The effects of induced magnetic fields on the larvae of the American Oyster, Crassostrea virginica

by

Steven A. HARTMAN

The American Academy of Ocean Sciences, 501 Fifth Avenue, New York, N.Y. 10027

and

George CLAUS

Offshore/Sea Development Corp., 99 Nassau Street, New York, N.Y. 10038

INTRODUCTION

In 1600, William Gilbert first postulated the existence of a general magnetic field near the surface of the earth. At the north and south magnetic poles the magnetic lines of force are vertical, but at varied points between the poles the force becomes more parallel to the earth, and finally at the equator they are completely parallel to the surface of the earth. The earth's field intensity is approximately 0.7 gauss. There are, however, some exceptions, such as the area near the ore deposits at Sör-Varanger in Norway, where the field measures 1.4 gauss. The field of the earth can best be represented by a dipole (Liboff, 1965).

There is no reason te exclude magnetic fields as having a possible influence on life activities. It is, therefore, reasonable to assume that by changing this factor in various degrees, one might be able to observe a phenotypical change in an organism.

Early studies like those of Dubois (1886) showed that static magnetic fields have a defined effect on the orientation of different bacteria in their colonies. Slater had found in 1885 that magnetic fields have a strong influence upon the metamorphosis and development of insect larvae. Either the period required for metamorphosis was shortened, or the developed individuals showed several types of abnormality, depending upon the field strengths employed.

The literature dealing with the biological effects of induced static magnetic fields which exceed by at least a hundred times that of the earth is rather restricted. From the end of the last century until 1964, according to the very thorough bibliography of Gross (1964b), only 114 papers were published dealing with this subject, and an additional 18 have appeared during the past six years. Most of the work has centered around the investigation of the effects of magnetic fields on tissue culture cells, including malignant tumors, or on the protective action of magnetic fields in radiation damage. Only a few works deal with subjects directly applicable to the studies undertaken.

Brown and his associates (1960; Webb et al., 1961), working at the Marine Biological Laboratory at Woodshole, obtained some interesting results with mud snails (*Nassarius* sp.). They noted that the snails could detect differences in magnetic field intensities and orientation. Further tests by Brown (1962) involving planaria, indicated that these animals were able to detect different angular orientations of magnetic fields. Brown et al. say (1960:384): "Responsiveness to electrostatic fields may possibly be one of the normally contributing factors in the timing system of the extraordinary clocks of animals and plants." In 1961, Kholodov and Verevkina ascertained that the reflexes of marine fishes can be affected by the deployment of magnetic fields to the degree that their egg laying habits can be changed.

Gross (1964, 1964a) found an array of different effects produced on mice in varying magnetic fields. Some of these were: leucocytosis, inhibition of growth of tumor grafts, delay in fibroblast proliferation and fibrosis, delay in rise of antibody titer and a reduction of the peak titer level. He proposed that an inhibition of enzyme activity obeying the Michaelis-Manton equations was responsible for the results. Gross states (1964a:138) "We also postulate that magnetic fields act by distorting the bond angle or orbital of the para-magnetic molecules involved as enzyme or substrates in biochemical reactions. An external magnetic field distorts the orbital of the unpaired electrons and alters the fit of enzyme to substrate, thus disturbing and delaying the reaction." Although this work was carried out in a rather specialized case, tumor growth, the results would seem to point to the potential importance of studying the effects of an externally induced magnetic field on the various functions of life forms. Indeed, Palmer (1963, 1967) showed that *Volvox* exhibited an active behavioral response (as opposed to a purely physical attraction or repulsion) to an orientation change of an induced five gauss magnetic field. Similar effects were shown by Becker (in Gauquelin, 1967: 141), regarding the orientation of insects, which align themselves along the lines of terrestrial magnetic force.

