at 600 cps.

The species with the poorest sensitivity was the schoolmaster (*Lutjanus apodus*), with its lowest threshold from 300 to 500 cps. at about +10 decibels. At 1000 cps., the threshold curve rose sharply to almost 40 decibels.

The highest sensitivity of the blue-head (*Thalassoma*) was at 500 cps., at a pressure level of about +5 decibels, and that of the sea robin (*Prionotus*) was at 400 cps. at about +4 decibels.

The grunt (*Haemulon*) showed a remarkably clear distinction between the primary and secondary low-frequency thresholds. Its lowest thresholds were on the secondary curve from 200 cps. down, at a pressure of about −16 decibels. The highest sensitivity on the primary curve was from 400 to 600 cps., at about −8 decibels. The threshold rose to more than +40 decibels at 1100 cps.

The threshold curves for the blue-head (*Thalassoma*) appeared to be intermediate between the curve for *Lutjanus* and that for *Haemulon* in a number of respects. The secondary low-frequency curve dipped lower at 300 cps. (to +1 decibel) than that for *Lutjanus*, but it rose at 200 cps., not like the case of *Haemulon*. The entire primary curve was generally almost 10 decibels lower than that for *Lutjanus* and about 10 decibels higher than that for *Haemulon*. The high-frequency portion of the curve for *Thalassoma* did not rise so sharply as that for the other two species. The threshold at 1200 cps. was 35 decibels.

Despite the relatively large number of determinations for the beau-gregory (*Eupomacentrus*), the data below 500 cps. are confusing. Based on the results from the other species, we can assume the existence of a secondary low-frequency curve; otherwise we would be at a loss to explain the hump in the curve at 300 to 400 cps. As it is, the curves, as drawn in figure 19, are only approximations and extrapolations. The lowest threshold was at 500 cps. (−14 decibels), and at 1200 cps. the curve rose to about +33 decibels.

The data for the red hind (*Epinephelus*) were limited to a small number of highly variable determinations based on a single specimen. Thus the threshold determinations are to be considered preliminary for this species. A secondary low-frequency curve was not detected. The range of highest sensitivity was from 200 to 400 cps. at below −10 decibels. At 1000 cps., the threshold was about 32 decibels.

The data on *Prionotus* were also incomplete in that the highest frequency tested was 600 cps. From 200 to 600 cps. the thresholds were in the order of 3.5 to 7.4 decibels, and at 100 cps. the curve rose sharply to about 3 decibels. Although no specific data are available for the higher frequencies, some preliminary tests indicated the probability of a steeply rising curve.

How do the thresholds reported here compare with those determined by other workers? In the following review, the values given in the original papers all have been converted to the reference point of 0 decibel = 1 microbar (= 74 decibels above the 0.0002-microbar threshold for human hearing at 1000 cps.)

The majority of reports have been on members of the Cypriniformes (Ostariophysi), whose possession of a Weberian apparatus has apparently increased both the range and sensitivity of their hearing.

Although Stetter (1929) was one of the first to do any intensity studies, he gave no specific figures on sound levels. For the Characidae, von Boutteville (1935) reported a threshold of about −60 decibels (at 650 cps.). The same author determined a threshold of −40 to −45 decibels (at 650 cps.) for the eel (*Gymnotus*).

*Phoxinus laevis*, the Elritze, was widely used by European workers, and a threshold of −50 to −40 decibels at 258 cps. was reported by Diesselhorst (1938).

Among the catfishes, *Macrones* has a threshold of −60 decibels at 400–1500 cps. (Dorai Raj, 1960). The common bullhead (*Ameiurus nebulosus*) appears to have the lowest threshold of any species tested, according to Autrum and Poggendorf (1951). These authors report a threshold near the lower limit of human hearing (i.e., near −70 decibels) for all frequencies from 60 to 1500 cps. The figures were confirmed by Kleerekoper and Roggenkamp (1959), and they showed almost a straight horizontal line for the threshold values at different frequencies. The lowest thresholds were between 200 and 1800 cps. They found that if the swim bladder was damaged, the thresholds went up about 20 decibels at 750 cps., and hearing was greatly impaired at all higher frequencies. Damage to the lateral line nerve affected the sensitivity to frequencies below 400 cps. Thus the separate sensitivities of the inner ear and lateral line could be plotted, and the resultant curves show a remarkable resemblance to the double curves obtained by us for *Haemulon* and other species.

The upper frequency limits for these ostariophysines were all high: 4000 cps. for *Ameriurus* (Farkas, 1936), almost 6000 cps. for *Semotilus atromaculatus* (Kleerekoper and Chagnon, 1954), 7000 cps. for characids (von Boutteville, 1935), more then 8000 cps. for *Phoxinus* (von Frisch, 1938). Rough (1954) claimed positive responses from carp (*Cyprinus carpio*), up to 22,000 cps., but no intensity figures were given.

Although lacking a Weberian apparatus, the mormyrids and labyrinthine fishes possess air chambers directly coupled to the perilymphatic fluid and inner ear. The studies of Diesselhorst (1938), Stipetić (1939), and Schneider (1941) showed upper frequency limits of more than 3000 cps. for mormyrids, with a threshold of −50 to −40 decibels at 258 cps. (Diesselhorst, 1938). Schneider (1941) reported upper frequency limits of 4500 cps. for certain labyrinthines.

Upper frequency limits in most other non-ostaryophysine species are considerably lower. Tables 14 and 15 summarize the majority of reports.

TABLE 14

SUMMARY OF REPORTED THRESHOLD DATA IN TELEOSTS WITHOUT A WEBERIAN APPARATUS

| Genus and Family | Upper Frequency Limit, in Cycles per Second | Reference |
|---|---|---|
| *Gobius* (Gobiidae) | 800 | Dijkgraaf, 1949 |
| *Corvina* (Sciaenidae) | 1000 | Dijkgraaf, 1949 |
| *Corvina* (Sciaenidae) | 1500–2000 | Maliukina, 1960 |
| *Sargus* (Sparidae) | 1250 | Dijkgraaf, 1949 |
| *Anguilla* (Anguillidae) | 600 | Diesselhorst, 1938 |
| *Lebistes* (Poeciliidae) | 435[a] | Farkas, 1935 |
| *Lebistes* (Poeciliidae) | 2068 | Farkas, 1936 |
| *Mugil* (Mugilidae) | 1600–2500 | Maliukina, 1960 |

[a] 640 in young.

Deserving special mention is the report (in abstract) that *Holocentrus ascensionis* can respond to frequencies up to 8000 to 9000 cps. (Winn and Marshall, 1960), but no indication was given as to the method of testing or the intensities used. When the data presented in the present paper are considered, the figures given by Winn and Marshall must remain in doubt, at least until further details become available.

In this connection, it might be added that no data are available on the threshold of feeling in fishes. It may well be that responses to high frequencies, as reported by Rough (1954) in the carp and Winn and Marshall (1960) for squirrelfish, actually represent integumentary tactile reception. It appears to be of little value to test organisms at stimulus intensity levels far beyond the maximal intensities encountered by the animals in their normal environment. The fact that human subjects often can detect powerful ultrasonic signals is well known, but that they can do so is obviously not a function of the auditory sense.

Sound pressure thresholds for non-ostariophysines as given by other authors are listed in tables 14 and 15.

The figure given by Griffin (1950) for the sea robin (*Prionotus evolans*) was not considered a threshold, but it appears to fall close to the determinations made here for *P. scitulus*. Griffin obtained responses at a frequency of 100 cps. and a pressure level of 17 decibels (re 1 microbar), and our figures at this frequency average 17.2 decibels.

If the figures in tables 14 and 15 are at all comparable to the ones reported by us, apparently the thresholds are distinctly below those for the species tested here. The figures for *Holocentrus* as given are of the same order of magnitude as those for *Anguilla* by Diesselhorst (1938). It is likely that the frequencies used in the reports cited are in the most

TABLE 15

SUMMARY OF REPORTED THRESHOLD DATA IN TELEOSTS WITHOUT A WEBERIAN APPARATUS

| Genus and Family | Threshold (re 1 Microbar), in Decibels, at Cycles Per Second | Reference |
|---|---|---|
| *Anguilla* (Anguillidae) | −20–0, at 250 | Diesselhorst, 1938 |
| *Mugil* (Mugilidae) | −50, at 640 | Maliukina, 1960 |
| *Corvina* (Sciaenidae) | −45, at 320 | Maliukina, 1960 |
| *Corvina* (Sciaenidae) | −50, at 500–600 | Maliukina, 1960 |
| *Mullus* (Mullidae) | Below −30, at 450–900 | Maliukina, 1960 |
| *Gaidropsarus* (Gadidae) | −30, at 750 | Maliukina, 1960 |

sensitive range of the hearing of the animals.

