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Magnetic sense in the Japanese eel, *Anguilla japonica*, as determined by conditioning and electrocardiography

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Summary

Magnetosensitivity of the Japanese eel, *Anguilla japonica*, was examined by conditioning and electrocardiography. Marine eels, river eels and farmed eels were conditioned to an imposed magnetic field ranging from 12 663 nT to 192 473 nT parallel to the fish body, which was placed along the earth's west–east axis. Electrocardiograms were recorded with electrodes placed close to the fish body inside a PVC pipe shelter. After 10–40 conditioning runs, all the eels exhibited a significant conditioned response (i.e. slowing of the heart beat) to a 192 473 nT magnetic field and even to a 12 663 nT magnetic field, respectively equivalent to 5.92× and 0.38× the horizontal geomagnetic field (32 524 nT) at our laboratory. The west–east vector of the imposed magnetic field (12 663 nT) combined with that of the geomagnetic field and produced a horizontal resultant magnetic field of 21° easterly. Therefore, Japanese eel are magnetosensitive whether they are at sea, in the river or in the farm. Results of the present study were compared with those of past studies that showed no magnetic sense in the American eel, *Anguilla rostrata*, and the European eel, *Anguilla anguilla*.

Key words: magnetosensitivity, eel migration, conditioned response, magnetoreceptor, Japanese eel, *Anguilla japonica*.

Introduction

Long-distance migration of fishes by sensing features of the earth's magnetic field is still a puzzle despite much quantitative research on salmonids and anguillids. In the rainbow trout, *Oncorhynchus mykiss*, electrophysiological and behavioural responses to magnetic fields have been demonstrated, and a magnetite-based magnetoreceptor cell has been identified in a discrete layer of the olfactory lamellae (Walker et al., 1997; Diebel et al., 2000). On the other hand, telemetric tracking of the chum salmon, *Oncorhynchus keta*, in the North Pacific and of sockeye salmon, *Oncorhynchus nerka*, in a lake in Japan indicated that these fishes do not cue to magnetic fields when homing (Yano et al., 1995; Ueda et al., 1998). Yano et al. (1996) were unable to condition landlocked hime salmon, *O. nerka*, to imposed magnetic fields when an electric shock was used as an associated stimulus and thus concluded that hime salmon have no magnetic sense. However, a putative biomagnetic sense organ has been shown in the posterior part of the skull of adult marine sockeye salmon during homing migration (Sakaki and Motomiya, 1990). Indeed, a magnetic sense may exist and function only during the homing phase of the life cycle, and landlocked salmon that do not migrate may not develop the magnetic sense.

The American eel, *Anguilla rostrata*, could not be conditioned to magnetic fields (McCleave et al., 1971; Rommel and McCleave, 1973). McCleave and Power (1978) examined the turning behaviour of American eel elvers in an arena where the vertical magnetic fields could be manipulated and found no differences in behaviour under four different magnetic field conditions. However, the American eel showed directional preferences under natural geomagnetic and artificial magnetic fields in another study (Souza et al., 1988).

Japanese, European and American eels are different species, and each species comprises three populations: the marine population spends the entire lifetime at sea; the estuarine population migrates between freshwater and seawater; and the freshwater population grows in the river and migrates to the ocean for spawning (Tsukamoto et al., 1998; Jessop et al., 2002; Tzeng et al., 2000, 2002). Given the migratory life history of the anguillid eels, it is possible that marine eels cue to magnetic fields during spawning migration but freshwater eels do not. The present study examined the magnetic sense of Japanese eel captured at sea, from a river and from a farm.