Recently Schneider (1964), demonstrated that cockchafers respond to both magnetic and gravitational forces, exhibiting a positive reaction toward the invisible approach of a mass of lead weighing 80 lbs. or more. This would seem to be a direct confirmation of the experiments of Fehér and his co-workers (1943-1950, see Fehér, 1954), who showed that sprouting beans will react with either positive or negative tropisms towards masses of purified elements. This field effect of pure elements was confirmed by Hollerbach in 1953, but it failed to gain scientific acceptance because of the lack of any physical explanation of the phenomenon.

The investigation of the effects of magnetic fields on living organisms is still at a relatively primitive stage. It is possible that his area of endeavor will elucidate several biological mechanisms which have previously been inexplicable. The demonstration of a biomagnetic effect on the growth and development of organisms seems to be an important field of future research. On the basis of the studies already done, one could speculate that the setting and orientation of oyster larvae may also be affected by exposure to high magnetic fields.

That biomagnetism exists is no longer doubted, but the number of unquestionably proven cases is still relatively small. By employing large numbers of organisms under well defined conditions, and exposing them to rigidly controlled magnetic fields, one should be able either to demonstrate the influence of these forces on the growth and/or the orientation of the oyster larvae, or, conversely, to show that there is no such influence.

MATERIALS AND METHODS

To produce the desired high magnetic fields, a solenoid was fabricated by coiling 520 feet of enamel insulated 12 gauge wire around a 4 inch diameter cardboard cylinder. There was 403 turns around the cylinder, a number significant in determining the magnetic field strength produced. The solenoid was placed on a stand to hold it either in the horizontal or vertical position. The stand, made out of wood in order not to interfere with the field pattern, had an instrument panel with a volt and ammeter to register the field intensity. A Sola(R) solid state, low ripple, variable amperage power source with a range of 0-10 amps was used to energize the coil.

The free swimming larvae of the American oyster, *Crassostrea virginica* (Gmelin), were used for the experiments. The specimens were obtained through the courtesy of Mr. Phillip Campbell from Long Island Oyster Farms. They measured approximately 300μ across the hinge, being thus close to metamorphosis. According to Mr. Campbell, the larvae at this stage may set anytime between two to four days, a statement confirmed by Loosanoff et al. (1966). They found that laboratory grown larvae of this species will set at lengths varying from 275 to 315 μ.

Four plexiglas tanks were prepared to hold the oyster larvae, two to be inserted into the coil, while two identical ones served as the controls. Each tank was 18 inches long, cylindrical in shape, with a $3\frac{1}{2}$ inch interior diameter and a 1/8 inch wall thickness. Two of the tubes were sealed on one end so that they could be positioned vertically for those experiments in which the generated magnetic field was parallel to the gravitational force. The other two tubes were plugged on both ends with plexiglas and a circular window was cut in their center with a diameter equal to that of the interior of the tubes ($3\frac{1}{4}$ inches). A plexiglas ring, $\frac{1}{2}$ inch high, was pasted onto the rim of the window, thereby providing an area for aeration equal to that of the other tanks. These latter tubes were used in the horizontal position in the experiments where the effect of magnetic fields perpendicular to gravity were tested.

Moving air produced by two fans served to cool the system during the experiments. This arrangement maintained the temperature of the water in the coil at the desired level. Cooling became necessary because the coil running at 10 amps produced a maximal inside temperature of 1C4°F, which was above the tolerance limits of the larvae. The air from the fans passed between the inside of the cardboard cylinder and the outside of the plexiglas tank within the coil. The plexiglas acted as an insulator while the heated air between the coil and the insulator was removed by the passing air. The temperature of the control was maintained to equal that of the experimental tank by keeping it in a rheostatic water bath regulated by a probe inserted into the experimental tank.

In each tank for each experiment and for each control one liter of filtered and autoclaved sea water was used. In the initial experiments a concentration of 200 larvae/liter was employed. Culch of oyster shells was placed into each tank to facilitate setting. The total area of the top and bottom of the shells was about 30 cm^2, varying slightly from one experiment to another. In the subsequent experiments, however, the numbers of oyster larvae and the culch areas were halved in order to eliminate the accumulation of possible toxic waste products.

