Studies on the Effects of Thermal Additions on Selected Zooplankton Populations

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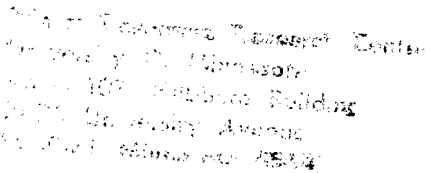

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

FOREWORD

This Bulletin is published in furtherance of the purposes of the Federal Water Resources Research Act of 1964. The purpose of the Act is to stimulate, sponsor, provide for, and supplement present programs for the conduct of research, investigations, experiments, and the training of scientists in the field of water and resources which affect water. The Act is promoting a more adequate National program of water resources research by furnishing financial assistance to non-Federal research.

The Act provides for establishment of Water Resources Research Centers at Universities throughout the Nation. On September 1, 1964, a Water Resources Research Center was established in the Graduate School as an Interdisciplinary component of the University of Minnesota. The Center has the responsibility for unifying and stimulating University water resources research through the administration of funds covered in the Act and made available by other sources; coordinating University research with water resources programs of local, State and Federal agencies and private organizations throughout the State; and assisting in training additional scientists for work in the field of water resources through research.

This Bulletin is number 84 in a series of publications designed to present information bearing on water resources research in Minnesota and the results of some of the research sponsored by the Center. This Bulletin is concerned with the effects of calefaction upon members of the animal portion of the Lake Superior ecosystem.

This Bulletin serves as the Research Project Technical Completion Report for the following Center project:

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Project Title: Thermal Pollution and Second Trophic Level Fauna of Lake Superior

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Publication Abstract:

The objective of this study was to examine the effects of calefaction upon members of the animal portion of the Lake Superior ecosystem. Additionally, certain species which are widely available were also utilized in order to establish a point of reference which could be used by workers in other parts of the country. The conventional approach to a problem of this sort has been to measure the effects of an effluent source on a specific community and attempt to understand the ecological interrelationships of the altered environment. The method described here utilized the establishment of an artificial segment of Lake Superior in a carefully controlled laboratory setting which could be modified at will in order to observe the impacts of each change upon the test organism or community.

Critical to this study was a method of observation of organism behavior which allowed remote monitoring of the population, but did not alter the environment of the test animals. Such a method was developed and is described in a succeeding section. This method enabled measurement of critical physiological and behavioral responses which provided a baseline for work with thermal additions.

INTRODUCTION

One of the single greatest water management problems of the present decade is the creation of large volumes of heated effluents by manufacturing processes and steam electric generating plants. The consequences of the release of heated waste water is becoming of increasing concern with the present rate of acceleration in the use of water for cooling. Authorities have estimated that by 1985 as much as one quarter of all surface waters in the continental United States will be used for cooling in manufacturing and power generation processes.

While it is clear that managerial decisions with regard to discharge of thermal effluents are eminent, regretably, all too little data are available to support this anticipated policy formulation.

Lake Superior is singular as the largest, coolest, and simultaneously the most oligotrophic body of water in North America. As a consequence, it is a very desirable source of cooling water, and potentially one of the most fragile with respect to the impact of thermal additions.

At the present time, there are more than sixty-five power generating facilities on or immediately adjacent to the Great Lakes. Of this total, six are nuclear-powered units. Since the apparent abandonment of plans for a breeder-reactor at Knife River, Minnesota by the Union Carbide Company, no nuclear-powered installations have been suggested for Lake Superior. However, as demands for cooling water continue to grow, Lake Superior will become an increasingly desirable resource because of its size and the generally low temperature of its waters.

The objective of this study was to examine the effects of calefaction upon members of the animal portion of the Lake Superior ecosystem. Additionally, certain species which are widely available were also utilized in order to establish a point of reference which could be used by workers in other parts of the country. The conventional approach to a problem of this sort has been to measure the effects of an effluent source on a specific community and attempt to understand the ecological interrelationships of the altered environment. The method described here utilized the establishment of an artificial segment of Lake Superior in a carefully controlled laboratory setting which could be modified at will in order to observe the impacts of each change upon the test organism or community.

Critical to this study was a method of observation of organism behavior which allowed remote monitoring of the population, but did not alter the environment of the test animals. Such a method was developed and is described in a succeeding section. This method enabled measurement of critical physiological and behavioral responses which provided a baseline for work with thermal additions.

This work is far from completed. Even at the time of this writing, efforts are going forward in studies dealing with the response of urythermal organisms to small increments of increased temperature. It is hoped that these studies and the data contained in this report will make some small contribution to the information base required for effective management of thermal discharges.

NON-INVASIVE REMOTE MONITORING SYSTEM FOR INVERTEBRATE POPULATIONS

General Considerations.

