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PERCEPTION AND EFFECTS ON LOCOMOTOR
ACTIVITY IN AMERICAN EELS AND ATLANTIC
SALMON OF EXTREMELY LOW FREQUENCY
ELECTRIC AND MAGNETIC FIELDS

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I. ABSTRACT

Possible effects of the proposed Sanguine communication system on American eels (Anguilla rostrata) and Atlantic salmon (Salmo salar) were investigated in three ways. Conditioned cardiac deceleration techniques demonstrated that both species are marginally sensitive to Sanguine level ELF (60 - 75 Hz) electric fields (0.007 - 0.07 V/m) but not to magnetic fields (0.5 gauss). Locomotor activity levels or diel patterns of the fishes were not affected by alternating 24 hr periods of exposure and non-exposure to the ELF electric or magnetic fields. Activity rhythms were not entrained (synchronized) by 1 hr exposures every 23 hr to ELF electric or magnetic fields. It is concluded that while at least these two species can probably perceive Sanguine electromagnetic radiation, their normal behavior is unlikely to be affected by such fields. Higher frequencies will probably result in less effect than lower frequencies.

II. INTRODUCTION

The feasibility of Extremely Low Frequency (ELF) communications has been under study by the United States Department of the Navy for a number of years (Libber 1970, Wait 1972). These studies, known as Project Sanguine, have claimed that ELF transmission can provide reliable, one-way communications with submerged submarines world-wide. Communication would be established using a buried antenna system.

Concern that ELF electromagnetic radiation from the antenna system might adversely affect organisms in the environment, coupled with general lack of knowledge of possible biological effects of ELF fields, prompted initiation of a variety of studies within a research matrix described by Libber (1970).

This paper reports results of three types of experiments designed, within the desired research matrix, to determine effects of ELF Sanguine intensity electromagnetic fields on two migratory fishes: Atlantic salmon (Salmo salar) and American eel (Anguilla rostrata). The specific objectives were to determine: (1) if these species could be conditioned to exhibit a cardiac rate deceleration in response to weak ELF electric or magnetic fields, (2) if their locomotor activity patterns in various light cycles would be changed by alternating periods of exposure and non-exposure to weak ELF fields, and (3) if their locomotor activity patterns in constant light conditions could be entrained by a periodic, brief exposure to an ELF electric or magnetic field.

Electric fields near the buried antenna of a functional Sanguine system are expected to be approximately 0.07 V/m, and magnetic fields approximately 0.2 gauss. Our experiments utilized 60 Hz or 75 Hz electric fields between

0.0007 and 0.7 RMSV/m or magnetic fields of 0.5 gauss.

Previous work in our laboratory has demonstrated that American eels and Atlantic salmon are sensitive to d.c. electric fields of 0.06 mV/m or less, but are not sensitive to d.c. magnetic fields of about 0.5 gauss (Rommel and McCleave 1972, 1973a). We have also described the basic locomotor activity patterns of Atlantic salmon in various light conditions (Varanelli and McCleave in press; Richardson and McCleave MS in preparation).

III. CONDITIONED CARDIAC RESPONSES TO ELF FIELDS

Introduction

The initial objective of our research was simply to determine if salmon and eels were able to perceive ELF electric and magnetic fields of approximately Sanguine intensity.

Classically conditioned cardiac deceleration was chosen as an appropriate method for meeting this objective for two reasons. First, it has become an accepted technique in fish sensory perception studies, since Otis, Cerf, and Thomas (1957) first demonstrated the phenomenon in goldfish (Carassius auratus). The method has been used in determining auditory thresholds in tautogs (Tautoga onitis) and goldfish (Offut 1968, 1971), visual discrimination in goldfish (McCleary and Bernstein 1959, Hester 1968), and factors involved in interocular transfer in goldfish (Schoel and Agronoff 1972). Second, we have previously used this technique in demonstrating sensitivity of salmon and eels to weak d.c. electric fields (Rommel and McCleave 1972, 1973a). Unconditioned cardiac deceleration has also been used to confirm electrosensitivity in sharks (Scyliorhinus canicula) and rays (Raja clavata) (Dijkgraaf and Kalmijn 1966, Kalmijn 1966).

Methods

Searun Atlantic salmon parr (16-22 cm total length) were obtained from the Craig Brook National Fish Hatchery at Orland, Maine and were held for 2-29 days before testing at 10 or $15 \pm 1^\circ$ C, depending on water temperature used during testing. American eels (31-57 cm total length), trapped in fresh and estuarine waters of Maine and New Brunswick, were supplied by a commercial dealer. The eels were held at 20° C for 1-42 days before testing at 20° C. All fish were held and tested in well water of measured resistivity between 2800 and 4500 ohm-cm.

To obtain electrocardiograms, electrodes were implanted in the fish under anesthesia with 2-phenoxyethanol, a 1:4000 solution for salmon and a mixture of crushed ice, water and anesthetic (2 liters ice, 1 liter H₂O, 3 ml anesthetic) for eels. During implantation salmon were kept in a pan of anesthetic solution but eels were restrained in air in a rubber tube exposing only the implantation area. The time for sufficient anesthesia varied from 2-5 min for salmon and 10-15 min for eels. Implantation took 2-8 min, averaging longer for eels than for salmon. Recovery of equilibrium in the fresh water of the experimental tank usually took less than 1 min for eels and 5 min for salmon.

Electrodes were implanted in eels the afternoon prior to testing and the fish remained in the aquaria overnight. Salmon usually did not survive if they were left overnight. Therefore the operation was performed early in the morning, then the fish were left undisturbed in the testing chamber for 1-1.5 hr before testing was started.

The cardiac electrodes consisted of two, 1 m long, teflon-coated, stainless steel wires (0.007 inch diameter) twisted together to prevent

induction of spurious electric signals. The electrodes were tied through the skin on the ventral surface just posterior to the pectoral fins. Then an extra loop was taken through the skin ventrally with each electrode to prevent it from pulling out. The insulation was scraped from the terminal 1-2 mm of each electrode. Then each electrode was implanted by hooking the wire tip in a #26 hypodermic needle, bending the wire back along the shank of the needle, inserting the needle and wire into the body, and withdrawing the needle leaving the J-shaped electrode in the eel. One electrode was implanted either just anterior or posterior to the pericardial cavity and the other 1 to 3 cm posterior to the first. Occasionally an electrode was actually lodged within the pericardial cavity. With salmon the electrodes were passed through the skin dorsally, passed around the body just posterior to the pectoral fins and tied through the skin ventrally to prevent slippage. The rest of the operation was the same as that for eels.

Immediately after implantation the fish was placed in one of two all-plexiglass aquaria 100 cm long, 30 cm wide and 37 cm deep. The twisted electrode wires were led upward from the dorsal surface of the fish to a clip above the aquarium. The fish were gently restrained by a long, narrow trough of rubber mesh suspended in the tank. The mesh distorted the electric or magnetic field very little but allowed the field to be presented parallel or perpendicular to the long axis of the fish's body.

To test sensitivity to electric fields, a pair of stainless steel plates, 100 cm by 30 cm were placed just inside each side or a 30 cm by 30 cm pair placed just inside the ends. These served as electrodes to impose a uniform weak field across or along the tank and a conditioning electric shock. The weak electric fields (60 and 75 Hz) were produced by an audio frequency oscillator and adjusted by a precision potentiometer ($0.0007 - 0.07 \text{ V/m} \pm 1\%$) and

verified with an oscilloscope before any experiments were performed. A blocking capacitor in series with the tank electrodes limited the d.c component to less than 0.1% of the applied a.c. field. Each presentation was monitored with a digital a.c. microammeter. A step-down transformer and variable resistor provided a 1-4 V a.c. shock across or along the tank. The shocking circuit was isolated from the weak electric or magnetic field circuit by a d.c. relay.

A horizontal magnetic field of 0.5 gauss ($\pm 1\%$) was produced by an audio frequency oscillator and an audio frequency amplifier connected to a Helmholtz coil of 100 turns of #20 copper magnet wire. The field where the fish was restrained was measured with a "Hall effect" magnetometer before an experimental series was started. During experiments current flow to the coils was monitored with an a.c. ammeter. One of the plexiglass aquaria was used with aluminum screen shocking electrodes substituted for the stainless steel plates.

The experiments were conducted with the aquaria in darkness in a light-tight chamber 2.3 m long, 1.5 m wide and 1.9 m high. The ceiling and walls of the chamber were covered by 12-15 cm of fiberglass wool insulation and 4.5 cm of insulation was placed beneath the floor. The aquaria rested on foam pads placed on stands whose legs rested in boxes of sand. Thus the sounds and vibrations present in the laboratory were damped, though not eliminated. Electrocardiograms were recorded on a Grass Model 7-B Polygraph.

Eighty training-testing trials were attempted on each fish. A trial consisted of the presentation of either the electric or magnetic field as the conditioned stimulus (CS) for two or three consecutive heartbeats followed by a momentary 1-4 V a.c. shock as the unconditioned stimulus (US). Each fish was presented with 10 trials separated by 60 to 90 sec then allowed to

remain 30 min undisturbed before the next ten trials and so on. Some fish pulled out their electrodes, the electrodes shorted out or the EKG was unreadable due to noise before 80 trials were completed. A minimum of 50 trials was selected as the smallest acceptable sample size for the statistical analysis.

The data from each fish were statistically treated separately. As an index of heartbeat rate, the interbeat interval was measured to the nearest 0.04 sec. The first two beats after the CS was turned on were designated the first and second test beats and the beat immediately prior to the first test beat was designated the test reference beat (Fig. 1). The change in rate from the reference beats to each test beat was calculated as a percentage. For example, the percent change in test beat two in Fig. 1 is $8/27$ or 30% and control beat two is $-1/28$ or -4%, the minus indicating an acceleration of heart rate. The percent change in test beats was compared to that of the control beats instead of zero because we could not be sure the mean percent change of a beat compared to a prior reference beat would be zero. Wilcoxon's signed ranks test at the 95% level was used to test the null hypothesis that there was no difference between the median percent change of control beats and the median percent change of test beats. The alternate hypothesis, that the median percent change in test beats was greater than the control beats, indicated conditioning, and thus sensitivity to the weak electric or magnetic field.

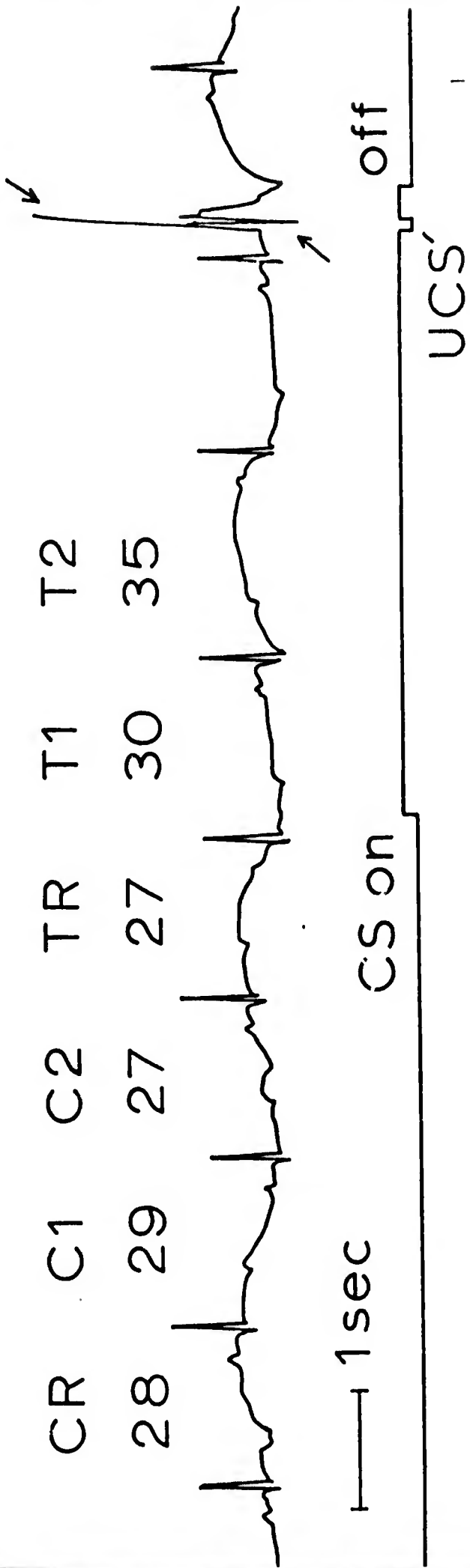
Salmon and eels were tested for sensitivity to the following electric fields:

1. Perpendicular to the body axis, 75 Hz, 0.07 V/m (Sanguine level)
2. Perpendicular to the body axis, 75 Hz, 0.007 V/m
3. Perpendicular to the body axis, 75 Hz, 0.0007 V/m

Fig. 1. EKG of an American eel during one training trial showing apparent conditioned cardiac deceleration, presentation of weak electric field (CS), unconditioned stimulus (UCS), and designation of test and control heart beats. CR = control reference beat; C1, C2 = control beats; TR = test reference beat; T1, T2 = test beats. Numbers are interbeat intervals (in millimeters at 25 mm/sec chart speed for original EKG record). Arrows mark artifacts caused by the UCS.

