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STUDIES OF MAGNETIC SENSITIVITY IN THE YELLOWFIN TUNA, *THUNNUS ALBACARES*

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN ZOOLOGY

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By

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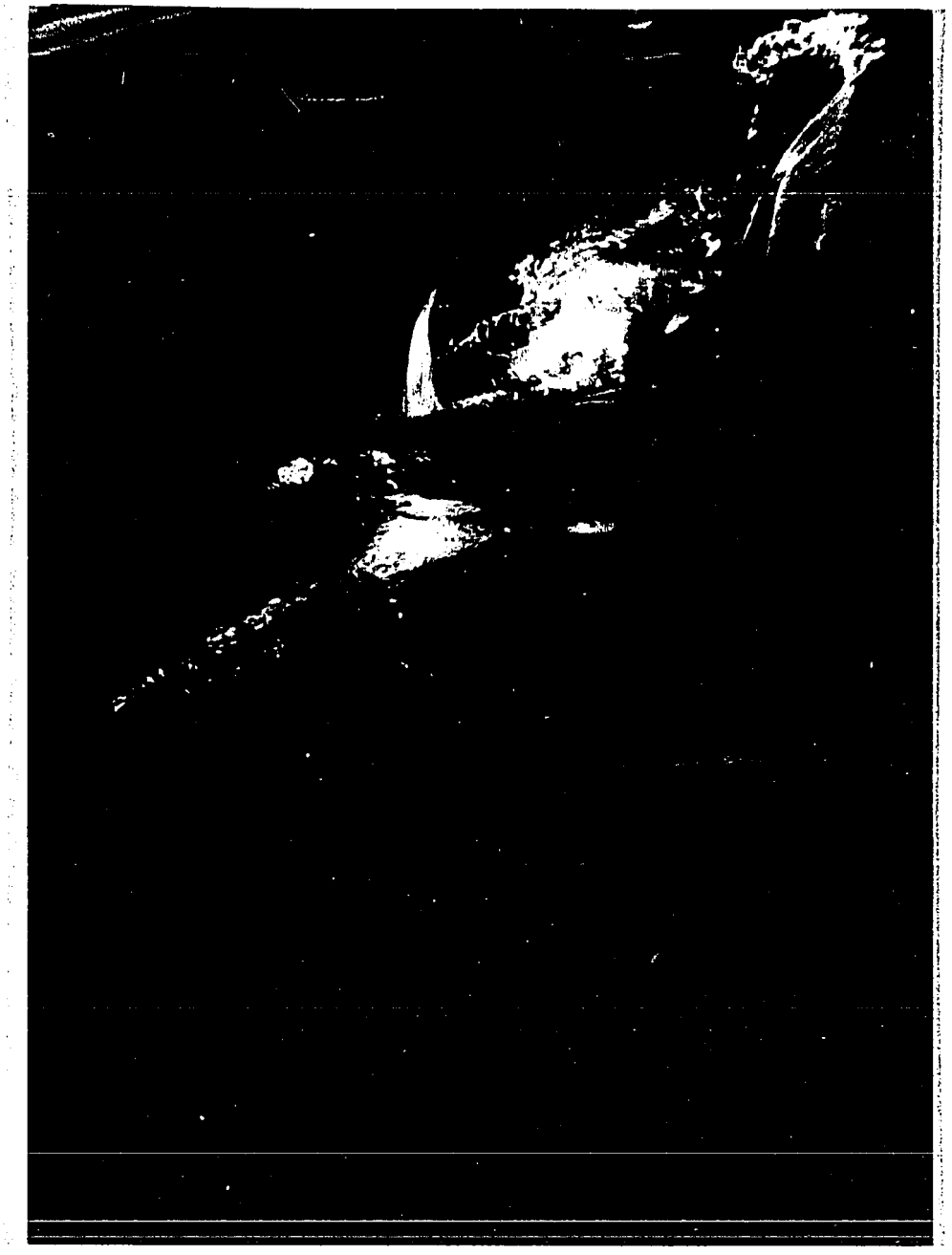
Frontispiece. A 50 kg yellowfin tuna breaks the surface to feed in a tank at the Kewalo Research Facility of the National Marine Fisheries Service Honolulu Laboratory.



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ABSTRACT

Despite considerable research the mechanisms used for navigation by pelagic marine fishes are not well understood. The geomagnetic field is a potentially important cue for navigation, but behavioral evidence that pelagic fishes respond to magnetic fields is limited. There have been two central problems in the study of magnetic sensitivity in animals. Both classical and behavioral conditioning experiments have either failed or produced inconsistent results and the site of magnetoreception is unknown.

A discrete-trials/fixed-interval training procedure was used to train yellowfin tuna, Thunnus albacares, to discriminate between two earth-strength magnetic fields. Five fish tested individually learned to discriminate between the fields. Two fish tested using double blind procedures also discriminated between the fields. Two other fish failed to discriminate between two magnetic fields in which the gradients of intensity were equal in absolute value and opposite in direction. The results suggest that the responses to magnetic fields by yellowfin tuna are neurally mediated.

Superconducting magnetometers were used to survey the tissues of the yellowfin tuna for concentrations of magnetic material and to analyze the properties of the material to

determine whether it was suitable for use in magnetoreception. Chemical analysis by X-ray diffraction spectroscopy of magnetic particles extracted from the tissue permitted unique identification of the magnetic material.

Concentrations of magnetic material were found reproducibly only in tissue contained within the dermethmoid bone of the skull of the yellowfin tuna. Detailed analyses of the magnetic properties of dermethmoid samples permitted the following conclusions: (1) the dermethmoid tissue probably contains only single-domain magnetite crystals; (2) the presumed magnetite crystals are organized into groups of interacting particles; and (3) the particles exhibit a narrow size frequency distribution with average length and diameter of approximately 50 and 40 nm respectively. Magnetic particles extracted from the dermethmoid tissue were uniquely identified as magnetite by X-ray diffraction. Crystals observed in the transmission electron microscope exhibited a narrow size range, averaging 45 x 38 nm, and a non-octahedral crystal form. Theoretical analyses show that groups of the crystals would be suitable for use in magnetoreception and suggest that a mechanoreceptor which monitors position or movement of the particle groups might serve to link the crystals to the nervous system.

Attempts to demonstrate nerves associated with the magnetite-containing tissue were unsuccessful. There is correlative evidence that pelagic fishes use the geomagnetic field in orientation in the open ocean. These observations and the results of experiments conducted in this study suggest that the possibility that pelagic fishes use the geomagnetic field to guide migrations warrants further experimental investigation.

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CHAPTER I

INTRODUCTION

Animal migration has long fascinated lay and scientific communities alike. Among migratory vertebrates the migrations of birds are far better known than those of other classes (Baker 1978). However, many species of fish migrate over equally large distances with precision often matching that of the birds (Harden Jones 1968, Leggett 1977, Hasler et al. 1978, Sund et al. 1981). Fishes usually migrate between feeding and spawning grounds (Harden Jones 1968, Stasko 1971). Thus migration directly affects fitness, and the sensory mechanisms that guide migration will be subject to intense selection pressure (Able 1980). In spite of the importance of guidance mechanisms to migration, the question of how fishes and other animals guide their movements is not completely answered.

The migratory orientation of fishes has been studied in both the field and the laboratory. These studies face the following experimental disadvantages: (1) stimuli available to experimental animals can not be fully controlled in field experiments, and (2) although control of stimuli is possible in laboratory experiments, migration can not occur. Consequently, only the responses to environmental stimuli made by migratory fishes can be studied in the laboratory. A theoretical basis for determining whether stimuli are likely

to be used in navigation is therefore required before beginning laboratory experiments. The biological limitations on the utility of the stimuli for navigation must then be determined by psychophysical measurement of the capacities of sensory systems responding to the stimuli.

This dissertation suggests four criteria for determining whether pelagic fishes can use different environmental stimuli to guide their migrations. The hypothesis that animals might use the geomagnetic field to navigate in the open ocean is evaluated using the criteria. The geomagnetic field easily meets criteria on suitability of the stimulus for use in navigation. However, evidence that pelagic fishes could and did use the geomagnetic field in navigation was lacking. Experiments were then carried out to demonstrate responses to magnetic fields in the yellowfin tuna Thunnus albacares and to determine how and where magnetoreception occurs in this species.

The cost and difficulty of studying the migratory behavior of fishes has caused our understanding of fish migrations to lag behind that of birds (Harden Jones 1968, Leggett 1977). Nevertheless, the economic importance of migratory fishes has resulted in considerable research effort being directed at understanding their movements (Harden Jones 1968). Fishery and field studies have

described the migrations of many species but analytical studies of the means by which fish guide their movements in the open ocean have begun only recently (Laurs et al. 1977, Smith et al. 1981). Laboratory studies have attempted to determine the responses of fish to environmental stimuli and to assess the abilities of their sense systems to guide their migrations. When combined, these approaches have proved fruitful in demonstrating that anadromous fishes use olfactory cues to return to their natal streams to spawn (Hasler et al. 1978). Comparable results for pelagic fishes are not yet available. However, field and laboratory studies carried out so far permit inferences about their navigational abilities and the capacities of their sensory systems to mediate navigation.

Fish tagging studies have shown that the migrations of pelagic fishes can not be explained by models of passive drift with ocean currents without incorporation of directional biases (Saila and Shappy 1963, Patten 1964, Seckel 1972, Leggett 1977). In the more spectacular cases of movements by individual fish (Hartt 1966, Mather et al. 1967, French et al. 1976), the distances travelled and the times taken require that the fish swim almost continuously in one direction at or close to their most economical speed (Stasko 1971). Spectacular movements by tagged fish parallel early observations of the homing movements of displaced

birds (Matthews 1953, Kenyon and Rice 1958) that led to extensive field and laboratory studies of bird migratory behavior. Results originally obtained with birds have frequently been confirmed by later studies of other animal groups (Able 1980). Although such comparisons must be made carefully, models of orientation and navigation developed for birds are relevant to consideration of orientation and navigation in fishes. Reference will be made to these studies where they assist in the interpretation of results of experiments with fishes.

The results of many independent studies of homing pigeons and migratory birds suggest that they navigate using a "map", which enables them to determine their position, and a compass, which enables them to set and maintain a course (Kramer 1953, Gould 1982a). Considerable redundancy in compass systems has been demonstrated (Able 1980). Analysis of orientation systems is therefore more complex because depriving an animal of one set of compass information may have no discernible effect on orientation (Emlen 1975, Able 1980). Map responses are still little understood and their sensory bases the subject of intense debate (Able 1980, Gould 1982a and subsequent comments by Benvenuti 1982, Papi 1982, Walraff 1982, with reply by Gould 1982b). However, the map and compass navigation hypothesis provides a conceptual framework for studying navigation by fishes and for

comparing navigational abilities among fishes and other vertebrates.

To conduct studies of navigation and migratory behavior in pelagic fishes, it is necessary to consider the environmental stimuli and the responses to them made by fish (Harden Jones 1968), and to select single stimuli from among the variety of stimuli available. Stimuli that have formed the bases of hypotheses for navigation by fishes include cues associated with water masses (currents, temperature, and dissolved chemicals; Harden Jones 1968), the position and movements of celestial bodies (Hasler 1971), gravity (inertial guidance; Barlow 1964, Kleerekoper et al. 1969), geoelectric fields (Kalmijn 1974), and the geomagnetic field (Tesch 1980).

The following criteria are suggested for determining whether environmental stimuli and the sensory systems mediating responses to them can be used in map and compass navigation by pelagic fishes: (1) that the available stimuli be defined and shown to vary over the distances covered by migrating fish in a way that provides information about position or direction; (2) that the stimuli detectable in the immediate environment of the fish provide information about the current position or swimming direction of the fish; (3) that the fish be sufficiently sensitive to the stimuli to be able to derive the navigational information

available; and (4) that the fish do indeed respond to the stimuli in the open ocean. Of the available stimuli, the geomagnetic field is a potentially important navigational cue that has been implicated on many occasions in navigation by birds (Able 1980). However, although the stimulus easily meets the first and second criteria above for use in navigation (see below), very little experimental evidence for magnetic sensitivity in fishes has been obtained. Therefore, the utility of the geomagnetic field in navigation by pelagic fishes can not be evaluated.

The geomagnetic field easily meets the first two requirements above for use in navigation. The dipole field (90% of the total field; IAGA 1975) varies systematically in the magnetic north-south direction. Intensity and angle of inclination of the field increase from about 27 microTesla (μT) to 70 μT (= 0.27-0.7 Gauss) and from 0° to 90° respectively between the magnetic equator and the magnetic poles. In the east-west direction systematic variations in the intensity, and in the angles of inclination and declination (the angle between the magnetic and geographic meridia) arise from the non-dipole component of the field, and from displacement of the magnetic from the geographic poles (Chapman 1951). Systematic and non-systematic variations in the field also arise from tectonic processes. Seafloor spreading away from mid-ocean ridges superimposes a

pattern of magnetic anomalies due to magnetization of new seafloor in the direction of the current magnetic polarity. Over geologic time, magnetic field polarity reversals lead to development of a pattern of anomalies that is roughly symmetric about the spreading zone. Submarine volcanism causes non-systematic variation in the geomagnetic field in a similar fashion. The field also varies in time. Diurnal fluctuations in intensity occur as a result of interaction between the solar wind and the upper atmosphere. Other periodic variations in the field arise from lunar and seasonal cycles (Garland 1979). Thus there is a great deal of navigational information available to animals with sufficiently sensitive magnetoreceptors.

The idea that animals might be able to navigate using the geomagnetic field has much intuitive appeal. A very early hypothesis for magnetic field navigation comes from Viguier (1882) who proposed a navigational system based on the intensity and the angles of inclination and declination of the geomagnetic field. One hundred years later different models for magnetic field navigation are still being proposed (Walcott 1980, Quinn 1982). In the intervening period evidence for the ability of animals, especially birds, to detect magnetic fields has accumulated (Gould 1982a). However, in many years of attempts, very few experiments have yielded consistent, repeatable, behavioral

or neurophysiological evidence that animals respond to earth-strength magnetic field stimuli (Griffin 1982). The evidence for magnetic sensitivity is now reviewed and the central problems arising from these studies identified.

Many species from different taxa are known to respond to one or more features of the geomagnetic field (Keeton 1971, 1972, Lindauer and Martin 1972, Wiltschko 1972, Wiltschko et al. 1981, Walcott and Green 1974, Martin and Lindauer 1977, Quinn 1980). These responses fall into two categories--responses to magnetic field direction and to magnetic field intensity. Magnetic compass responses include the vanishing bearings of homing pigeons (Walcott and Green 1974) and directional preferences of migratory species in orientation arena experiments (Wiltschko 1972, Tesch 1974, Quinn 1980). The postulated magnetic intensity, or "map", response (Gould 1980, 1982a, Moore 1980, Walcott 1980) refers to the apparent ability of homing pigeons to determine their position to within a kilometer or two using some feature related to geomagnetic field intensity. This response has been inferred from the vanishing bearings and homing speeds of birds released at geomagnetic field anomalies and during magnetic storms (Keeton 1969, 1971, 1972, Walcott 1978, 1980, Gould 1980, 1982a). The conclusion from these studies is that the responses to magnetic fields detected in homing pigeons are consistent with the abilities

required for navigation--an ability to fix position accurately and to set a compass course. The abilities of other species to navigate using the geomagnetic field remains unknown.

There are two central problems in the study of magnetic sensitivity in animals. The first is that all the behavioral results obtained so far are subject to methodological criticisms, are often unrepeatable, and tell little about the functioning of the sense. The second is that as yet it is unknown how and where in the bodies or organisms the magnetic field is detected (Able 1980). Thus it is difficult to design explicit experiments to obtain the necessary behavioral, anatomical, and neurophysiological proofs of the existence of the sense and to analyze its capacities.

Conditioning experiments can provide the necessary repeatability and power for unequivocal demonstration of the existence of the sense. However, attempts to condition animals to magnetic fields have largely failed (Able 1980). Where conditioning has been obtained (Reille 1968, Bookman 1977, Phillips 1977, Kalmijn 1978) the experiments have often been unrepeatable (Kreithen and Keeton 1974, Beaugrand 1976, Griffin 1982). These inconsistent results suggest that the experimental designs may have been inappropriate for demonstrating responses to magnetic fields (Ossenkopp and

Barbeito 1978). The roles of experimental situation, stimuli, response, and subjects in determining the outcome of magnetic field discrimination experiments are considered in Chapter II.

Among the hypotheses for magnetoreception that have been suggested are forms of electrical induction (Kalmijn 1974, Jungerman and Rosenblum 1980), optical pumping (Leask 1977), liquid crystal effects (Russo and Caldwell 1971), and biological superconductivity (Cope 1971, 1973). The transduction mechanisms suggested by these hypotheses would interact with the geomagnetic field to produce stimulus energies greater than the background thermal energy, kT. The receptor mechanisms should therefore be capable of depolarizing the membrane of a receptor cell and making detection of the geomagnetic field possible. However, none of the hypotheses explains both the commonly demonstrated compass responses to magnetic fields made by animals and the sensitivity to small variations in magnetic field intensity exhibited by homing pigeons and other birds (Southern 1978, Gould 1980, 1982a, Walcott 1980). Many also fail to provide evidence of receptor cells that behave in the required fashion (Cope 1973). Finally, magnetoreception is sometimes known to occur under conditions where special requirements of the hypotheses are not met (Quinn et al. 1981).

The possibility that the force exerted on magnetic particles could be transduced to the nervous system has been independently suggested by Ising (1945), Lowenstam (1962), and Keeton (1972). The movement of such particles in response to the geomagnetic field could easily be detected by mechanoreceptors such as hair cells (Keeton 1972). Support for the idea came with the discovery of the common magnetic mineral magnetite (also known as lodestone) in magnetotactic bacteria (Frankel et al. 1979, Frankel and Blakemore 1980), bees (Gould et al. 1978), birds (Walcott et al. 1979, Presti and Pettigrew 1980), and mammals (Zoeger et al. 1981). Theoretical analyses (Kirschvink and Gould 1981, Yorke 1979, 1981) show that where the magnetite is present in a suitable form and in sufficient quantities, it could provide the basis for a very sensitive magnetoreceptor system capable of deriving information about direction and intensity of the geomagnetic field. The appeal of this hypothesis is that it can theoretically explain the general responses of animals to magnetic fields as well as the high sensitivities inferred for the pigeons and other birds (Kirschvink and Gould 1981, Yorke 1981). The hypothesis also makes specific predictions concerning the operation of magnetoreceptor organelles and the constraints they place on behavior (Kirschvink and Walker in review, Chapter III).

The magnetite-based magnetoreception hypothesis also provides a basis for a search for the site of magnetoreception. The studies of biogenic magnetite in vertebrates and invertebrates report localization of the mineral in small regions of the body (Gould et al. 1978, Walcott et al. 1979). Studies have attempted to identify the mineral *in situ* and to test for an association between the magnetite and nervous tissue. However, such studies have failed to achieve this goal because they did not uniquely identify the ferric mineral (Kuterbach et al. 1982, Baker et al. 1983).

There are several reasons why magnetoreceptor organelles have not been previously identified by discovery of sensory structures followed by explanation of their function. The first is that no large, specialized accessory structures are required for magnetite-based magnetoreception (Chapter III). The small size and total volume of the crystals will make them very hard to detect *in situ* (Kirschvink et al. 1982) and the relevant histological techniques may preclude simultaneous demonstration of magnetite and nervous tissue (see Chapters III and IV). Thus failure to identify magnetoreceptor organelles does not necessarily rule out their existence.

The experiments reported in this dissertation seek to demonstrate repeatable responses to magnetic field stimuli,

in conditioning experiments and to show suitable physical and neural bases for these responses in the yellowfin tuna. Reasons for the failure of previous attempts to condition animals to magnetic fields are analyzed and, an orthodox discrimination learning procedure is adapted to test for magnetic sensitivity in the yellowfin tuna. The research also investigates the magnetite-based magnetoreception hypothesis. The primary tests of this hypothesis are behavioral and physiological. However, there are tests on the properties of the magnetite crystals that bear on their origin and suitability for use in magnetoreception. Histological studies attempt to demonstrate the presence of neural tissue in the magnetite-containing tissue of the yellowfin tuna.

Considerations of the nature of the stimulus, the sense, and the likely use made of it are important in the choice of techniques and their adaptation for use in these studies (Chapters II and IV). The properties of the magnetic material dictate care in adapting the paleomagnetic techniques for its detection, analysis, extraction, and separation from contaminants (Chapter III). As many of the methods used in this study had not been used previously to study magnetic sensitivity and its physical and neural bases, they received considerable attention during this study.

CHAPTER II

BEHAVIORAL RESPONSES TO MAGNETIC FIELDS

II.1 INTRODUCTION

Behavioral responses to magnetic fields by animals have been detected in three ways--in field experiments, and from unconditioned and conditioned responses in the laboratory. Field experiments have been conducted primarily with homing pigeons. The most commonly used measure of behavior is the vanishing bearing, the direction in which the bird is last seen or detected from the release site. Birds have also been radio-tracked from the release site to the home loft (Gould 1980). Unconditioned responses are ususally the spontaneous preferences for orienting in one direction exhibited by migratory animals in laboratory orientation arenas (Kramer 1951). Conditioned responses involve the animal learning to produce different responses to magnetic field stimuli by associating them with different outcomes--usually positive or negative reinforcements. The major findings of these studies are reviewed below.

Much of what we know about magnetic field sensitivity has come from field experiments with homing pigeons. The earliest experiments tested the compass response by manipulating the magnetic field within the heads of

experimental birds (Keeton 1972). Walcott and Green (1974) trained birds to home from release sites roughly west of their loft. They subsequently released the birds with electromagnetic coils mounted on their neck and head. Although all birds vanished in the direction of the home loft on sunny days, their vanishing bearings were influenced by the magnetic field within their heads. On cloudy days, those birds with the direction of the vertical component of the magnetic field reversed (north up) flew in the opposite direction from birds perceiving the normal direction of the vertical component (south up). These experiments indicated that the birds used the direction of steepest inclination of the field to indicate the direction of the magnetic pole (Walcott and Green 1974).

Three sets of field experiments suggest that the homing pigeons also possess a magnetic map. The small (30-3000 nanoTesla (nT; 1 nT = 1 gamma)) fluctuations in the total intensity of the geomagnetic field associated with magnetic storms and magnetic anomalies seriously affected the vanishing bearings of pigeons released at unfamiliar sites (Keeton et al. 1974, Walcott 1978). The magnitude of these effects was far greater than if the magnetic compass alone was being disturbed (Gould 1980). Radio tracks of birds homing after release at magnetic anomalies showed that the birds were initially disoriented and that once they had

achieved homeward orientation they avoided other anomalies, travelling over areas of smooth magnetic field intensity gradients (Gould 1980, 1982a). These results implied sensitivity to fluctuations of 10-30 nT in the total intensity of the geomagnetic field and suggested that the magnetic map is at least partly without backup systems (Gould 1980, 1982a, Walcott 1980). However, it is not clear whether the birds were responding to the absolute intensity of the geomagnetic field, the gradients in the intensity, or both (Gould 1982a).

Related observations on pigeon homing suggest that the map is learned, does not require visual cues, and that it is accurate to within a few kilometers. Pigeons without previous flight experience around the home loft appeared unable to place themselves and so to home when released at a distance from the loft (Keeton and Gobert 1970). Experienced pigeons fitted with frosted contact lenses showed initial homeward orientation, flew typical homeward paths, and circled or landed within a 1-2 km radius of the home loft (Schmidt-Koenig and Schlichte 1972, Schmidt-Koenig and Walcott 1978). Taken together these results suggest that the pigeons learn the position of the loft to within a few kilometers and that they do not require visual cues for homing except for their final approach to the loft (Michener and Walcott 1967). If the map response that is presumed to

make homing possible is based on magnetic field intensity, the homing accuracy shown by birds with reduced vision implies a sensitivity (± 10-20 nT) very similar to the sensitivities estimated from the effects of magnetic storms and anomalies on homing (Gould 1982a).

Unconditioned responses to magnetic fields have been used to demonstrate and analyze magnetic sensitivity in several different species. The simplest experiments are those where response to a novel stimulus is recorded (Kalmijn 1978). However, such experiments are of limited use beyond suggesting that animals can detect magnetic fields. Many migratory animals brought into the laboratory exhibit a spontaneous preference for orienting in a particular direction and some have shown the ability to use magnetic field cues to do so (Wiltschko 1972, Tesch 1974, Quinn 1980). In experiments manipulating the horizontal and vertical components of the magnetic field, sockeye salmon, Oncorhynchus nerka, fry responded to magnetic field polarity (Quinn et al. 1981) whereas birds and sockeye salmon smolts responded to the inclination of the field (Wiltschko 1972, Quinn and Brannon 1982). The axial responses detected in birds are corroborated by the field experiments of Walcott and Green (1974). However, no comparable experiments have been carried out with fish.

Indirect evidence for the ability to learn to use magnetic field cues has come from orientation arena experiments with European robins (Erithacus rubecula). The directional preferences exhibited by these birds during their period of migratory restlessness (Zugenruhe) were temporarily abolished in magnetic field intensities outside their previous experience (Wiltschko 1972). After a few days experience at elevated or lowered intensities the birds were again able to make their directional preferences, but were unable to orient at field intensities interpolated between those in which they had been tested. These results suggested that sudden, large changes in magnetic field intensity upset compass orientation in the birds, a finding consistent with the field experiments of Keeton (1972). However, the birds appeared able to overcome the effect of altered intensity on their magnetic compass, possibly through learning (Wiltschko 1972, 1978).

Although both field and laboratory experiments suggest that animals can learn to use magnetic field stimuli, classical and instrumental conditioning techniques have almost universally failed to demonstrate responses to magnetic fields (Ossenkopp and Barbeito 1978). Reille (1968) reported successful heart rate conditioning to magnetic field stimuli in homing pigeons. However, both Kreithen and Keeton (1974) and Beaugrand (1976) were unable to repeat

this result. Using a choice technique, Bookman (1977) reported conditioning homing pigeons to respond to earth-strength magnetic fields. This experiment also turned out to be unrepeatable (Griffin 1982, Walcott personal communication 1982). Phillips (1977) and Kalmijn (1978) reported conditioned responses to magnetic fields in salamanders and elasmobranchs respectively. These experiments have not been shown to be unrepeatable and suggest that further attempts to condition animals to magnetic fields would be useful.

Thus there is evidence that vertebrates can detect and use magnetic fields and that they can learn to use magnetic field stimuli. However, orientation arena experiments are subject to criticism on methodological grounds (Emlen 1975) and laboratory conditioning experiments are largely unsubstantiated. Field experiments, which can not exclude involvement of other sensory mechanisms in migratory or homing orientation, therefore provide the best evidence available for magnetic sensitivity.

The failure of magnetic field conditioning experiments has been given two interpretations: either animals have no useful sensitivity to magnetic fields (Griffin 1982) or experimental approaches have been inappropriate (Ossenkopp and Barbeito 1978, Able 1980). The following review of the nature of magnetic field stimuli, the likely use of the

magnetic sense, the species studied, and the conditioning techniques used suggest a number of reasons why conditioning experiments have mostly failed and what approaches may provide repeatable results.

The geomagnetic field is variable in space due to systematic latitudinal variations in field intensity, inclination, and declination. Spatial and temporal variations also result from tectonic activity, periodic events, and polar reversals (Garland 1979). These properties of the field have almost certainly influenced the evolution of the magnetic sense.

As discussed above some feature related to magnetic field intensity could form the basis of a magnetic map (Gould 1980, 1982a, Moore 1980, Walcott 1980). Use of these features of the geomagnetic field requires sensitivity to very small (<30 nT or 0.05%) changes in field intensity. These variations in the field have to be detected against a background of fluctuations arising from diurnal events, and from magnetic storms and anomalies. The background fluctuations can be many times the relevant signal, up to 5% of the total intensity or 100 times the required minimum sensitivity. Thus determination of position using magnetic field intensity requires highly sensitive receptors that can

detect a very weak signal embedded in considerable background noise.

The dipole property of the geomagnetic field provides magnetic compass information. During polar reversals, which have occurred at mean intervals of about 330,000 years over the last 44 million years, the dipole field may disappear completely, leaving a non-dipole field of about 10-20% of the normal field (Phillips 1977, Garland 1979). Following reversal, the magnetic poles reside in opposite hemispheres, so that magnetic field polarity provides information contradictory to the information available prior to reversal. Inclination of the field does not present contradictory information following reversal as it merely indicates the direction of the nearest magnetic pole. However, inclination will provide contradictory information about absolute direction on opposite sides of the equator. Thus a magnetic compass must be either evolutionarily flexible to accommodate the effects of magnetic field polarity reversal (Quinn et al. 1981) or behaviorally flexible to accommodate the contradictory information available from inclination on opposite sides of the magnetic equator.

The presumed function of a magnetic sense is to allow animals to orient themselves in space. The individual organism can be considered a point detector that apparently

needs to be able to detect spatial variations in the field to determine its position (Gould 1980, 1982a). Because it is a scalar quantity, spatial variations in magnetic field intensity can only be detected by sampling the field at different points in the environment. This implies that animals must be free to move to determine their position. There appears to be no similar requirement for deriving compass information. However, compass courses must be monitored during directed movements. Movement therefore appears very important in responses to magnetic fields and laboratory experiments that restrict movement may inappropriately constrain the use of the sense.

There are a number of reasons why the species commonly used for studies of animal learning may be unsuitable for use in magnetic field conditioning experiments. The sensitivity to magnetic field intensity inferred for homing pigeons (Gould 1980, 1982a, Walcott 1980) suggests that, to be able to use a magnetic sense for map responses, animals must make routine movements of more than a few kilometers. Species such as the goldfish and the white rat have long been domesticated. Even in the feral state, any movements these animals make are likely to be restricted to a familiar area that they have learned to recognize visually and perhaps through scent marking or other non-visual cues (Baker 1978). The homing pigeon does routinely cover

distances that would make possession of a magnetic sense advantageous and should therefore be a good model. However, pigeons and other birds have not performed well in magnetic field conditioning experiments, even where movement was permitted or required (Bookman 1977, Griffin 1982). It is therefore possible that other species may be better subjects for magnetic field conditioning experiments.

For pelagic fishes migrating in the open ocean there are probably relatively few environmental cues that are of use in guiding migrations (Tesch 1980). The shortage of alternative cues, the distances to be covered between feeding and breeding grounds (Sund et al. 1981), and the energetic costs of covering these distances (Sharp and Dotson 1977) suggest that the magnetic sense of pelagic fishes should be well developed. Pelagic fishes could therefore be good subjects for appropriately designed magnetic field conditioning studies. The disadvantages of using pelagic fishes are that the conditions under which they can be maintained in the laboratory, and their anatomy, physiology, and behavior are far less well known than those of other fishes. These drawbacks greatly increase the difficulty of any conditioning experiments (Kling 1971).

The approaches used to study magnetic field conditioning in animals can be summarized as follows. Fields

were uniform and movement was restricted, limiting the subject's ability to sample the magnetic field environment (Meyer and Lambe 1966, Reille 1968, Kreithen and Keeton 1974). Magnetic field polarity was the most commonly used discriminative stimulus (Phillips 1977, Kalmijn 1978, Griffin 1982). The subject animal was usually required to make a choice between two alternate responses and, with one exception (Meyer and Lambe 1966), multiple responding was not required (Bookman 1977, Kalmijn 1978, Griffin 1982). The discussion of magnetic field stimuli and their likely use above suggests that conditioning procedures that limit movement are inappropriate. As will be discussed below (see II.2.3.1), requiring single responses and using choice procedures are also inappropriate for testing whether animals can distinguish magnetic field stimuli.

In summary, studies of birds demonstrate a magnetic compass that forms part of a redundant system of compasses (Keeton 1969, 1971, 1972, Wiltschko et al. 1976, 1981). The intensity, or gradients in intensity, of the earth's magnetic field appear to contribute to the map response of homing pigeons (Gould 1980, 1982a). Learning has been implicated in the use of the map by pigeons (Keeton and Gobert 1970, Gould 1980) and has also been inferred from laboratory experiments (Wiltschko 1978). Laboratory studies of unconditioned responses to magnetic fields by birds have

clear methodological weaknesses (Emlen 1975, Ossenkopp and Barbeito 1978, Able 1980) and, in spite of the evidence for learning in both field and laboratory experiments, magnetic field conditioning studies have usually failed. Thus it is not yet possible to accept the hypothesis of magnetic field detection by animals without reservation. However, before rejecting the hypothesis it must be shown that experiments that are not subject to methodological criticism fail to produce repeatable results.

The experiments reported here attempted to use sensitive and robust methods to detect unconditioned and conditioned responses to magnetic fields in the yellowfin tuna. An easily quantified conditioned behavior was used to test for unconditioned responses to magnetic fields. The experiments observed changes in the pattern of performance of the conditioned response in the presence of a novel magnetic field stimulus. Conditioning experiments using both unitary and choice procedures sought to obtain accurate performance from the fish by (1) presenting as general a pair of discriminative stimuli as possible, (2) requiring multiple responding, and (3) positively reinforcing responses to one of the stimuli and negatively reinforcing responses to the other. These experiments sought to determine whether the fish did in fact possess a

conditionable magnetic sense and to compare the results obtained from different conditioning techniques.