In order to measure the responses of organisms to environmental stress in an adequate fashion, it was necessary to develop an electronic system capable of performing a continuous monitoring function of minute crustaceans over a long period of time. To provide significant advantages over existing methodology, a new system designed for remote surveillance or monitoring of any living organism must possess at least the following characteristics:

1. The system must eliminate the necessity for surgical implantation and the potential for alteration of organism response.

2. The system must avoid the addition of restrictive weight to the organism.

3. The system must not, as a result of its operation, generate or cause altered or unnatural activity.

4. The system must be able to provide continuous information for extended periods of time.

5. The system must offer a high degree of accuracy with a mechanism provided for "hard copy" data capable of storage for ultimate retrieval.

6. The system cannot be dependent for observation upon any parameter which will effect physiologic function or organism behavior.

7. The system must offer a wide latitude of options providing for as wide a latitude of application as possible.

When these general or basic characteristics are considered, another series of criteria must also be met. Interestingly, these criteria for remote monitoring of organisms in freshwater are identical to those required for sensing of the earth's environment from remote platforms, i.e., the use of satellite imagery. For a given system, these requirements include:

1. The system must be able consistently to monitor minute characteristics with a high degree of accuracy.

2. The system must be capable of undergoing periodic confirmation of data until a characteristic signature for each new parameter is established.

3. The resolving power of the system must be defined.

4. The system must be able to establish the position of the organism under surveillance in time and space with precision.

5. The system must consistently produce an acceptable signal to noise ratio.

It is believed that these twelve requirements are admirably satisfied in the design of the system utilized in this study. The only major limitations that appear to exist are 1) the necessity to limit the maximum distance between sensing electrode pairs, and, 2) the fact that the system cannot be applied to marine environments because of salinity.

Description of the Remote Monitoring System.

Historical considerations. Non-invasive remote sensing of physiologic and locomotor activities of aquatic species has been a long-sought research goal. Earlier studies designed to assess physiological parameters associated with behavior of the aquatic organism have either seriously altered the environment of the test population (Harris and Wolfe, 1955; Harris and Mason, 1958), or have been dependent for physical observation upon the chief parameter of concern, light. (Harris and Wolfe, 1955; Harris and Mason, 1958; Bainbridge, 1952; and Viaud, 1951). To overcome these obstacles, thermistor monitors have been used (Hausner and Enright, 1966), and has simple amplification of organism induced bioelectric potentials (Camougis, 1960, Groor, et al., 1971; Drummond, et al., 1973, Goodman and Weinberger 1971, Heath, 1972). This study reports a new technique for remote non-inhibiting monitoring of aquatic invertebrate populations using a modified impedance conversion system.

Initial studies in mammalian systems which utilized impedance conversion were conducted in mammalian systems to monitor the movements of erythrocytes within blood vessels (Fricke, 1933; Liebman, 1970; Nyboer, 1970). Based on knowledge of their observations, it was reasoned that the use of impedance conversion might hold particular promise for remote non-invasive monitoring of the movements of freshwater invertebrate populations. Available literature indicated only one recent reference to the use of this technique in invertebrate populations, i.e., the determination of cardiac activity in Orthopterans (Miller, 1973).

Theory and description of the system. By definition impedance is the resistance to the flow of alternating current and may be more specifically defined as the apparent total opposition to current flow in an alternating current circuit corresponding to the true resistance in a direct current. Impedance is equal to the ratio of the root mean square (rms) electromotive force in the circuit to the root mean square current produced by it, and is usually expressed by the notation:

$$Z = \sqrt{R^2 + iX^2}$$

where A = Impedance

R = Ohmic resistance, and

iX = Reactance

Reactance is further defined as the opposition to the flow of an alternating electric current caused by the inductance and capacitance in the circuit. Reactance, therefore, may be defined by the equation:

$$iX = \frac{1}{2\pi fc}$$

where

f = Frequency, and

c = Capacitance

Thus, any change occurring in either the ohmic resistance (R) or reactance (iX) would of necessity induce changes in impedance (Z).

If a dielectric is thrust into a weak electric field, induced surface charges appear which tend to weaken the original electric field within the dielectric. This weakened field is manifested as a reduction in potential difference between the plates of a charged capacitor when the dielectric is moved between the plates.

Since aquatic invertebrates moving in such a field, acting as dielectrics, would oppose or alter the flow of alternating current, it was reasoned that the resulting change in capacitance (c) and hence in reactance (iX) would induce changes in impedance (Z), an easily measurable entity. Further, if the system was sufficiently sensitive, it would be possible to observe organisms resting in a plane between electrode pairs from the induced changes in impedance caused by appendage and respiratory movements of the organisms.