4. Perpendicular to the body axis, 60 Hz, 0.07 V/m
5. Parallel to the body axis, 75 Hz, 0.07 V/m
6. Parallel to the body axis, 60 Hz, 0.07 V/m

Both species were also tested for sensitivity to 0.5 gauss horizontal magnetic fields of 75 Hz and 60 Hz.



Results

The cardiac conditioning experiments indicate that eels are sensitive to Sanguine level 75 Hz electric fields perpendicular to their body axis and that salmon are probably marginally sensitive to the ELF fields tested (Table 1). A total of 47 eels were tested with 75 Hz electric fields perpendicular to their body axis and the following showed a significant ($p < 0.05$) cardiac deceleration (examples in Table 2): 6 of 19 at 0.07 V/m (Sanguine level), 5 of 19 at 0.007 V/m and 0 of 9 at 0.0007 V/m. Response curves for these three groups substantiate the Wilcoxon's test results (Fig. 2a, b, c). Eleven eels were tested for sensitivity to 0.07 V/m, 60 Hz fields applied perpendicular to their body; only two eels were conditioned, but there probably is little difference in sensitivity to 60 and 75 Hz fields. None of the eels tested responded to 0.07 V/m fields applied parallel to their body axis (Fig. 2d); 8 were tested at 75 Hz and 3 were tested at 60 Hz.

Two types of control experiments were performed with eels. Six fish were tested with the CS but without the US, to determine if there was a consistent natural cardiac deceleration in response to the onset of the weak electric field, or if there were any spurious signals that might be produced in the circuit to which the fish might respond. One eel showed a significant cardiac deceleration and we consider this possible due to chance. Seven eels were tested with the ELF source unplugged but with the US functional, to examine the possibility that the animals were responding to some cue other than the electric field, or that the US alone could produce decelerations that would appear to be a conditioned response. None had a significant deceleration.

Fewer salmon than eels had significant cardiac decelerations in 75 Hz

Table 1. Number of American eels and Atlantic salmon showing conditioned cardiac deceleration to 75 and 60 Hz electric fields, either perpendicular or parallel to the long axis of the body, at various field intensities. Significance criterion is Wilcoxon's Signed Ranks Test at 95% level.

Field intensity (V/m)	Field characteristics			
	Perpendicular		Parallel	
	75 Hz	60 Hz	75 Hz	60 Hz
	<u>EELS</u>			
0.07	6 of 19	2 of 11	0 of 8	0 of 3
0.007	5 of 19	-	-	-
0.0007	0 of 9	-	-	-
	<u>SALMON</u>			
0.07	2 of 9	2 of 8	0 of 8	0 of 4
0.007	0 of 4	-	-	-
0.0007	0 of 1	-	-	-

Table 2. Responses of American eels to 75 Hz electric fields (in water of 2800-3800 ohm-cm). Field applied perpendicular to the body axis for 80 trials. Statistically significant cardiac decelerations at 95% (*) and 99% (**) levels determined by Wilcoxon's Signed Ranks Test. (Large standard deviations expected in fish heart rates.)

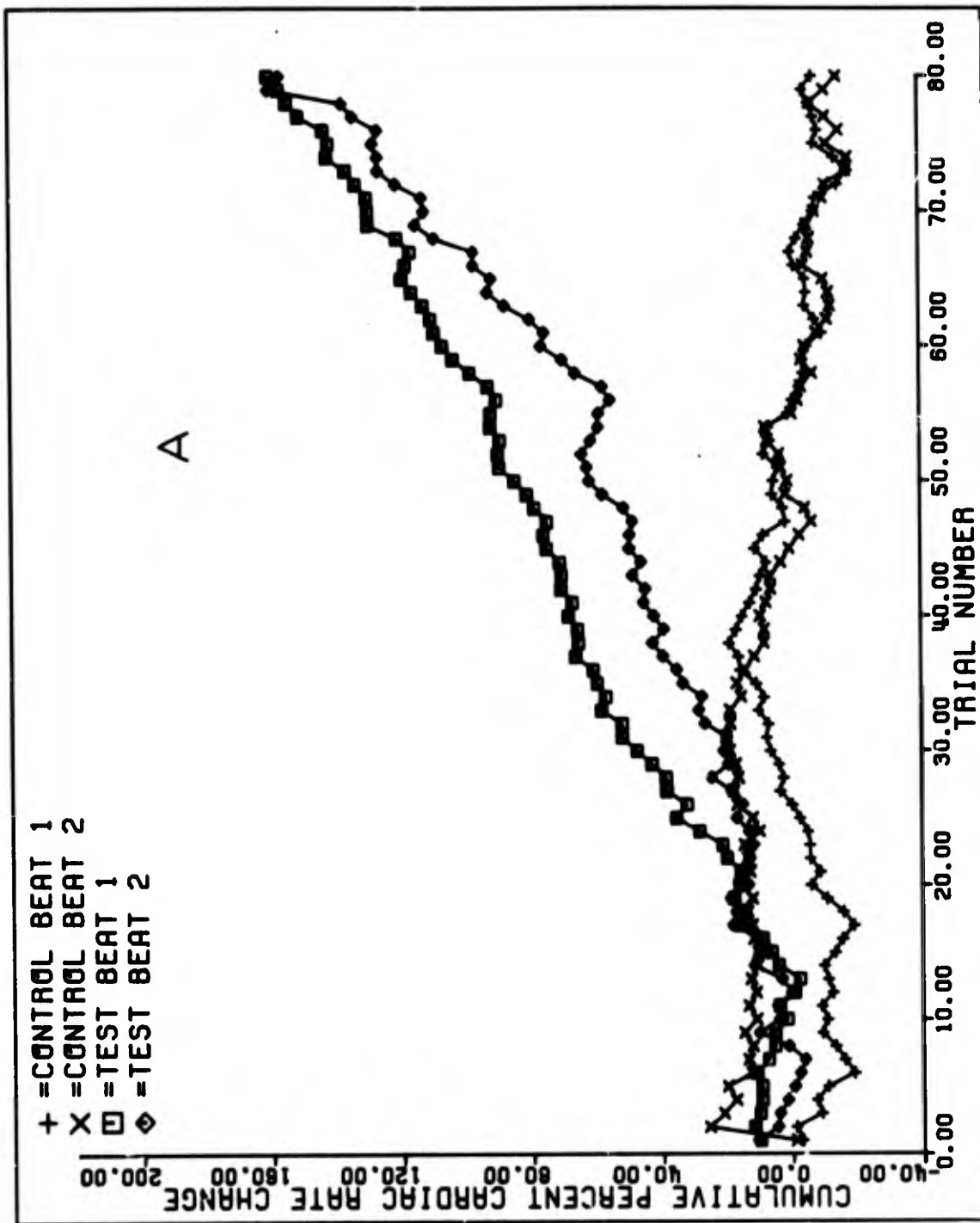
Electric current density ($\mu\text{A}/\text{cm}^2$)	% Change control beat 1 Mean \pm s.d.	% Change test beat 1 Mean \pm s.d.	% Change control beat 2 Mean \pm s.d.	% Change test beat 2 Mean \pm s.d.
0.07 V/m				
.219	-0.8 \pm 18.6	5.2 \pm 28.4	2.5 \pm 37.9	9.4 \pm 36.2*
.219	-1.0 \pm 16.7	4.5 \pm 15.7*	0.9 \pm 18.7	4.0 \pm 17.7
.206	-0.4 \pm 13.5	1.5 \pm 14.8	-0.7 \pm 12.4	-2.0 \pm 12.8
.205	1.5 \pm 25.4	5.3 \pm 21.9	-2.8 \pm 20.6	8.1 \pm 47.4*
.205	2.6 \pm 17.2	3.8 \pm 19.1	0.9 \pm 13.9	3.1 \pm 15.6
0.007 V/m				
.020	-1.3 \pm 10.4	7.9 \pm 23.2**	1.4 \pm 11.4	9.4 \pm 23.0**
.020	1.0 \pm 22.3	8.6 \pm 27.9*	4.5 \pm 28.2	6.4 \pm 24.1
.020	2.0 \pm 18.9	2.7 \pm 23.2	2.8 \pm 24.6	2.8 \pm 31.6
.022	1.4 \pm 13.7	2.8 \pm 15.0	0.3 \pm 15.3	2.6 \pm 15.1
.022	1.4 \pm 16.5	4.3 \pm 20.1*	-0.2 \pm 19.9	5.9 \pm 24.1**
0.0007 V/m				
.002	-3.3 \pm 12.0	-1.9 \pm 12.3	-0.8 \pm 16.3	0.5 \pm 16.6
.002	1.2 \pm 10.4	2.4 \pm 12.7	1.5 \pm 10.0	2.0 \pm 11.3
.002	0.8 \pm 7.5	0.6 \pm 9.3	0.7 \pm 9.4	0.0 \pm 8.8
.002	0.5 \pm 10.6	1.8 \pm 9.2	-0.3 \pm 10.6	-0.8 \pm 10.0
.002	-0.6 \pm 15.4	2.7 \pm 15.2	0.1 \pm 12.0	4.4 \pm 22.0