Materials and methods

Eels for experiments

Ten Japanese eels, *Anguilla japonica* Temmic et Schlegel (47.2–76.0 cm in total length), that were caught from the East China Sea by purse seines were obtained from the Shibushi Station of the Japan Sea Farming Association and from a commercial fish dealer in Kagoshima. The eels were kept for 2–3 months in an indoor glass aquarium with filtered

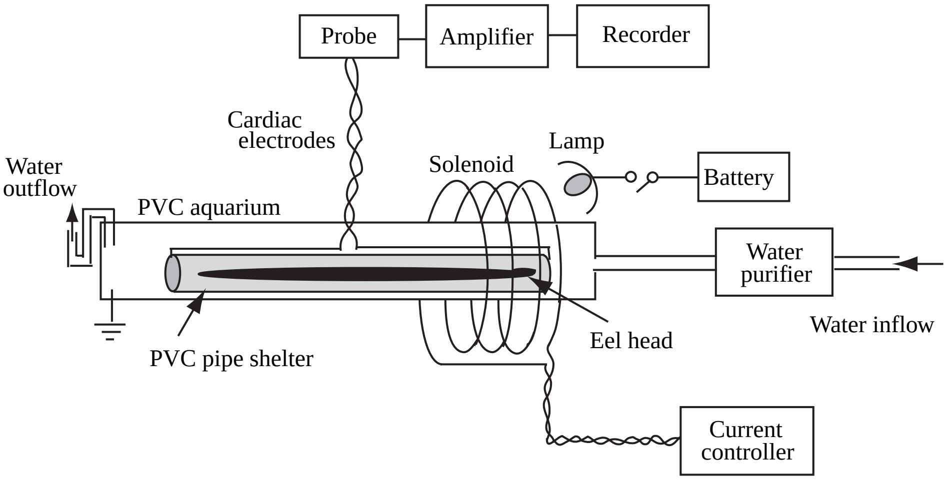

Fig. 1. Experimental apparatus used for conditioning the Japanese eel, *Anguilla japonica*, to artificial magnetic fields.

seawater. They were given fresh fish but did not feed at all. These marine eels had gonads in the early stages of maturation. Four Japanese eels (53.0–60.6 cm in total length) were trapped in freshwaters in the Sendai River and Izaku River, Kagoshima during an ecological survey by the Kagoshima Prefecture Fisheries Research Laboratory. They were kept in an indoor glass aquarium with filtered freshwater for three weeks without food. These specimens showed no gonad maturation. Five Japanese eels (45.3–55.5 cm in total length), also with immature gonads, were obtained from a commercial freshwater farm in Kagoshima 1–2 days before the experiment.

The eels were handled according to methods prescribed by the Kagoshima University's Guide for the Care and Use of Laboratory Animals.

Experimental apparatus

The experiment was carried out in darkness in the laboratory of the Faculty of Fisheries, Kagoshima University. The eels were tested in a PVC aquarium (20 cm wide × 105 cm long × 20 cm deep) placed on a vibration-proof table surrounded by a black curtain, behind which the investigators worked. The test eels were provided with shelter in PVC pipes (either 46 cm long × 3.6 cm in diameter or 72 cm long × 5.8 cm in diameter, depending on eel size) (Fig. 1). The aquarium, pipe and eel were placed in an east–west orientation (the eel faced east).

The test eels from the river and the farm were allowed more than 1 h to acclimate to the holding conditions in the PVC aquarium. The marine eels were directly transferred from seawater into freshwater in the PVC aquarium and allowed 2 h to acclimate. The eel heart beat was monitored by electrocardiogram (see below), and the conditioning tests were started only after the heart beat had become stable (in terms of the time between successive QRS waves). Being euryhaline, the marine eels showed no irregular behaviour when transferred directly into freshwater and the heart beat rate became stable within 30 min.

Flow-through freshwater was continuously supplied to the PVC aquarium through a water purifier (CW-101; NGK, Nagoya, Japan). Water temperatures in the aquarium ranged from 23.6 to 29.0°C during the eel conditioning experiments.

Generating magnetic fields

Around the PVC aquarium, a solenoid 35 cm in diameter was constructed with 74 turns of teflon-coated copper wire (0.3 mm-diameter wire) in a single layer. The head of the test eel was at the centre of the solenoid. By passing direct current through the solenoid, a magnetic field parallel to the fish body was produced. Thus, inside the solenoid, magnetic north was east when the solenoid was turned on. The magnetic field produced was monitored with a small compass placed on the solenoid.