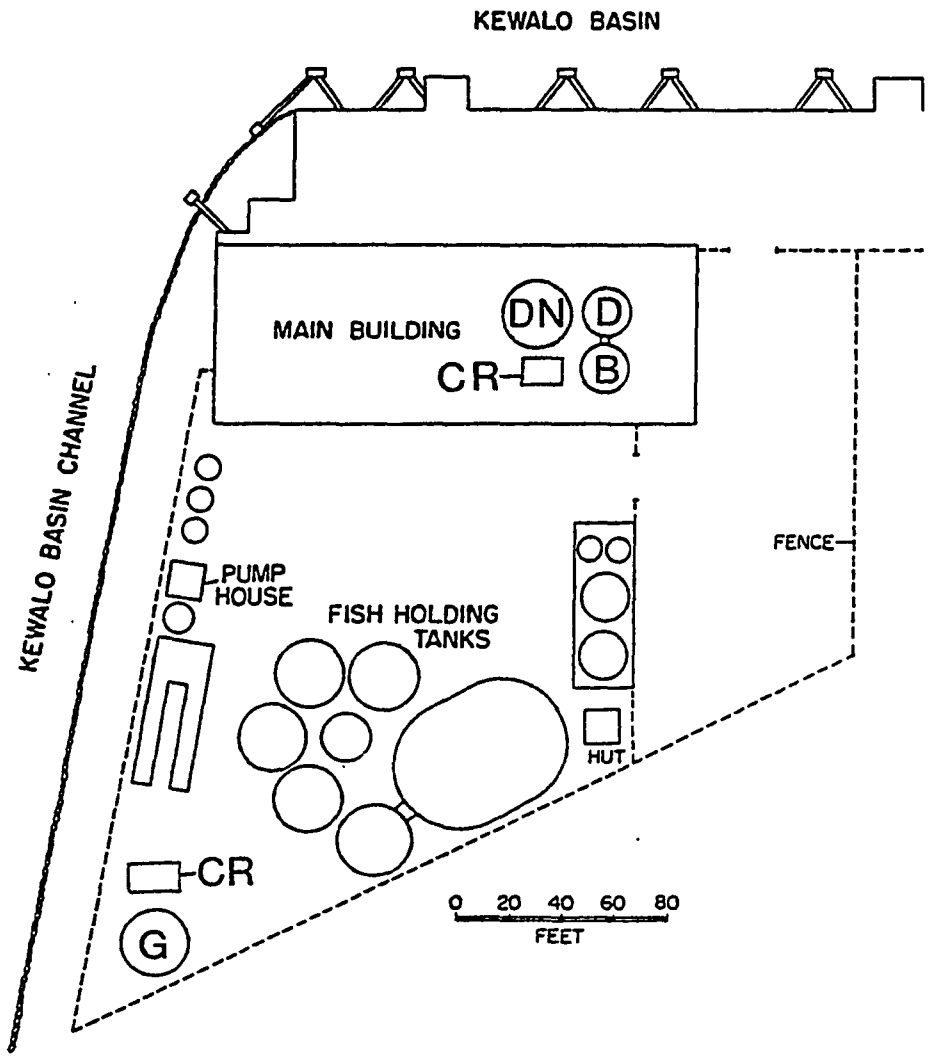
II.2 MATERIALS AND METHODS

II.2.1 Experimental facilities, apparatus, and animals

The Kewalo Research Facility of the National Marine Fisheries Service Honolulu Laboratory routinely maintains live tunas in shoreside tanks for behavioral and physiological studies. Over the 25 years of operation of the facility, techniques for the maintenance and handling of the fish have developed to the point where some species can be kept in captivity for several years and can be kept individually in experimental tanks for periods of many weeks. There has been a parallel development of techniques for study of the behavior and physiology of captive tunas (Queenth and Brill 1983). Thus many of the problems of initiating conditioning studies (Kling 1971) in tunas have been overcome.

Experimental tanks used in these studies were constructed of fiberglass or plywood and were 6 m in diameter by 0.75-1.0 m in depth. The absence of metal in the tank construction limited distortion of the magnetic fields in the tanks to that produced by iron in adjacent

Figure 2.1. Schematic map of the Kewalo Research Facility indicating locations of experimental tanks (modified from Queenth and Brill 1983). Abbreviations used: CR: control room; DB: dumbell tank; DN: Do-nut tank.

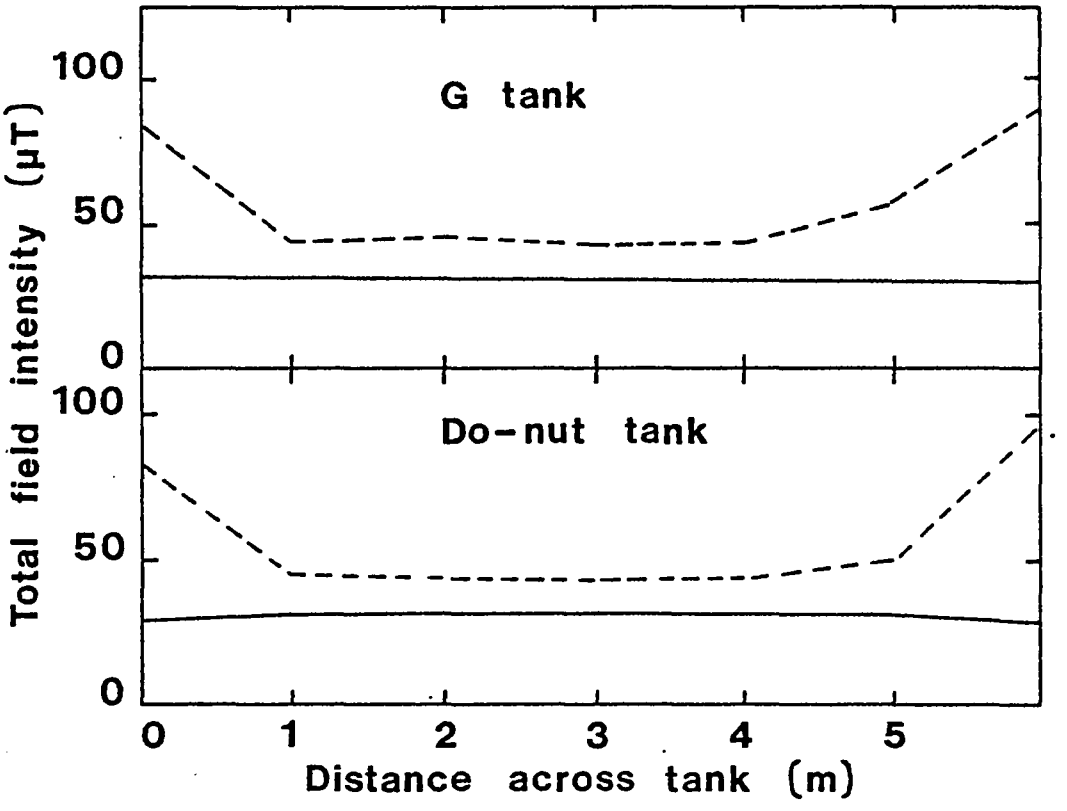

KEWALO BASIN
MAIN BUILDING
DN
D
B
CR
KEWALO BASIN CHANNEL
FENCE
PUMP HOUSE
FISH HOLDING TANKS
HUT
CR
G
0 20 40 60 80
FEET

structures. Tanks used in the experiments were "G" tank, the Do-nut tank (simple cylindrical tanks), and the dumbell tank (a pair of tanks linked by a short tunnel through which the fish could be easily trained to swim (Figure 2.1)). A direct current passed through 100 turns of #18 AWG magnet wire wrapped around the perimeter of the tanks induced a vertical magnetic field in each tank. The artificial fields were non-uniform, adding from 10 μT (= 0.1 Gauss) in the center to 50 μTat the edge of the tanks (Figure 2.2) when current was passed through the coils). The non-uniformity in the altered fields resulted from an edge effect that made the fields stronger near the wires than in the center of the experimental tanks.

Thus the differences in the magnetic fields used in the conditioning experiments were as follows. The local Hawaiian field was uniform throughout the tanks. That is, inclination (38°; USNOO 1966), declination (11° 30' E; USNOAA 1981), and total intensity (approximately 30 μT; field measured with Develco fluxgate magnetometer) were the same at any point in the area occupied by the fish. The altered field introduced significant radially oriented gradients of inclination and intensity as well as changing the intensity and inclination of the field at all points in the tanks.

Because tunas must swim continuously (Magnuson 1973) they can only be conditioned to produce responses involving

Figure 2.2. Map of total field intensity in microTesla (μ T) under normal and altered field conditions within G and the Do-nut tanks when direct current was passed through the coils surrounding the tanks. Solid lines: normal fields; broken lines altered fields. Currents passed G: 0.95 A; Do-nut: 0.8 A.


G tank
Do-nut tank
Total field intensity (μT)
100
50
0
100
50
0
0
1
2
3
4
5
Distance across tank (m)

movement. The response apparatus used in the conditioning experiments was a pipe frame lined with vinyl or plastic sheet. The conditioned response required the fish to swim through a 60-x30-cm opening cut in the sheet lining the frame. This response could not be produced outside the context of the experiment and provided an easily measurable bit of behavior (Bitterman 1966).

Experiments were run from control rooms that were physically isolated from the tanks. Mechanical and electrical linkages in the control rooms operated the response apparatus and feeders, and direct current power supplies generated the altered magnetic fields used in the experiments. The fish were observed through small viewing ports and their responses recorded manually. In the unconditioned response experiments, the behavior of the fish was monitored automatically by micro-computer.

Fish used in the experiments were juvenile yellowfin tuna (40-50 cm fork length) held in schools in outdoor holding tanks at the Kewalo Research Facility. For testing fish were moved individually (procedure described in Queenth and Brill 1983) to one of the experimental tanks and allowed to acclimate for two days. During this time they were fed from a feeder mounted at the side of each tank (Jemison et al. 1982). The fish had no difficulty approaching the

feeder, which dropped food into the water approximately 1 m from the edge of the experimental tank.

II.2.2 Unconditioned response experiments

As part of temperature preference experiments at the Kewalo Research Facility, yellowfin tuna were conditioned to shuttle between the two halves of the dumbell tank in anticipation of a food reward given according to a variable ratio schedule. Banks of photocells in the 60-x120-cm tunnel recorded the passage of the fish and a micro-computer monitored the intervals between passes by the fish through the tunnel. An electromagnetic coil mounted at the side of the tunnel and switched on randomly for one inter-pass interval (IPI) induced a magnetic field anomaly in the tunnel. This anomaly extended over a small area so that the fish probably could not detect it until committed to swimming through the tunnel. The effect of the anomaly on the behavior of the fish was determined by comparing the time taken for the fish to swim through the tunnel after it had passed through the anomaly with the mean interval between passes where no anomaly had been present.

II.2.3 Conditioning experiments

II.2.3.1 Experimental strategy

Learning in animals is detected as a relatively permanent change in behavior resulting from conditions of practice (Kling 1971). Thus, in discrimination learning experiments, some measurable bit of behavior is modified by the experience of differential reinforcement of response to the discriminative stimuli. In unitary, or go-no go, discriminative training procedures a single, generalized response is defined and then either positively or negatively reinforced under different stimulus conditions. Discrimination learning in such experiments is measured by comparing the readiness with which the response is expressed between the stimulus conditions (Bitterman 1966). In choice procedures, two discrete responses that can not be produced together are defined. In one stimulus condition one of the responses is rewarded and the other punished. In the alternate stimulus condition the consequences of the two responses are reversed. Discrimination is detected from the choices the animal makes between the alternate responses under the different stimulus conditions (Bitterman 1966).

The first hypothesis to test in magnetic field conditioning experiments is that animals can distinguish

between different magnetic field stimuli. Most of the published magnetic field conditioning experiments have used choice procedures in which movement by the subject was limited. Magnetic fields are pervasive stimuli that can only be presented singly in experimental situations. As discussed above the ability to sample the magnetic field at different points in space and time appears important in the use of the magnetic sense by animals. Discriminative training procedures should therefore permit the subjects freedom of movement and sufficient time to sample the magnetic field before or during responding. Training procedures should also be appropriate for use with singly presented stimuli. The approach adopted first in these studies was to use a unitary conditioning procedure. A single response requiring movement of the whole body of the fish was defined and rewarded under one magnetic field condition but not under another. The use of a discrete-trials/fixed-interval procedure (Woodard and Bitterman 1974; see below) permitted the subjects time to sample the field and to produce multiple responses during trials, a procedure that sharpens discrimination (Bitterman 1976). Thus the measure of behavior compared between the discriminative stimuli was the rate of performance of the conditioned response.

The magnetic fields used in these experiments provided changes in angle of inclination, magnetic field intensity,

and the gradients in inclination and intensity of the magnetic field. Spatial variations within the altered field, and differences between the patterns of spatial variation in the two test fields, were considerable and the fish could conceivably monitor any or all of the varying features in making the discrimination. The unitary procedure and the discriminative stimuli used in these experiments therefore provided as general a pair of stimuli as possible for the fish to distinguish in as simple a testing procedure as I could devise.

The unitary response experimental procedure using the rate measure was, however, unsuitable for detailed analysis of the capacities of the magnetic sense of the yellowfin tuna. To estimate the smallest changes in magnetic fields the yellowfin tuna could detect required development of apparatus that provided uniform fields and a different training procedure. The approach that was adopted was to modify the coil around the Do-nut tank to provide a more uniform altered magnetic field stimulus and to test a choice conditioning procedure.

Because magnetic field stimuli can only be presented singly, the procedure is defined as a successive rather than a simultaneous choice procedure (Bitterman 1976). Successive choice procedures are generally more difficult than simultaneous choice procedures and may be impossible for

animals to learn when stimuli are not salient (Mackintosh 1974, Bitterman 1976). Choice conditioning experiments using magnetic fields are therefore likely to be very difficult. However, because the only reports of successful behavioral conditioning using magnetic fields used choice procedures (Bookman 1977, Phillips 1977, Kalmijn 1978), it is important that these procedures be tested and evaluated. Because of the anticipated difficulty of the experiments, I took measures to maximize the chances of demonstrating discrimination in the choice experiments. These measures sought to enforce accuracy of responding and used responses that required movement and allowed the fish time to sample the magnetic field in the tank before responding.

II.2.3.2 Unitary discriminative training procedure

After the acclimation period, the fish began training in 1-2 hour sessions held once daily. Training was conducted between 0800 and 1600 with each fish being trained at approximately the same time each day. The pipe frame was lowered into the water and the fish enticed through the frame using a bait hanging in front of the opening. The bait was removed as the fish struck at it and became committed to swimming through the frame. After the fish passed through the frame it was rewarded with a piece of food (cut smelt).

By the end of the first session, the fish usually began to swim through the frame spontaneously and all fish responded freely after the second training session. On the third day's training, the fish was required to make up to three passes per reward. On the fourth day, the fish made up to five passes per reward and the frame was periodically retracted from the tank for several minutes.

The fish was then shaped to respond for fifteen 30 second trial periods in each training session. The pipe frame was lowered into the tank and the fish allowed to respond freely for 30 seconds. The first response after 30 seconds brought food from the feeder and the frame was retracted for an inter-trial interval (ITI). The ITI, selected randomly from a set of cards specifying different ITI lengths, averaged three minutes duration in this pre-training period. This pre-training procedure led to the fish establishing a rate of performance of the conditioned response and receiving a certain number of rewards per unit time.

To ensure that the fish only gained experience with receiving food in association with the correct stimulus, the magnetic field that was later to become the positively reinforced stimulus (designated S+) was presented simultaneously with the hoop during pre-training. That is, if the altered field was to become S+ during discrimination

testing, the field was switched on at the same time as the pipe frame was lowered into the tank and switched off at the end of the trial when the frame was removed. Alternatively, if the altered field was later to become the negatively reinforced stimulus (designated S-) the fish gained no experience with that field at any stage during the pre-training period.

When the fish had attained stable rates of responding, generally after two days, discrimination testing began. In discrimination testing, a trial began with simultaneous presentation of the pipe frame and either the positively or negatively reinforced stimulus. All responses by the fish in the 30 second trial were counted. In S+ trials, the fish was positively reinforced with a piece of food at the first response after 30 seconds. In S- trials, a 10 second penalty timer started at the end of the 30 second period. If the fish responded before the 10 seconds had elapsed, the timer was reset. The timer was reset by each subsequent response until either the fish failed to respond for 10 seconds or until a total of 30 seconds of penalty time had elapsed. Response to S- was thus penalized by extending the trial without any possiblity of the fish obtaining food for producing the response (Woodard and Bitterman 1974). After the reinforcement had been given the pipe frame was retracted for a variable ITI (mean 90 seconds) after which

another trial sequence began. An important feature of the discrete-trials/fixed-interval training procedure is that the rate of response to the stimuli is measured during a trial period in which the fish receives no reinforcement for responding. The possibility that the fish might discriminate the reinforcement is thus excluded.

The fish were given 30 trial sessions held once daily. In any trial session the S+ and S- were presented in equal numbers in a quasi-random order with no more than three S+ or S- trials in succession (Gellerman 1933). Reduction of the ITI to a mean of 90 seconds resulted in the fish receiving about the same amount of positive reinforcement per unit time as in the pre-training. To ensure that any difference in response to the two magnetic field stimuli was not due to some differential effect of the fields on the general behavior of the fish, for example, disorientation caused by the altered field, testing was balanced by training different fish with either the normal Hawaiian field or the altered field as S+.

In a subsequent experiment, designed to exclude possible observer-related cues and to examine the effect of manipulation of the paradigm on learning by the fish, fish were tested using double blind procedures. The penalty timer was set at 15 seconds with a total allowable penalty time of

up to 90 seconds. In the double blind procedure two people working in different rooms operated different components of the experiment. Communication was by signal light and a simple code. The first person, (the field controller) operated the trial procedure, determining the beginning and end of trials, and the presentation of the discriminative stimuli. The second person (the apparatus controller) was directed by the first to raise and lower the pipe frame and, at the end of each trial, to deliver positive or negative reinforcement. The apparatus controller recorded the responses made by the fish during the trials and signalled the field controller when each command had been executed. The apparatus controller was given no knowledge of the field conditions and which was S+ and S-, whereas the field controller had no knowledge of events under the control of the apparatus controller.

A second manipulation of the experimental procedure involved use of a different pair of discriminative stimuli in the same double blind procedure. As developed, the testing procedure used the normal Hawaiian magnetic field as one of the discriminative stimuli. This field was present at all times outside the experimental training sessions and so may have adversely influenced the behavior of the fish during discrimination testing. An attempt was therefore made to use as discriminative stimuli two magnetic fields that

were outside the previous experience of the fish and that could only be detected during the experiments. A second altered field was generated by reversing the direction of the current through the coil around G tank. The fields added to the background field were of equal intensity but were opposite in their inclination and in their gradients of intensity and inclination. As a result total intensity and inclination at any point in the tank differed between the two experimental fields. However, the gradients in intensity in the tank were the same, although of opposite sign.

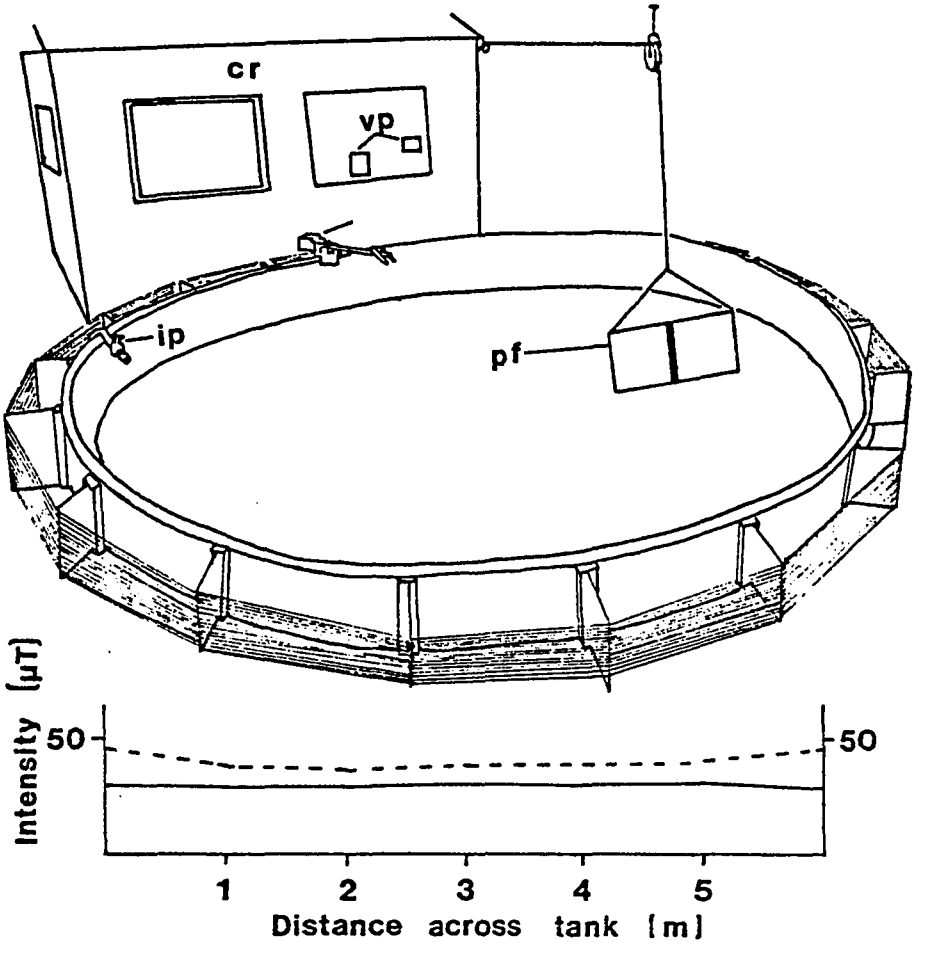
Some criteria for recognition of discrimination learning by the fish were necessary. In these experiments, the experience of differential reinforcement of the response contingent on the S+ and S- was expected to modify the performance of the response by the fish. The quantitative change in the behavior detected was a maintained difference in the response rates to S+ and S-. Qualitative changes in behavior included differences in swimming patterns between S+ and S- trials, for example, how close the fish stayed to the pipe frame. Both the quantitative and qualitative changes in behavior were considered in determining whether or not the fish were distinguishing between S+ and S-.

II.2.3.3 Successive choice training procedure

To reduce the edge effect in the vicinity of the coil around the Do-nut tank, the wires were wound as a long solenoid on formers that extended 60 cm outside the perimeter of the tank (Figure 2.3). This substantially reduced the gradients of magnetic field intensity in the tank (Figure 2.3). The response apparatus was the same pipe frame modified to present the fish with a pair of openings to swim through. Each opening in the frame was paired with positive reinforcement when one magnetic field was presented and with negative reinforcement when the other magnetic field was presented.

In pre-training the fish was baited through the side of the frame designated correct for the magnetic field presented in that trial. When the fish began to respond spontaneously, it was allowed a progressively increased number of errors before correction by being baited through the correct opening (Bitterman 1966). The basic hoop training required for this experimental procedure was complete after one training session. In discrimination testing, the fish scored a hit by swimming through the opening paired with the field presented and a miss by going through the other opening. Training sessions lasted twenty trials with the discriminative stimuli being presented

Figure 2.3. A. Schematic drawing of the Do-nut tank (not to scale) indicating relationships of feeder, control room, choice response apparatus, coil modified to provide a uniform magnetic field. Abbreviations used: cr: control room; f: feeder; ip: water inlet pipe; pf: pipe frame; vp: viewing ports. B. A map of the fields measured in the tank (measured in μT; 0.6 A current passed to generate the altered field). Solid line: normal field; broken line: altered field.


cr
vp
ip
pf
Intensity [μT]
50
50
1
2
3
4
5
Distance across tank [m]

according to the same rules as in the unitary response experiments.

Systematic attempts were made that sought to determine the stimulus presentation, choice apparatus, positive and negative reinforcements, trial procedure, and signals that would lead to most accurate responding by the fish. Configurations of the pipe frame used included a uniform background with upper and lower openings, a black opening beside a white opening, and a white surround divided by a vertical black bar. The ITI was two minutes except when self-paced trials (Kling 1971) were used. A bridging light and buzzer were used to signal a hit and a miss respectively. Time outs, removal of the pipe frame from the water for a short period before allowing correction after an incorrect response by the fish, and a fixed ratio of responses, a minimum number of correct responses before positive reinforcement was given and the trial ended, were used to enforce accuracy by increasing the cost of a miss.

II.3 RESULTS

II.3.1 Unconditioned responses

The presence of the magnetic field anomaly caused a transient alteration in the rate at which fish shuttled

between the two halves of the dumbell tank. The IPI immediately after the fish had first swum through the anomaly was usually double the mean IPI for the session up to that point. The coil producing the anomaly was switched off immediately after the fish had passed through it. The untested IPI's immediately following a tested IPI were close to the mean untested IPI for the session, suggesting that the fish were disturbed by the anomaly when they first encountered it but settled down to normal shuttling behavior when they did not encounter it on subsequent passes through the tunnel. Successive passes through the anomaly showed progressively less effect on the IPI. For all but one fish the anomaly had no detectable effect on shuttling behavior after the first day's testing. The shuttling behavior of this fish continued to be disturbed by the presence of the anomaly on test days up to a week after the first tests (Table II.I). These results suggested that, with this exception, the fish were at first disturbed by the anomaly but that they later paid no attention to it. From these results it can be inferred that the fish were able to detect the presence of the magnetic field anomaly in the tunnel.

II.3.2 Unitary response experiments

In the pre-training period the fish established reasonably stable baseline response rates. At first the fish

Table II.I. -- Unconditioned responses to magnetic fields. Effect of swimming through an intermittently presented magnetic field anomaly on subsequent shuttling behavior in the dumbell tank in a yellowfin tuna. The time between passes through the tunnel (the inter-pass interal (IPI)) between the two halves of the tank is measured in seconds. Untested IPI's refer to those IPI's in which the fish did not swim through the anomaly and tested IPI's refer to the interval between a pass through the anomaly and the subsequent pass through the tunnel.

Date	Untested ± s. d. (N)	Tested ± s. d. (N)
IV/29/80	7.2 ± 4.0 (33)	8.5 ± 3.0 (4)
IV/30/80	5.8 ± 1.3 (19)	13.9 ± 8.1 (10)
V/5/80	6.5 ± 3.3 (20)	13.8 ± 6.3 (6)
Total	6.5 ± 0.7 (72)	12.1 ± 3.1 (20)

responded at low rates but over the course of two training sessions their response rates appeared to reach asymptotic levels. These rates differed among individual fish because of the different sizes of the fish relative to the opening in the pipe frame and the different approaches to the frame employed by each fish (Figures 2.4-2.8). Fish that swam in circles and approached the frame predominantly from one direction established lower rates of response than fish that approached it freely from both sides in figure 8 patterns centered on the frame. Maintenance of the fish on the pre-training regime beyond the second day saw no further increase in the response rate. Thus for all fish tested for discrimination the pre-training period was restricted to two days.

All of the fish used in development of the testing procedure showed some separation of response rates to S+ and S- during discrimination testing. Results for three fish tested with slightly different pre-training experience, different penalty times, and ITI's are presented in Figure 2.4. Two of the fish showed a large initial decrease followed by a recovery in response rates. The third fish showed a smaller drop in response rates and a difference in response rates to S+ and S- almost immediately after discrimination testing began. All these experiments ended in the fish jumping out of the experimental tank and dying. As

Figure 2.4. Response acquisition and discrimination performance for fish tested during development of the unitary testing procedure. Each point is the mean of five pre-training (closed circles, blocks 1-6), or S+ (closed squares) and S- (open circles) (blocks 7 *et seq.*) trials for each fish trained.

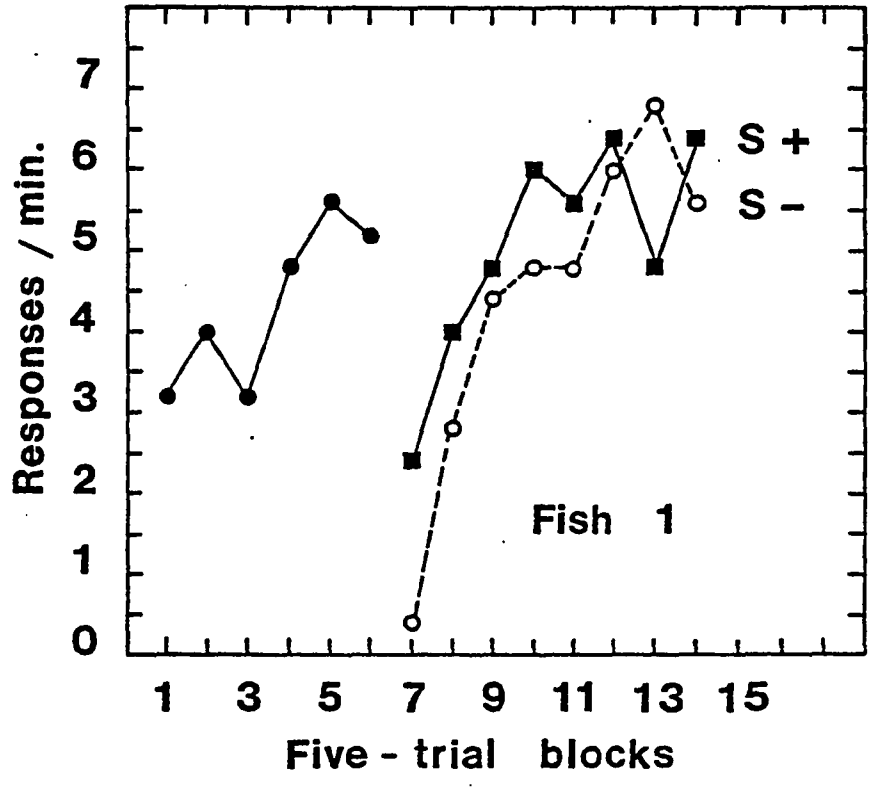

Responses / min.
0
1
2
3
4
5
6
7
1
3
5
7
9
11
13
15
Five - trial blocks
S +
S –
Fish 1

Figure 2.4 (Continued)

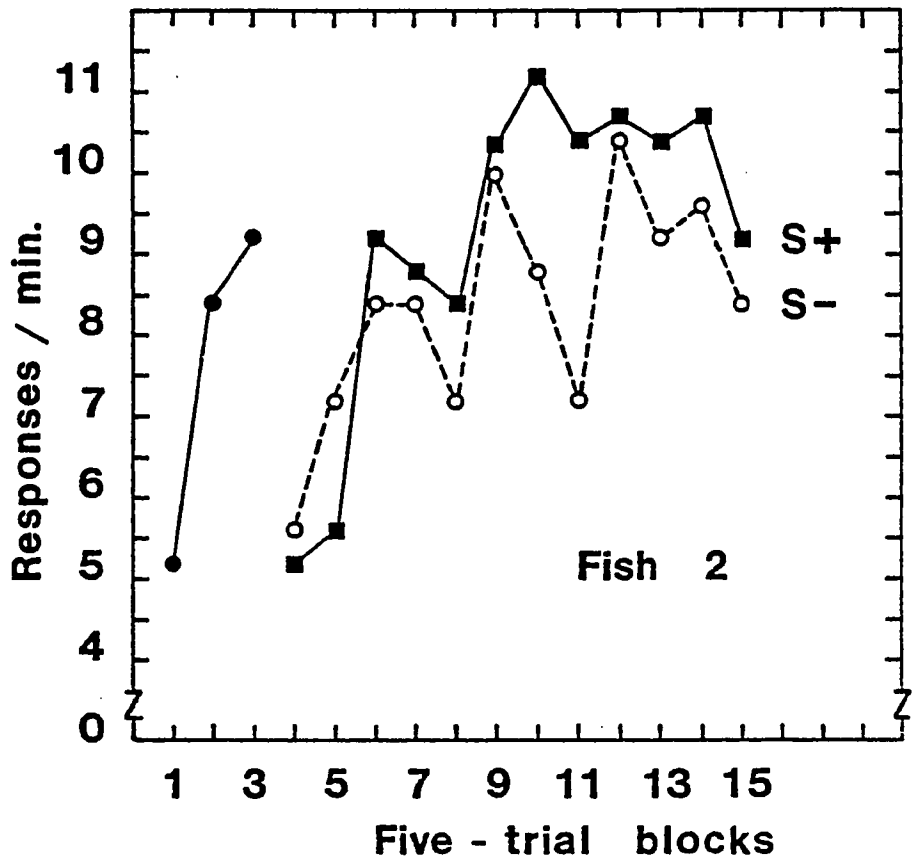
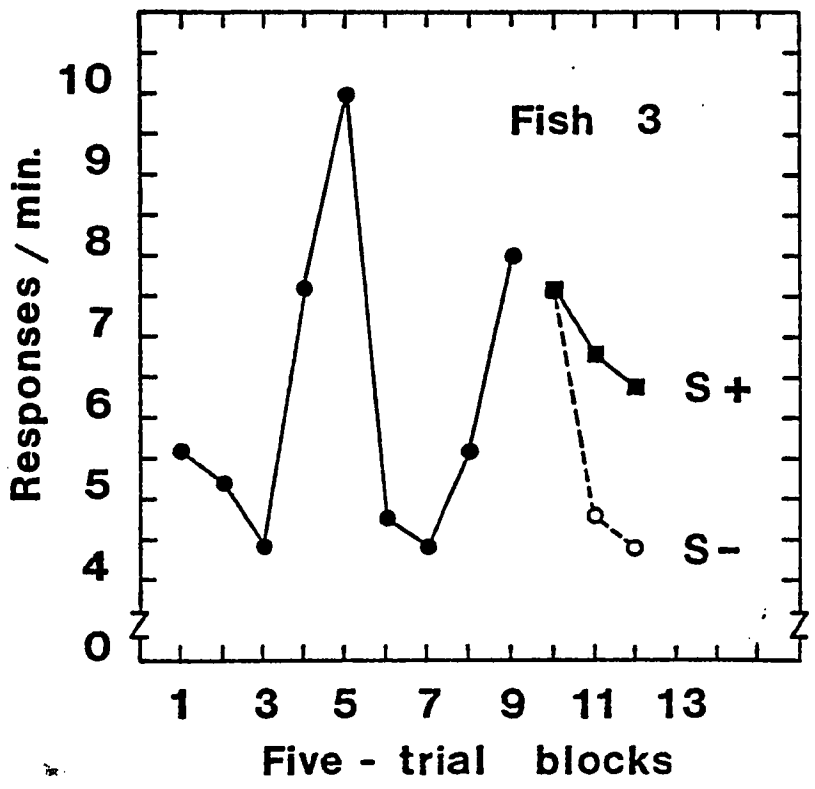


Figure 2.4 (Continued)



a result of these experiments the maximum allowable penalty used in the procedure finally adopted was set at 30 seconds.

For five fish tested in the first series of experiments, discrimination between the two magnetic fields became evident after two 30-trial sessions. During the first two days of testing (blocks 7-12 in Figure 2.5), response rates to the two stimuli fluctuated about each other. By the third day, all fish produced higher rates of response to the positively than to the negatively reinforced stimulus and continued to do so for the remainder of the experiments. These were of varying length depending on the health of the fish and the use subsequently made of them. Initiation of testing was followed by an increase in mean response rate (Figure 2.5). During testing, overall response rate declined slightly although response rates during S+ trials remained close to the maximum.