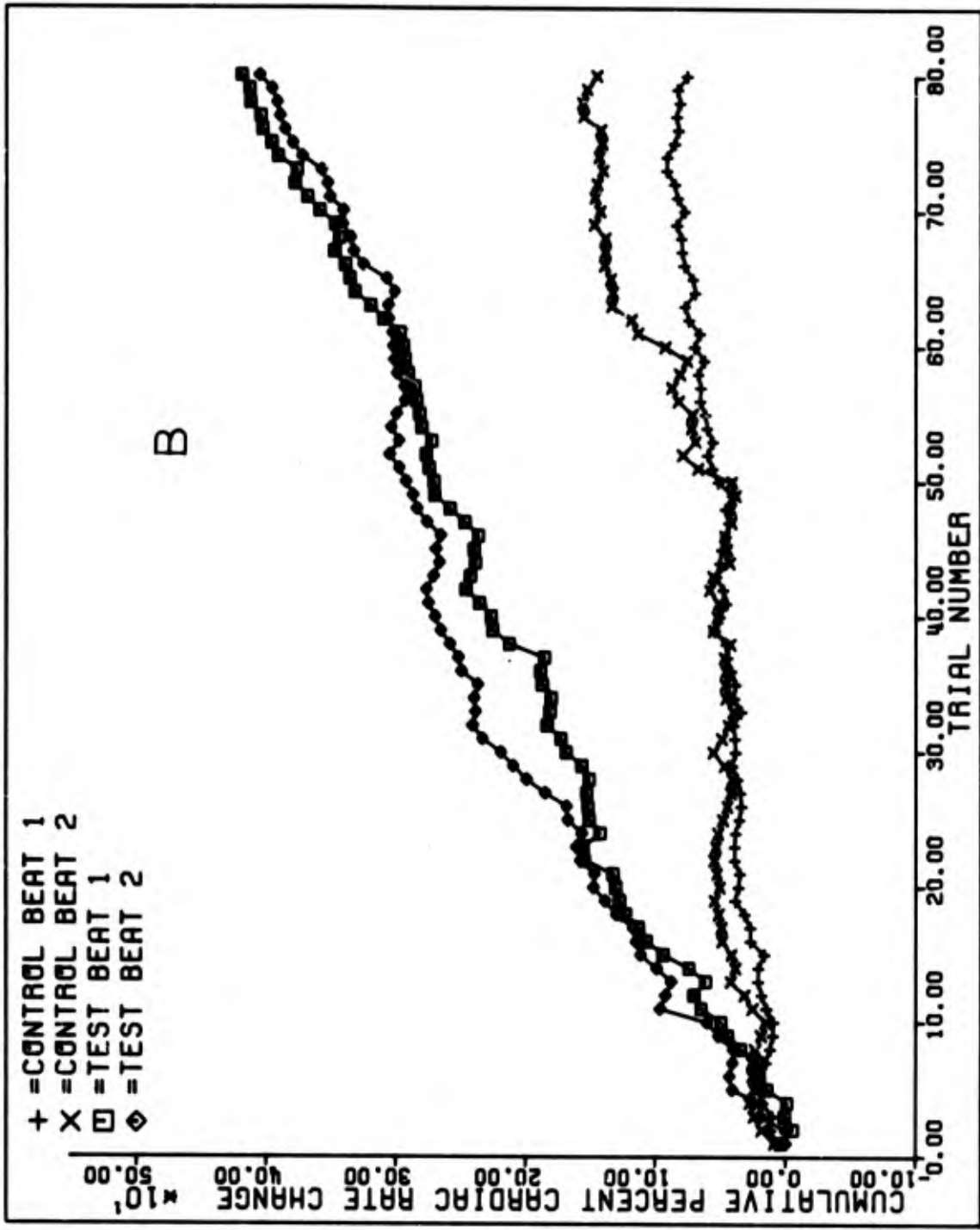
Fig. 2 a,b,c,d. Cumulative cardiac response of American eels to ELF electric fields of various intensities: (a) 0.07 V/m, 75 Hz, perpendicular; (b) 0.007 V/m, 75 Hz, perpendicular; (c) 0.0007 V/m, 75 Hz, perpendicular; (d) 0.07 V/m, 60 and 75 Hz, parallel. Each point is a mean value for all fish tested at that particular intensity.

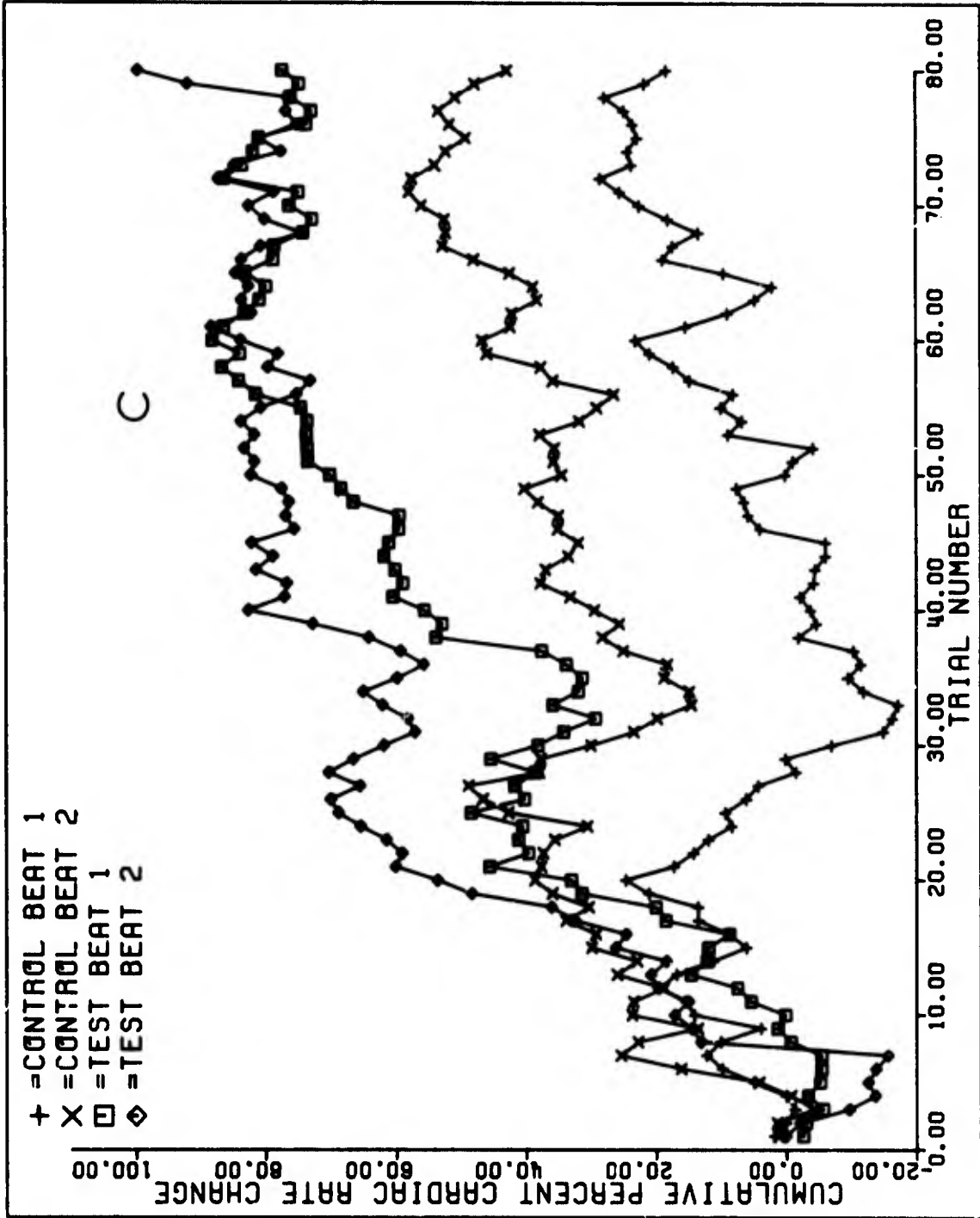
fields applied perpendicular to their body axis, only 2 of 9 at 0.07 V/m (Fig. 3a), 0 of 4 at 0.007 V/m and 0 of 1 tested at 0.0007 V/m. Eight salmon were tested for sensitivity to 0.07 V/m, 60 Hz fields applied perpendicular to their body axis; only two showed significant decelerations. The results of tests with fields applied parallel to the body axis were identical for salmon and eels. Of the eight salmon tested at 75 Hz and the four at 60 Hz (Fig. 3b), none had significant decelerations. No control experiments were conducted with salmon because the electric circuit was identical to that used for the eel control experiments and so few salmon were conditioned.

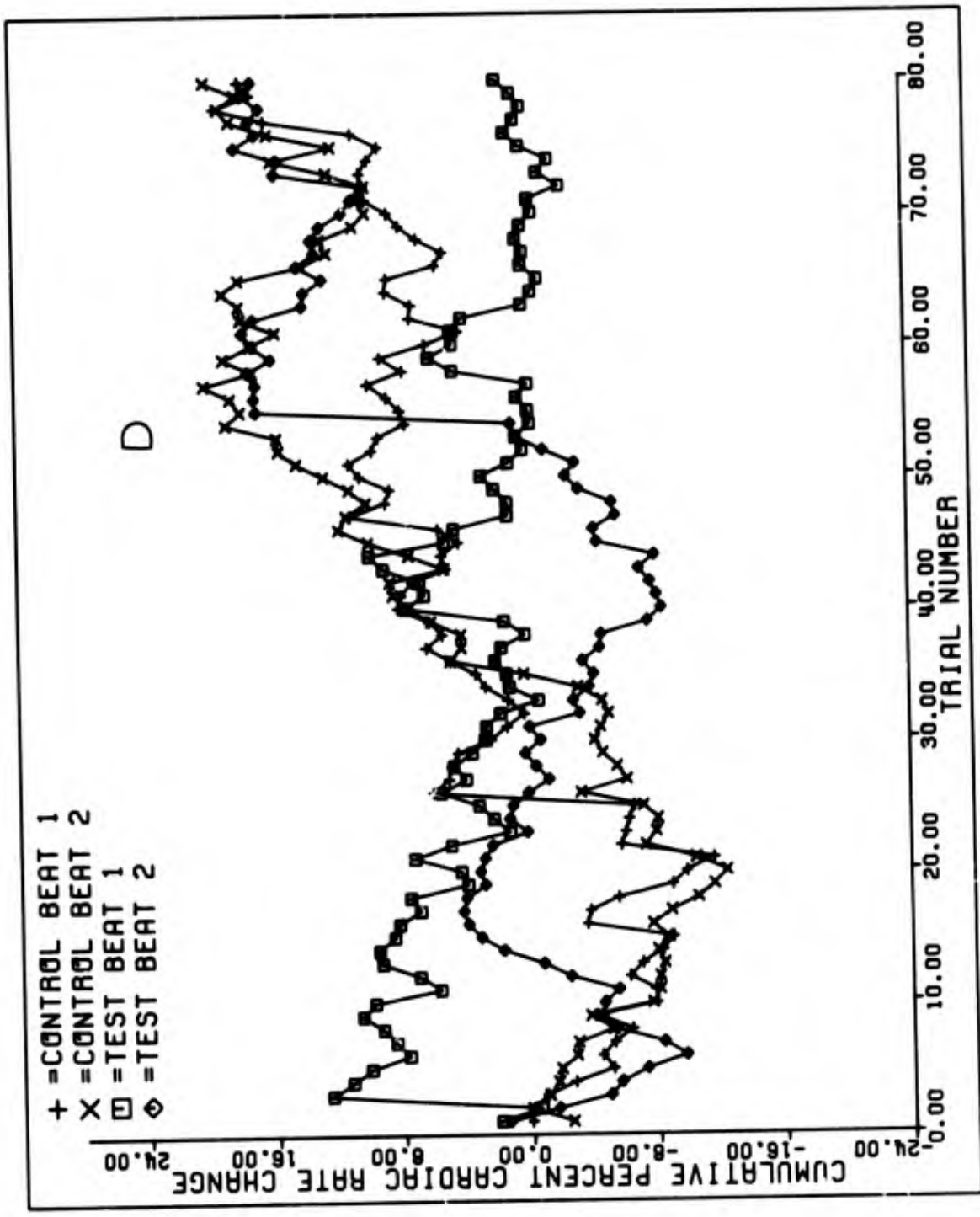
Only two of the sixteen eels and none of the twelve salmon tested for sensitivity to ELF magnetic fields had significant cardiac decelerations. Eight eels were tested at 75 Hz and eight at 60 Hz and one in each group had a significant deceleration, which was probably due to chance. Seven salmon were tested at 75 Hz and five at 60 Hz.

