

PRODUCTION OF SINGLE-DOMAIN MAGNETITE THROUGHOUT LIFE BY SOCKEYE SALMON, *ONCORHYNCHUS NERKA*

BY MICHAEL M. WALKER^{1,*}, THOMAS P. QUINN^{2,†},
JOSEPH L. KIRSCHVINK¹ AND CORNELIS GROOT²

¹*Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA* and ²*Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada*

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Summary

Although single-domain particles of biogenic magnetite have been found in different species of pelagic fishes, nothing is known about when it is synthesized, or about whether the time during life when it is produced is correlated with the development of responses to magnetic field stimuli. We have investigated production of biogenic magnetite suitable for use in magnetoreception in different life stages of the sockeye salmon, *Oncorhynchus nerka* (Walbaum). Sockeye salmon were chosen because responses in orientation arenas to magnetic field stimuli have been demonstrated in both fry and smolt stages of this species.

We found significant quantities of single-domain magnetite in connective tissue from the ethmoid region of the skull of adult (4-year-old) sockeye salmon. The ontogenetic study revealed an orderly increase in the amount of magnetic material in the same region of the skull but not in other tissues of sockeye salmon fry, yearlings and smolts. The physical properties of this material closely matched those of magnetite particles extracted from the ethmoid tissue of the adult fish. We suggest that single-domain magnetite particles suitable for use in magnetoreception are produced throughout life in the ethmoid region of the skull in sockeye salmon. Based on theoretical calculations, we conclude that there are enough particles present in the skulls of the fry to mediate their responses to magnetic field direction. By the smolt stage, the amount of magnetite present in the front of the skull is sufficient to provide the fish with a magnetoreceptor capable of detecting small changes in the intensity of the geomagnetic field.

Other tissues of the salmon, such as the eye and skin, often contained ferromagnetic material, although the magnetizations of these tissues were usually

* Present address: Békésy Laboratory of Neurobiology, University of Hawaii, 1993 East-West Road, Honolulu, HI 96822, USA.

† Present address: Fisheries Research Institute, WH-10, University of Washington, Seattle, WA 98195, USA.

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more variable than in the ethmoid tissue. These deposits of unidentified magnetic material, some of which may be magnetite, appear almost exclusively in adults and so would not be useful in magnetoreception by young fish. We suggest that tissue from within the ethmoid region of the skull in pelagic fishes is the only site yet identified where magnetite suitable for use in magnetoreception is concentrated.

Introduction

In previous work, we have demonstrated the presence of large numbers of biochemically precipitated, single-domain particles of the magnetic mineral magnetite in tissue contained within the dermethmoid bone of the skull of yellowfin tuna, *Thunnus albacares*, and within the ethmoid region of the skull of adult chinook salmon, *Oncorhynchus tshawytscha* (Walker *et al.* 1984; Kirschvink *et al.* 1985). Their uniform size, shape and chemical composition, consistent anatomical position within and among species, and organization into chains, which could possess sufficient magnetic moment to align with the geomagnetic field against the randomizing effects of thermal buffeting (Kirschvink, 1983; Kirschvink & Walker, 1985), indicated that the particles were suitable for use in magnetoreception. These results are consistent with behavioural responses to magnetic field stimuli in the tuna (Walker, 1984) and also in juvenile sockeye salmon (Quinn, 1980; Quinn *et al.* 1981; Quinn & Brannon, 1982) but differ from two previous magnetometry studies that failed to detect magnetic material other than contaminants in either sockeye salmon fry (Quinn *et al.* 1981) or smolt-stage fish from a variety of salmonid species (Ueda *et al.* 1986).

In the experiments reported here, we attempted to elucidate further the role of the magnetite by describing the distribution and abundance of magnetic material in different tissues of sockeye salmon adults, smolts, yearlings and newly hatched fry. There have been reports that magnetite is produced during specific life stages (Gould *et al.* 1978; Jones & MacFadden, 1982) or that older animals are more magnetic than young ones (J. G. Mather, personal communication). Sockeye salmon were selected for study because experimental studies have indicated different responses to magnetic field direction in two different life stages, fry and smolts (Quinn, 1980; Quinn & Brannon, 1982). Thus, we sought evidence indicating whether the age and size of the fish when the particles are produced are correlated with the development of responses to magnetic field stimuli. We sought also to understand why the presence of magnetite was not demonstrated in the previous studies of juvenile salmon (Quinn *et al.* 1981; Ueda *et al.* 1986).