All the fish completed at least 130 trials. An analysis of variance comparing S+ and S- response rates over the 13 S+ and S- five-trial blocks plotted in Figure 2.5 yielded an $F_{(1,4)\ \text{stimuli}} = 8.4543$, $p = 0.0438$ (Table II.II). One interaction term, stimuli by blocks, yielded an $F_{12,\ 48} = 2.8776$, $p = 0.0046$. All other comparisons within the analysis, including a test for a difference in behavior between experimental tanks (not shown in Table II.II), did not approach significance. Thus the analysis showed a main

Figure 2.5. Response acquisition and discrimination learning in five yellowfin tuna. Each point is the mean of five pre-training (closed circles, blocks 1-6) or S+ (closed squares) and S- (open circles) (blocks 7-19) trials for all fish tested.

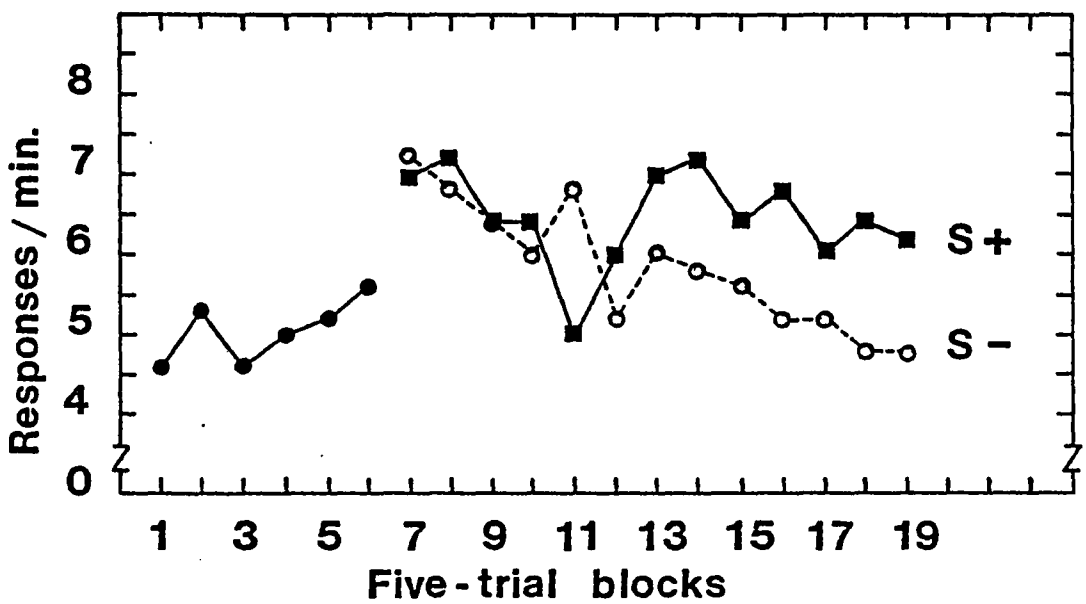

Responses / min.
0
4
5
6
7
8
1
3
5
7
9
11
13
15
17
19
Five-trial blocks
S+
S−

Table II.II -- Magnetic field discrimination learning. Results of analysis of variance comparing S+ and S- response rates for five yellowfin tuna. Abbreviations used: b: blocks; s: stimuli; s: subjects; t: trials.

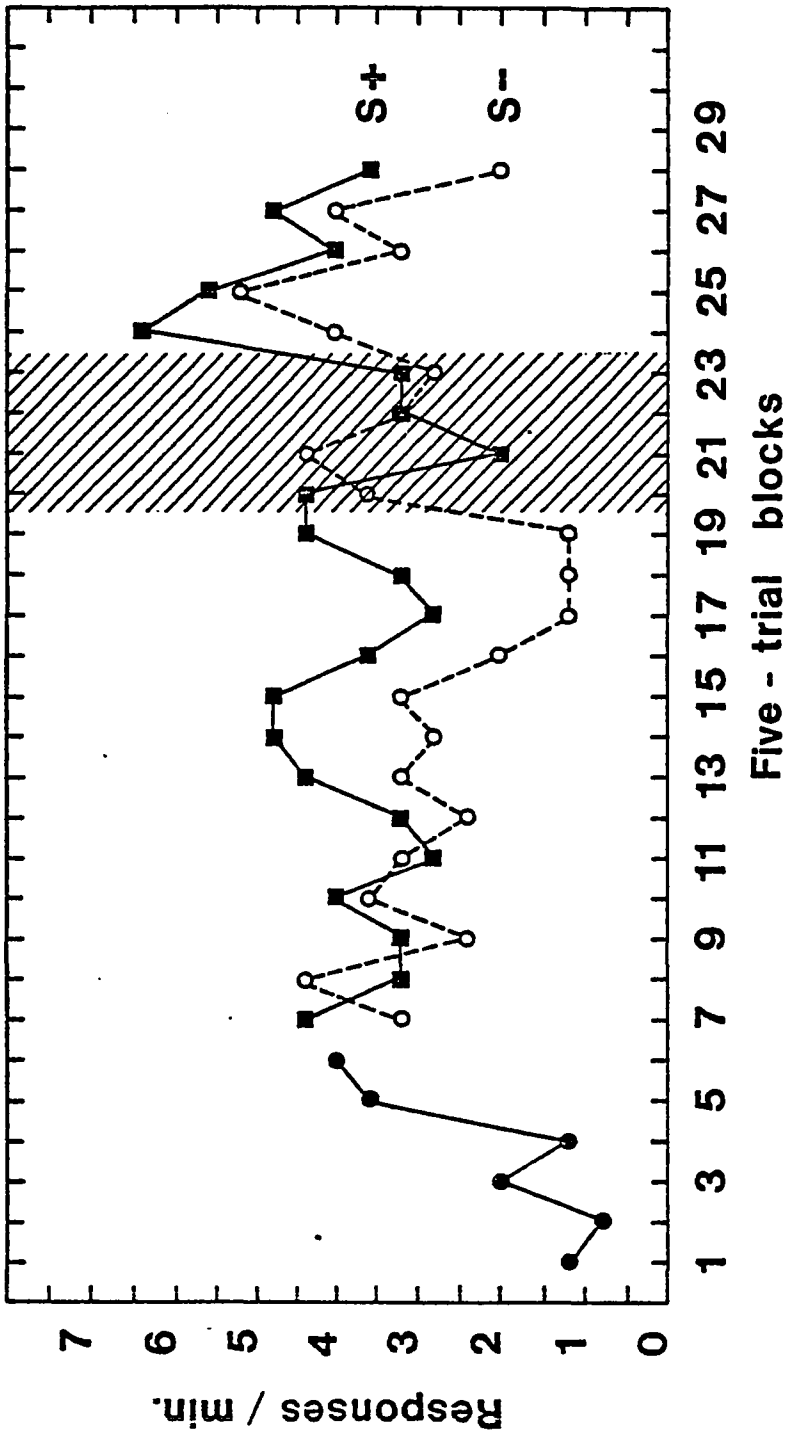
Source	d. f.	Mean square	F	Probability
Total	649			
Blocks	4			
s	1	13.8846	8.4543	0.0438
ss	4	1.6423		
b	12	3.3087	0.6881	0.7544
sb	48	4.8084		
sb	12	3.4046	2.8776	0.0046
ssb	48	1.1831		
t	4	1.3054	1.1538	0.3675
st	16	1.1313		
st	4	2.6731	2.2011	0.1151
sst	16	1.2144		
bt	48	1.3379	0.9855	0.5072
sbt	192	1.3576		
sbt	48	1.4722	0.9595	0.5534
ssbt	192	1.5344		

treatment effect due to the discriminative stimuli. The significant stimuli by blocks interaction showed that the main effect was due to a change in the response rates to S+ and S- during discrimination training, that is, that learning occurred during the experiments.

To test whether the fish were responding to possible equipment or observer-related cues, control trials were conducted with one fish. One of the wires connecting the power supply to the coil around the tank was disconnected and all procedures followed as before. The response rates during positively and negatively reinforced trials fluctuated randomly about each other during this period (Figure 2.6). When the circuit between the power supply and the coil was reestablished, the fish was again able to produce higher response rates to the positively than to the negatively reinforced stimulus (Figure 2.6). However, the separation between response rates was less than before the control trials were conducted.

These experiments revealed a clear conditionable response to magnetic field stimuli in the yellowfin tuna. Three problems were posed by the results. The first was that the differences between response rates to S+ and S- were small and that the variability in response rate was high relative to the maximum response rates achieved. The second was that the unitary procedure using rate as a measure of

Figure 2.6. Response acquisition, discrimination learning, and control tests for one yellowfin tuna. Each point is the mean of five pre-training (closed circles, blocks 1-6) or S+ (closed squares) and S- (open circles) (blocks 7-28) trials. Shaded area indicates control trials.


S+
S-
Responses / min.
0
1
2
3
4
5
6
7
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Five - trial blocks

discrimination can not be used to analyze the capacities of the magnetic sense of the yellowfin tuna. The third problem was that, although it was not confirmed by the analysis of variance, there appeared to be a difference in responding between the two tanks. For example, the mean difference between S+ and S- responding over the 13 five-trial blocks for fish tested in the Do-nut tank was twice that for fish tested in G tank. I followed a dual approach to dealing with these problems. First, I used one tank (the Do-nut tank) for development of a testing procedure that permitted determination of the threshold sensitivity of the yellowfin tuna to changes in magnetic fields (see II.3.3). Second, I attempted to obtain more robust responses from fish tested in G tank by altering the levels of negative reinforcement used in all subsequent experiments, which were also run using double blind procedures.

In the double blind experiments, the penalty timer was set at 15 seconds and the fish were allowed to accumulate more penalty time (up to 90 seconds) than in previous experiments. From this approach it was expected that the response rate would have to drop further than in the previous experiments for the penalty timer to time out regularly. Increasing the total allowable penalty time provided greater penalties for continued responding by the fish and greater opportunity for the penalty timer to time

out. The cost of response to S- was thus substantially increased, and it was hoped that better separation of response rates and more stable behavior would be obtained.

The fish had little difficulty in learning the discrimination in spite of the extensive precautions taken to remove observer-related cues in the double blind experiments. The mean difference between S+ and S- response rates over the first 13 five-trial blocks of discrimination testing (blocks 7-19 in Figure 2.7) was almost double the mean difference between response rates to S+ and S- in the first series of experiments (Figure 2.5). In addition, discrimination became evident considerably earlier, by the end of the first 30-trial session, than in the previous experiments. However, the variability in responding was very high. I attribute the variability in responding to poor environmental control around G tank. Although covered, G tank was more exposed than the Do-nut tank. Its roof leaked during rain and birds nested underneath the cover. Both birds flying over the tank and rain were associated with increased variability in responding by the fish.

The second set of double blind experiments attempted to obtain improved responding by providing discriminative stimuli that were not present in the tank at any times other than during experimental trials. As noted above these fields were obtained by reversing the direction of the current

Figure 2.7. Response acquisition and magnetic field discrimination learning in two yellowfin tuna tested in double blind experiments. Each point is the mean of five pre-training (closed circles, blocks 1-6) or S+ (closed squares) and S- (open circles) (blocks 7-24) trials.

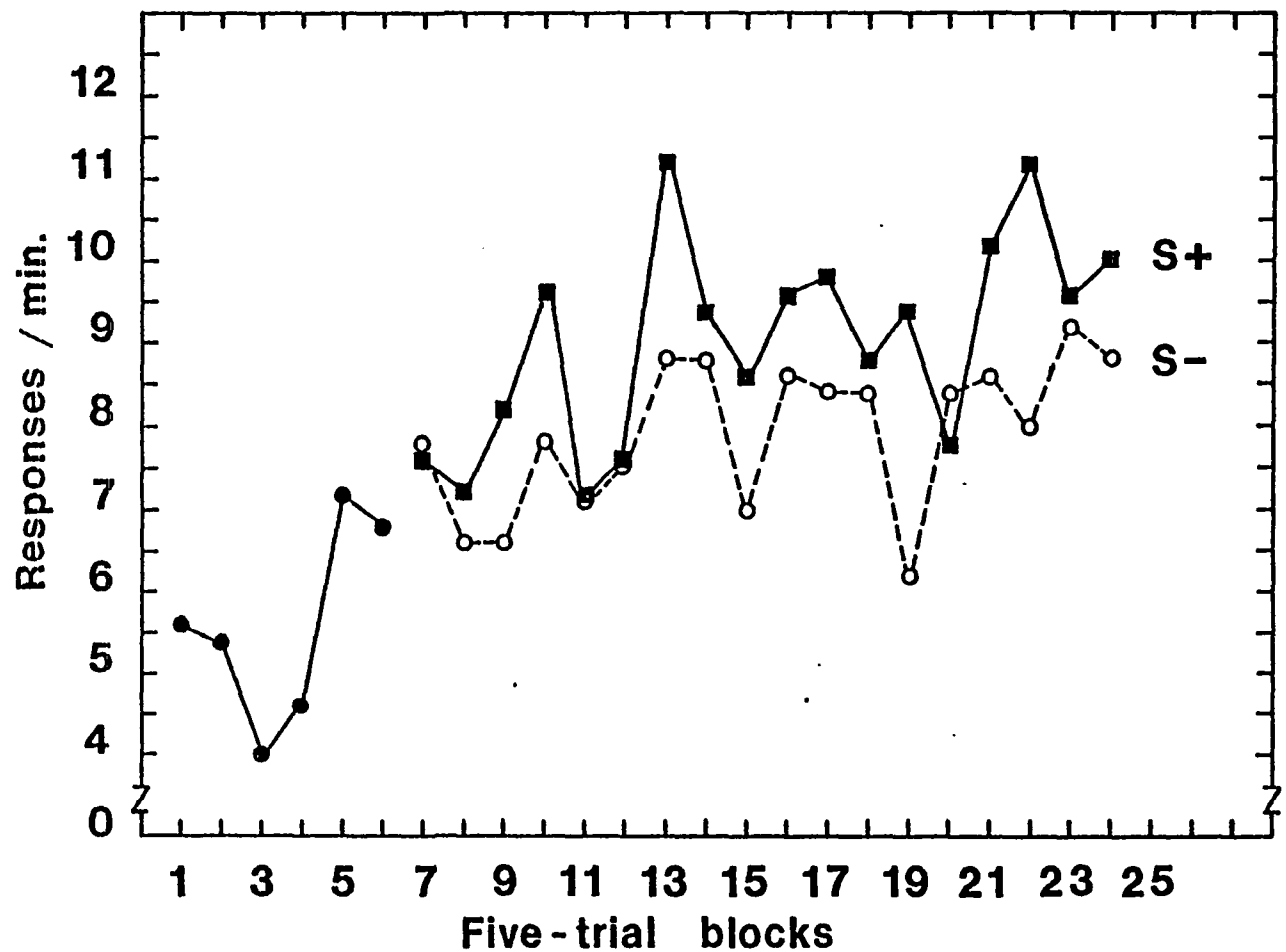
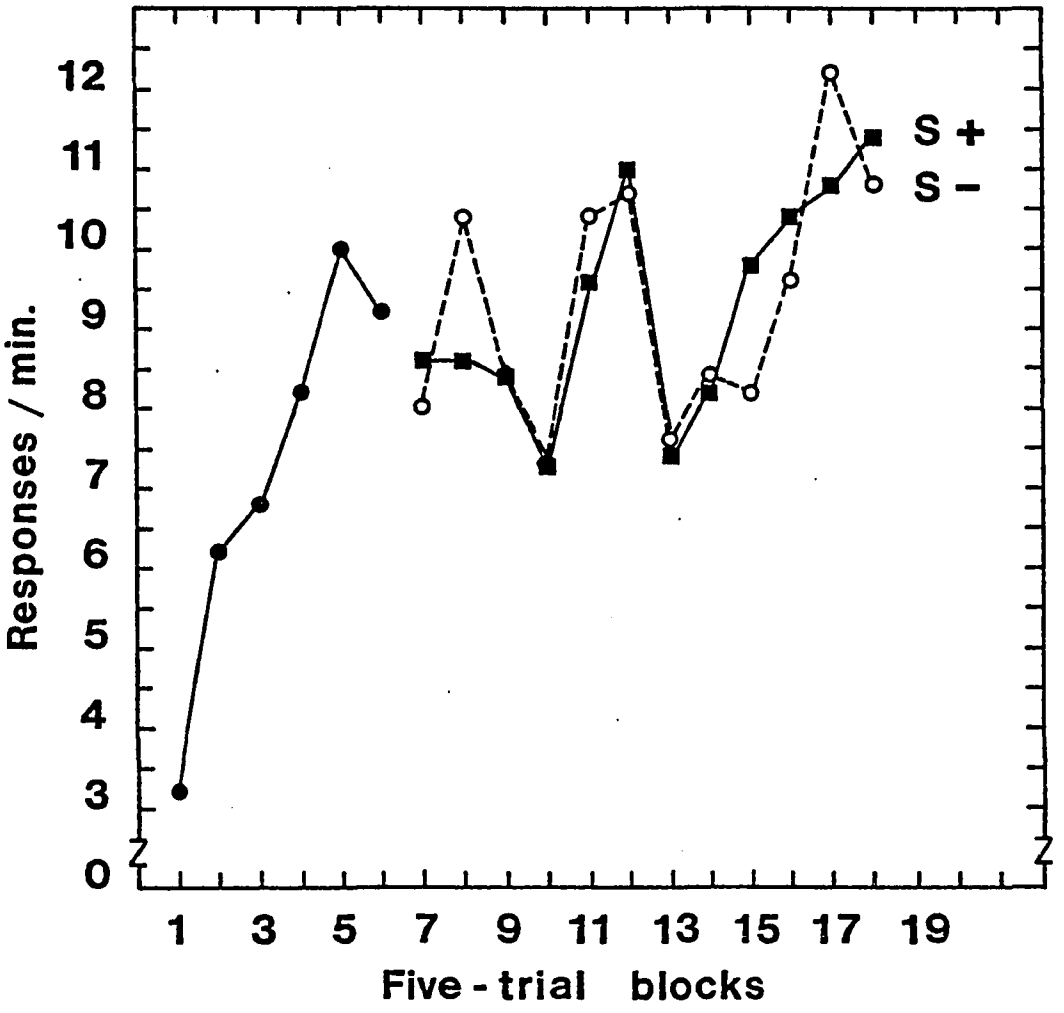

Responses / min.
12
11
10
9
8
7
6
5
4
0
S+
S-
1 3 5 7 9 11 13 15 17 19 21 23 25
Five-trial blocks

Figure 2.8. Response acquisition and discrimination performance in two yellowfin tuna tested using the same double blind procedures as in Figure 2.7 but with different discriminative stimuli. Each point is the mean of five pre-training (closed circles, blocks 1-6) or S+ (closed squares) and S- (open circles) (blocks 7-18) trials.


Responses / min.
0
3
4
5
6
7
8
9
10
11
12
1
3
5
7
9
11
13
15
17
19
Five - trial blocks
S +
S –

flowing through the coil around the tank. At no stage during testing did either of the fish tested show evidence of discrimination (Figure 2.8). It seems unlikely that two fish would fail to respond in this procedure when seven had previously done so in procedures that were either identical or differed only in the amount of penalty time allowed. It therefore appears that the fish could not distinguish between the two fields used in these experiments whereas they could distinguish between the fields used in all the previous experiments.

II.3.3 Successive choice experiments

Despite extensive attempts to obtain consistent, accurate responding in the choice experiments no fish attained accuracy greater than 75% correct over 20 trials and none were able to maintain this level of responding from one training session to the next. Table II.III lists the fish used, procedures tested, and the outcomes of experiments run over a period of 15 months. The experiments did not test all possible combinations of the procedure. Experience gained during the testing permitted exclusion of some combinations as being almost certainly unsuitable. For example, requiring the fish to go either over or under a central bar in the pipe frame presented no ambiguity arising from direction of approach to the frame. However,

Table II.III. -- Results of tests with choice procedures attempting to condition yellowfin tuna and kawakawa (fish 1, 5-7, 10, 15) to magnetic field stimuli. Best response is measured over one testing session of 20 trials. Terms in columns refer to format of pipe frame presented, nature of discriminative choice, and length of inter-trial interval (ITI). An ITI of 0 indicates self-paced trials (see text).

Fish #	Pipe frame	Choice	ITI	Best response
1	black	up/down	2 min.	75%
2	"	"	"	50%
3	"	"	"	75%
4	"	center/wall	"	70%
5	"	"	"	60%
6	black/white	"	"	60%
7	"	"	"	60%
8	"	"	"	55%
9	"	"	"	55%
10	"	"	"	55%
11	"	"	"	40%
12	"	"	0	60%
13	"	"	"	65%
14	white/black bar	right/left	"	70%
15	"	"	"	70%

this procedure was almost immediately discarded because the fish swam at different depths at different times and took up spatial biases related to swimming depth. Similarly, the fish quickly biased toward frame openings with light surrounds over those with dark surrounds.

The fish could not be trained when a fence designed to force them to approach the frame from one direction only was placed in the tank. Consequently, there was ambiguity in the procedure arising from the freedom given to the fish to approach the frame from different directions. In attempts to reduce ambiguity fish were given the opportunity to use external spatial cues, such as proximity of the openings in the frame to the wall or the center of the tank, to assist discriminative choice. The fish apparently failed to use these cues, nor could they resolve the ambiguity when the discrimination procedure required that they pass to the right or left of a central bar in the pipe frame. As a result the fish generally took up persistent spatial biases.

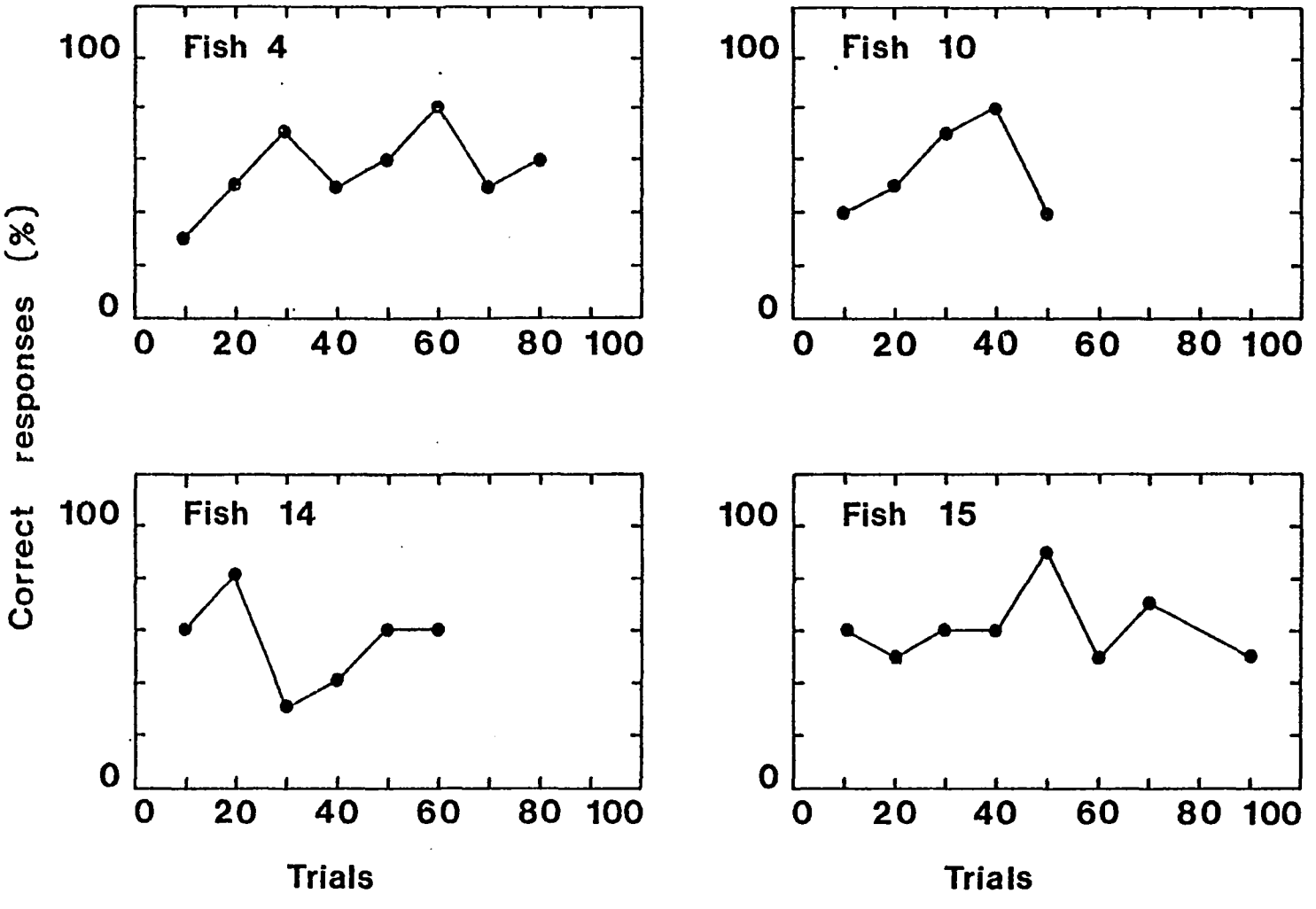
Several procedures were used in an attempt to enforce accuracy of responding. These included time outs, removal of the pipe frame from the water for a short period before allowing correction after an incorrect response, and fixed ratios of response, setting a minimum number of correct responses before positive reinforcement. These procedures appeared to be negatively reinforcing but did not greatly

increase accuracy of responding. The use of self-paced trials (Kling 1971) in which the fish initiated each trial by swimming around a stake at the opposite side of the tank from the pipe frame led to substantially increased activity by the fish but not to increased accuracy of responding.

The fish rapidly learned the experimental procedure (within 20 trials) and appeared to make the discrimination early in testing (Figure 2.9). Accuracies were as high as 13 correct responses out of 15 within some sessions and most fish made long runs of correct responses at some stage in the first 60-100 trials. However, in all cases high accuracies attained in one session were not repeated the next. This decline in accuracy was usually followed by development of a bias to one opening or other of the pipe frame.

Results of experiments with 15 fish (Table II.III) led to the following observations. The fish were able to make the discrimination to levels of accuracy of 75%. The discrimination was acquired very quickly, generally in 60-100 trials given in 20 trial sessions held once daily (Figure 2.9). The trial sessions were relatively short and data acquisition very rapid. However, the fish did not attain accuracies approaching 100%, as is desirable if this technique is to be used for analyzing the capacities of the

Figure 2.9. Discrimination performance in successive choice experiments in three yellowfin tuna and one kawakawa (identification numbers from Table II.III). Data are plotted as percent correct choices within successive blocks of ten trials. Expected result from random guessing is 50%.


Fish 4
Fish 10
Fish 14
Fish 15
100
0
0 20 40 60 80 100
Correct responses (%)
Trials

magnetic sense. Further, all the fish that did achieve statistically significant responding (15 correct responses or better in one training session) failed to make the discrimination in subsequent sessions. This result is attributed to the low cost of responding in the later compared with the earlier stages of the experiment, when the fish were still unpractised at swimming through the two openings in the pipe frame.

II.4 DISCUSSION

The behavioral experiments reported here demonstrated unconditioned and conditioned responses to magnetic fields in the yellowfin tuna. The mix of procedure and stimuli used were probably responsible for the success of the unitary experiments where the choice experiments failed. The separate influences of these components of the unitary experiments on their success and reasons for the failure of the choice experiments are discussed below. I then consider supplementary observations that bear on the features of the discriminative stimuli likely to have been used by the fish in making the discrimination. Observations made during the experiments also permit inferences concerning the magnetic sensory mechanism.

One yellowfin tuna was consistently disturbed by swimming through the anomalous magnetic field induced in the tunnel between the two halves of the dumbell tank (Table II.I). All the other fish tested, including some kawakawa, were disturbed by the stimulus once to a few times as indicated by the changes in their subsequent shuttling behavior.

The unconditioned response to magnetic fields in yellowfin tuna detected in these experiments is akin to that reported by Kalmijn (1978). There, sharks swimming laps around a circular tank were presented with a magnetic field anomaly at one edge of the tank. The fish altered their course, swimming into the center of the tank, as they encountered the anomaly. In contrast, Quinn (1980) demonstrated specific unconditioned responses to magnetic fields in sockeye salmon using orientation arenas. In these experiments, lake migrating sockeye salmon fry made directional responses to magnetic fields that were appropriate for their direction of migration along the axis of their nursery lakes. Subsequent experiments (Quinn and Brannon 1982) demonstrated similar responses in sockeye salmon smolts leaving nursery lakes for the migration to the sea. Taken together, these unconditioned responses to magnetic field stimuli suggest that migratory fishes

routinely detect or monitor magnetic fields in their environment.

The unconditioned response experiments suggested that the yellowfin tuna were able to detect novel magnetic field stimuli, leading me to initiate the conditioning experiments. Theoretical arguments and previous reports (Kreithen and Keeton 1974, Bookman 1977) suggested a requirement for freedom of movement for use of magnetic field stimuli. The experimental procedure chosen was thus required to provide a sensitive test of the ability of yellowfin tuna to distinguish between different magnetic field stimuli using a conditioned response that permitted freedom of movement.

I chose to adopt a discrete-trials/fixed-interval discrimination training procedure developed for pigeons and goldfish. This technique permits many more trials in a short time with equal if not better sensitivity than traditional free operant training methods (Woodard and Bitterman 1974). As well as sharpening discrimination by requiring multiple responses (Bitterman 1976), the technique used rate as a measure of discrimination. The advantages of rate as a measure are that it can vary widely and rapidly in response to changes in experimental conditions, behavioral baselines are stable, and small scale variability in behavior is easily accommodated (Kling 1971). Thus, for poorly

understood stimuli, such as magnetic fields, rate of response in a procedure such as the one used here provided a robust and sensitive test of the ability of yellowfin tuna to distinguish the stimuli.

In unitary discriminative training, many stimuli are present during both S+ and S- trials. These common stimuli may possess sufficient associative strength to maintain response rates at asymptotic levels (Bitterman 1976). For example, the fish in these experiments responded to initiation of trials by swimming rapidly to the pipe frame as it entered the tank. The fish often made their first pass through the frame before it had come to rest, with differences in responding between S+ and S- trials only becoming evident from the subsequent response pattern. Clearly, the fish strongly associated the presence of the pipe frame with food. Measures were therefore necessary to reduce the associative strengths of common stimuli if response to S- was to be brought below the performance ceiling.

The multiple responding required by the discriminative training procedure was not sufficient alone to suppress S- responding by the fish. It was found in experiments during development of the procedure that, unless the opening in the pipe frame was small relative to the size of the fish,

discrimination was either initially weak or did not appear at all. The penalty time used as a means of providing negative reinforcement in S- trials in the first series of experiments (Figure 2.5) was probably the minimum necessary to demonstrate the discrimination. The use of a longer allowable penalty time in the double blind experiments (Figure 2.7) resulted in a clear improvement in discrimination over the first set of experiments. In some S- trials, the second fish trained in the double blind experiments avoided the frame from the beginning of the trials. The fish remained on the opposite side of the tank for large parts of the trials, making only an occasional foray to swim around or through the frame. This observation suggested that the association between the presence of the frame and food was considerably reduced for fish tested in the double blind experiments compared with the fish tested in the first series of experiments. Thus the improvement in response predicted for these experiments was obtained, although the behavior was still variable, presumably because of the poor environmental control around G tank.

These experiments showed how, by appropriate manipulation of the associative strengths of common and discriminative stimuli, production of a generalized unitary response can be brought under the control of magnetic field stimuli. The change in response rates to S+ and S- in Figure

2.5 was shown to be due to a learning effect by the analysis of variance (Table II.II). The experiments also demonstrated two phenomena commonly associated with absence or loss of control by the discriminative stimuli. Fish that either lost or failed to make the discrimination (Fish 1 in Figure 2.4, Figure 2.8) showed evidence of temporal discrimination. In these fish, responses tended to be concentrated around the end of trials. During S- trials, response rate quickly declined with time after 30 seconds with the result that the penalty timer frequently timed out. Apparently, the fish did not associate the magnetic field stimuli with differential reinforcement. Instead, they attended to the time elapsed from the initiation of trials and learned to reduce their response rate if positive reinforcement was not given after 30 seconds.

The fish tested in the control experiment showed a response decrement as a result of non-differential reinforcement of the discriminative stimuli. The fish made a good initial discrimination (Figure 2.6). During the control trials, the magnetic field in the tank was associated with both positive and negative reinforcement. When the pair of discriminative stimuli were again presented the separation of response rates was smaller than before the control trials were conducted (Figure 2.6), suggesting that responding

after the control trials had been modified by the experience gained by the fish during the control trials.

Despite its success the unitary procedure as used in these experiments does have clear limitations. The use of a "whole body" response prevented enforcement of an observing response and caused the rates of response produced by the fish to be low compared with rates obtained using conditioned responses such as hitting a target (Woodard and Bitterman 1974). Consequently the scope for change in response rate was low and the variability in responding high compared with the performance ceiling. The task also appeared to become easier for the fish during the course of the experiments. Consequently, the results from several fish had to be discarded because the fish apparently began to make the discrimination but failed to maintain it, presumably because the associative strengths of common stimuli came to outweigh those of the discriminative stimuli (e.g. Fish 1 in Figure 2.4).

Although the evidence from these experiments tends to support the hypothesis that freedom of movement is necessary for magnetic field discrimination, this has yet to be established experimentally. The only other published behavioral conditioning experiment that both used a unitary procedure and required multiple responses used apparatus that restricted movement (Meyer and Lambe 1966).

Conditioning of birds to magnetic fields using a choice procedure in a flight tunnel was either unsuccessful (Griffin 1982) or was only statistically significant if the birds fluttered while moving down the tunnel to make the choice of responses (Bookman 1977). Kalmijn (1978) and Phillips (1977) allowed their subjects freedom of movement in making their discriminative choices and both obtained statistically significant responding. However, these results have not been repeated and there is not yet sufficient evidence to show that movement is essential to magnetic field detection. Adaptation of the unitary testing procedure for a species capable of making both stationary and whole body responses could resolve this point.