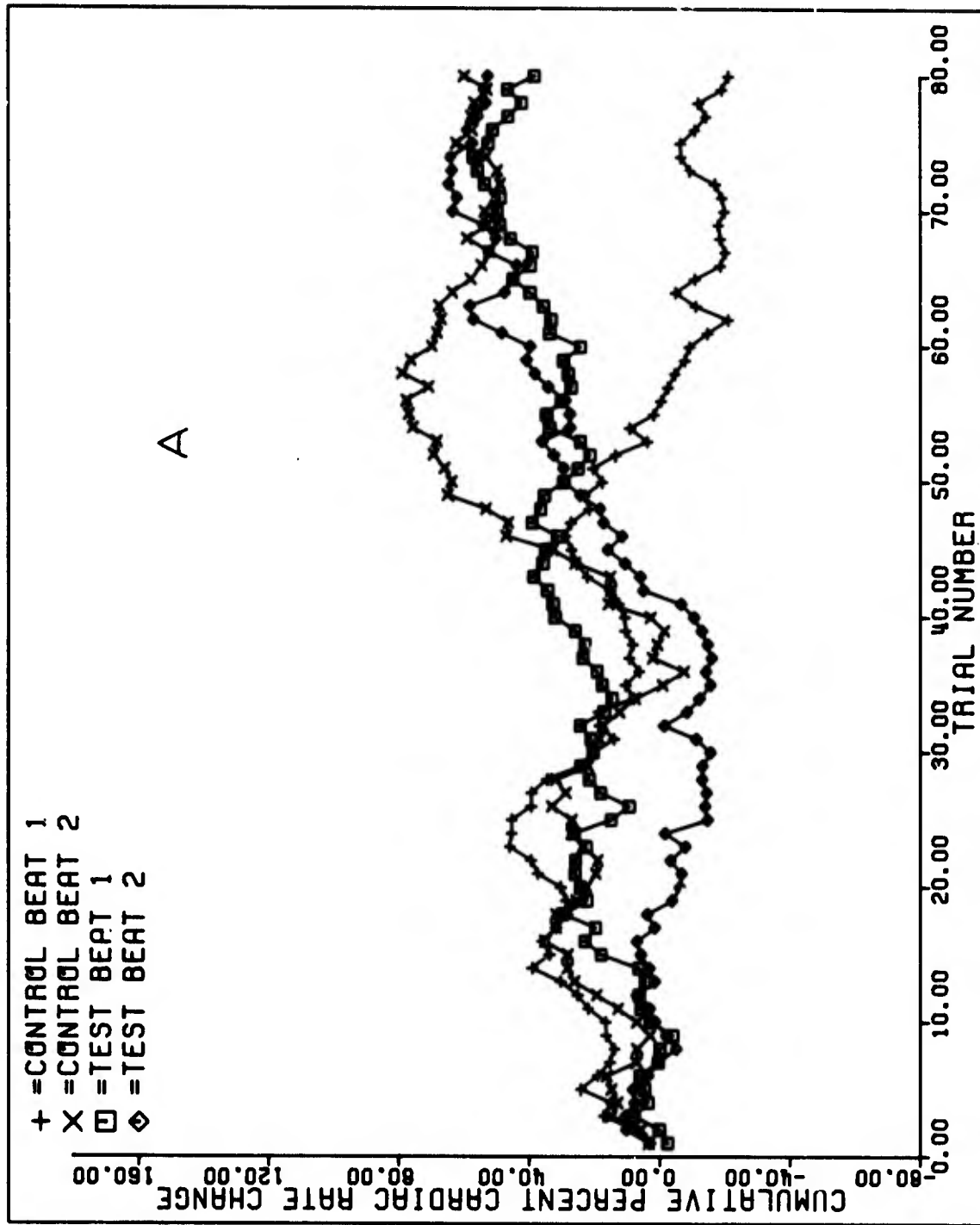
Fig. 3 a,b. Cumulative cardiac response of Atlantic salmon to ELF electric fields: (a) 0.07 V/m, 75 Hz, perpendicular; (b) 0.07 V/m, 60 and 75 Hz, parallel. Each point is a mean value for all fish tested at that particular set of conditions.

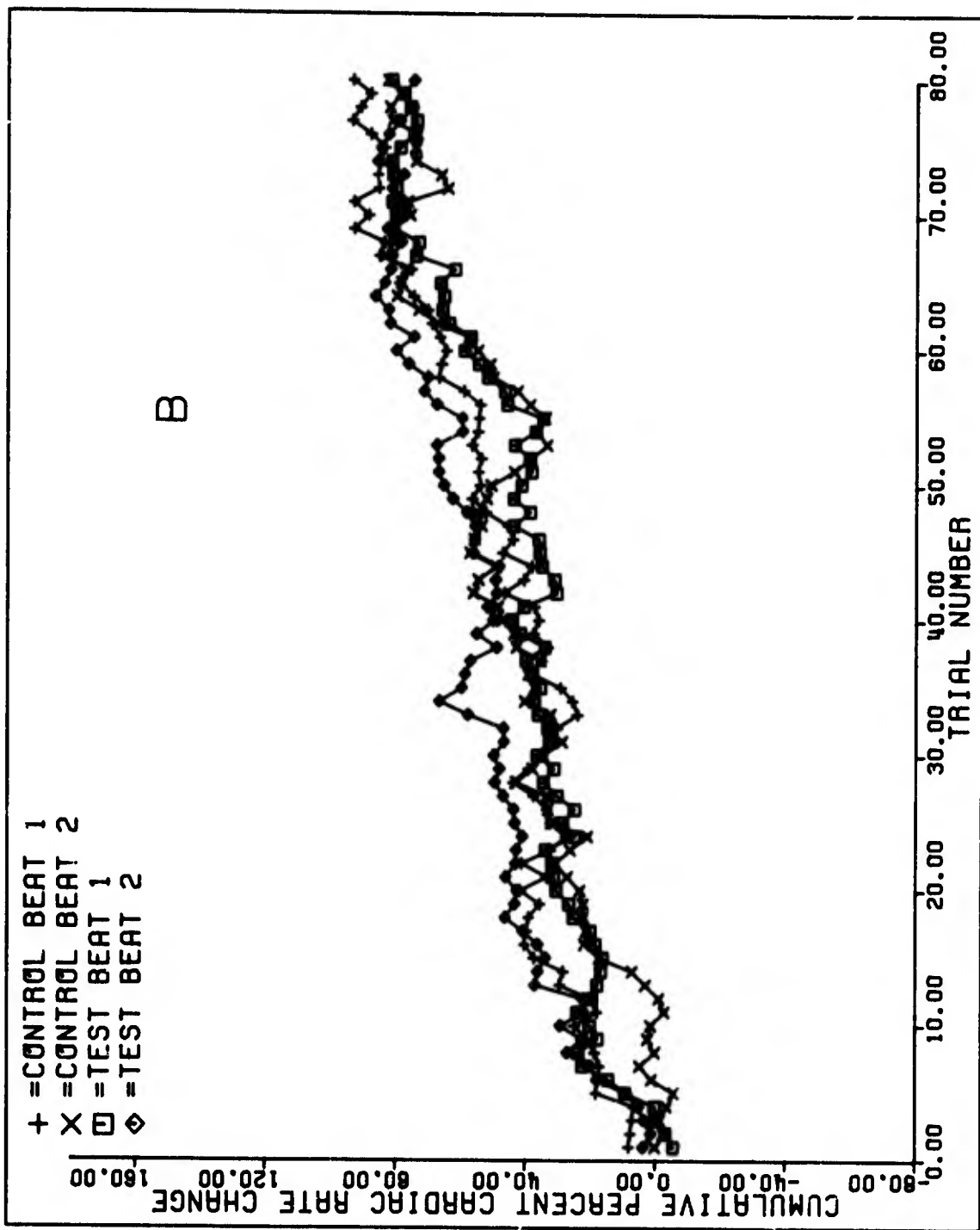












IV. LOCOMOTOR ACTIVITY PATTERNS IN ELF FIELDS

Introduction

The second step in examining whether a potential environmental contaminant will affect higher animals is to determine if the factor affects biological processes without conditioning. Even if a stimulus can be perceived (a premise in some doubt from the results of section III), it may not alter the normal behavior of the animal. Thus it was desirable to examine the possibility that locomotor activity patterns of salmon and eels were affected by exposure to ELF electric and magnetic fields. Locomotor behavior is a complex process which could potentially be affected by ELF fields at a number of levels.

Locomotor activity in fishes has previously been used as the measured response in studies of biological clocks (Davis 1964, Davis and Bardach 1965, Nelson and Johnson 1970, Kleerekoper, Taylor, and Wilton 1961, Müller 1970) and effects of low levels of natural chemicals (Kleerekoper and Mogensen 1963, Kleerekoper et al. 1961, Bardach, Todd and Crickmer 1967) and chemical pollutants (Davy and Kleerekoper 1973, Davy, Kleerekoper and Gensler 1973, Kleerekoper, Waxman and Matis 1973, Kleerekoper et al. 1972).

While the electromechanical method of recording locomotor behavior in fishes which we used is much less sophisticated than some (e.g. Kleerekoper et al. 1969), it has been used to determine diel patterns in American eels (Bohun and Winn 1966) and diel patterns in Atlantic salmon parr (Varanelli and McCleave in press, Richardson and McCleave MS in preparation).

Methods

Between June 1972 and October 1973, 12 experiments were conducted involving 96 Atlantic salmon and 48 American eels. Usable records were obtained from 88 salmon and 47 eels. Searun Atlantic salmon parr between 13 and 20 cm in total length were obtained from the Craig Brook National Fish Hatchery in East Orland, Maine. Most salmon were immature but a few were precocious males. Eels, 25-50 cm total length, were obtained from a commercial dealer in Passadumkeag, Maine. Salmon were put into individual activity chambers less than 24 hours after they arrived at our laboratory. Some eels, however, were held in tanks for several days (up to 17) before an experiment started.

In each experiment the locomotor activity of 12 fish was recorded for 10 days in a 12 hr light, 12 hr dark photoperiod (LD 12:12) except in one experiment of continual darkness (DD). Four fish served as controls (no ELF field), four were exposed to the electric or magnetic field for 24 hr periods every other day beginning with day 1, and four were exposed every other day beginning with day 2. Lights were automatically turned on at 0600 and off at 1800 EDT, and fields manually turned on or off at 1200 EDT.

The activity chambers were circular channels 10 cm wide by 27 cm deep. The channels were built from two concentric polyethylene cylinders, 47 cm and 27 cm in diameter. Air was bubbled into the center of the inner cylinder and circulated into the channel through holes in its inner wall. Two activity chambers were housed in each of six light-tight water baths. The chambers were water-tight and opaque so that fish in the same water bath had no visual or chemical contact with one another. Water was circulated through the baths from a refrigeration unit which maintained the temperature at $15 \pm 1^{\circ}$ C.

Temperature was continuously recorded on a strip chart from a thermistor in one of the chambers.

Weak a.c. electric fields were produced by an audio frequency oscillator and the output adjusted to provide the desired current flow. Eels were exposed to 0.07 V/m ELF fields and salmon were exposed to either 0.07 V/m or 0.7 V/m. Four aquaria were wired in series. Frequencies (60 and 75 Hz) were calibrated with an oscilloscope. An a.c. ammeter continuously monitored the current flow. A blocking capacitor in series with the tanks limited the d.c. component to less than 1 millimicroampere for an a.c. current of 0.5 milliamperes. Water conductivity was measured at the beginning and end of each experiment. Four control tanks were fitted with electrodes and wired just as the experimentals, but the oscillator was adjusted to zero.

The same aquaria were used, without electrodes, for magnetic field experiments. Three 2-m diameter coils (100 turns) were constructed to simultaneously expose four aquaria to a weak magnetic field. The field was produced by an audio frequency oscillator whose output was amplified by a stereo amplifier. The output of one channel energized the middle coil. The other channel energized the two end coils. Frequencies (60 and 75 Hz) were checked with an oscilloscope and an a.c. magnetometer before the experiments began. Current flow to the coils was checked once daily.