The generalized system developed for this study consisted of a rectangular chamber constructed of plexiglass. A bipolar arrangement of stainless steel grid electrodes (20 mesh/inch; 0.023 gauge wire, type 304) were cemented on opposing walls. These electrodes were then connected by shielded cable to a Brush Impedance Coupler (Model 13-4307-06) which utilized an excitation frequency of 50 KH$_z$, and a maximum excitation current of 1.5 milliamperes. The signal output from the impedance converter was then amplified and monitored on Brush 440 multi-channel recorders, a cathode ray oscilloscope and a 10 watt audio monitoring system. Figure 1 shows a generalized block diagram of the arrangement of system components.

A wide variety of electrode pair combinations were tried. A complete discussion of this portion of the study appears in a subsequent section. However, in order to yield maximum sensitivity and minimize interference from extraneous radio frequency, it was learned that the most consistent results could be achieved if the distance between electrode pairs exceeded the overall body length of the test organism by approximately 1.5 to 1.75 times. These values would tend to place the organism closer to one electrode grid than the other, maximizing the signal transmitted to the impedance unit. With this arrangement of electrode grid pairs, it was possible to detect both gross and minute movements of the test organism by adjustments of the level of sensitivity of the amplifying system. This process is discussed in detail in a later section.

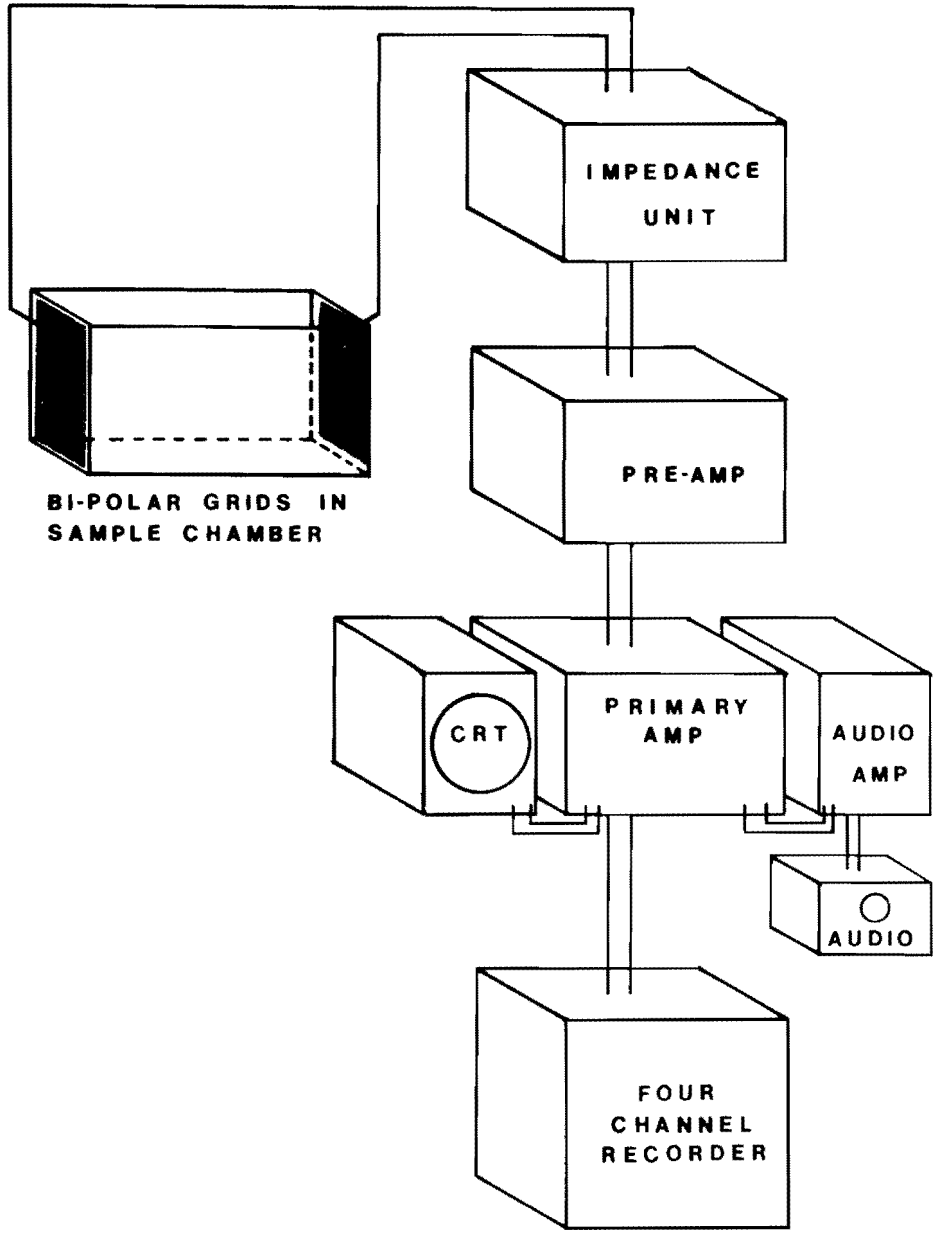

Figure 1. Generalized block diagram showing the arrangement of components in the Impedance Conversion System used for remote sensing of aquatic crustacean activity.

