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THE EFFECT OF EXPERIENCE ON THE DEVELOPMENT OF RESPONSES TO LOW-FREQUENCY SOUNDS IN THE LEMON SHARK (NEGAPRION BREVIROSTRIS)

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INTRODUCTION

The observations of spearfishermen that sharks frequently appear in the vicinity of speared, struggling fish (Hass, 1959) stimulated behaviorists to investigate the sensory factors that may be involved in eliciting approaches of sharks to potential prey. Low-frequency sounds produced by the struggling fish were suggested as the primary attractive stimulus in view of the rapidity with which the sharks appeared and the fact that their appearance from directions upcurrent eliminated olfactory stimulating substances from consideration. It was considered likely that these sounds were attractive because of their association with the presence of potential prey and that they are involved in eliciting attack behavior by sharks. Nelson (1967), Nelson and Gruber (1963), Banner (1968a,b), Myrberg (1970, 1971), Myrberg *et al*. (1969) all at the University of Miami undertook a series of investigations of low-frequency sound as an attractive stimulus, utilizing several species of sharks in field and laboratory studies.

I. Characteristics of Sounds Effective in Shark Attraction

The initial stages in the investigation of sound as a sensory factor in shark attraction were concerned with establishing conclusively whether sound could, in fact, attract sharks. Clark (1959) in an instrumental conditioning experiment observed that lemon sharks (*Negaprion brevirostris*) responded to the sounds of a submerged bell. Nelson and Gruber (1963) in a field study successfully attracted large sharks by playing back previously recorded sounds of a struggling fish. It then remained to establish whether there were differences in attractiveness of different types of sounds to sharks of various species. Further, it was necessary to specify the frequencies, temporal patterns (i.e. pulse length, repetition frequency etc.), and energy distribution of the sounds that proved to be most attractive.

Nelson and Gruber (1963) showed that pulses of noise with frequencies between 20 and 60 Hz attracted sharks while frequencies between 400 and 600 Hz did not. Non-pulsed noise in the 20 to 60 Hz range, on the other hand, was not attractive. Apparently, a pattern of irregular pulses was necessary for low-frequency sounds to be attractive to sharks. The sounds used by Nelson and Gruber were artificially produced by a noise generator, recorded on tape and played back through an underwater transducer. In spectral composition these sounds resembled the recordings they had made of a speared, struggling grouper (*Mycteroperca* sp.) in that most of the acoustic energy was distributed below 100 Hz. Myrberg et al. (1969) showed that sharks were attracted by irregularly pulsed, "overdriven," clipped sine waves of frequencies from 55 to 500 Hz and by bands of filtered noise in octaves from 25 to 1000 Hz. On the other hand, overdriven frequencies higher than 1000 Hz and pure tones of 55 to 1500 were not effective in attracting sharks although it is known that sharks are capable of detecting pure tones of low-frequency (Wisby et al., 1964). Wisby and his co-workers found that lemon sharks (Negaprion brevirostris) showed an unconditioned bradycardia when presented with pure tones of frequencies 7.5 to 100 Hz. The response consisted of a momentary slowing of the heartbeat and was more pronounced at frequencies between 7.5 and 40 Hz than at higher frequencies. Failure to attract sharks with pure tones might be attributed to a lack of biological significance for the sharks or to the possibility that pure tones may even be aversive since the bradycardia was also seen in severely frightened animals.

Nelson and Johnson (1970) attempted to compare the effectiveness of recorded, artificially-produced low-frequency sounds with "natural" ones in attracting sharks. The latter had been recorded from a group of bonefish (Albula sp.) that had been speared and stampeded in a small enclosure. Peak acoustic pressure was between 50 and 150 Hz. The artificial sounds consisted of octave bands of noise at frequencies between 50 and 200 Hz. Attraction was observed with both types of sound, but the data were insufficient to draw firm conclusions as to their relative effectiveness. The subjective impression of these workers was that the natural recorded sounds elicited slightly stronger responses than did the artificial ones.

II. Threshold Determinations

The studies cited above had shown that sharks could hear low-frequency sounds and be attracted by them. In addition, an indication had been obtained of what constituted an attractive sound. The next step was to specify with greater precision the true hearing ability of sharks. In order to measure the maximum sensitivity to sounds over a range of representative frequencies, Kritzler and Wood (1961) used approach-conditioning techniques on a bull shark (*Carcharinus leucas*). They found that the maximum sensitivity relative to spectrum-level ambient noise occured in the 400 to 600 Hz range. A graphic representation of such threshold intensities at various frequencies is termed an audiogram. To date, audiograms for only two species of sharks have been produced; one for the bull shark and one for the lemon shark (*Negaprion brevirostris*). Nelson (1967) found that lemon sharks could hear best around 40 Hz and were unable to hear frequencies above 640 Hz or below 10 Hz. However, some problems were raised by Banner (1972) in connection with the threshold values determined by Nelson. Before considering these problems it is necessary to discuss briefly the concept of thresholds; the characteristics of sound transmission in water and the effect of these characteristics on thresholds.

In their work, Kritzler and Wood (1961) defined the threshold as half the difference in decibels between the lowest intensity stimulus that evoked an acceptable response and the next lower intensity at which it did not. The animal was subjected in successive trials to signals of decreasing intensity but the decrease in number of dB per step was not specified. Nelson (1967) on the other hand, utilized a modified "staircase" method of arriving at thresholds. He presented sounds that decreased in intensity 5 dB at each successive trial. When the animal no longer responded, Nelson increased the intensity for the next trial. The lowest value at which an unmistakable response occurred was considered the threshold. The results represent an average of threshold values obtained in three ways: by approach conditioning, avoidance conditioning, and utilizing the "arousal response" exhibited in these sharks. Tavolga and Wodinsky (1965) have discussed the concept of thresholds in connection with their work on the auditory capacity of a teleost, the blue-striped grunt (Haemulon sciurus). They viewed a threshold as a statistical concept, i.e. as that stimulus-intensity level at which a positive response can be expected in 50 percent of the trials presented. They further pointed out that some measure of inter- and intra-individual variability of thresholds is necessary to arrive at more objective means of establishing sensory capacities. The shark audiograms were obtained using only one or a few animals while Tavolga and Wodinsky successfully obtained audiograms on 18 individuals. With this larger number of subjects, Tavolga and Wodinsky were able to state reliably the limits of accuracy of the thresholds they obtained and also to identify the sources of variability.

There are two components of a sound propagated in water that are of importance in threshold determinations with aquatic animals. One is pressure fluctuation and the other is particle displacement. The effects associated with particle movement attenuate with distance from the source much more rapidly than do those associated with pressure variation. The distance from the source at which the attenuation of displacement equals the attenuation of pressure is defined as the limit of the near field and is generally accepted by most investigators as one sixth of a wavelength. Beyond this, in the far field, pressure and displacement attenuate at the same rate. For a dipole source under ideal conditions, the rate of attenuation is the reciprocal of the distance from the source (Harris and van Bergeijk, 1962). At distances of less than a wavelength, particle motion of large amplitude is present and is of a relatively greater magnitude than are the effects of pressure variation. For this reason, if thresholds are taken with a pressure detector close to the source, the measurements of signal intensity obtained will underestimate true signal intensity. Banner (1972) re-examined the data obtained by Nelson (1967) and found that the signal intensity values of thresholds had not been corrected for the near-field effect. With these corrections the lowest signal intensity was found to be 20 dB, rather than 12 dB, above spectrum-level ambient noise.

The importance of the near-field effect is evident in the threshold determinations of young lemon sharks made by Nelson (1967). The distances involved were less than a wavelength so that large-amplitude particle motion components of greater magnitude than pressure components must have been present in the signal and therefore have been detected by the sharks. If it is true, as has been stated by Harris and van Bergeijk (1962), that the lateral line and inner ear sensory apparatus are particle-motion detectors, then the component of the signal relevant to the sharks was not measured by the pressure hydrophone used as a signal monitor. The signal intensity given as the threshold by Nelson must have been lower than that actually detected by the sharks.

At close range it is technically very difficult to measure pressure and particle motion independently. Nevertheless, Banner (1967) attempted, in the laboratory, to obtain separate, independent measurements of pressure and particle motion in the course of determining thresholds of lemon sharks. He used pressure hydrophones and a specially constructed accelerometer that measured particle motion. From his analyses, Banner concluded that the sharks had responded to displacement effects rather than pressure, but he did note that sensitivity to pressure was not ruled out.

Even though sharks do not have a gas bladder that can act as a pressure transducer, they may be capable of detecting high-level sound pressure of intensities that could be encountered in the near field. A mechanism by which this may occur was outlined in a recent paper by Tester et al. (1972) who described the morphology of the ear of three species of *Carcharinus* and noted the presence of an oval, membrane-covered perforation in the chondrocranium, the *fenestra ovalis*. This structure is in close proximity to a duct that connects the sacculus with the posterior semicircular canal in which the *macula neglecta* is situated. The macula neglecta, as well as the sacculus macula, have been shown by Lowenstein and Roberts (1951) to be extremely sensitive to vibration in the ray (*Raja clavata*). Since the endolymphatic ducts connected with the sacculus open to the exterior by means of the endolymphatic pores in the skin, the suggestion was made that these ducts, together with the fenestra ovalis, comprise a sound-conducting mechanism directed at the macula neglecta and the sacculus macula. Details of the operation of this postulated mechanism await further investigation.

In a further attempt to assess the relative magnitudes of pressure and particle-motion effects, Banner (1968) measured the particle velocity of ambient noise in shallow water (.25 to .45m in depth) by using a refraction geophone as a velocity detector. Velocity was used because it is independent of frequency whereas displacement is a decreasing function of frequency. Banner compared velocity measurements taken with the geophone with pressure measurements taken with the hydrophone over a range of frequencies of 20 Hz to 320 Hz. Mean spectrum-level noise measurements of velocity with the geophone were 30 dB above pressure values at 20 Hz and this difference decreased as the frequency increased. Banner was thus aware that through its masking effects, ambient noise would have considerable influence on thresholds of young lemon sharks in their typical shallow-water habitat and would decrease the distance from a source that sharks could detect a given sound. Velocity measurements with a velocity detector such as a geophone must be viewed with caution, however, because geophones are extremely directionally sensitive and even slight movement of the instrument will give widely variable readings. It is very difficult to mount such an instrument with sufficient stability to prevent movement and at the same time avoid picking up extraneous vibration through the mount. Moreover, velocity measurements are affected by the direction of particle motion which, in turn, is affected by water depth. In shallow-water layers, motion is primarily vertical (Banner 1972; Schuijf et al., 1972) while in deep water, particle velocity is primarily in the radial direction, and this complicates the comparison of field studies done in water of different depths.

It is apparent from the preceding considerations that truly accurate measurement of the hearing ability of sharks is extremely difficult because of the intervening factors of ambient noise, complications due to the characteristics of sound transmission in shallow water, and the lack of a satisfactory displacement transducer to measure near-field effects. The audiograms produced for sharks have not yet approached the accuracy of those produced for teleosts and so must be regarded as provisional. Nevertheless, it has been clearly established that sharks could be attracted by certain types of low-frequency sound and this calls for further investigation of the behavioral responses of sharks to such sounds.

III. Behavioral Responses to Low-Frequency Sounds

The behavioral responses described by Nelson and Gruber (1963) consisted simply of a direct approach by the sharks which swam in a straight line toward the transducer and veered away at distances from 1 to 12 m. The sharks then either departed from the area or circled near the limit of visibility. This behavior was observed in mature sharks of four genera, *Carcharinas*, *Sphyrna*, *Negaprion* and *Galeocerdo*. In immature lemon sharks, Banner (1968a) observed a somewhat different type of response. When recordings of broad-band pulsed noise were played to them, these sharks approached in groups of two to six, increasing speed as they neared the transducer, and then they circled it actively. The sharks remained in the area for as long as three minutes or until the sound ceased, and on three occasions the transducer was bitten. This contrasts with the observations of Nelson and Gruber (1963) who never observed an attack and found that the sharks did not remain in the area long after the initial presentation of sound.

These differences in behavior between adult and immature sharks may possibly be due to the inexperience of the young sharks. Nelson (1969) noted that among predatory animals caution tends to increase with maturity and experience. Young predators learn to recognize which prey animals are too large, powerful or dangerous to be overcome successfully and learn to attack the weak, young, and injured, all of which are more easily captured. Nelson noted that young sharks typically display less caution toward a diver than do older individuals of the same species. They come closer and are generally the first to approach speared fish or baited hooks. Young sharks might, therefore, be more likely than adults to bite an inappropriate object such as the transducer but this likelihood might be influenced by the level of excitement and the number of sharks present. Nelson et al. (1972), on an occasion when an exceptionally large number of sharks (35 in a 3-minute period) was present, did observe adult sharks biting and striking a transducer. These investigators believed that the high level of excitement was caused in part by some kind of social facilitation.

In another field study, Banner (1972) observed that young lemon sharks were attracted by a large number of recorded, biological sounds. Positive responses consisted of an approach to the transducer and an arc, circle or repeated circles around the transducer. This behavior was similar to that observed in previous studies, but Banner did not report any attacks on the transducer in these experiments.

IV. General Observations on Sharks

In the preceding sections I have discussed the relationship of sound to shark behavior and some of the special problems associated with studies on underwater sound. Now, a more general consideration of the habits of sharks may provide a background that will help give some perspective on the possible role of sound in the life of the shark.

A. Population Structure and Distribution

A large portion of the data that relate to the distribution of the more than 250 species of sharks has been obtained from commercial fishery statistics. For this reason information is more abundant in species that are now or have been commercially valuable. The Family Carcharhinidae includes many large species that were formerly sought for their liver oil and some that are still commercially valuable and so data on this family are more available than on most other families of sharks.

As a group, sharks are widely distributed all over the world with only a few species being restricted to a given area. An example of the latter is Carcharinus galapagensis, the Galapagos shark, which seems to be found only near oceanic islands (Garrick, 1967). Even though many species are widespread, local populations of a given species may be identified and Springer (1967) has compiled data on shark populations (chiefly carcharhinids) of inshore waters along the east coast of the United States. Populations of sharks in these waters have strongly developed migratory patterns and from his data Springer has constructed an hypothetical population that outlines the main features of the distribution of species found on the East Coast. Individual species, of course, may deviate more or less from this hypothetical construct. The following account is paraphrased from Springer (1967).

In the hypothetical population, young are born in the spring or early summer on nursery grounds in shallower water than is normally frequented by adults. Adult males rarely if ever visit nursery grounds and adult females do so only if gravid and remain in the grounds only long enough to give birth. Adult females are inhibited from feeding while in the nursery grounds but may resume feeding shortly after their departure. The young remain in the nursery area while feeding and growing to sexual maturity unless forced to leave by extremes in seasonal temperature changes. In this case the young may form migratory aggregations composed of individuals of both sexes of approximately the same size. The extent of the migration of such aggregations is generally much less than that of adult groups, and the young are usually not found near the adults of the same species. Adults and young may be separated geographically or may occupy different depths in the water column.

The adults segregate by sex for migration. Aggregations of males stay in deeper, cooler water than do aggregations of females. These migrating groups of adults may cover great distances. Adult males move inshore to mate in the spring. The water in these inshore areas, especially along the Gulf coast, may be cooler than more outlying waters due to temperature inversions. Springer believes that this temperature factor may be involved in bringing the sexes together in mating areas.

In this hypothetical population, there is spatial separation between mature males, mature females and between adults and young. In those species such as the sandbar shark (Carcharhinus milberti) that have an extensive geographical range, the difference between the ranges of adults and young may be considerable. At terminal points on migration routes, the aggregation of adults may not be distinct except that males do not enter nursery areas. The nursery areas of some of the better-known species such as the bull-shark (Carcharhinus leucas) are well defined. Bull shark nursery areas are found in brackish water estuaries such as the mouth of the Mississippi River on the Northern Gulf of

Mexico and in estuaries along the coast of the Carolinas.

A population structure that does not conform to Springer's hypothetical construct is seen in the marbled catshark (Galeus arae), a small species of the family Scyliorhinidae. Bullis (1967) studied sex and maturity relationships in this species and showed that a population in the Caribbean had four distinct characteristics: Adult males were distributed evenly over the depth range of the species (160 to 400 fathoms); immature and adolescent males were restricted to the shallow end of the depth range; mature females were concentrated in the middle to deeper parts of the depth range; and immature and adolescent females were restricted to the middle depths. These data were obtained from catches taken in depths of 200 to 320 fathoms. Bullis stated that the distinctive characteristic of this pattern of distribution is the sexual segregation of the immature and adolescents rather than of adults. Further, he hypothesized that gravid females do not move into nursery areas to give birth but stay in the middle to deeper strata and that the newborn pups swim up to shallower depths on their own. Apparently, the young females segregate and move into the middle depth ranges which is their area of maximum abundance thus leaving the young males to continue on upward.

Bass et al. (1973) have described the distribution and population structure of Carcharhinus leucas, the bull shark, off the coast of Natal, South Africa. Their data are based on the results of a tagging study conducted along the coast and in a saline lake connected with the sea by a channel. The habit of bull sharks to invade fresh water in such places as Lake Nicaragua (Thorson, 1971), the Zambesi R. (Bass, et al., 1973), and the Mississippi R. (Springer, 1967) is well known. A characteristic not previously mentioned in the literature but documented by Bass et al. (1973), was the occurrence of C. leucas in hypersaline water. Lake St. Lucia is an H-shaped body of water 40 km long and 20 km wide connected with the sea by a narrow channel 20 km long. During droughts the waters of this lake may reach salinities of 100‰, because evaporation rate exceeds the inflow of fresh water from rivers entering the system. The results of the tagging study indicated that C. leucas in the lake may be found in salinities of up to 50‰, but these sharks apparently cannot tolerate higher salt concentrations. Normal salinity in sea water is approximately 35‰.

The sharks found in the St. Lucia system were predominantly juveniles and those adults present were gravid females and these were captured not far from the sea. Individuals caught in the estuary and narrow channel had open or newly-healed umbilical slits and were 60 to 90 cm long, thus indicating that the St. Lucia system is a nursery area. The pattern of distribution that emerges here is that the young are born in the estuary and narrow channel and subsequently move into the saline lake where they feed and grow. The larger individuals in the lake then presumably move back through the channel, out to sea and become sexually mature.

Some sexual segregation is present in this population. Animals in the channel that were less than 90 cm long were predominantly males. The larger sharks (more than 100 cm) caught in the same area were predominantly females. In the lake proper there were approximately equal numbers of both sexes of larger sharks. The number of small sharks found here was so small that Bass and his co-workers could not determine whether they were sexually segregated.

Seasonal variation in abundance may be present in this population, but Bass stated that the data did not permit definite conclusions. There were some indications that a greater number of small sharks (90 cm and less in length) were present in summer than at other seasons in the St. Lucia system. In the sea off Natal on the other hand, no seasonal trends were observed. It is possible that these populations of C. leucas differ in migratory tendency from those observed by Springer (1967) off the Florida and Carolina coasts.

In their monograph on the genus Carcharhinus, Bass et al. (1973) referred to an earlier tagging study that concentrated on C. obscurus, the dusky shark. In the Indian Ocean off the east coast of southern Africa this species is definitely migratory. Recovery of tagged individuals showed that some of them may travel at speeds as high as 31.9 miles per day although the average of migratory sharks was between 3 and 10 miles per day. The nursery area of C. obscurus was apparently located along the southern Natal coast where the smallest sharks (80 to 90 cm) were found in approximately equal sex ratio. Gravid females, or those that had recently given birth, were also found in this area. Larger immature sharks tended to move out of this area in groups consisting predominantly of females migrating northward into northern Natal and southern Mozambique waters and predominantly male groups moving southward to southern and southeastern Cape coasts. The mating area appeared to be located in the Mozambique channel since this is the principal range of the adults of both sexes. Moreover, females with early embryos were found slightly to the south in northern Natal waters, thus indicating that mating may have taken place in Mozambique waters. Bass considered remarkable the fact that while sexual segregation occurred in immature individuals in this species, it was not complete, that is, those groups occurring in northern waters contained two-thirds females and one-third males while those in southern waters had two-thirds males and one-third females.

A recent paper provides some data on a little-known, large species of non-carcharhinid shark. Gubanov (1972) conducted a long-line survey in two areas of the northwestern Indian Ocean in which he concentrated on Alopias vulpinus, the thresher shark. This species inhabits waters less than 5000 m deep and tends to be concentrated at levels from 180 to 265 m in these waters. No sharks were caught at levels above 90 m so Gubanov discounts reports that thresher sharks leap out of the water while feeding (Coles, 1915). However, Limbaugh (1963) reported the occurrence of small specimens of this species (4-6 ft long) off La Jolla, California. One was observed to have jumped out of the water but most were seen at depths of 60 to 90 ft. This shark also exhibits sexual segregation. Gubanov noted that the catches of sharks in the area off the east coast of Somalia that were obtained during the period of January through May consisted of 93.8% females. Of these animals 93.3% were gravid. On the other hand, the catches obtained in the second area of study, near the Maldive Islands, consisted of only 21.3% females and only one was gravid. It is reasonable to assume then, that the region off the eastern coast of Somalia is a nursery area. The pattern of vertical distribution tends to reinforce this conclusion. The sharks off Somalia inhabit shallower water than those near the Maldive Islands and so it is concluded that females in the former area ascend to the upper layers to give birth.

Springer (1950) has provided some information on the distribution and population characteristics of the lemon shark (Negaprion brevirostris). This species has not been caught at depths greater than 50 fathoms and is most often encountered in shallow waters. It is found in the Caribbean region, the Gulf of Mexico especially off South Florida, and it may occur in the Atlantic as far north as Chesapeake Bay. It also occurs in the tropical eastern Pacific. Young lemon sharks are more abundant in shallow water than adults and are taken in water less than 10 fathoms. The waters of the Florida Bay region appeared to be the primary nursery area, since new-born pups and gravid females containing full-term embryos were taken here. Adults are found in groups of predominantly one sex but the segregation of the sexes is much less clear-cut than in other carcharhinids such as C. milberti. Springer (1950) did not state whether sexual segregation occurs in young lemon sharks.

McLaughlin and O'Gower (1971) have carried out a comprehensive study on the Port Jackson shark (Heterodontus portusjacksoni). This species is a member of the family Heterodontidae, known as the horn sharks, which is among the most primitive groups of sharks alive today. A comparison of distribution and population structure of this shark with that of the more advanced carcharhinids should be interesting.

The Heterodontidae are all oviparous sharks, less than 1.5 m long and are primarily inshore forms. McLaughlin and O'Gower (1971) studied a population in the Sydney area over a period of 5 years. They utilized SCUBA apparatus to make underwater observations and also carried out a tagging program. Their results indicated that there is a well defined breeding season which starts in late July and early

August with the influx of mature females accompanied by some males on to inshore reefs. Mating probably occurs at this time. Breeding females remain on the reefs in sheltered areas such as caves, deep fissures, and trenches. Most mature males remain in deeper water during this time. Females deposit 10 to 16 eggs, mainly during August and September, on shallow, sheltered reefs in depths of a few meters. The eggs are anchored by tendril-like flanges on the egg cases that catch or hook in rock crevices and fissures and this is where the embryos develop. Young sharks emerge from the eggs after approximately one year and move into nearby bays and estuaries that apparently are used as nursery areas. The young do not tend to remain on the hatching grounds after emergence from the egg. The juveniles remain in the nursery area in mixed groups of males and females but some may migrate to deeper water in summer. At the onset of sexual maturation, juveniles move to deeper water and segregate into groups of males and females to form a separate adolescent population. After several years, upon attaining sexual maturity, these sharks become part of the adult population.

Adult males depart from inshore waters at the end of the breeding season in September and October. Migrating adults may either move southward along the coast or move offshore into deeper, cooler water. Some sharks may return to the reefs in March or April but no significant numbers arrive until the beginning of the breeding season in late July.

In comparison, it seems that the breeding season of the carcharhinids reviewed here is less sharply defined than that of Heterodontus portusjacksoni. Springer's hypothetical carcharhinid population breeds in the spring or early summer, a time span of 4-5 months, in which the number of females giving birth may vary widely. In the Port Jackson shark, breeding activity reaches a peak rather suddenly in the interval of 2 months and then drops off quite abruptly. In both the Port Jackson shark and carcharhinids, the juveniles are not sexually segregated while on the nursery grounds. At the onset of sexual maturity in both groups, the sharks segregate into male and female populations which are spatially separated from each other and from the young.