Activity was recorded electromechanically. Two plastic probes were suspended in opposite sides of each swim channel. The ends of the probes hung 2-4 cm off the bottom. The upper portion of each probe was attached to a piece of copper braid which hung through a carbon ring. When a fish moved the probe, contact was made with the carbon ring completing an electric circuit. This caused one count to be registered by an automatic counter. The two probes from each chamber were wired in parallel to one counter. The counters printed ten times per hour. Counter tapes were removed once each

day and the activity counts were coded directly for computer processing. A visual activity record was simultaneously produced for each fish by means of an event recorder wired in parallel with the counters.

Fish were not fed during the experiments to eliminate inducement of activity cycles based on feeding. Experiments were carried out in a basement laboratory which was usually entered only once each day at 1200 EDT. Efforts were made to isolate the fish from as many environmental disturbances as possible.

Initially light was provided by two 20 W fluorescent bulbs mounted on the lid of each water bath, one bulb directly over each chamber. This provided a range of illumination between 2.8 and 8.8 lux at the water surface. In later experiments the fluorescent fixtures were replaced by one 60 W incandescent bulb in each water bath (1-15 lux, depending on the position of the tanks).

Plots of the hourly activity for each fish over the course of each day were obtained with the aid of a computer. Composite plots encompassing all ten days were also produced for each fish. These plots were visually examined to determine the nature of individual activity patterns. Data from fish with similar patterns was combined to produce plots illustrating typical patterns.

Activity records were analyzed for periodicity by the periodogram method of Enright (1965). This method is appropriate for serially correlated data because it does not require random independent observations. The periodogram method calculates amplitudes for a series of possible period lengths within the range of interest. The amplitude in this case was the standard deviation of the hourly means of activity. The hourly means used for each amplitude were drawn from an arrangement of the data based on the assumption that a

given period existed. If a true periodicity exists, the amplitude produced by the proper assumed period length will be clearly larger than the other amplitudes. Computer calculated amplitudes were obtained for period lengths taken every tenth of an hour between 3.0 and 33.0 hours.

Results

Eels. The activity patterns of the eels tested in a 12:12 photoperiod were primarily night-active, light-change-active or erratic (Table 3). Light-change-active refers to peak activity when the lights were switched on or off. The total activity of these fish during the lighted portion of the day was about equal to their activity during the dark period. The predominate type of activity of fish exposed to electric fields was erratic or nocturnal while that of fish exposed to magnetic fields was nocturnal. In the control group the largest number of fish were active at light changes. These results are different from those of Bohun and Winn (1966) who found eels to be exclusively night-active.

Using the periodogram analysis we found only 8 of 47 eels in an LD 12:12 photoperiod to have an activity period near 24 hours (Table 4). Five of the fish with 24 hr periodicity were nocturnal and the other three were active at light changes. The greatest number of eels with a 24 hr periodicity are from the group exposed to magnetic fields. There are, however, fish with 24 hr periodicity in electric experimental and both control groups. Three other eels had weak activity period peaks somewhat greater than 24 hr (Table 4), but the majority of the fish showed no definite activity periodicity.

Table 3. Numbers of eels with various activity patterns in LD 12:12 or DD with or without electric or magnetic fields.

Activity pattern	Experimental conditions			
	LD	Control	Electric	Magnetic
Nocturnal		2	5	10
Diurnal		2	0	0
Light change		8	2	3
Erratic		4	8	3
DD				
"Nocturnal"		1	0	1
"Diurnal"		3	2	1
Erratic		4	6	6

Table 4. Period lengths and corresponding activity patterns of eels exhibiting periodicity with or without electric or magnetic fields in 12:12 light-dark conditions.

Experimental conditions	Period length (hr)	Activity pattern
Controls, Electric	24.0	Light change
	24.0	Light change
Controls, Magnetic	24.0	Nocturnal
	25.7	Nocturnal
	26.8	Light change
Experimental, Electric	23.9 (60 Hz)	Nocturnal
	28.8 (75 Hz)	Erratic
Experimental, Magnetic	23.8 (75 Hz)	Light change
	24.0 (75 Hz)	Nocturnal
	24.0 (60 Hz)	Nocturnal
	24.0 (60 Hz)	Nocturnal

The daily percentages of the total activity indicates that eels did not consistently increase or decrease their locomotor activity when either the electric (Fig. 4) or magnetic field (Fig. 5) was imposed. Also, the pattern and level of activity was very similar to that of the control animals (Figs. 4 and 5). The percent of total activity for days one and two was higher for eels exposed to 75 Hz electric fields. However, this was not higher than values for the controls for the 60 Hz electric fields; therefore it is probably due to normal variation in activity. There was no consistent decline in activity over the ten day period, although the means for fish in the electric field experiments were somewhat higher for days one and two.

When compared to either control groups or days the field was off, eels exposed to electric or magnetic fields did not consistently increase or decrease total activity during the period when the field was on. Six of 15 eels exposed to electric fields were more active on days when the field was on than they were on days when it was off, and 7 of 16 eels exposed to magnetic fields were more active on days when the field was on than they were when the field was off. One fish had a 248% increase in activity during the days it was exposed to an electric field, however, another had an increase of 177% during days when the electric field was off, and a control fish was 150% more active on even days than on odd days which indicate these values are all within normal variation.

Fig. 4. Percent of daily activity of American eels occurring on each day of a ten day experiment. Each point is a mean of counts taken from eels exposed to 60 (a) or 75 Hz (b) electric fields. Lines connect groups exposed to the same sequence of days field on vs. days field off.

○=FIELD OFF
■=FIELD ON
X=CONTROLS

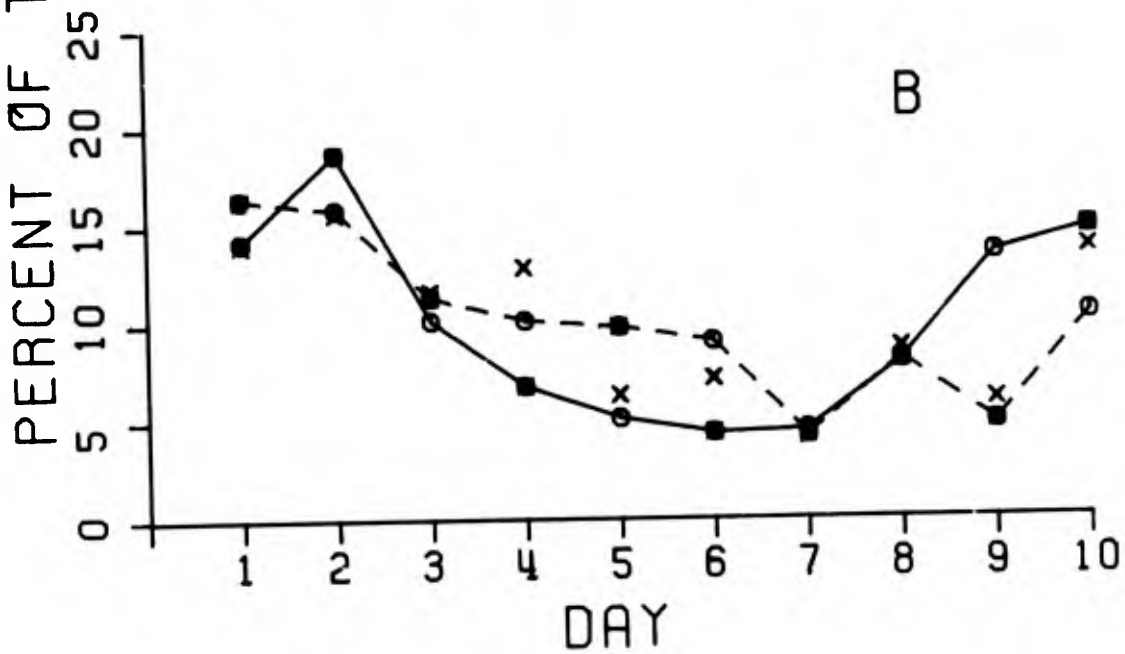
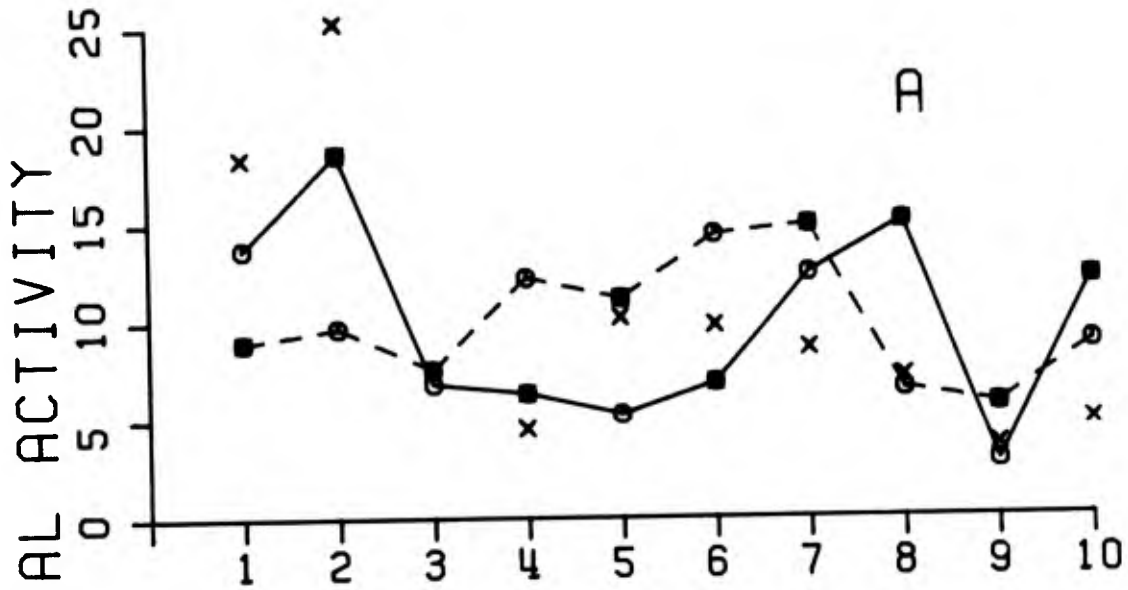
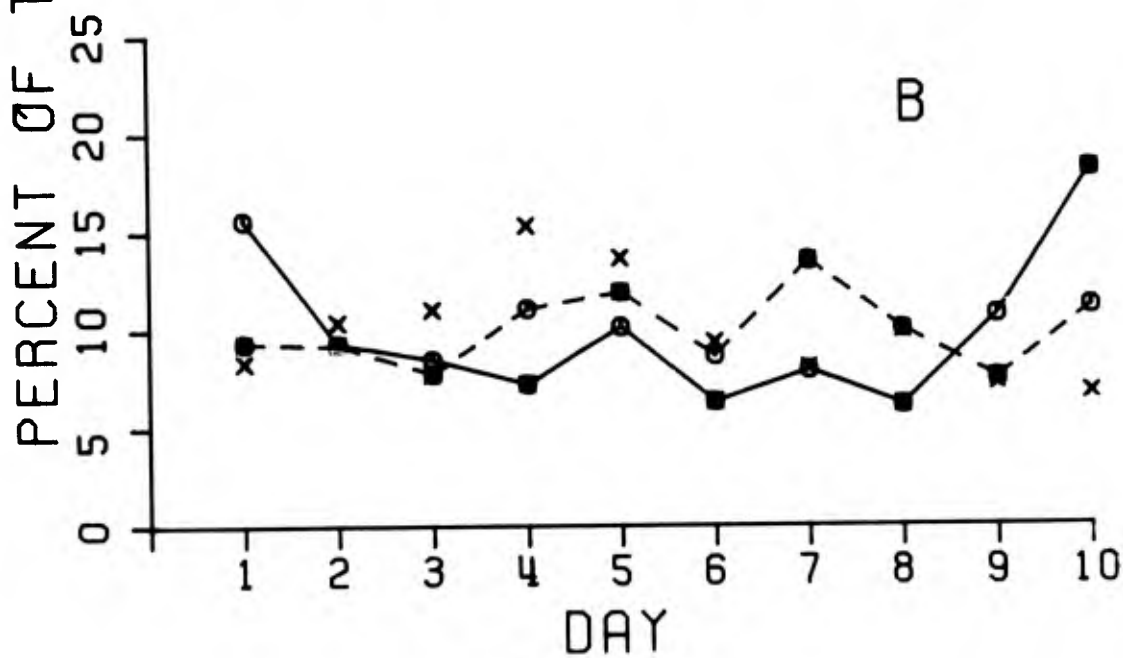
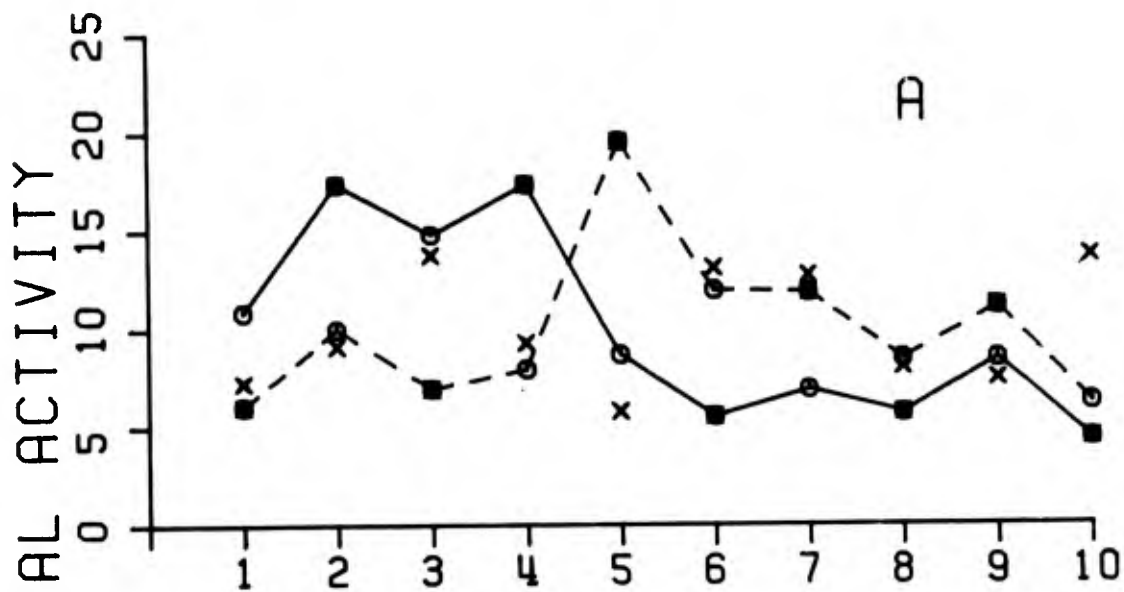