The magnitude of the imposed magnetic fields was varied by changing the electric current from 0.05 A to 0.76 A and was calculated by the formula of Biot and Savart (Jackson, 1999):

$$B = \mu_0 \times Ir^2/2R^3 \,,$$

where B is magnetic flux density (T), μ_0 is magnetic permeability (4×10^{-7} in vacuo), I is electric current (A), r is radius of solenoid (m), and R is distance from a coil to an observation point (m).

The magnetic fields varied from 192 473 nT to 12 663 nT during the tests on eels. These magnetic fields were from 5.92× to 0.38× the horizontal geomagnetic field of 32 524 nT (measured with an Overhauser effect magnetometer; GSM-19-MC; GEM Systems Inc., Richmond Hill, Ontario, Canada) at our laboratory. The solenoid produced a horizontal west–east vector that combined with the earth's south–north vector for a resultant field redirected 80° to 21° easterly from the geomagnetic north, and the resultant magnitude was 187 298 nT to 34 611 nT at the respective directions (Fig. 2).

Conditioning of eels to magnetic fields

Classical conditioning was done on the Japanese eel to determine its sensitivity to a magnetic field. The method depended on establishing a conditioned response, in this case a change in the heart beat of the eel when exposed to a magnetic field (the conditioning stimulus) accompanied by flashes of light. A light flash is a commonly used stimulus that scares and stresses fishes (Kawamura et al., 2002). The light flash in this experiment came from a halogen lamp placed in front of the solenoid; the light intensity was 7300 lux at the head of the test eel.

A conditioning run consisted of exposing an eel to an artificial magnetic field of 192 473 nT for 10 s, with three light

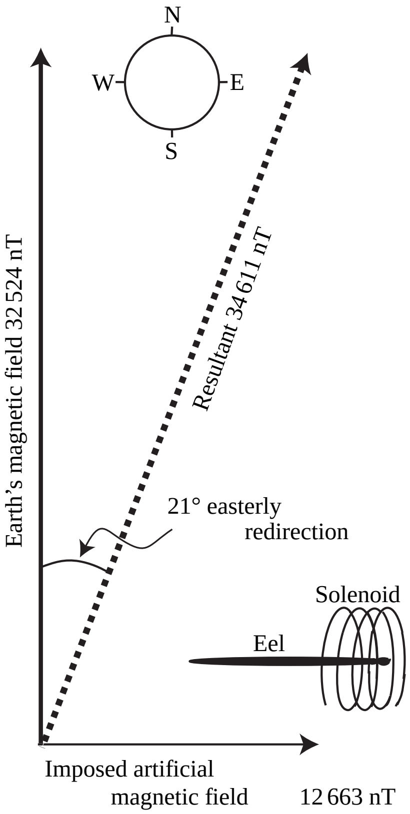

Fig. 2. The experimental apparatus produced a resultant magnetic field due to the combination of the geomagnetic field and the imposed magnetic field. The eel and the solenoid are placed in a west–east direction. When the artificial magnetic field is varied to 12 663 nT, the intensity of the resultant field becomes 34 611 nT redirected 21° easterly. The 80° redirection is not shown in this figure.

flashes at 1 s intervals during the latter 5 s. One set of 10 conditioning runs (at irregular intervals of 1–5 min) was done on each eel. 1 min after a set of conditioning runs was finished, a conditioning test was done on the same eel to record the conditioned response – i.e. a change in the eel heart beat (see below). A conditioning test consisted of re-exposing the eel to the same magnetic field (192 473 nT) for 10 s but without the three light flashes. Eleven heart beats were recorded before the conditioning test to measure 10 interbeat intervals (mean and confidence interval) for each eel. Only four heart beats were recorded during the conditioning test because the conditioned response was evident as soon as the magnetic field was turned on and it was enough to measure three interbeat intervals.