In contrast to carcharhinids, the Port Jackson shark forms separate populations of adolescent sharks that are sexually segregated. Distinguishable adolescent populations may be due to the slow growth rate and prolonged period of adolescence of the Port Jackson shark.

In summary, it seems that sexual segregation is the rule in shark populations. In some, it is the adults which are segregated and in others, the young and immature. Segregation of the young from adults seems also to be the case in most, if not all, species. As large sharks tend to be the chief predator of small sharks, this appears to be an adaptation that serves to prevent cannibalism (Gubanov, 1972). The habit of young sharks to form aggregations of one size may also be an adaptation that prevents cannibalism. If the sharks in a population are all of the same size, then swimming speed, jaw size, and the capability to inflict damage would all be similar so that any one shark, should it be attacked, would be capable of either escaping or defending itself as effectively as any of the others.

The factors responsible for migratory movements of shark populations have not been definitely determined, but seasonal temperature changes seem to be involved. No evidence exists on the effect of photoperoid changes on migratory movements. Salinity variation does not seem to be a significant factor. In females, reproductive state may in some way provide endogenous stimuli that causes them to migrate to nursery areas. The extent to which a given species migrates may vary according to geographical area. Apparently, C. leucas is much more strongly migratory in Atlantic and Caribbean waters than in the Indian Ocean off the east coast of Africa.

B. Social Behavior

1. Schooling or Aggregating Behavior

It is doubtful whether sharks form schools of the same type as seen in bony fishes, that is they do not form a group of fishes swimming closely together in a specific spatial arrangement with regular motion. Most accounts of shark aggregations merely note the presence of large numbers of animals that appeared to form a cohesive group but nothing has been mentioned about coordinated, specialized behavior (Springer, 1967). Many of these aggregations were assumed to be migrating. A report of somewhat unusual behavior was cited by Springer (1967). The observer was watching an aggregation of over 200 sharks, 2 to 4m in length from a helicopter. One group of 15 to 20 sharks in this population formed a head-to-tail circle of about 50 ft diameter and milled about for 15 or 20 minutes.

Stead (1964) in his discussion of the school shark (Galeorhinus australis) noted that this species is almost always encountered in large groups. He noted the experience of handline fishermen having their bait stolen and hooked fish taken by large bodies "of these ravenous little monsters." Stead further noted that a close relative of G. australis, the soupfin shark (Galeorhinus zygopterus) of American waters also occurs in large aggregations. These sharks could be caught in large numbers in one locality at one period and this may be the reason that the fins and oil of this species were exploited commercially rather than any intrinsic superiority of these products.

Nurse sharks (Ginglymostoma cirratum) form aggregations, sometimes of 10 to 20 individuals, in which they lie on the bottom in close contact. They may lie with snouts on one anothers' flanks or pectorals or with heads and tails together (Lineaweaver and Backus, 1970).

The factors that cause sharks to aggregate are not known but many aggregations may be the result of the individual sharks all responding to a localized environmental stimulus. Other aggregations may be caused by some type of mutual attraction between members of the species group such as the nurse sharks and are not primarily caused by environmental factors.

2. Dominance Relationships

Allee and Dickinson (1954) investigated the possible occurrence of dominance-subordinance relationships in the smooth dogfish (Mustelus canis). They noted that dominance hierarchies occur in all classes of vertebrates except the Agnatha, Amphibia and Chondrichthys. Social dominance orders in the Class Osteichthys are well-developed and so Allee and Dickinson stated that the stimulus for their investigation was the "tension" between lack of information and the logical expectation of the existence of social dominance orders in cartilaginous fishes.

Allee and Dickinson conducted observations on 16 individuals of different sizes in a concrete tank 4 m square. During observation periods they recorded the activity of the animals and attempted to determine the frequency with which each shark avoided contact in head-on approaches to other sharks. The problem of distinguishing avoidance turns from spontaneous, casual turns was noted and it is obvious from the description of the observations that considerable subjectivity exists in their method of collecting data. Nevertheless, Allee and Dickinson stated that the smaller sharks avoided the larger ones more often than the reverse. They gave data on the smallest size difference that is associated with avoidance by a smaller shark as a 6.7% difference for small animals and a 7.4% difference for the largest animals of the size range.

These workers were unsuccessful in their attempts to promote active competition between sharks for food morsels. Even after starvation no competition was observed but the sharks became "increasingly aggressive in their search for food and as a result, activity in the tank increased markedly."

Clark (1963), in reporting on some peripheral observations during an instrumental conditioning study on lemon sharks, noted that there was a suggestion of dominance in the relationship of the two test animals, a male and female. It seemed that the female always deferred to the male in obtaining the food reward. The data were inadequate to determine whether this was related to sex and since both animals were the same size, about 3 m long, dominance due to a size difference could be eliminated as a cause. Adult male and female lemon sharks as a rule are about the same size in contrast to some other carcharhinids, such as C. milberti, in which the female may be larger (Springer, 1950, 1960).

During the mating season in many sharks, the male may inflict wounds on the female during courtship, presumably to enlist the cooperation of the female in copulation (Springer, 1967) but in non-breeding situations there is scant evidence to indicate a dominance relationship based on sex.

Limbaugh (1963) noted in field observations on shark populations of Clipperton Island that there was a "nip order" among the Galapagos shark (*Carcharhinus galapagensis*), the silvertip shark (C. *albimarginatus*), and the blacktip shark (C. *limbatus*). "The larger sharks within a species dominate the smaller ones. Among the sharks of nearly the same length, the whitetip reef shark [silvertip] very clearly dominated. The Galapagos shark dominated the blacktip shark, which seemed almost afraid of its own shadow." Limbaugh did not indicate what the behavioral criteria for the definition of dominance were, nor did he describe typical encounters between sharks in which evidence of dominance was manifested.

Springer (1967) noted what appeared to be interspecific dominance in a mixed population of sharks feeding near a vessel at the surface. These individuals were all of about the same length but when a whitetip (*Carcharhinus longimanus*) and a silky shark (C. *falciformis*) attempted to seize the same morsel, the silky shark gave way. If the competition was between two whitetip or two silky sharks neither would give way and "some physical contact and buffeting resulted." The criterion here apparently was that a subordinate shark would defer to the other shark in competing for food. Springer also observed that hammerhead sharks have a special status because sharks of other species tend to stay at a distance from them even if there is no great size difference.

In general, the evidence on the existence of social orders such as dominance hierarchies or dominance-subordinance relationships is not convincing. The existing evidence is not substantiated and needs further corroboration. The type of social interaction observed in some of the studies cited may be different from that observed in some groups of Osteichthyes and may represent a lower level of biosocial organization.

C. Reproductive Behavior

Accounts of mating behavior directly observed in sharks are very scarce in the literature. One such account is furnished by Clark (1963) in which a male and a female lemon shark were observed copulating while in a restraining enclosure. This incident was first observed at midnight and the sharks were described as swimming in perfect synchrony slowly around the enclosure in counterclockwise circles with the posterior half of their bodies in close contact. In fact, they were so close that Clark (1963) described their appearance to be that of "a single individual with two heads." The animals apparently remained *in copula* the rest of the following day and all through the night. Subsequent observations in the ensuing week were made but no further copulation was seen. On one occasion, however, the male was seen to sink to the bottom in a curled position and remain there for up to 4 minutes. While in this position the left clasper was visible and had an enlarged and slightly pink appearance. Another shark in which copulation has been observed is the horn shark (Heterodontus francisi). Dempster and Herald (1961) reported this incident which occurred at the Steinart Aquarium in a display tank. In this case and in all the copulations observed subsequently, the right clasper was used exclusively. The male bit and held the female by the left pectoral fin and curved his body over the female's back ahead of the second dorsal fin. Using the second dorsal spine as an anchor, the male inserted the right clasper into the cloaca of the female and made rhythmic motions with the middle part of his body during the entire time of copulation, that is, about 35 minutes. Bolau (1881) described mating behavior in Scyliorhinus caniculus. He observed that the right clasper was used and upon examination of the male after copulation found that the right clasper was swollen whereas the left clasper was of normal size. In this species there appears to be much greater bodily contortion than in H. francisi. The male more or less wraps himself around the female at her cloacal region with his ventral aspect uppermost, slightly twisted, with his head laid over his own caudal peduncle. Copulation in this species lasts about 20 minutes during which the female lies quietly. The respiratory gill movements of the male rise in frequency from 38 per minute initially, to 56 per minute at the end of copulation.

From these few observations it is apparent that there is great variability in the duration of copulation, up to 36 hr in the large carcharhinids, and around a half hour in the smaller heterodontids and scyliorhinids. Use of a single clasper at a time seems to be the rule in all three of the species cited and there appears to be a right or left preference in the clasper used. However, observations on so few species do not permit generalization to all sharks in regard to these characteristics. Moreover, the conditions of captivity may have resulted in mating behavior which differs from that normally exhibited in the natural habitat. Nevertheless, the occurrence of certain behavioral tendencies in such widely separated groups as carcharhinids and heterodontids may indicate that they are typical.

V. Experiential Factors in the Development of Feeding Responses

A. Elasmobranchs

Although the feeding of sharks on large prey, including man, has fascinated people for centuries, no accurate observations were ever made until recently.

Moss (1972) examined the feeding mechanism of carcharhinid sharks and noted observations on typical feeding behavior of adults in this family. "Relatively unmotivated sharks tend to circle intended food objects slowly for a considerable period of time before feeding. A pass is finally made directly at the prey which may be first bumped with the snout. Hard, inanimate objects encountered in this fashion usually elicit no further response. Whole or cut fish are attacked following a bump; sometimes on the same pass, but usually after a rapid circle . . . highly motivated sharks . . . tend to circle the food fewer times and swim faster with more erratic motions. Bumping behavior is not often observed . . . rather the approach is direct and rapid, the jaws are often gaped prior to reaching the food and the animal often swims in a stiff, jerky manner with the gill covers and jaws flexing spasmodically." Moss also observed that whenever a food object is taken into the jaws, the animal shakes its head from side to side and swims rapidly away.

A sequence of feeding motions that differs from the pattern in carcharhinids has been found in Scyliorhinus caniculus, a small species in the catshark family Scyliorhinidae. Kalmijn (1971) observed the behavior of adult individuals in experiments in which he tested their ability to detect bioelectric potentials that are produced by prey animals. The prey in these experiments was a plaice

(Pleuronectes platessa) covered over with an agar plate and buried in the sand. Although this arrangement removed mechanical, visual and chemical cues, the shark was nevertheless able to direct an accurate attack on the buried plaice. These and other experiments indicated that the ampullae of Lorenzini were the sensory receptors involved in the detection of weak electric fields generated by the physiological activities of the prey and that an attack could be elicited by this stimulus in the absence of any other stimuli. The character of the attacks elicited by electrical stimuli alone clearly showed that the shark was able to localize the source of the electrical field. The response to prey without a covering agar chamber consisted of a sudden, smooth, well-aimed turn toward the prey from a distance of 15 cm or less. When directly over the buried plaice, the shark removed the sand over the prey by sucking it up and expelling it through his gill slits. Then the shark took the plaice between his jaws and swam away, "wildly shaking his head to tear the prey to pieces small enough to swallow." The response was identical in tests in which the plaice was covered by the agar chamber to those in which it was merely buried in the sand but, of course, the shark was prevented from capturing the prey in tests with the agar chamber.

Young individuals of large fishes are frequently utilized in physiological experiments, but very few behavioral data have ever been obtained for captive sharks, large or small. However, some evidence obtained in a conditioning experiment on young bull sharks and lemon sharks (Wright and Jackson, 1964) indicated that sharks three months old can be more easily conditioned to press a target for food than can adults. Wright and Jackson compared their results with those of Clark (1959) on two adult lemon sharks and noted that the adults required six weeks to acquire the response whereas the juveniles in their own study acquired the response in only three weeks. In both groups, one training period per day was conducted for a total of five per week. The data are presented in such a fashion that the individual number of trials cannot be compared in these two studies but it can be stated that the adults contacted the target with their snouts 617 times during the training period of six weeks compared with 82 times for the juveniles during a three-week training period.

A study carried out by Tester and Kato (1966) on visual target discrimination in blacktip (Carcharhinus melanopterus) and grey reef sharks (C. menisorrah) also provides data on the learning ability of juveniles. These investigators utilized a shock-avoidance conditioning technique rather than food reinforcement. They found that there was great variation in learning ability between individuals and that only two of the blacktips successfully learned to avoid shock by remaining away from the end of the tank that displayed the "negative" target. The problem for the shark was to discriminate between a negative target, i.e. one associated with shock at one end of the tank, and a neutral target associated with no shock at the other end. The tests were designed to test the ability of the sharks to discriminate form, orientation, and color by presenting to them square, rectangular and circle shapes in various combinations. Targets of different colors versus a grey neutral target were also tested. While only two sharks learned to avoid shock, most of the others did learn to associate the negative target with shock in at least one of the discrimination problems. The sharks manifested the acquisition of this association by sudden dashes out of the negative end of the tank, sudden swirls or changes in swimming pattern or increased excitability when the negative target appeared. The fastest rate of learning (comparing rates in the same problem) was demonstrated by a grey reef shark which learned to discriminate a horizontal from a vertical rectangle after 54 minutes of training in three sessions. The slowest acquisition of this discrimination was shown by a blacktip who required 158 minutes of training in 11 sessions to learn the response. A valid comparison between the learning ability of young blacktip and grey reef sharks cannot be made from these data, however, because only two grey reef sharks were tested compared with eight blacktips.

These studies indicate that juvenile sharks can be conditioned with both negative and positive reinforcement and that in some individuals the rate of learning is quite rapid. A comparison of the available evidence on the learning ability of adults and juveniles indicates that young sharks may be somewhat more readily influenced by experiential factors than adults.

B. Teleosts

Without entering into a discussion of the extensive literature on experiential effects on teleost behavior, it may be well to mention a few selected examples of studies illustrating the effect of experiential factors on teleost fish in order to provide a comparison with elasmobranchs.

Ware (1971) observed the effect of experience on predation in adult rainbow trout (*Salmo gairdneri*). He found four stages in a feeding response: positioning, approach, seizure, and ingestion. The "prey" used in this study were cylindrical pellets of chicken liver blanched to a uniform white color. Inexperienced fish required several days of exposure before these behavioral stages appeared. One can assume, although it was not explicitly stated, that the chemical characteristics of the food pellet caused the fish to recognize it as edible in the initial exposures to this type of food. Ware measured the distance from which inexperienced fish attacked the food object and found an increase with experience to a maximum of twice the initial distance. After 6 days of training, no further increase was observed. Vision was presumably the dominant sensory modality at this time. Ware considered this increase in reaction distance and an increase in the rate of food uptake to be an indication that a "search image" was being formed through a learning process. Use of the term "predation" to describe the process of food uptake in this experiment seems inaccurate since the food did not resemble a natural prey organism in form and exhibited no movement. Activity on the part of a prey organism might have had an effect on the responses of the fish that would permit differentiation from responses to inanimate food objects.

A study that provides some data on the feeding behavior of newly hatched fish was conducted by Braum (1964). He analyzed the early feeding behavior of northern pike (*Esox lucius*) and two species of *Coregonus*. The first feeding movements were apparently oriented visually. The larvae would orient the head and move the eyes to fixate on the prey object--copepods or cladocerans--then bend the body into a sinusoid shape and lunge forward snapping the mouth and "sucking the prey in." Braum maintained that these movements are "innate" but he did observe improvement in the percentage of successful attacks with increase in experience. In this study, no attempt was made to experimentally modify the early experience of the fish. Bryan (1973), however, did provide evidence on the effect of modifying early experience of rainbow trout fry (*Salmo gairneri*). He was interested in the effect of early feeding history on the food selectivity of these fish. Bryan also investigated whether the food preferences of parental stocks had any effect on food selectivity of the progeny. By this experimental design, he was apparently attempting to assess the relative importance of experiential factors and inheritance in the determination of food preferences.

Two major types of fry were tested: One group was hatched from domestic hatchery stock cultured at least 4 generations and the other was hatched in the laboratory from eggs of adults native to local lakes. In one experimental series, groups of these two strains of fry were subjected to training with brine shrimp, tubifex, mayfly larvae, daphnia and hatchery food, that is to say, each group was fed only specific food for a given period of time. The groups were then tested by presenting the fish with a choice of food and counting the number of each type of food item taken. These counts were made either directly or the stomach contents were analyzed after the experiment. Bryan used the term "training bias" to refer to a preference for the food a fish had eaten previously when given a choice between familiar and novel foods. He suggested that there may be a relationship between training bias and "food imprinting" of the type referred to by Hess (1962). Bryan, in his experiments, found that training biases occurred in both domestic and wild stocks when trained and tested with non-live food. When trained with live food, the trout selected the faster-moving prey (brine shrimp rather than tubifex) regardless of previous training experience in some experiments. In others, the trout selected the live food on which they had been trained. In a second experimental series, naive fry of both domestic and wild strains were tested as to their food preferences with no training. These groups were presented with mayfly larvae, daphnia, and hatchery food. The results indicated that there seemed to be no relationship between food preferences of parents and progeny. Both domestic and wild strains selected mayfly larvae, daphnia and hatchery food in decreasing order of preference. An additional, peripheral, finding was that these fry consumed more food when tested in a group than when tested singly.

In this species, as with young northern pike, vision seems to be the dominant sense by which food was sought, although Bryan believed that olfactory cues may have sensitized the trout to visual cues. No mention was made of the possible involvement of lateral line sense or hearing. Since moving prey seemed to elicit more feeding than dead prey organisms or non-living food, the possible role of the lateral line in the detection of low-frequency vibrations that may be produced by the prey should not be ignored.

Other studies (Le Brasseur, 1969) have shown that the previous feeding experience of juvenile chum salmon (Oncorhycus keta) influenced their choice of food later. Fish were fed 2 species of copepod (Calanus plumchrus and

Pseudocalanus minutus) and one species of euphausiid (*Euphausia pacifica*). Those fish "trained" on euphausiids --the largest-sized prey--selected it in choice tests. All fish, with and without training experience, selected Calanus (of intermediate size), avoided Pseudocalanus (the smallest prey) and to some limited extent selected euphausiids. Apparently, the very large and very small prey were less readily captured than the intermediate-sized Calanus and, in fact, the young fish may be adapted to this prey size since Calanus forms a large part of the plankton biomass of the natural habitat of juvenile chum salmon. The basis of the preference for Calanus by the young fish in these experiments may be that the presentation of prey of suitable size caused prey to be selected in proportion to its abundance rather than its palatability. Le Brasseur stated that other prey of organisms of the same size as Calanus may be selected to the same extent if sufficiently abundant.

Beukema (1968) studied predation in the three-spined stickleback (*Gasterosteus aculeatus*). He observed the predatory behavior of individual fish in a specially constructed aquarium divided into hexagonal, interconnecting cells which formed a maze. In experiments, fish became increasingly efficient with experience in searching the maze for prey. If a new type of prey were introduced, the "risk," or probability of being eaten of the prey, rapidly increased after an initial delay. Beukema termed this delay period the time of "searching image formation." If a new type of prey were introduced that was extremely palatable, the fish avoided the previous prey and selected the highly palatable prey. This selectivity was present even when the fish were at high hunger states and Beukema believed that it indicated a learning process that involved "the refusal of Tubifex worms at high hungers in the expectation of the more palatable pieces of Enchytraeus."

In summary, it can be seen that in adult teleosts, experiential factors can modify food preferences. These preferences are apparently labile and may be modified by such factors as palatability, size, and abundance of prey. Other characteristics of the prey such as movement and behavior also may be involved. Juvenile and newly-hatched fry are influenced in their later choice of food by their initial feeding experience. Apparently, food preferences of the parents are not significant in the preferences of the progeny. In addition, the character of the predation process changes in that there is an increase in efficiency of prey capture with experience. The species studied in the above-cited investigations were primarily visually oriented predators but these results may have relevance to species in which other sensory modalities are dominant.

The experimental studies on elasmobranchs that are available in the literature have not been concerned with experiential factors in feeding behavior in the same way as have studies on teleosts. Elasmobranch studies, such as the instrumental conditioning work cited, have centered on sensory capacities and learning ability with the underlying goal of elucidating the nature of stimuli that may elicit feeding in elasmobranchs. Many of the feeding studies in teleosts are concerned with determination of the factors that affect rate of food uptake, food selectivity, and the nature of predatory behavior. For this reason, the results of these two groups of studies are not comparable in their specific details. Nevertheless, it may be stated that elasmobranchs are capable of conditioned learning and that experiential factors, especially in the young, may be involved in modifying later feeding behavior. As is well known, teleosts have been utilized as subjects in conditioning studies for many years and the learning ability of several species is well established. Although a subjective impression is gained from the few studies that have been carried out, that sharks learn more slowly and are capable of less complex behavior than teleosts, no definitive experiments have been performed on the degree to which learning processes differ between elasmobranchs and teleosts. It is possible that the tests that have so far been utilized to determine the learning ability of sharks, do not provide optimal conditions in which a shark can demonstrate true learning ability. Few data exist on the effect of stress on shark behavior or learning ability and accurate measures of stress are not easily obtained. The effect of captivity on animals as large as sharks (Clark, 1963) is undoubtedly different from the effect on the small-sized teleosts that are commonly used in instrumental conditioning studies. For these reasons, it is, as yet, premature to make a rigorous comparison of learning processes in these two groups.

VI. Embryonic Experiential Factors in Feeding Responses

The studies referred to so far, with the exception of Braum's (1964) study, have been concerned with experiential factors in the external environment affecting the animal during the period of life after hatching or birth. The investigators did not devote attention to the influence of internal, interoceptive, experiential factors at any stage of life nor did they consider external experiential factors during prenatal or prehatching periods. For a complete understanding of the processes of the development and expression of feeding behavior in fishes information of this type, which is becoming increasingly available in higher vertebrates, is essential. The reasons for the lack of studies providing this kind of information are probably technical. In ovoviviparous elasmobranchs work on the effect of pre-natal experiential factors is difficult because these animals do not reproduce readily in captivity and gravid females are not readily obtained. In oviparous elasmobranchs this type of study would be more easily carried out but there are still difficulties in getting the eggs to hatch and the young to survive.

A study illustrating the above method of approach is relevant here. Abu Gideiri (1969) investigated the development of behavior in *Tilapia nilotica*. While not specifically concerned with feeding behavior, his observations provide some pertinent information on the ontogeny of movements that may be considered precursors to feeding movements. Abu Gideiri observed spontaneous embryonic behavior from the time fertilized eggs were taken into the mouth by the parents through hatching until four days post-hatching. He found that this behavior can be divided into four stages: 1. *Myogenic stage* during which the stimulus causing myofibrils to contract lies within the muscle itself. 2. *Neurogenic stage* when somatic motor neurons have reached muscle fibers and initiate contractions. 3. *Reflexogenic stage* at which an external stimulus produces responses and 4. *Swimming stage* in which elaborate structural development has occurred and precise responses are produced. Movements which possibly are related to later feeding responses were seen at 103 hours of embryonic life when slight irregular jaw movements occurred. These movements occurred first only on one side or the other and were accompanied by body movements. Shortly afterward, opercular contractions, also irregular, occurred in accompaniment with body movements. Irregular, coordinated movements of the jaw and operculum then occurred but only when there was movement in the axial musculature. At 121 hours, full coordination was achieved, jaw and opercular movements were emancipated from body movements, and irregular individual jaw and opercular movements were no longer seen. Hatching occurred after 123 hours. Feeding movements per se were observed in 3-day-old larvae. "When a pin suspended from a thread was dipped into the water in front of a larva, it snapped vigorously at it making a quick jump forward and touching the pin. After each bite the larva retreated 2-3 cm backing away with its head still oriented to the needle." Abu Gideiri correlated the observed behavior with the state of development of the nervous system at the different stages and found that the jaw movements occurred in the swimming stage which began at 103 hours. Further, he studied the effect of stimulating the embryo by means of a mounted human hair from the 37 hour stage up to hatching and found that responses occurred at different times in different parts of the body. The order of appearance of responses was: anterior half of trunk, oral region of head, pectoral fins, opercular region, and eyes. This was correlated with the maturation of sensory connections with the spinal cord and the proliferation of internuncial neurons. The stimuli Abu Gideiri administered were not quantified however, nor were they systematically applied so that the possible influence of such stimulation in the maturation of structure was not established. These stimuli were presented merely to determine the tactile sensitivity of the embryos during the various developmental stages.