A variety of aquatic organisms has been tried with this experimental system ranging in size from the copepod *Limnocalanus macrurus*, some 600 microns in average body length through dragonfly naiads of the genera *Anax* and *Somatochlora* of more than 3 to 5 centimeters in total body length.

DESIGN OF EXPERIMENTAL CHAMBERS

General Principles.

As was noted in the introductory section, impedance conversion technique has been used as a standard procedure for certain medical and physiologically related problems. To date, no application of this method had been made to invertebrate organisms residing in the aquatic environment.

Since most aquatic invertebrates are small relatively active forms, a series of chambers were required in order to allow for a complete range of motion of the organism and to retain the test animal in fairly close proximity to one or both of the sensing electrodes.

Characteristically, impedance measurements in mammalian species are made with implanted needle electrodes. Initial studies in chambers constructed to receive needle electrodes proved fruitless. Figure 2 shows an early column 25 x 10 x 105 mm. (ID) which was constructed to monitor copepod and cladoceran movements. This method of construction was abandoned since the needle electrodes, when used in sequence, acted as antenna resulting in the amplification of high levels of radio frequency (RF) interference and raising the signal to noise ratio to an unsatisfactory level. This masking effect is shown in Figure 3. Several other chambers utilizing needle electrodes were constructed with similar limited success. In these installations, even the use of shielding or the construction of a Faraday Cage did not allow for the reduction of RF signals to an acceptable level.

Studies by Spoor and Drummond (1972) reported success using stainless steel grids in a direct current system utilizing amplified EKG potentials. It was reasoned that such success in a direct current system might also have applicability to an alternating current system of increased sensitivity.

A series of test and organism specific chambers were then constructed using type 304 stainless steel wire cloth as sensing electrodes. These wire grid electrodes were cemented with a non-toxic plastic polymer to opposing walls of the chamber. While other configurations were tried, this arrangement of electrodes seemed to provide the most stable and consistent results. Coaxial cable was utilized to construct five-pin shielded leads to further minimize extraneous RF signals or noise from poor electrical contacts.

Test results from the use of experimental chambers constructed according to this pattern gave excellent results. From this arrangement, it was possible to monitor both gross locomotor activity and smaller appendage movements of a variety of crustacean invertebrates including *Daphnia sp.*, *Mysis relicta* and *Limnocalanus macrurus*. Figure 4 shows the differences in the characteristic base signature between *Daphnia* and *Mysis*. Identification of various portions of signature for *Daphnia* and *Mysis* is provided in Figures 5 and 6 respectively.

The distance between grids was found to be a critical factor in the reception of clear, accurate signals. It was learned that signal input could be maximized if the distance between electrode pairs exceeded the overall body