Fig. 5. Percent of daily activity of American eels exposed to 0.5 gauss magnetic fields. Other conditions same as Fig. 4.

○=FIELD OFF
 ■=FIELD ON
 X=CONTROLS



Salmon. No obvious differences in the level of activity of Atlantic salmon parr resulted from exposure to either electric or magnetic fields. The number of fish showing more activity counts when a field was applied (32) was close to the number showing more activity when no field was applied (28). The amount of activity for some fish was almost equal for days the field was on and days the field was off. The variation in activity counts for field off - field on was no greater for experimental fish than for controls (Fig. 6).

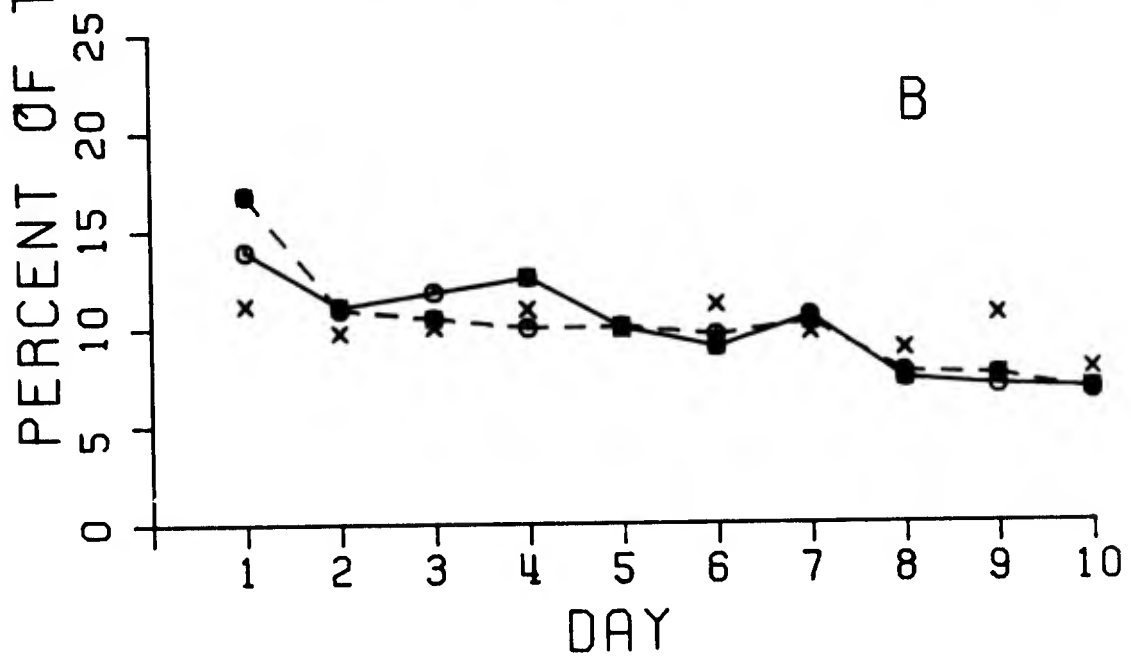
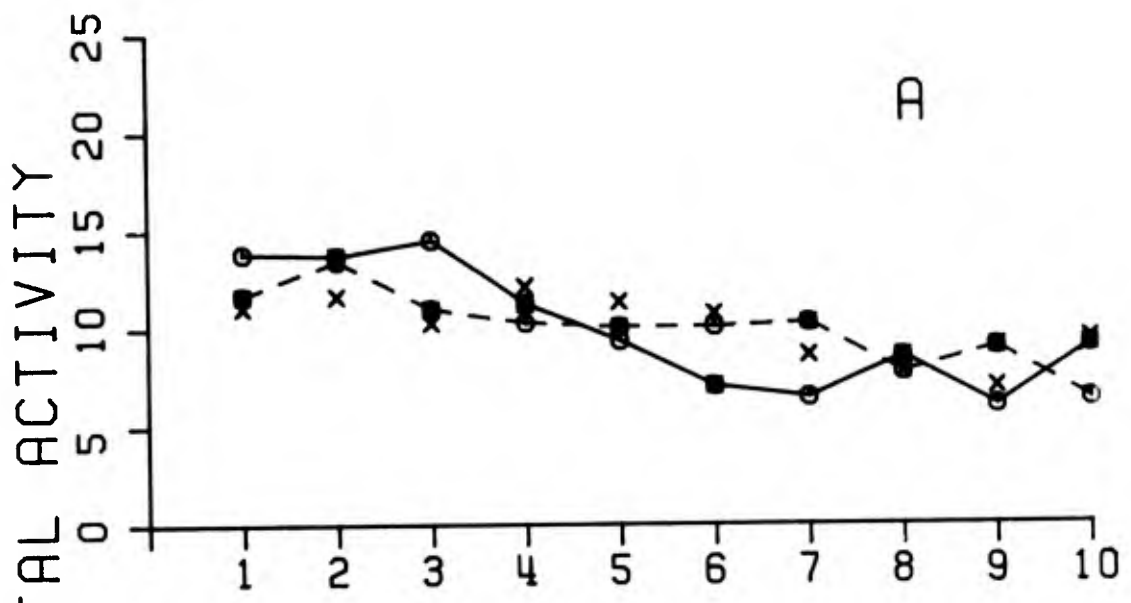
No differences in patterns of activity associated with ELF electric or magnetic field exposure could be detected. Fish subjected to a light-dark cycle exhibited one of three typical patterns of locomotor activity, light-active (35), dark-active (20) or light-change active (32). These patterns were determined from daily activity plots.

Periodogram analysis demonstrated that all three LD groups displayed 24.0 hr periodicity (Fig. 7). The light-change group was characterized by a distinct secondary maximum at 12.0 hr. Only one of 88 fish exposed to the light-dark cycle was aperiodic. Fish in the DD experiment were aperiodic with activity distributed throughout the day in sporadic bursts.