A significant slowing down of the heart beat was obtained at 192 473 nT, so the magnitude of the imposed magnetic field was reduced in three steps to 12 663 nT during the conditioning tests to see if the Japanese eel still responded to small redirected resultant magnetic fields. For six marine eels, the smallest magnetic field used was 12 663 nT, equivalent to 0.38× the south–north horizontal geomagnetic field at Kagoshima, and redirection was 21° easterly.

Recording and measuring eel electrocardiograms

Electrodes are usually implanted close to the heart to record the electrocardiogram uncontaminated by muscle potentials and movement artefacts. However, the eel heart has a high electromotive force, and electrocardiograms could be recorded by electrodes placed in the water but not in the fish's body (Yamamori et al., 1971). Thus, in this study, the eel heart beat was recorded while the test eel rested inside a PVC pipe shelter – that is, the eel experienced minimum handling, no anaesthesia and, presumably, little or no stress. Electrodes (1.5 m-long teflon-coated copper wires, 1 mm diameter) were attached to the two ends of the PVC pipe and twisted together and connected to the probe of an amplifier (Ab-601G; Nihonkoden). The electrocardiograms were recorded with a thermal array recorder (RTA-4100, Nihonkoden).

Ten heart beats before the conditioning test and three heart beats after the test were measured for interbeat intervals. For the statistical analysis, the interbeat intervals were normalized by logarithmic transformation following the formula of Kawamura et al. (1981): normalized interval=$\log_{10}(1+T)$, where T is the raw value of the interbeat interval (s). Each test interbeat interval was then compared with the mean pre-test interbeat interval (by t-test).

The cardiac deceleration ratio was computed to assess the strength of the conditioned response (Northmore and Yagar, 1974). This ratio was defined as: (test interbeat interval – mean pre-test interbeat interval)/mean pre-test interbeat interval, and was computed from the raw values of the interbeat intervals. In this paper, the largest value of the interbeat intervals during the test (either T1, T2 or T3) was used in computing the maximum cardiac deceleration ratio.

Results

Electrocardiograms show the typical heart beat response of Japanese eels after a set of conditioning tests (Fig. 3). A statistically significant (at 99% confidence level) conditioned response to magnetic fields, i.e. slower heart beat or wider interbeat interval, was recorded for 10 marine, two riverine and five farmed Japanese eels during tests done 1 min after 1–4 sets of 10–40 conditioning runs. The lowest intensity of the magnetic field that elicited the conditioned cardiac response was 12 663 nT. Six of the 10 marine eels were conditioned to magnetic fields after only one set of runs, one eel after two sets, two eels after three sets, and one eel after four sets. All five farmed eels and two river eels showed the conditioned response after only one set of runs. In two other river eels, the response was unclear, confounded by muscle potentials and movement artefacts.

The highest cardiac deceleration ratios ranged from 0.11 to 2.60 in 10 marine eels, from 0.10 to 1.47 in two river eels, and from 0.16 to 2.27 in five farmed eels (Fig. 4). The ratios varied widely even at the same imposed magnetic field (the correlation coefficients between the intensity of imposed magnetic field and the highest cardiac deceleration ratios were not statistically significant by the F-test). The variation