Materials and methods

Samples

We obtained heads from adult Fraser River sockeye salmon caught by purse seine in the coastal waters between Vancouver Island and the British Columbia mainland. We also obtained samp

yearlings and smolts, reared under controlled conditions at the Rosewall Creek Hatchery on Vancouver Island. Sockeye salmon eggs are fertilized in the autumn, and embryos hatch in winter but remain buried in the gravel until their yolk sacs have been absorbed (in spring). The fry sample consisted of fish (mean standard length = 2.5 ± 0.13 cm) from the Weaver Creek population (part of the Fraser River system) that had not begun to feed. The yearlings (mean standard length = 11.0 ± 0.86 cm) and smolts (mean standard length = 22.2 ± 2.89 cm) were from Fulton River, a tributary of Babine River in Central British Columbia. As yearlings, sockeye salmon normally feed in lakes, and the term smolt designates the stage when they migrate to the ocean. When sampled, the yearlings and smolts used here were about 13 and 25 months post-fertilization, respectively.

Procedure

Heads from five adult fish were dissected using glass knives and non-metallic tools in a magnetically shielded, dust- and magnetic-particle-free clean laboratory at the California Institute of Technology. The laboratory and procedures used for conducting non-magnetic dissections and avoiding contamination of the samples are described elsewhere (Kirschvink, 1983; Walker *et al.* 1984, 1985; Kirschvink *et al.* 1985). Tissue samples removed from each head were washed in glass-distilled water, cleaned ultrasonically, washed again, and then rapidly frozen in liquid nitrogen to prevent physical rotation of any magnetic particles present.

Prior to measurement each sample was demagnetized by an alternating field (Af) with an initial amplitude of 100 milliTesla (mT) decaying to zero over a period of 5 s. Each sample was then exposed to a series of unidirectional magnetic pulses of approximately 1 ms duration that increased progressively in intensity up to 1000 mT. The isothermal remanent magnetization (IRM) acquired after each magnetization step was measured in a superconducting (SQUID) moment magnetometer (Fuller *et al.* 1985). Each sample was then Af demagnetized in fields increasing in steps up to 100 mT and the moment retained after each demagnetization step was measured in the magnetometer. Tissues sampled in the adult fish included eye, brain, the olfactory rosette and attached olfactory nerve, ossified bone from the orbital region of the skull, cartilage from the parietal and ethmoid areas of the skull (Gorshkov *et al.* 1979), and connective tissue that occupied internal spaces within the cartilage of the ethmoid region of the skull. (Its superficial appearance, and location in the ethmoid region, suggested that the latter tissue was similar, although not anatomically identical, to magnetite-containing tissue from within the dermethmoid bone of the skull in the tuna.) One sample of the eye was also examined using the ARM modification of the Lowrie-Fuller test for single domains (King *et al.* 1982).

In addition to the samples of adult (probably 4-year-old) salmon caught at sea, we examined tissues sampled from the sockeye salmon yearlings and smolts. However, because of the small size of the yearlings, it was not possible to separate the ethmoid from the frontal or the parietal from the occipital areas of the skull. The skulls of these fish were divided simply into anterior and posterior sections.

Other samples taken from these fish included eye, skin, bone from the orbital region of the skull and, in the smolts, the ethmoid and parietal regions of the skull. In the fry, subdivision of the skulls and bodies of the fish was not possible. Consequently, only the eyes, skulls and caudal regions of the fry were measured. Because the moments acquired by the fry were weak, the heads and bodies of these fish were pooled and measured in groups of 10 or more individuals.