Studies of sensory factors (Aronson et al. 1967; Bryan, 1973; Beukema, 1968; Clark, 1959; Tester and Kato, 1966; Ware, 1971; Wright and Jackson, 1964) that were concerned with the early feeding movements and instrumental capabilities of predatory fishes have centered chiefly on vision with relative neglect of olfaction and audition. Some investigations have been carried out (Cahn and Shaw, 1965; Iwai, 1967) on the structure, development and function of lateral-line cupulae in teleost larvae. These cupulae are jelly-like projections that are attached at the basal end to a naked or free neuromast. The cilia of the sensory hair cells that are part of the neuromast organ protrude into the jelly-like mass and are subjected to shear forces when the cupula is displaced. This displacement presumably sets off afferent nerve impulses in branches of the Facial (VII) and Vagus (X) nerves that innervate the hair cells. Water flow and other types of water motion are forces that may cause the cupulae to move back and forth. These neuromast organs are distributed about the head and body in a bilaterally symmetrical fashion and the organs on the head are distributed precisely in three dimensions so that the fish may localize a source of turbulence (Cahn and Shaw, 1965). Iwai (1967) noted that in many species, cupulae are present in prehatching embryos and in his investigations he observed cupulae being displaced in prehatched, live embryos of the goby Tridentiger. Other species such as the goldfish (Carassius auratus) did not show cupulae until after hatching and in still others (Japanese dace, Tribolodon) cupulae became visible only after four days. This indicates that at least in some species the lateral line may be an avenue of stimulation while still in the egg and the possibility exists that this stimulation is involved in the development of subsequent locomotory and feeding movements. Iwai indicated that marine teleosts produce larvae in which the cupulae are well developed and functional at hatching and he correlated this with the immediate darting and swimming action of such pelagic forms. In contrast, freshwater teleosts with a large yolk sac still present at hatching tend to lie on the bottom after hatching.

In elasmobranchs, anatomical evidence exists (Tester and Kendall, 1967; 1968; 1969) that embryos of two ovoviviparous sharks (Carcharhinus menisorrah and Sphyrna lewini) have well developed lateral line canal neuromasts and free neuromasts distributed in "pit organs" over the body. These organs are innervated by branches of the lateralis branch of the vagus nerve (X). It is possible that stimulation of these organs while in utero may play a part in their subsequent function as a prey detector in localizing the source of water displacements in the near field. Tester and

Kendall (1968, 1969) have shown that in canal organs, cupulae are present which may serve to provide a surface whereby fluid motion in the canals can displace the hairs of neuromast sensory cells. Apparently, these cupulae are continuously secreted by supporting cells adjacent to the sensory cells of the neuromast and cupular material may be intermittently or continuously sloughed off.

A group of sharks in which the lateral line organs as well as other sense organs may be stimulated before birth is the Odontaspididae or sand sharks. In the sand tiger (Odontaspis taurus) a pair of young are produced, one in each oviduct. Springer (1948) has detailed the process by which the young obtain nourishment while in the uterus. Several specimens of gravid females became available for examination and Springer found a large number of egg capsules (60-70) along with embryos in the uteri. Dissection of an embryo revealed a large amount of yolk-mass present in the stomach and intestine and digestion appeared to have taken place. Springer concluded that the embryos were feeding on eggs contained in the egg capsules that were present in the oviducts and uteri. Each egg capsule contained an average of 19 ova and two intact ova were found in the stomach of one embryo. On one occasion, Springer observed an active embryo while dissecting a gravid female. When he inserted his hand into the slit-open oviduct he "received the impression that I had been bitten. What I had encountered was an exceedingly active embryo which dashed about open-mouthed inside the oviduct." A second embryo was observed to display well-coordinated swimming motions directed at keeping its dorsal side uppermost and it also kept its mouth open. Respiratory movements were not observed. No shell membrane was present to sheathe the embryo so uterine fluid had direct access to the lateral line organs. The absence of yolk sac attachment scars in near-term embryos implies that they depend on pseudoplacental connections or non-ingested yolk for nourishment only in the earlier stages of development and subsequently assume a free-living mode in the oviduct. Initially, the embryos are oriented head forward in the oviduct but may reverse their orientation prior to birth. Springer noted that this may be an adaptation that prevents the rough, backward-directed denticles from abrading the oviducal walls since there is no sheath covering the embryo. Apparently, this species "sends forth into the world not only large, well-developed and even experienced young but may send them forth with a full stomach." Some evidence (Springer, 1948) indicates that this oviphagous habit may also occur in thresher shark embryos (Alopias vulpinus).

VII. Internal Experiential Factors on Feeding Responses

A paper concerning an example of an internal experiential factor on feeding in fishes may be mentioned here. Ware (1972) investigated the effect of "hunger" on predation in adult rainbow trout (Salmo gairdneri). He calculated regression equations to describe the decline in rate of food uptake with increase in degree of satiation. Presumably the fish were "hungrier" at the beginning of a test than at the end when they had consumed substantial quantities of food. Ware concluded that the decline in rate was due to the fact that the fish took longer to manipulate the food as they approached satiation, i.e. the prey might be taken into the mouth and rejected several times before being actually swallowed. This study was more concerned with the formulation of mathematical models describing feeding rates than with the qualitative influence of "hunger" on feeding behavior but it does illustrate the influence of an internal factor on adults.

A study by Blaxter (1965) provided some data on the influence of duration of food deprivation on rate of food uptake in herring larvae (Clupea harengus). He noted that the rate of food consumption was high as long as the last feeding was 5 hours or more before a test. The initial rate (in the first 2-3 minutes) was essentially similar in larvae that had been deprived of food for periods of 5 to 18 hours.

Bryan (1973) noted that different measures of "hunger" and food "preferences" have different characteristics and therefore the terms must be used with reference to specific circumstances and may only apply to other situations which are very similar. Hunger levels can be defined relative to the time length of food deprivation prior to an experiment or in terms of percent of satiation if the maximum food ration in a typical feeding session is known. Ware found that the duration of food deprivation had no effect on the degree of food selection in trout with training bias. He does not mention differences in behavior relative to different levels of hunger in these fish.

Another example of a type of internal factor that may be involved in feeding behavior is the case of the absence of food intake in female sharks when in the nursery grounds. Gravid females, those about to give birth, and those which have recently given birth do not feed (Springer, 1967). Presumably the endocrinological state of the female associated with this phase of reproduction can be implicated in the lack of feeding responses. Of course, external stimuli such as olfactory-stimulating substances released when the young emerge with an inhibitory effect cannot be ruled out.

A similar condition is found in mouthbrooding teleosts among Tilapia. After spawning, during the time in which eggs are held in the mouth by the parent, feeding is inhibited. Apparently the experience of spawning is necessary for the inhibition of feeding because immature Tilapia will pick up eggs and swallow them, thus failing to distinguish eggs from food (Thomas, 1973).

VIII. Learning and Habituation

Some of the evidence cited above implies that in sharks and teleosts there may be periods in the life of the young animal when external experiential factors involved in such processes as learning may be more effective in modifying behavior than at later periods (Aronson *et al.*, 1967; Bryan, 1973; Wright and Jackson, 1964). Whether or not this period of greater susceptibility to experiential factors bears any relation to the so-called critical period of higher vertebrates is unknown.

There are some indications that rather rapid learning takes place in sharks under the conditions of the field play-back experiments referred to earlier (Myrberg *et al.*, 1969) but the effects that were observed seemed to be transitory. In these experiments the sharks failed to respond to the test sounds after a number of successive presentations of three-minute sound periods separated by three-minute periods of no sound. The sharks may have learned after repeated approaches that these sounds did not indicate the presence of prey and with the absence of reinforcement, extinction occurred. Myrberg observed this in five species of sharks when sounds were presented over a three-hour period. After a period of one hour without sound, the sharks were again responsive.

The decrease in responses observed by Myrberg was also found by Nelson and Johnson (1970, 1972) and has been referred to as "habituation." These investigators use this term to refer to the decrease in response intensity as measured by the numbers of sharks approaching. This is a much different meaning from that commonly used in behavioral studies. The usual definition of habituation refers to a response decrement to repeated or continuous stimulation of the same experimental subject (Hinde, 1970), but in these studies there was no assurance that the same animals had appeared at the test site or that they had been subjected to repeated stimulation.

IX. Summary

Recent investigations have shown that low-frequency sound is an effective stimulus in the attraction of sharks. The characteristics of the sounds most effective in attracting sharks have been established and these characteristics were found to be ones associated with potential prey and thereby related to feeding stimuli. Observations on the behavioral responses of adult and juvenile sharks showed that there were some differences between them. The possibility that these differences are influenced by the degree of experience of the sharks was considered, and a brief survey was made of the literature concerning various types of experiential factors in the development of feeding responses in both elasmobranchs and bony fishes.

Feeding behavior in elasmobranchs is not well understood. Additional detailed analyses of feeding responses in adult animals will be necessary to characterize accurately the form of feeding behavior found in the adult of any given species. This might be considered to establish the nature of an end-point of the developmental processes that occur in a species-typical environment and lead to the characteristic feeding behavior of the species.

In adult elasmobranchs the sensory modalities of olfaction, gustation, vision, electrical sense, and general mechanoreception (touch) have been implicated in feeding behavior. A few studies have been performed on young elasmobranchs as well, also indicating their involvement in feeding behavior. However, studies concerning the influence of external experience, mediated through these sensory modalities, on the development of feeding responses of sharks are lacking. Little information is available on the relative importance of each sensory modality during different developmental periods of the young shark.

Experimental investigations on sensory factors in early feeding behavior of elasmobranchs and teleosts have been primarily concerned with vision. Few such studies provide evidence on the role of olfaction and there is even less evidence on the role of audition or electrical sensitivity.

The evidence that is available on the development of feeding responses concerns bony fishes. Much of this work is related to motivational analyses or the formulation of mathematical models and is not primarily concerned with developmental issues (Colgan, 1973; Ware, 1972). Studies on teleost fishes are cited (Abu Gideiri, 1969; Braum, 1964) that illustrate the type of information necessary for a complete understanding of the development of feeding behavior. The work cited above is a preliminary step but is only concerned with maturational processes and the correlation of nervous structure with behavior. It does not consider the reciprocal influences between structure and behavior and the effect of various types of experiential factors on structure (Gottlieb, 1970).

X. Objectives of Study

This study is an attempt to determine the relationship between the feeding experiences of naive sharks and their response to low frequency sounds. The availability of a litter of young lemon shark pups that had been taken from a moribund female provided an opportunity to modify the early feeding experience of animals that had never experienced a species-typical post-natal environment. It was our objective to determine the effect of depriving the young sharks of any access to the stimulation of living prey on their responses to low-frequency sounds.

I decided to utilize as a sound generator a mechanical device that simulated the action of a struggling fish by the oscillation of a plastic flipper at frequencies below 20 Hz. In the field studies cited earlier, low-frequency sounds in the range from 20 to 1000 Hz were generated by means of electronic apparatus. It seemed possible that a mechanical device which operated at frequencies below those used in field play-back studies would provide a signal with hydrodynamic components more like the water disturbances actually produced by struggling fish.

A secondary objective was to determine whether there were any differences in the responses of these sharks to mechanically produced signals with and without large-amplitude hydrodynamic components and, further, to compare their responses to mechanically produced sounds with those to electronically produced sounds.

These sounds were presented in such a manner as to minimize response decrements of the kind observed by other investigators in free ranging sharks. The limitations encountered in field studies could be overcome in a laboratory situation in which a more accurate estimate of response decrement is possible because individuals could be recognized thus making close observation of each shark possible.

MATERIALS AND METHODS

Experiments were conducted in an outdoor, circular, cement tank 4.5 m in diameter containing seawater from 1 m to 1.2 m deep. The bottom was slightly sloped toward a drain located approximately at the center. The mechanical sound-producing device, called the Ichthyo-Uro-Simulator Mk III (IUS), was mounted on a wooden platform over the center of the tank. The platform was constructed of two parallel 2 x 8 planks upon which 2 x 4 crosspieces were nailed in a ladder-like fashion. Spacing between crosspieces was large enough to permit a relatively unobstructed view of the whole tank but nevertheless could provide support for two observers as well as the IUS (Fig. 1). At the center of the tank, the bottom was painted blue. This circle had a radius of 46 cm and a thin circular ring of the same color was painted 91 cm from the center. This ring marked the limit of the scoring area of the tank within which behavioral data was taken (Fig. 2). Closeness of approach could then be estimated accurately merely by scoring whether the shark crossed the ring or the central area. The sharks most commonly swam near the wall of the tank in clockwise circuits. Occasionally they crossed over the width of the tank. They also frequently described small circles approximately 1 m in diameter and figure-eight patterns. For the most part, however, an entry into the area inside the ring marked a deviation from the accustomed path (Fig. 9).

I. Ichthyo-Uro-Simulator

Three models of this mechanical sound-producing apparatus were constructed. Mk I was made of brass and wood but was too small and of insufficient strength to withstand the heavy loads produced by the oscillatory motion of the flipper in the water. Mk II was substantially larger and more sturdy and was used in a pilot study but problems developed with extraneous noise produced by the linkages and shaft bearings. Mk III, the present version shown in Fig. 3, was constructed of Plexiglas with aluminum shafts. Plexiglas was used because it was virtually transparent underwater and (Fig. 4) thus reduced the likelihood that sharks would orient to the apparatus visually rather than acoustically, and because it would not rust after exposure to salt water. The simulated caudal fin or flipper was made of 3-mm flexible transparent vinyl plastic. Other materials of less flexibility did not provide a fish-like action.

The overall length of the IUS was 91 cm; the shaft support that extended vertically into the water was 61 cm long and 10 cm wide. The pulley shaft platform was 30.5 cm wide and 30.5 cm long. The shaft struts were 25 mm x 51 mm x 102 mm blocks of Plexiglas, cemented in place. Holes were drilled in these blocks on 76-mm centers to accept sealed, saltwater-proof ball bearings. The aluminum flipper shaft and pulley shaft were 76 mm from the surface of the strut support and pulley platform. The plastic flipper was attached to the flipper shaft by means of a Plexiglas clamp that held the flipper material and this in turn was bolted to the aluminum shaft by one 6-32 bolt. The flipper with its holder could thus be conveniently removed from the shaft and, if necessary, the flipper itself could be removed from the holder.

A Plexiglas clevis was attached to the upper end of the shaft by means of a setscrew. A series of holes in the clevis permitted changing the amplitude of lateral movement. The Plexiglas linkage arm, which was bolted on one end to the clevis and on the other end to an eccentric was 3 mm thick, 25 mm wide and 279 mm long. Ball bearings were recessed into the Plexiglas here also, since this linkage was the major source of extraneous noise. The eccentric was also constructed of Plexiglas and was secured on the pulley shaft by a setscrew. Power for the machine was provided by a 1/3 hp electric motor and V-belt. Three frequencies of oscillation were permitted by the 3-speed V-belt pulley on the pulley shaft, 11 Hz, 14 Hz, and 22 Hz at a motor speed of 1760 rpm.

A mounting clamp of Plexiglas was constructed which permitted the apparatus to be fastened to a 2 x 4 or any similar support in the manner of an outboard motor (Fig. 5). This clamp ran in a track on the reverse side of the pulley shaft platform and could be set in any position by a 3/8" eyebolt used as a setscrew. The flipper could thus be lowered into the water to any desired depth.

The electric motor was bolted to a small plywood base. This base in turn was attached to another plywood board by means of a single bolt. The motor could then be rotated 360° into any position so that its pulley could be lined up with the drive pulley of the IUS. The motor mount was then shock mounted to the observation platform with a C-clamp and foam rubber (Fig. 1). The observation platform itself rested on foam rubber where it contacted the cement rim of the tank. Rubber was also used in clamping the IUS to the observation platform. In this manner, noise from the vibration of the electric motor was minimized.

The plastic flipper was square with a total area of 120 cm^2 and an amplitude of lateral movement of 7.5 cm. During tests, irregular pulses of sound were produced by means of a hand-held on-off switch. Pulse length and interpulse intervals were irregular and ranged from 0.5 to 5 sec.

I attempted to monitor the sound pattern in the test tank with a pressure hydrophone. Use of a displacement detector such as a geophone was precluded because it proved to be impossible to mount it so that it would be directionally stable and yet not the susceptible to extraneous vibration. Because of the shallowness of the water and the complexity of the reverberation patterns it seemed advisable to try to map the sound field. A grid framework of 1/2" square lumber was set up across the tank marking hydrophone positions (Fig. 1). The hydrophone output signal was fed into an oscilloscope, and the trace produced at each hydrophone position was photographed with an oscilloscope camera (Fig. 6). Sounds were also recorded on tape and later analyzed with a Sonagraph (Kay Electric Co.). Complex, extremely variable patterns were present, including bursts of noise of up to 8 kHz in frequency that were produced by the mechanical linkages (Fig. 7). The oscillation frequency of the flipper as determined from the oscillogram, was 12.5 Hz.

II. Electronically Produced Sound

I conducted a series of tests composed of two sets of trials in which I used a J-9 underwater sound projector to produce sound. In one set, the signal consisted of 10 Hz sounds, generated by an audio oscillator, plus broad-band switching transients. In the other set the signal consisted only of the 10 Hz sounds. The response of sharks to broad-band noise "clicks" of 75-100 msec duration and high amplitude, irregularly pulsed sine waves of 10 Hz was tested. Sounds were turned on and off with a gain control to avoid the switch transients that were not wanted with the high-amplitude sine waves. In this series, the J-9 was suspended from the observation platform at the same position occupied by the IUS with its major axis directed at a 45° angle from the length of the platform. An approximation of the ambient noise and signal level was obtained with a pressure hydrophone (Chesapeake Instruments) placed 30 cm from the tank wall. Ambient noise levels were generally high (30-34 dB below a microbar) and there was considerable 60-Hz noise induced from buried electrical cables in the area. Signal levels ranged from -28 dB to -30 dB re μb as detected by the pressure hydrophone.

III. Experimental Animals

The animals used as subjects in the present study were all members of a single litter. A gravid female lemon shark had been caught on the shark line regularly set near the Mote Marine Laboratory, Sarasota, Florida. At the time of arrival at the holding facilities of the Laboratory, the female was moribund so the unborn shark pups were removed from the uteri. Of the 20 in the litter, 13 survived and these were maintained in a circular cement tank 14 feet in diameter identical with the test tank described. A continuous flow of sea water was maintained in both holding and test tanks, but the flow was interrupted in the test tank during tests.

The animals were fed cut smelt daily, prior to and during the first series of tests. At the time testing began, the animals were two months old and approximately 50 to 65 cm in length (Fig. 8). After the completion of this series of tests, they were fed live fish 7-12 cm in length (*Mugil cephalus*, *Lagodon rhomboides*, *Sardinella macrophthalmus*) seined from a nearby bay. In order to facilitate capture by the sharks, these fish were damaged by diagonal cuts in the lateral body musculature, thus reducing their swimming efficiency. Undamaged fish were able to elude capture so the sharks would have starved to death. Some fish that were not damaged severely enough were able to recover and lived in the tank throughout the remainder of the study.

After a period of two weeks of feeding on live fish exclusively, the sharks were tested in the second series. During the remainder of the study the animals were fed both live and fish and cut smelt.

IV. Experimental Procedure

Preliminary tests conducted in 1970 indicated that a 3-minute trial was adequate to score responses but not so long that habituation of the fish would be unduly accelerated. Each trial was bracketed by a 3-minute observation period that served as control during which behavioral responses were recorded. A 3-minute inter-trial interval, during which responses were not recorded, separated control and trial periods so that residual effects from the previous trial would be less likely to influence the succeeding one.

Initially, tests were conducted on one shark at a time in order to make the scoring of responses possible by a single observer. Animals were selected from the holding tank and transferred by dip net to the test tank located approximately 46 m away. After each test, the animal was marked by notching the pectoral fin and was returned to the holding tank. In this way animals already tested could be recognized and not be tested again for at least 13 days, and habituation to the sound stimuli could be restricted within one test period.

As experiments progressed, it became obvious that a shark tested in isolation seemed more subject to sudden erratic changes in velocity and direction as compared with its behavior in a group. On the basis of exploratory experiments, I decided then to test three sharks at a time and enlist the aid of an additional observer. Testing sharks in groups seemed to cause them to behave more normally and to reduce their excitability.

Four types of sounds, all irregularly pulsed, were presented: 12.5-Hz sounds with large amplitude hydrodynamic components produced by the plastic flipper; 12.5-Hz sounds lacking large-amplitude hydrodynamic components, produced by the machine with the flipper removed; broad-band "clicks"; and high-amplitude, 10-Hz sine waves projected with the J-9 transducer (Table 1).

The responses of a single shark to the two types of sound produced by the IUS were observed in the initial series. After all 13 animals had been tested, 17 days after the start of the experiments, feeding with live fish was commenced, as described above.

After two weeks of feeding on live fish, one shark at a time was tested again. The results of the first test with a single shark in this new series indicated definite attraction to the stimulus. The animal repeatedly approached and circled the IUS. This type of response had been absent in the experiments of Series No. 1.

In subsequent tests with single sharks, as previously mentioned, it became obvious that the disturbing effects of being alone in the test tank overshadowed all others. After this, groups of three sharks were tested and all the remaining tests were performed with this size group.

Sounds with large-amplitude hydrodynamic components were presented during a test session, followed by a session with the flipper removed. The order of presentation was then reversed for the next day's testing to make it possible to check for order effects. This procedure was selected because the disturbance caused by lifting out the IUS and removing the flipper to alternate the types of sound presented might have unduly alarmed the sharks. Electronically produced sounds were presented on the same schedule as mechanically produced sounds; that is, three minutes of sound, a 3-minute inter-trial interval in which no responses were recorded, and a 3-minute control observation period. Broad-band noise plus 10-Hz sine waves was the stimulus presented in the first set of trials utilizing electronically produced sound (Fig. 10) and this was followed by a set of trials with 10-Hz sine waves alone. During all tests the otherwise continuous flow of sea water was interrupted to reduce the level of ambient noise.

In all tests six trials were run per session separated by six control observation periods and six 3-minute inter-trial intervals. This permitted two sessions per day with the same shark or group of sharks, one in the morning and one in the afternoon or evening. Frequency of crossing the ring or moving over the center area with a pectoral fin or with the head as far back as the base of the pectorals was scored. Crossing the ring was termed an "A" response and moving into the center area a "B" response. Notes on behavior exhibited while in the scoring area were taken and general observations on the swimming speed, direction, and level of excitement were also recorded. Four qualitative categories of behavior were abstracted from these observations: (1) <u>Initial oriented turn</u> - this was defined as a sudden, right-angle turn toward the signal source at the instant of signal onset and subsequent approach within ring or central area; (2) <u>Investigative approach</u> - defined as a turn toward the signal source any time after signal onset and subsequent approach within the ring or central area with a decrease in velocity and orientation of the head toward the signal source; (3) Circle - defined as at least one-half of a circuit around the signal source inside or outside of center area but within the ring; (4) Avoid - defined as a sudden change in velocity and/or direction with rapid darting away from the signal source after an initial approach. Frequency of A or B responses permitted localization of the sharks in space, and the four qualitative categories are descriptive of the response and reveal aspects of the responses that otherwise would not have been recorded. In the remainder of this paper, Initial Oriented Turns will be referred to as IOT responses, Investigative Approaches as IA responses, and Avoids as Av responses. Circles will not be abbreviated.

RESULTS

I. Behavior of Sharks Captured in the Field

Preliminary experiments were conducted in August 1970 with immature sharks approximately 65-75 cm in length of unknown age that were captured in the field. Experiments with these sharks indicated that they could be attracted by sounds produced with the IUS Mk II. In one experiment, a single shark confined within a rectangular net enclosure 4.5 m by 7.3 m in a small lagoon made investigative approaches to the IUS from distances of 4-5 m. During one trial, the shark came from the bottom at the deep end of the enclosure (2-3 m in depth), approached directly, and veered off at a distance of 1.5 to 2 m from the machine. This behavior was comparable to that observed by Banner (1972) in young lemon sharks.