A number of attempts were made to adapt the conditioning procedure reported by Bookman (1977) for use with both yellowfin and kawakawa. As noted above, this successive choice procedure is recognized as a very difficult discrimination problem for animals (Mackintosh 1974, Bitterman 1976). It is, however, potentially much more powerful than the unitary procedure reported here and so was deemed worth trying.

The difficulties experienced in using the choice procedure support the hypothesis that unitary response procedures are more appropriate than choice procedures for

use with magnetic field stimuli. They also suggest that the reports of successful conditioning of animals to magnetic fields in choice experiments should be viewed with caution. However, the observed pattern of accurate responding during the early stages of the choice experiments carried out in this study does suggest that animals can be conditioned to magnetic field stimuli in appropriately designed choice experiments. The greater power of such techniques compared with the unitary procedure used here suggests that attempts to develop procedures more suitable for detailed analyses of magnetic sensitivity should continue.

Two reasons for the success of the unitary experiments arise from the nature of the tuna as subjects. The response used to compensate for the requirement that tunas must swim continuously may have been advantageous because it gave the fish the opportunity to sample the magnetic field at different times and at different points in the tank during the trials. The high swimming speeds the tunas can maintain made it possible for them to attain relatively high rates of performance of the generalized conditioned response. This capability increased the sensitivity of the conditioning technique by allowing a wide scope for change in response rates during the experiments compared with the response rates that other, less active fish species might achieve.

Although they have proven useful so far in demonstrating conditioned responses to magnetic fields, yellowfin and other tunas may not be ideal subjects for magnetic field conditioning studies. The size of the fish and the tanks required to house them make it difficult to obtain good control of the environment and stimulus, and resulted in apparent inter-tank differences in experimental results. There were also differences in performance between individuals. Because of their varying sizes, different fish found the task more or less difficult. Thus it was necessary to adjust the task to each fish tested by making the opening in the frame larger or smaller. A task that was of uniform difficulty for all fish was impossible under these conditions.

A further difficulty in working with tunas is that the fish are under stress in captivity and are difficult to maintain alone in experimental tanks. More specific experiments, for example those that require handling of the fish for impairment of sensory function, would be impossible with tunas because of their fragility (Queenth and Brill 1983). For these reasons, other species may be found to be more suitable for magnetic field conditioning studies as the techniques are developed further.

The stimuli used in the unitary experiments provided the fish with magnetic fields varying in several features.

It is not possible to state with any certainty which feature or features the fish used to make the discrimination. However, predictions can be made from consideration of the information they provide and their likely relative importance to fish navigating in the open ocean.

The magnetic field stimuli differed from each other in their angles of inclination, total intensity, and the gradients in these two features in the experimental environment. Of these, the angles of inclination and the gradients in them are probably the least likely to be used by the fish. In the field, angles of inclination vary only slowly and appear to be used by birds primarily for determining the direction of the magnetic pole in setting compass courses (Walcott and Green 1974). Although gradients in inclination were very large in the artificial fields, they did not indicate any shift in the direction of the magnetic pole. Total magnetic field intensity is also unlikely to be important because intensity of the altered field was not uniform throughout the experimental tanks. This leaves the gradients in intensity as the likely key stimulus feature used in discrimination by the fish.

Other evidence for the importance of gradients of intensity in discrimination by the fish came from the experiments in which reversed fields were used as

discriminative stimuli. The fields differed in intensity and inclination at any point in the tank. The gradients in the angle of inclination have been discounted above as a likely cue. The gradients in intensity in the tank were equal (high near the edge and low near the center of the tank) although opposite in sign (increasing and decreasing to 0 from the edge to the center of the tank). Two fish were completely unable to discriminate between the two magnetic fields when others tested under the same conditions but with different fields made good discrimination responses. Although other interpretations are possible, the very large differences in intensity gradients between the normal and altered fields may have made discrimination easier for fish presented with these fields as discriminative stimuli.

The experiments so far permit two inferences about the means by which the yellowfin tuna detect magnetic fields. The first is that magnetoreception is neurally mediated. It could be argued that the discriminations shown by the fish in the first experiments merely resulted from some differential physiological effect of the fields on the behavior of the fish. Training different fish with either the altered or the normal field as S+, and the highly significant learning effect interaction in the analysis of variance (Table II.II) exclude this explanation. In addition, the rapidity with which the fish were able to make

the discriminatory decision in each trial, particularly in the choice experiments, is characteristic of neural rather than non-neural processes.

A second inference that can be made from these experiments concerns the magnetic field transduction mechanism. I saw no evidence that the induced electrical fields associated with the presence or absence of water currents in the experimental tanks, or the rate at which the magnetic field was changed, affected discrimination by the fish. Similarly, Quinn et al. (1981) concluded that sockeye salmon fry and smolts must be able to detect magnetic fields in the absence of water flow, in both fresh and salt water, and in the dark. From these behavioral observations it seems unlikely that these teleost fishes detect magnetic fields by electrical induction (Kalmijn 1978) or through optical pumping (Leask 1977). However, the observations are consistent with the magnetite-based magnetoreception hypothesis considered in Chapter III.

In summary, the behavioral studies demonstrated repeatable responses to magnetic fields in yellowfin tuna. The unitary response procedure gave immediate evidence of discrimination when it was first tested and required relatively little adaptation for use with the tuna in the apparatus used. Limitations on the technique, its use with other species, and its application to more sophisticated

studies of magnetic sensitivity arise from the freedom of movement permitted the subjects. The choice procedure absorbed considerably more effort in development than the unitary procedure, produced tantalizing evidence of discrimination by the fish, but yielded no repeatable results. There are reasons contributing to this failure that arise from the technique itself. However, it will be important in future research to continue to test training procedures that will provide the means to analyze the capacities of the magnetic sense in detail.

The success of the experiments is attributed to use of a species considered very likely to benefit from possession of a magnetic sense using appropriate magnetic field stimuli in an appropriate testing procedure. The results suggest that magnetic field detection is neurally mediated and that it can be analyzed as can other sensory modalities. Future work must seek to repeat these results, refine the procedures, develop tests for responses to individual components of the geomagnetic field, and begin analyzing the magnetic field transduction mechanism.

CHAPTER III

PHYSICAL BASIS FOR MAGNETIC SENSITIVITY

III.1 INTRODUCTION

Two general categories of behavioral responses by organisms to magnetic fields have been defined above (Chapter I). The first of these is the magnetic compass response detected in both pro- and eukaryote groups. Magnetotactic bacteria and algae are passively rotated into alignment with the external magnetic field by the chains of magnetite crystals within their cell bodies. The organisms then swim along the field lines to reach their preferred habitats (Frankel et al. 1979, Lins de Barros et al. 1981). Most of the metazoan groups that have been shown to possess a magnetic compass respond to the angle of inclination of the geomagnetic field (Wiltschko 1972, Walcott and Green 1974, Quinn and Brannon 1982). Such responses imply reference to a gravitational component (horizontal or vertical) in use of the magnetic compass (Kirschvink 1982).

The second category of responses to magnetic fields by animals includes responses that appear to monitor total magnetic field intensity, a scalar. These responses are more complex and more sensitive than compass responses and have been implicated in determination of both time and position

(Martin and Lindauer 1977, Gould 1980, 1982a, Moore 1980, Walcott 1980).

For map and compass navigation using the geomagnetic field, some feature related to magnetic field intensity is considered most likely for determination of position. Determining position by monitoring the very small spatial fluctuations in other field features requires very accurate information on body orientation (Kirschvink 1982). For example, Quinn (1982) proposed a magnetic map based on a bicoordinate grid of the angles of inclination and declination of the geomagnetic field. Measurement of the very small changes in these angles (about 0.01^{o}/km change in inclination in the N-S direction) depends on highly accurate determination of the directions of reference vectors--a gravitational component for inclination and geographic north for declination. It is highly unlikely that the gravity receptors could monitor the organism's own orientation relative to gravity with sufficient accuracy to measure inclination with the required precision (Kirschvink 1982). Similarly, it is very unlikely that animals can determine the direction of the geographic pole accurately enough to use declination as the second component of a navigational grid (Adler 1963).

Relative to the geomagnetic field a living organism occupies but a point in space. No spatial variations in the

field are detectable within or across the body of the organism. Nor can there be any differential transmission of the field from the outside to the inside of the body of the animal (Ossenkopp and Barbeito 1978). However, animals appear to respond to both the vector and scalar features, and to the even smaller variations in these features, of the relatively weak geomagnetic field (Martin and Lindauer 1977, Southern 1978, Gould 1980, 1982a, Walcott 1980). Clearly, some highly sensitive sensory system that can transduce these weak stimuli to the nervous system must have evolved in at least the homing pigeons and other species showing sensitivity to small fluctuations in the geomagnetic field.

In sensory systems some form of energy is converted by receptor cells into electrical signals. Receptor cells usually respond best to a specific type of stimulus. Accessory structures that may be associated with the receptors are arranged to channel these particular forms of energy to the receptors while at the same time excluding others. Thus sense organs define the limits of sensitivity and determine the range of stimuli that can be perceived (Kuffler and Nicholls 1976).

In any sensory system, particularly one detecting very weak stimuli, the stimulus energy has to be detected against the background thermal energy, kT, where k is the Boltzmann

constant and T is the absolute temperature. The energy of interaction between the incoming stimulus and the receptor must therefore be greater than kT (4.14×10^{-14} erg at 300°K) for a signal to be detected (Jungerman and Rosenblum 1980, Kirschvink 1982).

Any hypothesis seeking to explain geomagnetic field sensitivity in animals must provide a mechanism by which the action of the geomagnetic field can bring about orderly displacement of the electrical potential of a receptor cell membrane. That is, the geomagnetic field must act on the magnetoreceptor cell with a neural coupling energy greater than kT. The mechanism must also explain the general compass and intensity responses and the very high sensitivities inferred for detection of changes in magnetic field intensity (Martin and Lindauer 1977, Gould 1980, 1982a). Finally, the hypothesis should make testable predictions concerning magnetoreceptor operation. Some of the magnetoreception hypotheses that have been proposed, and the evidence for and against them, are reviewed below.

The earliest plausible mechanism for magnetoreception was suggested to be some form of electrical induction arising from movement of a conductor through the geomagnetic field. At least five detection systems have been proposed. The best supported is the special case of the electrically sensitive fishes, in particular the elasmobranchs. Kalmijn

(1974) predicted that elasmobranchs would be sensitive to earth-strength magnetic fields through detection of the electrical fields induced by their own or the water's movement through the earth's magnetic field. These fields are well within the measured capacities of the ampullary electroreceptors of the elasmobranchs and could permit the fish to detect both the direction and total intensity of the geomagnetic field (Kalmijn 1974). The importance of this hypothesis lies in there being known receptors that appear to behave in the fashion required by the hypothesis (Andrianov et al. 1974, Brown and Ilyinsky 1978), and in the behavioral responses to magnetic fields in elasmobranchs demonstrated by Kalmijn (1978).

The difficulty with electrical induction as a general means of detecting magnetic fields is the requirement for a completed circuit for induction to occur. In the elasmobranchs, the conductive external medium is part of the electrical circuit; in air, the resistance of the external medium is too high to form part of a conductive loop. The second plausible magnetoreception mechanism based on electrical induction accounts for magnetic field detection in vertebrates by postulating a circuit contained within the body of the animal (Jungerman and Rosenblum 1980). The system requires closed conducting loops that will conduct current when the organism moves through the external

magnetic field, carrying the loops with it. For detection to be possible at physiologically reasonable levels requires loops at least 3 mm in radius with conductors 1 mm in diameter (Jungerman and Rosenblum 1980). These dimensions are similar to those of the semicircular canals, and Jungerman and Rosenblum (1980) consider the possible use of the labyrinth in magnetoreception worth investigation.

The third electrical induction mechanism involves the electrostatic field that will build up on the surface of a bird flying through the atmospheric electric field (Stewart 1957). The electrostatic field will cause the feathers to twist, with the torque on the feathers being dependent on the interaction between the electrostatic field on the bird and the geomagnetic field. As with the electrical induction system of Jungerman and Rosenblum (1980) this system has the weakness that, although receptors that behave in the fashion required by the hypothesis are possible, they are not known (Ossenkopp and Barbeito 1978).

Two semiconducting crystals of the same substance but with different impurities make up a P-N semiconductor. The glial and neuronal membranes of the central nervous system have an ordered (liquid crystal) structure and possess different electrical properties. They may therefore display semiconductor properties, including directional control of

current flow, power amplification, and susceptibility to induction (Russo and Caldwell 1971). As yet this magnetic field transduction hypothesis lacks evidence for the semiconductor properties and for receptors which respond to electrical induction occurring in such semiconductors.

An even less plausible magnetic field transduction mechanism based on induction involves biological superconductivity, the ability to conduct electrical current without generation of heat and therefore without resistance. Superconduction is dependent on Josephson junctions--two layers of superconductor separated by a thin layer of dielectric across which electrons and electron pairs can pass. Weak magnetic fields affect the ease with which electrons cross the dielectric and the hypothesized magnetic field transduction mechanism easily provides for sensitivity to weak magnetic fields (Cope 1973). However, superconduction is only known to occur in inorganic materials below 20^{o}K and there is as yet no evidence that superconduction will be found in biological systems (Kirschvink and Gould 1981). In addition, it is not clear from Cope's (1973) analysis whether the proposed transduction system could detect very small magnetic field fluctuations (<50 nT) against the much larger (50 μT) geomagnetic field.

Leask (1977) proposed a transduction mechanism in which interactions of paramagnetic molecules with the geomagnetic field could be amplified by electromagnetic radiation. Paramagnetic molecules possess unpaired electrons whose spins will interact weakly with an external magnetic field. This interaction makes them more susceptible to certain wavelengths of electromagnetic radiation, from which they will preferentially absorb energy to enter an excited electron energy state. The electrons will then decay back to their original energy state with photon emission. Magnetoreception then reduces to a special case of photoreception (Leask 1977). This optical pumping mechanism is considered unlikely because demonstration of any effect of direct current magnetic fields on the electroretinogram of turtles required fields at least 20-200 times the strength of the geomagnetic field. In addition, the effects occurred only after transitions from light to darkness (Raybourn 1983). The optical pumping hypothesis also has the experimentally determined weakness that magnetoreception by cave salamanders and sockeye salmon can occur in the dark (Phillips 1977, Quinn et al. 1981).

As noted above all the known responses to magnetic fields by organisms fall into two simple classes--responses to vector direction and scalar intensity. This applies to such disparate groups as magnetotactic bacteria and algae,

and both vertebrates and invertebrates in which magnetoreception has been demonstrated. The common characteristic of the magnetic field transduction mechanisms outlined above is that they explain at best some of the responses for the groups for which they were developed; they are not generally applicable, as might be hoped for such simple and widespread responses. Almost all the hypotheses do not explain the sensitivities to very small magnetic field fluctuations demonstrated in behavioral experiments (Martin and Lindauer 1977, Southern 1978, Gould 1980, 1982a, Walcott 1980) and most of them fail to provide evidence for receptors that are known to behave in the required fashion. Where suitable receptors are identified (Kalmijn 1974), closer examination shows that they do not respond to magnetic field stimuli in the fashion required (see Chapter V).

In 1975 Blakemore reported an easily manipulated magnetotactic response in bacteria. Blakemore was able to control the movement of the bacteria using magnetic fields, and to reverse the direction they moved by remagnetizing them. This response was later shown to be due to magnetite crystals deposited inside the bacterial cells (Frankel et al. 1979). The crystals were responsible for alignment of the axis of the individual cells with the magnetic field vector, and, through direction of the swimming movements of

the bacteria, for bringing them into their preferred habitats (Frankel and Blakemore 1980).

This discovery spurred a renewed search for magnetic minerals in the bodies of metazoan organisms. The development of superconducting magnetometers for paleomagnetic research made possible the detection and characterization of very small amounts of magnetic material in rock samples (Collinson 1975, Goree and Fuller 1976). This new equipment was soon applied to biological samples and led to the discovery of magnetite in the bodies of bees and homing pigeons (Gould et al. 1978, Walcott et al. 1979). The single major objection to serious consideration of the hypothesis that magnetic minerals might be the basis for magnetoreception (Ising 1945, Lowenstam 1962, Keeton 1972) was thus removed.

The sections that follow review the basic principles of magnetism relevant to this study (physical constants are defined in the Appendix), the ferromagnetic magnetoreception hypothesis, and paleomagnetic techniques and their adaptation for use with biological samples. Special attention is given to recognizing magnetic contaminants and distinguishing them from true biologic deposits that may be used in magnetoreception. The experiments carried out and reported in later sections attempted to demonstrate clearly that yellowfin tuna produce magnetite crystals in a form and

in sufficient numbers to provide these fish with a highly sensitive magnetoreceptor system capable of detecting both direction and intensity of the geomagnetic field.

III.2 Basic principles of ferro- and ferrimagnetism

The magnetic properties of matter arise from the movement of charged particles (electrons) in space. The magnetic moment of an unpaired electron, called the Bohr magneton (μ_B), is a fundamental unit of magnetism and is 9.27×10^{-17} erg/Tesla (Kittel 1976). The orientation energy arising from its interaction with the 50 μT geomagnetic field is given by $-\mu_B B\cos\Theta$ where B is the field and Θ is the angle between the vector directions of the field and the Bohr magneton. The maximum interaction energy of the Bohr magneton with the geomagnetic field is 4.14×10^{-21} erg, which is about 10^{-7} of the background thermal energy at physiological temperatures (4.14×10^{-14} erg at 300°K). Some organic molecules are paramagnetic, that is, they possess unpaired electrons and will therefore interact with magnetic fields (Leask 1977). However, their interactions with the geomagnetic field are so small that they could only be detected in living systems through statistical effects involving detection of the interactions of vast numbers of the molecules with the external field.

In ferromagnetic materials the crystal lattice forces the moments on unpaired electrons into parallel alignment. They then sum linearly and the moment of a crystal is simply $N.\mu_B$ where N is the number of Bohr magnetons present in the crystal. The magnetism of magnetite is a subclass of ferromagnetism known as ferrimagnetism. The crystal lattice has an inverse spinel structure in which the moments of Bohr magnetons in alternate crystal layers are aligned antiparallel to each other. Oxygen atoms in the crystal lattice are arranged in slightly distorted cubic close packing and the crystal lattice contains both tetrahedral and octahedral iron coordination sites in the ratio 8:1. One eighth of the tetrahedral sites are occupied by Fe^{3+} and half the octahedral sites are occupied by equal numbers of Fe^{2+} and Fe^{3+} ions (Kirschvink and Gould 1981). As a result of this arrangement, the numbers of Bohr magnetons in the layers are not equal and the difference between these numbers leaves the magnetite crystal with a net magnetic moment (Kittel 1976). The magnitude of the moment is dependent on the number of ions contained in the lattice, and therefore on the size of the particle. In all other respects the material behaves as a true ferromagnet, including the presence of a Curie (called Neel) temperature.

A crystal of magnetite will spontaneously magnetize throughout its volume to the saturation magnetization for

magnetite (4.8×10^5 A/m). The direction of magnetization relative to the crystal axes is a complex function of the external field present at the time the crystal forms as well as its shape and crystallographic orientation. The magnetic dipole moment (μ) of the crystal which develops will be VJs where V is the volume of the crystal and Js is the saturation magnetization of magnetite, unless the net moment is reduced through the formation of multiple domains (see below).

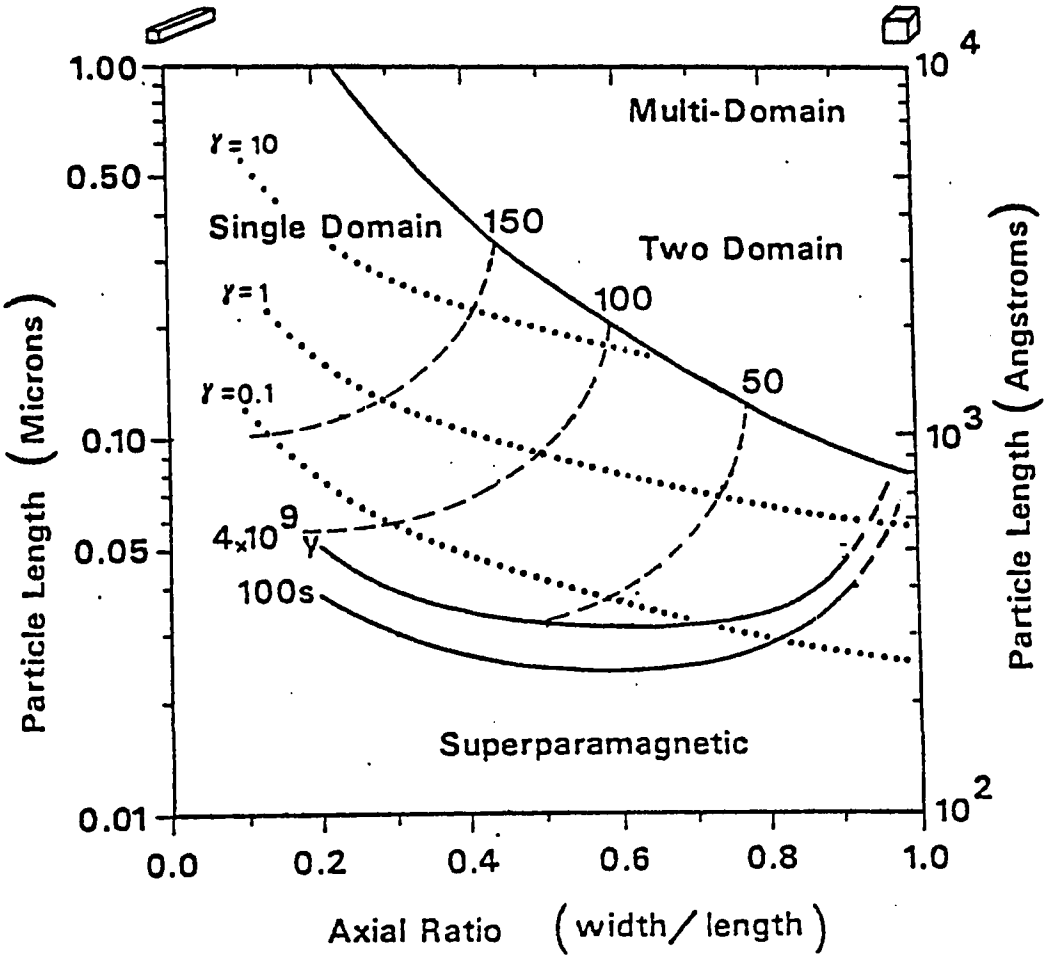
The stability of magnetization of magnetite crystals depends on their size, shape, and the absolute temperature. The characteristic relaxation time required for the magnetic direction of an isolated, uniformly magnetized particle to change spontaneously is proportional to $e^{VJsHc/2kT}$, where Hc is the microscopic coercivity of the particle (Néel 1949, see below). As can be seen from Figure 3.1 slight changes in the size and shape of small particles (<30 nm) can greatly affect their stability of magnetization. The direction of the moment vector of a very small particle (<<30 nm) will wander constantly relative to the crystal axes. Such particles are classed as superparamagnetic because their moments will statistically track an applied magnetic field without movement of the particles. From the expression describing the relaxation time of an isolated, uniformly magnetized particle it can be seen that the stability of

magnetization of superparamagnetic particles can be increased by reducing T in kT. This property can be exploited in their detection.

Demagnetizing fields build up within large magnetite crystals and cause the parallel alignment of electron spins to collapse into two or more regions, known as domains, with moments pointing in opposite or non-parallel directions. When two equally sized domains are formed within one crystal, their moments cancel and the net moment of the particle arises from electron spins in the wall between the domains. Within the wall, the direction of magnetization rotates through 180° from one domain to the other, giving rise to pseudo-single domain behavior. The net moment of the pseudo-single domain and multi-domain particles is far less than would be the moment of uniformly magnetized particles of the same volume. Although the magnetization of multi-domain magnetite particles is stable in the geomagnetic field, the particles are easily remagnetized through realignment of the domain walls (Kirschvink 1983).

Magnetite crystals that are between the sizes of superparamagnetic and multi-domain particles are called single-domains and are uniformly magnetized throughout their volume. The direction of magnetization of single-domain particles exhibits shape anisotropy. That is, the north- and south-seeking poles of the particle will tend to lie at the

Figure 3.1. Magnetic stability diagram for rectangular parallelipipeds of magnetite (composite of results from McElhinny 1973, Butler and Banerjee 1975, and Kirschvink and Gould 1981). Solid lines represent the theoretically and experimentally determined boundaries between multi-domain (m. d.), single-domain (s. d.), and superparamagnetic (spm) crystal sizes. The boundary between spm and s. d. behavior is temperature- and size-dependent. The solid lines between the spm and s. d. regions indicate the relaxation times for particles of different sizes and shapes at 300°K. The lower line gives a relaxation time of 100 seconds (spm behavior) and the upper line 4×10^9 years (s. d. behavior). The three dotted lines crossing the s. d. and spm areas represent contours of equal grain volume and hence constant magnetic moment. Each contour is scaled such that $\mu B/kT$ ($= \gamma$) is respectively 0.1, 1, and 10 at physiological temperatures in the geomagnetic field. Broken lines in the s. d. field are equal particle coercivity contours of 50, 100, and 150 mT. From these curves the size, shape, and energy of interaction with the geomagnetic field of magnetic particles of known coercivity can be inferred.


1.00
0.50
0.10
0.05
0.01
0.0
0.2
0.4
0.6
0.8
1.0
10 4
10 3
10 2
Multi-Domain
Single Domain
Two Domain
Superparamagnetic
γ = 10
γ = 1
γ = 0.1
150
100
50
4x10 9 γ
100s
Particle Length (Microns)
Particle Length (Angstroms)
Axial Ratio (width/length)

opposite ends of the long axis of the crystal (Kirschvink and Gould 1981). The moment vector of the particle can be aligned in either of the two possible antiparallel directions in the long axis of the particle. However, shifting the direction of magnetization away from the long axis of the crystal is energetically unfavorable, making the direction of magnetization stable at normal temperatures (300^{o}K) in normal magnetic fields.

Thus the magnetic properties of magnetite vary directly as a function of crystal size and shape, as illustrated for isolated parallelipipeds on the stability diagram in Figure 3.1. The particle length and the axial ratio, the ratio of width to length for a given grain, are plotted on the ordinate and the abscissa respectively. Superparamagnetic and single-domain grains are uniformly magnetized throughout their volume to magnetite's saturation magnetization. In single-domain grains the direction of magnetization is extremely stable and can remain unchanged at 300^{o}K for all of geologic time (>4.5 x 10^{9} years; Kirschvink and Gould 1981). In superparamagnetic grains, the direction of magnetization will change without movement of the grains in response to thermal agitation and external fields. As noted above the magnetization of multi-domain particles is stable but such particles are easily remagnetized. Thus superparamagnetic, single-domain, and multi-domain particles

show different magnetic stability properties. These are used by paleomagnetists to distinguish among the different classes of magnetite grains.

One commonly used measure of the magnetic stability of a ferromagnetic particle is its microscopic coercivity. The magnetic moment of a magnetite particle is energetically constrained to lie in the long axis of the particle unless it is forced out of alignment by a strong magnetic field. The coercive field of the particle is the minimum intensity of an external magnetic field required to flip the moment from one stable orientation to the other. Because (1) the moment of a ferro- or ferrimagnetic particle is dependent on the number and arrangement of Bohr magnetons in the crystal lattice and (2) the stability of the particle magnetization is dependent on the size and shape of the crystal, the coercive field can be used to place constraints on the identity and to estimate the size, shape, and number of particles of a magnetic mineral present in a sample.

III.3 The magnetite-based magnetoreception hypothesis

To function in magnetoreception magnetite crystals must be linked to the nervous system so that the energy of their interaction with the geomagnetic field causes displacement of the electrical potential of the membrane of a receptor

cell. The information so transmitted to the nervous system must be able to mediate the responses to both the scalar and vector properties of the field. The discussion that follows demonstrates (1) that the physical properties of single-domain magnetite particles detected in homing pigeons and honey bees (Gould et al.1978, Walcott et al. 1979) could mediate responses to both magnetic field direction and intensity, and (2) that such responses could easily achieve the sensitivities to small fluctuations in magnetic field intensity estimated from behavioral experiments.

The critical first step in developing the magnetite-based magnetoreception hypothesis is demonstration of the necessary stimulus energy. The interaction energy, E, between a magnetic particle with moment, μ, and the geomagnetic field, B, is:

$$E = - \mu . B \cos \Theta ,$$

where Θ is the angle between the vector directions of $\vec{\mu}$ and $\vec{B}$ (Kirschvink and Gould 1981). For a 50 nm cube of magnetite the dipole moment is:

$$\begin{aligned} \mu &= VJs \\ &= 1.25 \times 10^{-22}\ m^3 \times 4.8 \times 10^5\ A/m \\ &= 6 \times 10^{-17}\ Am^2 \\ &= 6 \times 10^{-14}\ \text{electromagnetic units (emu).} \end{aligned}$$

This moment interacts with the 50 μT geomagnetic field with an orientation energy (converted to ergs: 1 emu = 1 erg/Gauss) of $3 \times 10^{-14} \cos\Theta$ erg. This energy is about 0.7 x kT at 300°K.

Thus the interaction energies of the single domain magnetite particles found in metazoan organisms are of the same order of magnitude as the background thermal energy, kT. Magnetite particles larger than about 50 nm will preferentially align with the geomagnetic field. They will, however, be subject to thermal buffetting from the surrounding medium at physiological temperatures. Consequently, their vector directions will wander randomly around the vector direction of the external field in a type of Brownian motion (Kirschvink 1981a). It is intuitively obvious that, although the instantaneous alignment of individual particles in a population of grains will be imperfect, the mean alignment of the particle moment vectors will be in the direction of the applied field. This can be demonstrated mathematically using μB/kT, the ratio of magnetic to thermal energies for single-domain particles.

The randomly wandering magnetic moment of an individual single-domain grain will have a three dimensional angular dispersion about the external field vector given by the Boltzmann distribution $e^{\mu B \cos\Theta / kT}$ (Kirschvink 1981a). The projection of $\vec{\mu}$ on $\vec{B}$ is given by $\mu \cos\theta$ (Figure 3.2). The

average alignment of the particle is then $\cos\Theta$ averaged over the Boltzmann distribution or:

$$\langle\cos\Theta\rangle = \int_{\text{surface of sphere}} \cos\Theta\, e^{\mu B\cos\Theta/kT}\, d\Omega \Big/ \int_{\text{surface of sphere}} e^{\mu B\cos\Theta/kT}\, d\Omega ,$$

where Ω is the solid angle described by the distribution of $\vec{\mu}$ about $\vec{B}$, and the term on the right hand side of the expression is the integral definition of an average. Solving:

$$\begin{aligned}\langle\cos\Theta\rangle &= \coth(\mu B/kT) - kT/\mu B \\ &= L(\mu B/kT) \\ &= L(\gamma)\end{aligned}$$

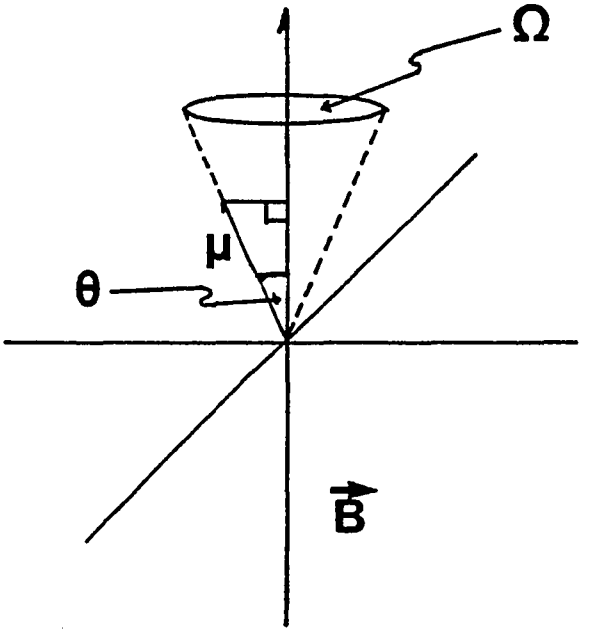
where $\gamma = \mu B/kT$. This is the definition of the Langevin function, L, which describes the accuracy of alignment of freely rotating magnetic particles in an external field (Kittel 1976). Figure 3.2 shows the shape of the curve defined by the Langevin function for different values of γ. For small γ ($\gamma << 1$) the average alignment is poor and $L(\gamma)$ is well approximated by $\gamma/3$; as γ increases beyond 1 $L(\gamma)$ approaches 1 asymptotically and is approximated by $1 - 1/\gamma$ (Kittel 1976).

The accuracy of estimation of the vector direction of $\vec{B}$ will depend on γ, and so on the moment, μ, of a magnetic particle when B and T are constant. For a small single-

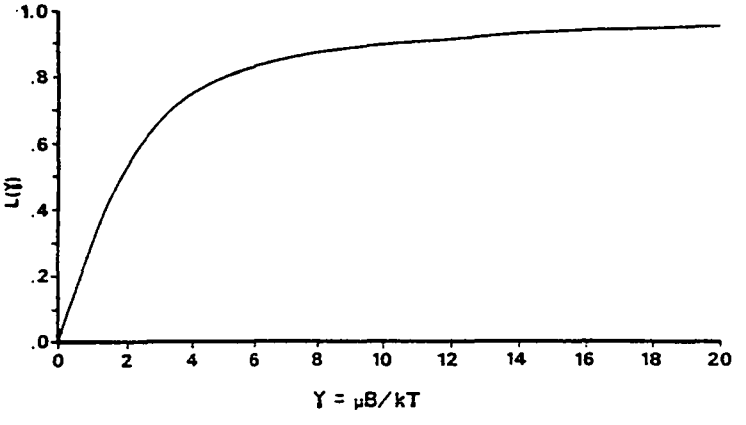
Figure 3.2. A. Projection of the moment, μ, of a single-domain magnetite particle on the direction of an external field, $\vec{B}$. The vector component of $\vec{\mu}$ parallel to the direction of the external field is given by $\mu\cos\Theta$ where Θ is the angle between $\vec{\mu}$ and $\vec{B}$. Ω is the solid angle described by the random wandering of $\vec{\mu}$ about the direction of the external field.