In order that the threshold figures determined here and in the earlier literature can be properly compared, it is necessary to know such details as the methods of measuring intensities, the sound insulation used (if any), the frequency response of the monitoring equipment, and the distance of the sound source from the monitoring hydrophone.

Kritzler and Wood (1961) attempted to determine a complete audiogram in a shark (*Carcharhinus leucas*). Their data, based on positive reward conditioning, range in threshold values from 10 decibels (re 1 microbar) at 100 cps. to a low level of about −15 decibels at 400 to 600 cps., to more than 10 decibels at 1400 cps. In consideration of the fact that the shark has no swim bladder and therefore receives all sounds either through direct conduction to the inner ear or by way of the lateral line system, these low thresholds are quite remarkable and may indicate that an air chamber need not function as the main transducer in sound reception, and that the acoustical difference between the water medium and the bone or cartilage of the neurocranium may be sufficient to permit low-frequency detection (i.e., below 1500 cps.). However, it should be noted that the response criterion used by Kritzler and Wood was a subjective judgment on the part of the observer, and these data would bear confirmation.

Also, in an elasmobranch, Dijkgraaf (1963) conditioned the dogfish (*Scyliorhinus canicula*) to respond to a tone of 180 cps. The method was that of classical conditioning, and the results indicated a threshold at about 30 to 40 decibels.

Despite the apparent accuracy of Maliukina's (1960) data, she gave no details as to the actual mechanics of the experiment. No information was given on the sound-measuring equipment, nor what, if any, sound insulation was used.

The method of sound measurement used by Diesselhorst (1938), Dijkgraaf (1949), and others was to compare the test signals with their own auditory thresholds. Diesselhorst used a loudspeaker outside the test aquarium, while Dijkgraaf used tuning forks in contact with the aquarium wall. Diesselhorst's signal generator was calibrated so that, once his own threshold values were determined for the test frequencies, he was able to calculate the intensity of the actual test signal. Dijkgraaf determined his auditory thresholds by placing his ear against the wall of the aquarium. Griffin (1950) attempted to duplicate Diesselhorst's experimental conditions, but, in addition, to measure the sound pressures with a calibrated hydrophone and sound-level meter. He concluded that Diesselhorst's determinations were accurate within about 10 decibels. The main factor that must taken into account is the transmission loss through the water-glass-air interfaces. At a water-air interface, sound generated under water has a critical angle of about 13 degrees from the normal. Beyond this point all the energy is reflected. More than 99.9 per cent of sound impinging directly on the surface is reflected (Vigoureux, 1960; Horton, 1959). Griffin (1950) estimated that in Diesselhorst's (1938) and von Boutteville's (1935) experiments, only 0.012 per cent of the sound generated by the external loudspeaker reached the inside of the aquarium. None of these early investigators took into account the reflection, reverberation, and standing waves within the aquarium.

As is pointed out above, the acoustical resistance of water is much greater than that of air. With the use of the equations given by Albers (1960), the acoustical intensity in air of 1 microbar of sound pressure is about $2.3 \times 10^{-9}$ watts per square centimeter, whereas at the same pressure the intensity in water would be only about $6.7 \times 10^{-13}$ watts per square centimeter. At equivalent intensities, sound pressures in water are almost 60 times those in air. The sound-pressure threshold in human beings is usually taken at 0.0002 microbar, or 74 decibels below 1 microbar, with an intensity of $10^{-16}$ watts per square centimeter. The same intensity in water gives a sound pressure of almost 38.5 decibels below 1 microbar. If the figures given by Diesselhorst (1938), Maliukina (1960), and others are correct, then apparently the true intensity thresholds in many fishes are actually considerably lower than those for human hearing in air. A threshold of −70 decibels, as reported for *Ameiurus* by Autrum and Poggendorf (1951), would be equivalent in terms of intensity to −98.5 decibels in air! When the

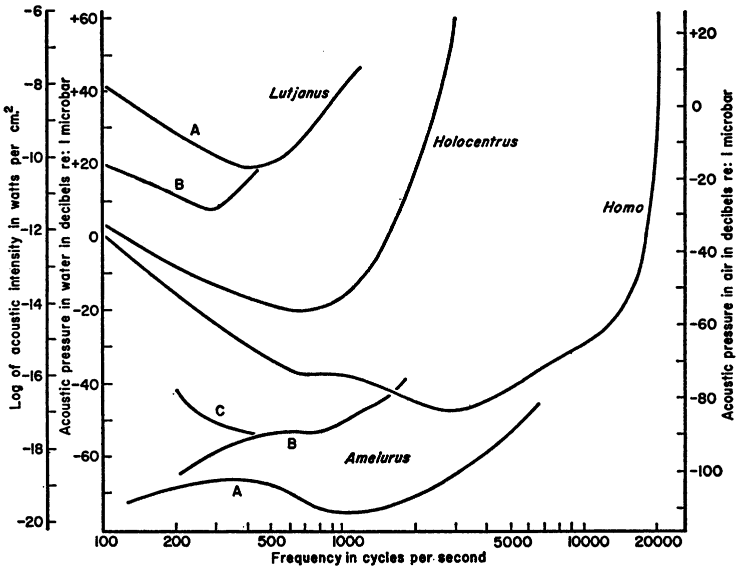

FIG. 23. Composite graph showing comparisons among auditory thresholds in the schoolmaster (*Lutjanus*) and squirrel fish (*Holocentrus ascensionis*), as described in this report; the human audiogram according to Sivian and White (1933); and the auditory thresholds of the catfish (*Ameiurus nebulosus*) as given by Autrum and Poggendorf (1951) and Kleerekoper and Roggenkamp (1959). All these curves are plotted against the extreme left-hand ordinate—acoustic intensity in watts per square centimeter. The acoustic pressures in water are on the left ordinate, and the equivalent pressures in air (against which the human thresholds are plotted) are on the right-hand ordinate. See text for discussion.

general intensity levels of ambient noise, even in calm weather, are considered, threshold levels such as these would seem to make the animals inordinately sensitive to noise of little or no informational value.

Figure 23 presents the sort of comparison made in the above paragraph. The auditory threshold curve in human beings is based on that used in most textbooks. It is the minimum acoustic field audiogram of Sivian and White (1933). It is plotted against the right-hand ordinate, with the sound pressure expressed in terms of decibels re 1 microbar. The equivalent acoustic intensity is shown on the extreme left-hand ordinate in terms of the logarithm (base 10) of the energy in watts per square centimeter. Two of the species tested here, the squirrelfish (*Holocentrus ascensionis*) and the schoolmaster (*Lutjanus apodus*), are plotted on the same graph. The thresholds of these aquatic animals are plotted against the extreme left-hand ordinate of acoustic intensity. Parallel to this scale is the equivalent acoustic pressure in water in decibels re 1 microbar. Curves A and B for *Lutjanus* are the primary and secondary threshold values, respectively. For comparison, the data on the catfish (*Ameiurus*) are also included. Curve A is the audiogram reported by Autrum and Poggendorf (1951). Curve B is copied from the work of Kleerekoper and Roggenkamp (1959) and represents the effect on the audiogram of *Ameiurus* after damage to the swim bladder. Curve C is the result of the additional damage to the lateral line nerve.

The primitive nature of the sound generating and measuring equipment used by these earlier works, the factors presented above, and the somewhat ambiguous nature of positive responses by their experimental animals all lead us to believe that the previous reports on auditory thresholds in fishes badly need re-evaluation and confirmation. Griffin (1950) pointed out the need for acoustical controls and unequivocal responses, as well as indicating the importance of accurate, under-water sound measurements in order that threshold determinations can be made with precision and dependability.

## SIGNIFICANCE OF SECONDARY LOW-FREQUENCY THRESHOLDS AND PLATEAUS

The presence of secondary low-frequency thresholds in our data is of considerable interest. In most cases, they appeared after considerable training and testing in the low-frequency range. The clearest example is that of *Haemulon*, in which the entire shape of the audiogram curve became changed when the secondary curve was detected. The absence of the secondary curve in our data on *Epinephelus* is probably not significant, since these determinations were based on a single specimen and a relatively small number of threshold determinations. The absence of a secondary curve is certainly significant in *Holocentrus* and *Equetus*, since some of the animals were retested almost daily over a period of more than two months.