Another preliminary series of tests was conducted with the IUS oscillating at 8 Hz, this time with two sharks held in a rectangular fiberglass tank 2 m by 3 m in a small shed. These sharks had also been captured in the field, at the same time as the one referred to above. During control observation periods, these sharks exhibited a typical swimming path that consisted of clockwise or counterclockwise circles near one or the other end of the tank. Swimming velocity typically was quite slow (20 to 25 cm per second). During the tests, swimming velocity increased, and positive responses were scored when deviations from the circular path occurred with subsequent investigative approaches to the machine, sometimes as close as .3 m. The IUS was mounted on the long side of the tank approximately at the middle in these tests. Positive responses were quite easy to detect with this arrangement when the flipper was attached. With the flipper detached, only two positive responses were scored during 9 trials while 54 positive responses were scored with the flipper attached during 11 trials (Table 2). There was no indication of any investigative approach during the control observation periods. These results indicate that immature lemon sharks with early feeding experience presumably typical for the species, responded positively to sounds with large-amplitude hydrodynamic components produced with the IUS Mk II. The lack of response to sounds with no large-amplitude hydrodynamic component may indicate a lack of attractive significance but may also be due to the observer's inability to detect subtle responses with the scoring technique used. These preliminary experiments were not sufficiently refined to measure adequately responses to this type of sound.

II. Behavior of Sharks Born in Captivity

A. Sharks With No Experience in Capturing Living Prey - Series No. 1

Lemon sharks approximately two months old with no experience in feeding in a natural habitat were tested in June and July, 1972 for their response to the IUS Mk III in the experimental situation described in the section on Materials and Methods. Each shark was tested alone in the tank. The behavior of these sharks contrasted markedly with that observed in the preliminary experiments of 1970 (Table 3). The predominant action observed in the initial trials of a test upon signal onset was a startle response or, if no immediate reaction occurred, swimming velocity frequently would increase as the trial progressed. Occasionally, the animal would collide with the machine especially if it was swimming rapidly. Swimming velocity was extremely variable, both within trials and between trials, and also, from day to day. Some animals swam rapidly through the entire test, others slowed down as the test progressed, and still others swam slowly initially and increased speed toward the end of the test. Estimates of velocity consist of timed circuits around the perimeter of the tank expressed as cm per second. Velocity ranged from 88 cm/sec to as slow as 20 cm/sec in which cases the shark seemed almost to hang in the water with pectoral fins at a high angle of attack. The impression that the sharks spent the majority of the time swimming outside the scoring area is reinforced by the low frequency of A and B responses observed.

The median number of A and B responses for each day's test was tabulated. These medians were used as variates in the Wilcoxon Matched-Pairs Signed-Ranks test (Siegal, 1956) for differences between control observation periods and sound periods. Since I used a one-tailed test, the level of significance was .025 rather than .05. The median of these medians was then tabulated to show the central tendency of the Series and these are given in Table 3. The median number of A responses with the flipper attached for animals in this series was 1.25. With the flipper removed, that is, in tests without large-amplitude hydrodynamic components in the sound produced, the median was 1.0. There was no statistically significant difference in median number of A responses between tests with flipper attached and those with flipper removed. The median number of B responses observed was zero. Even on those few occasions when a shark would cross over the ring it would not approach closely enough to the IUS to be within the central area. Comparison of the number of A and B responses in signal-on periods with control observation periods shows no statistically significant difference at the .025 level with the Wilcoxon test. This holds whether the flipper was attached or removed. The low numbers of B responses seen during control periods is correlated with a low level of random crossings of the tank.

A total of four Av responses were recorded during control observation periods of all tests and these occurred when the shark was within the central area and was about to collide with the flipper support of the IUS, when it made a short turn just before doing so. This lends support to the opinion that the animals could not see the machinery very well. Underwater photographs seemed to corroborate the supposition that the machine was hardly visible (Fig. 4).

The frequencies of the qualitatively distinguishable behavior that occurred in tests with both types of sound were low also (see Table 3). With the flipper attached, no IOT or Circles were observed, and a total of 5 IA and 22 Av were recorded. With the flipper removed, no IOT or IA were seen but 1 Circle and 9 Av occurred. In trials with the flipper removed, there was no significant difference in Av responses at the .025 level between treatment and control, but with the flipper attached, there were significantly more Av responses than in the control observation periods at the .025 level.

One behavioral pattern seen on several occasions during tests, control periods, and inter-trial intervals alike consisted of the shark swimming next to the wall of the tank with snout elevated sometimes with the head out of the water, to the level of its eyes. On rare occasions, a shark might rest on the bottom for periods no longer than a minute. Very frequently sharks would swim in small circles of about 1 m in diameter or describe a figure-eight path over a 1.5 m section of the tank. These patterns might be interspersed with periodic circuits around the perimeter of the tank near the wall.

1. Analysis of Results - Series No. 1

The results of the first series of tests utilizing the IUS Mk III as the sound source indicate that sounds with and without a high-amplitude hydrodynamic component were not attractive to sharks with atypical feeding experience. All thirteen animals were tested one at a time. At no time did the responses of the sharks indicate any significant degree of attraction. This lack of response cannot wholly be attributed to excessive distress on the part of the sharks since a single shark tested after feeding on living prey did give evidence of being attracted to these sounds. Furthermore, some sharks tested in the first series did not exhibit this behavior and seemed to behave in as normal a manner as any of the sharks captured in the field or as those which responded positively in subsequent experimental series.

The behavior which I interpreted as signs of distress were: very rapid swimming around the perimeter of the tank; a quick, jerky type of swimming motion; protrusion of the head out of the water up to the tank sides and startle reactions, i.e. sudden changes in velocity or direction of swimming when they occurred more often than once or twice during the trial.

The fact that the frequency of Av responses is essentially the same with sounds produced without the flipper and in control observation periods indicates that they result from chance encounters with the IUS or reactions to stimuli not detected by the observer. The occurrence of many more Av responses with the flipper attached may indicate that sounds with large amplitude hydrodynamic components are of a level of intensity that is aversive. However, there was no statistically significant difference between the median number of A responses recorded with flipper attached and removed. Apparently, when a shark did happen to cross over the ring, it was more likely to be startled by sounds with large-amplitude hydrodynamic components than by sounds with these components absent.

B. Sharks With Experience in Capturing Living Prey

1. Series No. 2A

After a period of two weeks of being supplied with live fish once a day, as described above, a single shark was again tested for responses to both types of sound produced by the IUS. The response was quite dramatic in that the animal repeatedly circled around the flipper scoring a median of 4 A responses and 1.5 B responses with the flipper attached compared with a median of 2 A responses and 0 B-responses in the control observation periods (Table 4). There was no difference in frequency of A and B responses between signal on with the flipper removed and control observation periods. In subsequent tests with a single animal, however, the shark frequently protruded the snout up the tank wall and while some positive responses were scored its behavior was quite erratic. Since the readiness or ability of the animals to respond positively to any kind of stimulus while exhibiting this behavior seemed in doubt, a series of tests was undertaken to determine whether an animal in a group would respond more reliably than a single animal and if so, to discover the minimum group size that would still produce this effect. On the basis of these tests, to be described later, I decided that a group of three sharks would be the optimum size to use as the experimental group.

2. Series No. 2B

This series of experiments was performed on groups of three sharks with two observers scoring responses. The non-parametric Wilcoxon Matched-Pairs Signed-Ranks test was again used to test the significance of differences between treatment and control medians. Results of tests with sounds having large-amplitude hydrodynamic components that were produced with the flipper attached showed no significant difference in the number of A responses at the .025 level (Table 5a) even though the sharks crossed over the ring slightly more often while the flipper was attached than during control observation periods. There was no significant difference between the median number of A responses scored in signal-on periods and control observation periods with sounds produced without the flipper. In these sounds large-amplitude hydrodynamic components were lacking. The median number of B responses scored on each test with the flipper attached was also greater than in control observation periods but the difference was not significant at the .025 level. No significant difference between median number of B responses scored with flipper removed and control observations was observed. Apparently the tendency to approach within the scoring area was only slightly greater with the flipper attached than with it removed when compared with control observation periods.

The total number of responses in three of the four qualitative categories was lower with flipper removed than with it attached (Table 5b). In the Circles category, however, the frequencies were the same. The response frequency was quite low for IOT responses both with flipper attached and removed. Only a single IOT response was recorded while the flipper was removed, and with the flipper attached, 12 positive responses were observed in 12 of the 66 trials. The frequency of IA responses was considerably higher with both types of sound. Positive responses occurred in 40 of the 66 trials with a total number of 85 with the flipper attached. In comparison, there were 28 responses, occurring in 17 trials, with the flipper removed. However, the Wilcoxon test shows no significant difference at the .025

level in a one-tailed test when comparing the levels of both IA and Av responses to sounds produced with the flipper attached and with it removed. On the other hand, if the two tests that were performed in the evening are not included (see page 93), the Wilcoxon test shows a greater level of response in these two categories to sounds produced with the flipper attached. This may indicate a time-of-day effect on response level which will be more fully discussed later.

On one occasion the level of excitement was extremely high. In this instance the flipper was attached and the sharks rapidly approached, circled the IUS repeatedly and took several bites at the flipper. Similar behavior was reported by Banner (1968a) in young lemon sharks when presented with broad-band, pulsed noise. The greater number of IA responses recorded with the flipper attached may indicate a greater attractive value of sounds with high-amplitude hydrodynamic components, but the closeness of approach to the source of sound was not significantly greater than with flipper removed, as shown by comparison of the median number of B responses (Table 5b). The number of Av responses scored with flipper attached was also much greater than with flipper removed, 93 compared with 32. If sounds with no high-amplitude hydrodynamic components are less attractive, fewer IA responses would be expected and further, if the lack of these components constitutes a stimulus of lower intensity, fewer Av responses would be expected. However, responses may be affected by diurnal variation in sensitivity so that an increase in responses to low-intensity stimuli and a decrease to high-intensity stimuli may occur.

During control observation periods, responses in all four qualitative behavioral categories were almost completely absent. Two Av, no IOT, three IA responses and four Circles occurred in 66 trials. During test No. 3, in which the two Av responses were scored, the sharks for unknown reasons suddenly darted rapidly around the tank in a very excited manner but after half a minute calmed down. No disturbance that might have caused this behavior was apparent.

3. Analysis of Results - Series No. 2B

The second series of tests showed a slight but statistically non-significant difference in A and B response to sounds produced with the flipper compared with sounds produced with it removed. Sharks crossed over the ring only slightly more often with flipper attached than in control observation periods but they did not come into the central area any more often than they did during control observation periods. The type of sound produced with the flipper removed was apparently not at a level of attractiveness sufficiently great to cause the sharks to deviate significantly from a random swimming pattern since there was no statistically significant difference between the median number of both A and B responses recorded during signal on periods and control observation periods.

A possible explanation for the apparent lack of difference between A and B response frequency during signal-on periods and controls is as follows: Sharks tended to exhibit uniform behavior for short but discrete periods of time and then would suddenly change into a different pattern for another discrete time period (see page 94). In many cases, during control observation periods, a shark would make several small diameter circles of low velocity while passing over the ring or central area and each crossing of the ring would have to be scored as an A response even though it was clear that the shark was not orienting to the IUS or responding to any external stimuli. These non-random "blocks" of behavior tended to falsely elevate the A and B response level during control observation periods.

Consideration of the four qualitative categories of behavior gives a somewhat different picture from the conclusions derived from the measures of position in space. The total number of responses was lower with the flipper removed in three of the four categories. However, the same number of Circles occurred with both types of sound. Even though the A and B response frequency was non-significant with flipper attached and removed, the qualitative categories show some degree of attractive significance for these sounds. Hence, A and B response frequency by itself is an inadequate measure of level of attractiveness of sounds.

In examining the qualitatively distinguished categories of behavior, it is apparent that there were a greater number of IA responses than any of the other approach-type responses. This was true whether the sound was produced with or without the flipper but, again, the level of responses was lower to sounds produced without the flipper. The high IA response frequency is in contrast to the low frequency of the IOT category which had the lowest response level of the three approach-type responses. The significance of this result may be that these sounds do not have a level of attractiveness high enough to cause an immediate change in swimming direction (IA response) upon the first instant of stimulus presentation in the majority of trials. However, these sounds may be sufficiently attractive to cause a more gradual increase in response strength later on in the trial so that the animal would subsequently approach to investigate the stimulus source more often and with increasing level of arousal. As previously stated, there was one occasion when the level of attractiveness of the sounds produced with the flipper seemed comparable with the results that other workers have obtained with broad-band noise. In this particular incident, the sharks rapidly approached, circled the IUS repeatedly, and snapped several times at the flipper. Under optimum conditions then, sounds with high-amplitude hydrodynamic components produced by mechanical means can approach the level of attractiveness of electronically produced, broad-band noise while sounds without these components seem less attractive to sharks under most conditions.

With regard to the Av category, the response frequency was highest of all categories with the flipper attached. The apparent contradiction of the simultaneous occurrence of high numbers of IA and Av responses may be explained by a pattern in which the attractiveness of the signal causes the shark to approach to the area within the ring, but, upon approaching even closer, encounters a rapidly increasing signal intensity that soon reaches a level producing an avoidance response. This would be in accordance with the Approach-Withdrawal theory of Schneirla (1959). Welker (1961) has come to similar conclusions in his study of exploratory behavior in mammals. Sounds produced without the flipper were apparently less attractive so fewer approaches were made. The lower number of Av responses may be related to a lower level of signal intensity so that even chance excursions into the immediate area of the IUS caused fewer startles or avoidance responses.

C. Behavior of Sharks When Presented With Electronically Produced Sounds - Series No. 3A and B

The series of tests just described took place over a period of two and one-half weeks. After 11 tests, the possibility arose that habituation could have occurred since all of the animals had been tested at least twice in the second series. Moreover, the sharks seemed to be very easily startled even in their home tank. Two sharks of the original 13 for unexplained reasons jumped out of the home tank. The series was therefore terminated, and after two weeks the behavior of the sharks seemed to be more normal. A new series of tests composed of two sets utilizing sounds projected with a J-9 underwater loudspeaker was then begun. In the initial set of tests, broad-band noise "clicks" produced by means of switching transients were presented together with 10-Hz sine waves produced by an audio oscillator. In the subsequent test set, high-amplitude sine waves alone were used. At this time, nine sharks were remained of the original 13 since two had been sacrificed for other purposes.

1. Series No. 3A

The results in the initial set with sounds containing broad-band noise are shown in Table 6. The Wilcoxon test shows a significantly greater number, at the .025 level, of B responses with signal-on than in control observations. There was no significant difference between signal-on and control in the number of A responses. The four qualitative categories showed a high level of response in these sharks (Table 6). A total of 18 IOT responses were recorded during 12 trials; 17 IA responses and 12 Circles occurred. The responses in the Circle category occurred repeatedly and rapidly. The sharks were frequently so close to the transducer as to make repeated circles within the margin of the center area. A total of 14 Av responses was recorded. The responses of these sharks to sounds containing broad-band noise are comparable to those observed in play-back experiments with unrestrained sharks in a normal environment. The greatest number of responses occurred in the IOT category which indicates a level of attractiveness high enough to cause an immediate, almost instantaneous diversion from the swimming path. As shown by the B response frequency, the sharks approached more closely to this type of sound than to either the mechanically produced sound or 10-Hz sound alone.

2. <u>Series No. 3B</u>

A second set of experiments in this series was performed utilizing only high-amplitude, 10-Hz sounds produced by the audio oscillator. Substantially fewer IOT responses were scored with these sounds alone than with broad-band sounds (Table 7). During 17 trials 5 were recorded, as compared with 18 during 12 trials of Series No. 3A. The number of IA responses recorded with 10-Hz sounds alone was 11 during 17 trials which was also fewer than the 17 recorded with broad-band plus 10-Hz sounds. A total of 15 Circles and 13 Av responses were recorded for 10-Hz sound alone while 12 Circles and 14 Av responses occurred with the broad-band, 10-Hz combination. In control observation periods for both of these sets of tests, only a single IA response occurred. No responses were noted in the other three categories. In Series No. 3B also, the number of B responses was significantly greater, at the .025 level (in a one-tailed test) with signal on than during control observation periods as shown by the Wilcoxon test.

3. Analysis of Results - Series No. 3A and B

The responses in Series No. 3B were much less intense than in Series No. 3A although the number of B responses in both series were significantly greater than in control observation periods. Proportionately fewer IOT responses were recorded with this type of sound than in the first set. The same is true for the other qualitative behavioral categories which indicates a much lower level of attractive significance for 10-Hz sounds alone than with 10-Hz sounds presented together with broad-band noise. Due to the low level of response in the qualitative categories with this latter set of tests, a rigorous comparison cannot be made with the other types of sound regarding relative frequency in these categories. It seems clear that both broad-band plus 10-Hz sounds and 10-Hz sounds alone can attract sharks but the level of attractiveness is greater when broad-band components are present. This is in agreement with the results of Banner (1972) who found a greater number of responses to broad-band noise than to low-frequency noise or to any of the sounds of biological origin tested.

The results of this series of tests utilizing electronically produced sounds corroborated the results obtained by other workers on juvenile lemon sharks. Series No. 3A utilizing the combination of broad-band noise and 10-Hz sounds indicated a very high level of attractiveness even though the transducer was not actually bitten as had been reported by Banner (1968a).

III. Responses to Tethered, Struggling Fish

The following tests referred to on page 74 were initiated to determine the sharks' responsiveness to a natural stimulus and also to determine whether a facilatory group effect on responsiveness existed. A small fish 6-8 cm in standard length restrained by a 2 m length of monofilament fish line through the dorsal musculature was presented to the shark in trials of three minutes' duration. The latency of first mouth contact with the fish was measured. Two sharks were tested in this manner. One shark responded to the tethered fish and attacked it in less than a minute. The other swam rapidly in circuits around the tank with periodic velocity changes and attacked after two minutes. On the second trial with this animal, the tethered fish jumped spasmodically as the shark approached which caused the shark to dart rapidly away. For the next few trials the shark remained unresponsive and on the seventh trial finally attacked the tethered fish after two minutes. Previous experiments with tethered fish conducted in 1970 indicated that the latency of the majority of attacks in normally behaving sharks is less than one minute (median of

30 seconds for 17 trials; three trials with latencies greater than one minute). These attacks consisted of a swift approach and final rush with rapid snapping of the jaws and they occurred even in extremely murky water with visibility no greater than .3 m to .5 m. Presumably, latencies of less than one minute indicate the normal degree of responsiveness and if the animals showed significantly larger latencies this would be indicative of some factor decreasing the normal level of responsiveness. It thus seemed that latency of attack would be a reliable measure with which to evaluate the sharks' degree of distress and/or readiness to respond. The two tests with single sharks gave widely divergent results thus indicating that even with a natural stimulus, great variability in responsiveness is present so the possibility of a salutory effect on the sharks' responsiveness of being tested in a group therefore seemed worthy of consideration.

We then initiated another series of experiments to determine whether there was, in fact, any effect on the sharks' behavior by being tested in a group rather than alone. Latency to first mouth contact was measured. A group of seven sharks was tested in the original holding tank and a group of six in the test tank. One hour after the group of six was introduced into the test tank, they were tested. The group in the home tank was tested as soon as possible after the group in the test tank. The median latency for sharks in the home tank was 17.5 seconds and 63.5 seconds in the test tank for three trials each (Table 8). The following day five trials each were administered to a group of ten sharks in the home tank and three in the test tank. The median latency for the home tank was 25 seconds and 30 seconds for the three sharks in the test tank. These latter results indicate that a group as small as three is at least as ready to respond to stimuli as the group of six, even after being subjected to the stress of transferral to the test tank. The similarity in latency of the group of three in the test tank compared with the group of ten in the home tank is noteworthy since it indicates that the small group has about the same degree of responsiveness as the large group. As one might expect, the latencies tended to be lower in the home tank since these animals had not been subjected to stress. On the basis of these exploratory tests it was decided that a group of three sharks was the maximum that could be accurately observed by two observers and still not be too small for a calming, group effect to prevail. This effect may be related to the typical habit of young sharks to remain in groups in nursery areas of the natural habitat. An isolated shark may be more vulnerable to predation and hence, isolation could be aversive to young sharks.

The results of these tests with groups of sharks showed not only a much smaller latency but a significant lessening of signs of distress. The drop in latency cannot be attributed to greater probability of chance encounter with the tethered fish on account of greater number of sharks in the tank. This is supported by the fact that the median latency for a group of ten was 25 seconds compared with a latency of 30 seconds for a group of three which is not significantly different. It would have been advantageous to have tested groups of three sharks before they had experience with living prey as a double check on the effect of distress on results in Series No. 1, but this was not possible since I did not become aware of the group effect until after the sharks had fed on live prey. To determine whether each group of sharks to be tested was not unduly distressed, a tethered fish was now presented before each test. If the latency of attack was great, thus indicating lack of readiness to respond these animals would not have been tested. Testing was begun after the effects of presentation of the tethered fish had subsided. As it occurred, however, in no case did any of the test groups have a latency too great to permit testing.

IV. Behavioral Habituation

In the present study, all tests were conducted with the same set of subjects so it was assumed that there might be some response decrement after repeated presentation of test stimuli. I attempted to minimize this with a testing schedule that would allow a long time period between tests of the same individual or group and to select a number of trials per test that would permit the collection of sufficient data but not cause a large decrease in responsiveness from first trial to last trial. An accurate appraisal of habituation rate cannot be established from these data since by design the response decrement was kept to a minimum.

Examination of the number of A responses recorded with flipper attached and flipper removed for sharks having no experience capturing living prey shows a non-significant number of responses when compared with control periods (Table 3). No evidence for a significant response decrement over trials or over tests can be seen. Sharks experienced in capturing living prey also do not show a significant decrease in response from first trial to last in any given test. There are significant differences between test days as shown by the F test in a one-way analysis of variance of number of A responses with the flipper attached, but there is no consistent trend in the differences (Tables 9 and 10). This is also true with the flipper removed. It therefore appears that unknown factors were producing substantial variations in the sharks' responses and no trend, such as that expected if habituation were the major factor, can be detected.

The results of the test series utilizing electronically produced sound do not permit conclusions to be drawn about habituation over the series since there were an insufficient number of test days to provide a valid comparison. Examination of the data for response decrements from initial to final trials, however, shows a significant difference in number of B responses in tests with broad-band sounds plus high-amplitude 10-Hz sine waves. The Kolgomorov-Smirnov one-sample test shows a significant difference at the .05 level in a two-tailed test (Table 11). The number of A responses recorded with this type of sound was not significantly different, at the .05 level, even though somewhat fewer were recorded in final trials. This may reflect a tendency of the sharks not to come as close to the stimulus source in the last trials as in the initial trials but still to be sufficiently attracted to cross the ring with about the same frequency throughout the test day.

Three of the four qualitative categories of behavior show no significant difference at the .05 level. These were the IOT, IA, and Av responses. Only the Circles show significant decrements at the .05 level.

Series No. 3B in which high-amplitude, 10-Hz sine waves alone formed the stimulus show different results (Table 12). Here, both A and B responses have a significant decrement from initial to final trials. However, as with broadband sounds in the qualitatively distinguished responses, 10-Hz sine waves show no significant decrement in either IOT or IA responses. The number of Av responses decreased significantly but there was no significant decrement in the number of Circles scored. In this set, the only qualitative category that showed a significant decrement at the .05 level was the Av response. This is in part due to the low, fairly constant level of response in these qualitative categories so that whatever decreases might be present are not large enough to be statistically significant. At the same time there is a decreasing tendency to approach within the scoring area as shown by the decrement in number of A and B responses.

A decreasing number of responses with repeated trials because of the absence of a reinforcer such as food is a consequence of utilizing an unconditioned response as the behavioral measure. Examination of the data for trends of decreasing number of responses showed negative results in the first series of tests (Series No. 1) when considering trials in a test or tests in a series. In Series No. 2, the fact that no significant decrement is noted over trials but significant differences are noted between test days may indicate that unknown factors produced substantial variations in activity and/or response level. Such variation as that expected if habituation were the predominant factor was absent. As previously stated, no significant decrement in the number of A responses was recorded from initial to final trials in Series No. 3A with electronically produced sound containing broad-band sound in the signal.

Series No. 3B in which 10-Hz sine waves alone were the stimulus, shows a significant decrement in both A and B responses from initial to final trials. The indication seems to be that with the testing schedule used in this study, habituation as measured by decrease in response strength from the initial value is present with large-amplitude 10-Hz sine waves as the stimulus. Apparently the test schedule was successful in minimizing the degree of habituation with the other types of stimulus to levels below significance.

V. Effect of Order of Presentation and Time-of-Day on Responses to Mechanically Produced Sounds

Since the order of presentation of the two types of mechanically produced sounds might influence the level of response of the sharks, I used a counter-balanced order of presentation. If sounds produced with the flipper attached were presented first, then the following test day sounds produced with the flipper removed were presented first. The response level when the particular sound was first could then be compared with the level when it was presented second. The total number of responses in three of the four qualitative behavioral measures taken together on each test day were the variates used in this comparison. I was mainly interested in whether the sharks spent more time in the scoring area with any particular order of presentation and not specifically in what they did when in the test area.