B. The Langevin function plotted against γ, the ratio of magnetic to thermal energies for single-domain magnetite crystals. The accuracy of alignment of the crystals ($L(\gamma)$) increases rapidly up to values of about 6 and increases asymptotically thereafter. The accuracy of behavioral responses to magnetic field direction mediated by magnetite-based magnetoreceptors should at first increase rapidly with external field intensity. Beyond a certain point further increase in external field intensity should not lead to greater compass accuracy (modified from Kirschvink 1981a).

A



B



domain particle this alignment is not very good (Yorke 1979). The accuracy of estimation of the vector direction of $\vec{B}$ can, however, be increased by using more than one receptor. Use of multiple receptors provides independent samples of the distribution of particle moment vectors about $\vec{B}$. From the central limit theorem, the accuracy of estimation of $\vec{B}$ will increase by $1/\sqrt{N}$ where N is the number of receptors. Yorke (1979) and Kirschvink (1981a) show that only a few hundred magnetite-based magnetoreceptors would be necessary to produce a very accurate magnetic compass system.

The same hypothetical magnetite-based magnetoreceptors can generate a signal dependent on the intensity of the geomagnetic field. In the discussion above the magnetic field intensity, B, was assumed to be constant. However, the geomagnetic field is variable in time and space and the behavior of magnetite-based magnetoreceptors will be affected accordingly. The sizes of magnetite particles in organisms, and hence their moments, appear to be constant, and temperature will be assumed constant within the body of the individual organism for the purpose of calculating the sensitivity of the hypothesized magnetoreceptor system. Thus in the relation $\mu B/kT$ only B varies. The wandering of the particle moments, and therefore the variance of the Boltzmann distribution, will depend only on B. The root mean

square angular deviation of a dispersion of single-domain magnetite particles about B is $(2kT/\mu B)^{1/2}$ (Kirschvink and Gould 1981). Assuming that the magnetoreceptor system can detect a change in mean particle alignment greater than the r.m.s. deviation, the theoretical minimum sensitivity to intensity changes, $\Delta B/B$, is $(2kT/N\mu B)^{1/2}$ where N is the number of magnetoreceptor organelles (Kirschvink and Gould 1981). Using cubic crystals 100 nm on a side and setting N equal to 10^6 crystals $\Delta B/B$ is 0.00082 or about 40 nT, well within the ranges of sensitivity inferred for the homing pigeons and honey bees (Gould 1980, 1982a).

Sensitivity of magnetite-based magnetoreceptors can be further increased by integrating over time as well as over multiple receptors. Temporal integration can readily be incorporated into the equation for $\Delta B/B$ above by defining I, the interval over which integration occurs, and τ, the rotational response time of the particles. The τ is dependent on the moment of inertia of the crystals and the viscosity of the medium. From the central limit theorem accuracy of estimation of $\Delta B/B$ will also increase as the reciprocal of $(I/\tau)^{1/2}$. The theoretical sensitivity then becomes:

$$\Delta B/B = (2kT/N\mu B(I/\tau))^{1/2}.$$

For 100 nm crystals in a medium with viscosity 10 times that of water, τ will be about 35 milliseconds (Kirschvink and

Gould 1981). Integration of the intensity-dependent signal arising from an array of magnetite-based magnetoreceptors over 100τ (3.5 seconds) will thus lead to a tenfold increase in the theoretical sensitivity to changes in intensity.

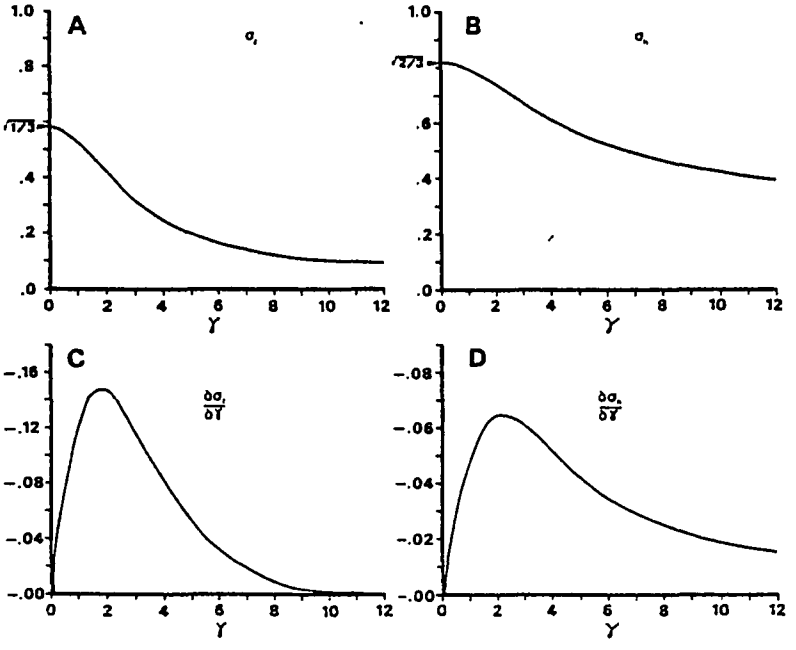
By a similar argument, particles used to monitor magnetic intensity would in theory respond to small variations in temperature. Fluctuations in the body temperature of the organism could therefore affect the operation of intensity receptors. For example, if body temperature increased from $300^{\circ}K$ to $305^{\circ}K$, the variance of the crystal alignment would increase by 1.7%. Position determined from total intensity could be up to 150 km in error. However, threshold sensitivity to changes in total intensity would be raised by less than 1%. Thus, although they would increase the uncertainty of measurement of total field intensity, temperature effects will probably not prevent detection of small fluctuations in intensity.

The magnetite-based magnetoreception hypothesis permits two predictions concerning the moments of compass and intensity receptor organelles. The first is that the optimal moment for a magnetic compass receptor is about 6. Inspection of the Langevin function plotted in Figure 3.2 shows that, for small γ, alignment accuracy for magnetite-

based magnetoreceptor organelles is poor. Beyond γ values of about 6 the alignment accuracy does not increase greatly and there is probably no advantage to possessing magnetite particles with moments that will give γ values greater than 6 in the geomagnetic field (Kirschvink 1981a). Using a slightly different treatment for deriving the intensity-dependent signal, Kirschvink and Walker (in review) show that the optimum moment for intensity receptors is $\gamma = 2$ (Figure 3.3). The different γ values for optimal detection of the vector and scalar properties of the geomagnetic field suggest the possibility of different receptor systems. More important, however, is the fact that these values of γ depend on the use of single-domain particles with very restricted size ranges (see Figure 3.1). Thus the single-domain particles detected in the homing pigeons and honey bees are not only capable of being used for magnetoreception, but they also fall in the size range best suited for such a purpose.

The above derivation shows that magnetite-based magnetoreceptors can theoretically account for both the commonly observed responses to magnetic fields shown by animals. The basis of the mechanism is (1) the information about $\vec{B}$ provided by the energy of interaction between the crystals and the geomagnetic field and (2) the information about B provided by the effect of thermal buffetting on the

Figure 3.3 A, B. Plots of the Langevin function variance parallel and perpendicular to external field direction against γ, the ratio of magnetic to thermal energies of single-domain magnetite crystals. C, D. Plots of the first derivative of the Langevin variance parallel and perpendicular to external field direction. The Langevin variance declines with increasing γ and, for magnetite-based magnetoreceptors, will be dependent on external field intensity. It follows that receptors monitoring some component of this variance will be most sensitive to changes in intensity when the change in variance with intensity is a maximum. C, D show that the maximum rates of change of variance with γ, and so with intensity, occur at about γ = 2. Behaviorally measured thresholds to magnetic sensitivity should follow the form of these plots. Threshold sensitivity should increase rapidly with external field to a maximum at about 50 μT and decline thereafter (Figure from Kirschvink and Walker in review).


A
B
C
D
1.0
.8
.6
.4
.2
.0
-.16
-.12
-.08
-.04
-.00
0
2
4
6
8
10
12
γ

crystals as they seek alignment with $\vec{B}$. The only requirement for operation of the magnetite-based magnetoreceptors is that B not be very small, nor more than a few times the intensity of the geomagnetic field (Yorke 1979).

The magnetite-based magnetoreception hypothesis also makes clear the reasons why biologic magnetite and magnetite-based magnetoreceptors could not have been discovered until now. The crystals take up very little space (each crystal is approximately 10^{-16} cm^3) and no large accessory structures are required for their use in magnetoreceptor organelles. Consequently, structures involved in magnetoreception that might have attracted attention and required explanation have not been detected in the past. Ising first proposed involvement of magnetic particles in magnetoreception in 1945. However, the small size and small moments of the particles prevented their discovery until the advent of superconducting magnetometers and the subsequent development of techniques for detection and characterization of biologically produced magnetic minerals.

III.4 Strategy and techniques for detection, extraction, and characterization of biogenic magnetite

III.4.1 Introduction

The primary difficulties in working with the deposits of biogenic magnetite in metazoans are that (1) very small amounts of material, dispersed in tissues, are involved, (2) the methods used to detect and analyze the magnetite are indirect, (3) and contaminants can be present in the samples before dissection and can enter at any time during dissection and measurement. Magnetite crystals in the abdomens of bees and in the heads of homing pigeons (Gould et al. 1978, Walcott et al. 1979) are submicroscopic (<100 nm), occupy a combined volume of 10^{-9} to 10^{-8} cm^3, and have a mass of 1-100 nanograms. In large organisms (up to 100 kg or more), detecting such quantities of magnetite from its magnetic properties depends on the crystals being highly concentrated in small, recognizable structures and not uniformly dispersed throughout all the tissues. Extraction and recovery of the crystals likewise depend on their being sufficiently concentrated to be magnetically detectable.

The failure to recognize contaminants and the influence they can have on results of biomagnetic studies has greatly hindered progress in understanding the origin and functions

of biogenic magnetite. Magnetite is a common industrial pollutant and can often find its way onto the external body surface or into the gut of higher animals (Kirschvink 1983). A typical 100 nm crystal of the type found in the honey bees and homing pigeons has a moment of about 0.5 fAm^2 whereas a 10 μm dust-sized particle may have saturation moments of up to 500 pAm^2. The moment of the multi-domain particle is well within the 1-10 pAm^2 sensitivity limits of the superconducting magnetometers currently in use, whereas the moments of 10^3 to 10^4 of the single-domain particles must be aligned to be detectable. Other ferro- or ferrimagnetic contaminants are frequently present within the laboratory environment, particularly in paleomagnetic laboratories where rock samples tend to leave fine dust behind them that is often rich in magnetic contaminants. The ease with which contaminants can enter at all stages of biomagnetic studies dictates that not only must procedures to minimize the risk of contamination be adopted but also that contaminants and true biochemical precipitates must be distinguished. It is therefore necessary to identify those properties that are likely to be unique to biogenic magnetite compared with other magnetic minerals.

Interest in biogenic magnetite focuses on its potential use in the transduction of the geomagnetic field to the nervous system. The magnetite-based magnetoreception

hypothesis assumes that the physical properties of the crystals are of primary importance and predicts that single-domain crystals are the most likely form of magnetite for use in magnetoreception. This constraint should result in a restricted size frequency distribution of the magnetite particles. Magnetite particles suitable for use in magnetoreception should therefore have coercivities greater than the coercivity of multi-domain magnetite (<20 mT) and less than the theoretical maximum for single-domain magnetite (300 mT; McElhinny 1973). Although superparamagnetic particles of biogenic magnetite have been detected (Gould et al. 1978) it is not possible to detect them without special facilities. Thus, in searching for magnetite suitable for use in magnetoreception, I was primarily attempting to distinguish between single-domain and multi-domain particles.

There is as yet no evidence that magnetite in the gut or the environment could enter the bloodstream and be transported to the places it has been detected. This suggests that any such particles used for magnetoreception must be produced within the bodies of the organisms themselves, presumably by enzyme catalysis. The specificity of enzyme pathways would be expected to result in biogenic magnetites containing few of the impurities associated with geologic magnetites or the metals used to harden iron alloys

(Lowenstam and Weiner 1983). Magnetite particles suitable for magnetoreception can therefore be reasonably expected to possess physical and chemical properties distinguishing them from their geologic and synthetic counterparts.

Magnetite or magnetic material without apparent magnetoreceptive function has been detected in a variety of tissues in different species (Lowenstam 1962, Presti and Pettigrew 1980, Kirschvink et al. 1982). Except in the chitons, where magnetite is used to harden radular teeth (Lowenstam 1962), the function of these deposits is unknown. Hypotheses are that the deposits store excess iron or that they may be of pathologic origin (Lowenstam and Weiner 1983). It is therefore more difficult to predict characteristics that will distinguish them from other magnetites. Eventually it will be important that attention be given to these anomalous deposits because they may predate the use of magnetite for magnetoreception (Kirschvink and Gould 1981).

The discussion below reviews the techniques developed so far for detecting and characterizing biogenic magnetite that is suspected to be used in magnetoreception. Procedures for avoidance of contamination and of specific tests for contaminants that I have conducted are included.

III.4.2 Magnetometry studies

III.4.2.1 Laboratory and sample preparation

Accidental contamination of samples is a major problem in the search for biogenic magnetite (Jones and MacFadden 1982). When working in a paleomagnetic laboratory I sought to minimize the risks of contamination by thoroughly scrubbing the walls, roof, and floor, and lining them with thin polyethylene sheets. The recent development at the California Institute of Technology of a clean laboratory specifically designed for biomagnetic studies eliminated many of the contamination problems previously experienced (Kirschvink 1983). However, when using the laboratory I still found it necessary to clean all surfaces regularly.

Non-magnetic tools are essential in carrying out dissections in preparation for measurements in the magnetometer. Typical metal dissection tools, such as bone saws, scalpels, and forceps, can leave trails of highly magnetic particles behind them. Even tools made from non-ferrous metals such as aluminum or copper often contain small ferromagnetic inclusions sufficient to prevent their use in dissections (Kirschvink 1983). Magnetic particles left in tissues by these tools can easily be detected but

can only be identified from extensive tests of their magnetic properties.

Wood, plastic, and glass are the materials most suitable for tools used in dissections. I found glass microtome knives convenient for dissection and easily obtainable from electron microscopy laboratories. Disposable wooden chopsticks were ideal for handling tissue samples of the sizes measured in the magnetometer. Although they would acquire magnetic moments, frequent washing and replacement of the chopsticks minimized the risk of their picking up and transferring contaminants to samples. Some contact with the samples by non-disposable equipment was inevitable. To minimize contamination of samples, this equipment was frequently washed in glass distilled water, and was periodically cleaned ultrasonically in either glass distilled water or 6N HCl.

The risk of contamination was further reduced if dissections were made from whole carcasses rather than sections that had been reduced in size using metal saws or knives. Saws appear to inject magnetic particles well into tissues (M. Fuller personal communication 1982). These may be dispersed further during dissection and their presence and contribution to tissue moments can not be determined other than by extensive testing. I also found during the study that juvenile or subadult animals with incompletely

ossified bones were easier to dissect than adults. To gain access to tissues and organs within the skull of adult fish often required considerable force. However, in juvenile fish the smaller size of target structures often made them hard to dissect cleanly. Thus, although my dissection techniques were effective, they made accurate localization and identification of magnetic structures difficult.

After tissue samples had been dissected and washed in distilled water, several of their magnetic properties were of interest. However, before a tissue sample could be measured it had to be frozen so that any small magnetic particles present were immobilized. Otherwise, in the null field environment of the magnetometer, the orientation of magnetic particles suspended in a viscous medium would be randomized by Brownian motion, and any moment due to alignment of the particles lost.

Important magnetic properties of the samples included the natural remanent magnetization (NRM), the saturation isothermal remanent magnetization (sIRM), and the rate at which magnetization was acquired or lost in progressively increased inducing or randomizing fields. The NRM is the moment of a sample that has not been exposed to any inducing field. The sIRM is the moment acquired by the sample after it has been exposed to a large (>300 mT) inducing field.

Although the physical orientation of the particles in the frozen sample can be random, their moment vectors will be realigned by a large inducing field so that they all have a positive component in the direction of the applied field. The moments of the particles in the sample will then sum vectorally to produce a stable remanent moment, the sIRM. The magnitude of the sIRM in samples therefore provides an estimate of the amount of magnetic material present in a sample.

In my early work I attempted to induce the sIRM in samples using a cobalt-samarium magnet. Unfortunately, it was difficult to obtain homogeneous magnetization of large samples due to rapid decay of field strength with distance from the magnet. Inhomogeneous magnetization can lead to underestimates of the amount of magnetic material present, making it possible to miss potentially important magnetic structures. An air core solenoid delivering homogeneous inducing fields of up to several Tesla proved very reliable in uniformly magnetizing samples for measurement in the magnetometer. This solenoid also made possible the progressive magnetization of samples in coercivity studies (Kirschvink 1983).

Great care was necessary in the choice of sample holders for magnetometry experiments. The mylar, glass, or polyethylene plastic tubes commonly used by paleomagnetists

were adequate for use with biological samples although they would acquire magnetic moments if exposed to strong magnetic fields. Samples therefore had to be magnetized separately from these holders and then loaded for measurement (Jones and MacFadden 1982). This made it difficult to maintain the same orientation of the sample to components of the process (solenoids and magnetometer detection coils) in repeated measurements.

Two simple methods of attaching samples to a holder that maintained the orientation of the sample relative to the different instruments used in making measurements were developed during the study. A magnetized, frozen sample was attached to the moistened end of a white cotton thread and lowered vertically into the magnetometer. If thoroughly cleaned, the thread did not show any NRM and could be used for repeated measurements such as alternating field (AF) demagnetization. However, the "clean" thread could acquire a moment if exposed to strong fields. A more effective holder was found to be a hook made of quartz glass fiber. The hook was inserted into the unfrozen tissue and left within it throughout all the measurements. Control experiments conducted with the quartz fiber hook attached to an ice cube showed that the fiber possessed no natural moment and did not acquire a moment even in strong inducing fields. With this holder and the air core impulse or AF solenoid mounted

in line on the magnetometer, the measurements could be automated and the time spent handling and measuring samples in IRM acquisition and AF demagnetization experiments minimized. The disadvantage of the quartz fiber hook was its fragility. The fiber broke easily and samples were often lost in the magnetometer as a result.

III.4.2.2 Sample measurements

Superconducting magnetometers were developed to measure the direction and magnitude of the remanent magnetic moment of samples at room temperature (Kirschvink 1983). The insertion of a sample with a weak remanent moment causes a persistent current to flow in the superconducting loop that acts as a flux transformer. The current signal is then amplified by the detection system and converted to a digital output giving the moment detected in two or more axes (Goree and Fuller 1976).

Paleomagnetists have developed a range of techniques using superconducting magnetometers that can be adapted for identifying and characterizing the properties of biogenic magnetite. In this study, the most important of these techniques was measuring the coercivity spectrum of particles present in magnetic samples. The range of applied fields over which a sample acquires or loses magnetization

is dependent on the coercivities of the magnetic particles present in the sample. The coercivity spectrum can therefore eliminate a variety of minerals like hematite and goethite and can give information on the size and shape of any magnetite fraction present in samples.

Two methods are available for determining the coercivity spectrum of particles present in a sample: progressive IRM acquisition and AF demagnetization. Progressive IRM acquisition begins by taking a sample that either has no NRM or has been demagnetized, and exposing it to stronger and stronger inducing fields of known intensity. Because magnetite has a theoretical maximum coercivity of 300 mT (McElhinny 1973), magnetite-containing samples should not continue to acquire magnetization beyond this intensity of inducing field unless the samples also contain high coercivity contaminants.

Alternating field demagnetization is essentially the reverse of IRM acquisition. In a saturated sample all the particle moments are aligned with their north-seeking poles in the hemisphere centered around the direction of the applied field. The sample is placed in a solenoid in a magnetically shielded area and exposed to a sinusoidally oscillating magnetic field that slowly decreases in amplitude. The moments of particles with coercivities less

than $B\cos\Theta$, where B is the peak intensity of the applied field and Θ is the angle between the axis of the oscillating field and the long axis of the individual particles, will track the external field as it oscillates. As B decreases towards 0, the oscillating moments will come to rest with equal probabilities in one or other of their stable orientations. After each demagnetization step in this procedure, the moment of the sample will arise only from particles with coercive fields greater than $B\cos\Theta$. The coercivity spectrum is thus determined by measuring the retained moment after exposing the sample to alternating fields with progressively increased peak intensities.

Although the use of the cotton thread or quartz fiber techniques described above made these iterative measurements very easy, they made it impossible to correct for the effect of the angle between the direction of the crystal axes and the applied fields. This caused slight overestimation of the coercivities of the particles. However, for distinguishing single-domain magnetite from either multi-domain particles or high coercivity contaminants, such as hematite, this error could be ignored.

III.4.3 Extraction and characterization of biogenic magnetite

Much can be learned about the nature and organization of the magnetic material discovered in biologic samples using its bulk magnetic properties as discussed above. However, it is eventually necessary to extract the material from the sample and apply a range of techniques to its identification and characterization. Areas of highest concentrations of magnetic material must be accurately identified if sufficient quantities of material are to be obtained for analysis. I did this by magnetometry studies that exhaustively sampled the tissues of the yellowfin tuna until I could reliably locate tissues containing high magnetic remanence. I was able to identify one specific and relatively small structure, the dermethmoid bone, that was always magnetic. My subsequent experiments showed that the magnetite was concentrated in tissue contained in a sinus within the dermethmoid bone. Extraction experiments were aimed at isolating and purifying the magnetic material from this tissue.

Extraction of the magnetic material immediately produces a substantial amount of information that assists in its identification. For example, magnetite is the only ferromagnetic mineral that is optically black in fine powder

(Kirschvink and Gould 1981). Color therefore makes it possible to distinguish magnetite and maghemite, which can not be separated by coercivity studies. However, tests are still necessary to identify and demonstrate the biological origin of the magnetic material and to exclude the possibility that contaminants may have entered during dissection and extraction.

X-ray diffraction and electron diffraction patterns uniquely identify minerals from their crystal structures and have been used to identify magnetite found in magnetotactic bacteria (Towe and Moench 1981). However, diffraction patterns are not conclusive proof of the origin of magnetite particles extracted from tissues and care is necessary in their interpretation. Pure, fine-grained magnetite powders such as those predicted for use in magnetoreception will ideally give sharp, unambiguous diffraction patterns. Streaking of the spots or lines in X-ray and electron diffraction patterns could arise from more than one source. Towe and Moench (1981) suggested that vacancy defects in the crystal structure could have caused streaking of an electron diffraction pattern taken from single-domain magnetite particles isolated from magnetotactic bacteria. Multi-domain or coarse particles present as contaminants could also be expected to give streaked diffraction patterns (K. M. Towe, L.-C. Ming, personal communications 1982, 1983). Therefore

tests that will demonstrate the origin of the particles are still required. Among these tests is measurement of the size and shape of particles, which can be done independently in TEM. Thus although electron diffraction is a more cumbersome technique than X-ray diffraction, it does provide a conclusive test of the identity and origin of the particles when carried out in conjunction with determination of their size and morphology.

III.4.4 Histological studies

A number of studies have sought to detect histologically the presence of magnetite in tissues that had previously been shown to contain magnetic material. Kuterbach et al. (1982) demonstrated iron-containing cells in whole mounts of the abdominal segments of honey bees and Baker et al. (1983) have shown bands of tissue containing ferric iron situated 5 μm below the sinus bones of humans. Although it is not actually stated, a reasonable inference from the report of Baker et al. (1983) is that the material that took up stain in the Perl reaction (Hutchison 1953) may be the magnetic material previously detected, that it may be involved in magnetoreception, and that it may well be magnetite.

It is highly unlikely that magnetite crystals suitable for magnetoreception could be detected in histologic sections using iron staining techniques. The crystals are submicroscopic, occupy a miniscule total volume, and, unless they were very densely aggregated, could not be detected from the quantity of stain they would take up. Aggregations of single-domain magnetite particles dense enough to be detected by iron staining procedures are likely to interact too strongly with each other and with the geomagnetic field to be suitable for use in magnetoreception (see III.3, III.7).

A simple chemical test that can readily be inserted into iron staining procedures can neatly demonstrate whether ferric iron present in tissue sections could arise from magnetite. Sodium dithionite ($Na_2S_2O_4$, also known as sodium hydrosulfite) is a strong reducing agent that will reduce ferric iron minerals to soluble ferrous iron (Mehra and Jackson 1958). Kirschvink (1981b) showed that, whereas magnetite powder was stable for at least one month in citrate-bicarbonate buffered sodium dithionite solution, maghemite, goethite, and hematite powders dissolved in 24-48 hours at 20-25°C. Thus ferric iron present in histological sections should be leached out after two days in sodium dithionite solution unless it is magnetite.

III.5 METHODS

III.5.1 Magnetometry studies

The magnetometry experiments in this study were carried out in three separate series. The first series of experiments was carried out in October 1980 and May 1981 in the paleomagnetics laboratory at the Hawaii Institute of Geophysics. The purpose of these experiments was to survey the tissues of the yellowfin tuna for inducible magnetic remanence. The walls and floor of the laboratory were thoroughly washed and covered with plastic dropcloths. The cobalt-samarium magnet used to produce the IRM was placed in a plastic bag to prevent magnetic particles on the surface of the magnet from contaminating samples. To prevent stray fields from affecting sample measurements the magnet was kept well away from the magnetometer. The sample holders and ice made from the distilled water used to wash samples were tested for both natural and saturated remanence.

All dissections were carried out in the clean area. Representative tissues and organs from three yellowfin tuna were dissected, washed, frozen, and magnetized by momentarily bringing them close to the cobalt-samarium magnet. The samples were then measured in the magnetometer. To determine whether or not samples were magnetic, it was

necessary to remove the contribution of background noise in the magnetometer to measured sample moments. A signal-to-noise ratio for each sample was calculated by dividing the signal from the sample by the mean of the background noise measured in the magnetometer before and after each measurement. Thus, to be considered magnetic, a sample had to have a signal-to-noise ratio greater than unity. For one of the fish measured in these first experiments, each sample was weighed after measurement. Intensities of magnetization for samples from this fish were calculated by dividing the moment of each sample by its mass.

The second series of experiments was conducted in the paleomagnetics laboratory at the California Insitute of Technology (CIT). Procedures for laboratory cleaning and sample preparation were as previously described. The purpose of these experiments was to obtain coercivity estimates by progressive AF demagnetization of the magnetic tissue from the yellowfin tuna. Samples were magnetized and then demagnetized in alternating fields increased in steps from 5 to 25 mT.

The third series of experiments was carried out in February 1983 in the specially developed magnetic clean laboratory at CIT. Special procedures in these experiments were regular cleaning of all surfaces in the laboratory and testing of the distilled water and all holders used in

repetitive measurements. The magnetic tissues (the dermethmoid bones) from four fish were attached to quartz fiber hooks and their NRM determined in the magnetometer. They were then subjected to progressive magnetization using the air core impulse solenoid. After saturation the samples were progressively demagnetized using alternating fields. To test whether magnetic particles within the dermethmoid bone were free to rotate, the saturated dermethmoids from seven fish were allowed to warm to room temperature and their moments remeasured at five minute intervals.

III.5.2 Magnetite extraction and characterization

Once magnetic structures had been identified, it was a simple matter to dissect and combine a number of them for magnetite extraction. In this way I was able to treat the dermethmoid tissues of up to five yellowfin tuna at once. The tissues were ground with a little distilled water in a glass tissue grinder or in a test tube using a non-magnetic pestle. Breakup of the tissue released fat and oil droplets into suspension. These were removed by adding ether to the suspension and shaking vigorously. After the aqueous and ether phases had separated, the ether was decanted and replaced. The ether extraction procedure was repeated until the aqueous phase became clear.

After ether extraction the suspension was centrifuged, the aqueous supernatant removed, and 5% millipore filtered sodium hypochlorite solution (commercial bleach) added. The mixture was centrifuged and the hypochlorite replaced periodically until no further digestion occurred. When digestion was complete the suspension was centrifuged and the supernatant replaced with distilled water. This washing procedure was repeated at least five times. A white residue associated with the magnetic material remained after tissue digestion. Treatment with buffered EDTA (pH 7.1) carried out in similar fashion to the hypochlorite digestion freed the crystals so that they could easily be sorted from the residue under a dissecting microscope.

To remove the magnetic particles for analysis, I held them in suspension using a cobalt-samarium magnet held to the side of the test tube and then pipetted them onto a microscope slide coated with xylene-based cement. The water in which the crystals were pipetted onto the slide was allowed to dry and a second layer of cement applied. The crystals were thus sealed in a cement sandwich which could be cut out, removed from the slide, and placed in the beam of a mini-Debeye Scherrer X-ray camera. I exposed the crystals to 48 hours Mo K_{α} X-radiation, developed the film, and measured the band pattern.

For electron microprobe analysis, I pipetted the crystals onto clean microscope slides and allowed them to dry completely. I then transferred the crystal aggregates to slides coated with epoxy resin, cured the resin, polished the crystals, and coated them with carbon. The electron microprobe analyses determined the elemental composition of the mineral and permitted tests of the purity and origin of the magnetite crystals. I analyzed for oxides of Fe against a magnetite standard and for oxides of rare earth metals such as titanium and manganese, which are commonly found as impurities in geologic magnetites. I also chose to analyze for calcium in an attempt to determine how closely the residue remaining after hypochlorite digestion was associated with the particle aggregates.

The very small amounts of material obtained by digestion made it impossible to use the approach developed by Towe and Moench (1981) for preparing the crystals for transmission electron microscopy (TEM). I therefore used a method developed by R. S.-B. Chang (personal communication 1982) for obtaining dispersed crystals that compensated for this limitation. The crystals were pipetted onto carbon-coated film supports on copper mesh grids and dispersed in a 100 mT alternating magnetic field. The grids were then air dried and prepared for examination in TEM.

III.5.3 Histological studies

To test whether ferric iron was present in the dermethmoid tissue, I stained sections using the Prussian Blue technique (also known as the Perl reaction; Hutchison 1953). This technique was then modified to determine whether or not stained material was magnetite by pre-treating the sections for 48 hours with a solution of sodium dithionite. The dermethmoid and similar skull sinus tissues from two yellowfin tuna were fixed and sectioned as described in Chapter IV. For the first experiment, mounted sections were deparaffinized in xylene and hydrated to water through a descending series of alcohols. The sections were then stained for five minutes in an equal parts by volume mixture of 4% solutions of potassium ferrocyanide and hydrochloric acid, washed in distilled water, and counterstained with Nuclear Fast Red. The sections were then rapidly dehydrated in absolute alcohol and, after two xylene baths, mounted in Eukitt.

For the second experiment, sections were hydrated to water. The slides were then partially immersed for 48 hours in a 2.2% solution of citrate-bicarbonate buffered sodium dithionite solution prepared according to Kirschvink (1981b). After washing in distilled water, the iron staining procedure was completed as in the previous experiment.

III.6 RESULTS

III.6.1 Magnetometry studies

My first studies set out to determine whether magnetic material is consistently localized at any point in the body of the yellowfin tuna. All the recognizable tissues and organs that could be extracted from the bodies of three fish were examined for sIRM in the superconducting magnetometer. Tissues and organs sampled in all fish included bones of the body and skull, skin, viscera, sense organs and swimming muscles. Although subsequent magnetometry studies focused on the magnetic tissue, different samples were measured in all fish. The background noise in the magnetometer was less than 50 pAm^2 throughout the studies. If the background signal drifted more than 10% during measurements, samples were remeasured. Sample holders were also measured between all measurements to ensure that their contribution to magnetic moments were minimal.

The sizes of the tissue samples caused a scaling effect on the signal-to-noise ratios. The null hypothesis for these experiments was that no tissue contained selectively concentrated magnetic material. If it is assumed that any magnetic particles are uniformly dispersed, a bulky sample such as a whole eye should acquire a high signal-to-noise

ratio compared with a small piece of skin or brain. For this reason the intensity of magnetization, or the relative concentration of magnetic material in each sample, was calculated by dividing the moment of each sample by its mass. This could potentially cause a reverse type of scaling from the signal-to-noise ratio by making a very small sample with a signal-to-noise ratio barely greater than unity appear to be intensely magnetized. Consequently, I chose to recognize only those tissues that gave both high signal-to-noise ratios and high intensities of magnetization as being magnetic.

The first series of experiments showed that most tissues of the yellowfin tuna were not magnetic. Six tissue samples showed neither high signal-to-noise ratios nor high intensities of magnetization (Table 3.1). Seven other samples had moments less than 10 times the background noise in the magnetometer. Because of the scaling effect caused by their small mass, some of these samples showed high intensities of magnetization. Cardiac muscle and eye samples acquired high moments. However, their intensities of magnetization showed that this was due partly to their large size relative to other samples. Subdivision and remeasurement of the eye samples showed that the moments they acquired were not associated with the lens, retina, or optic nerve. Because these samples were either clearly non-

Table III.I. -- Magnetic tissues of the yellowfin tuna. Saturation moments, signal-to-noise ratios and intensities of magnetization for tissue and organ samples from different yellowfin tuna. Variability estimates are standard deviations and numbers in parentheses are the numbers of samples measured for the saturation moments and intensities of magnetization. Intensity estimates for many samples came from one fish only; X indicates no data. Signal-to-noise ratios were calculated by dividing the mean saturation moment by the maximum background noise (50 pAm^2) in the magnetometer. Samples are grouped into those that were clearly non-magnetic, those that were magnetic from their signal-to-noise ratio or their intensity of magnetization only, and those that were magnetic from both measures.

Sample	Mean moment (pAm^2) ± S.D. (N)	S/N	Intensity (pT) ± S.D. (N)
Liver	105.0 ± 134.4 (2)	2.1	1.8
Pyloric caecum	49.6 ± 50.0 (3)	1.0	3.5
Intestine	14.5 ± 20.5 (2)	0.3	4.8
Red muscle	184.0 ± 274.7 (3)	3.7	3.5
White muscle	155.0 ± 211.2 (6)	3.1	5.7 ± 5.3 (3)
Brain	36.4 ± 50.3 (5)	0.7	7.5

Table III.I. (Continued.)