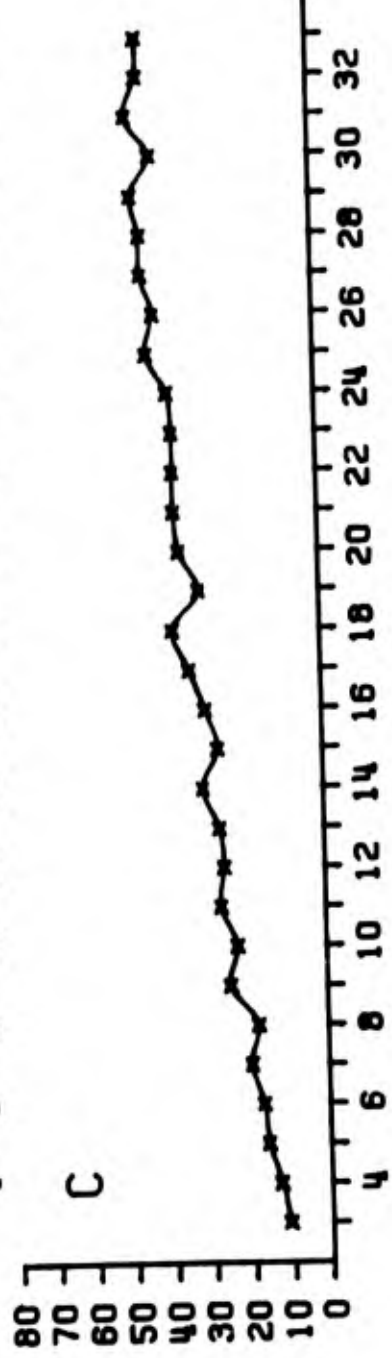
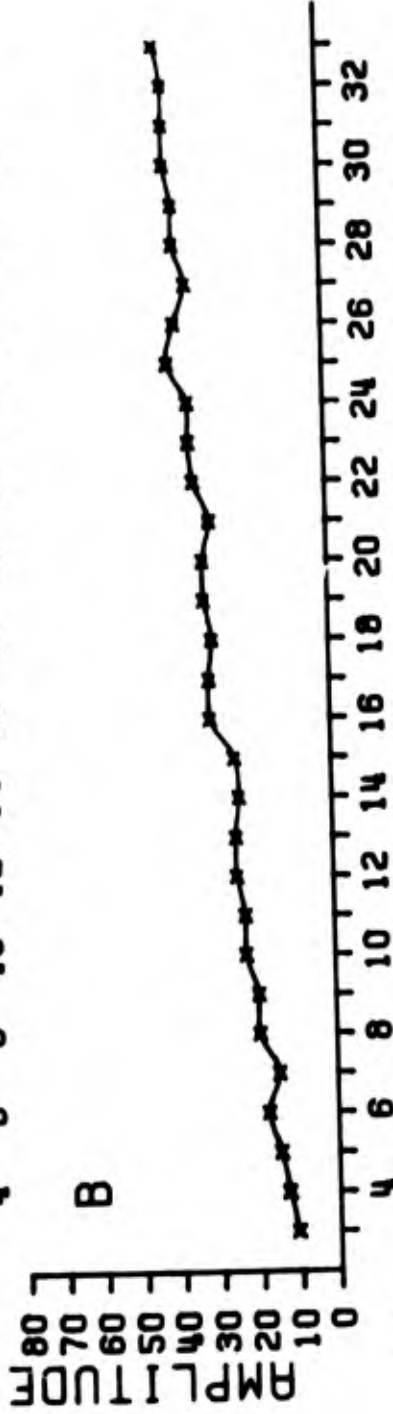
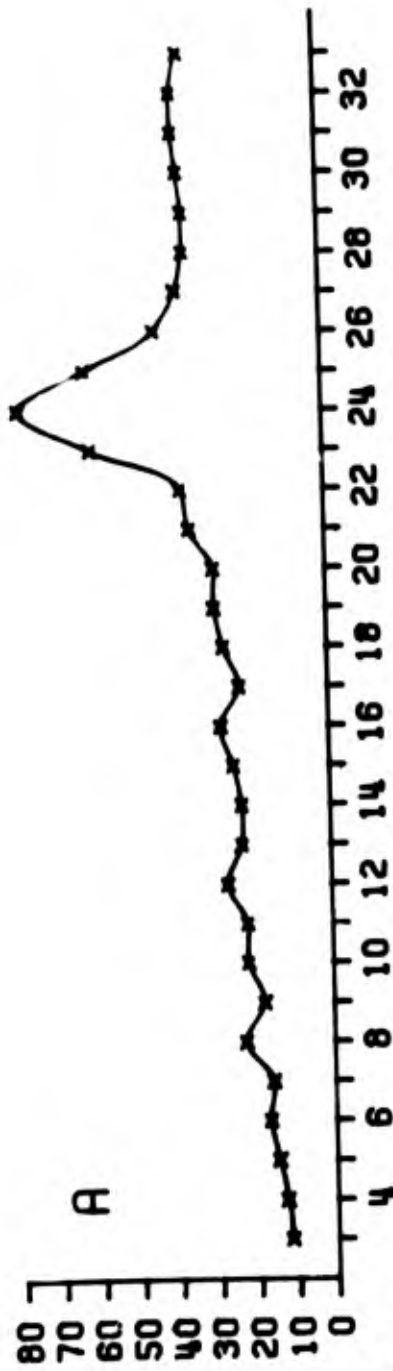
Data from experimental fish in the same behavior class were combined to produce plots illustrating typical patterns shown during field exposure and non-exposure. Composite plots were also drawn for the control fish of each behavior type. This procedure was applied for each experimental condition: LD 12:12 and 0.07 V/m, LD 12:12 and 0.7 V/m, DD and 0.7 V/m, LD 12:12 and 0.5 gauss. In all cases the activity patterns shown on days of field exposure did not differ from patterns shown on days of non-exposure or from patterns of control fish (Figs. 8,9,10).

Fig. 6. Percent of daily activity occurring on each day of a ten day experiment with Atlantic salmon. Each point is a mean of counts taken from salmon exposed to 75 Hz magnetic (A) or 75 Hz electric (B) fields. Lines connect groups exposed to the same sequence of days field on vs. days field off.

○=FIELD OFF
■=FIELD ON
X=CONTROLS



- Fig. 7. (a) Periodogram of salmon exposed to LD 12:12.
(b) Periodogram of salmon in DD with 23 hr periodic exposure to 7.0 mv/cm.
(c) Periodogram of salmon in DD, control.



PERIOD LENGTH, HR

Fig. 8. Composite of light-change-active salmon exposed on alternate days to 0.5 gauss (a) days field off (b) days field on and (c) control fish.

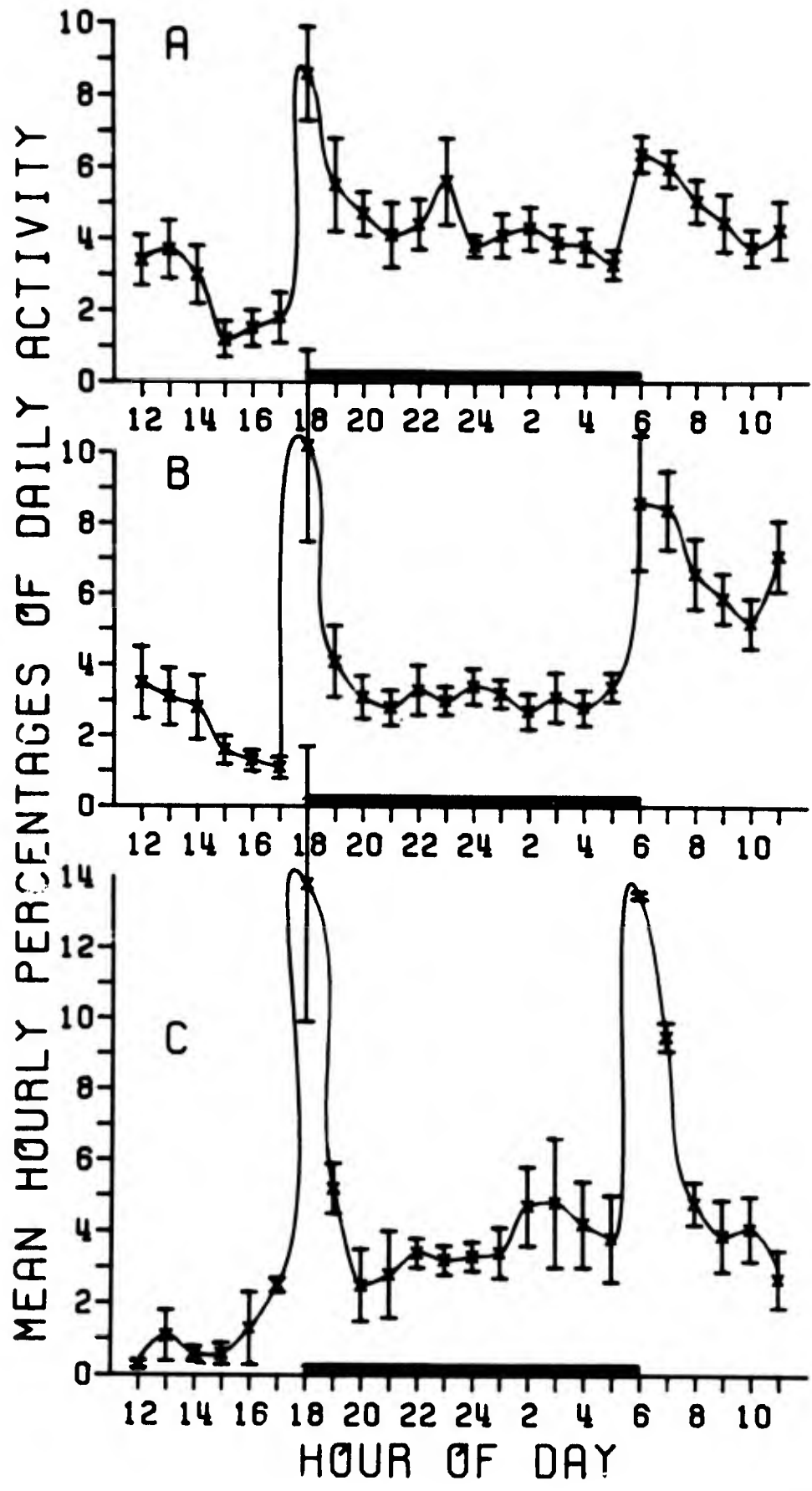


Fig. 9. Composite for dark-active salmon in LD 12:12 with alternate day exposure to 0.5 gauss (a) days field off (b) days field on and (c) control fish.

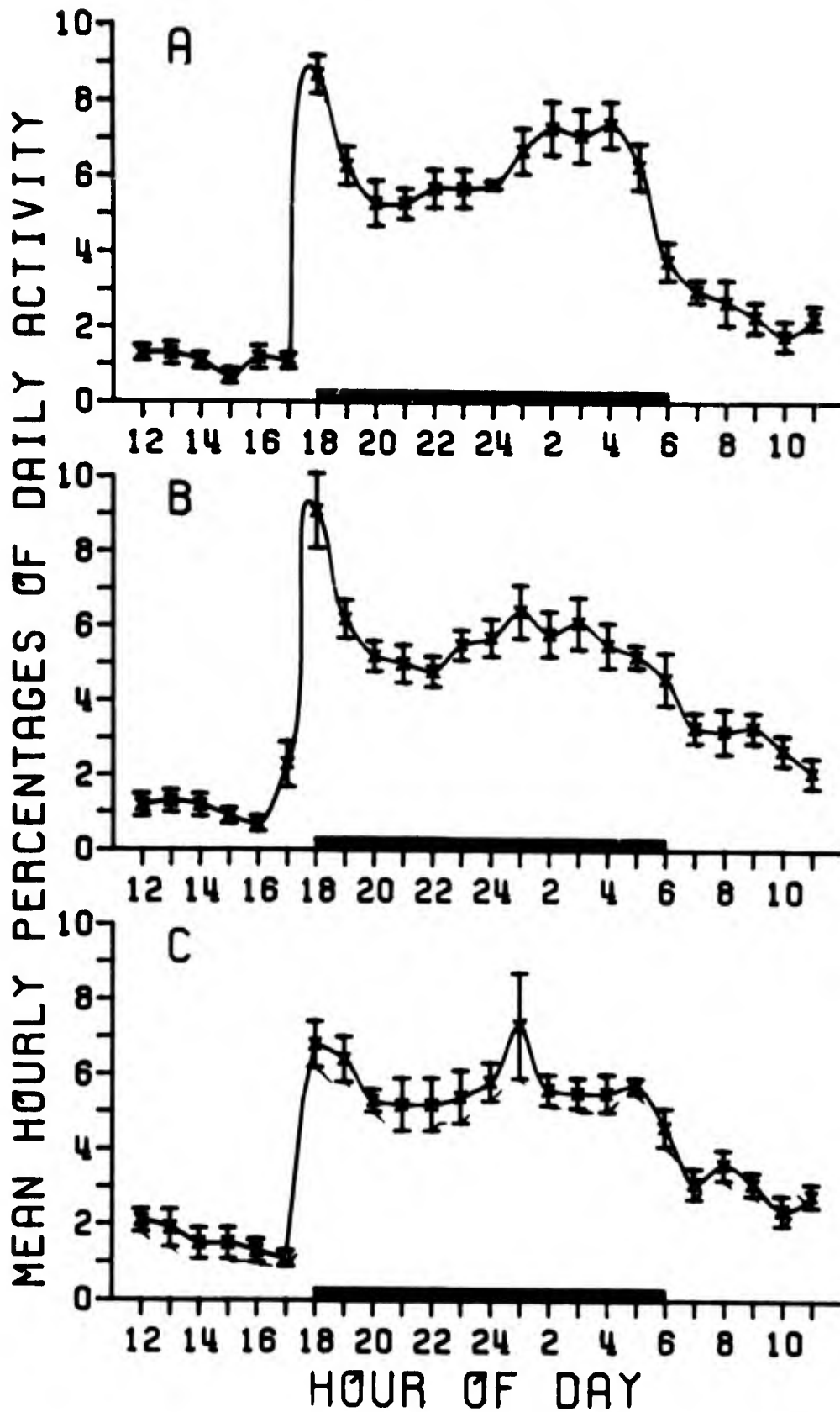
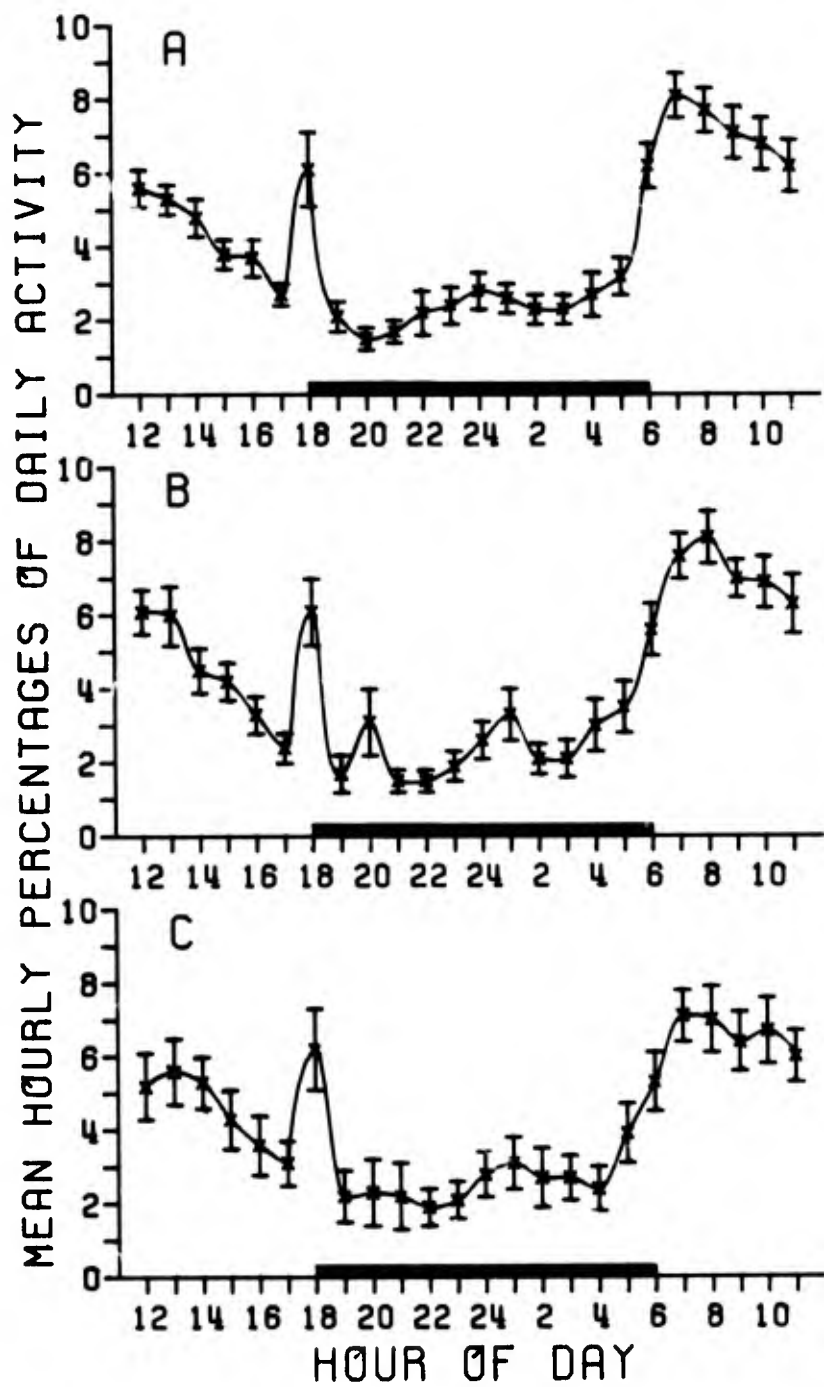