What does the secondary low-frequency curve indicate? We conclude that it shows there are two mechanisms or two sensory modalities operating to receive low-frequency vibrations and that the two receivers have thresholds of about 20 decibels apart. There is, of course, the possibility that only one receptor is involved, with two separate central neural mechanisms of different levels of excitation, but such a conclusion would appear to be untenable since there is no neurophysiological evidence to support it.

Evidence exists, however, to support the possibility of multiple sound receptors in fishes. Three systems are to be considered as potentially responsive to low frequencies: the general cutaneous sense, the lateral line, and the inner ear.

Experimental work on the possible functions of the cutaneous tactile sense in low-frequency detection is sparse. Parker and van Heusen (1917) were among the earliest workers to propose a cutaneous sense in fishes capable of receiving vibrations. Manning (1924) showed an increase in responsiveness produced by strychnine. This drug, used on goldfish, presumably increased the sensitivity of the integumentary sense organs. In sound localization studies on *Phoxinus*, von Frisch and Dijkgraaf (1935) demonstrated that the animals could detect the direction of the sound source only if the distance was small and the sound intense. They voiced the suspicion that an integumentary sense was being used. Later, von Frisch (1938) was more positive in stating that frequencies from 16 to 129 cps. were received by a cutaneous sense, although he did not eliminate the possible role of the lateral line. However, Reinhardt (1935) obtained positive directional responses from both *Phoxinus* and *Ameiurus* to low frequencies despite the destruction of the inner ear and lateral line. In *Gobius paganellus*, Dijkgraaf (1949) determined the upper frequency limit as 800 cps., and he postulated that this species detected sound mainly through a cutaneous receptor. Dorai Raj (1960), studying the catfish (*Macrones gulio*), obtained positive responses to frequencies down to 15 cps. in animals the lateral line nerves of which had been severed.

The functions of the lateral line have been investigated by many workers, and functions including chemical sense, temperature sense, and sound reception have been only some of the many ascribed to this structure. Parker (1902, 1918) proposed that the lateral line was sensitive to low-frequency vibrations and, especially, to shock waves in the water. Rode (1929) claimed a sensitivity of 2 to 60 cps. for the lateral line. Dijkgraaf (1933, 1947b, 1952) demonstrated that the lateral line can detect water movements and is used in avoidance of both moving and stationary obstacles. This organ effectively extends the range of tactile sense, termed by Dijkgraaf the "Ferntastsinn." The use of action potentials from the lateral line nerve showed its response to vibrations up to 150 cps. in frequency (Schreiver, 1935, 1936), and also that it reproduces frequencies microphonically up to 180 cps. (Suckling and Suckling, 1950). This microphonic activity was studied further by Jielof, Spoor, and de Vries (1952) and by Kuiper (1956). Essentially, Kuiper confirmed most of Dijkgraaf's observations and concluded that the lateral line is a tactile receptor and that "alternating water currents produced by the object are the stimuli for the organ." Harris and van Bergeijk (1962) described the response of lateral line organs to displacement of water produced by the "near-field" effect of a nearby sound generator.

In a recent review of lateral line function, Dijkgraaf (1962) emphasized the fact that the lateral line is primarily sensitive to the "damming phenomenon" which is a local displacement effect produced by a moving object. In agreement with Harris and van Bergeijk (1962), he stated that the effective stimulus was displacement rather than pressure. Repetition or rhythmicity of the stimulus appears not to be significant, and therefore Dijkgraaf eliminated the lateral line as being a low-frequency sound detector. He cited evidence that the destruction of the lateral line and inner ear does not eliminate responses to low-frequency sound. There is, however, a considerable body of evidence that the lateral line does respond to vibrations, and Kuiper (1956) even stated that the cupula is "an ideal vibration perceptor." Furthermore, in many of the ablation experiments, only the lateral line nerve (vagus branch) was cut, and the cephalic canals were still presumably functional. The evidence on the response of lateral line cupulae to water displacements seems unequivocal, and, within the mechanical limits of the receptor, the cupulae should respond equally well to rhythmic vibrations as to non-rhythmic flow phenomena, and, indeed, Harris and van Bereijk (1962), Suckling and Suckling (1950), and many others have shown such response to be true. Dijkgraaf (1962) also eliminated the functioning of the lateral line in the detection of locomotor currents. We fail to see the difference between water displacements produced by "moving obstacles" and those produced by the hydrodynamics of fish locomotion. Certainly the extent to which integumentary receptors participate in displacement detection is not known, but, on the basis of physiology, mechanics, and behavior, the lateral line functions as a short-range subsonic receptor.

Since it has been clearly established that the lateral line detects water displacements of both a rhythmical and a non-rhythmical nature, it is evident that it must be capable of sound detection in the low frequencies. Especially in view of the sparse evidence on general cutaneous sense, we must accept the idea that the lateral line and the integumentary tactile receptors constitute a single system as far as low-frequency sound is concerned.

Most authors agree that the portions of the teleost inner ear that are the specific sound receptors are the sacculus and lagena. Probably the earliest reports of any validity are those of Manning (1924) and von Frisch and Stetter (1932). Pearson (1936) described the central connections of nerves from the inner ear and postulated that the coarse fibers from the saccular root transmit sonic stimuli. Von Frisch (1938) described the connection of the Weberian ossicles in *Phoxinus* as transmitting vibrations from the swim bladder to the saccular otolith—the sagitta. He also stated that the lagenar otolith (asteriscus) can receive sonic stimuli by way of bone conduction. In *Lebistes*, which lacks a Weberian apparatus, Farkas (1938a, 1938b) reported that the sagitta is the otolith that receives vibrations through the fenestra sacculi. The most definitive work was that of Dijkgraaf (1949 and 1952) who demonstrated that the auditory function of the inner ear resides in the sacculus and lagena. Action potential techniques were used by Zotterman (1943) to detect the sonic reception of the macula sacculi. Similar results were obtained by Lowenstein and Roberts (1951) in elasmobranchs, but in addition they were able to detect action potentials from the utriculus as well. No one has yet been able to separate the sacculus and lagena in terms of their function in sound reception. The role of the lagena, then, has not been clarified. For the present, we assume that the inner ear constitutes a single mechanism by which the fish receives sound.

From the above discussion, it appears likely that two mechanisms are involved in the reception of low-frequency sound: the inner ear and the lateral line-integumentary system. Such a possibility is in agreement with the conclusions based on the presence of primary and secondary low-frequency thresholds as described here. The threshold curves for frequencies above 500 cps. are almost certainly representative of the sensitivity of the inner ear mechanism. The primary low-frequency curve is a logical continuation of the curve for the same mechanism. We conclude, therefore, that the secondary curve represents the sensitivity of the lateral line-integumentary receptors. It must be realized, however, that this conclusion is tentative in that it is based on only indirect evidence; considerable further investigation is required to support it.

Some support for this interpretation can be derived from the data presented by Kleerekoper and Roggenkamp (1959) on the catfish (*Ameiurus*). In this case the damage to the lateral line produced a clear increase in the thresholds for low frequencies.

As noted above, direct contact of the hydrophone with the insulating material in the experimental tanks resulted in a 2-decibel rise in the sound level that was received. Quite possibly direct integumentary reception was involved in cases in which the experimental animal was touching the rubberized hair. Suckling (1962) found that brushing the dorsal fin spines or any scales distant from the lateral line organs resulted in an increase in action potentials from the lateral line nerve. Possibly, therefore, with increased training, the animals learned that low-frequency sound detection by way of the lateral line was more efficient.

From the aspect of central nervous mechanisms, it is remarkable that, with two receptors for the same class of stimuli, the more sensitive and efficient is not used immediately. If we accept the conclusions of the above paragraphs, it appears that many of our experimental animals utilized the inner ear mechanism first and, after additional training, switched to the more sensitive low-frequency detector. Our data indicate that this switch does not occur with predictable regularity.

A roughly parallel situation exists in the combination of rod and cone receptors in the retina. Here are two distinct receptor systems for the same class of stimuli, and their thresholds overlap. The dark adaptation studies of Blough (1956, 1958) have shown that, when the thresholds are tracked by means of the staircase technique, the cone threshold is reached first. At this point there is a temporary plateau, followed by another drop to the level of the rod threshold (Blough, 1961). This phenomenon appears similar to the plateau effect that our studies have demonstrated in auditory sensitivity of fish, and its existence strengthens our hypothesis that two sensory systems are involved at the low frequencies.