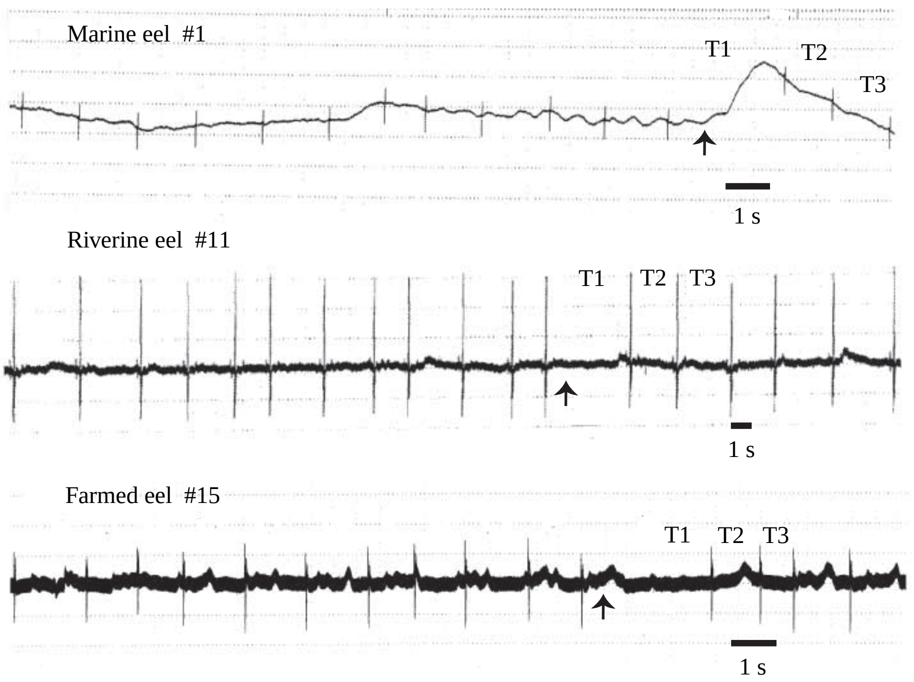

Fig. 3. Typical electrocardiograms showing the heart beat response of Japanese eels after a set of conditioning tests. Horizontal bars mark the time scale. T1–T3 are the test heart beats. Arrows represent the onset of the artificial magnetic field.

was not due to the magnitude of the imposed magnetic field nor the number of conditioning runs (Wilcoxon–Mann–Whitney test, $P>0.10$) (Siegel and Castellan, 1988) but was probably due to individual differences in physiological condition. These statistics meant that the maximum cardiac deceleration ratio was not a good indicator of the strength of the conditioned response of Japanese eels to magnetic fields. Thus, no such comparison was made among the marine, riverine and farmed Japanese eels in this study.

The Japanese eel could detect a 21° easterly shift in the horizontal magnetic field (Fig. 2). The solenoid produced horizontal vector south–east, which combined with the earth's vector for a resultant field redirected horizontally 21° easterly with 34 611 nT in resultant magnitude (6% increment) at the centre of the solenoid where the test eels were placed.

Discussion

The present study clearly showed that Japanese eels, *Anguilla japonica*, have a magnetic sense, whether they are at sea, in the river or in farms. That they could be successfully conditioned by magnetic stimuli indicates the presence of magnetoreceptors, which have yet to be localized in the body. The magnetosensitivity varied among individual eels. No significant correlation was found between the intensity of the magnetic field and the maximum cardiac deceleration ratio. Probably, the eel's magnetic sense has a low threshold sensitivity, even less than the ambient geomagnetic field, and the response no longer increases at higher intensities.

In the present study, Japanese eels responded to a 12 663 nT east–west horizontal geomagnetic field equivalent to 0.38× the north–south horizontal geomagnetic field at Kagoshima. This result indicates that Japanese eels have higher sensitivity to magnetic fields than other migratory fish that have been studied. More significantly, Japanese eels have sufficient sensitivity to detect existing environmental magnetic fields. In earlier experiments, investigators applied magnetic field stimuli much higher than the ambient magnetic field. East–west magnetic fields of 50 000 nT and 100 000 nT were used for American eel (Souza et