This was also the reason for excluding the Av category because the animals might have left the scoring area in performing an avoid response. The statistical test used was the Mann-Whitney-U test because there was an odd number of test days which did not permit the use of the Wilcoxon test. The Mann-Whitney-U test determines the probability that two groups of scores are samples from the same population or alternatively, that one group of scores is from a population stochastically larger than another. Response levels were very low in tests with sounds produced with the flipper removed so to permit comparison with responses to sounds produced with the flipper attached. Total number of responses in each test day were utilized rather than the median number. The results of the Mann-Whitney-U test were identical whether using median or totals for sounds produced with the flipper attached so it seemed valid to use totals.

The test shows that there is no significant difference at the .05 level between the total number of qualitative responses scored with the flipper attached whether presented first or second. No order effects are in evidence with these sounds. The test does show a significantly greater number of these responses at the .05 level with the flipper removed when presented second.

The majority of the treatments performed second took place in the afternoon and evening and those performed first took place in the morning or at noon. The possibility exists that effects may be present that are due to the time of day when the experiments took place. On several occasions when evening presentations were made there was a very high level of response, one that subjectively seemed higher than what had been seen in the morning or mid-day. For example, the occasion when the sharks attacked the IUS took place in the early evening at around 7:20 p.m.

Springer (1950) in observations on the feeding habits of lemon sharks, noted that the species may be caught on baited hooks more successfully at night. Presumably, this indicates that feeding activity is somewhat greater at night than during the day and therefore responsiveness to food-associated stimuli may be greater at night. To test for time-of-day effects, the previously mentioned comparisons were made with the Mann-Whitney-U test but the scores were segregated into a.m. and p.m. groups including those scores obtained in the evening. In this case the test shows that there were greater numbers of responses in the morning to sounds produced with the flipper attached than in the afternoon and evening. On the other hand, there were significantly more responses, at the .05 level, in the afternoon and evening to sounds produced with the flipper removed than in the morning. This seemingly paradoxical result may be explained by the possibility that sensitivity was low in the morning and a high level of stimulus intensity, i.e. with large-amplitude hydrodynamic components, was necessary to elicit a response at that time. In the afternoon and evening sensitivity may have increased and the high signal level that formerly elicited responses became aversive and the lower signal level provided by sounds without large-amplitude hydrodynamic components then produced responses. There may be enhanced sensitivity during periods of greater feeding activity thus producing greater response to the lower intensity sounds in the evening. In order to establish this point conclusively a more accurate measure of relative signal intensity of these types of sound in a more controllable acoustic environment is necessary.

VI. General Observations on Experimental Subjects

The behavior of the captive juvenile lemon sharks used in this study during periods when no stimulus was being presented was characteristically uniform for discrete time periods. For example, if a shark began to swim in small circles it would tend to continue to do so for 2-3 minutes and then might stop and begin swimming in clockwise circuits around the tank. It would then continue with this pattern for another few minutes and then go into still another pattern of activity. This constancy of swimming pattern for discrete periods made it easy to obtain rather accurate measurements of swimming velocity by timing circuits around the tank.

Among the infrequent behavioral acts noted was a sudden, wide opening of the mouth accompanied by a convulsive expansion of the gill openings. This behavior may be similar to the behavior described by Myrbert *et al*. (1971) as "yawning" and "gill puffing," in free-ranging silky sharks (*Carcharhinus falciformis*). Another behavior I occasionally observed was "rolling." In this maneuver the shark rotates its body on its long axis through a 90° arc while swimming and after 1-2 m regains normal, upright orientation. If the animal were close to the bottom when initiating this maneuver, one pectoral fin sometimes dragged on the bottom. Occasionally, the animal rolled its body completely around through 360°. Myrberg *et al*. (1971) noted behavior in *C. falciformis* that may be similar. They observed that silky sharks occasionally would move about in groups of two or three, swimming parallel and simultaneously carry out such maneuvers as sharp turns. At these times (when swimming parallel) there might be a "sudden tilting of the body of one individual sideways, the dorsal aspect being directed at the other individual." This lasted from 3-5 seconds and then the normal position was regained and the shark moved off with the other individual. The significance of this behavior is unknown.

On two separate occasions, one of which occurred during a trial with the flipper attached in Series No. 2B, I observed regurgitation in one individual of the test group. The other two sharks immediately started swimming rapidly all over the tank at high velocity with sudden direction changes. After a few minutes, all the sharks returned to their normal swimming velocity. On neither of these occasions was any recognizable food regurgitated but the regurgitant consisted of a strand of greenish slimy material which floated in the water.

VII. Summary of Results

Experiments conducted with immature lemon sharks captured in the field indicated that they could be attracted with mechanically produced sounds such as those generated by the IUS. This was true of a shark restrained in a pen in which the tide could flow in and out and also of sharks confined in tanks. Sounds with large-amplitude hydrodynamic components proved to be attractive but the attractiveness of sounds without large-amplitude components was not clearly established.

The results of experiments in Series No. 1 with young lemon sharks deprived of the experience of feeding in a natural habitat indicated that sounds produced by the IUS were not attractive but in many instances were aversive. These sharks' responses differed markedly from the responses of sharks captured in the field in that they exhibited a much higher frequency of avoidances. Sounds with large-amplitude hydrodynamic components present were more likely to elicit an avoidance response than sounds with these components absent.

The experiments of Series No. 2A in which animals had been provided with the experience of capturing living prey for two weeks revealed that sounds produced by the IUS were attractive but that testing a single shark at a time produced highly variable results. Series No. 2B in which three sharks at a time were tested showed reduced variability and confirmed that the sounds produced by the IUS now had attractive significance. On one occasion the IUS was attacked and bitten. Sounds with large-amplitude components present elicited more responses than sound with large-amplitude components absent as revealed by analysis of the four qualitative behavioral categories. A steep intensity gradient apparently was present that produced many avoidance responses in addition to the approach responses noted. An explanation has been offered to account for the lack of statistical significance of differences in A and B responses between signal-on periods and control periods. This is related to the sharks' habit of exhibiting uniform, non-random "blocks" of behavior patterns for discrete time periods that were apparently independent of external stimulation. When these patterns occurred in the scoring area during control periods the A and B response frequency was elevated but was not related to IUS signals.

The test signals in Series No. 3 were produced electronically and were of two types. In Series No. 3A broadband noise "clicks" together with 10-Hz sine waves were presented with a J-9 underwater loudspeaker. Sharks came significantly closer to the J-9 during signal-on periods than in control periods as indicated by the B response frequency. Similarly, the four qualitative measures of behavior indicated that these sounds were highly attractive and the responses of the sharks were comparable to those of unrestrained sharks in play-back experiments in a normal environment. The signals in Series No. 3B were high-amplitude 10-Hz sine waves without broad-band noise. While these sounds also were attractive, the response level was lower than that in Series No. 3A. The presence of broad-band noise in the signal greatly enhanced its attractiveness to these sharks.

Tests in which a tethered, struggling fish was used as a stimulus to determine the level of responsiveness of sharks showed great individual variability even with such "naturally produced" signals. This variability in degree of responsiveness was reduced by testing sharks in groups rather than singly. Groups of three sharks had approximately the same degree of responsiveness as measured by latency to attack the tethered fish as did groups of ten sharks.

No significant response decrement or habituation from initial to final trials in a test was observed in

Series No. 1 and 2B. There was considerable variation in response level from day to day in Series No. 2B, however, but no consistent downward trend that might indicate habituation could be detected. In series No. 3A the number of B responses dropped. Some habituation with these electronically produced signals was thus indicated over trials but there were insufficient data to determine whether habituation may have occurred over the series.

Analysis of the data in Series No. 2B to detect differences in response level that may be associated either with order of presentation of signals or with time-of-day of presentation was performed. A higher response level to lower-intensity sounds produced without the flipper if presented second was discovered. Further analysis indicated that these effects may have been due to the time of day when presented rather than order of presentation. The response level was higher to high-intensity signals if presented in the morning and on the other hand, the response was higher to low-intensity signals if presented in the afternoon or evening. An hypothesis to explain these results has been offered which postulated daily variation in sensitivity to acoustic signals conditioned by variation in feeding activity.

DISCUSSION

I. Introduction

The following discussion will begin with an examination of the results of the present study in the light of other evidence on the biology of young lemon sharks. First to be considered is the species-typical responses of juvenile lemon sharks to artificially produced, low-frequency sounds and their species-typical feeding habits. Secondly, the responses of sharks deprived of species-typical feeding experiences will be compared with the responses of these same sharks after they had experienced the capture of living prey. The initial responses of naive sharks to injured, living prey will also be noted and related to their responses to low-frequency sounds. The results of the present study will then be related to the possible role of low-frequency sounds in normal feeding behavior.

The discussion will then turn to a consideration of the experimental technique of experiential deprivation and the type of experiments generated by this technique. The kind of experiments that are carried out in any investigation are strongly influenced by the theoretical viewpoint of the investigator. A brief consideration of some approaches to the study of experiential effects that have been used in the past may be helpful. Two theoretical approaches to the problem of analyzing the effects of experiential deprivation will be presented. Some experimental work that is concerned with these effects at the behavioral level will be presented to illustrate the two approaches. Then a few studies will be cited that provide examples of the effects of stimulus deprivation at lower levels of organization, that is, at the level of sense organs and neural elements. The examples will be selected from work on mammals, birds and teleost fish in order to provide a broad comparison with elasmobranchs.

Having considered the effect of the absence of certain kinds of stimulation, the discussion will then be concerned with the effect at the behavioral level of the presence of stimulation. Some of the formulations that were useful to other investigators in the study of higher animals will be discussed. These formulations refer to the characteristics of a stimulus, the characteristics of a response, and the nature of associations between stimulus and response and an attempt will be made to apply them to elasmobranchs.

Finally, some of the unanswered questions that were raised in the present study will be outlined and suggestions for further study will be made.

II. The Significance of Feeding Experience

A. Sharks With Species-Typical Feeding Experience

The preliminary experiments conducted in 1970 indicated that juvenile lemon sharks that presumably had species-typical early experience exhibited an unconditioned approach response to sounds produced mechanically by means of the Ichthyo-Uro-Simulator Mk II. Their responses resembled those reported by other investigators for young lemon sharks, adult lemon sharks, and other species as well, when presented with various types of electronically produced sounds. The attractive significance of these sounds is presumably related to their association with potential prey.

Banner (1972) noted that the stomach content of young lemon sharks was predominantly whole mullet (*Mugil* sp.) and he concluded that these sharks are primarily active predators rather than scavengers. In the past some reports on the gut contents of sharks, especially tiger sharks (*Galeocerdo cuvieri*), have led to the impression that these animals are predominantly scavengers. These reports may have been based on the stomach contents of animals that were members of "accessory" populations in the terminology of Springer (1963). Shark fishermen whose catch consisted of such animals found that these sharks had a wide variety of materials in their stomachs including garbage, artifacts, tin cans, etc. Members of accessory populations apparently have different food habits from members of "principal" populations in that those sharks taken from the principal populations had stomach contents of fresh fish and could only be caught with fresh fish as bait. The normal experience of young lemon sharks presumably would be to attack and capture active prey in shallow water environments. Some degree of selectivity is present since the proportion of mullet in their stomachs was much greater than the proportion of mullet in the area studied by Banner. It is an advantage to a predator to be able to detect and localize the source of any type of signal or disturbance produced by potential prey. An approach response to such signals is likely to place the predator in the vicinity of the prey and facilitate prey capture. It is not as easy to see what advantage a detection system of this type would have to an animal that is primarily a scavenger and feeds on dead organisms and organic matter.

B. Sharks Deprived of Species-Typical Feeding Experience

The deprivation of sharks from normal post-natal experience prevented them fron encountering natural feeding situations. In the artificial feeding situation provided, that is, presentation of cut fish to the sharks in their home tank, visual, tactile, gustatory and olfactory stimuli were not removed. Presumably the sharks were able to see the pieces of fish settling to the tank bottom and were able to detect the blood and body fluid emanating from the pieces of fish and so recognized them as food objects. These sharks were induced to feed on cut fish on the basis of the visual, tactile, gustatory and olfactory information present and in the absence of any known auditory information such as that produced by prey organisms in a natural feeding situation.

Since the animals were not individually isolated but maintained as a group, the stimuli provided by group behavioral interactions were not absent. Auditory stimuli produced by the sharks themselves in the act of feeding were present and may have had a positive influence on food consumption. However, this influence would only be present after feeding had already been initiated by other stimuli.

The stimuli that were present and the total experiential and maturational events that occurred in these sharks until the time of testing did not cause the sharks to be attracted to sounds produced by the IUS; in fact, the responses of the sharks indicated that these sounds were mostly aversive.

C. Sharks Experienced in Capturing Live Prey But Without Species-Typical Feeding Experience

In initial presentations of live, damaged fish these sharks exhibited startle reactions and withdrawal responses but subsequently recognized the fish as prey and pursued them. It is possible that the high degree of novelty of the auditory stimuli and water disturbances produced by the irregularly swimming fish caused the stimulus to be aversive.

This will be more fully discussed below.

Comparison of the responses recorded in Series No. 1 with those in Series No. 2 indicated a greater tendency to approach close to the signal source after the animals had experienced the capture of living prey. The sounds with high-amplitude hydrodynamic components that had been mostly aversive to the sharks became attractive after the experience of capturing live prey as shown by responses in the four qualitative behavioral categories. These responses resembled the ones exhibited by sharks tested in the preliminary experiments of 1970 that had had normal early feeding expierence. The inference can be made, therefore, that sounds produced by the IUS are attractive as a result of association with stimuli encountered in feeding experiences. The experience of the pursuit and capture of irregularly swimming prey appear to have been essential for these sounds to be attractive to immature sharks previously deprived of species-typical feeding experience.

D. The Significance of Responses to Low-Frequency Sounds in Feeding Behavior

Banner (1972) attempted to relate the attractiveness of various recorded sounds to their association with potential prey of the sharks. His results indicated that the relative attractiveness of any particular sound was not related to the relative importance as prey of the animal that produced it. For example, the most attractive sounds, other than broad-band pulsed noise, were those produced by catfish, and catfish are not normally preyed upon by young lemon sharks. This indicates that sharks respond to a rather broad range of frequencies. The characteristic that all these sounds had in common was their pulsed nature which seemed to be more important than their frequency composition, although Banner did not use frequencies below 20 Hz. The fact that broad-band, pulsed noise was more effective than any of the sounds of biological origin further indicated the non-specificity of the stimuli eliciting an approach response. This may be related to the similarity of pulsed, broad-band noise to sounds produced by an accelerating or turning school of fish. Such a fish school could represent prey to a shark and an approach response to sounds of this type would increase the probability of placing a shark in a feeding situation and the response is, therefore, adaptive.

Feeding in sharks is largely opportunistic and of a low order of efficiency. Springer (1960) noted that carcharinid sharks are relatively ineffective in catching uninjured fish in open water or even in finding such slow-moving objects as crabs. Springer (1950) also observed that young lemon sharks are frequently found among schools of mullet but are not efficient in capturing them. They "work very hard at it and if the mullet are abundant enough the sharks get a satisfactory fare." Approach responses to generalized stimuli would thus increase the probability of survival if in a significant proportion of instances, the stimulus producers, in fact, turned out to be suitable prey.

These responses to generalized sound stimuli may be due to the possibility that sharks are not able to discriminate frequencies very well. Nelson (1967) found that young lemon sharks were only capable of discriminating one-half octave differences in pure tones. In sharks, the upper frequency limit for response to sounds is 1000 Hz. It has not been established whether the lack of response to frequencies higher than 1000 Hz is due to inability of the sensory apparatus to detect them or to some form of stimulus filtering, either peripherally or centrally.

When the shark is close enough to the sound source, other stimuli may be necessary to elicit a complete feeding response and/or to prevent a withdrawal or avoidance response. The generalized nature of the sound stimuli effective in producing attraction make it unlikely that sharks actively seek out specific types of prey, but once a feeding situation is encountered, some degree of prey selection may be exercised on the basis of information provided by other sensory modalities.

III. Experiential Deprivation

A number of experimental techniques are currently in use to assess the effect of experience on behavior. In some of these studies the investigator sets out to determine whether the environment or the genotype is more important in given behavioral acts or sequences and emphasizes one or the other side in the nature-nuture dichotomy. The isolation experiment is frequently used in such a study and the appearance of certain behavior in the apparent absence of learning or previous exposure to conspecifics is cited as proof of the "inborn," "innate," or "predetermined" nature of the behavior (Moltz, 1965). An investigator who does not view the development of behavior in terms of the innate vs learned dichotomy may use the isolation experiment to determine that certain types of experience, e.g., learning, are or are not necessary for the appearance of a given behavior. It may also provide a stimulus for further research by indicating that other types of experience are occurring at other stages of development.

Too often, in isolation experiments, the previous history of the animal is inadequately specified and stimulative events in earlier ontogenetic stages prior to the beginning of the experiments are manifested during the experiment. For example, in the work of Fuller (1967) on dogs, the experimental animals used in experiential deprivation studies were puppies removed from their mothers at the age of 21 days before which time they cannot survive independently. The experience of the puppies until this age is undoubtedly not uniform and depends on litter size, age and experience of the mother, and other factors. These differential experiential factors may influence later behavioral tendencies in isolation experiments. In deprivation-type experiments, it must be stated as clearly as possible which forms of stimulation and experience the animal is being deprived of and which stimuli are present.

A. Theoretical Formulations in the Investigation of Experiential Effects

We will now consider some theories generated by deprivation-isolation experiments and attempt to relate them to the results of the present study and other work on elasmobranchs. Fuller (1967) discussed two theories propounded by other investigators and advanced one of his own in relation to his work on dogs. He referred first to the theory of Thompson and Heron (1954) derived from Hebb's (1949) concept of the necessity of stimulation for the occurrence of perceptual learning. Isolation then would be the passive withholding of essential information. Secondly, he referred to the theory of Lessac (1965) which states that under the conditions of stimulus deprivation, established perceptual mechanisms deteriorate. Fuller's own theory, which he called the emergence-stress model, attempts to account for the disruption of behavior observed when isolates are introduced into the test arena. "The excessive arousal in an organism exposed to a myriad of unfamiliar stimuli is assumed to produce overload in the neural systems underlying many forms of behavior." The isolated animal in Fuller's view becomes habituated to the low-level stimulation of isolation, and its sudden emergence into a condition of high stimulation produced the "disrupted" behavior. The level of stimulation here is influenced by its novelty and its complexity. Fuller's theory "plays down the role of experience in guiding perceptual learning . . . and . . . places more emphasis on intrinsic forces in behavior development than accords with much current psychological thought." These considerations introduce the concepts of arousal and habituation as well as novelty and complexity of stimulus as factors that influence the development and expression of behavior. Although these concepts have been used in reference to mammals they may, nevertheless, be useful in the study of the behavior of animals that are at a less complex psychological level, for example elasmobranchs.

In comparing his results with those of Thompson and Heron, Fuller concluded that their theory had not been substantiated because isolation, while producing many animals that did poorly in learning tasks, also produced some excellent learners. Moreover, in a few animals, simple orientation, approach and manipulative behavior patterns appeared "instantaneously" at the time these animals were given their first arena test. Fuller emphasized the variability of his results and cited it as grounds for rejecting the Hebb-Thompson-Heron theory.

An opposing viewpoint to that of Fuller has been provided by Riesen (1961) who has analyzed the problem of the effect of stimulation on behavior development at two different levels of organization. Riesen first considered the effect of restricted stimulation on neural elements including peripheral sense organs. Secondly, he discussed the stimulation required for the appearance of various behavioral capacities and set up four categories of adaptive responses based on the type of antecedent experience required.

1. Innate activities and responses. In this category Riesen included mammalian postural reflexes, the startle response, nystagmus of vestibular origin, reflexes with autonomic mediation including the arousal reaction, and reflexes such as pupillary, palpebral, and pinna responses. He also considered the capacity of stimulus generalization to be an innate behavioral mechanism that follows from the properties of the particular receptor system involved. By the use of the term "innate" he does not imply that these responses are to be considered impervious to prior stimulation but only that they can be elicited by the very first stimulation of the appropriate receptor.

2. Perceptions requiring sensory preconditioning. In this category Riesen attempted to separate sensory learning from motor learning and cited experimental work to illustrate this. Withholding patterned visual stimulation

by rearing cats in the dark or in diffuse light has different effects on two sensory processes. Where only visual intensity discrimination is required in orientation and approach to a light source, the deprived animals perform readily. "This discrimination transfers promptly from a single eye used in training to the contralateral eye even when that eye has never received light stimulation previously." In movement and pattern vision tests, however, both initial learning and interocular transfer are significantly retarded. In these tasks the sensory information must be integrated through space and time, and apparently the mechanism responsible for this integration requires patterned light stimulation for proper functioning.

3. Perception dependent upon prior sensory-motor and motor-sensory associations. Included in this category are latent learning, spatial delayed response, and shifts in egocentric localization. These are all involved in the spatial orientation of animals. An example is the inability of cats reared in unpatterned light to discriminate moving from non-moving two-dimensional figures. Moreover, cats that did receive patterned light stimulation but only while restrained in a holder were able to coordinate their eyes, to make normal pursuit movements with eyes and head, and to orient well to moving persons while restrained, but were still unable to discriminate moving from non-moving two-dimensional figures. Apparently, these animals while moving

toward the choice point and to the surface onto which the stimuli were projected during tests, were unable to distinguish self-initiated from externally produced movement across the visual field. In this case, the prevention of the motor experience of locomotion while receiving the patterned light stimuli was sufficient to impair the development of the mechanisms underlying this type of movement discrimination.

4. Acquired response-response units. This category includes motor integrations of the type in which less and less detailed sensory control is present as the response develops, for example, as in "kinesthetic memory" or movements in which sensory control has been experimentally eliminated. An example of the latter is the autonomous rhythms of eye and the head movements that develop when cats or dogs are reared in darkness.

The position taken by Riesen (1961) is that stimulation is a requirement for growth and function and that deprivation of stimulation will have different effects at different ages of the organism. The extent and duration of deprivation are also important in that the effects of shorter periods of deprivation may be reversed by stimulation while longer periods may produce effects that can be incompletely reversed or are irreversible. Riesen reviewed a number of deafferentiation studies performed on the visual systems of fetal and infant mammals and concluded that there are both temporary and long-term effects with a more serious degeneration of structure the younger the animal is. The literature was inconclusive on the reversibility of prolonged deprivation. The theory of Lessac (1965) mentioned above is not inconsistent with the view of Riesen. Continued stimulation seems necessary to prevent deterioration at the neural level and also, presumably, at the behavioral level.

In summary, it may be said that the degree of influence of experiential factors in the development of perceptual mechanisms and of behavior in general that are discovered in any particular investigation may be a function of the responses selected as the experimental variables. Measurement of gross responses may not reveal subtle differences in behavior that arise from the differential functioning of one or more mechanisms in a given behavioral act. The method of approach suggested by Riesen would permit the detection of subtle differences in behavior produced by these mechanisms.

B. The Effect of Stimulus Deprivation on the Development and Function of Neural Elements and Sense Organs

The theoretical position that the role of experience in perceptual learning is less important than "intrinsic forces" does not seem to be substantiated by some recent studies on birds and mammals. Cynader *et al*. (1973), in a neurophysiological-behavior study on cats, investigated the receptive field properties of the visual cortex of animals reared in stroboscopic illumination. Under these conditions the animals were deprived of experience of visual movement, but not of visual form from birth to six months of age. Single neural units were recorded in area 17 of the cortex, and their orientation selectivity was determined when a unit fired differentially in response to flashed slits of light with different spatial orientations. Units in strobe-reared cats showed greatly reduced direction selectivity compared with normally reared cats, and some units in strobe-reared cats responded only to strobe flashes.

The altered functional characteristics in the visual system at the neural level that were produced by this type of early experience undoubtedly have significant effects at the behavioral level.