Before each experimental series two batches of oyster larvae were acquired from the Laboratories of Long Island Oyster Farms (Oyster Bay, N.Y.), in two separate flasks of one liter of sea water, and were transported in an air conditioned car to the laboratories, in order to maintain constant temperatures. Two hundred larvae from each flask were randomly selected and transferred into the experimental and control plexiglas tubes, respectively.

After transferring the larvae into the plexiglas tanks they were fed. Larval feeding habits are rather specific and the methods of feeding have to be exact in order to avoid either malnutrition of the animals or pollution of the tanks. In the preliminary tests *Monochrysis lutheri* Droop (Droop, 1955), a Chrysophycea, was used as the food source, while during the main experimental series *Dunaliella tertiolecta* Butcher (Butcher, 1959), a Chlorophycea, was employed. This alga has to be fed to the oysters at the rate of 300 organisms/hour/larva. Since feeding was carried out only once a day, 7200 algal cells had to be supplied for each oyster (or 1,440,000 at each feeding in the experiments which utilized 200 larvae). During the later tests in which larval numbers were halved, the amount of food organisms introduced was also reduced by one-half. To determine the exact quantity of algal cells to be fed daily, the cells were centrifuged in a calibrated tube, and their packed volume established and correlated with microscopic counts obtained on 0.1 ml of the original culture. New batches of algae were used for each new experiment.

Temperature readings on the tanks were taken as often as feasible, usually 12 to 14 times every 24 hours. The instrumental readings on the panel of the power source were frequently compared to those on the coil stand, in order to ascertain that the proper and desired amperages were continuously present.

At the completion of each test the larvae were transferred into one liter flasks and immediately observed for any effect. The numbers of surviving animals were established through microscopic determinations, while the culches were investigated for sets.

A total of 12 experimental series were conducted, each consisting of an actual test and a control. At the two selected field strengths the effects of both exposure time (from two to four days) and vertical and horizontal coil positions were tested. Three preliminary experiments served to establish the proper configuration and functioning of the equipment. Since these preliminary tests led to high

Experimental Series	Amperes	Field Strength in weber/m^2	Direction of Field	Duration of Experiment in Days	Number of Larvae	Temperature Extremes in ^{o}C	Area of Cutch in cm^2	Number of Sets	Counts of Mortality	Percentage of Mortality
Experiment No. 1 Control No. 1	10	5.8×10^{-3}	Horizontal	4	200	25-28 25-26	28.0 32.0	0 1	200 20	100 10
Experiment No. 2 Control No. 2	10	5.8×10^{-3}	Horizontal	4	200	25-28 24-25	28.0 30.0	0 1	200 18	100 9
Experiment No. 3 Control No. 3	10	5.8×10^{-3}	Horizontal	4	100	25-28 24-25	15.5 16.0	0 2	100 14	100 14
Experiment No. 4 Control No. 4	10	5.8×10^{-3}	Horizontal	3	100	25-27 25-26	14.0 16.0	0 1	100 15	100 15
Experiment No. 5 Control No. 5	6	3.5×10^{-3}	Horizontal	4	100	24-25 24-25	15.0 16.0	0 2	100 16	100 16
Experiment No. 6 Control No. 6	6	3.5×10^{-3}	Horizontal	3	100	24-25 24-25	15.0 16.0	0 2	16 16	16 16
Experiment No. 7 Control No. 7	6	3.5×10^{-3}	Horizontal	2	100	25-26 25-25	15.5 14.5	0 0	8 6	8 6
Experiment No. 8 Control No. 8	10	5.8×10^{-3}	Vertical	4	100	24-25 25-25	17.0 16.5	6 3	94 13	94 13
Experiment No. 9 Control No. 9	10	5.8×10^{-3}	Vertical	3	100	25-26 25-25	14.5 17.5	7 3	93 15	93 15
Experiment No. 10 Control No. 10	6	3.5×10^{-3}	Vertical	4	100	24-25 25-25	15.5 16.0	8 4	20 13	20 13
Experiment No. 11 Control No. 11	6	3.5×10^{-3}	Vertical	3	100	25-26 25-25	15.0 16.5	9 4	18 11	18 11
Experiment No. 12 Control No. 12	6	3.5×10^{-3}	Vertical	2	100	25-26 25-25	17.0 16.0	6 1	10 9	10 9

TABLE 1

mortality rates in the controls, their results are not reported here.