Sample	Mean moment (pAm^2) ± S.D. (N)	S/N	Intensity (pT) ± S. D. (N)
Gill	95.0 ± 143.4 (6)	1.9	20.6
Skin	41.7 ± 80.1 (6)	0.8	35.7
Peduncle tendon	120 ± 169.7 (2)	2.4	41.4
Frontal bone	202.0 ± 129.0 (6)	4.0	103.6 ± 86.9 (2)
Pectoral fin	325.0 ± 427.0 (2)	6.5	62.5
Posterior brain case	375.0 ± 455.0 (6)	7.5	X
Dorsal fin	400.0 ± 628.5 (5)	8.0	X
Cardiac muscle	500.0 ± 707.1 (2)	10.0	4.5
Eye	1242.5 ± 1052.6 (4)	24.9	13.7 ± 14.1 (2)
Dermethmoid bone	1320.6 ± 867.5 (15)	26.4	127.0 ± 86.7 (7)

magnetic, appeared magnetic from one measure only, or were not reproducible in different fish, it seemed unlikely that they would come from a sensory organ. For this reason I focused my work on tissues that acquired large reproducible moments.

Only the dermethmoid bone (Figure 3.4) gave high values for all measures of magnetization used in all fish examined (Table III.I). A scatter diagram plotting signal-to-noise ratio against intensity of magnetization for the data presented in Table III.I clearly identified the dermethmoid bone as the most magnetic sample measured. Subdivision and remeasurement of the dermethmoids from a number of fish suggested that the magnetic material was contained in tissue in a sinus within the dermethmoid bone.

The finding that tissue from within the space formed by the dermethmoid bone of the skull was magnetic led me to focus further magnetometry studies on this tissue. The second series of experiments set out to determine the coercive field of the magnetic particles using AF demagnetization of the sIRM acquired by the dermethmoid tissue. The experiments showed that samples of dermethmoid tissue from yellowfin tuna lost 50% of their remanent magnetization in peak alternating fields of 22.5 mT (Figure 3.5). It can be inferred from this experiment that the

Figure 3.4. Lateral and dorsal views of the skull of Thunnus sp indicating bones measured for magnetic remanence. Abbreviations used: D: dermethmoid bone; Pt: parethmoid bone; Pf: pineal foramen; Pr: parietal bone. Figure modified from Gibbs and Collette (1971).

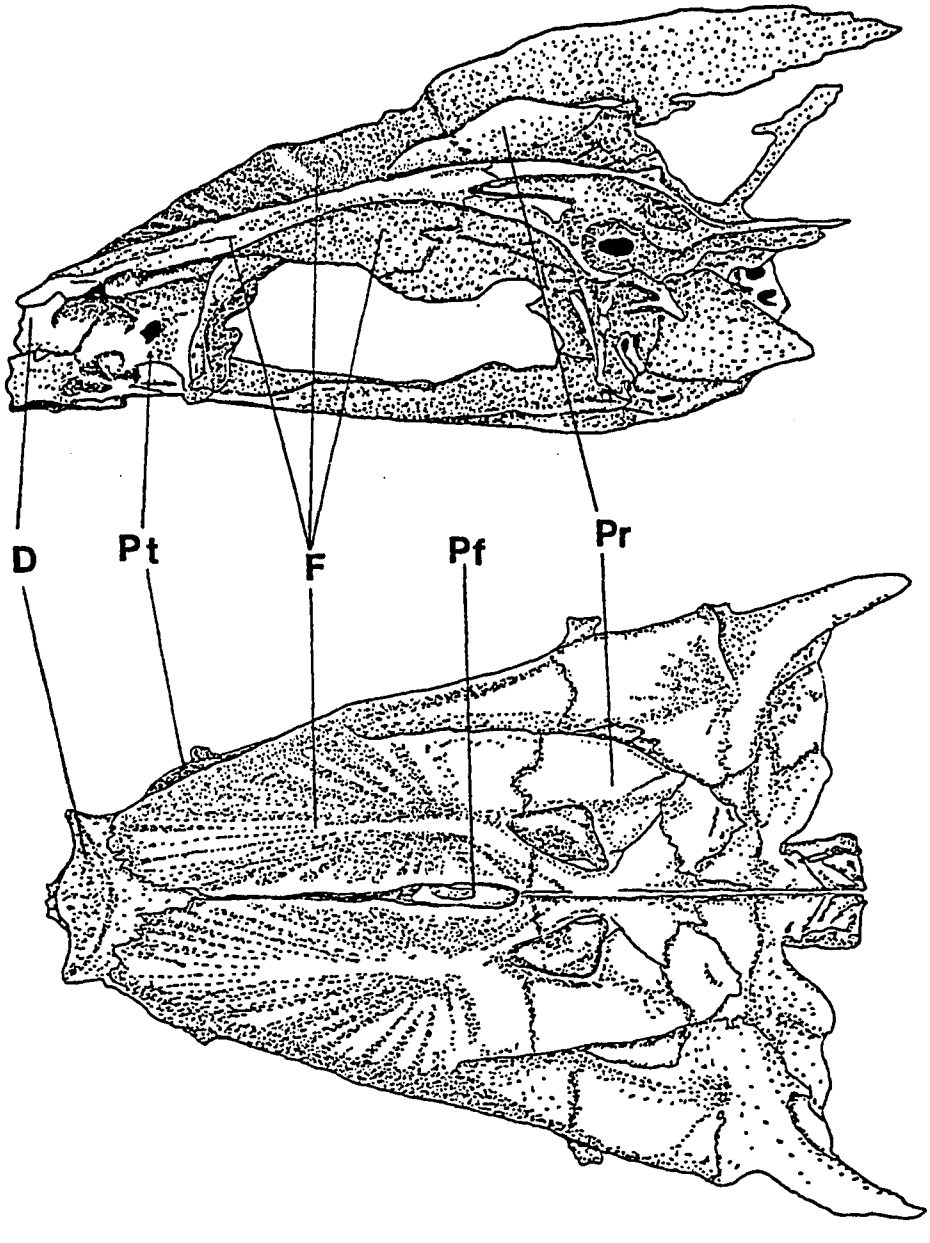
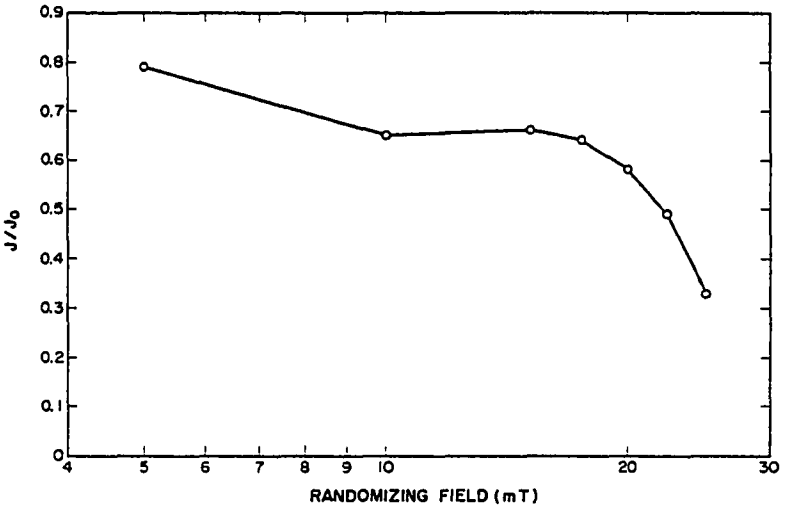

D
Pt
F
Pf
Pr

Figure 3.5. Progressive alternating field demagnetization of the dermethmoid bones of three yellowfin tuna. The ratio of the moment (J) retained after each demagnetization step and the saturation moment (J_o) is plotted against the strength of alternating field used.

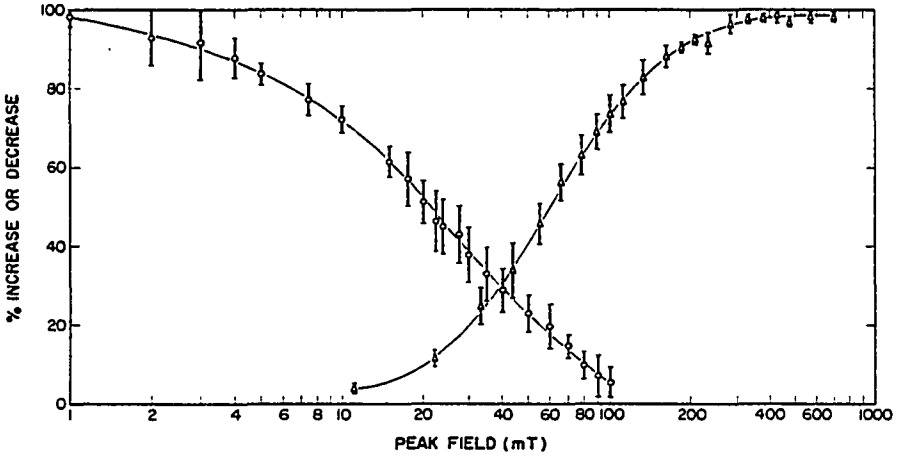

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
J/J₀
4
5
6
7
8
9
10
20
30
RANDOMIZING FIELD (mT)

dermethmoid tissue contains a population of single-domain grains of magnetite with a median coercivity of 22.5 mT. Inspection of the Butler-Banerjee diagram (Figure 3.1) suggests that the crystals are 50-70 nm in length with an axial ratio of 0.9.

The third series of experiments provided more detailed coercivity measurements. Four dermethmoid samples that had been demagnetized were washed, refrozen, and subjected to progressively increasing magnetic fields in the air core impulse solenoid. After each magnetization step their moments were remeasured to monitor IRM acquisition and the data plotted as a percentage of the saturated moment. The samples were then progressively demagnetized, remeasured after each demagnetization step, and the data plotted as a percentage of the saturated moment.

Plotting the results of IRM acquisition and AF demagnetization experiments together can reveal much about the nature of the magnetic particles in the sample and their magnetic micro-environment. Where all the particles are similar sized single-domains uniformly dispersed throughout a sample, the two curves will intersect at 50% magnetization and be mirror images of each other over the same ranges of intensity of the applied fields. If the crystals are sufficiently close to one another, their own magnetic fields

Figure 3.6. Progressive magnetization and demagnetization of the dermethmoid tissues of four yellowfin tuna. The 100% represents the saturated moment and 0% the natural remanent moment of the tissues. Vertical bars indicate standard errors. The ordinate and abscissa of the intersection point of the two curves are 30% and 40 mT respectively.


% INCREASE OR DECREASE
100
80
60
40
20
0
1
2
4
6
8
10
20
40
60
80
100
200
400
600
1000
PEAK FIELD (mT)

will act on neighbouring particles to inhibit IRM acquisition and to aid AF demagnetization (Cisowski 1981). Thus asymmetry of the curves about the 50% magnetization point indicates the existence of interactions between the particles. Cisowski (1981) found that, in samples containing single-domain magnetite, the shift towards higher coercive fields in the IRM aqcuisition curve and the shift to lower coercive fields in the AF demagnetization curve were almost exactly the same. As a result, the abscissa of the intersection point of the two curves is independent of the interaction effect and provides an estimate of the median coercive field of the particles.

The 50% magnetization point from the IRM acquisition experiments provided an estimate of about 60 mT for the median coercive field of the magnetic particles present in the dermethmoid tissue of the yellowfin tuna (Figure 3.6). This was considerably larger than the estimate obtained from the previous AF demagnetization experiments. The abscissa of the intersection point of the IRM acquisition and AF demagnetization experiments is 40 mT, which is about midway between the estimates of coercivity obtained from the two methods. The difference between the median coercivity estimates obtained from the two methods demonstrates the existence of significant inter-particle interactions within the dermethmoid tissue.

Cisowski (1981) showed that the ordinate of the point of intersection (designated R) of IRM acquisition and AF demagnetization plots for dispersions of single-domain magnetite grains ranged from 0.5 for completely non-interacting grains to 0.27 for the very closely associated grains in chiton teeth. The R value of 0.3 (or 30% magnetization) obtained from the two plots for the yellowfin tuna (Figure 3.6) suggests that the crystals are less tightly packed than the crystals in chiton teeth but that they are still sufficiently close to interact significantly. A possible explanation for this interaction is that the crystals are organized into chains such as have been observed in the magnetotactic bacteria (Frankel et al. 1979, Balkwill et al. 1980).

Further conclusions that can be drawn from these data concern the identity and size frequency distribution of the magnetic particles. The magnetic particles present in the dermethmoid tissues of four yellowfin tuna exhibited a narrow range of coercivities, causing the samples to acquire virtually all of their remanence in applied fields of less than 200 mT (Figure 3.6). This rules out the presence of many other ferromagnetic minerals such as hematite and some metallic iron alloys, which will continue to acquire IRM in fields up to at least 1000 mT. Two observations indicate the absence of the multi-domain magnetite particles detected in

the dura mater membrane of Pacific dolphins by Zoeger et al. (1981). These were the flatness of the AF demagnetization curve below peak fields of 10 mT and the apparent absence of any sudden loss of remanence as the ethmoid tissues warmed through magnetite's isotropic point (see below; Zoeger et al. 1981). Maghemite can not yet be excluded as a source of remanence. However, it is reasonable to infer that the remanence in the dermethmoid tissue of the yellowfin tuna was predominantly produced by single-domain magnetite particles. Inspection of Figure 3.1 shows that magnetite particles with coercivities of 40 mT will be approximately 50 nm long and 40 nm in diameter.

The distribution of coercivities, and hence of size frequency, of the presumed magnetite particles in the dermethmoid tissue is very narrow when compared with those observed in geologic magnetite samples. Geologic magnetites commonly exhibit wide ranges of coercivities and log-normal size frequency distributions (Kirschvink and Lowenstam 1979). This pattern results from the crystal growth process, which leads to faster growth rates in large crystals than in small ones. Thus the coercivity spectrum for magnetic particles in the dermethmoid tissue of the tuna helps to distinguish them from geologic magnetite.

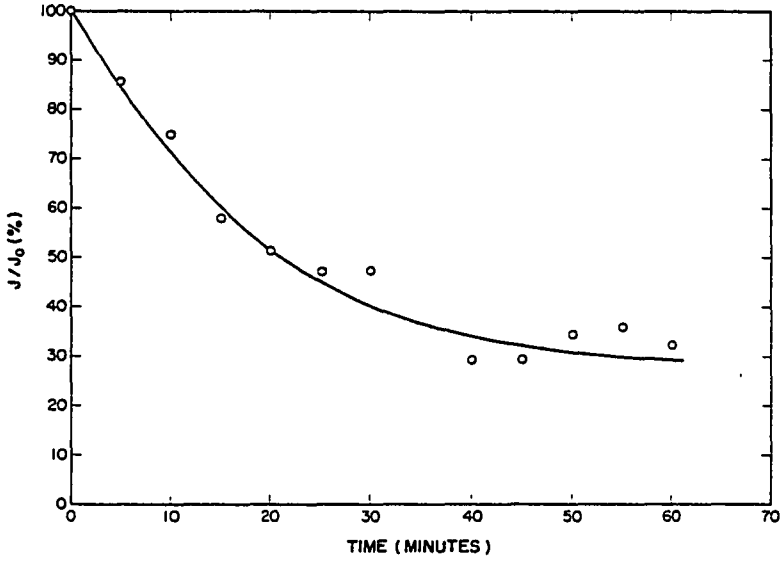
The frozen dermethmoid tissues of 7 yellowfin tuna showed no natural remanent magnetization (moments 3-30

pAm^2). These samples were magnetized and allowed to warm to room temperature. All showed an exponential decay with time in the moment they retained (Figure 3.7). This suggested that the orientation of the magnetic particles was randomized by thermal agitation as the tissue thawed. However, not all the moments decayed completely to the point where they could not be detected against the background signal in the magnetometer. From these two observations I conclude that the crystals are at least partly free to rotate.

III.6.2 Magnetite identification and analysis

The extraction techniques permitted a number of distinctive assays for magnetite on the polycrystalline aggregates extracted from the dermethmoid tissue of the yellowfin tuna. The particles were black, both to the naked eye and when viewed under a dissecting microscope. This excluded maghemite as a possible source of the remanence in the dermethmoid tissue and strongly suggested that the only magnetic mineral present was magnetite. In an attempt to determine whether normally non-magnetic tissues also contained finely dispersed magnetic material, a large sample (about 10 g) of the white muscle of one fish was digested using the same techniques. No magnetic particles were

Figure 3.7. Loss of remanent magnetization with time on warming from liquid nitrogen temperature (77°K) to room temperature (293°K) in the dermethmoid tissues of seven yellowfin tuna. Only two measurements were taken at 35 min.


100
90
80
70
60
50
40
30
20
10
0
J/J0 (%)
0
10
20
30
40
50
60
70
TIME (MINUTES)

obtained, presumably because any particles present in the swimming muscle must have been present in concentrations too small to be extracted using these techniques.

X-ray diffraction, the technique used to identify the crystals, depends on the interaction between the collimated X-ray beam, the ions in the mineral, and their orientation in the crystal lattice. The beam enters the sample and is scattered at angles characteristic of the position of each ion in the lattice. The scattered beam is detected by X-ray photographic film which, after development, shows a pattern of concentric arcs. The distance of each arc from the center is characteristic of the structure and composition of the sample crystals. From these distances are calculated the distances (known as d-spacings) between adjacent ions in the unit cell of the lattice.

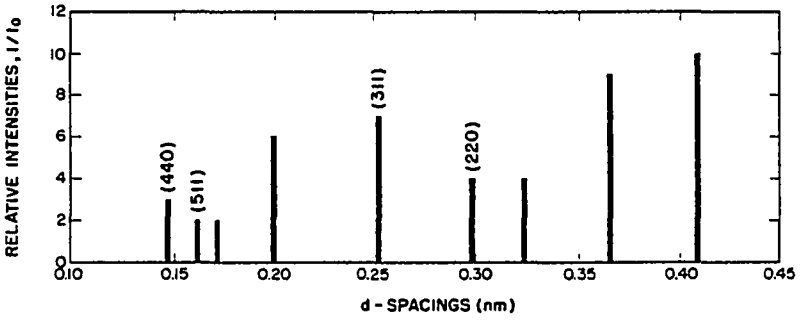
The X-ray pattern obtained from the magnetic particles extracted from the dermethmoid tissue of the yellowfin tuna uniquely identified the magnetic mineral as magnetite. The pattern was too streaky and faint to print directly. The data are therefore presented as measured, using a standard method of graphic interpretation of X-ray diffraction data (Figure 3.8). From their d-spacings and relative intensities the fourth, fifth, eighth, and nineth lines recognized in the pattern corresponded to reference planes (indicated in parentheses in Figure 3.8) for the lattice of magnetite

(Joint Committee on Powder Diffraction Standards (JCPDS) 1974). The four values for the lattice parameter, a_o, calculated from the measured d-spacings are all approximately equal, with a mean of 0.8358 ± 0.004 nm. This is sufficient to conclude that the magnetic mineral present in the sample was magnetite (reference lattice parameter = 0.8396 nm; JCPDS 1974).

A number of the diffraction lines in the pattern did not come from magnetite nor from any other ferromagnetic mineral. These lines clearly arose from some other crystalline material in the sample. An extensive search through reference X-ray patterns did not identify any other minerals that could have produced some or all of these extra lines. Consequently, their source remains unknown.

The Cameca MBX electron microprobe used in this study compares the composition of a prepared sample with a standard of known composition. For a mineral such as magnetite, where more than one valence state of an element are present, one valence state only is assayed. Identification of the mineral is then by stoichiometry. The microprobe operates by bombarding the sample with a beam of electrons, causing ions in the sample to give off X-rays characteristic of each atom and its valence state. The X-rays are then analyzed by the machine to give a quantitative

Figure 3.8. X-ray diffraction data for magnetite extracted from tissue contained within the dermethmoid bone of the yellowfin tuna. Vertical lines indicate relative intensities of lines in the diffraction pattern. Numbers in parentheses indicate lines associated with magnetite and the crystal plane giving rise to each line.


RELATIVE INTENSITIES, I/I_0
12
10
8
6
4
2
0
(440)
(511)
(311)
(220)
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
d - SPACINGS (nm)

(± 2%) estimate of the amount of each element tested in the sample. The magnetite standard (NMNH 11487) used in this study came from the Minas Gerais mine in Brazil and is recognized as an unusually pure geologic magnetite (M. O. Garcia personal communication 1981).

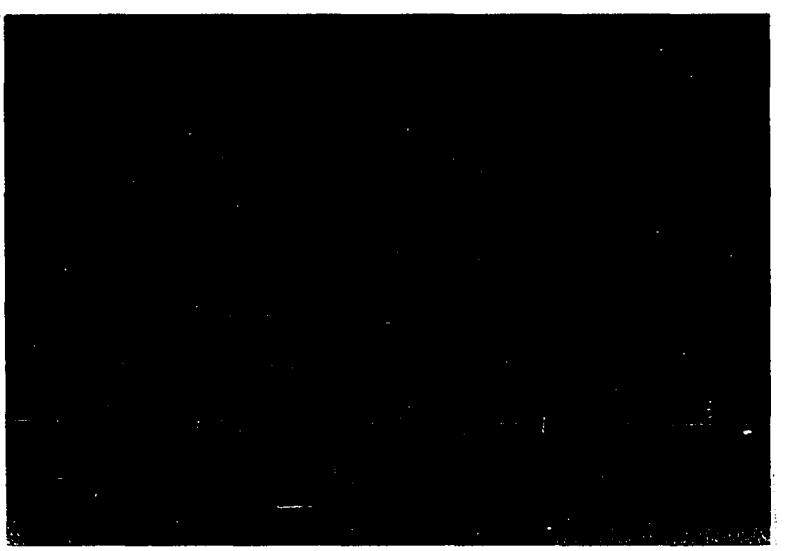
The magnetite extracted from the tuna was shown by the electron microprobe analysis to contain very few of the impurities present in the standard (Table III.II). The sample contained almost no measurable titanium or manganese, which are common components of geologic magnetites. The crystals also contained no measurable chromium, excluding many possible synthetic ferromagnetic materials. The calcium content in the sample was low, suggesting that the residue with which the crystals were associated after extraction was largely separated by the aggregation procedure. The high variability in the estimated FeO composition of the sample relative to the standard resulted from one reading, where it is believed surface irregularity prevented quantitative analysis. With this reading excluded, the measured FeO composition was close to the reference value and the variability in the estimate substantially reduced.

Under TEM, isolated magnetite particles averaged 45 ± 5 nm in length and 38 ± 5 nm in diameter (Figure 3.9). These dimensions fall within the single-domain stability field for magnetite (Figure 3.1) and their sizes and axial ratios are

Table III.II. -- Electron microprobe analyses of magnetite particles isolated from yellowfin tuna.

Oxide	Magnetite standard (NMNH 11487)	Weight (%) of sample
FeO	90.9	86.3 ± 7.7
TiO_2	0.2	0.0 ± 0.0
Cr_2O_3	<0.25	0.0 ± 0.0
MnO	<0.0	0.2 ± 0.1
CaO	--	0.2 ± 0.0
Total	91.4	86.7

Figure 3.9. Free magnetite grains extracted from the dermethmoid tissue of the yellowfin tuna. (Scale line is 100 nm; transmission electron micrograph courtesy R. S.-B. Chang, California Institute of Technology).

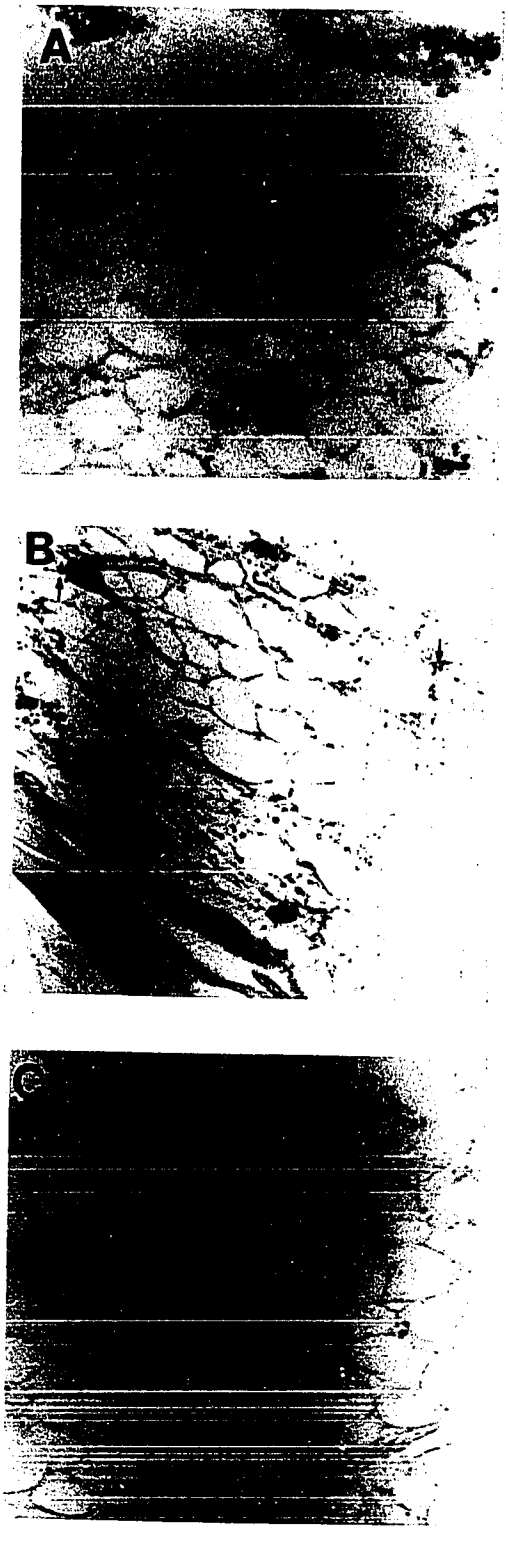


consistent with the particle coercivities measured in whole tissues. The crystals did not conform to the morphology shown by geologic or synthetic magnetite. Magnetite belongs to the cubic system and synthetic and geologic magnetite crystals all show an octahedral crystal form. The magnetite grains extracted from the tuna appear to be cylinders and may be hexagonal in cross-section (Figure 3.9). They are therefore similar in general form to the particles found in the magnetotactic bacteria (Towe and Moench 1981), although considerably smaller in size.

III.6.3 Histological studies

Sections of tissue from sinuses in the skull of the yellowfin tuna stained positively for ferric iron in the presence of acidic potassium ferrocyanide (Figure 3.10). The dermethmoid tissue took up far more stain than tissue from other skull sinuses, which were mostly stained around bones. The iron was present in cells both as densely stained granules and as a diffuse coloration of of the cytoplasm of cells. The size of the stained regions of the cells was too large to be consistent with the sizes predicted for the magnetite particles likely to be present in the tissue. In the second experiment sections that had not been immersed in the dithionite solution stained positively for ferric iron whereas those that had did not (Figure 3.10). From these

Figure 3.10. Ferric iron detected using the Prussian blue technique (also known as the Perl reaction) in A: the dermethmoid tissue and B: tissue from a similar sinus in the parethmoid bone in the yellowfin tuna. C. Adjacent section from the same slide as A. C was immersed in citrate-bicarbonate buffered sodium dithionite solution for 48 hours prior to staining with the Prussian blue procedure whereas A received no such treatment. Cells staining positively in the Prussian blue reaction appear dark (indicated by arrows in A and B; magnification = 120X in all three micrographs).


A
B
C

observations it is obvious that magnetite could not have been the form of ferric iron taking up the stain.

III.7 DISCUSSION

The magnetometric and mineralogical analyses reported here showed that yellowfin tuna contain single-domain magnetite selectively deposited in tissue contained within the dermethmoid bone of the skull. The size, shape, and arrangement of the crystals closely matched theoretical constraints established for use of the crystals in magnetoreception. The experimental results permit both refinement of the magnetite-based magnetoreception hypothesis and further predictions on the likely organization and operation of magnetite-based magnetoreceptors. From the magnetite-based magnetoreception hypothesis and the known behavioral responses of yellowfin tuna and other species to magnetic fields I (1) propose specific behavioral experiments to test the predictions of the hypothesis, (2) argue that data from previous behavioral experiments are consistent with the hypothesis, and (3) attempt to show that previously inexplicable results of behavioral experiments can be explained by the hypothesis. The biomineralization of magnetite in the yellowfin tuna has important implications concerning the possible origin and evolution of magnetoreception in the metazoa. These issues

will be discussed separately and then considered together to provide an overall picture of magnetite biomineralization and the possible involvement of magnetite in magnetoreception. The purpose of this approach is to identify the most important areas for future research.

Discovery of single-domain magnetite in a variety of metazoan groups provides the basis for a general magnetoreception mechanism suitable for use in both aquatic and terrestrial environments. Single-domain magnetite with properties very similar to those found in the tuna has been found in other pelagic fishes from different orders (Kirschvink et al. in review, Walker et al. in review). In other vertebrates single-domain magnetite or some magnetic material has been found in the anterior dura mater membrane or in association with the ethmoid area of the skull (Walcott et al. 1979, Mather and Baker 1981, Zoeger et al. 1981, Perry 1982, Baker et al. 1983).

These findings do not demonstrate the site of magnetoreception. However, all these observations of the presence in the same part of the body of magnetite or magnetic material that may be suitable for magnetoreception are consistent with the trend to cephalization of the special senses so evident in the vertebrates (Hyman 1942). Simple demonstration of the presence of magnetite or magnetic material in the same place in different individuals

magnetic material in the same place in different individuals and in different species does not, however, contribute greatly to the understanding of magnetoreception. It is important to develop a magnetite-based magnetoreception hypothesis that makes testable predictions concerning the operation of such magnetoreceptor organelles.

The magnetite-based magnetoreception hypothesis is appealing because it can theoretically account for the responses to magnetic fields exhibited by such diverse groups as the magnetotactic bacteria and algae (Frankel and Blakemore 1980, Lins de Barros et al. 1981), bees (Lindauer and Martin 1972), fishes (Quinn 1980, Quinn et al. 1981, Quinn and Brannon 1982), and birds (Keeton 1971, 1972). In the unicellular organisms and honeybees, the hypothesis has been tested experimentally (Kalmijn 1981, Kirschvink 1981a). Both Kalmijn (1981) and Kirschvink (1981a) show that the responses of magnetotactic bacteria and honey bees to magnetic field direction are quantitatively defined by the Langevin function in magnetic fields of different intensities. The measures of behavior used were swimming velocity in the bacteria and horizontal dance accuracy in the honeybees. Behavioral responses to magnetic fields by organisms therefore appear amenable to tests of their hypothesized ferromagnetic basis.

The experimental results obtained in this study permit refinement of the magnetite-based magnetoreception hypothesis and further predictions on receptor organization and operation. Theoretical analyses by Yorke (1979, 1981) and by Kirschvink and Gould (1981) considered the likely functioning of magnetoreceptors based on the single-domain crystals then known to occur in the magnetotactic bacteria, honeybees, and homing pigeons. The biophysical analysis above (III.3) assumed that the magnetic properties of the magnetite crystals and the energy of their interactions with the geomagnetic field are of primary importance in their hypothesized use in magnetoreception. The analysis predicted that single-domain crystals will interact most efficiently with the geomagnetic field in magnetite-based magnetoreception.

The crystals found in the dermethmoid tissue of the yellowfin tuna conformed very well to the prediction concerning their size. The particles detected in the magnetometry studies were shown by coercivity and TEM studies to be single-domains approximately 45 nm in length (Figures 3.6, 3.9). From these dimensions, it is easily shown that approximately 8.5×10^7 particles are necessary to produce the mean sIRM observed in the dermethmoid tissue. The energy of interaction of these particles with the geomagnetic field will be about 0.1 kT (Figure 3.1). This is

too small for use of the crystals individually in magnetoreception. To achieve coupling energies with the geomagnetic field large enough for detection by the nervous system, the crystals must be organized into interacting groups. Depending on the number of crystals in the groups, the maximum theoretical sensitivity of a magnetite-based magnetoreception system in the yellowfin tuna can easily be calculated (Kirschvink and Gould 1981, Yorke 1981).

At this stage I consider chains of crystals more likely than other possible arrangements because of the different inter-particle interactions likely to occur when the crystals are in arrangements other than chains. If particles are arranged in bundles with the layers organized such that their vector directions are antiparallel, their moments will cancel, and the arrays will tend to behave as multi-domain particles. If the layers are arranged in parallel, the interactions between like poles in adjacent layers will be very large and may impede magnetoreceptor operation. In chains, the particle moments will sum linearly and the crystal groups will exhibit the inter-particle interactions detected in the coercivity studies. If the magnetic particles are closely apposed, the chains will act as magnetic units (Yorke 1981).

A possible form for magnetite-based magnetoreceptors can now be described. The crystals are likely to be

organized into chains of interacting particles which may be up to a few μm in length. For optimal compass and intensity reception, two chain lengths (60 and 20 particles respectively) should be present. The decay with warming of the IRM acquired by the dermethmoid tissue of the yellowfin tuna indicates that the particle groups are at least partly free to rotate. These results are consistent with the use of a mechanoreceptor that detects the position or movement of the chains in magnetoreception. Based on the magnetite-based magnetoreception hypothesis developed above and assuming optimal use of the 8.5×10^7 particles detected in the tissue, it can be shown that there could be 1000 compass and 4.247×10^6 intensity receptors in the dermethmoid tissue. The smallest change in magnetic field intensity ($\Delta B/B$) that could theoretically be detected with this arrangement is 0.00049 or about 25 nT. Allowing reasonable integration times this sensitivity could be improved to 10 nT or less.

The theoretical analysis above (III.3) yields three testable behavioral predictions (Kirschvink and Walker in review). The first is that the magnetic sense organ should comprise separate receptor systems for detecting direction and intensity of the geomagnetic field. The second is that the accuracy of the compass response should be quantitatively defined by the Langevin function (Figure 3.2; Kirschvink 1981a), that is, the accuracy of the compass

response should increase asymptotically with external field strength. The third prediction is that the threshold sensitivity to changes in magnetic field intensity will be defined by the first derivative of the equation for the r.m.s. deviation of the crystals' alignment in the intensity receptors. Plotted against external field strength the sensitivity should increase to a maximum at about 50 μT and decline monotonically thereafter (Figure 3.3; Kirschvink and Walker in review). These predictions are testable with currently available behavioral conditioning procedures.