Results

We found inducible remanent magnetization in all tissues except the brains of the adult fish (Table 1). The saturated remanent moments permitted estimates of the amount and, after consideration of coercivity data (see below), of the number of particles of magnetic material in each sample, whereas the intensity of magnetization allowed us to determine whether the concentration of magnetic particles differed among tissue samples. There were two- to 82-fold and two- to 34-fold variations among individuals in saturated remanent moments and intensities, respectively, for the different tissues. Only in samples of the ethmoid tissue was the range of variation relatively low and the same (sixfold) for both measures of magnetization. A similar pattern was revealed by coefficients of variation in the saturated moments and intensities of magnetization calculated for the different tissues (Table 1). Although the coefficients of variation were often lower for other tissues than for the ethmoid tissue, only in the ethmoid tissue were the coefficients of variation the same for both measures of magnetization. These patterns suggested that there were similar amounts of magnetic material present in similar concentrations among individual fish in only the ethmoid tissue samples.

Acquisition and loss of remanence data provide information on the coercivity, and so on the identity and domain state, of magnetic particles present in a sample. These data are typically plotted as curves showing the spectrum of particle coercivities present in a given sample (e.g. Cisowski, 1981; Walker *et al.* 1984; Kirschvink *et al.* 1985; Stolz *et al.* 1986). In this study, four pieces of information were derived from the curves (Table 1): (1) an estimate of the average coercivity (H_{rc}) of the particles present; (2) an estimate of R , the ratio of the remanence at H_{rc} and the saturation remanence; (3) a measure of the variability among individuals in the coercivity spectra for each tissue; and (4) information from the form of the curves on the likely domain states of magnetic particles present in the different tissue samples.

The intersection point of the Af demagnetization and IRM acquisition curves provides an estimate of the average remanent coercivity (H_{rc}) of particles present in a sample (Cisowski, 1981). The presence of inter-particle interactions in the sample can be detected from R , the ratio of the remanence at H_{rc} to the saturation remanence. For isolated single domains, R will equal 0.5; when single domains interact significantly, R will fall below 0.5 (Cisowski, 1981). Variation in coercivity spectra among individuals for a given tissue sample is detected through increasing standard errors about any given point on the curves (for example, see Figs 1, 2). In

Table 1. Mean saturated remanent moment (with coefficient of variation expressed as a percentage in parentheses), mean intensity of magnetization (with coefficient of variation in parentheses), mean coercivity (Hrc), ratio (R) of remanence at Hrc and saturation remanence, and ratio of the estimated standard error and percentage magnetization at Hrc for tissues of sockeye salmon adults (A), smolts (B), yearlings (C) and fry (D)

	Saturated remanent moment (pAm ²)	Intensity of magnetization (pT)	Mean coercivity (Hrc) (mT)	Remanence at Hrc/saturated remanence (R)	Error at Hrc/% remanence at Hrc
A Adults					
Tissue					
Skin	1126 (44)	222 (95)	44	0.28	0.1
Orbital bone	1122 (53)	493 (131)	52	0.33	0.2
Eye	7441 (155)	176 (151)	28	0.33	0.3
Ethmoid cartilage	366 (43)	281 (77)	46	0.31	0.1
Ethmoid tissue	933 (74)	130 (75)	44	0.31	0.1
Parietal cartilage	594 (93)	52 (66)	49	0.33	0.2
Olfactory rosette	242 (28)	173 (44)	51	0.30	0.2
B Smolts					
Tissue					
Skin	123 (61)	156 (94)	41	0.29	0.2
Orbital bone	106 (21)	71 (48)	47	0.37	0.2
Eye	140 (79)	28 (53)	35	0.30	0.1
Ethmoid region	281 (34)	96 (31)	40	0.27	0.1
Posterior skull	269 (50)	33 (34)	40	0.28	0.1
C Yearlings					
Tissue					
Skin	103 (64)	322 (42)	*	*	*
Anterior skull	148 (44)	191 (42)	42	0.33	0.3
Posterior skull	312 (77)	141 (102)	24	0.26	0.2
D Fry					
Tissue					
Eye	5	*	24	0.30	*
Pooled skulls	3	*	42	0.33	*
Caudal regions	23	*	29	0.16	*

Remanent moments for the tissues from the fry were obtained by dividing the total moment by the total number of samples of tissue present in each pooled sample (one sample measured per tissue type).