An additional observation of significance is that the sequence of frequencies used to test the animals has an effect on the threshold determinations. The effect was particularly evident in the data on the beau-gregory (*Eupomacentrus*). When the fact that the avoidance response involves not only sensory but perceptual factors is considered, it is really not surprising that length and sequence of testing should affect the threshold data.

## THE FUNCTION OF THE SWIM BLADDER IN SOUND RECEPTION

According to Griffin (1950, 1955) and Pumphrey (1950), a fish is essentially transparent to water-borne sound, and its only acoustical discontinuity is the swim bladder (or other gas chamber). Sound reception under water requires the presence of a transducer constructed of material very different in acoustical properties and density from the surrounding medium. Air bubbles are known to be excellent reflectors and resonators (Horton, 1959; Meyer, 1957), and certainly the swim bladder can serve efficiently as a transducer. Marshall (1951) and Jones and Pearce (1958) have shown that fish swim bladders are effective sonic reflectors and that 50 per cent or more of impinging sound energy is returned by the bladder, while a smaller percentage is reflected by the rest of the body of the fish. However, some other portions of the fish, such as the skull, may also serve as acoustical discontinuities and thus permit sound reception by bone conduction. The swim bladder still appears to be the most obvious and efficient sonic transducer that the fish possesses. Harris and van Bergeijk (1962) consider swim bladders as aquatic middle ears which transform pressure waves into near-field displacements.

If we accept the above contention, fishes with swim bladders should have better hearing than those without. Furthermore, those species in which the swim bladder is acoustically coupled to the inner ear should have the highest auditory sensitivity and broadest range.

As reviewed above, the Cypriniformes

apparently possess the lowest auditory thresholds and highest upper frequency limits. The auditory capacities of these fishes are undoubtedly enhanced by the Weberian apparatus which couples the auditory signal received by the swim bladder to the inner ear in a manner analogous to the operation of the middle ear ossicles in mammals. Other air chambers can serve in similar fashion as, for example, the branchial cavity in the labyrinthine fishes (Schneider, 1941).

Among non-ostariophysines, in a number of forms the swim bladder has anterior extensions which are either coupled directly to the perilymphatic fluid (as in many clupeids) or attached to the occipital region of the neurocranium (Froese, 1938). Wohlfahrt (1938) described long, thin, anterior extensions of the swim bladder in herrings. These terminate in gas-filled capsules enclosed in bone and coupled to the perilymph by an elastic "fenestra."

Although satisfactory auditory thresholds have not been reported for any clupeid fishes, probably their auditory sensitivity is high.

Our data have shown that *Holocentrus ascensionis* has a low threshold and broad frequency response spectrum, probably related to the contiguity of the anterior end of the swim bladder to the skull, as described by Nelson (1955). However, *H. vexillarius*, with the same swim-bladder construction, exhibits a higher auditory threshold, and *Equetus*, with no such specialization, possesses a much lower auditory threshold.

Species with reduced swim bladders or without swim bladders should have poor hearing. The evidence is sparse. Bull (1928) was unable to condition a blenny (*Blennius*) to respond to sound. In *Gobius*, Dijkgraaf (1949) showed an upper frequency limit of only 800 cps., and he postulated that most sound reception in this species took place through lateral line or cutaneous tactile senses. Tavolga (1958) demonstrated the inability of *Bathygobius* to discriminate its mating sounds from other low-frequency noises.

Superficially, there appears to be little difference in basic structure between the swim bladder of *Haemulon*, that of *Epinephelus*, and that of *Lutjanus*, yet *Lutjanus* has a threshold at 500 cps., some 15 decibels higher than the others. *Eupomacentrus* has a swim bladder of the thin-walled type, tightly wedged in against the dorsolateral body wall, and its auditory-threshold curve is only a few decibels higher than that of *Holocentrus*. The smallest swim bladder of the six species tested here was that of *Thalassoma*, of which the general-threshold curve falls somewhere between that of *Haemulon* and that of *Lutjanus*. To assess the exact degree of acoustical coupling between the swim bladder and the inner ear in any of these forms is difficult, and probably other factors enter into auditory sensitivity in addition to just the shape and position of the bladder. The construction of the inner ear would certainly be one such factor. What should be investigated are the general acoustical properties of swim bladders and their degree of connection with the inner ear through the musculature, vertebral column, and skull. Some correlations may be discovered which will clarify the function of the swim bladder as an under-water microphone, if, indeed, it has any such function in many species.

Furthermore, the validity of the statement that the fish is acoustically transparent is open to question. To test this statement, some direct measurements are needed as to the acoustical coupling and transmission of sound through the skin, skull, vertebral column, and swim bladder as well. If a swim bladder with good coupling to the otic region were a requirement for under-water hearing, then species such as the blue-head and sea robin should be virtually deaf, and sharks should be completely insensitive to all except low frequencies detectable by the lateral line. The data of Kritzler and Wood (1961) certainly demonstrate that hearing in sharks is as good as that of many teleosts, and Dijkgraaf (1963) showed that sectioning of the acoustic nerve in the dogfish reduced the sensitivity at 180 cps. by about 20 decibels.

## CHARACTERIZATION OF THE ACOUSTIC STIMULUS

Questions arise as to the exact nature of the stimulus produced by the under-water speakers used here, the nature of the stimulus received by the sense organ, and which sense organ is stimulated. For purposes of this discussion we can assume that the sound source is a pulsating sphere. In such a case, the acoustic energy produced is measured in terms of ergs or watts per square centimeter, and the surface is taken to be a sphere surrounding the sound source. It is evident that the energy flow through this spherical surface is inversely proportional to the square of the distance from the center of the sound source, which is the commonly known inverse square law for transmission loss due to divergence or spreading (Albers, 1960). Sound energy, i.e., intensity, is directly proportional to the square of the acoustic pressure, according to the equation

$$I = p^2/\rho c,$$

in which $I$ equals the intensity in ergs per square centimeter, $p$ equals the pressure in dynes per square centimeter (=microbars), and $\rho c$ equals the acoustic radiation resistance of water (approximately 150,000 grams per square centimeter per second). Pressure, therefore, is inversely proportional to the distance from the sound source. Empirically, however, such a proportion obtains only in an infinite medium and at distances well over one wave length.

A sound field actually consists of energy that can be measured in two different ways: as pressure or as displacement. Harris and van Bergeijk (1962) have shown that both these factors must be considered in the characterizing of an under-water sound stimulus, especially at short distances and low frequencies. They showed that water displacement produced by a pulsating sphere involves two factors. One is the propagated pressure wave, i.e., the "far-field," and the other is the "near-field" displacement effect. In the far-field, the displacement amplitude and distance are linearly related, and the pressure at a given frequency is also directly proportional to the displacement according to the equation

$$p/\rho c = 2\pi f d,$$

in which $p$ equals the pressure in dynes per square centimeter, $\rho c$ equals the acoustic resistance of water, $f$ equals the vibration frequency in cycles per second, and $d$ equals the displacement of water in centimeters.

The corrected formula for displacement and pressure (Harris, personal communication) would include the near-field effect:

$$d = \frac{p}{i2\pi f \rho c}\left(1 + \frac{\lambda}{i2\pi r}\right).$$

The symbols represent the following:

$d$ = displacement amplitude in centimeters
$p$ = acoustic pressure in dynes per square centimeter (= microbars)
$i$ = square root of $-1$ (representing a 90° phase lag)
$f$ = frequency in cycles per second
$\rho$ = density of water (taken as 1 gram per cubic centimeter)
$c$ = velocity of sound in water (approximately 150,000 cm. per second for sea water; Tschiegg and Hays, 1959)
$\lambda$ = wave length of sound ($= c/f$)
$r$ = distance from the center of the pulsating sphere

The first factor in the above equation is the displacement produced by the far-field propagated wave. Note that the second factor, which is the correction for the near-field effect, is dependent on wave length and distance. At distances of less than one wave length, this factor becomes increasingly significant. Since wave length and frequency are inversely related, the near-field effect becomes of greater importance at the low frequencies.

In the near-field effect, the displacement amplitude varies approximately according to the equation (Harris and van Bergeijk, 1962)

$$d = A^2 D/r^2$$

for a pulsating sphere of radius $A$. The increase in the radius during pulsation is $D$, and the distance from the center of the sphere is $r$. It follows that at short distances the displacement is inversely proportional to the square of the distance (as opposed to the far-field effect, in which displacement is inversely proportional to the distance). It should be noted that the near-field displacement itself produces a pressure wave and that displacement and pressure are two representations of the same energy.