An interesting question considered earlier but that also should be raised here, is how permanent are the effects produced by early experience that differs from normal. Marler _et_ _al_. (1973) have provided some information in a study on the effect of continuous noise on hearing and vocal development in canaries. Two groups of males were raised to 40 days of age in continuous noise. One group was then deafened surgically and the other left to mature in soundproof chambers. Surgically deafened birds developed significantly fewer song syllables than intact birds. Other groups raised in noise to sexual maturity at 200 days sang at first like surgically deafened birds but gradually increased their repertoire. The effect of the noise was to prevent auditory feedback from the birds' own songs but with an ancillary effect of damage to the birds' hearing, especially at the higher frequencies. Birds prevented from hearing their own songs were unable to develop a normal song repertoire but if subsequent auditory feedback was permitted at least a partial recovery occurred. This study illustrates that at least some animals at the level of psychological complexity of birds require patterned auditory stimulation for the development of normal vocal behavior. Moreover, it shows that the effects of abnormal stimulation are manifested both at the sense organ level and at the neural level. If physical damage to the sense organ is not severe, the presentation of normal stimuli does permit some recovery of normal function at the neural level.

A neurophysiological study by Sharma (1972) on the visual projections in goldfish illustrates a certain capacity of sensory systems to retain normal function in spite of extensive, surgically-produced neural damage. Sharma removed a rostro-caudal strip of optic tectum and constructed electrophysiological maps 1 month and 4-5 months after ablation. After 1 month he found no response from units in the visual field that corresponded to points in the ablated tectum. After 4-5 months, however, he found that the entire visual field had become redistributed over the remaining tectum and had compensated for the missing segment. It would be interesting to determine the behavioral consequences of these neural redistributions. The effect of stimulation on this phenomenon was not conclusively demonstrated, although Sharma did crush the optic nerve contralateral to the ablated tectum. He maintained that there were no differences between maps of fish with intact or damaged optic nerves. A behavioral study is required to determine the extent, if any, of functional differences produced by stimulations while these neural redistributions are taking place.

The above studies on mammals, birds and teleost fish indicate that function has a pronounced effect on structure in that structural changes induced by deprivation of normal stimuli and by surgery can be reversed to various extents by the restoration of normal stimuli. At the neural level, this would seem to lend further support to the proposition that there is a reciprocal relationship between structure and function (Gottlieb, 1970).

There are very few deprivation studies on elasmobranchs but the work of Graeber and Ebbesson (1972) and Graeber _et_ _al_. (1973) may be mentioned here. These investigators studied visual discrimination learning in normal and tectal ablated nurse sharks (_Ginglymostoma_ _cirratum_). Although this is not a stimulus-deprivation study, it does provide information on the ability of sharks to recover from neural deficits. Normal sharks, in the course of learning color and shape orientation discrimination, were seen to manifest some of the stereotyped behavior seen in animals at a higher level of psychological complexity. This was the so-called position preference and vicarious trial and error observed in mammalian studies. After bilateral tectal ablation, naive sharks were able to learn the same discriminations as did normal ones within the same range or number of trials to acquire the responses. These results were interpreted to represent some type of recovery of visual function and cast doubt on the assumption that tectal ablation in sharks causes blindness. Retinal projections in sharks may not be confined to the tectal area but may "distribute to other areas in [a manner in] common with many other vertebrates." These other areas in the shark brain may perform the integrative functions necessary to promote learning of these discriminations.

IV. The Effect of Stimulation on the Development of Behavioral Responses

A. Characteristics of a Stimulus

1. Novelty

The behavioral consequences of a stimulus cannot always be explained entirely on the basis of its purely physical characteristics such as intensity and duration. In an earlier section we referred to the concepts of novelty and complexity of a stimulus. The novelty of a stimulus is a function of the past experience of the animal that is receiving it and the efficiency of the memory-storage mechanisms of the animal. It may perhaps be safely assumed that the concept of novelty has relevance only to those stimuli that after being mediated by the peripheral sensory apparatus, leave trace effects in the central nervous system integrative mechanisms. Welker (1961) in his discussion of exploratory and play behavior in animals made use of the concept of stimulus novelty. While "play behavior" as it is recognized in mammals and birds does not occur in elasmobranchs, exploratory behavior does occur and may be identified as movements of the animal that provide the sensory apparatus with new and different stimuli. This has relevance to shark behavior since it is quite characteristic of sharks to swim slowly about in a random fashion subjecting stimulus objects that are encountered to tactile, olfactory, gustatory, auditory and visual examination (Banner, 1972; Springer, 1967). One of the factors that governs the type of response an animal makes is the degree of novelty. Welker has postulated that if the degree of novelty is very high, the animal may withdraw. If the stimulus is not novel at all or very low in novelty, the animal may ignore the stimulus object. At intermediate degrees of novelty, the animal may approach and continue to examine the stimulus object with its other sensory modalities. The concept of novelty is of special relevance in young animals. There may be a period of maximum plasticity and adjustment to novel stimuli when approach-withdrawal thresholds are established. Experience during this period would significantly influence what types of stimuli will, in the future, elicit withdrawal and what types will elicit approach. Young sharks are typically more prone to approach novel stimuli if their arousal level is within certain limits than are adults. If harmful consequences result from approaches to a particular novel stimulus the probability of subsequent approaches to this stimulus is decreased. If beneficial consequences result, then the probability of subsequent approaches would increase. In this way the young animal learns to avoid certain stimuli and approach others. In cases when the consequences of an approach are neutral, that is neither beneficial nor harmful, the animal may exhibit approach behavior until the degree of novelty has dropped too low. Further approach responses may then be eliminated. In this way non-adaptive responses would gradually be eliminated and adaptive responses maximized until a certain repertoire of responses had been established, a repertoire characteristic of the adults of the species and relevant to certain classes of stimuli. Any stimuli that do not fall into these classes may then be ignored.

The manner in which experiential factors may assist in the differentiation of the feeding response in young sharks is to alter the thresholds of relatively simple approach-withdrawal mechanisms that were established in earlier developmental stages (Schneirla, 1957). Initially, thresholds for withdrawal are raised and stimulation can then attain threshold levels for approach responses. The auditory modality would probably be of prime importance in establishing either approach or withdrawal in the initial phase of the feeding response while olfaction would have more immediate control over the level of arousal. Once approach responses have been established, higher order processes may become involved. These may produce trace effects that increase the probability of approach to certain stimuli and decrease it for others.

The sharks in the present study may have avoided live, damaged fish upon their initial presentations because the degree of novelty of the stimulus configuration provided by the fish was too great and hence was aversive. Visual, olfactory, and auditory stimuli of specific kinds at the intensities presented may have had too great an impact to be attractive. In subsequent presentations, stimuli presumably of the same intensity range as initially may then have a gradually decreased novelty until arousal levels were produced that permitted pursuit and capture of the damaged fish. Fiske and Maddi (1961) have proposed that the level of central nervous activation produced by a stimulus is a measure of its impact. This impact, in turn, is a function of the (1) intensity, (2) variation from previous stimuli, and (3) meaningfulness of a given stimulus. Novelty would enter into the formulation of Fiske and Maddi by affecting item (2); in their view, variation refers to differences from previous stimulation of both intensity and specific type of stimulus. Novelty then would be equivalent to variation in type of stimulus. These proposals will be discussed further in relation to impact of a stimulus.

2. Stimulus Complexity

Most natural situations involve constellations of stimuli. The number of elements in addition to the relative novelty of each of the elements in the stimulus array determine the complexity of the stimulus situation (Welker, 1961). The degree of stimulus complexity influences responses of animals in different ways depending on the psychological level of the animal. Presumably, animals with higher levels of psychological organization can attend to more complex stimuli and make a greater number of different types of responses to stimuli than can animals with lower levels. Nevertheless, the way in which stimulus complexity exerts its effect may be similar at all psychological levels. Stimulus complexity affects the degree of novelty and therefore, influences the rate of habituation. A more complex stimulus will maintain its novelty value for a greater number of repeated presentations than a simple stimulus (Welker, 1961). The animal may tend to explore complex stimulus configurations which contain many elements by subjecting each individual stimulus element in the array to examination with all of its sensory equipment. When it has examined all the elements of the array the first element may be examined again. This represents a "recovery from habituation" in the terminology of Welker (1961) and retards the overall rate of habituation to the stimulus array. It is possible that excessive degrees of complexity may be aversive and would produce withdrawal or disrupted behavior as has been suggested by Fuller (1967). The three concepts of novelty, complexity, and habituation are interrelated and are different aspects of the attractive significance of stimuli.

The complexity of the stimulus array represented by a damaged irregularly swimming fish was considerably greater than the stimulus produced by the IUS. The sensory modalities of vision, olfaction, gustation, audition and touch comprised the "natural" stimulus while only audition comprised the mechanically produced stimulus. If the reinforcing properties of eating the fish could be eliminated, in order to create truly comparable conditions, habituation would be expected to occur more rapidly with the simpler stimulus. In spite of the relative lack of complexity of the stimulus, however, it did produce a level of arousal such that the sharks attacked the IUS in the absence of the other stimulus types.

3. Stimulus Intensity

The intensity of a stimulus of any type has great effect on the nature of responses. There may be an optimal range of intensity for a given stimulus which if exceeded will produce either no response or will change the sign of a response, that is, if the response is normally an approach it will change to withdrawal.

Stimulus intensity with auditory signals is important in determining the nature of the response in young sharks because the near-field effect is considerable at the low frequencies that are attractive. This effect attenuates rapidly so that the signal exhibits a steep intensity gradient. At high intensities, an animal might detect the signal at distances where vision would not be involved and it could orient to the source by means of its displacement detectors, the lateral line organs. If the animal, in continuing to approach the source, comes close enough, it might encounter such a steep intensity gradient at high signal levels that the withdrawal threshold would be exceeded and the animal would withdraw in spite of the attractive significance of the sound. Sounds produced by the IUS that contain large-amplitude, hydrodynamic components seemed, frequently, to produce this type of withdrawal.

B. Characteristics of a Response

1. Arousal

The term arousal has been defined by Fiske and Maddi (1961) as the external manifestation in different parts of the body of central-nervous-system activation. The level of CNS activation is influenced by the total impact of all the stimuli impinging upon the organism at any point in time and may vary from moment to moment. A further consideration of the factors influencing impact is in order. Impact as defined by Fiske and Maddi (1961) is a function of the intensity, variation, and meaningfulness of a stimulus. Both variation and meaningfulness are terms that have relevance to the experience of the animal prior to the time of stimulation and affect the level of CNS activation produced by the stimulus. For example, a constant stimulus of uniform intensity such as a continuous, low-frequency noise has low variation and would have less effect in changing the level of CNS activation in a shark than a pulsed, low-frequency noise. The varying temporal and intensity characteristics of pulsed noise would result in a greater impact of the stimulus. Meaningfulness refers to the degree of association of a stimulus with an adaptive response on the part of the animal. A stimulus of low meaningfulness would be one to which the animal makes no response such as the lack of response of young lemon sharks to the sounds of crabs crushing hard food (Banner, 1972). These sounds may not be associated with beneficial consequences (i.e. providing food) for an approach response and so are neutral. If any tendency to approach such sounds upon the first encounter existed it would not have been reinforced upon subsequent encounters and the sound would have been eliminated from the class of attractive stimuli. A stimulus of high meaningfulness would be one that, in a high proportion of cases, is associated with a characteristic response on the part of the animal. Johnson and Nelson (1973) have described a characteristic behavior in sharks that can be elicited by a diver charging a shark (*Carcharinus menisorrah*) that is partially restricted from withdrawal. This behavior consists of laterally exaggerated swimming, rolling or spiraling including four postural elements; lifting of the snout, dropping the pectoral fins, arching of the back and lateral flexing of the body. Johnson and Nelson believed this behavior to be a defensive threat posture and possibly of value in normal social encounters such as dominance situations or territorial defense. In this case, the stimulus of an approaching large object such as a diver or other large shark would have high meaningfulness and would produce elevated arousal. This indicates a high level of CNS activation.

The level of CNS activation as measured by arousal has a strong influence on the response made to succeeding stimuli and is in part responsible for the variation in behavior sometimes noted in experimental situations. It has been postulated (Fiske and Maddi, 1961) that each task or activity in an animal's behavioral repertoire has a characteristic level of activation which is necessary for maximally effective performance. Activation levels above or below this value result in poor performance or disrupted behavior. The animal's behavior then would be directed so that it would modify its activation level toward the optimal value for the task at hand. In the discrimination studies on nurse sharks (Aronson, et al., 1967; Graeber and Ebbesson, 1972) too-low levels of activation might have caused the shark to lie on the bottom and fail to respond to stimuli; too high activation levels might have produced rapid swimming about and failure to attend to the stimuli present. The young lemon sharks in the present study exhibited such behavior and failed to respond to the same stimuli at high activation levels to which they did in fact respond at lower, more optimal levels of activation.

In the view of Fiske and Maddi, each stage of an organism's daily activity cycle has a characteristic normal level of activation, and in the absence of specific tasks, the organism behaves so as to maintain this normal level of activation. They further postulate that negative affect results when the level of activation differs from normal and positive affect results when the activation level is shifted toward normal. At the psychological level of elasmobranchs it is rather difficult to evaluate positive and negative affect, but the so-called threat posture described by Johnson and Nelson (1973) may be indicative of affective state in sharks. At least, these investigators believed that this behavior is not related to predatory behavior or normal food capture and that it may indicate some shark attacks are not food stimulated.

2. Behavioral Habituation

Another characteristic associated with responses is habituation. This was referred to earlier in connection with the response decrements observed in the field play-back studies on sharks.

The term habituation, as used in behavioral studies, is not a precisely defined category but usually refers to a response decrement to repeated or continuous stimulation. Hinde (1970) examined some of the ways in which the term should be used and its limitations. He believes it most important to avoid application of the term to cases in which the response decrement can be ascribed to the intervention of a neuronal refractory period, sensory adaptation, or effector fatigue. Hinde then listed five ways to test for the presence of any of these three factors. A second important limitation pointed out by Hinde is that the decrement should not be the result of a "consummatory stimulus" in which the animal encounters a new stimulus as a consequence of the response. The example he gave was a case in which an infant monkey on avoiding a withdrawal-producing stimulus showed a response decrement as a result of contact with its mother.

Hinde (1970) pointed out that although most experimental situations in which habituation was considered, involved repeated presentations of the stimulus, some involve continuous stimulation. There are at the present time insufficient data to determine how the properties of the habituation produced by these two kinds of stimuli differ.

The extent of recovery from habituation may determine to a large extent the selectiveness of the responses in an organism. If habituation to certain stimuli is of long duration or if extinction occurs, the number of different types of stimuli that elicit responses may decrease as the animal gets older. Certain responses may be more susceptible to recovery than others and these would remain in the behavioral repertoire longer, perhaps even for the rest of the life of the animal. Approach responses by sharks to olfactory stimulating substances such as the blood and body fluids of fish could be included in this category. Even if short-term habituation to these stimuli has occurred, there is survival value in the recovery of the response since it is important in detecting food. Another example is the startle response of sharks to sudden disturbances. Habituation in specific circumstances may occur but the recovery of the response is necessary for the animal to survive by avoiding its enemies. Startle responses mainly involve vision and hearing and are produced by the sudden appearance of large objects in the visual field, quick movement of an object, or sudden, loud sounds. Of course, tactile sense could also involve startle responses since a shark that is suddenly hit will dart away.

The temporal pattern of stimulation has great effect on the rate of habituation in addition to the complexity of the stimulus. Welker (1961) noted that the duration, frequency, and recency of a stimulus are all factors affecting the rate of habituation. Prolonged durations of signal lead to a greater habituation rate than short periods of duration; a high frequency of repetition produces greater reduction in response than does low frequency; and short intervals between successive stimuli produce faster habituation than long intervals. The effect of massed trials in accelerating habituation depends on the frequency of presentation. In Welker's view, repeated stimulation decreases the degree of novelty and this decrease in novelty produces a decrease in arousal and, therefore, reduction in response results.

Recovery from habituation, once habituation has occurred, depends on the same three factors that influence the rate of habituation. Shorter periods of signal duration upon retesting an habituated animal would retard the rate of recovery less than long periods; longer intervals between trials and between sessions of stimulus presentation would produce a response rate closer to the initial value than would short intervals.

The schedule of stimulus presentation used in the present study was designed to minimize habituation. An accurate assessment of habituation rates in lemon sharks is not possible from the data obtained but an experiment to provide this kind of information may be suggested. A stimulus of known attractiveness such as pulsed, broad-band noise could be presented to groups of lemon sharks on a schedule designed to test the effect of different numbers of presentations of a given duration on response decrements from initial values. Other comparable groups could be tested on a schedule in which different time intervals between successive trials would be utilized. When significant response decrements had been obtained, one group of lemon sharks could be re-tested after a given time interval, another at a longer interval, a third at a still longer interval, and so on in order to determine the recovery time of the response. If a sufficient number of animals were available, different groups could be presented with different lengths of stimulus duration to determine the effects of longer and longer duration on response decrement. One series could include continuous stimulation presented until responses ceased.

C. Stimulus-Response Associations

1. Critical Periods

Considerable effort has been devoted to the investigation of the effect of certain types of stimulation during discrete periods in the life of young animals. These time intervals have been termed critical periods and it has been found that stimulation at ages after these periods is less effective or ineffective in promoting the development of a particular characteristic response. A well-known example is the following response of ducks and chickens to moving objects in their visual field in the first few days after hatching. When this response has developed, it is exhibited upon almost every presentation of the imprinting stimulus. The concept of critical periods in behavior development when applied to phenomena other than imprinting has been criticized by Schneirla and Rosenblatt (1963). With reference to their work on the social behavior of kittens, they stated, "at all age periods social approaches preliminary to feeding behavior undergo a course of development in the litter situation significantly different from the behavior of kittens reared under conditions of isolation and fed from an 'artificial mother'. No evidence was found for any time interval in which the different conditions of rearing failed to produce a pattern . . . that was significantly different from that in normally reared kittens. . . ." They further stated that the concept of critical periods differs from Schneirla's theory of behavioral development which emphasizes the fusion of "maturation (growth-contributed) and experience (stimulation-contributed) processes at different stages in ontogeny . . . and that the contribution of maturation and experience . . . as well as the interrelations of these contributions may differ greatly according to stage in any animal."

There is no evidence that a phenomenon of imprinting such as that seen in birds and mammals, or such sharply defined behavior as following a stimulus object exists in young elasmobranchs. The degree of variability in type of response to any given stimulus is high in these animals, and the strength of association of a response with a stimulus is not necessarily high in a large number of behavioral situations. There is some indication, for example, in the present study, that the type of experience a young elasmobranch has in the early stages of life affects its later behavior, including the effects of stimuli experienced at later ages. The extent of these effects and their permanence remain to be established.

In sharks, different components of the feeding response may be formed at different stages as the system of maturational and experiential elements progresses through its characteristic levels of organization. In some shark species, the development of the components necessary for the actual ingestion of food is completed while still in the uterus. In the Odontaspididae (Springer, 1948), one shark embryo will devour all the other eggs in the uterus. The stimuli necessary to elicit this response may be gustatory and olfactory. The role of visual and auditory stimuli is difficult to speculate upon but is probably not one of primary importance. In any case, the nervous system is sufficiently well developed so that afferent impulses from sensory receptors carry relevant information to integrative centers in the brain with the resultant motor response of opening and closing the jaws and swallowing potential littermates.

The degree of completeness of neural organization exhibited in ovoviviparous sharks at such an early stage in the developmental sequence is relatively high but may vary somewhat from species to species since most sharks do not exhibit this type of intra-uterine behavior. In some other species, nutrient material other than that provided by the yolk mass is supplied in the uterine fluid but the details of the process of ingestion of this fluid are not known.

The elements in the maturational-experiential system that are necessary for the expression of a positive response to sounds such as those produced by the IUS, apparently exert their effect later in the sequence of development than elements primarily involved in the process of ingestion. During the two months prior to the beginning of testing in this study, near-term sharks could be induced to feed on cut smelt soon after removal from the uteri of the mother. An experiment that might have been performed had circumstances permitted is to segregate the newborn sharks into two groups. Both groups would have been presented with sounds produced by the IUS immediately after birth and subsequently during a test series long enough to establish conclusively the nature of their responses. After this time, one group would be given the opportunity to feed on live fish for a two-week period and the other would have continued to be maintained on cut fish of the same species. Both groups would then have been tested again. The control group could be tested at periodic intervals until sexually mature. In this way, the effect of stimuli associated with the capture of live fish on earlier stages than those in the present study could be evaluated and the long-term maturational effects together with the effects of altered experience on the response to sounds could be observed without the intervention of stimuli produced by live prey. Experiments on still earlier stages involving intra-uterine behavior would depend on the availability of gravid females and would pose formidable technical obstacles.

2. Behavioral Plasticity at the Elasmobranch Level

It is commonly accepted that elasmobranchs have a lesser degree of behavioral plasticity than teleosts and higher vertebrates, and support for this assumption is drawn from the fact that elasmobranch brains are less complex than higher vertebrates (Aronson *et al.*, 1967). The instrumental studies previously cited on learning and discrimination ability in sharks indicate that there may be less of a difference in plasticity than was previously supposed in spite of the relative neuroanatomical simplicity of elasmobranchs. Recent evidence provided by Ebbesson (1972) also indicates that previous conceptions of neural organization in elasmobranch brains may have to be revised. With the Nauta method, Ebbesson traced fiber tracts in the brains of nurse sharks. He found that, contrary to the prevailing view of the entire telencephalon as being olfactory in nature, that only a relatively modest portion of the telencephalon is directly related to olfaction. A restricted ventrolateral region receives olfactory tract projections and no evidence of contralateral projections was noted. Projections from the thalamus to a caudal cell group in the telencephalon were definitely described for the first time. The organization of the diencephalon was also shown to be different than had previously been believed as well. Telencephalic and retinal inputs to the hypothalamus were revealed. Retinal input to the thalamus was large and inputs from spinal cord, optic tectum, and cerebellum were shown to exist. In the cerebellum, Ebbesson found no evidence on a direct connection to reticular and motor nuclei of the medulla and spinal cord. Only when the lateral cerebellar nucleus was lesioned were fibers traced outside the cerebellum proper. These findings show that in some brain areas that were thought to be organized in a fundamentally different manner, sharks really have more in common with the higher vertebrates than was previously believed.

V. Further Studies on Experiential Effects in Elasmobranch Behavior

A. Permanence of Experiential Effects

The degree of long-term influence of early experience on behavior in sharks could be profitably investigated. The results of the present study imply that the influence of abnormal early feeding experience in producing the lack of response to low-frequency, mechanically produced sounds can be overcome by subsequent quasi-normal experience in capturing live prey. These effects seem to be rather readily reversible. A study that may be suggested is to determine the persistence of the effects of early experience by employing noxious stimuli in conditioning experiments. If a group of sharks such as those in the present study could be obtained, they could be segregated into groups and the animals of each group could be subjected to low-frequency, broad-band noise paired with an electrical shock at specified, different ages for each group. During an intervening time period the animals would be fed on non-living food and maintained without experimental stimulation and, subsequently, a schedule of testing could be initiated. In this schedule, low-frequency, broad-band noise could be presented to the animals of the different groups at different time intervals since the last training session. This would determine whether the age when first stimulated and the time interval since the last previous stimulation are correlated with the conditioned avoidance response to auditory stimuli.

B. The Effect of Sensory Deficits on Behavior and Structure

Although technically difficult, an experiment to test for the necessity of the inner-ear hearing mechanism to detect low-frequency sound and the effect of sensory deficit on brain structure may be suggested. Sharks could be conditioned to approach a broad-band noise source. After reaching criterion, a technique could be utilized in which Streptomycin is used to impair the function of the hair cells in the sacculus and utriculus of the inner ear that are involved in hearing. One group of sharks could receive this treatment unilaterally, another bilaterally, and a third control group sham treatment. Subsequently, the three groups could be retested in the approach conditioning paradigm to determine the existence of behavioral deficits. After behavioral tests had been completed, the animals could be sacrificed and their brains sectioned to determine whether there were any histological differences among the three groups. Another possible approach, that is, to try to create lateral line deficits is not suggested because the probability of being able to create a complete deficit in the hair cells of the saccular and utricular maculae may be greater than to do so in the neuromasts of the lateral line. Free-standing neuromasts and pit organs are also connected to the lateral line nerve and since these receptors are so widespread on the body one may never be sure that a true lateral line deficit is in fact, created (Tester and Nelson, 1967).

These experiments could be performed on animals of different ages to determine whether there were differences in behavioral and structural deficits due to the duration and/or the age of onset of sensory impairment.

SUMMARY AND CONCLUSIONS

Investigations in recent years have shown that low-frequency sounds are attractive to several species of inshore and pelagic sharks. The characteristics of the sound most effective in attracting sharks were established in these investigations. Sounds below 1000 Hz of an irregularly pulsed nature proved to be the pattern that was most attractive but these investigators did not employ sounds below 20 Hz so the response to this frequency range was unknown.