* Samples were too weakly magnetized to permit estimation of the values of sample parameters.

Posterior skull in B and C corresponds to parietal cartilage in A. Ethmoid region in B and anterior skull in C correspond to the combined ethmoid tissue and cartilage of A.

an attempt to quantify the level of variation in coercivity spectra, we estimated the ratio of the variability and the percentage magnetization at the intersection point, H_{rc} , of the Af demagnetization and IRM acquisition curves. We first calculated the mean of the standard errors at the four points nearest the intersection point. This mean value was then divided by the percentage magnetization at the intersection to estimate a value for the ratio at H_{rc} for each sample type. Differences in the sizes and domain states of magnetic particles among tissues are determined from qualitative differences in the IRM acquisition and Af demagnetization curves. A sample in which there is a wide range of particle sizes will have a broad coercivity spectrum, which will be indicated by acquisition and loss of remanence over a wide range of fields. In contrast, a pure sample of uniformly sized particles will have a very narrow coercivity spectrum and will acquire and lose remanence over a narrow range of fields. For example, the curves for single-domain magnetite from magnetotactic bacteria rise and fall steeply in the region immediately around H_{rc} and are almost flat outside this region (Stolz *et al.* 1986).

All but one of the tissues had values of H_{rc} and R around 40–50 mT and 0.30–0.35, respectively (Table 1). These values are very similar to those found in the dermethmoid tissue of yellowfin tuna (Walker *et al.* 1984) and the ethmoid tissue of chinook salmon (Kirschvink *et al.* 1985) and are consistent with the presence of interacting single domains of magnetite in these tissue samples. The estimates of the ratio of the standard error and the percentage magnetization at

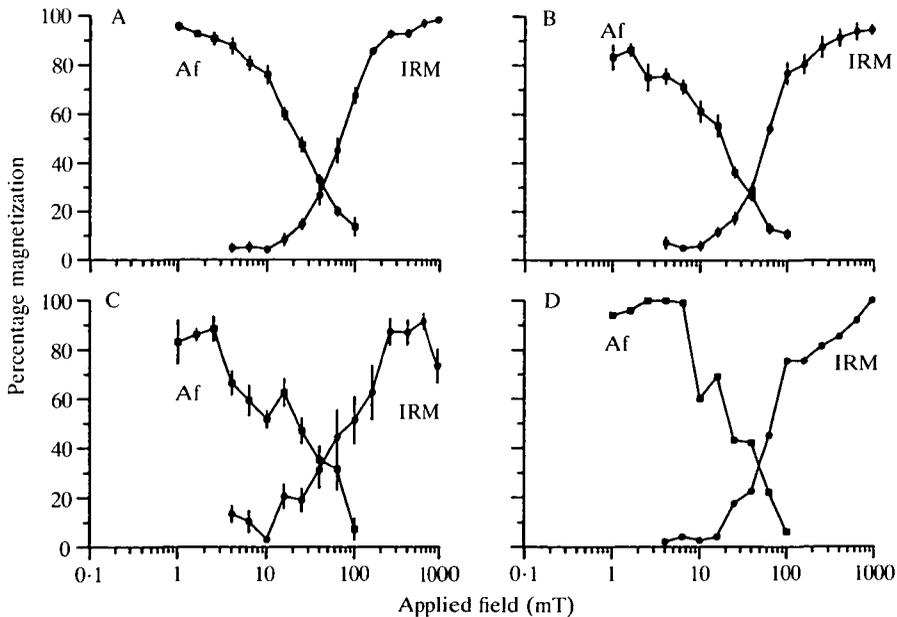


Fig. 1. IRM acquisition and Af demagnetization curves for the ethmoid tissues of sockeye salmon adults (A) and smolts (B), for the anterior skull of sockeye salmon yearlings (C) and for the skulls of sockeye salmon fry (D). (A–C) Mean values \pm S.E.M.

Hrc (Table 1) range from about 0.1 (for the skin, the ethmoid cartilage and the ethmoid tissue) to 0.3 (for the eye).