The first prediction is testable using the unitary conditioning procedure developed in Chapter II. A factorial design is suggested in which separate experiments test for independent responses to magnetic field direction and intensity. The tests would require subjects to discriminate between magnetic fields with different vector directions but the same total intensity, and between fields with the same vector direction but different total intensities. The second prediction is testable using the procedure developed for testing sun-compass orientation by Hasler et al. (1958). The accuracy of a directional response should increase asymptotically with magnetic field strength and should conform to the Langevin function (Figure 3.2). To test the third prediction suitable unitary or choice conditioning procedures must be adapted to test for the smallest changes

in magnetic field intensity the subjects can detect. This threshold sensitivity to changes in intensity should vary with field strength in the manner shown in Figure 3.3.

Although explicit tests of the magnetite-based magnetoreception hypothesis have not been conducted for fishes, some indirect evidence for the hypothesis is available. Quinn et al. (1981) state that the magnetoreceptor of the sockeye salmon must be able to function in the dark, in salt- and in freshwater, in the absence of water flow, and be evolutionarily adaptable to magnetic field reversals. Magnetoreception in the sockeye salmon must apparently also occur without the use of such highly sensitive electroreceptors as the ampullae of Lorenzini of elasmobranch fishes (Quinn et al. 1981). Behavioral observations on the effects of electrical fields on magnetoreception in the yellowfin tuna (II.4) are, like the constraints established by Quinn et al. (1981), compatible with the magnetite-based magnetoreception hypothesis. However, magnetic induction can not yet be excluded as a possible magnetic field transduction mechanism because all the repeatable behavioral responses by animals to magnetic fields involve at least some movement by the animals (Wiltschko 1972, Phillips 1977, Quinn 1980).

An interesting outcome of the magnetite-based magnetoreception hypothesis is its ability to explain at least some of the effects of externally placed magnets or coils on the responses of homing pigeons (Keeton 1972, Walcott and Green 1974). A strong external field applied to the head of a pigeon will align all the magnetite crystals within the head of the bird. Because the position of the experimental field source is constant relative to the body of the bird, the alignment of the particles relative to the body of the bird will be constant, regardless of the direction the bird faces or flies. The compass information the crystals provide the bird will therefore be useless, and the compass response should be abolished. If the intensity of the applied field matches that of the geomagnetic field but reverses its vector direction the compass response should not be abolished but will cause the birds to take up vanishing bearings 180° away from the vanishing bearings of control birds.

The intensity response will not necessarily be abolished by external magnets or coils. The experimental field will cause a change in the value of the magnetic to thermal energies (γ) of magnetite-based magnetoreceptor organelles. The thermally driven variance of the orientation of the particle groups about $\vec{B}$ would consequently change, reducing the sensitivity of the receptors if their moments

are optimal for use in the geomagnetic field. A damped response of the intensity receptors to changes in the geomagnetic field would be expected in this case. Since the experimental portion of the total field available to the bird is constant, regardless of the orientation of the bird, it is also possible that the bird could "see through" the experimental field to monitor total intensity of the geomagnetic field as well as detecting changes in the total field perceived. Thus, where access to alternative directional cues is not restricted, the ability of homing pigeons to return to their loft need not necessarily be destroyed by the application of strong magnetic fields to their heads. A corollary of this analysis is that the damping effect of the experimental fields on sensitivity to small changes in total intensity should be related to the intensities of the experimental fields.

Two outcomes of the work with yellowfin tuna suggest that the biomineralization of the magnetite crystals is very important in their use in magnetoreception. An important implication of the biophysical analysis (III.3) is that the size, shape, and arrangement of the magnetite crystals within the hypothesized magnetoreceptor organelles will have been subject to selection and will be under close genetic control. In different species, this should lead to convergence on receptors of similar magnetic moments, even

though the magnetite crystals within the receptors can be of different sizes and chain lengths in different species. More important is the observation that particle size should be similar among individuals within species. Because freely growing crystals will take up log-normal size frequency distributions, the control over particle size must be achieved during the formation of the crystals themselves.

A second important outcome from the work with the tuna is demonstration of the need to test for contaminants at all stages of the research and for consistency of the results obtained using different techniques. This presupposes the ability to distinguish contaminants from true biochemical precipitates using the full range of techniques available. Although biogenic origin of deposits in magnetic tissues can be reasonably inferred from the bulk magnetic properties, extraction is necessary for definitive demonstration that magnetite found in tissues is indeed biogenic. The property of the magnetite crystals extracted from the yellowfin tuna, and also from the bacteria (Towe and Moench 1981, Matsuda et al. 1983), that sets them apart from their geologic and synthetic counterparts is their non-octahedral crystal form, a property not essential to the magnetite-based magnetoreception hypothesis. The observation that biogenic magnetites have distinctive crystal morphologies suggests that the key to distinguishing biogenic magnetite from other

magnetites lies in the process of magnetite biomineralization. In this vein, the purity of the magnetite extracted from the tuna suggests a biological origin. An even more distinctive test could be comparison of the oxygen isotope ratio of biogenic magnetite with those found in geologic and synthetic magnetites (Lowenstam and Weiner 1983, see below).

The process of biomineralization of magnetite in the yellowfin tuna must be considered in the light of other biomineralization processes. A spectrum of biomineralization processes have been identified by Lowenstam and Weiner (1983). One end member of the spectrum, termed "biologically induced mineralization", arises from the interaction of biologically produced metabolites and external cations. At the opposite end of the spectrum is the process termed "matrix-mediated biomineralization", in which minerals are deposited in preformed organic structural frameworks (Lowenstam and Weiner 1983). Soluble acidic proteins appear to be responsible for crystal nucleation and growth in matrix-mediated biomineralization (Weiner et al. 1983). The organic framework is usually formed before deposition of the mineral (Towe and Lowenstam 1967) and will even take place independently in the absence of the mineral precursors (Balkwill et al. 1980). These observations imply genetic

control of the shape of the matrix and so of the size and shape of the crystals.

Minerals formed under matrix-mediated control often exhibit a disequilibrium between their isotope ratios and those found in minerals formed in the external environment (Lowenstam 1981). The isotope disequilibria occur as a result of the participation of the different isotopes of an element at different rates in the chemical reactions leading to biological mineral formation. Thus the ratio of ^{16}O to ^{18}O in biogenic magnetite may be significantly different from those found in geologic or synthetic magnetites. Oxygen isotope disequilibria may therefore be a useful diagnostic tool for distinguishing biologic from non-biologic magnetites in the absence of the cells or tissues from which the biogenic forms came (see below).

Matrix-mediated biomineralization in the bacteria dates from at least the early Precambrian. It is significant that two of the three matrix-mediated biominerals known from that time are iron minerals (Lowenstam and Weiner 1983). In the metazoa matrix-mediated biomineralization is recognized earliest in the calcium minerals used to form skeletal structures. The calcium mineralization processes appear similar in different phyla, although it is unknown whether the process is similar to that occurring in the bacteria (Weiner et al. 1983). Similarity of biomineralization

processes led Lowenstam and Weiner (1983) to two alternative interpretations of the origin of matrix-mediated biomineralization in the metazoa. Either matrix-mediated biomineralization appeared independently in the late Precambrian, after the divergence of the metazoan phyla, or it dates from a long but as yet undetected history in the Precambrian (Lowenstam and Weiner 1983).

Magnetite, presumably formed by matrix-mediated biomineralization has now been identified in phylogenetically distant metazoan groups. From arguments similar to those advanced by Lowenstam and Weiner (1983) two interpretations of the appearance of magnetite in metazoan groups can be offered. Either biogenic magnetite arose in the metazoa early in the Precambrian, predating the differentiation of the metazoan phyla, or it arose from multiple independent origins in the late Precambrian, after the divergence of the metazoan phyla. If it becomes possible to distinguish metazoan from bacterial and geologic magnetites on the basis of physical or chemical properties, it may become possible to resolve this problem of interpretation by identifying biogenic magnetites with clear metazoan origins from before the late Precambrian.

Lowenstam and Weiner (1983) suggest that the ability of bacteria to produce magnetite evolved in the early

Precambrian as a means of iron storage in the reducing environment of that time. In bacteria possessing magnetite crystals, the ability to perform directed swimming responses may have provided selective advantages for magnetotaxis even before the advent of an oxygen-rich environment. In the metazoa, it seems necessary to postulate selection operating on an association between magnetite crystals and sensory nerves as the likely origin of magnetite-based magnetoreception. This assumes that magnetite played some other, prior role in the bodies of metazoan organisms (Kirschvink and Gould 1981).

Although the dermethmoid tissue of the yellowfin tuna was the only tissue that was magnetic in all fish examined, other tissues sometimes showed either high signal-to-noise ratios or intensities of magnetization (Table III.I). Some of these probably arose from the presence of contaminants. However, the possibility that some of them were magnetic because they contained true biochemical precipitates can not be excluded. The lack of consistent localization of these deposits in the yellowfin tuna and other vertebrates (Presti and Pettigrew 1980, Kirshvink et al. 1982) argues against their use in magnetoreception. Study of these deposits and the conditions under which they form may elucidate the early functions of magnetite in the metazoa.

The magnetometric techniques developed so far permit characterization of the bulk properties of concentrations of magnetite in organisms. It is also possible to conduct analyses of polycrystalline aggregates and isolated crystals of magnetite extracted from magnetic tissues. An important conclusion arising from the work is that, although it is relatively easy to detect the presence of magnetic material in an organism, it is far more difficult to determine its origin and what, if anything, it does.

Similar arguments apply to the ferric iron detected in histological sections of the tissues from the skull sinuses of the yellowfin tuna. Although all these tissues appeared to be hemopoietic (see Chapter IV), the dermethmoid tissue took up far more stain than the other tissues. It is therefore possible that part of the iron-staining material in this tissue is unrelated to the production of blood but is involved in the storage of iron prior to precipitation of magnetite, or in the hardening of bones (Bassett et al. 1974). Like the magnetometry data, these results illustrate the need for hypotheses that predict the characteristics of biologically formed magnetic minerals and the need for techniques that will distinguish them from other magnetic and non-magnetic iron compounds.

In summary, my attempts to obtain empirical support for the magnetite-based magnetoreception hypothesis permit

several conclusions and indicate likely productive areas for further research. The yellowfin tuna produce interacting single-domain grains of magnetite in tissue contained within the dermethmoid bone of the skull. This material is ideal for use in magnetoreception and is almost identical in bulk magnetic properties to that found in other pelagic fishes (Walker et al. in review). The size, shape, and composition of the particles is closely controlled. Consideration of the magnetite biomineralization process suggests that metazoa may have been producing magnetite since before the phyla diverged in the late Precambrian. These conclusions correlate with the similarity of behavioral responses to magnetic fields in the different metazoan phyla, raising the possibility that magnetoreception developed as or before the phyla diverged. These conclusions do not prove the case for magnetite-based magnetoreception. They do, however, provide a consistent background that lends plausibility to the hypothesis.

CHAPTER IV

NEURAL BASIS FOR MAGNETIC SENSITIVITY

IV.1 INTRODUCTION

The conditioning experiments reported in Chapter II permitted the important inference that the behavioral responses to magnetic fields by yellowfin tuna are neurally mediated. In addition, there is direct and indirect evidence from these and other behavioral experiments that is compatible with the existence of magnetite-based magnetoreceptor organelles in both vertebrates and invertebrates. A hypothesis that the responses to magnetic fields demonstrated in the yellowfin tuna are based on the crystals of magnetite found in the dermethmoid tissue therefore seems reasonable. The results of experiments testing for a physical basis for magnetic sensitivity permit the predictions that magnetite-based magnetoreceptor organelles in the yellowfin tuna will be based on crystal chains that are 1-3 μm in length and at least partly free to rotate. Such receptors would transform magnetic fields into mechanical stimuli for transduction to the nervous system. The following paragraphs examine reports of the responses of the nervous system to magnetic fields and present arguments suggesting why the neural basis for magnetoreception has not been identified by such an

approach. An examination of the magnetite-based magnetoreception hypothesis then suggests a number of reasons why magnetite-based magnetoreceptors will be very difficult to detect using conventional histological techniques.

Attempts to make recordings of neural responses to magnetic field stimuli have sought to record from receptors or organs which do not appear to be specialized for magnetoreception. Although responses to magnetic field stimuli have been obtained in these experiments, the results suggest that the sensory systems tested would not normally mediate responses to magnetic fields.

Changes in electrical activity of single cells in response to changes in magnetic fields have been detected in the pineal of the guinea pig. By summing the number of action potentials produced by the individual cells over 256 1 second time blocks, Semm et al. (1980) were able to show depression of activity in the cells when they presented a 50 μT magnetic field stimulus. Responses of the cells to reversed polarity fields and cessation of magnetic field stimuli varied between cells. However, the latency of response to changes in external fields was always at least two minutes. This is too long to explain behavioral responses by yellowfin tuna and the other species that

respond rapidly to magnetic fields (II.3, Walcott and Green 1974). Thus, although the pineal cells in the guinea pig responded to earth-strength magnetic field stimuli, they are unlikely to mediate behavioral responses to magnetic fields.

Neural responses to magnetic field stimuli have been recorded from the electroreceptor system of an elasmobranch, the Black Sea skate (Trygon pastinaca). Fields changing at a minimum rate of 2 Gauss/sec. and movement of the fish or the water through constant fields of at least 8.5 Gauss caused responses by individual neurons in the anterior lateral line nerve and in the acousticolateralis region of the dorsal medulla oblongata (Andrianov et al. 1974, Brown and Ilyinsky 1978). The voltages induced by the stimuli used ranged between 0.04 and 40 μV. They therefore fell within the range of sensitivity of ampullary electroreceptor cells (Clusin and Bennett 1979a). However, the minimum rate of change of magnetic fields necessary to stimulate single units (2 Gauss/sec.; Andrianov et al. 1974) is many orders of magnitude greater than the changes in electrical fields generated by the animal swimming through the magnetic field of the earth. The static fields used by Brown and Ilyinsky (1978) were 15-30 times the intensity of the geomagnetic field and produced transient action potential activity. By 40 seconds after stimulus onset the afferent nerve activity had returned to baseline levels. Thus, demonstration of

responses by elasmobranch electroreceptors to magnetic fields required either rates of change of fields or movement of the fish or water through magnetic fields that were far greater than the animal could encounter in the ocean.

Failure of known sensory systems to respond to appropriate magnetic field stimuli suggests that a separate magnetoreceptor system must exist if behavioral responses by animals to magnetic fields are to be explained. Although magnetoreception hypotheses have suggested many different receptors, no systematic search for the receptors predicted by the hypotheses has been reported. Such a search is essential if necessary conditions for the existence of a true magnetic sense are to be satisfied.

New receptor systems have been discovered in one of several ways: (1) discovery of some structure whose function is unknown; (2) by chance while seeking to make recordings from other receptor systems; and (3) by search for the neural basis for previously demonstrated behavioral responses to known stimuli (Bullock 1974). The amount of biogenic magnetite required for magnetite-based magnetoreception could not have been detected until the development of superconducting magnetometers and the techniques for their use with biological samples. The hypothesized magnetoreceptor organelles require no large accessory structures to function and will fit into a very

small volume. In neurophysiological experiments both the external magnetic field and the preparation are usually static. Under such conditions the activity of individual magnetite-based magnetoreceptors will be random as the orientation of particle groups continually changes under the influence of thermal agitation. After integration, the signal from magnetite-based magnetoreceptors is likely to be relatively stable in an immobilized preparation. Thus magnetite-based magnetoreceptors are unlikely to have been discovered under the first two scenarios above; a specific search for the neural basis for magnetoreceptor organelles is therefore necessary.

The information available from the geomagnetic field, the likely use made of it by animals, and the magnetite-based magnetoreception hypothesis suggest that very little neural circuitry will be required for detection of magnetic fields. The geomagnetic field presents only a limited amount of information to animals. Features of the field that might be used in orientation by animals include the inclination, declination, total intensity and gradients in the total intensity of the field. It is highly unlikely that spatial variations in the geomagnetic field could be detected across the body of the animal. Magnetoreceptors will therefore detect the same features of the field no matter where they occur in the body of the organism. Assuming a sensitivity to

magnetic field changes of 1-10 nT, an organism will have to travel 500-5000 m to detect the systematic latitudinal changes in total magnetic field intensity. Although sensitivity to magnetic field intensity gradients may be higher, it is clear that magnetic field information reaching magnetoreceptors varies only slowly in time as the animal moves about its environment.

The total number of receptors involved in magnetite-based magnetoreception will depend on the arrangement of the crystals in their interacting groups. If the magnetite-based magnetoreception hypothesis holds, it can be assumed for the purposes of calculation that the particles are likely to be arranged in groups or chains best suited for responses to intensity and direction of the geomagnetic field. Under these conditions, optimal use of the 8.5×10^7 crystals in the dermethmoid tissue of the yellowfin tuna sets a limit of 3 to 5×10^6 receptors. The signal arising from each individual receptor will provide a sample from a random distribution of the orientations of magnetite crystal groups. Determination of the direction and intensity of the geomagnetic field requires sampling from multiple receptors and across time. From these properties it can be inferred that much of the integration of magnetic field direction and intensity signals coming from magnetite-based magnetoreceptors can occur at the receptor level.

Thus nerve fibers arising from magnetite-based magnetoreceptor organelles are likely to be few in number and may not be required to transmit information rapidly. It is therefore possible that nerve fibers involved in magnetoreception are not myelinated and nerve tracts not large enough to have attracted immediate attention in the past. Nor would they necessarily have been detected easily had they been specifically sought, because no site of magnetoreception could be identified. The consistent localization of magnetite in the dermethmoid tissue identifies a likely site and provides a clear focus for a search for the neural basis for magnetoreception in the yellowfin tuna.

Magnetite crystal groups suitable for use in magnetoreception will at best be barely detectable in light microscopy using iron staining techniques. Although it may be possible to stain the magnetite crystals and nerve fibers simultaneously, the small size of the crystal groups will make them very difficult to detect, particularly against a background of silver stain used to detect nerve fibers. In initial studies the most efficient approach for demonstrating magnetoreceptor organelles is therefore to demonstrate the presence of magnetite and neural tissue at the same site. An attempt to demonstrate histologically the presence of magnetite in the dermethmoid tissue of the

yellowfin tuna has been reported in Chapter III. The experiments reported below set out to identify nerves that might be responsible for carrying magnetic field information and to demonstrate the presence of nerve axons in the dermethmoid tissue of the yellowfin tuna.

IV.2 METHODS

IV.2.1 Dissections

To determine what nerves might be responsible for magnetoreception in the yellowfin tuna I conducted a series of dissections of the dermethmoid region. Skin and muscles over the anterior skull were removed. Beginning at the dermethmoid bone, bones were then picked apart, taking care not to disturb nerves lying beneath or within them. When nerves were detected they were traced out to identify them as fully as possible.

IV.2.2 Histological studies

Two live yellowfin tuna held at the Kewalo Research Facility were exsanguinated and fixed by transcardiac perfusion with Alcohol:Formalin:Acetic acid fixative solution. The ethmoid regions of their skulls were quickly dissected out, the dermethmoid cavity pierced to allow entry

of fixative, and the samples placed in alcoholic Bouin's solution. After decalcification in the Bouin's solution over several weeks, the samples were subdivided and embedded according to the following procedure: dehydration for three hours in three changes of 95% ethanol, followed by two hours in three changes of absolute ethanol; 1 1/2 hours in three changes of xylene, followed by three hours in three changes of Paraplast. The samples were then embedded in Paraplast and sectioned at 12 μm.

Sections were mounted on cleaned microscope slides coated with Mayer's albumen. To improve adhesion the dry sections were warmed, covered with a sheet of hardened filter paper, and pressed onto a slide using a printer's roller. This procedure reduced loss of sections during the long incubation times at 37° C necessary for the nerve staining procedure used.

The Holmes (1943) silver staining procedure as modified by Sobkowicz et al. (1973) to improve delipidation was used to test for the presence of neural tissue in the dermethmoid tissue sections. Sections were deparaffinized in xylene and passed through absolute ethanol to a mixture of equal parts by volume of absolute ethanol and chloroform. The slides were then passed through three changes of chloroform over 1 1/2 hours and returned to the ethanol/chloroform mixture for

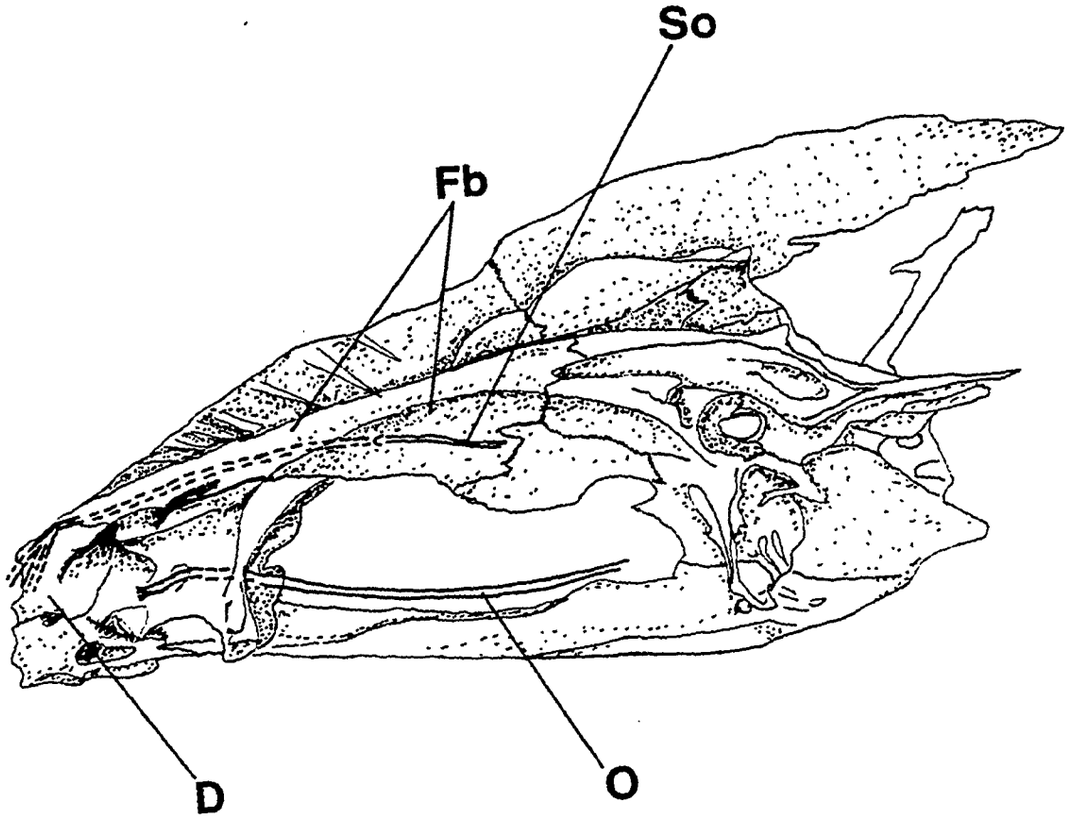
four hours. Following delipidation in the chloroform treatments, the sections were hydrated to water, immersed for two hours in the dark in a 20% solution of silver nitrate, rinsed in three changes of distilled water, and placed in the impregnating solution overnight at 37° C. After impregnation, the sections were reduced in hydroquinone, toned in 0.5% gold chloride solution, and the silver stain developed in 2% oxalic acid solution. Fixation was in 5% sodium thiosulphate solution, after which the sections were washed, dehydrated through absolute ethanol, cleared in xylene and mounted in Eukitt.

IV.3 RESULTS

IV.3.1 Dissections

The gross dissections of the anterior skull of the yellowfin tuna revealed two major nerves, the olfactory and supraophthalmic trunks, in the ethmoid region. The olfactory nerve courses craniad from the olfactory bulb in the brain to the olfactory rosettes, which are situated laterad from the parethmoid bones (Figure 4.1). The olfactory nerve bundle runs in the midline below the eyes and divides immediately posterior to the ventrolateral corner of the parethmoid bones before entering the olfactory capsule. The terminal nerve is presumed to be associated with the

Figure 4.1. Approximate courses of the olfactory and supraophthalmic trunk nerves in the region of the frontal, dermethmoid, and parethmoid bones of *Thunnus sp.*. Figure modified from Gibbs and Collette (1971).


So
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olfactory nerve (Demski and Northcutt 1983) although it was not specifically identified in the dissections.

The supraophthalmic trunk nerve carries branches of the trigeminal, facial, and anterior lateral line nerves. The trunk courses craniad from roots in the brainstem, passing over the orbit and along a lateral canal formed in the frontal bone (Figure 4.1). Although small branches leave the trunk at several points above the eye, no major branching occurs until the region of the dermethmoid bone. At the junction of the nasal, parethmoid, and dermethmoid bones the nerve divides into three or four branches which course ventrolaterad and anteriad. Uncertainty about the exact number of branches arises from the difficulty of dissection in the area where branching occurs. The junction of the frontal, ethmoid, and nasal bones forms the socket for the olfactory capsule and the seat for attachment of ligaments from the maxillary bone, which is involved in the protrusion of the upper jaw. Consequently, the area contains tendons and, in one area, heavily pigmented tissue. The area was very difficult to dissect cleanly, and branches of the nerve were often detected in some dissections but not in others.

In two dissections it appeared that a branch from the supraophthalmic trunk ran from the junction of the frontal and ethmoid bones into the dermethmoid tissue. The "branch" could not, however, be traced out to any specific parts of

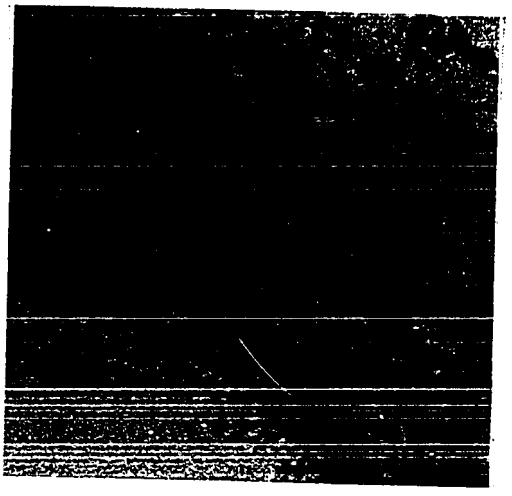
the tissue, and could not be found in any other fish. Thus it can not be concluded with any certainty that any of the nerves identified in the dissections do innervate the dermethmoid tissue.

In the dissections, the dermethmoid tissue was found to be well vascularized and, except in fish that were known to have starved, contained large amounts of fat. Other, similar sinuses in the parethmoid bones were continuous with the dermethmoid sinus and contained very similar tissue. The only difference between these and the dermethmoid tissues was their color. The parethmoid tissues were generally white, whereas the dermethmoid tissue tended to be pink or light red. These tissues in the sinuses within the skull of the yellowfin tuna appeared superficially similar to bone marrow. However, such marrow is unknown for teleost fishes although it has been reported for ganoid fishes (Andrew 1965).

IV.3.2 Histological studies

Of several silver stains tested, the Holmes technique provided the most reliable staining of the dermethmoid tissue. Most of the tissue contained fat and a large number of small blood vessels (Figure 4.2). This is consistent with a hemopoietic function of the tissue from this and other

Figure 4.2 A. Dorsolateral region of the dermethmoid tissue of the yellowfin tuna. Dorsal is at the top of the frame (X 120). B. Mid-ventral region of the dermethmoid tissue showing collagenous and reticular fibers (X 300). C. Lateral edge of dermethmoid tissue showing red blood cells in a capillary, fat cells, surface epithelium, and associated connective fibers (X 1000). Abbreviations used: cf: collagenous fibers; fc: fat cell; rbc: red blood cell; rf: reticular fibers; se: surface epithelium.



skull sinuses (Andrew 1965). The tissue also contained abundant connective tissue fibers which usually took up at least some of the stain. The presence of such fibers complicated the search for nerves and made necessary the adoption of criteria for distinguishing between neural and connective fibers in the dermethmoid tissue.

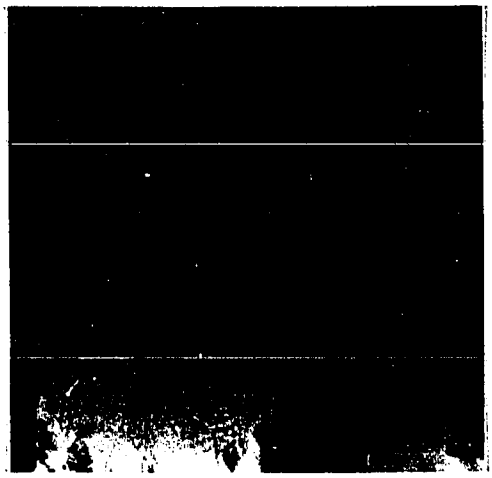
Bloom and Fawcett (1968) described three types of connective tissue fibers. Collagenous fibers are strands 1-12 μm in thickness and of indefinite length. Collagen takes up eosin in hematoxylin-eosin staining and is tinted tan to brown in silver stains (Bloom and Fawcett 1968). Elastic fibers are difficult to identify and do not stain with silver. They are smaller and less variable in size than collagenous fibers and form networks in tissues where they occur. Reticular fibers form networks around adipose cells and support the endothelium of capillaries and the endoneurium of nerves. Key features of reticular fibers are that they form the fibrous support tissue in hemopoietic organs and stain more intensely with silver methods than do typical collagenous fibers (Bloom and Fawcett 1968). If the dermethmoid tissue is hemopoietic, reticular fibers are very likely to be abundant in the dermethmoid tissue and, in searching there for nerves, it will be particularly important to distinguish between them and any nerve axons present.

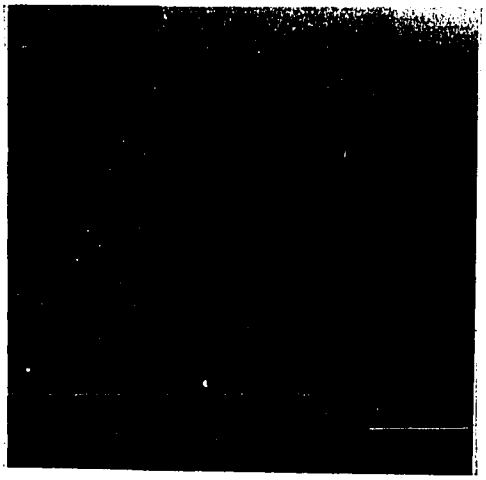
As noted above, operation of magnetite-based magnetoreceptors requires no large accessory structures, relatively little neural circuitry, and very little space. Therefore, the hypothesized receptors are unlikely to be dispersed throughout the dermethmoid tissue but are more likely to be localized within a small portion of the tissue. Differentiation of tissue associated with the receptors is to be expected, particularly if secondary receptors such as hair cells are responsible for stimulus transduction. At the individual fiber level it will be difficult to distinguish between reticular and nerve fibers. However, it should be possible to identify nerves from their intensity of staining, branching patterns, and aggregation into bundles coursing towards the central nervous system. Consequently, the following criteria for identifying nerve fibers in the dermethmoid tissue were adopted: (1) staining of the fibers should be intense compared with other fibrous material in the same sections; (2) fibers should be present in some organized form in tissue that could be differentiated for sensory function; and (3) at some point fibers should coalesce into larger fibers and bundles that leave the dermethmoid tissue. Obviously the first criterion is relative and will be most applicable to sections from the same slide and, to a lesser extent, to slides from the same staining batch.

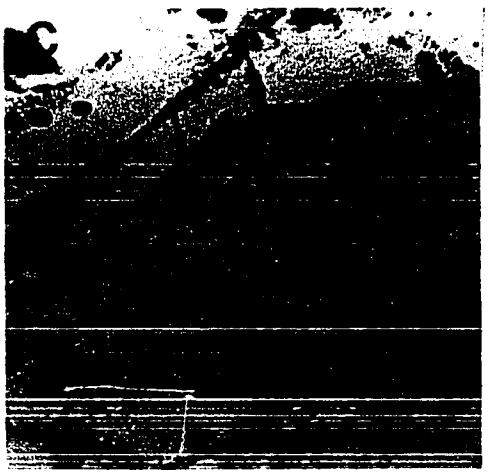
Despite numerous attempts it was not possible to obtain definitive identification of nerve axons within the dermethmoid tissue. Collagenous fibers in the tissue were only moderately stained and many exhibited the wavy course commonly seen in such fibers when not under tension (Bloom and Fawcett 1968). Reticular fibers were also evident. The fibers branched irregularly and could be found throughout the dermethmoid and other skull sinus tissues (Figure 4.2 A). They formed networks around the fat cells, around blood vessels, and at the basement of the epithelium bounding the lateral and ventral surfaces of the dermethmoid tissue (Figure 4.2 B, C).

Intensely staining fibers that may have been nerve axons were located at the dorsolateral surface of the dermethmoid tissue. The tissue containing the fibers did not contain the abundant fat cells and small blood vessels observed elsewhere in the dermethmoid tissue. Instead, the fibers ramified extensively throughout a small area of the tissue (Figure 4.3). The fibers stained more intensely and had greater diameters than the clearly identifiable reticular fibers in the same sections (Figure 4.3). Because the sectioning procedure often caused damage to the tissue in this region the space occupied by the fibers and the tissue in which they were found may be greater than appeared in the sections.

Figure 4.3 A. Isolated intensely staining fibers (arrow) located at the dorsal surface of the dermethmoid tissue (X 300). B. Intensely staining fibers (arrow) ramifying in tissue laterad from the fibers shown in A (X 300). C. Apparent coalescence of the intensely staining fibers into bundles (arrow) oriented toward the dorsolateral corner of the dermethmoid tissue (X 300).







Evidence was found for aggregation of the fibers into bundles. The isolated fibers shown in Figure 4.3 A were located in the anterior medial portion of the dermethmoid tissue. In sections from the same slide these fibers were concentrated into relatively small bundles (Figure 4.3 C) situated laterad and caudad from the highly branched fibers shown in Figure 4.3 B. The bundled fibers were oriented toward the junction of the dermethmoid, frontal, and nasal bones. Further laterad and caudad I located what could have been a bundle of the fibers in section. This bundle was associated with a small blood vessel which was known from dissections to exit the dermethmoid tissue at its posterior, dorsolateral corner. Unfortunately, because of damage to sections the bundle associated with the blood vessel and the fibers associated with the dorsal surface of the dermethmoid tissue could not be shown to be linked.