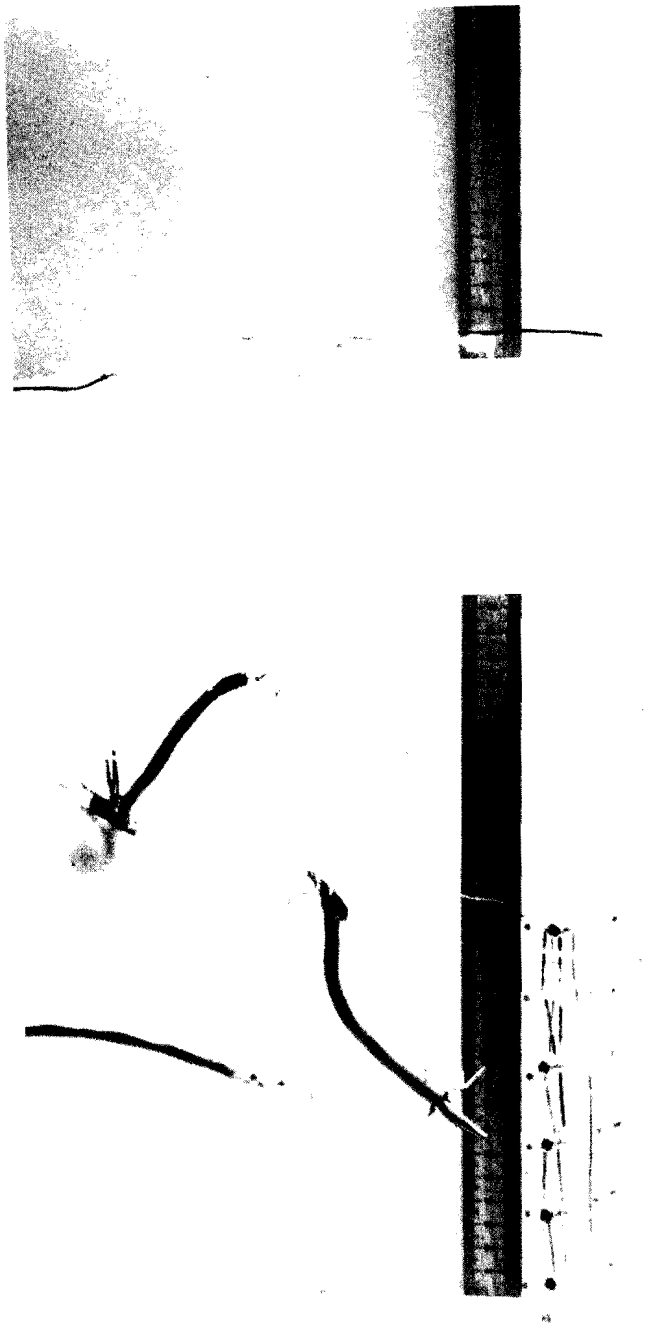

Figure 2. Early needle-electrode chambers (right, both top and bottom) as compared with later grid models (left, both top and bottom). Single chamber experiments (top) led to the development of columns (bottom) to observe vertical movements.

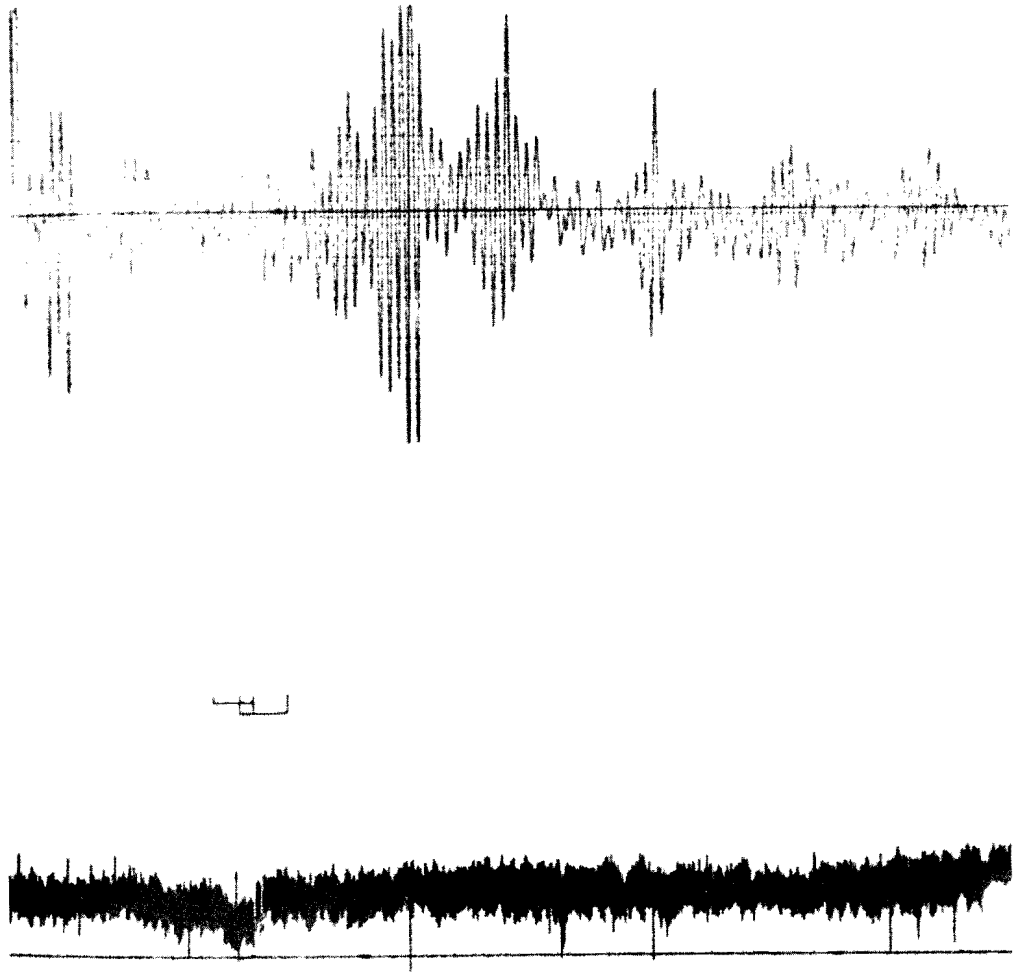

Figure 3. Masking effects of radio frequency (RF) interference (top) and extraneous unfiltered $60H_z$ signal (bottom).