Fig. 10. Composite for diurnal salmon exposed on alternate days to 0.07 V/m (a) days field off (b) days field on and (c) control fish.



V. ENTRAINMENT OF CIRCADIAN RHYTHMS BY ELF FIELDS

Introduction

Our third objective was to determine if a circadian locomotor activity rhythm could be entrained by brief, periodic exposure to an ELF field.

Light is the most well known entraining stimulus for biological rhythms, but other periodic stimuli may serve to entrain rhythms. For example, feeding (Davis and Bardach 1965) and chemical odors (Kleerekoper et al. 1961) can serve as a circadian entrainment stimuli for fishes.

Methods

The test chambers and electromagnetic apparatus used in the entrainment experiments were the same as those used in the locomotor activity experiments. The procedure for these experiments, however, was slightly modified from that of the activity experiments. Fish were maintained for 10 days in either constant darkness or constant light. Every 23 hours 4 of the 12 fish were exposed to an ELF field for one hour, then 4 other fish were exposed for one hour and 4 fish (controls) were never exposed to an ELF field. Eels were exposed to 0.07 V/m ELF fields and salmon were exposed to 0.7 V/m. In one experiment, salmon were exposed to a 23:1 photoperiod rather than an ELF field stimulus. Usable records were obtained from 84 salmon and 24 eels.

The method of grouped-tau-comparisons (Binkley, Adler and Taylor, 1973) was applied to the periodogram data from the entrainment experiments. This method is used to determine if a signal has entrained a rhythm when the

activity records contain much noise or when the entrained oscillation has a low amplitude. It is assumed that the period lengths of a synchronized group of organisms will better approximate the entraining cycle than those of the non-synchronized group. Hence, the variance in the period lengths for the experimental group will be smaller than the variance for the control group if entrainment has occurred. Two ranges were chosen with the test period length, 23 hours, as the median value (20.5-25.5 hr and 18.0-28.0 hr). The period length having the maximum amplitude was determined for each individual within each range for a particular set of experimental conditions. The variances of the period lengths of the control and experimental fish in each range were statistically compared using a homogeneity of variance F test (Binkley et al. 1973, Steele and Torrie 1960).

Results

Eels. The periodograms from the 16 eels exposed to the electric or magnetic fields did not indicate a 23 hr period matching that of the stimulus. Two fish exposed to electric fields had indistinct activity periods of about 28 hr, however, this is not close to the period of the stimulus and one of the control fish had an activity period of about 27 hr which indicates these are not due to the electric field stimulus. The grouped-tau-comparisons test (Binkley et al. 1973) also showed that no significant differences existed between control and experimental groups.

There was little evidence that the eels maintained a natural activity pattern that might have been established before they were placed in constant

conditions. Two fish were active during the hours corresponding to night and five were slightly more active during hours corresponding to daytime, however, the remaining 17 had no apparent pattern of daily activity.

Salmon. No periodicity resulted from recurrent exposure at 23 hr intervals to an electric field, magnetic field, or light stimulus. Periodograms of experimental fish and of control fish kept in constant conditions were similar in showing a lack of rhythmicity (Fig. 7).

The locomotor activity of all fish exposed to electric fields, 18 in DD and 36 in LL, was aperiodic. All 11 fish exposed to magnetic fields with constant light were also aperiodic. Of the 11 fish exposed to magnetic fields and a 12:12 LD cycle, 9 were light change active and 2 were nocturnal. However, the activity periods were between 23.9 and 24.0 hours not the expected 23 hours if entrainment to magnetic fields had occurred.

The grouped-tau-comparisons test (Binkley et al. 1973) was applied to the periodogram amplitude data for both electric and magnetic experiments in DD and in LL. No significant differences existed between control and experimental groups. A lack of entrainment on the part of fish subjected to a recurring light stimulus was surprising because this species had shown marked responses to lights being turned on or off in 12:12 light-dark cycles.

VI. DISCUSSION

Results of our cardiac conditioning experiments suggest that the threshold of perception for 75 Hz electric fields by American eels is about at Sanguine level or perhaps lower by a factor of ten. The threshold for Atlantic salmon is probably about Sanguine level or perhaps slightly higher.

This is in distinct contrast to thresholds for American eels and Atlantic salmon to d.c. electric fields reported previously (Rommel and McCleave 1972, 1973a). Their results suggest d.c. thresholds about 10^2 lower for eels and 10^4 lower for salmon. These differences in sensitivity may be attributable to a real difference in sensitivity to d.c. and ELF fields, or to some difference in conduct of the d.c. and ELF experiments.

There is some evidence to suggest the latter. We conducted a small series of d.c. electric field experiments, not reported here, in which we did not find the threshold to be as low as reported by Rommel and McCleave (1972, 1973a).

There is also evidence to support the belief that eels and salmon are much less sensitive to ELF electric fields than to d.c. fields. According to Bullock (1973) most of the non-electrogenic electroreceptive fishes, at least those with ampullary type electroreceptors, are sensitive to electric fields between 0.1 and 10 Hz and sensitivity falls off rapidly above 10 Hz. The fall in sensitivity screens out higher frequency noise.

Weakly electrogenic fishes that use electric signals they produce to electrolocate or communicate are sensitive to ELF fields of 50 to 2000 Hz depending on the species (Bullock 1973).

Most of the non-electrogenic animals probably use their electroreceptive abilities in preying (Kalmijn 1971, Peters and Bretschneider 1972) or perhaps

in electro-orientation (Rommel and McCleave 1973 a,b, Roth 1969). In either case the d.c. fields are the necessary ones to be sensed. The swimming movements of the fish are sufficient to convert the d.c. signals into ones of 0.1 to 10 Hz (Bullock 1973).

Neither the work of Rommel and McCleave (1973a) nor the present cardiac experiments provides evidence for a d.c. or ELF magnetic field sensitivity in eels or salmon. In all cases in the literature in which sensitivity to a magnetic field is shown, the sensitivity is actually to the electric field produced by motion of the magnetic field.

Despite the fact that both eels and salmon can apparently sense electric fields of Sanguine intensity we found no evidence that their locomotor activity levels or patterns were affected by exposure to such fields. In our experiments levels up to ten times greater than those expected near a Sanguine antenna failed to produce detectable activity changes.

Greenberg (1973) has pointed out the difficulty of demonstrating that a low level environmental contaminant will not have an effect on organisms in the environment. Additionally it is risky to extrapolate from selected organisms to the flora and fauna as a whole.

There are members of the families Anguillidae, Salmonidae and Ictaluridae, known to be sensitive to d.c. electric fields at or below Sanguine levels (Rommel and McCleave 1972, 1973a, Roth 1968, 1969), which inhabit areas under consideration as Sanguine sites. Since so few fishes have been tested for electrosensitivity there are undoubtedly other sensitive species. Since the electric field decreases in intensity rather slowly at increasing distances from the antenna, a relatively large area may exist in which fishes could detect the fields.

However, since we were unable to detect changes in activity patterns,

the ELF radiation from the Sanguine system probably would little affect normal behavior of anguillid and salmonid fishes. There are no weakly electrogenic fishes (that depend on ELF fields for normal activities) in North America, so use of the highest frequency compatible with the communication requirements of the Sanguine system would serve to minimize any effect on the fishes.

VII. ACKNOWLEDGMENTS

Dr. Sentiel A. Rommel, Jr. assisted greatly with ideas, design of experiments, and construction and testing of apparatus during the early stages of the research. Mrs. Carole C. Varanelli also assisted initially. We thank Mr. Gerald Crommett who supplied all our eels, and Craig Brook National Fish Hatchery for supplying the salmon. Computing and Data Processing Services, University of Maine, provided computer and keypunch time.

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Locomotor activity levels or diel patterns of the fishes were not affected by alternating 24 hr periods of exposure and non-exposure to the ELF electric or magnetic fields. Activity rhythms were not entrained (synchronized) by 1 hr exposures every 23 hr to ELF electric or magnetic fields. It is concluded that while at least these two species can probably perceive Sanguine electromagnetic radiation, their normal behavior is unlikely to be affected by such fields. Higher frequencies will probably result in less effect than lower frequencies.

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