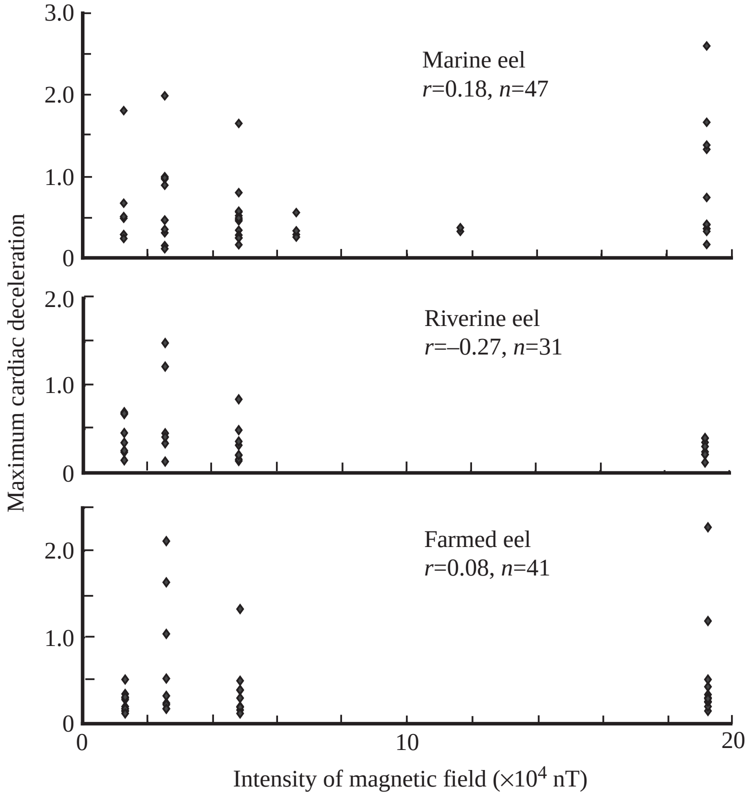

Fig. 4. Relationship between intensity of magnetic field imposed and maximum cardiac deceleration of Japanese eels. r, correlation coefficient; n, number of conditioning tests in which significant cardiac deceleration was recorded.

al., 1988), and a vertical magnetic field with a peak intensity of 125 000 nT (an artificial field of 70 000 nT added to the earth's magnetic field of 55 000 nT) was used for rainbow trout (Walker et al., 1997). For yellowfin tuna, *Thunnus albacares*, a non-uniform vertical magnetic field of 10 μT to 50 μT was used against a background 30 000 nT (Walker, 1984). It is possible that these fishes would have sensed lower magnetic fields if these had been presented to them.

Given that Japanese eels are sensitive to magnetic fields, they could use the geomagnetic field as a cue for their long-distance migration to the spawning area. The spawning area of Japanese eels has been found in the North Equatorial Current, west of the Marianas (Tsukamoto, 1992). After 5–8 years (Tzeng et al., 2002) growing at sea, off eastern and northeastern Asia, adult eels migrate thousands of kilometres to the spawning area.

In contrast to the Japanese eels, American eels (*A. rostrata*) showed no conditioned response to a magnetic field applied parallel to the body or to a reversal of the vertical magnetic field (McCleave et al., 1971; Rommel and McCleave, 1973). The difference in the conditioned response to magnetic fields between American eels and Japanese eels may simply be due to the recording method (electrodes were implanted in the body of the American eel under anaesthesia). American eels did perceive, and responded to, magnetic fields during another set of tank experiments, in which the eels showed a preference for a northeast direction under the earth's magnetic field (50 000 nT) and for a southeast direction under the –50 000 nT and –100 000 nT fields (Souza et al., 1988).

Magnetic fields are relatively simple stimuli with two dimensions – direction and intensity – and it is not clear which is more important to Japanese eels for orientation. Direction may be a more critical cue for migrating fishes since direction can change rapidly in space and time as the body moves from side to side during swimming. Its sensitivity to a 21° easterly shift in the horizontal magnetic field could well guide Japanese eels.

In most fishes, behaviour depends on more than one source of stimulation, and often the stimuli operate sequentially (Blaxter, 1988). The presence of more than one orientation system has been shown in migrating *O. nerka* fry in a lake (Quinn, 1980). Rommel and McCleave (1972) demonstrated that American eels are sensitive to electric fields of 0.167×10^{-3} μA cm^{-2} applied perpendicular to the body axis in freshwater, and that this intensity is within the range of naturally occurring oceanic electric fields. Fricke and Kaese (1995) suggested that different hydrographic regimes could be used as a rough orientating mechanism in eel migration in the ocean. Thus, magnetosensitive eels may also use geoelectric stimuli and hydrographic regimes as orientating cues during migration.