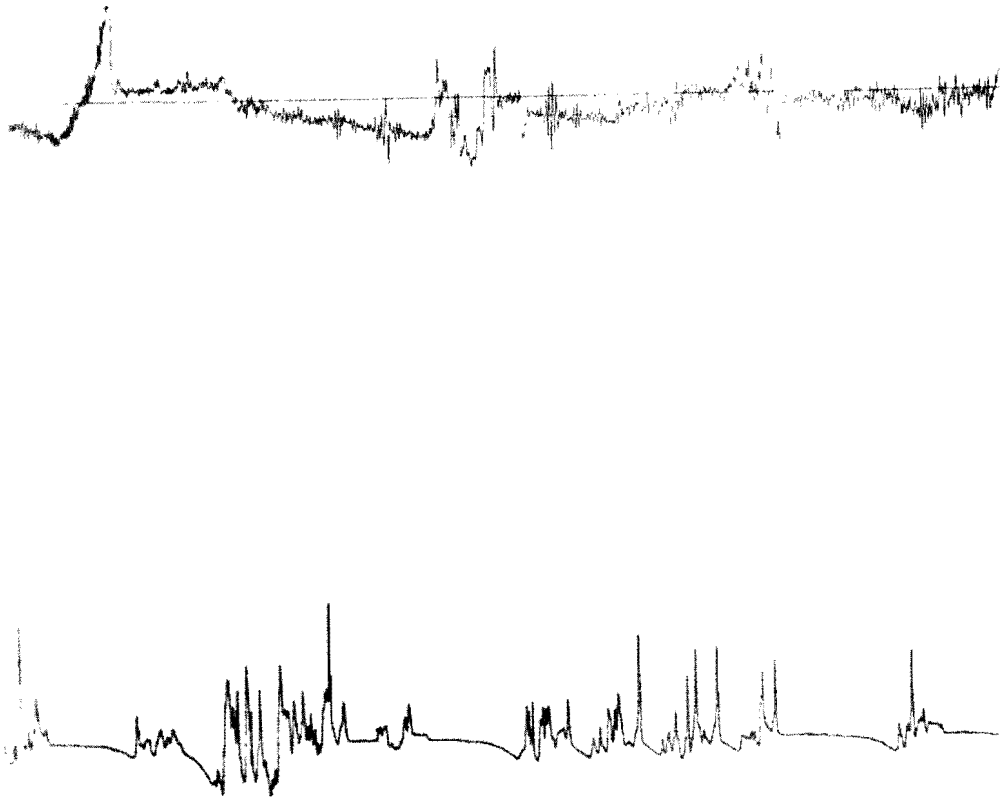

Figure 4. Characteristic impedance signatures of *Daphnia* (top) and *Mysis* (bottom).

Figure 5. Characteristic impedance signature for *Daphnia*. Major spikes represent gross whole body movements and lesser peaks are the response to second antennae movements. (Sensitivity 0.005; chart speed 5 mm/sec.).

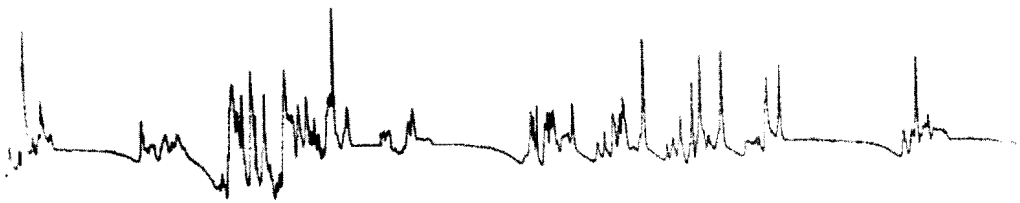

Figure 6. Characteristic impedance signature for *Mysis relicta*. Major spikes represent whole body movements, smaller peaks were caused by tail movements. (Sensitivity 0.05, chart speed 25 mm/minute).

length of the test organism by approximately 1.5 to 1.75 times. These values tend to place the organism closer to one electrode grid than the other, maximizing the signal transmitted to the impedance unit. Grids placed closer to each other than the range indicated above confined the organism too severely, restricting its natural movements. Further, sensing grids located in close proximity increased the probability of gross motor movements in physical contact with one of the grids producing massive recorder-pen deflections when a high sensitivity setting was used.