RESULTS

The experiments produced some unexpected results which are compiled in Table 1, together with the values for the various experimental parameters. In all those series in which a field strength of 5.8 x 10^{-3} webers/m^2 was used, 100% mortality of the larvae occurred. That this could be attributed to the prolonged exposure to high magnetic fields became evident from a comparison of the results of experiments nos. 2 and 3 with those of experiments nos. 4 and 6. Because of the possibility that 200 larvae/tube produced large quantities of toxic metabolites resulting in the eventual death of the organisms, the larval number was halved in experiment no. 3. Even in this case, however, employing otherwise identical conditions, 100% mortality was found. When exposure time was decreased but field strength remained the same, as in experiment no. 4, still no larvae survived. In experiment no. 5, when field strength was decreased but the exposure period was retained, 100% mortality likewise occurred. Therefore, only a decrease of field strength with a concurrent decrease of exposure time resulted in a mortality rate comparable to that of the controls (experiment no. 6). Further decrease of exposure time (experiment no. 7) had no effect on either setting or survival.

In the above discussed seven experiments the coil was in the horizontal position, producing a magnetic field perpendicular to the force of gravity; while in the remaining experiments it was changed into the vertical position, resulting in a field parallel to that of gravity. The geometry of the arrangement was such that the direction of the field was the same as that of gravity.

At a field strength of 5.8 x 10^{-3} webers/m^2, at exposure times of three and four days (experiments nos. 8 and 9) this position resulted in only 93-94% mortality in comparison to 100% obtained under identical conditions with perpendicular magnetic fields (experiments nos. 1, 2, 3 and 4). It was noteworthy that the number of sets under this condition was more than double that of the controls. A reduction of the field strength to 3.5. x 10^{-3} webers/m^2 (experiment no. 10), with parameters the same as in experiment no. 8, resulted in approximately normal mortality but in a significantly increased number of sets. In contradiction with the results obtained under the same conditions with the horizontal coil (experiment no. 5), where no setting and 100% death rate occurred, a reduction of exposure time in the vertical position no longer affected the survival rate of the organisms, but produced even at 48 hours exposure to the field a high increase in the number of sets. Thus, the last two experiments (nos. 11 and 12) of the vertical series differed significantly in results from the two comparable horizontal tests (nos. 6 and 7) in that, in the former, setting seemed to be stimulated by the magnetic field, while in the latter, the field apparently inhibited the process.

No deviations from expected normal mortality rates or numbers of sets were shown by any of the 12 controls.

DISCUSSION

From the above experiments the following generalized patterns can be deduced. Exposure to a 5.8×10^{-3} webers/m^2 horizontal field, even for three days, completely inhibits setting and has a 100% lethal effect on the larvae. At the next tested level (3.5×10^{-3} webers/m^2), although no setting occurs in the horizontal field, the survival rate is dependent upon exposure time. Three days exposure permits normal survival rates to occur, while four days results in 100% mortality. It seems that the lethal exposure time lies between seventy-two and ninety-six hours. Exposure to vertical fields at both field strengths showed a favorable influence on setting. Although the exposures to higher strength fields induced a higher mortality rate, even four days exposure to the lower field strength permitted the survival of the organisms.

Thus, two significant points emerge from the experiments. First, the oyster larvae are highly sensitive to exposure to magnetic fields. Secondly, the larvae react differently when the induced field in relation to the force of gravity is perpendicular or parallel.

Previous investigations, conducted mainly with micro-organisms, have not succeeded in demonstrating appreciable biological effects from electromagnetic fields in the ranges employed in the present study. Fields of 100,000 webers/m^2 or even higher were necessary to produce recognizable damage or death of test organisms. Therefore, it seems rather important that the larvae of the American oyster are so sensitive to magnetic fields that even three days exposure to 5.8×10^{-3} webers/m^2 will cause a 100% mortality in their population. It appears that oyster larvae could be utilized for detecting magnetic field variance.