The fibers and the tissue in which they were found were closely associated with the dorsal dermethmoid bone. Despite an extensive search, comparable areas could not be found at the ventral and lateral surfaces of the bone, which generally contained large concentrations of connective tissue fibers and none of the intensely staining fibers (Figure 4.2 A-C). However, because the intensely staining fibers could only be detected when sectioning separated them from the bone, their relationship with the bone is

uncertain. Further study will be necessary to determine whether or not the fibers and the associated tissue are involved in osteogenesis or in neural activity.

IV.4 DISCUSSION

The results reported here represent a preliminary investigation into the hypothesized neural basis of magnetite-based magnetoreception. Fibers in the dermethmoid tissue of the yellowfin tuna stained intensely in the Holmes silver technique and may have been neural tissue. However, the fibers could not be identified as nerve axons and no association between the fibers and the magnetite crystals was established. In the following discussion it is therefore important to consider other possible means by which magnetoreception might occur.

Several pieces of evidence support the tentative identification of fibers at the dorsal surface of the dermethmoid tissue as nerve axons. The first is the relatively intense staining of the fibers when compared with the staining of clearly identifiable collagenous and reticular fibers in the same sections (Figures 4.2 A, 4.3 C). Because both the reticular fibers and the intensely staining fibers can be demonstrated together, comparison of intensity of staining as an aid to distinguishing neural

from non-neural fibers seems valid. The second piece of evidence is the presence of the fibers only in a small portion of the dermethmoid tissue rather than being dispersed throughout the whole tissue. The fibers ramify throughout the part of the dorsal surface but do not enter the fatty material making up most of the dermethmoid tissue (Figure 4.2 A, 4.3 B). Finally, the fibers coalesce into a bundle, which may later become associated with a blood vessel that exits the dermethmoid complex in the region where major branching of the supraophthalmic nerve occurs.

Against this evidence for the presence of nerves must be weighed the absence of unequivocal evidence for a nerve entering and ramifying in a portion of the dermethmoid tissue that is clearly differentiated for sensory function. This is due partly to the close association of the tissue containing the fibers with the dorsal dermethmoid bone, and partly to the difficulty of dissecting in the ethmoid region. If the nerve fibers are not myelinated they will be difficult to detect against the background of fatty tissue in dissections. Use of a decalcification method less damaging to membranous tissue than Bouin's solution, and sectioning the tissue from different angles may assist in obtaining good preparations in further histological studies. It will also be necessary to conduct specific tests to exclude other possible explanations for the role of the

tissue and the fibers. Thus, until more substantial evidence comes to light, it can not be concluded that the dermethmoid tissue contains nerve axons.

The dissections in the region of the ethmoid bones identified one sensory and two mixed nerves that could potentially supply the neural basis for magnetoreception. Mechanoreceptors are associated with all three nerve systems. For a variety of reasons I consider the anterior lateral line nerve most likely to be involved in magnetite-based magnetoreception in the dermethmoid tissue of the yellowfin tuna. The magnetite crystals present in the dermethmoid tissue are organized into interacting groups which were predicted to be 1-3 μm in length. These magnetic units (sensu Yorke 1981) are at least partly free to rotate. For magnetoreception, the important properties of the crystal groups are their mean alignment and the variance about their mean alignment with the geomagnetic field. Receptor cells must therefore be capable of accurately monitoring the position and movement of the crystal arrays. The membrane of a primary mechanoreceptor cell is unlikely to be able to accommodate freely rotating groups of such large dimensions (Kirschvink and Gould 1981). It is also difficult to conceive how other types of primary mechanoreceptors, such as those associated with the trigeminal and facial nerve systems, could accommodate the

crystal groups while allowing them the freedom to rotate. Some other receptor configuration therefore seems necessary.

On rotation through 90° in response to the geomagnetic field, a 1 μm magnetite crystal array will describe an arc approximately 0.8 μm in length. The displacement response curve produced by *in vitro* micromanipulation of the tips of hair bundles in the sacculus of the bullfrog has an apparent width of about 1 μm (Hudspeth and Corey 1977). Thus the range of movement of the tips of hair cell bundles, the sensitivity of such cells to forces of the same order as the background thermal energy, kT (Hudspeth 1983), and the consistent association of hair cells in the acousticolateralis system with structures transforming weak environmental stimuli to mechanical stimuli all suggest that they could provide ideal receptors for use in magnetite-based magnetoreception. If so, the involvement of the acousticolateralis system in magnetoreception would be consistent with its involvement in other forms of spatial orientation in fishes (e.g. Lissman 1958, Kalmijn 1971, Schuijf and Hawkins 1983).

In conclusion, the failure to demonstrate unequivocally the presence of nerves inside the dermethmoid tissue forces consideration of alternative hypotheses. One possibility is that the crystals are merely produced within the dermethmoid tissue and then moved elsewhere to participate in

magnetoreception. The amount of magnetite required to provide sensitivity sufficient to enable the fish to distinguish between the magnetic fields used in the discrimination learning experiments is far less than the fish actually produce. In addition, magnetoreception will not necessarily be affected by the site where magnetoreception occurs in the body of the organism. Thus the dispersed receptor system based on muscle spindles suggested by Presti and Pettigrew (1980) is feasible and would not necessarily be detectable in a superconducting magnetometer. Therefore the hypothesis that the magnetite is merely produced in the dermethmoid tissue and transported elsewhere to participate in magnetoreception in the yellowfin tuna can not be excluded.

Two observations suggest that a dispersed receptor system is unlikely. The first is the effect on orientation of magnets or electromagnetic coils placed on the heads of homing pigeons. Little distortion of magnetic fields occurs outside the region of the head in such experiments yet the effect of the experimental fields is marked (Keeton 1972, Walcott and Green 1974). Second, the sample of yellowfin tuna white muscle digested in the extraction experiments yielded no detectable magnetic particles, despite its large size. The hypothesized association of magnetite particles with muscle spindles or other mechanoreceptors commonly

associated with muscles therefore lacks corroborative evidence at this time.

The second alternative hypothesis is that the magnetite crystals are not involved in magnetoreception at all. Although it is not confirmed from previous reports, a likely function of the dermethmoid and other tissues in sinuses in the skull of the yellowfin tuna is blood formation (Andrew 1965). Since other major hemopoietic tissues, such as the spleen, were not magnetic (unpublished data), the selective precipitation of magnetite crystals in the dermethmoid tissue of the yellowfin tuna appears unrelated to the production of blood. Deposits of magnetite apparently unsuited for magnetoreception appear to occur only irregularly in the tissues where they are found (Presti and Pettigrew 1980). Because magnetite is always present in the dermethmoid tissue of the yellowfin tuna, it is unlikely to have a solely pathologic origin or to be used solely as an iron dump (Baker et al. 1983, Lowenstam and Weiner 1983).

These hypotheses concerning the role of the magnetite crystals can not yet be excluded. However, none provide convincing explanations of the selective deposition of magnetite crystals in the dermethmoid tissue of the yellowfin tuna. The failure to obtain definite identification of nerves in the dermethmoid tissue of the

yellowfin tuna in this study should therefore not be sufficient reason to reject the hypothesis that magnetite-based magnetoreception occurs within the dermethmoid tissue of the yellowfin tuna. Considerable further research will be necessary to determine how the magnetite crystals may be linked to the nervous system.

CHAPTER V

DISCUSSION

V.1 The magnetic sensory mechanism

Four criteria for determining whether an environmental cue can be used for navigation by pelagic fishes were suggested in Chapter I. The geomagnetic field easily met criteria concerning stimulus variation over long and short distances that could be used in navigation. However, evidence that pelagic fishes could and did detect the geomagnetic field was lacking. This research addressed the third criterion for use of the geomagnetic field in navigation by pelagic fishes: that they are sufficiently sensitive to magnetic field stimuli to be able to derive the navigational information available from the geomagnetic field. Evidence for the existence of a magnetic sense and a physical basis for a magnetic sense organ in the yellowfin tuna was obtained. The discussion that follows describes important problems raised by the results that are not addressed in previous chapters and which must be resolved if the third criterion is to be met. Of particular importance is the mechanism by which the geomagnetic field is detected. The electrical induction and magnetite-based magnetoreception hypotheses are compared and experimental tests to distinguish between them described. Finally, the

available literature is surveyed for correlative and experimental evidence that pelagic fishes respond to magnetic field stimuli in the open ocean.

This research was conducted in three discrete areas and sought to satisfy three necessary conditions for the existence of a magnetic sense and sense organ in the yellowfin tuna. These conditions were (1) the existence of reproducible behavioral responses to magnetic fields; (2) a suitable physical basis for the responses; and (3) an association between the physical basis and neural elements of a magnetic sense organ. The first of these conditions was satisfied and a potentially ideal physical basis for the behavioral responses was identified and analyzed. However, no evidence for links between the physical basis and the necessary neural elements of a sense organ could be demonstrated. It will be necessary in future studies to show that the magnetite crystals are linked to receptor cells, that the receptor cells transmit magnetic field information to the central nervous system, and that the animals respond to the stimuli with appropriate behavior.

Proving that the magnetite crystals are linked to receptor cells is perhaps the most difficult experimental problem arising from this research. It can easily be shown that a saturation remanence of 10^4 pAm^2 can be produced by

as little as 20 parts per billion of magnetite in a 1 cm^3 sample. The size and organization of the magnetite crystals make their detection in situ and any association they may have with receptor cells very unlikely in conventional histological studies. The scarcity of magnetite and abundance of other forms of ferric iron in the dermethmoid tissue of the yellowfin tuna make a search for iron using scanning electron microscopy impractical, and not sufficiently specific to identify the magnetite fraction (Kuterbach et al. 1982). A combination of methods therefore seems necessary to increase the chances of detecting the crystals using transmission electron microscopy (TEM).

Magnetite crystals are very electron dense. Their morphology and organization predicted from the magnetometry experiments should make them easily recognizable in situ in TEM. However, the problem of low concentration of the magnetite crystals in the dermethmoid tissue of the yellowfin tuna is compounded by the very small volume of tissue sampled in TEM sections. A possible means of increasing the chances of detecting the crystals is to prepare 1 mm^3 tissue samples for TEM studies and, following fixation, measure their saturation moments in a superconducting magnetometer. Magnetic samples could then be sectioned exhaustively. The risk with this approach is that cells would be lysed by the freezing and thawing associated

with measurement of the tissue IRM in the magnetometer. This risk is outweighed by the likely return of information from detection of the crystals in any association with cellular material.

Once an association between the magnetite crystals and receptor cells has been identified, determination of the higher projections of the magnetoreceptor system should eventually be amenable to conventional histological techniques. Demonstration of the higher projections should make it possible to use neurophysiological techniques to demonstrate transmission of magnetic field information from the receptors to the central nervous system.

It is as yet unknown what is an appropriate stimulus to present and what nervous system responses to magnetic field stimuli will be. Two general approaches to the study of neural responses to stimuli are possible, and the choice between them depends on the experimental situation. The first approach involves the use of coarse stimuli and a search for crude responses. The second is to present stimuli comparable to those available to the subject in the course of its normal activity and then to record responses of single units to the stimuli. Averaged evoked potentials and multi-unit activity in response to coarse stimuli are easy to record compared with single unit activity, especially when the site of activity is not precisely defined (Bullock

et al. 1982). The approach most likely to yield results in first attempts to record neural responses to magnetic field stimuli is therefore to record evoked responses in higher brain centers, or multi-unit activity in afferent nerves carrying magnetic field information.

It is likely to be some time before the neurophysiological experiments described above can be carried out. Behavioral experiments therefore seem more likely to test the hypothesis that there is a ferromagnetic basis for magnetoreception in the near future. Such tests will depend on development of robust conditioning procedures. In the interim, more evidence has accumulated for the magnetite-based magnetoreception hypothesis than for most other magnetic field transduction hypotheses--an ideal physical basis for the sense exists, and there is both direct and indirect behavioral evidence in species from different taxa for a ferromagnetic basis for magnetoreception (Kirschvink 1981a, Quinn et al. 1981). Receptors that behave in the fashion required by the hypothesis are known to exist, but no receptors linked to the physical elements of the hypothesized sense organ have been identified.

The only magnetic field transduction hypothesis for which receptors that behave in the fashion required by the

hypothesis have been identified is the electrical induction hypothesis of Kalmijn (1974). Difficulties with the hypothesis have been cited at several points in previous chapters. In the following discussion elasmobranch receptor operation is briefly described. The potential utility of geoelectrical fields in navigation by pelagic fishes is then evaluated using the four criteria suggested in Chapter I. Based on the properties of the electroreceptor cells I argue that the electroreceptor system of elasmobranchs is very unlikely to be used for magnetoreception. I then devise tests to distinguish between the electrical induction and magnetite-based magnetoreception hypotheses.

The electroreceptors of elasmobranchs are tonic, low frequency receptors located in the ampullae of Lorenzini at the end of long canals. The canals are filled with a highly conductive jelly and are distributed around the head. The electroreceptor system comprises an epithelium containing a single layer of receptor and supporting cells within the blind ampullary swelling at the end of each canal. The supporting cells generate a transepithelial potential that is short circuited by the jelly filling the ampullary lumen. The potential causes a small inward current carried by calcium ions to flow continuously across the lumenal surfaces of the receptor cells (Bennett and Clusin 1978). Superimposed on this continuous current flow are small

oscillations caused by the successive depolarization and repolarization of the membranes of the receptor cells. The oscillations appear to be generated by the action of the inward current and fast, voltage-sensitive or calcium-activated outward potassium currents in both the basal and lumenal membranes of the receptor cells (Clusin and Bennett 1979a, b). When a small, steady current is applied across the ampullary epithelium, the transepithelial voltage exponentially approaches a new potential that is proportional to the applied current. The transepithelial voltage and current continue to show oscillations about their new levels.

The oscillations in the transepithelial current are the basis for electroreception. They are responsible for tonic activity of the receptor cells and cause tonic post-synaptic potential and impulse activity in the associated afferent nerves. Small excitatory and inhibitory voltage stimuli (as low as 1-5 μ V) increase and decrease the mean current flow across the epithelium and damp, but do not suppress, the oscillations (Clusin and Bennett 1979a). Post-synaptic responses of the afferent nerves reflect the different rates of transmitter release by receptor cells at the different transepithelial current levels.

Thus currents flowing in the receptor cells act to maintain the transepithelial potential at a steady mean

value that depends on the background electrical field. Superimposed oscillations in the current flow then mediate electroreception. This mechanism keeps the receptor cells poised near threshold over a wide range of background electrical fields. However, there are limits to the range over which accommodation can occur. The oscillations of the receptor cells are suppressed by stimuli greater than 300-500 μV (Clusin and Bennett 1979a). Clusin and Bennett (1979b) suggest that such suppression occurs by direct deactivation of outward potassium conductances in the basal membranes of the receptor cells, leading to maintained depolarization and transmitter release.

The electroreceptor mechanism of elasmobranchs provides for maintained incremental sensitivity over a wide stimulus intensity range. Summation of voltage drop along the length of ampullary canals, and integration across multiple receptors can presumably lead to the 10-15 nV/cm threshold sensitivities demonstrated in behavioral and electrophysiological experiments (Bullock and Corwin 1979, Kalmijn 1982).

The basis for magnetic field detection by electrical induction is the movement of a conductor through a static magnetic field. A marine elasmobranch swimming at 1 m/s in the geomagnetic field will generate electrical field

gradients of up to 500 nV/cm in its ampullary canals (Kalmijn 1974). The stimulus intensity will be at a maximum when the fish is moving in the east-west direction, and determination of direction using electrical induction appears relatively simple (Kalmijn 1974). Determination of the total intensity of the geomagnetic field is also feasible using electrical induction, making determination of position using geomagnetic field intensity theoretically possible (Kalmijn 1978).

Although the geomagnetic field is relatively static, water masses are not. Unique determination of position and direction using electrical field stimuli is therefore complicated by the variable velocities of both the fish and the water mass in which it swims (Tesch 1980). For example, an elasmobranch fish swimming in still water at 1 m/s in the magnetic east-west direction in Hawaiian latitudes will generate electrical field gradients of approximately 400 nV/cm. If the fish turns through 90°, the portion of the total electrical field due to the horizontal component of the geomagnetic field will vanish, giving a net electrical field of about 300 nV/cm. The net field will vary as the cosine of the angle between the swimming direction of the fish and magnetic east or west. In the open ocean, movement of the water mass in which the fish swims will contribute to the available electric field stimuli. Determining direction

using electrical fields may not be impossible as the total electrical field will reflect the net direction of movement of the fish. Thus the fish merely needs to determine the directions of greatest and least electric fields to determine magnetic east and north respectively. In the example above, a shift in swimming direction of 5^{o} away from east-west will lead to a 4 nV/cm change in electrical field gradient, which is close to the behaviorally measured threshold sensitivity to electrical field stimuli for elasmobranchs (Kalmijn 1982). The fish could therefore determine magnetic direction to within 5^{o} to 10^{o} using its electroreceptors.

Thus determination of direction would be possible using ampullary electroreceptors and induced electric fields. However, accurate determination of position is easily shown to be beyond the capacities of the electroreceptor system of elasmobranchs unless they possess an as yet undetected means of greatly increasing their sensitivity to induced electrical fields. In the example above, an elasmobranch swimming north will generate electrical field gradients of about 300 nV/cm in its electroreceptors. Assuming a systematic variation of 5 nT/km in total geomagnetic field intensity in the magnetic meridian, the systematic variations in electrical field gradient stimuli available to the fish will be about 3 fV/cm. Such stimuli are at least

six orders of magnitude smaller than the behaviorally measured threshold sensitivities of elasmobranchs to electrical fields (5-10 nV/cm; Kalmijn 1982). The fish could therefore detect its position in the magnetic meridian with an accuracy of about ± 100 km.

From the studies of the map sense of pigeons, a sensitivity to changes in total geomagnetic field intensity of ± 10 nT has been inferred (Gould 1980, 1982a). Position in the magnetic meridian can therefore be fixed to within 1-2 km. Thus, to approach this ability to fix position using their ampullary electroreceptors, the elasmobranchs would have to have some means of greatly increasing their sensitivity to electrical field stimuli. However, the systematic variations in geoelectrical fields in the immediate environment of the fish are likely to be swamped by local variations in electrical fields. Such local variations would arise from variable water and fish velocities and could cause the local electrical field variations to be at least one to two orders of magnitude above the measured threshold sensitivities (Kalmijn 1982). It is therefore unlikely that electrical field stimuli could be used as a navigational cue because the fish are probably not sufficiently sensitive to detect the systematic variations in the stimuli in their immediate environment

against the much greater background variations in the stimuli.

The responses of ampullary electroreceptor cells argue against their use in magnetoreception. Sources of electrical fields in the immediate environment of the fish include fields induced by the movements of the fish and the water in which it swims, fields arising from other organisms, and miscellaneous fields arising from a variety of sources (Kalmijn 1974). As noted above, the currents flowing in the cells vary according to the level of maintained electrical field stimuli, thus permitting detection of small, varying stimuli against the larger background. For example, Brown and Ilyinsky (1978) showed that Black Sea skate electroreceptors fail to respond after 20 seconds to electrical fields induced in water flowing at 24 cm/sec through an 8.5 Gauss magnetic field. Electroreceptor cells are evidently tuned to respond not to static or steady electrical fields produced from movement of the fish or the water through the geomagnetic field, but to the low frequency, low amplitude electric fields put out by other organisms in the immediate vicinity of the fish.

So far magnetoreception by electrical induction has only been considered in connection with the elasmobranch fishes. Evidence from a variety of sources almost completely excludes induction as a means of magnetoreception in teleost

fish such as the yellowfin tuna. In species from six teleost orders, Bullock et al. (1982) failed to find evidence for electroreception using averaged evoked potentials to record neural responses to electrical field stimuli. These observations are correlated with the absence of the octavolateral nuclei responsible for electroreception in the brains of most teleosts (Bullock et al. 1982) and of the large ampullary canals required for electroreception in salt water. Finally, behavioral observations are compatible with magnetite-based magnetoreception but not with the use of electrical fields in magnetoreception in the yellowfin tuna and sockeye salmon (Chapter II, Quinn et al. 1981).

Despite this body of evidence against the use of electrical induction by elasmobranchs and other fishes to detect the geomagnetic field, the existence of both conditioned responses to magnetic fields and potentially suitable receptors in the elasmobranchs (Kalmijn 1978) have brought the hypothesis considerable acceptance (Gould et al. 1978, Walcott et al. 1979, Jungerman and Rosenblum 1980, Kalmijn 1982). It is therefore necessary to devise explicit tests that will distinguish between the electrical induction and magnetite-based magnetoreception hypotheses.

A possible approach to determining whether organisms detect the geomagnetic field directly or by induction

involves testing counterpredictions of the induction and ferromagnetic magnetoreception hypotheses. From the first derivative of the Langevin variance it was predicted that sensitivity to magnetic field intensity changes will be maximum in earth-strength fields in magnetite-based magnetoreception (Figure 3.3; III.7). The threshold sensitivity in earth-strength fields will be much less than 1 μT and may be as low as 1-100 nT (III.3, Kirschvink and Gould 1981, Yorke 1981).

The electrical fields available to elasmobranch fishes will depend on their own swimming velocity and direction in still water. At a mean velocity of 1 m/s an elasmobranch will generate near threshold electrical field gradients of 10 nV/cm in a 1 μT magnetic field. The fish should therefore be able to detect changes of 1 μT in a background field ranging from a few μT to at least 1 mT. Thus the basic distinction between the two hypotheses is that the magnetite-based magnetoreception hypothesis predicts high sensitivity to magnetic field intensity changes over a narrow range of fields whereas the electrical induction hypothesis predicts relatively low sensitivity maintained over a wide range of magnetic fields. Testing these counterpredictions will depend on the development of conditioning techniques that permit testing for threshold sensitivity to magnetic field intensity changes.

The second means for excluding the electrical induction hypothesis involves separating magnetic from electric field stimuli. Electrical fields associated with magnetic fields could be masked by presenting either a static field sufficiently large to saturate elasmobranch electroreceptors or randomly varying electrical fields that will scramble any electrical field information provided by magnetic fields. The fish could then be tested for conditioned responses to magnetic fields in the presence of these electrical field stimuli.

Successful conditioning in such masking experiments would only show that electrical field information is not essential to magnetoreception. The test of counterpredictions of the two hypotheses suggested above would show that electrical field information either is or is not used when it is available. However, the masking experiments could be conducted with the testing procedure used to demonstrate behavioral responses to magnetic fields in the yellowfin tuna and may therefore be the more tractable of the two approaches for some time.

V.2. Responses by pelagic fishes to magnetic fields in the open sea

There is a serious lack of evidence for use of the geomagnetic field by pelagic fishes in the open sea. This is attributed to the relatively recent initiation of analytical studies of the movements of individual fish at sea and to the absence of experiments testing for involvement of the geomagnetic field in orientation. An early report on the movements of skipjack tuna observed that the fish appeared to move from island to island in the Izu and Ogasawara island chains (Kawasaki and Asano 1962). A parsimonious explanation for this and other, similar observations is that the association between tuna schools and island groups is entirely passive. That is, the fish move from food source to food source, forming aggregations in areas where food is locally abundant, as indeed it is around island chains (Gilmarten and Revelante 1974). It is worth noting, however, that the submarine ridges associated with island chains will have distinct magnetic field signatures. Fish with the requisite sensitivity to magnetic field variations could conceivably associate the magnetic anomalies along submarine ridges with localized food sources. Moving along such ridges could reasonably be expected to bring the fish to such food concentrations, much as aggregation at localized temperature

fronts brings albacore into forage-rich areas around coastal upwelling off California (Laurs et al. 1977).

This tentative hypothesis can be supported by observations on the movements of schools of skipjack tuna approaching the New Zealand fishery. Habib (1978) reported that skipjack entering the New Zealand summer fishery for the first time were first observed in surface schools over the Kermadec, Lord Howe, Norfolk, and Reinga ridges to the north of New Zealand. The schools moved rapidly southwards along the ridges and apparently did not feed until they reached the area of the fishery (Habib personal communication 1981). The implication from these observations is that the association between the fish and submarine ridges is not dependent on the presence of food concentrations in the immediate vicinity of the fish.

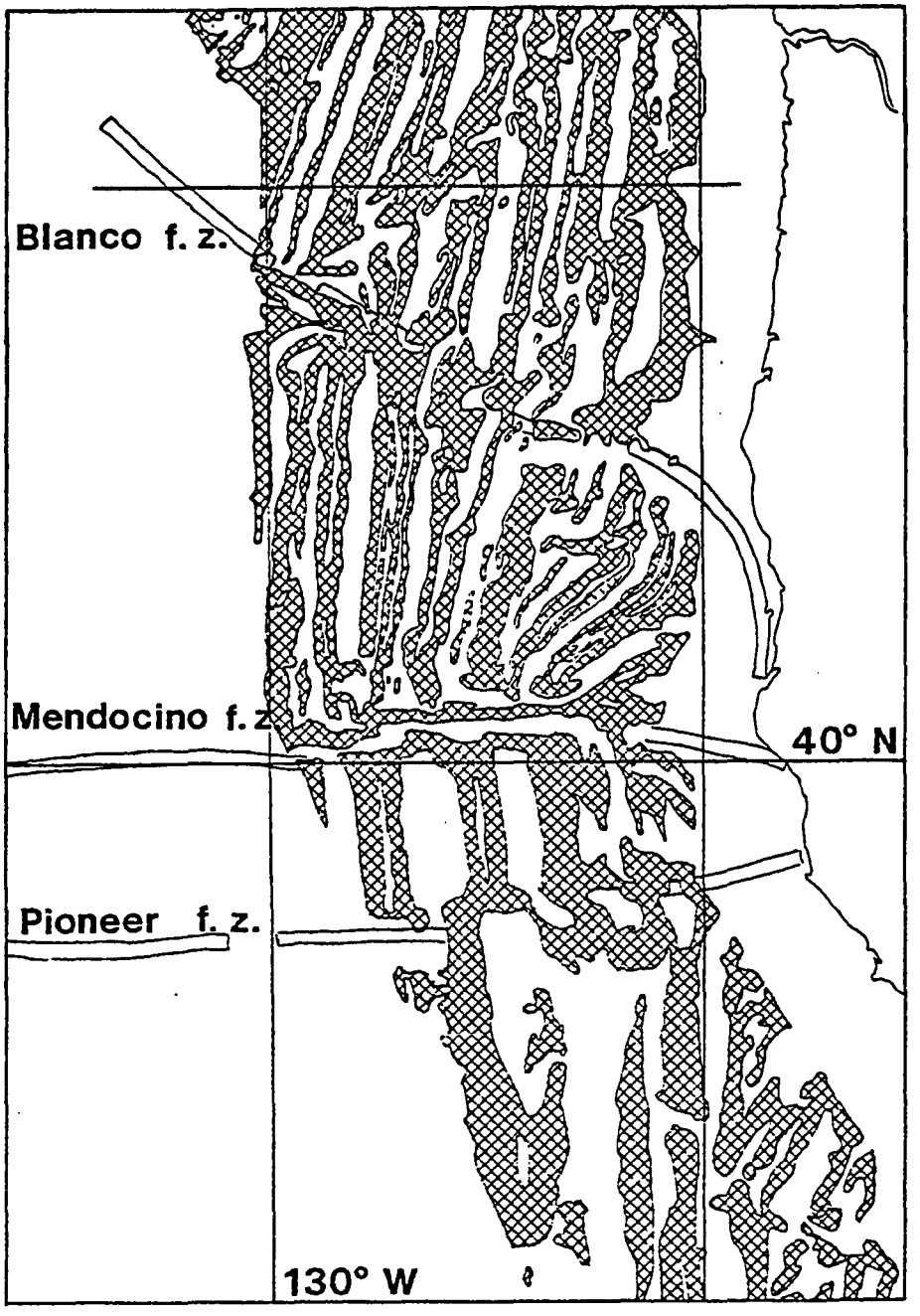
In contrast, albacore tuna respond to the Mendocino fracture zone as if it were a barrier to north-south movements, although habitat and forage are adequate on both sides of the fault. Albacore are found both to the north and south of the fracture zone but rarely cross it. Laurs (1979) reported that, in the year of tagging, only 0.8% of recoveries of fish tagged directly to the north of the fracture were made to the south of it and only 0.4% of recoveries of animals tagged on the south side were made to the north. During subsequent years, after migration by the

fish to the central or western Pacific and back, crossover recoveries were 6.5% and 4.5% for fish tagged to the north and south of the fracture respectively (Laurs 1979).

The Mendocino fracture zone extends westward from Cape Mendocino for a distance of several thousand kilometers. The slope of the scarp averages about 7-10° but can be as much as 18-24° (Menard and Dietz 1952). The seafloor to the south of the scarp is about 750 m deeper than that to the north and the water depth over the fault is never less than 2000 meters. Although the scarp will affect oceanographic conditions at the surface, these are not likely to provide obstacles to such highly migratory fish as albacore.

The Mendocino fracture zone is an extensive transform fault. Instead of the predominantly north-south pattern, which is maintained across other fault systems in the area, magnetic lineations arising from sea floor spreading form an extended band running east-west at the Mendocino fracture (Figure 5.1). An albacore tuna approaching the fracture from the north or the south would therefore encounter changing magnetic fields. If these fish do interpret magnetic lineations as submarine ridges, and follow them until they find food, the change in the magnetic lineation pattern at the Mendocino fracture zone would prevent the fish from passing the fault, but would lead them either to the east or

Figure 5.1. Magnetic lineations off the west coast of North America. Positive anomalies are indicated by cross-hatched areas. Note the extended positive anomalies running east-west at the Mendocino fracture zone. Magnetic anomaly patterns are obtained from proton precession magnetometer measurements of the total intensity by removal of a geomagnetic reference field and adjustment to an arbitrary baseline. Figure traced from U. S. Department of Commerce, NOAA, National Ocean Survey Chart 9000 M: Magnetic lineations in the Pacific Ocean (1971).


Blanco f. z.
Mendocino f. z.
40° N
Pioneer f. z.
130° W

west. The hypothesis that the fish will travel with the magnetic grain could therefore explain the low frequency of recoveries of fish on the opposite side of the fault from which they were tagged. The weaknesses in this hypothesis are (1) that it is almost certainly too simplistic to explain what the responses of the fish to the fault actually are, and (2) that following magnetic lineations can not explain the very long east-west migrations that the albacore make each year.

Although it is still correlative, more detailed information on the possible use of the geomagnetic field for map and compass navigation comes from tracking experiments with individual fish. A variety of experiments have shown that different pelagic fishes make diurnal horizontal movements onto and off shallow banks where they feed (Yuen 1970, Sciarotta and Nelson 1977, Carey and Robison 1981). The skipjack tracked by Yuen (1970) made 25-106 km nocturnal excursions from Kaula bank in several different directions, returning to the bank to feed at about the same time each morning. These movements led Yuen (1970) to suggest that the fish knew where it was relative to the bank and possessed the ability to navigate.

Several studies have shown that pelagic fish can maintain compass courses for extended periods. Atlantic salmon (Salmo salar) tracked by Smith et al. (1981)

maintained relatively steady compass headings for periods of up to 21 hours regardless of the flow of tidal currents. Similarly, a swordfish tracked by Carey and Robison (1981) maintained a steady compass course over a period of several days, during which it left the area of Cape Hatteras, crossed the Gulf Stream, and entered the Sargasso Sea. Although none of these tracking experiments provides direct evidence that the fish used the geomagnetic field in navigation, all the fish tracked apparently determined their position or course independently of topographical features of the sea floor, celestial cues, and water currents.

There is one published report of an attempt to test experimentally for geomagnetic orientation by pelagic fish. Westerberg (1982) fitted an Atlantic salmon with an electromagnetic coil that presented a field of 100 μT (= 1 Gauss) in a square wave with equal on and off intervals. The fish was then tracked and its turning behavior and swimming speeds monitored. The behavior of the fish was significantly correlated with the on- and offsets of the coil. The experiment did not provide clear evidence that magnetic or electric fields are used by migrating pelagic fish and is subject to other possible interpretations. However, it does show that experiments manipulating perceived magnetic fields can be carried out on free pelagic fishes. The possibility that pelagic fishes do indeed detect and use the geomagnetic

field in navigation is therefore worth serious experimental investigation. The key difficulty to be overcome in such experiments will be detecting the effect of experimental magnetic fields on orientation against a background of spontaneous changes in behavior when the likely destination of the fish and the effect of the experimental fields on orientation are unknown.

In summary, this research has produced a body of evidence that yellowfin tuna possess a functioning magnetic sense. The fish responded to magnetic fields in discrimination learning experiments and possess an ideal physical basis for such responses. They may also possess a suitable neural basis for magnetic sensitivity. This possible neural basis for magnetoreception requires considerable further study. Theoretical arguments described the operation of hypothetical magnetite-based magnetoreceptors and predicted that they could easily provide the fish with sufficient sensitivity to make geomagnetic navigation possible. During this research considerable effort was spent in development of methods in the two main areas of the study. I anticipate that, as interest in biogenic magnetite and the magnetite-based magnetoreception hypothesis grows, methods will be developed and refined during attempts to confirm the results obtained in this study.

APPENDIX

Physical constants, and the mathematical relations among them, used in magnetism and in development of the magnetite-based magnetoreception hypothesis.

Magnetic moment

1 electromagnetic unit (emu) = 10^{-3} Ampere.meter^2

For a grain of volume, V, (cm^3) and saturation magnetization Js (emu/cm^3), the moment μ = VJs.

1 emu = 1 erg/Gauss.

sIRM = $N.\mu/2$ where N is the number of particles of moment, μ, present in a sample.

Magnetic intensity and magnetization

1 Gauss = 1 emu/cm^3

= 10^3 Ampere/meter

= 10^{-4} Tesla.

Energy relations

1 erg = 1 emu.1 Gauss (or E = - .B).

kT = 4.14 x 10^{-14} erg where k is Boltzmann's constant (1.38 x 10^{-16} erg/^{o}K) and T is the absolute temperature (300^{o}K).

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