Single pair grids were used in a variety of size and shape configurations with little or no interference. However, in vertical series, it was found that extraneous interference increased proportionately with an increase in grid size. Further, if vertical grid sequences were situated in too close proximity to each other interference also increased.

The chamber constructed for vertical movement studies of mysids was 47.0 mm. square internal dimension. Effective grid sizes for this column ranged from 10 x 47 mm. to 50 x 47 mm. with a minimum vertical distance of 30 mm. between each successive grid pair. This distance between grids was a critical factor since the sphere of influence above and below each grid pair was approximately 10 mm. Grid pairs placed within 20 mm. vertically of each other caused the entire column to act as an antenna, obviating the receipt of acceptable signals from the test organisms. However, if vertical grid spacing was carefully chosen, the test organism could be continuously followed throughout the length of the column. Thus, judicious placement of electrodes allowed the animal to migrate from the sphere of influence surrounding one grid pair into that of the next grid pair without either the generation of unnecessary interference or the creation of "dead spots" between electrodes where detection would be avoided.

The maximum grid size used in vertical movement experiments was 30 x 100 mm. This arrangement produced excellent signal clarity. This length of grid allowed the construction of a sizable column with vertically stacked electrodes to monitor vertical movement of organisms. This column will be discussed in detail in a subsequent section.

. Studies of Respiration in Response to Diminished Oxygen.

Smaller chambers were also produced to study specific problems relative to thermal phenomenon. A minute chamber was constructed to monitor the incubation times of eggs of various aquatic species (see Figure 7). Yet another sequence was used to monitor respiratory activity of dragonfly (Odonata) naiads of the general *Anax* and *Somatochlora*, some 3 to 5 cm. in body length. Using the generalized arrangement of elements shown in Figure 1, it was reasoned that since one of the major consequences of increasing temperature in the aquatic environment is a dimunition of available dissolved oxygen, that data should be available with regard to oxygen consumption of at least some of the larger species in the Lake Superior Basin.

As a consequence, it was decided that dragonfly naiads would be utilized because of the following characteristics:

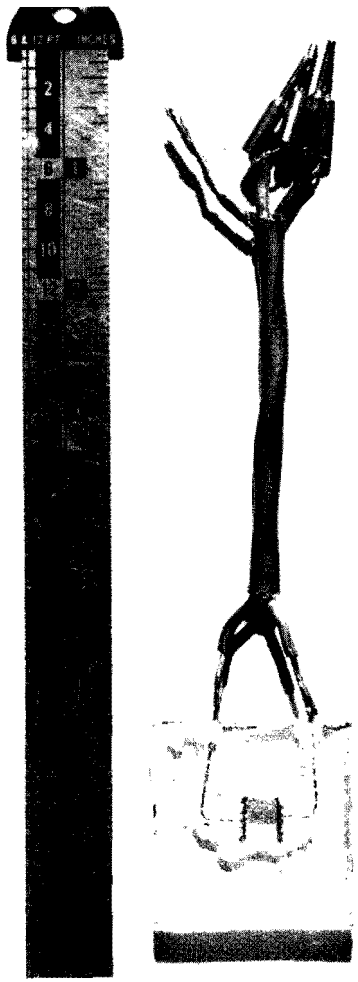

Figure 7. A minute chamber designed to monitor emergence behavior of aquatic species. Internal dimensions of this chamber are 10 x 8 x 6 mm.

1. Because immature dragonflies have rectal gills, their respiratory cycle can be easily observed by pulsations of the abdomen.

2. Dragonfly naiads occur naturally in impoundments, streams and tributaries of the Lake Superior Basin.

3. Early electrochemical (polarographic) determinations of oxygen consumption for related species was available for comparison.

Studies of the respiration rates of both *Anax sp.* and *Somatochlora sp.* were undertaken. Figure 8 shows the respiratory activity of *Anax sp.* shortly after the organism was introduced into the test chamber. This and subsequent recording chart strips are read in right to left sequence. The total peak to peak impedance signal generated by the respiratory movements of the test organism is equivalent to 100 millivolts. This total is divided on this chart and subsequent recordings unless otherwise specified to yield 0.5 percent of total signal per chart division (sensitivity = 0.005). It is clear from this recording that the signal to noise ratio even at this high sensitivity is quite acceptable. In this sensitivity range, unfortunately, gross whole body movements can cause off-scale pen deflections from the center base-line (Figure 9). However, a minimum of data are sacrificed because of the system's automatic reset mechanism. In the instance demonstrated in Figure 9, a total of nineteen seconds of data were lost. Losses of this order can be tolerated since the entire run was of more than four hours duration.