Some of these investigations indicated that there were behavioral differences between adults and the young of a species in their response to low-frequency sounds. It was hypothesized that these differences may be influenced by the experience of the young sharks and that this was a subject that required further investigation.

In the present study I was able to utilize a litter of young lemon shark pups taken from a moribund, gravid female as experimental subjects. This provided an opportunity to control and modify the early experience of animals and observe the effect on their responses to low-frequency sounds and feeding behavior.

The sounds presented in this study included frequencies below 20 Hz and were produced by a mechanical device called the Ichthyo-Uro-Simulator that oscillated a flexible plastic flipper in a manner similar to the caudal fin of an injured, struggling fish. In this way signals were produced that had dynamic components more like the signals produced by a struggling fish than electronically produced signals.

Three types of sound were used as the stimulus in this study: (1) Sounds produced by the IUS with the flipper attached that contained large-amplitude hydrodynamic components, (2) sounds produced by the IUS with the flipper removed without large-amplitude hydrodynamic components, and (3) electronically produced sounds presented with a J-9 underwater transducer.

1. Preliminary studies conducted in 1970 indicated that immature lemon sharks (Negaprion brevirostris) captured in the field and maintained under laboratory conditions were attracted by sounds produced by the Ichthyo-Uro-Simulator Mk II. These sounds contained large-amplitude hydrodynamic components. The results of tests utilizing sounds without such components were not clear in these preliminary studies.
2. Immature lemon sharks born in captivity and lacking species-typical early feeding experience showed a lack of attraction to sounds produced by the Ichthyo-Uro-

Simulator Mk III. Their responses differed from those seen in immature lemon sharks captured in the field that presumably had species-typical feeding experience.

3. Sharks born in captivity that were given the opportunity to pursue and capture living prey for a two-week period were again observed in their responses to sounds produced by the IUS Mk III. The results of these tests showed significant attraction of sharks to sounds with and without large-amplitude hydrodynamic components but the response level was lower to sounds without large-amplitude hydrodynamic components. There was evidence that the level of attractiveness of sounds with large-amplitude hydrodynamic components could approach that of the pulsed, broad-band noise as reported by other workers. It is concluded that experience in the capture of live prey may be essential for sounds produced by the IUS to be attractive to these sharks.
4. The responses of these sharks to electronically produced sounds known to be attractive to juvenile lemon sharks in the field were observed. Pulsed, broad-band noise was highly attractive as well as pulsed, 10-Hz sine waves. The level of response to broad band noise appeared similar to that described in field play-back experiments by other workers. The response level to 10-Hz sine waves while significantly positive, was lower than with broad-band noise as the stimulus. The

behavior of these sharks in response to electronically produced sounds of known attractive significance can be regarded as similar to sharks observed in a natural environment.

5. Use of the term habituation in behavioral studies was discussed. In conformity with accepted usage, application of the term under conditions other than those in which repeated or continuous stimulation is presented to the same individual subjects throughout the experiment is regarded as inappropriate. In addition, the criteria of measurement of habituation rate must be clearly specified. The schedule of stimulus presentation used in this study was designed to minimize habituation. There was no evidence for significant decrements in responses to any of the sounds presented in this study except high-amplitude, 10-Hz sine waves.
6. Examination of the data of the second series of tests in which sounds produced with and without large-amplitude hydrodynamic components were the stimulus, reveals no effect that can unequivocally be ascribed to order of presentation. An effect is present, however, that may be correlated with the time of day at which the sounds were presented. Apparently those trials performed in the evening with sound that had no large-amplitude hydrodynamic components had a slightly greater response level than those performed at other times of

day. Sounds with large-amplitude components present produced more responses in the morning. An hypothesis is advanced to account for this result.

7. In the course of this study it was discovered that there was a greater tendency for a shark isolated in the test tank to exhibit behavior such as elevated swimming velocity; protrusion of snout up tank walls and sudden velocity and/or direction changes than when it was a member of a group. This behavior is interpreted as signs of distress and during periods when it is exhibited the animal is unresponsive to stimuli. The effect of the group in reducing the occurrence of this behavior was noted.
8. The response of young sharks to low-frequency sounds was related to their feeding behavior. These sounds resemble those that may be produced by potential prey organisms and an approach to the source of such signals may place a young shark in a feeding situation. The generalized nature of these signals plus other evidence indicates that sharks have poor sound discrimination ability and probably do not actively seek out specific types of prey on the basis of sound signals.
9. Several theoretical formulations in the study of experiential influences on behavior and structure were reviewed. The proposition that "intrinsic factors" are the most important factors influencing behavioral

development was regarded as a less productive concept than the view that there is a reciprocal relationship between structure and function in behavioral development and that experiential and maturational factors are fused in a system that progresses through characteristic levels of developmental organization. An example of an experimental approach utilizing this concept was given and was related to the results of the present study.

In conclusion, it may be stated that a review of recent evidence on elasmobranch behavior and sharks in particular indicates that certain concepts about the behavioral capacities of these animals may have to be revised. New anatomical evidence indicates that sharks have more in common with "higher" animals in some brain structures than was previously thought. On this basis then, the uncritical acceptance of the proposition that the behavior of animals at the psychological level of sharks is primarily "innately" determined is regarded as inappropriate. A further study of the degree of influence of different types of experiential factors on shark behavior is desirable.

TABLES

TABLE 1. Treatments Presented

A. Mechanically produced	a.	Sounds with large-amplitude hydrodynamic components (Flipper attached 12.5 Hz oscillation frequency)
	b.	Sounds with no large-amplitude hydrodynamic components (Flipper removed 12.5 Hz oscillation frequency)
B. Electronically produced	a.	Broad-band noise to 8 kHz plus high-amplitude sine waves (10 Hz)
	b.	High-amplitude sine waves (10 Hz)

TABLE 2. Responses of Sharks Captured in the Field to IUS Mk II During Preliminary Experiments of 1970

	Flipper Attached		Flipper Removed	
Trials	Approach	Startle	Approach	Startle
1	2	1	0	0
2	7	2	0	1
3	0	0	0	0
4	9	1	0	0
5	2	1	0	0
6	1	0	0	1
7	3	2	1	0
8	15	1	0	0
9	7	1	1	1
10	5	1		
11	3	1		
Total	54	10	2	3

TABLE 3. Series No. 1. Responses of Sharks Deprived of Normal Feeding Experience. Tests with Single Sharks.

Position in Space	Flipper Attached	Control Obs. Pd.	Wilcoxon Test	Flipper Removed	Control Obs. Pd.	Wilcoxon Test
Median A-Response	1.25	0.75	ns*	1.0	1.0	ns*
Median B-Response	0	0	ns*	0	0	ns*
Qualitative Measures (totals)						
IOT	0	0	--	0	0	--
IA	5	0	na+	0	0	--
Circle	0	0	--	1	0	na+
Av	22	3	sig**	9	1	ns*

* H_0 is accepted, there is no difference at the .025 level in a one-tailed test. .05 is the level of significance in a two-tailed test of the Wilcoxon statistic.

** H_0 is rejected, H_1 is accepted: the treatment is significantly greater than the control at the .025 level.

+ Test was not applicable since the obtained number of differences between pairs (N) was less than the minimum value of N in tables for the Wilcoxon statistic.

TABLE 3 (continued)

A-Response: Crossing over the ring

B-Response: Entering the central area

IOT : Initial Oriented Response

IA : Investigative Approach

Circle : Circle

Av : Avoid response

(See text, page 65 for complete description)

TABLE 4. Series No. 2A. Responses of a Single Shark Deprived of Normal Feeding Experience, but Experienced in Capturing Living Prey

Position in Space	Flipper Attached	Control Obs. Pd.	Flipper Removed	Control Obs. Pd.
Median A-Response	4	2	1	1
Total A-Response	23	7	9	6
Median B-Response	1.5	0	0.5	0.5
Total B-Response	14	2	3	5
Qualitative Measures (totals)				
IOT	2	0	0	0
IA	7	0	1	0
Circle	7	0	0	0
Av	1	0	0	0

TABLE 5a. Series No. 2B. Responses of Sharks Deprived of Normal Feeding Experience, but Experienced in Capturing Living Prey. Tests with Groups of 3 Sharks

Position in Space	Flipper Attached	Control Obs. Pd.	Wilcoxon Test	Flipper Removed	Control Obs. Pd.	Wilcoxon Test
Median A-Response	12.25	10.0	ns*	11.25	10.5	ns*
Median B-Response	3.75	3.25	ns*	3.5	3.25	ns*
Qualitative Measures (totals)						
IOT	12	0	na+	1	0	na+
IA	85	3	sig**	28	0	sig**
Circle	34	4	sig**	33	0	sig**
Av	93	0	sig**	32	2	sig**

* H_0 is accepted, there is no difference at the .025 level in a one-tailed test. .05 is the level of significance in a two-tailed test of the Wilcoxon statistic.

** H_0 is rejected, H_1 is accepted: the treatment is significantly greater than the control at the .025 level.

+ Test was not applicable since the obtained number of differences between pairs (N) was less than the minimum value of N in tables for the Wilcoxon statistic.

TABLE 5b. Series No. 2B. Comparison of Shark's Responses to Sounds Produced with Flipper Attached and with it Removed

Position in Space	Flipper Attached	Flipper Removed	Wilcoxon Test
Median A-Response	12.25	11.25	ns*
Median B-Response	3.75	3.50	ns*
Qualitative Measures (totals)			
IOT	12	1	na+
IA	85	28	ns++
Circle	35	33	ns*
Av	93	32	ns++

* H_0 is accepted, there is no difference at the .025 level in a one-tailed test. .05 is the level of significance in a two-tailed test of the Wilcoxon statistic.

\+ Test was not applicable since the obtained number of differences between pairs (N) was less than the minimum value of N in tables for the Wilcoxon statistic.

++ Eliminating the two sets of trials performed in the evening gives a significantly greater number of responses at the .025 level with flipper attached (see Table 13). With these trials included H_0 of no difference is accepted.

TABLE 6. Series No. 3A. Responses of Sharks to Broad-band Noise Plus 10-Hz Sine Waves

Position in Space	Signal	Control Obs. Pd.	Wilcoxon Test
Median A-Response	12	9.5	ns*
Median B-Response	3.5	1.5	sig**
Qualitative Measures (totals)			
IOT	18	0	sig**
IA	17	1	sig**
Circle	12	0	na+
Av	14	0	sig**

* H_0 is accepted, there is no difference at the .025 level in a one-tailed test. .05 is the level of significance in a two-tailed test of the Wilcoxon statistic.

** H_0 is rejected, H_1 is accepted: the treatment is significantly greater than the control at the .025 level.

+ Test was not applicable since the obtained number of differences between pairs (N) was less than the minimum value of N in tables for the Wilcoxon statistic.

TABLE 7. Series No. 3B. Responses of Sharks to High-amplitude 10-Hz Sine Waves

Position in Space	Signal on	Control Obs. Pd.	Wilcoxon Test
Median A-Response	11	9	ns*
Median B-Response	4	1	sig**
Qualitative Measures (totals)			
IOT	5	0	na+
IA	11	0	na+
Circle	15	0	sig**
Av	13	0	sig**

* H_0 is accepted, there is no difference at the .025 level in a one-tailed test. .05 is the level of significance in a two-tailed test of the Wilcoxon statistic.

** H_0 is rejected, H_1 is accepted: the treatment is significantly greater than the control at the .025 level.

\+ Test was not applicable since the obtained number of differences between pairs (N) was less than the minimum value of N in tables for the Wilcoxon statistic.

TABLE 8. Latency of Attack on Tethered, Struggling Fish

Subjects	Latency in Seconds Median	No. of Trials
Group of 4 Sharks Captured in the Field (normal latency)	30.0	17
Single Shark #1 Born in Captivity	59.0	1
Single Shark #2 Born in Captivity	180	7
Group of 7 Sharks Born in Captivity (Home Tank)	17.5	3
Group of 6 Sharks Born in Captivity (Test Tank)	63.5	3
Group of 10 Sharks Born in Captivity (Home Tank)	25.0	5
Group of 3 Sharks Born in Captivity (Test Tank)	30.0	5

TABLE 9. *Analysis of Variance of A Responses to Sounds Produced with Flipper Attached. Test for Response Decrement From Initial to Final Trials Series 2B*

	SS	df	MS	F
Between trials	175.07	5	35.01	1.27*
Error	1655.13	60	27.58	
Total	1830.20	65		

@ = .05 Table F value = 2.37

* Accept H_0: No difference between trials at the .05 level.

TABLE 10. Analysis of Variance of A Responses for Sounds Produced with Flipper Attached. Test for Response Decrement over Series

	SS	df	MS	F
Between tests	988.6	10	98.86	6.48*
Error	839.8	55	15.26	
Total	1828.40	65		

@ = .05   Table F value = 1.99

* Reject H_0; Accept H_1: A difference between tests exists at the .05 level.

Test Number	1	2	3	4	5	6	7	8	9	10	11
Mean Number of Responses	15.8	10.8	15.8	9.0	9.0	8.5	13.8	17.8	10.8	9.0	6.7

TABLE 11. Kolgomorov-Smirnov D Statistic. Test for Response Decrement from Initial to Final Trials of Series 3A. Broad-Band Noise + 10-Hz Sine Waves

Trials	1	2	3	4	5	6	Table D .05	Obs. D Value	No. Responses
A Res.	.03	.01	.09	.05	.04	0	.11	.09	145
B Res.	.22	.25*	.25	.19	.11	0	.17	.25*	64
IOT	.16	.17	.17	.11	.11	0	.31	.17	18
IA	.13	.17	.40	.23	.06	0	.41	.40	10
Circle	.67*	.67	.50	.33	.17	0	.43	.67*	9
Av	.26	.24	.29	.19	.10	0	.35	.29	14

* Significant difference between theoretical and observed cumulative frequency distribution

TABLE 12. Kolgomorov-Smirnov D Statistic. Test for Response Decrement from Initial to Final Trials of Series 3B. High-Amplitude 10-Hz Sine Waves

Trials	1	2	3	4	5	6	Table D .05	Obs. D Value	No. Responses
A Res.	.02	.07	.12	.13*	.04	0	.10	.13*	184
B Res.	.01	.08	.19*	.16	.06	0	.16	.19*	75
IOT	.43	.27	.10	.33	.17	0	.56	.43	5
IA	.13	.17	.40	.23	.07	0	.41	.40	10
Circle	.23	.27	.23	.13	.03	0	.34	.27	15
Av	.48	.52*	.52	0	0	0	.35	.52*	14

* Significant difference between theoretical and observed cumulative frequency distribution

TABLE 13. Mann-Whitney-U Test for Time-of-Day and Order-of-Presentation Effects on Qualitative Responses to Mechanically Produced Sounds in Series 2B

	Flipper Attached			Flipper Removed		
Test	H_o	H_i	P	H_o	H_i	P
1st vs 2nd	1st=2nd	$p(\text{1st}>\text{2nd})>\frac{1}{2}$	.396**	1st=2nd	$p(\text{1st}>\text{2nd})<\frac{1}{2}$	.015*
AM vs PM	AM=PM	$p(\text{AM}>\text{PM})>\frac{1}{2}$	.008^{+}	AM=PM	$p(\text{AM}>\text{PM})<\frac{1}{2}$	.032*
AM vs PM +Eve.	AM=PM+Eve	$p(\text{AM}>\text{PM+Eve})>\frac{1}{2}$	.036^{+}	AM=PM+Eve	$p(\text{AM}>\text{PM+Eve})<\frac{1}{2}$	.032*

* H_o is rejected; H_i is accepted at the .05 level: PM (or 2nd) is greater than AM (or 1st)

$^{+}$ H_o is rejected; H_i is accepted at the .05 level: AM is greater than PM

** H_o is accepted; at the .05 level there is no difference between treatments

Fig. 1 Mounting platform of the Ichthyo-Uro-Simulator

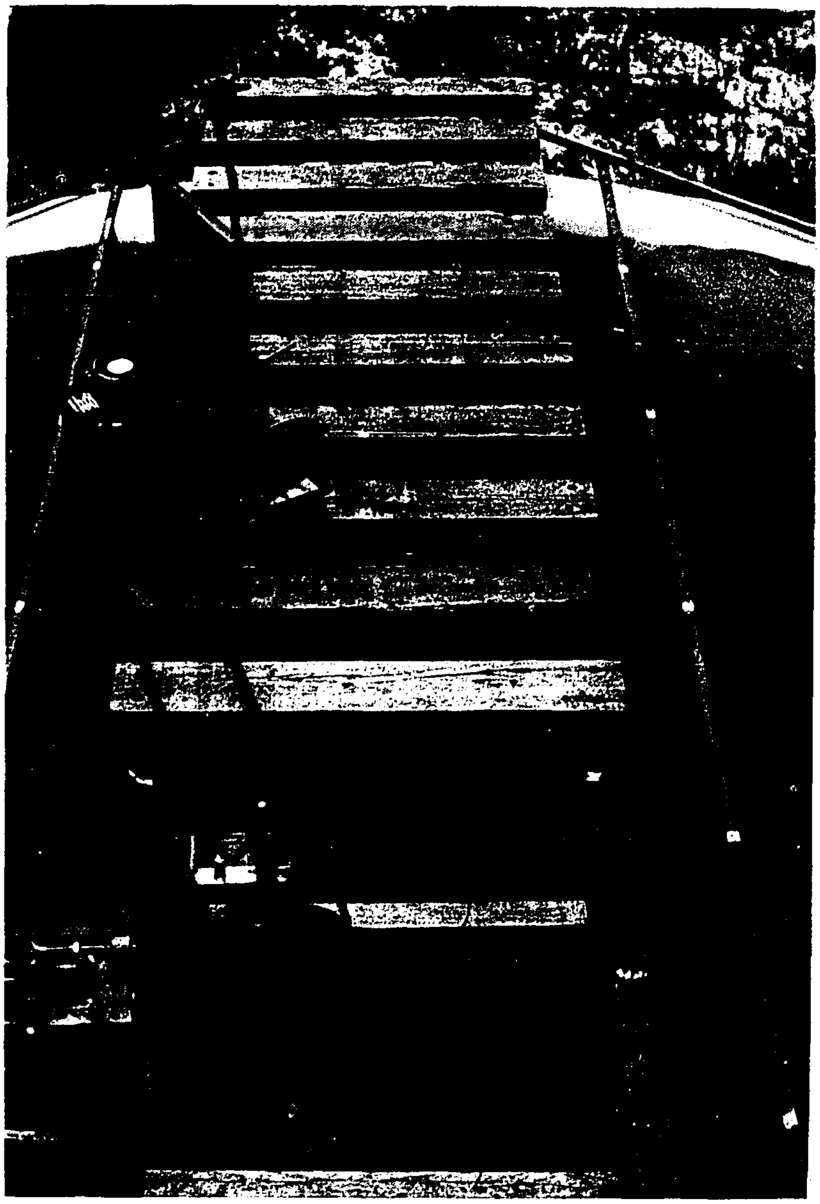


Fig. 2. Experimental tank showing the scoring area marked on the tank bottom and the grid framework used to position the hydrophone.

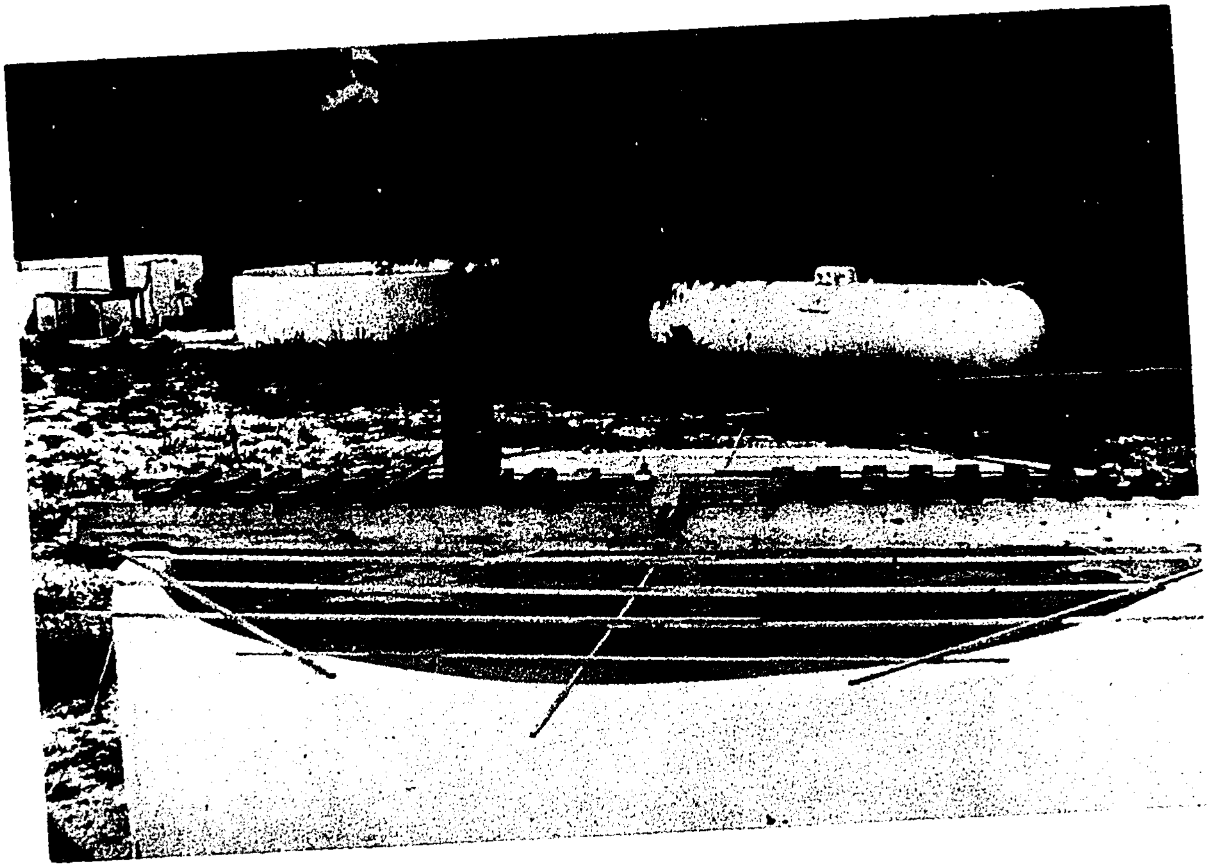


Fig. 3. Drawing of the Ichthyo-Uro-Simulator Mk III.

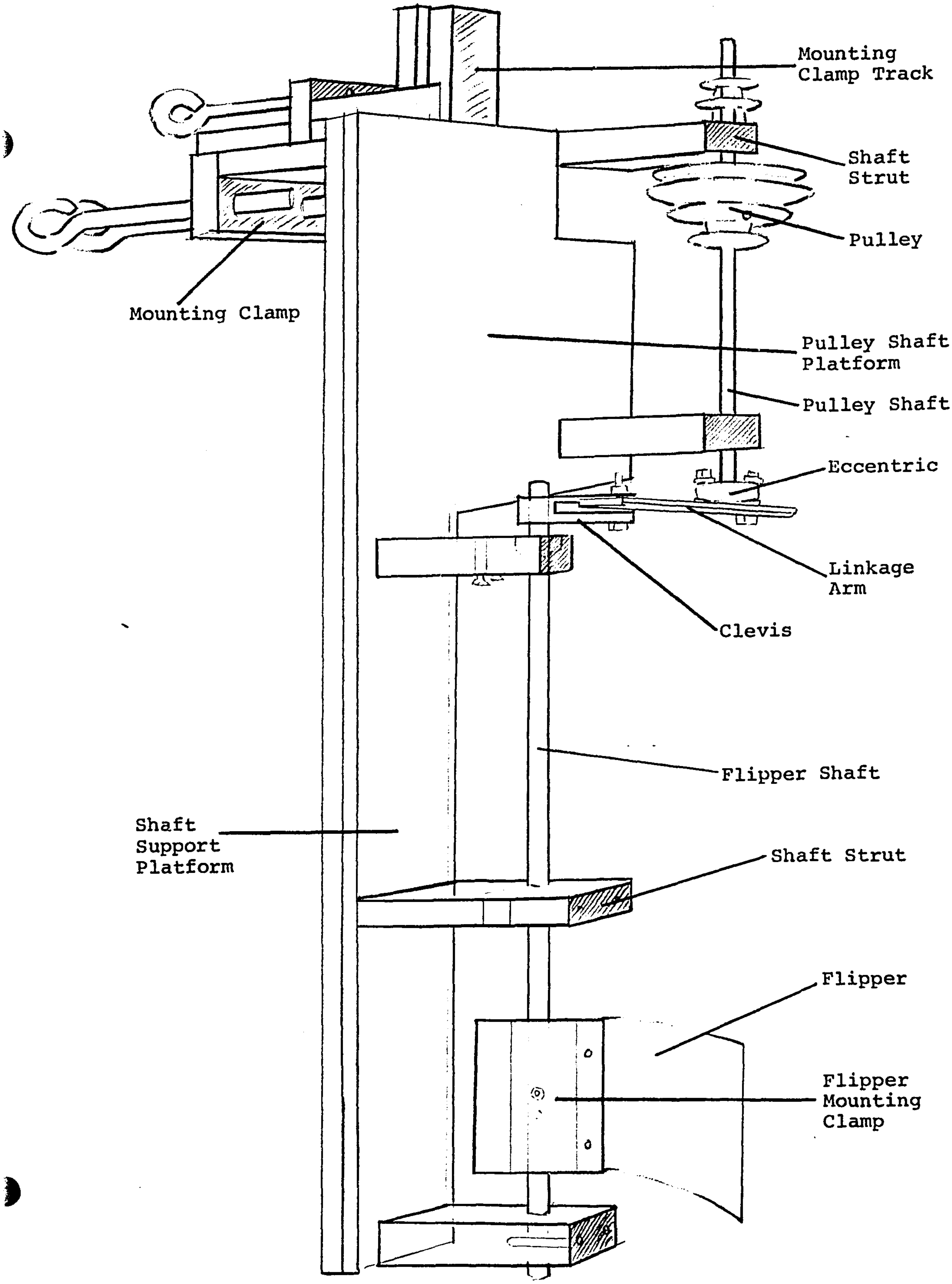

Mounting Clamp Track
Shaft Strut
Pulley
Mounting Clamp
Pulley Shaft Platform
Pulley Shaft
Eccentric
Linkage Arm
Clevis
Flipper Shaft
Shaft Support Platform
Shaft Strut
Flipper
Flipper Mounting Clamp

Fig. 4. Underwater view of the Ichthyo-Uro-Simulator.