Among the samples taken from adult sockeye salmon, the narrowest and least variable coercivity spectrum is that shown for the ethmoid tissue in Fig. 1A. The ethmoid tissue acquired and lost little of its sIRM below 10 mT or above 100 mT. Consequently, the IRM acquisition and Af demagnetization curves both have pronounced 'shoulders' (Fig. 1A) like those seen in the magnetotactic bacteria (Stolz *et al.* 1986), yellowfin tuna (Walker *et al.* 1984) and chinook salmon (Kirschvink *et al.* 1985). As in the yellowfin tuna (Walker *et al.* 1984) and chinook salmon (Kirschvink *et al.* 1985), the variability in the coercivity spectra among individuals was low (see Fig. 1A; Table 1). The coercivity data are consistent with the presence of single-domain magnetite in the ethmoid tissue of the adult sockeye salmon. This interpretation has been confirmed by electron diffraction and lattice-imaging studies (Mann *et al.* 1988).

The broadest and most variable coercivity spectrum among the samples taken from adult sockeye salmon was that for the eye (Fig. 2A). The descending limb of the Af demagnetization curve for the eye is almost a straight line, indicating loss of remanence over the whole range of demagnetizing fields used (1–100 mT). The eye began to acquire measurable remanence (greater than the background noise in the magnetometer) by 5 mT and continued to acquire remanence up to 300 mT. The estimate of Hrc was 28 mT (Table 1), which was substantially lower than for

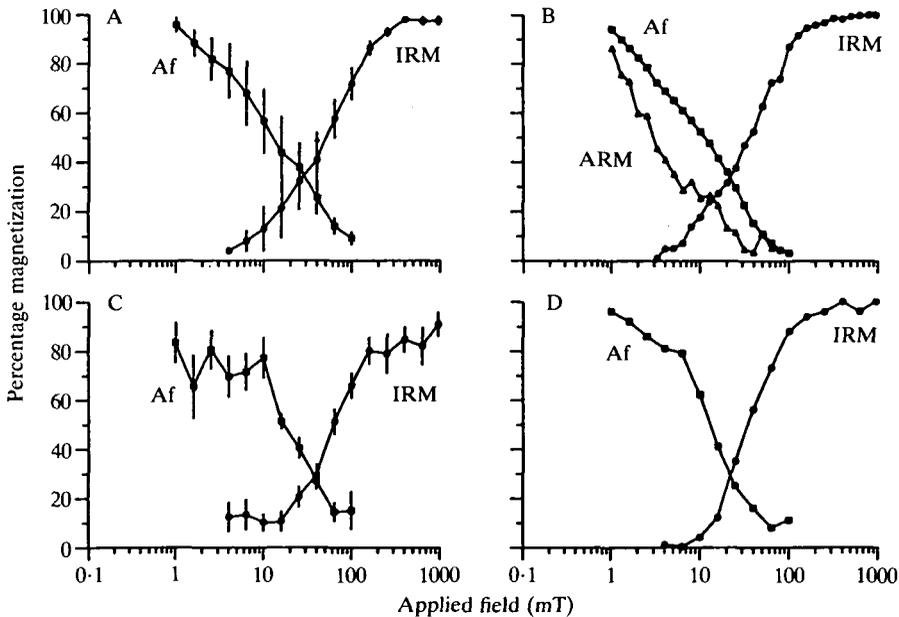


Fig. 2. IRM acquisition and Af demagnetization curves for the eyes of sockeye salmon adults (A), a single adult (B), yearlings (C) and fry (D). The curve for Af demagnetization of anhysteretic remanent magnetization (ARM) for the eye from the individual adult fish is shown in B. (A,C) Mean values \pm S.E.M.