Recent studies have demonstrated the importance of olfaction rather than magnetic sense in the migration of American eels and European eels. Tagged European eels were inhibited in either the visual, magnetic or olfactory sense, and it was observed that the group that had been made anosmic by injection of elastomer into the nasal cavity behaved differently from the control group and from the other experimental groups in that they showed irregular swimming behaviour, slower speed and no common direction (Tesch et al., 1991; Westin, 1990). Ultrasonic telemetry showed the importance of olfaction in the estuarine migration of silver-phase American eels – the anosmic eels with nares filled with petroleum jelly spent more time in the estuary whereas the control eels moved upstream and downstream with the tides (Barbin et al., 1998). In these studies, the perceived absence of a magnetic sense in the European eel and the American eel may be an artefact of the method used to induce anosmia, where boiling petroleum jelly (>150°C) was injected into the nares (Keefe, 1992). If these eels have magnetoreceptor cells in the nose, as does the rainbow trout (Walker et al., 1997), then the anosmia treatment may have damaged the magnetoreceptor cells or the nerves [the superficial ophthalmic ramus (ros V) of the trigeminal nerve] such that the eels could also not detect the geomagnetic field. The fine branches of the ros V nerve (ros V rami) that surround the nasal capsule form a complex network from which very fine processes penetrate the capsule wall and terminate in the lamina propria of the olfactory lamellae (Walker et al., 1997). This thin nerve structure might be very vulnerable to heat at high temperature.

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References

Barbin, G. P., Parker, S. J. and McCleave, J. D. (1998). Olfactory clues play a critical role in the estuarine migration of silver-phase American eels. *Env. Biol. Fish.* **53**, 283-291.

Blaxter, J. H. S. (1988). Sensory performance, behavior, and ecology of fish. In *Sensory Biology of Aquatic Animals* (ed. J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 203-232. New York: Springer-Verlag.

Diebel, C. E., Proksch, R., Green, C. R., Neilson, P. and Walker, M. M. (2000). Magnetite defines a vertebrate magnetoreceptor. *Nature* **406**, 299-302.

Fricke, H. and Kaese, R. (1995). Tracking of artificially matured eels (*Anguilla anguilla*) in the Sargasso Sea and the problem of the eel's spawning site. *Naturwissenschaften* **82**, 32-36.

Jackson, J. D. (1999). *Classical Electrodynamics.* 3rd edition. New York: John Wiley & Sons.

Jessop, B. M., Shiano, J. C., Iizuki, Y. and Tzeng, W. N. (2002). Migratory behaviour and habitat use by American eels *Anguilla rostrata* as revealed by otolith microchemistry. *Mar. Ecol. Prog. Ser.* **233**, 217-229.

Kawamura, G., Shibata, A. and Yonemori, T. (1981). Response of teleosts to the plane of polarized light as determined by the heart beat rate. *Nippon Suisan Gakkaishi* **47**, 727-729.

Kawamura, G., Anraku, K., Nakahara, M. and Shigesato, N. (2002). Conditioning of negatively phototactic red sea bream and guidance by light. *Nippon Suisan Gakkaishi* **68**, 706-708.

Keefe, M. (1992). Chemically mediated avoidance behavior in wild brook trout, *Salvelinus fontinalis*: the response to familiar and unfamiliar predaceous fishes and the influence of fish diet. *Can. J. Zool.* **70**, 288-292.

McCleave, J. D., Rommel, S. A. and Cathcart, S. L. (1971). Weak electric and magnetic fields in fish orientation. *Ann. N. Y. Acad. Sci.* **188**, 270-282.

McCleave, J. D. and Power, J. H. (1978). Influence of weak electric and magnetic fields on turning behavior in elvers of the American eel *Anguilla rostrata*. *Mar. Biol.* **46**, 29-34.

Northmore, D. P. M. and Yagar, D. (1974). Psychophysical methods for investigation of vision in fishes. In *Vision in Fish* (ed. M. A. Ali), pp. 689-704. New York: Plenum Press.