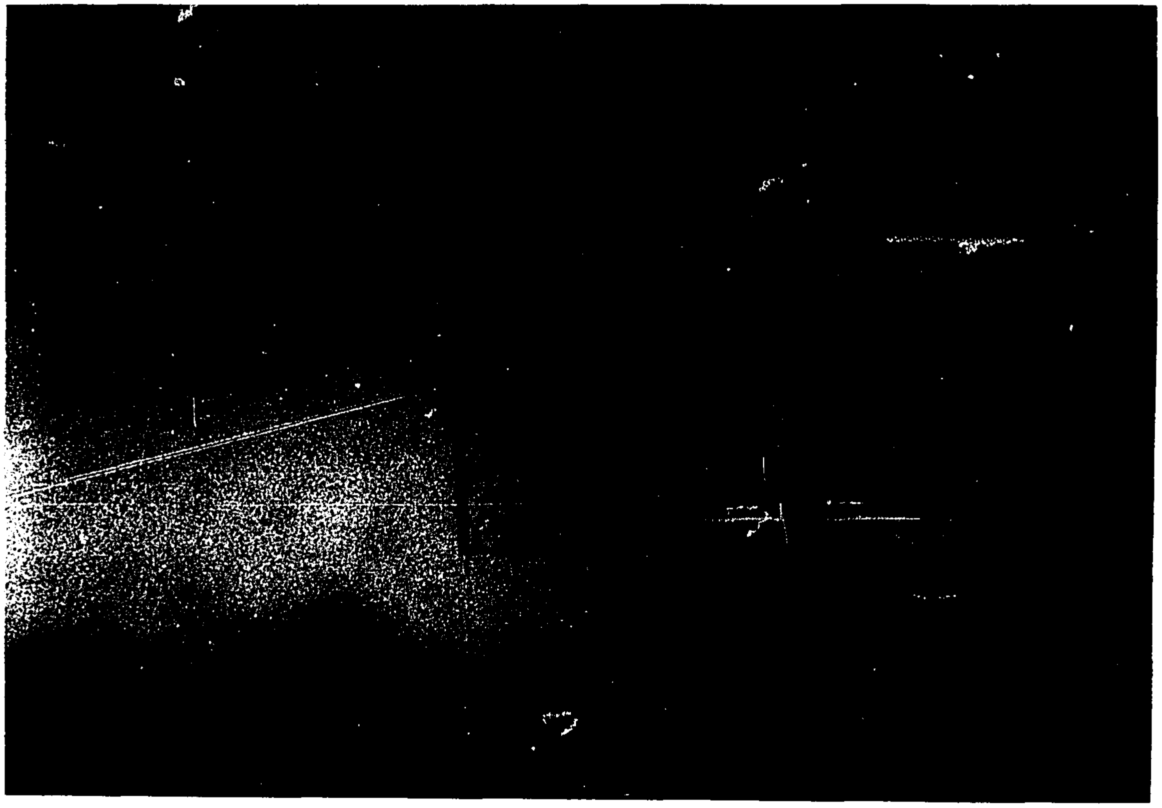


Fig. 5. Adjustable mounting clamp of the Ichthyo-Uro-Simulator.

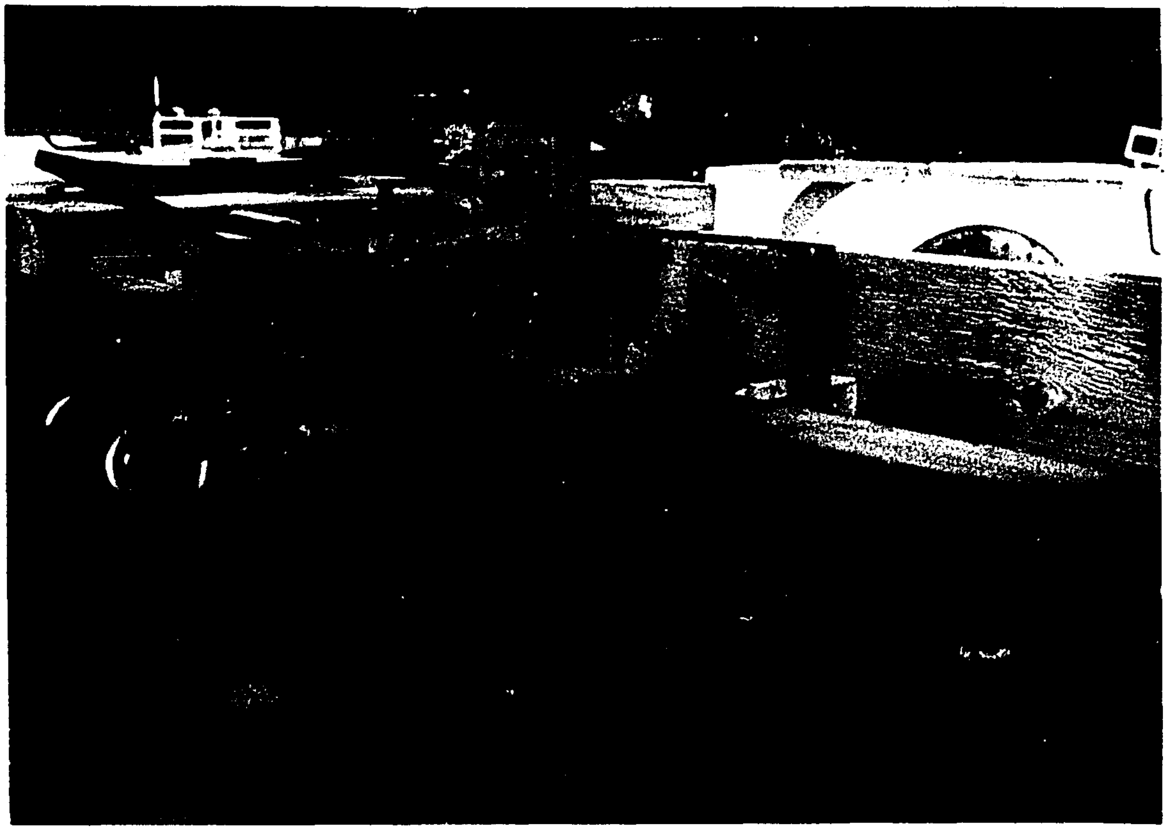
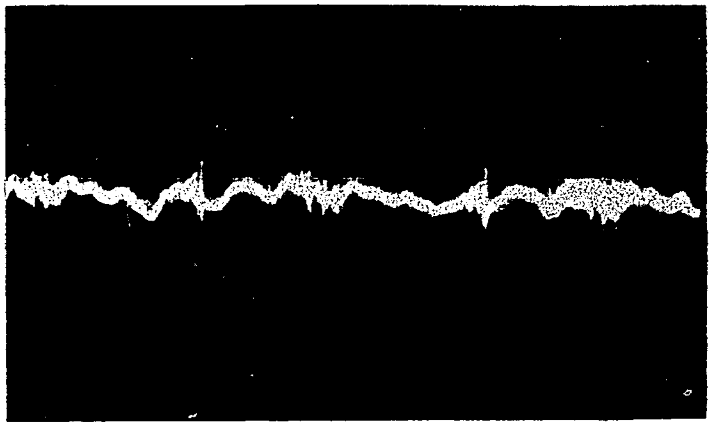


Fig. 6. Oscillograms of mechanically-produced sounds.

Upper trace-- Hydrophone placed .3 m away from the wall of the tank.

Lower trace-- Hydrophone placed at the wall of the tank.



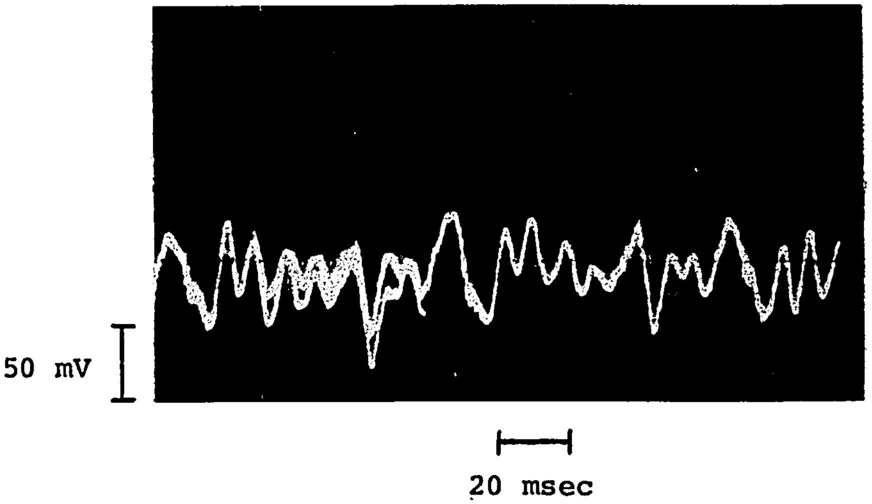

50 mV
20 msec

Fig. 7. Sonogram of mechanically-produced sounds.
Narrow bandwidth analyzing filter

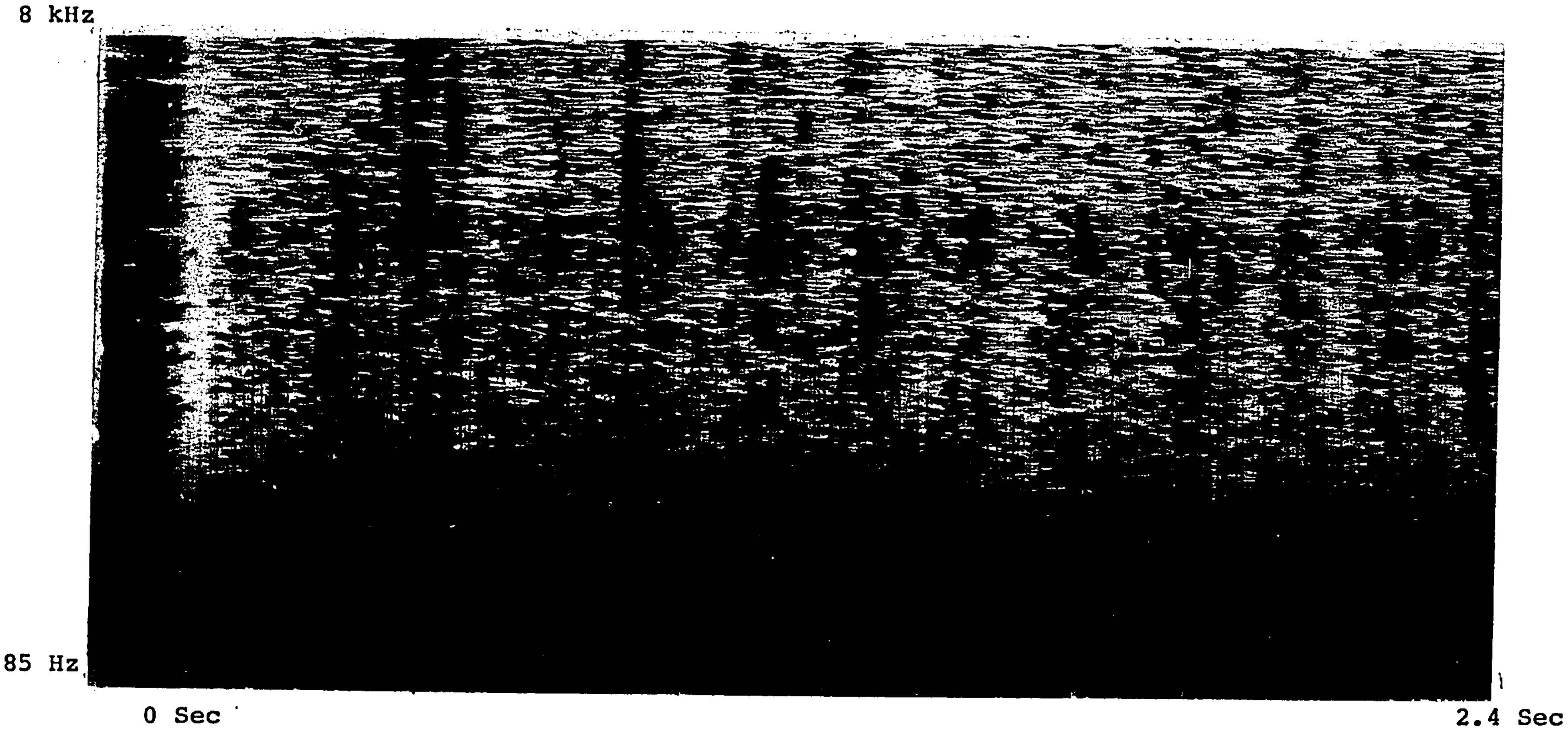

8 kHz
85 Hz
0 Sec
2.4 Sec

Fig. 8. Juvenile lemon shark viewed from the observation platform.

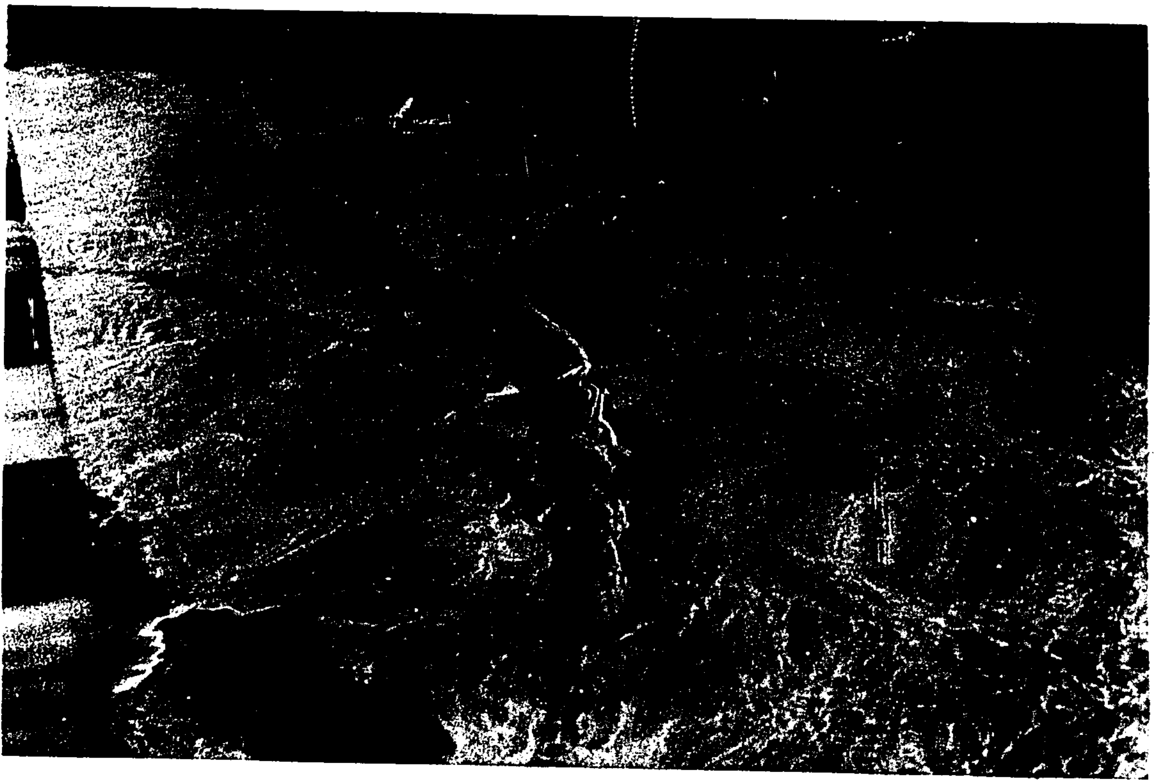


Fig. 9. Typical orientation while swimming of juvenile lemon shark.

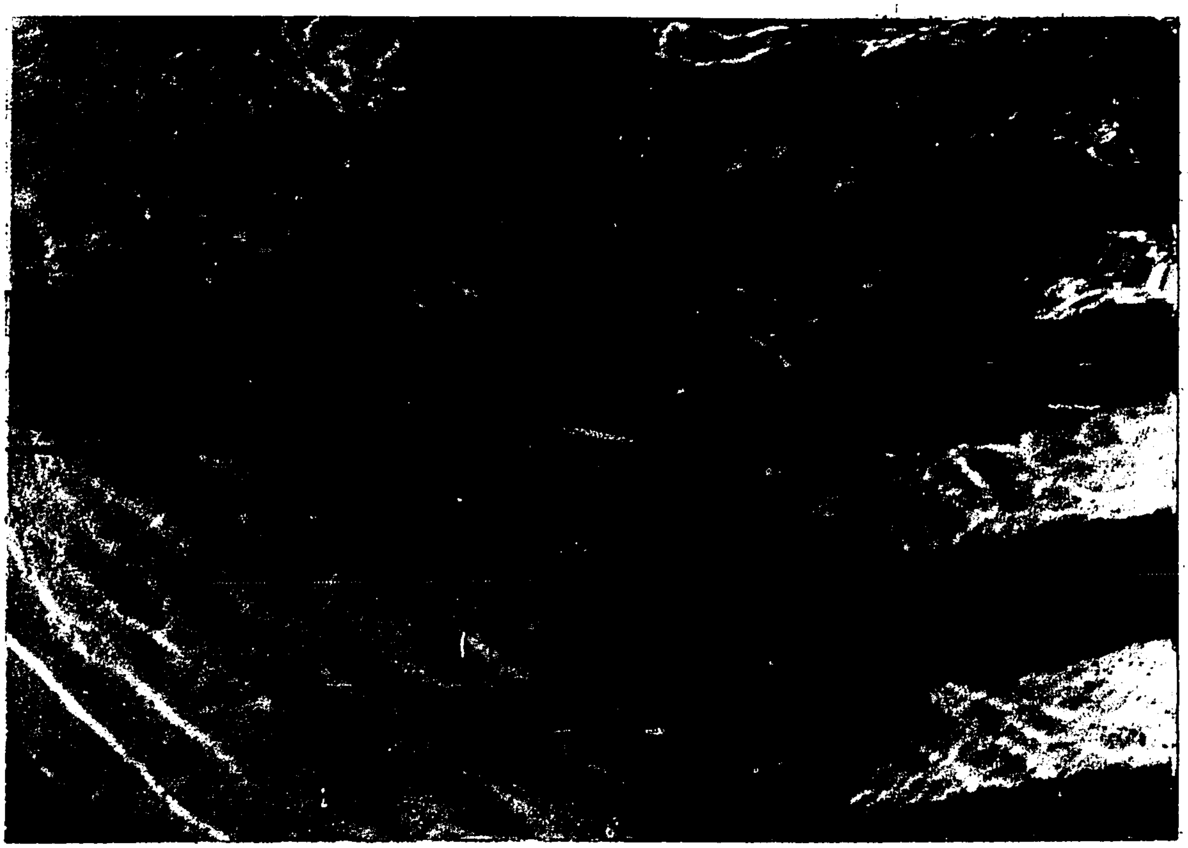
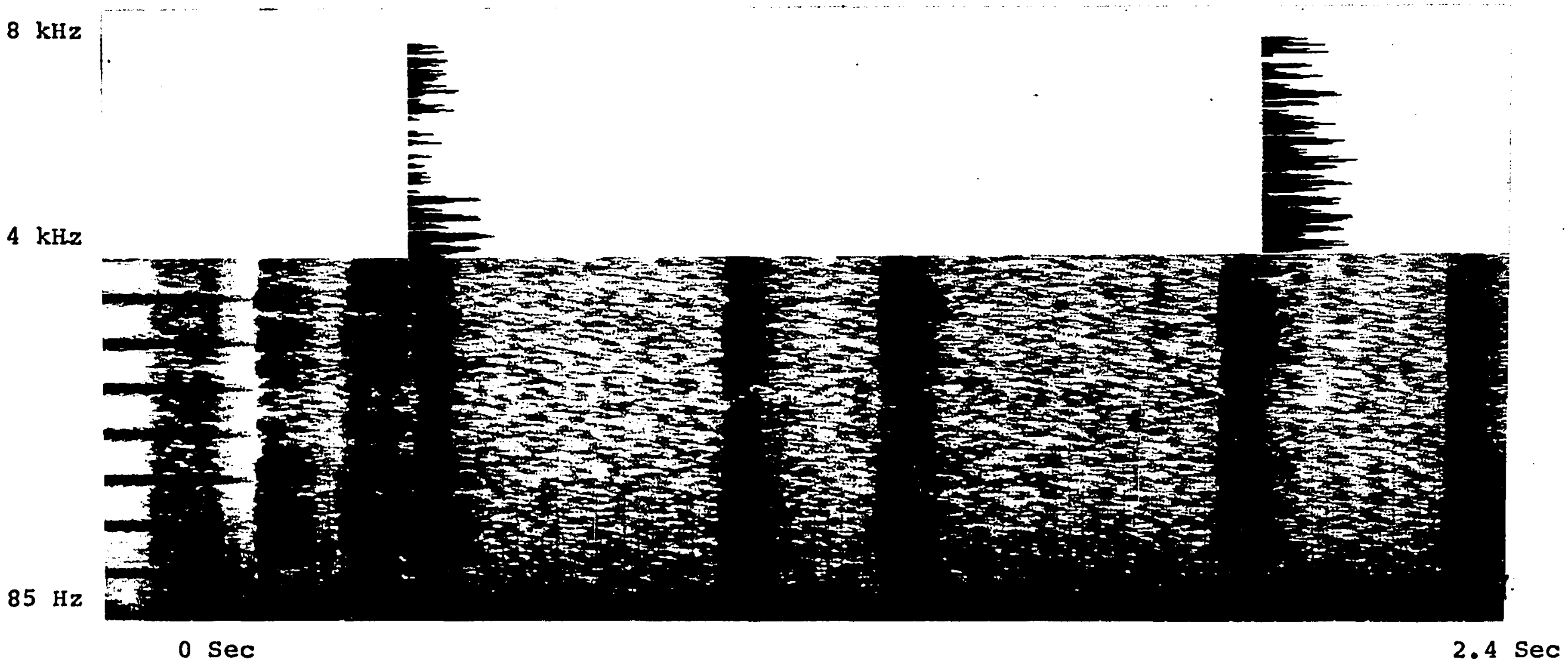


Fig. 10. Sonogram of electronically-produced sounds.
Narrow bandwidth analyzing filter.
Top part of sonagram shows a section through a pulse of sound which represents intensity as a lateral deflection to the right.


8 kHz
4 kHz
85 Hz
0 Sec
2.4 Sec

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