In the equipment used in the present study, the hydrophone was essentially a pressure receiving device and did not respond to water

TABLE 16

CALCULATED DISPLACEMENT AT THRESHOLD (IN ANGSTROM UNITS)

| | Frequency, in Cycles per Second | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 100 | 100[a] | 200 | 200[a] | 400 | 600 | 800 | 1000 | 1200 | 1600 | 2000 |
| *Holocentrus ascensionis* | 21 | — | 1 | — | 3 | <0.1 | <0.1 | <0.1 | <0.1 | 0.2 | 0.8 |
| *Holocentrus vexillarius* | 82 | — | 14 | — | 2 | 0.2 | 0.3 | 0.4 | 1 | — | — |
| *Haemulon* | 33 | 3 | 7 | 0.6 | 2 | 0.4 | 3 | 14 | — | — | — |
| *Lutjanus* | 1300 | 130 | 124 | 14 | 10 | 8 | 17 | 22 | — | — | — |
| *Thalassoma* | 207 | — | 44 | 8 | 3 | 2 | 4 | 7 | 10 | — | — |
| *Eupomacentrus* | 231 | 21 | 20 | 2 | 0.6 | 0.5 | 0.3 | 2 | 8 | — | — |
| *Epinephelus* | 15 | — | 1 | — | 0.5 | 2 | 2 | 9 | — | — | — |
| *Equetus* | 6 | — | 0.2 | — | <0.1 | <0.1 | — | <0.1 | — | — | 0.2 |
| *Prionotus* | 92 | — | 7 | — | 2 | 1 | — | — | — | — | — |

[a] These values are for the secondary low-frequency thresholds.

displacement *per se*. The acoustic pressures, as measured by our hydrophone, were produced entirely by the local water displacement of the near-field effect.

It is important to determine the displacement effect of the acoustic stimulus levels at the thresholds. This would be particularly significant for the secondary low-frequency curves, since we believe that these represent lateral line thresholds and since Harris and van Bergeijk (1962) have shown this organ to be primarily sensitive to near-field displacements.

Based on the equations for displacement and pressure given above, we can calculate the theoretical displacement amplitudes. For example, at a pressure of 1 microbar and a frequency of 100 cps. and for a distance of several wave lengths from the sound source, the displacement is approximately 1 A. ($=10^{-8}$ cm.). At distances of less than one wave length (for 100 cps. one wave length is 1500 cm.), the second factor, i.e., near-field, increases in value. At a distance of 1 cm., for example the displacement is about 240 A., and at 10 cm. it is about 25 A.

The threshold values, therefore, can be converted from pressure values to displacement amplitudes. One difficulty, however, is to determine the value of $r$ in the equation. On the basis of the behavior of most of the fish, we can assume an average distance from the sound source of 20 cm. Table 16 shows the calculated displacement amplitudes (in Angstrom units) for the average threshold values as obtained in the present study.

Jielof, Spoor, and de Vries (1952) stated that the resonant frequency of the lateral line cupular organ was about 100 cps. Kuiper (1956) found the highest microphonic output of a single cupula at about 75 to 100 cps. In a species of perch (*Acerina*) he determined the approximate threshold level of 25 A. as being the minimum displacement in the vicinity of the cupular hairs necessary for the production of a microphonic impulse. Harris and van Bergeijk (1962) estimated a value of 20 A. for *Fundulus*.

A comparison of the microphonic threshold and a response threshold is difficult to make. A large number of reacting cupulae may have some amplifying effect. There are certainly species differences in the number, distribution, canal enclosure, and sensitivity of the cupulae. The distribution of cupulae throughout the lateral line system may also act to pick up water displacements more efficiently. Lastly, the response of the animal in the avoidance apparatus involves perceptual as well as sensory factors.

What is the nature of the stimulus as received by the sense organ? In the human ear, a pressure wave impinges upon the tympanum, and it is clear that the received energy is in the form of pressure, since near-field displacement in the air medium would be negligible. Subsequently, however, this pressure wave must be translated and amplified into a displacement effect, because the sensory hair cells of the organ of Corti respond specifically to a mechanical deformation (von Békésy, 1960). The lateral line organs, according to Kuiper (1956) and Harris and van Bergeijk (1962), are directly displacement sensitive. As such, they can respond only to near-field effects when the displacement amplitude is sufficiently above noise level to be detected. The value of this noise level was calculated by Kuiper (1956). The displacement resulting from Brownian movement was given as about 3 A. (reported as 0.3 as a result of a typographical error). From table 16 it is evident that most of the displacement values below 400 cps. are significantly above the Brownian noise level. *Equetus* is a notable exception. However, only some of these values are significantly above the threshold limit of 25 A. as given by Kuiper (1956). In the higher frequencies, above 400 cps., none of the values approach Kuiper's threshold, and most give figures below his theoretical noise level.

It is certainly true that, like the human ear, the inner ear of a fish contains hair cells that respond to displacement. We are led to the conclusion, therefore, that, again as in the human ear, a pressure wave is received and converted into the endolymphatic displacements. The Weberian apparatus of the Cypriniformes can take the pulsations of the swim bladder and transmit these into the endolymph as do the middle ear bones of mammals. Harris and van Bergeijk (1962) suggested that the swim bladder can respond to pressure pulsations in the medium and convert these into a local near-field displacement effect. The studies of Dijkgraaf (1950) and Qutob (1960) indicate that the swim bladder may function as a pressure receptor. We suggest further that other structures, such as skull bones, vertebrae, and even scales, can produce significant displacements as a result of an impinging pressure wave, and, by means of lever-like or tension-spring arrangements, these displacements can be amplified or efficiently transmitted. Such displacements may then be of an order of magnitude large enough to produce a suprathreshold displacement of the endolymphatic fluid.

The data reported by Autrum and Poggendorf (1951) and Kleerekoper and Roggenkamp (1959) gave threshold values of below 0.001 microbar at 100 and 200 cps., equivalent to displacement values far below the level produced by Brownian movement. It seems hardly likely that the animals (*Ameiurus*) could detect a signal at a signal-to-noise ratio below unity. Harris and van Bergeijk (1962) provide at least one explanation for the data reported by Poggendorf (1952) and Autrum and Poggendorf (1951). The experimental tanks were very small and the area of the piston set in the floor of the tanks was large enough to produce a significant "jiggling" effect on the water and the fish. It is possible, therefore, that the fish were responding to water displacements and pressure changes produced in this way. The flat nature of the threshold curves obtained by this method shows an almost complete independence from frequency changes.

The report by Kleerekoper and Roggenkamp (1959) is not clear about the source of the control threshold data, as to whether these were derived directly from the Autrum and Poggendorf (1951) paper or were determined independently. Thus these data may be significant only in that they show a change in thesholds after lateral line and swim bladder damage.

The study of Dijkgraaf (1963) on *Scyliorhinus* used high-intensity sound at a low frequency, and the distance of the subject from the sound source was only about 5 cm. Thus the actual displacement effect may have been much greater than his sound-pressure figures indicated.

## TRAINING AND TESTING METHODS

All previous auditory studies on fishes have utilized either classical conditioning or instrumental reward training techniques, as defined by Hilgard and Marquis (1940) and Kimble (1961). In classical conditioning, the unconditioned stimulus is invariably presented to the subject whether or not he makes a response to the conditioned stimulus. Both beneficial (food) and noxious (electric shock) unconditioned stimuli have been used in a variety of test situations. Studies of audition in fish have used both types. Examples using food include the report of Autrum and Poggendorf (1951) and that of Kleerekoper and Chagnon (1954), and those using electric shock include the report by Froloff (1928) and that by Maliukina (1960).

In instrumental reward training, the reward is contingent on the response, i.e., on presentation of the conditioned stimulus, the subject must make the required response prior to receiving the reward. The reward can consist of food or the escape from a noxious situation. If no response is given, then no reward is delivered or the noxious stimulus continues. Many variants of this technique have been employed for sensory threshold studies: discriminative training, single trial measures, and response rate measures (Ash, 1951; Blough, 1956, 1958; Hilgard and Marquis, 1949). An example of the use of instrumental reward training for auditory studies in fishes is the report of Kritzler and Wood (1961).