the other samples. This result suggested the presence of magnetically soft, multidomain material, perhaps as contaminants that were not removed successfully by the ultrasonic cleaning given to all samples. The variability of the magnetic properties among individuals is shown by the high standard errors about the curves for the eye (Fig. 2A). The estimate of the ratio of the error and the mean percentage magnetization at H_{rc} for the eye was 0.3 (Table 1), the highest value of the ratio we obtained in the adult fish. The curves for IRM acquisition and Af demagnetization of the sIRM for the eye from one of the fish are shown in Fig. 2B. As in the pooled results, these curves indicated the presence of relatively low coercivity particles in the eye of this fish. We gave this sample an anhysteretic remanent magnetization (ARM) by Af demagnetizing it in the presence of a $200 \mu\text{T}$ ($= 2 \text{ Gauss}$) d.c.-bias field. The sample acquired a moment of $2 \times 10^{-9} \text{ Am}^2$, or one-thirtieth the sIRM ($6 \times 10^{-8} \text{ Am}^2$). The eye sample failed the Lowrie-Fuller (Lowrie & Fuller, 1971; King *et al.* 1982) test for the presence of single domains because the curve for Af demagnetization of the ARM fell well below rather than above the curve for the Af demagnetization of the sIRM (Fig. 2B).

Among the tissues from the adult sockeye not shown in Figs 1 or 2, the skin and the ethmoid cartilage also had coercivity spectra that were relatively narrow with low variability among individuals. As noted above, however, the variation in the total moments and intensities of magnetization did not suggest that these tissues contained similar concentrations of magnetic material in different fish (Table 1). The remaining tissues, the ossified bone and the cartilage from the orbital and parietal regions of the skull, respectively, and the olfactory rosette, acquired and lost remanence over a wide range of applied fields, and their coercivity spectra resembled the spectrum for the eye more than that for the ethmoid tissue.

Measurements of saturated remanent moment, intensity of magnetization and coercivity spectra, reported in Table 1B-D and Fig. 1B-D, showed that the heads of the juvenile sockeye salmon had magnetic properties similar to those found in the ethmoid tissue of the adults. There was only a two-fold range in mean peak moments and intensities among the ethmoid samples from the smolts and two- and threefold ranges in mean peak moments and intensities, respectively, from the anterior skulls of the yearlings. Coefficients of variation in the saturated remanent moments and intensities of magnetization for the anterior skulls of the yearlings and smolts were not only similar to each other but also among the lowest recorded within each age class. These results suggested that, as in the adults, there were similar concentrations of magnetic material in the anterior part of the skull of fish in each age class.

Determination of the median coercivity of magnetic material in tissue samples became increasingly difficult with decreasing age in the younger fish because the saturated remanent moments of their skulls were much lower than in the adult fish. Consequently, the background noise in the magnetometer made a greater contribution to the variation about the IRM acquisition and Af demagnetization curves, particularly for the yearling fish (Fig. 1C). The estimates of H_{rc} for the anterior skulls of the juvenile fish (Fig. 1B-D) all fell between 40 and 44 mT and

the estimates of R all were clustered around 0.30 (Table 1). The estimated errors at H_{rc} were also low, particularly for the smolts (Table 1). Taken together, these properties strongly suggested the presence in the juvenile fish of interacting single domains of magnetite. Although it was not possible to measure the ethmoids of the fry or the yearlings separately from other parts of the skulls, the consistency of their magnetic properties with those for the ethmoids of the smolts and adults strongly implied that the magnetic material present in the skulls of the fry and yearling fish was the magnetite ultimately found in the ethmoid tissues of the adults.

Many of the other samples from the juvenile fish were only weakly magnetized, if at all. In the yearlings, for example, the saturated remanent moments of the eyes were below the noise in the magnetometer, whereas those for the skin were greater than the noise but were not high enough to permit estimation of H_{rc} and R (Table 1). Where they could be made, estimates of H_{rc} in the juvenile fish ranged from 24 to 35 mT (eyes in the fry and smolts; Fig. 2C,D; Table 1) and from 24 to 40 mT (posterior skulls in the yearlings and smolts: Table 1). Estimates of R were between 0.16 (caudal regions in the fry) and 0.37 (orbital bone in the smolts), and the ratios of the standard error to percentage magnetization at H_{rc} were generally about 0.2 (Table 1).

Discussion

As well as describing the production of single-domain magnetite particles during life by sockeye salmon, our results investigate the development of the ferromagnetic hypothesis of magnetoreception and methods for testing its predictions. In the absence of anatomical or neurophysiological data, behavioural tests of the hypothesis provide the only means of demonstrating a connection between the magnetite particles and the nervous system.