It is probably even more significant that these organisms are affected not only by the field strength and duration of exposure, but also by its direction relative to the earth's gravitational field. It does not seem to be far fetched to presume a combined effect of the gravitational and the induced electromagnetic forces, bringing about the directional discrimination, as exhibited by the increased setting rate of the larvae when exposed to vertical magnetic fields. The large numbers of survivors in the animals while under the influence of fields parallel rather than perpendicular to that of gravity also indicate that no opposing forces acted upon the organisms in this configuration. In view of these facts it is tempting to speculate about a close interaction of the electromagnetic and gravitational forces, of which the oyster larvae seem to be highly sensitive indicators. The recent discovery of gravitational waves by Weber (1969), seems to further substantiate this statement regarding the possible interaction of electromagnetic and gravitational fields.

At the present time it is somewhat difficult to determine the exact biological cause of the observed phenomena.

That life manifests itself in electrical potential or potential charges was first postulated by Ingvar (1920), elaborated by Lund (1922), and more thoroughly substantiated by Gurwitsch (1922). The latter postulated that any life function, including death, creates measurable electrical fields, which interact with those of other organisms and those present in the environment. This hypothesis has been confirmed experimentally by Burr, who has written a series of articles on the subject, of which his 1932 contribution seems to be the most important. In 1935, he formulated his ideas into a philosophical theory on the nature of life in a joint publication with Northrop.

Burr's theoretical reasonings were not generally accepted, and until the early sixties his hypothesis regarding the electrodynamic nature of life seemed to be forgotten. At that time Piccardi began his investigations into the structure of water. In 1962, he described the extreme sensitivity of the structure of water to electric or magnetic fields. Since on the average water constitutes 65% of the bodies of living organisms, it is not surprising that even slight changes in the electrical or magnetic environment can influence profoundly the physiology and/or behavioral patterns of living things. These ideas are very close to those expressed by Brown (1962a:782):

> "Physiologists must recognize that organisms, even when shielded and screened from all ordinary factors to which they

have classically, been deemed sensitive, still are obtaining information about their rhythmic external environment on our planet."

Another hypothesis advanced by Gross (1964), postulates the distortion of molecular bonds within the chemical compounds of the cells. Such bond distortion may cause steric changes, either in enzymes or substrates, which prohibit their coupling mechanism. If enzyme action is inhibited, life functions can no longer exist. The fields employed in the present experiments, however, were too low to result in drastic bond distortions, thus, the observed effects were probably not caused by this mechanism. Indeed, it is more likely that interaction between fields, or influencing of the structure of body water might have caused the observed changes in the oyster larvae. At any rate, further investigations seem to be warranted, both in order to utilize the remarkable sensitivity of oyster larvae towards magnetic fields and to attempt to define the biological basis of this sensitivity.

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SUMMARY

The influence of high magnetic fields was tested on the larvae of the American oyster, at the period of their setting. Two field strengths (5.8×10^{-3} and $3.5. \times 10^{-3}$ webers/m^2), of two to four days of exposure, were employed in configurations either parallel or perpendicular to the earth's gravitational field. It was found that the magnetic field at the higher level resulted in 100% larval mortality while perpendicular to gravity, both at three and four days of exposures. The same field induced a 93% mortality together with an approximate doubling of sets above the normal controls while parallel to gravity at the same exposure times. Shorter exposures to this field were without effects. The lower magnetic field resulted in 100% larval mortality while perpendicular to gravity at four days of exposure but was without effect at shorter exposure times. When parallel to gravity it affected only 20%

mortality with a simultaneous doubling of sets at four days of exposure and had no lethal effect but still increased the number of sets at any shorter exposure time tested.

It seems that oyster larvae are sensitive indicators of the presence of higher magnetic fields and that their life functions are influenced not only by the strength of field employed but also by its direction. The underlying biological mechanism of this phenomenon is unknown.

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