The technique used in the present study was neither of the above. We have used what Hilgard and Marquis (1940) called "instrumental avoidance training," and evidently this is the only reported use of this technique in sensory investigations of fishes. Operationally, the technique consists of the paired presentation of a neutral and a noxious stimulus (electric shock). The avoidance response is generally preceded in the training sequence by escape training, in which the appropriate response simply turns off the shock. Since the conditioned stimulus is invariably paired with the unconditioned stimulus, the subject then learns that the conditioned stimulus is a signal for forthcoming punishment. The next step is for the subject to learn that an immediate response enables him to avoid the shock altogether. In the context of threshold determinations, once the subject has learned to avoid the shock by making the appropriate response to the conditioned stimulus, then the learned response becomes an objective index of the fact that the subject has received the stimulus. If a subthreshold stimulus is presented, then the learned response will not be made and the subject will be shocked and will make the learned escape response.

The processes involved in the acquisition and retention of the avoidance response have been and are focal points of theoretical controversy in psychology, and we avoid this area as being irrelevant to the purpose of this research. A general statement as to the theoretical basis of avoidance learning is, however, pertinent. The fundamental idea was expressed most simply and directly by Maier and Schneirla (1942), who stated that the avoidance response is the result of two stages of learning. The first is contiguity learning, in which the association of the conditioned and unconditioned stimuli is established by virtue of their pairing in time, and the escape response is basically the result of autonomic patterning. The second stage is a higher nervous process in which the subject learns to anticipate the unconditioned stimulus. This phase is termed "selective learning." Most recent reports have adopted this view of two-stage learning. Miller (1951), Mowrer (1961), Solomon and Wynne (1954), and others call the first stage in avoidance training the acquisition of a "fear" which later becomes transformed into an acquired drive.

In sensory threshold studies of mammals, three general avoidance procedures have been used. Culler, Finch, Girden, and Brogden (1935) described the method that used a leg flexion response by means of which the subject avoided a shock. Brogden and Culler (1937) used this technique for auditory studies. The second procedure has been the use of a rotating cage. Brogden and Culler (1936) described this method as one in which the subject had to turn the cage a predetermined amount in order to avoid a shock. The use of this method for auditory studies was reported by Ades, Mettler, and Culler (1939), and Meyer and Woolsey (1952). The third method, which is similar to the one used by us, was the shuttle box which required the subject to cross a barrier at the onset of the conditioned stimulus in order to avoid the shock. This method has been described by Warner (1932) and Horner, Longo, and Bitterman (1961), and its use for auditory studies in cats was reported by Butler, Diamond, and Neff (1957).

The avoidance response is a useful technique in psychophysical studies for a number of reasons, both theoretical and practical. It is superior to classical conditioning in that it is more properly an overt behavioral response, rather than being purely autonomic in origin. In our studies of teleost fishes, we have found that, once the optimal parameters of shock level and water level are known, most animals reach a 90 per cent response criterion within 150 to 200 trials (i.e., six to eight days). The responses are, for the most part, clear, unambiguous, and easily replicable. The resistance to extinction of an avoidance response has a great advantage in threshold studies, because numerous trials at suprathreshold stimulus levels can be run with little or no effect on the response. It appears that the avoidance response serves as its own reënforcer. Conversely, in trials at subthreshold levels, the additional escape training also aids in reënforcing the avoidance response. Although no actual comparisons of methods have been made, the threshold values obtained by the avoidance methods may be lower than those derived from positive reward techniques. According to the signal detection theory (Swets, Tanner, and Birdsall, 1961), experimental conditions can alter the results of psychophysical studies, particularly of sensory threshold determinations. In the avoidance technique, the animal is punished only at subthreshold stimulus levels, but is free to give positive responses ("false alarms") in the absence of a stimulus (viz., intertrial crossings). The subject, therefore, would operate as though it were primarily concerned with never missing an occasion when the conditioned stimulus is presented. One result is that the number of false positive responses increases, i.e., the number of such responses when no conditioned stimulus is present. In the avoidance situation these "false alarms" would be manifested by an increase in intertrial crossings. This increase was clearly shown by the observations reported here. A second consequence is that the threshold should be lowered, as compared to situations in which the subject attempts to keep the number of false positive responses to a minimum. The threshold shifts as predicted by signal detection theory may be expected to be small, in the order of 5 decibels.

In 1948, Dixon and Mood introduced a new psychophysical method for threshold determination which has come to be known as the "staircase" or "up-and-down" method. It is a modification of the classical method of limits. Guilford (1954) termed this method very efficient, particularly for locating threshold levels when there is no prior information as to the approximate stimulus intensities to be tested. Objections have been raised to the use of the staircase method in human studies because of the tendency in human observers to avoid repeating judgments, but it would seem that such an objection does not apply to lower vetebrates. Since the 1950's, this method has come into wide usage for animal sensory threshold determinations, particularly by psychologists influenced by B. F. Skinner. The method has been used with birds for the determination of visual acuity, dark adaptation, and color thresholds. The reports of Blough (1956, 1958) and Guttman and Kalish (1956, 1958) serve as examples of the utilization of the staircase method in sensory studies. However, we know of no comparisons between thresholds obtained by the staircase method and those obtained by the older and more standardized psychophysical methods, such as the methods of limits, average error, and constant stimuli.

## Relation of Sound Production to Hearing

Fish (1954) has shown that virtually any species of fish is capable of sound production. By her technique of electrical stimulation, sounds were elicited not only from organs specialized for sound production, but from general muscular contractions against a drum-like swim bladder. Our discussion is concerned, however, with the types of sounds for which there is evidence of some behavioral significance to the animals, whether the sound is produced by pharyngeal teeth, swim-bladder drumming muscles, or other mechanisms.

Sound production among the Cypriniformes is known in a few cases. Faint knocking sounds have been detected from minnows. Dijkgraaf (1932) described them in *Phoxinus*, and recent studies by Delco (1960), Winn and Stout (1960), and Stout (1960) have demonstrated the function of such sounds in *Notropis* as being related to species and sex discrimination. Klausewitz (1958) has described loud knocking sounds as part of aggressive behavior in a loach (*Botia*). Although the fresh-water bullhead (*Ameiurus*) is not known to produce any sounds, the marine ariid catfish (*Bagre* and *Galeichthys*) are known to be sonic (Burkenroad, 1931; Tavolga, 1960). In these forms, the sounds are produced by an "elastic spring" of bone attached to the swim bladder, and their occurrence appears to be related to nocturnal schooling (Tavolga, 1962). From what is known of the auditory sensitivity of this group of fishes, it is certain that the intensity and frequency of their sounds are within their range of hearing.

Most of the best-known sound producers have not been studied from the viewpoint of their auditory capacities. Recent reviews by Schneider (1961) and Maliukina and Protasov (1960) showed in general that few sonic species have been tested. Prominent in this category are the members of the Sciaenidae, the great choruses of which have been described by Knudsen, Alford, and Emling (1948) and by Dobrin (1947). These and other studies have established the role of sound production in sciaenids in spawning or pre-spawning behavior (Dijkgraaf, 1947a; Protasov and Aronov, 1960; Schneider and Hasler, 1960).

Nothing is known of the auditory system of the toadfishes, the powerful sound output of which has been investigated by Fish (1954), Fish and Mowbray (1959), Tavolga (1958, 1960), and Gray and Winn (1961), or of the codfish, of which the sonic capacities have been described recently by Brawn (1961). Of all the known sound-producing fishes in which the sound appears to have some behavioral significance, the only teleost in which auditory sensitivity has been tested, prior to the present report, is *Corvina* (Sciaenidae). In *Corvina*, as in most sciaenids, sound production is limited to the male and occurs during the spawning season. The sound, as described by Dijkgraaf (1947a), Shishkova (1958b), and Protasov and Aronov (1960), consists of low-pitched grunts or thumps, and occurs during feeding and in interindividual contacts as well as during breeding. As determined by Dijkgraaf (1949) the upper frequency limit of hearing in *Corvina* is about 1000 cps. Maliukina (1960) showed it to be considerably higher (1500–2000 cps.), and she also demonstrated thresholds of −45 decibels at 320 cps. and −50 decibels at 500 to 600 cps. It appears that *Corvina* rivals even the ostariophysines in its high sensitivity to sound.