As in tuna and chinook salmon, the ethmoid tissue in the adult sockeye salmon possessed magnetophysical properties consistent with the presence of large numbers of single domains of magnetite formed under close biochemical control of their size, shape and composition. Magnetic material extracted from the ethmoid tissue of the adult sockeye has been identified as single-domain magnetite (Mann *et al.* 1988). The particles occur in long chains that closely resemble the magnetosome from magnetotactic bacteria. This arrangement makes it possible for the particles to be used in magnetoreception because summation of their moments will give a net energy of interaction with the $50 \mu\text{T}$ geomagnetic field greater than the background thermal energy, kT . Our data also indicated the likely presence of magnetite in the skin and ethmoid cartilage of the adult sockeye salmon. The variability among individuals in the amount and concentration of the particles (Table 1) suggested that these tissues were not likely sites for a magnetoreceptor system (Kirschvink *et al.* 1985). In addition, the presence of magnetite in tissues other than the ethmoid tissue has yet to be confirmed by extraction of the material and analysis of its diffraction spectra.

The IRM acquisition and Af demagnetization characteristics of the ethmoid tissue in the adult sockeye salmon proved useful in demonstrating the probable presence of magnetite in the skulls of the juvenile fish. By pooling samples from many individuals when necessary, we obtained estimates of Hrc and R for the skulls of the fry, yearlings and smolts that were almost identical to those measured in the ethmoid tissue of the adult fish. It seems reasonable to conclude from these results that the magnetic particles detected in the skulls of the juvenile fish were magnetite. The data obtained make it difficult to draw conclusions concerning the nature of any magnetic material present in tissues outside the skull of the juvenile fish. Our data permit the suggestion, however, that single domains of magnetite suitable for use in magnetoreception are concentrated in the anterior skull of all the life stages of sockeye salmon examined.

Previous studies by Quinn *et al.* (1981) and Ueda *et al.* (1986) failed to identify single-domain magnetite in a variety of salmonid fishes. We attribute the differences between their results and ours to three features of our experiments: (1) use of a more sensitive instrument in a clean environment, (2) use of fixed or frozen samples, and (3) application of more advanced magnetic techniques to characterize the magnetic material detected in our samples. For example, the saturated remanent moments of many of the samples we measured, especially in the youngest fish (fry and yearlings), were below 200 pAm^2 and so would have been well below the 2000 pAm^2 noise level in the magnetometer used by Quinn *et al.* (1981). In addition, we have found that it is critical to fix or freeze tissue samples so that the moments of magnetic particles they contain will be aligned after magnetization. Otherwise, the orientations of magnetite particles suspended in a viscous medium will be randomized by thermal buffeting in the low-field environment of the magnetometer enclosure. Although it cannot be determined with certainty, loss of IRM through physical rotation of magnetite particles in unfixed samples measured at room temperature may have occurred in the study of sockeye salmon fry by Quinn *et al.* (1981). Finally, it is important to study coercivity of the samples (that is, to measure Hrc, R and the coercivity spectrum), in addition to the total moment and intensity of magnetization of the samples if the composition and domain state of magnetic particles present are to be characterized. Ueda *et al.* (1986) detected magnetic material in the heads and trunks of juvenile stages of a variety of salmonid fishes. Failure to measure the coercivity of the magnetic particles in their samples made it impossible to distinguish magnetite suitable for magnetoreception from other biochemical precipitates and external contaminants (Ueda *et al.* 1986).

We used analysis of ARM for the first time to demonstrate the presence of magnetically soft multidomain material in the eye of an adult sockeye salmon. Although this material was physically unsuited for use in magnetoreception and probably resulted from external contamination, at present we cannot rule out the biological precipitation of larger concentrations of magnetite such as those extracted from marine turtles by Perry *et al.* (1985). In future work, we plan to use analysis of ARM to assist the interpretation of coercivity spectra and for

investigation of the separation between grains in interacting assemblages of magnetite particles. In the presence of a systematically varied d.c.-bias field, the total ARM acquired by a sample is a sensitive indicator of the distance between interacting particles (Cisowski, 1981). The intergrain separation is of interest because it will affect the size of candidate magnetoreceptors and their detectability in any histological or ultrastructural studies of the ethmoid tissue.