Quinn, T. P. (1980). Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. *J. Comp. Physiol*. **137**, 243-248.

Rommel, S. A., Jr and McCleave, J. D. (1972). Oceanic electric fields: perception by American eels? *Science* **176**, 1233-1235.

Rommel, S. A., Jr and McCleave, J. D. (1973). Sensitivity of American eels (*Anguilla rostrata*) and Atlantic salmon (*Salmo salar*) to weak electric and magnetic fields. *J. Fish. Res. Bd. Can*. **30**, 657-663.

Sakaki, Y. and Motomiya, T. (1990). Possible mechanism of biomagnetic sense organ extracted from sockeye salmon. *IEEE Trans. Magnetics* **26**, 1554-1556.

Siegel, S. and Castellan, N. J., Jr (1988). *Nonparametric Statistics for the Behavioral Sciences*. 2nd edition. New York: McGraw-Hill.

Souza, J. J., Poluhowich, J. J. and Guerra, R. J. (1988). Orientation responses of American eels, *Anguilla rostrata*, to varying magnetic fields. *Comp. Biochem. Physiol. A* **90**, 57-61.

Tesch, F. W., Westerberg, H. and Karlsson, L. (1991). Tracking studies on migrating silver eels in the Central Baltic. *Meeresforschung* **33**, 183-196.

Tsukamoto, K. (1992). Discovery of the spawning area for Japanese eel. *Nature* **356**, 789-791.

Tsukamoto, K., Nakai, I. and Tech, W. V. (1998). Do all freshwater eels migrate? *Nature* **396**, 635-636.

Tzeng, W. N., Wang, C. H., Wickstrom, H. and Reizenstein, M. (2000). Occurrence of the semi-catadromous European eel *Anguilla anguilla* (L.) in Baltic *Sea. Mar. Biol.* **137**, 93-98.

Tzeng, W. N., Shiao, J. C. and Iizuka, Y. (2002). Use of otolith Sr:Ca ratio to study the riverine migratory behaviors of Japanese eel *Anguilla japonica*. *Mar. Ecol. Prog. Ser*. **245**, 213-221.

Ueda, H., Kaeriyama, M., Mukawa, K., Urano, A., Kudo, H., Shoji, T., Tokumitsu, Y., Yamauchi, K. and Kurihara, K. (1998). Lacustrine sockeye salmon return straight to the natal area from open water using both visual and olfactory cues. *Chem. Senses* **23**, 207-212.

Walker, M. M. (1984). Learned magnetic field discrimination in yellowfin tuna, *Thunnus albacares*. *J. Comp. Physiol. A* **155**, 673-679.

Walker, M. M., Diebel, C. E., Haugh, C. V., Pankhurst, P. M., Montgomery, J. C. and Green, C. R. (1997). Structure and function of the vertebrate magnetic sense. *Nature* **390**, 371-376.

Westin, L. (1990). Orientation mechanisms in migrating European silver eel (*Anguilla anguilla*): temperature and olfaction. *Mar. Biol.* **106**, 175-179.

Yamamori, K., Hanyu, I. and Hibiya, T. (1971). Electrocardiography of the eel by means of underwater electrodes. *Nippon Suisan Gakkaishi* **37**, 94-97.

Yano, A., Sato, A. and Sakaki, Y. (1995). Study of chum salmon, *Oncorhynchus keta*, movement in a disturbed magnetic field in the North Pacific Ocean using ultrasonic telemetry. *Salmon Rep. Ser.* **39**, 174-196.

Yano, A., Sato, A., Miyata, T., Mizutani, Y., Sakaki, Y., Kitamura, S., Ikuta, K. and Ogura, M. (1996). Behavioral tests for magnetic sensitivity of hime salmon (Kokanee: land-locked sockeye salmon *Oncorhynchus nerka*). *Nippon Suisan Gakkiashi* **62**, 911-919 (in Japanese with English abstract).