Of the nine species reported upon here, *Holocentrus ascensionis* clearly has a low threshold and the broadest frequency response. This species is also one of the most vociferous sound makers in the Bimini area (Moulton, 1958). Although the sounds appear to be related to territorial activity, the exact behavioral significance of them has not yet been established (Moulton, 1958; Winn and Marshall, 1960). The sounds of the squirrelfish and of the groupers, as described by Moulton (1958), have their dominant frequencies in the range below 300 to 400 cps. Although no figures are available on the intensity of these sounds, they certainly fall in the sensitive range of hearing of most fishes in the area. The sounds of European codfish (*Gadus callarias*), as described by Brawn (1961), are apparently of high intensity and very low frequency—in the region of 50 cps. Certainly such sounds would be in the range of sensitivity of the integumentary and lateral line organs, and they may be of sufficiently high intensity to affect the inner ear as well.

In this connection, it is noteworthy that an auditory threshold has been reported for another gadid fish, *Gaidropsarus mediterraneus*, of almost −30 decibels at 740 cps., by Maliukina (1960).

Low-frequency, non-harmonic sounds are characteristic of swimming movements of fishes. These have been described as "swimming sounds" and "hydrodynamic sounds" by Moulton (1960) and Shishkova (1958a). Such noises are commonly produced by fishes, particularly during rapid changes in direction or speed. The predominant frequencies are below 100 cps. and are probably mainly subsonic. It is not likely that these sounds can be detected through the inner ear, but the lateral line and associated organs would be admirably suited for reception of such stimuli.

There is little information available on the intensities of fish sounds. Under laboratory conditions, Fish (1954) reported sound outputs of more than 30 to 40 decibels from toadfish and sea robins at distances of less than 3 feet. The sonic intensities of individual fish can rarely be accurately measured under field conditions, but it is well known that nighttime choruses of sciaenids and marine catfish produce an ambient noise level higher than 30 decibels (Knudsen, Alford, and Emling, 1948; Tavolga, 1960).

## ECOLOGICAL CONSIDERATIONS

A recent installation of under-water, sound-detecting equipment at the Lerner Marine Laboratory has brought out some data pertinent to the study of auditory sensitivity in fishes. Tavolga and Steinberg (1961) described a conference which took place in connection with this installation. A report describing the facility (Smith, 1961) has included figures on ambient noise in the Bimini area. Monitoring over a period of some months (more than 48-hour continuous time spans) has revealed that noise levels off the west shore of Bimini in 17 fathoms of water averaged 1.7 decibels (re 1 microbar) and ranged generally from 0 to 4 decibels. Occasional, infrequent, short-duration peaks were encountered up to about 16 decibels. These most intense sound pulses were presumably produced by some form of marine life, probably fishes, close to the hydrophone. Man-made noise from distant shipping increased noise levels by about 3 decibels, and vessels passing almost directly over the hydrophone produced sound pressures of over 30 decibels.

In another report on this installation, Steinberg, Kronengold, and Cummings (1962) stated that, aside from cetacean sounds, "most of the recorded sounds contain major frequencies below 1000 cps." Steinberg (personal communication) informed us that the usual ambient noise levels and spectra off Bimini do not differ significantly from those reported by Knudsen (*in* Albers, 1960), and that most of the ambient noise is produced by surface waves.

In the shallow water of Bimini Bay, Kritzler and Wood (1961) reported an ambient noise level of −14 decibels. Much of the inshore and shallow-water noise is caused by snapping shrimp and by air bubbles rising from the substrata. This is mostly high-frequency sound (over 2000 cps.).

Knudsen, Alford, and Emling (1948) gave ambient noise measurements for a number of localities. Their figures, here transposed to a reference level of 1 microbar, include Long Island Sound, where the noise level varied from −8 decibels on a calm day to +9 decibels on a rough one. Off Fort Lauderdale, Florida, the figures ranged from −1.5 to +10.5 decibels. Most sea noise was found to be in the frequency range below 1000 cps. Sound pressures from marine life, especially seasonally spawning fishes, increased ambient noise levels to about 35 decibels. A single boat-whistle blast from a toadfish went up to almost 30 decibels, but this was at close range.

Figure 24 is a graphic comparison of auditory thresholds and ambient noise spectra. Curve 1 is the audiogram for *Lutjanus*, and curve 2 represents the data on *Holocentrus ascensionis* as presented in this paper. Curve 3 is the spectrum of noise in the experimental tanks. None of the threshold values determined in this work falls below this noise spectrum. Curves 4 and 5 are taken from the data of Knudsen (as reported by Albers, 1960) and represent the ambient noise spectra for ocean waters at sea state 6 (12–20-foot waves) and sea state 0 (calm), respectively.

The significance of these data in conjunction with the available information on auditory capacities of fishes is that fishes are virtually deaf to ambient noise at frequencies higher than 1500 to 2000 cps. Possibly high-energy outputs from long-range, echo-ranging equipment may be detected, but it seems hardly likely.

Ambient noise in the 800–1500-cps. range would certainly be detected by Cypriniformes, but most other fishes would not hear this sound. Some forms, such as *Holocentrus* and *Equetus*, would be likely to receive this portion of the ambient noise spectrum.

The 300–800-cps. range of noise would probably be detected by most marine fishes, with the possible exception of forms such as *Lutjanus* or *Prionotus* which would be sensitive only to occasional high-level peaks more than 10 decibels in intensity.

Noise in the frequency range below 300 cps. is likely to fall within the range of detection of the integumentary and lateral line senses of most species. In consideration of the way in which threshold differences at these frequencies were manifested in the present study, it appears that low-frequency sound must have a very different perceptual value to the fish.

"Water noise" (produced by air bubbles, water currents, and so on), crustacean noises,

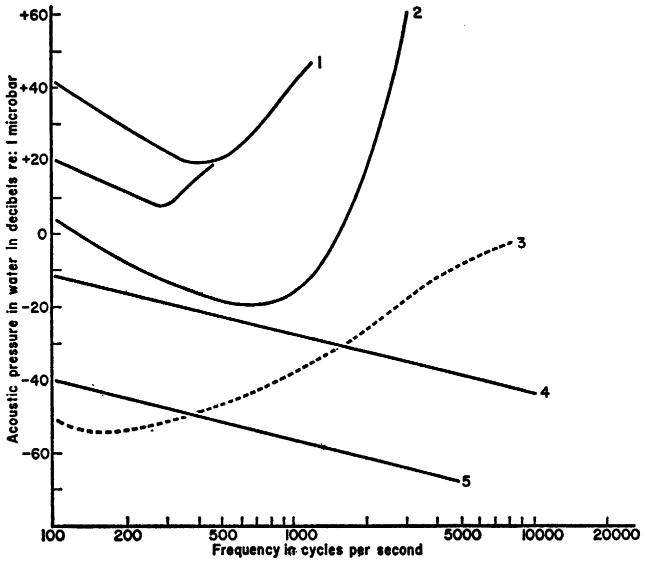

FIG. 24. Composite graph showing the auditory thresholds in *Lutjanus* (curve 1) and *Holocentrus* (curve 2) as shown in figure 23. These are compared to ambient noise levels and spectra. Curve 3 is the spectrum of noise levels in the experimental tanks used in the present report. Curves 4 and 5 are the spectra of sea noise (sea states 6 and 0, respectively) as based on the Knudsen curves given by Albers (1960).

and many stridulatory sounds of fishes are predominantly in the high-frequency range, and these would not be likely to form any part of the perceptual world of fishes. The noise produced by wave motion and the hydrodynamic shock waves from movements of fishes should fall into the range of sensitivity of fish hearing. Feeding sounds, and similar sounds produced by the gnashing or rubbing of jaw or pharyngeal teeth, are of low intensity and contain mostly high-frequency components. It is doubtful that these noises can be detected, even by ostariophysines, except in extreme high-intensity cases of fishes crushing shellfish, barnacles, or corals. Most fish sounds produced by swim bladder and associated structures are certainly within the frequency and intensity range of the highest sensitivity of most fishes tested. This fact is a remarkably good instance of the correlation of receptor and emitter organs.

The extremely high frequency, indeed the ultrasonic frequency, of the sounds of echo-ranging of cetaceans, as described by Kellogg (1961) and many other investigators, is well beyond normal detection by fishes. The echo-ranging clicks of cetaceans also contain a strong sonic component. Each click is actually a pulse of white noise. There is no published information on the exact spectrum and relative intensities of the various components of a cetacean click, but Sutherland and Dreher (1962) have reported measurements of up to 39 decibels re 1 microbar as sound pressures from echo-ranging sounds of captive porpoises. These same authors present spectrograms which indicate that the sound energy in the echo-ranging sounds at frequencies below 2000 cps. is a small percentage of the total acoustic pressure. The majority of cetacean whistles are at frequencies above 4000 cps. and at pressures below 20 decibels re 1 microbar. We conclude, therefore, that most marine fishes are probably not able to detect either the echo-ranging or the communicatory sounds of cetaceans. It is probable, however, that fishes can detect the low-pitched hooting and groaning sounds produced by some of the baleen whales (Mysticeti) (Schevill and Watkins, 1962). It is interesting to note that, in an apparently parallel situation, many nocturnal moths have evolved highly specialized ultrasonic detectors which enable them to escape the echo-ranging insectivorous bats (Roeder and Treat, 1961).