Our results permit two new behavioural predictions from the magnetite-based magnetoreception hypothesis. First, correlation of the amount of magnetite present in the ethmoid region of the skull with age and size in the sockeye salmon suggests that, if the particles are used in magnetoreception, there will be age-dependent limits on the sensitivity of the fish to the geomagnetic field. From the calculations of Kirschvink & Gould (1981), it is easily shown that the numbers of particles necessary to produce the mean moments for the ethmoids of the different life stages of the salmon are roughly 1×10^4 , 2×10^7 , 3×10^7 and 1×10^8 for the fry, yearlings, smolts and adults, respectively. These numbers of particles are more than sufficient to mediate directional responses to magnetic field stimuli exhibited by the sockeye fry and smolts (Quinn, 1980; Quinn *et al.* 1981; Quinn & Brannon, 1982). The numbers of magnetite particles present in the yearlings, smolts and adults are sufficient to mediate responses to small changes (100–1000 nT) in geomagnetic field intensity (Kirschvink & Gould, 1981; Kirschvink & Walker, 1985; Yorke, 1981), although behavioural evidence for such sensitivity has yet to be obtained.

The second prediction concerns the possible effect on magnetoreception of the magnetized wires with which cultured salmon are often tagged. These tags are inserted into the ethmoid region of the fry before they leave the hatchery. The magnetic field produced by a 1 mm piece of the wire decays as the inverse cube of distance, from extremely high values (>1 T) at the surface through earth-strength ($50 \mu\text{T}$) at about 3 mm to the range of normal geomagnetic diurnal variation (50 nT) at 30 mm from the wire. The field produced by the tag will be sufficient to increase significantly the magnetic to thermal energy ratio of magnetite particles within 5–10 mm of the wire. An increase in this ratio will cause a decrease in the variance of motion of the particles and should lead to a reduction in sensitivity to changes in magnetic field intensity (Kirschvink & Gould, 1981; Kirschvink & Walker, 1985).

Two suggestions for future work investigating the hypothesis of magnetite-based magnetoreception arise from this study. The first is to develop magnetic-field conditioning procedures to provide basic psychophysical data that test behavioural predictions of the hypothesis. Responses to magnetic field polarity should be reversed by remagnetization of the magnetite particles, whereas the accuracy of response to magnetic field direction should follow the Langevin function, that is it should increase rapidly with background field up to earth-strength ($50 \mu\text{T}$) and asymptotically beyond earth-strength (Kirschvink, 1981; Kirschvink & Walker, 1985). Threshold responses to magnetic field intensity should also depend on background field strength (Kirschvink & Walker, 1985) and, as noted above,

should depend on the age of the salmon. It seems unlikely that the orientation procedures used so far to investigate magnetic sensitivity in sockeye salmon (Quinn, 1980; Quinn *et al.* 1981; Quinn & Brannon, 1982) are powerful enough to provide such detailed psychophysical data. Thus, although magnetic-field conditioning studies have only rarely been successful with fish (Kalmijn, 1978; Walker, 1984), development of conditioning procedures is essential if responses to magnetic-field stimuli and their sensory basis are to be investigated in detail.

Second, there is a need for detailed histological and ultrastructural studies of the ethmoid tissue in the sockeye salmon and other fishes. The sockeye salmon smolts provide suitable material for such studies because of their small size and relatively high concentration of magnetic material in the ethmoid region. Although it must first be demonstrated that both the magnetite and nervous tissue can be detected in ethmoid tissue sections, ultrastructural studies of the hypothesized magnetoreceptors themselves should eventually be possible. It should be pointed out, however, that the volume fraction of the particles will still be very low (less than five parts per billion; Kirschvink *et al.* 1985), even in the sockeye salmon smolts. It therefore will be necessary to develop procedures to localize further the areas where magnetite is concentrated to improve the chances of detection of the particles in normal 100 nm thick transmission electron microscopy sections.

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