It is, of course, quite possible that fishes are capable of detecting high-frequency and high-intensity sounds through the general cutaneous sense. It is a well-established fact that the human ear can perceive frequencies far above the upper auditory limit of 17 to 20 kilocycles per second. The small difference in density between the tissues of a fish and its medium would be enough to enable a detection of even ultrasonic vibrations not only by surface neuromasts but by deeper receptor end-organs. Such a possibility would explain the results reported by Winn and Marshall (1960) of squirrel fishes that responded to signals up to 9000 cps., and by Rough (1954) of carp that reacted to frequencies of 22,000 cps. Extrapolation from the data obtained in the present experiment indicates that the acoustic pressures must be far above 50 decibels above 1 microbar for them to be sensed. Whether the reception of such high intensities constitutes "hearing" remains to be determined.

### LIMITATIONS OF THE PRESENT WORK

The avoidance conditioning method as used here severely limited the number of species that could be investigated. Thus far, both this study and that of Wodinsky, Behrend, and Bitterman (1962) have shown that any species of fish that can be trained to escape the shock by crossing the barrier will eventually learn to avoid the shock. Certain species are evidently not capable of solving the initial problem of escape. The following is a list of species, in addition to the nine reported here, that were capable of both escape and avoidance conditioning:

Sergeant major (*Abudefduf saxatilis*, Pomacentridae)
Night sergeant (*Abudefduf taurus*, Pomacentridae)
Striped parrotfish (*Scarus croicensis*, Scaridae)
Rainbow parrotfish (*Scarus guacamaia*, Scaridae)

The following is a list of species at Bimini in which neither escape training nor avoidance was found possible with the apparatus used:

Slippery dick (*Halichoeres bivittatus*, Labridae)
Bucktooth parrotfish (*Sparisoma radians*, Scaridae)
Frillfin goby (*Bathygobius soporator*, Gobiidae)
Spotted scorpionfish (*Scorpaena plumeri*, Scorpaenidae)
Queen triggerfish (*Balistes vetula*, Balistidae)
Cowfish (*Lactophrys quadricornis*, Ostriciidae)
Puffer (*Sphaeroides spengleri*, Tetraodontidae)
Porcupinefish (*Diodon hystrix*, Diodontidae)
Bahamas toadfish (*Opsanus phobetron*, Batrachoididae)

It is interesting to note that some species within the same families (Scaridae and Labridae) show a diversity in their behavior within this avoidance situation. In most cases, the reason for failure to condition certain forms was that their most frequent response to shock was to "freeze" and remain in a corner. Surprisingly, it was found that the plectognaths, as a group, were extremely sensitive to electric shock. Shocks up to 10 volts were required to get any sort of swimming response from these fishes, but, after a few repetitions, the subjects went into an immobile state and often died.

It is clear that, for many species, threshold determinations cannot be obtained with the avoidance technique. However, other objective methods such as classical conditioning or reward conditioning are available. In this connection, it would be of interest to try all the methods on the same species and see if threshold differences are produced by the use of different response criteria. It must be emphasized that in all this work we have been dealing with a response threshold as opposed to a sensory threshold, a distinction that has been described and discussed by Pollack (1961). For purposes of interspecies comparisons and ecological interpretations, this behavioral approach to the study of auditory capacities seems to us more useful than a physiological investigation of the properties of the receptor organs.

Another limitation of the present work has been that only single frequencies were used, and the sound was turned on abruptly, maintained throughout the trial, and abruptly terminated. Further experiments are indicated in which sounds of various harmonic and non-harmonic complexity could be used as the conditioned stimulus. Discontinuous bursts of sound, masking background noise, and discriminatory studies are some of the various studies to which this technique could be applied.

The equipment, particularly the underwater loudspeakers, limited the range of intensities and frequencies that could be tested. The effects of high-intensity, high-frequency sound could be tested. The extreme low frequency, subsonic, end of the spectrum still needs clarification. In this connection, extirpation techniques such as those used by Dijkgraaf (1949) and by Kleerekoper and Roggenkamp (1959) should be attempted in order to separate the two modalities that the fishes use at low frequencies.

# SUMMARY

SINGLE-TONE INTENSITY thresholds were obtained for nine species of marine teleosts. The following is a list of the species tested and their families:

- Squirrelfish, *Holocentrus ascensionis* (Holocentridae)
- Dusky squirrelfish, *Holocentrus vexillarius* (Holocentridae)
- Blue-head wrasse, *Thalassoma bifasciatum* (Labridae)
- Beau-gregory, *Eupomacentrus leucostictus* (Pomacentridae)
- Blue-striped grunt, *Haemulon sciurus* (Pomadasyidae)
- Schoolmaster, *Lutjanus apodus* (Lutjanidae)
- Red hind, *Epinephelus guttatus* (Serranidae)
- Slender sea robin, *Prionotus scitulus* (Triglidae)
- Cubbyu, *Equetus acuminatus* (Sciaenidae)

The technique was a modified shuttle-box utilizing avoidance conditioning. The animal was trained to swim across a shallow barrier upon hearing the test sound, and in so doing avoided a mild electric shock. The interval between the onset of the sound and the onset of the shock was 10 seconds. This technique resulted in clear objective responses to the test sounds. Most species took five to seven days, at 25 trials per day, to achieve a 90 per cent criterion of avoidance.

The test sounds were single-frequency tones produced by an under-water loudspeaker. The experimental tanks were soundproofed, and the sound pressures were monitored to an accuracy of ±1 decibel.

Once training was completed, the thresholds were obtained by lowering the sound pressure of the test tone with each successive avoidance in steps of 2 or 5 decibels. When the animal ceased to avoid, the sound pressure was raised at successive trials until avoidance recurred. By such alternative increase or decrease in sound levels, the threshold for that frequency could be determined. This is the so-called "staircase method" which has recently come into wide usage in sensory psychophysics. The threshold was calculated from these data as being the point at which a positive response can be expected 50 per cent of the time. Each animal was tested at several frequencies, and each point on the audiograms was replicated at least once for the same animal or other subjects of the same species.

For the nine species tested, there were a number of features in common. The lowest thresholds were in the 300–500-cps. range in most. The lowest thresholds in *H. ascensionis* and *Equetus* were almost an octave higher (600 to 800 cps.). The upper-frequency limits were about 1000 to 1200 cps. in all except *H. ascensionis*, in which the upper limit was 2800 cps. This species had the broadest frequency spectrum, with its highest sensitivity at 800 cps. (threshold value −24 decibels), and at 100 cps. it was +4 decibels. The highest sensitivity was in *Equetus*, with a threshold of −40 decibels at 600 cps. The species with the poorest sensitivity was *Lutjanus apodus*, for which the lowest threshold was +10 decibels at 300–500 cps. Other species exhibited intermediate values, and the shapes of the audiogram curves were all very similar.

In the testing of low frequencies, i.e., below 500 cps., some species showed a clear-cut change in sensitivity after considerable training and testing. All the data, especially for *Haemulon*, *Lutjanus*, and *Eupomacentrus*, pointed strongly to the existence of two systems of sound detection. We conclude that initially the fish used the less sensitive detector and, after additional experience, switched abruptly to a receptor at least 20 decibels more sensitive than before. On various morphological and behavioral grounds. this phenomenon is interpreted as the utilization of two sensory modalities: the inner ear and the lateral line. The lateral line system is known to be a low-frequency receptor and is particularly responsive to near-field displacement effects.

The generalizations and inferences that are drawn from this study are that marine fishes are virtually deaf to frequencies of more than 2000 cps., except for the possibility that such sounds exist in intensities verging upon the thresholds of pain receptors. The highest sensitivities are below 1000 cps. In a comparison of the characteristics of ambient sea noise with the audiograms of marine fishes, it becomes evident that fish are not sensitive to most normal ambient noise levels. The detection of low frequencies appears to be quite efficient to 100 cps. and lower, which lends weight to the idea that the lateral line functions as a short-range obstacle and moving-object detector.

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