Subsequent modification of the remote monitoring system has included the addition of a Clark Electrode for intermittent electro-chemical determination of dissolved oxygen. This modification of the sample chamber is shown in Figure 10. Note also that a stainless steel screen has been added to the chamber for use as a substrate by the insect. A secondary strip of stainless steel screen is interposed between the organism and the agitation system of the oxygen electrode, thus preventing damage to the organism, and retaining it between the impedance electrode pair. A series of test sequences was run using this system to analyze respiratory activity and oxygen consumption in native aquatic insects. Wherever possible, paired tracings at chart speeds of 1 mm./sec. and 5 mm/sec. were utilized. Figures 11 and 12 are records of one of these sequences which began at 8.1 mg./l. of dissolved oxygen, 99 percent of saturation, (shown from 6.6 mg./l. in Figure 11) and ran through 2.1 mg./l. of dissolved oxygen (25 percent of saturation).

Table I shows the general relationship of observed respiratory activity of levels of dissolved oxygen. In this study, data reported are taken from arithmetic means of the 1 mm./sec. tracings, except as otherwise noted. With a single exception, 2.2 mg./l., respiratory frequency demonstrated a consistent increase from a mean of 0.45 respiratory cycles per second (CPS) to a maximum of 1.4 CPS, an increase of more than 3.1 times the original level. Respiratory amplitude, with the exception of the 6.6 mg./l. value, was observed to undergo a concommitant decline from 19.5 scale units to 14.0 scale units (5.2 mg./l.02), increase to 18.5 scale units and subsequently diminish to one scale unit at the 2.1 mg./l. level of dissolved oxygen.

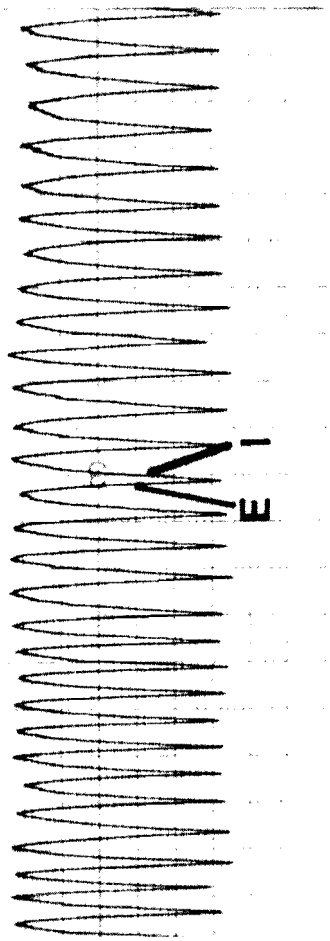

Figure 8. The respiratory activity of *Anax sp.* shortly after introduction into the test chamber. Chart strip reads RIGHT to LEFT. I = inspiratory fraction of the curve; E = expiratory fraction of the curve.

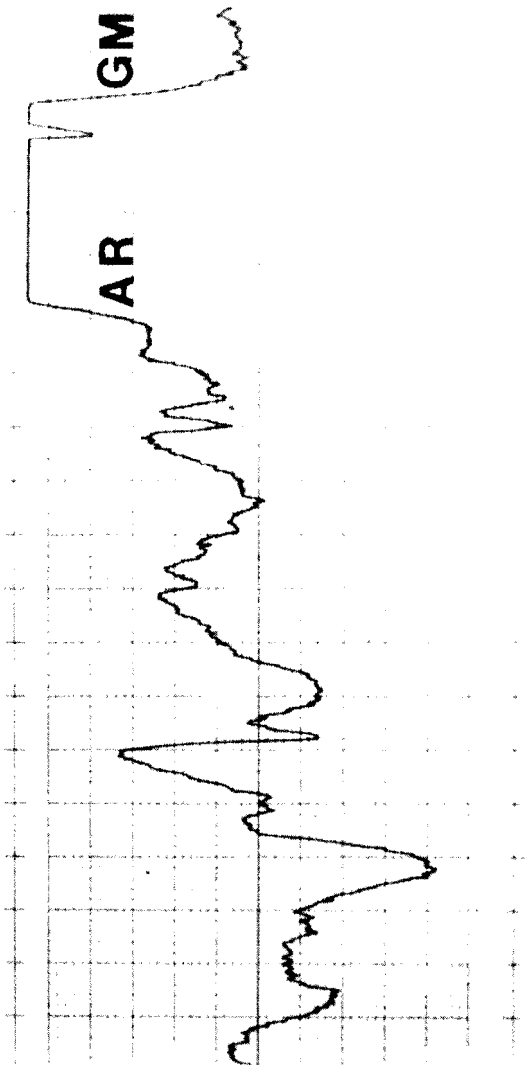

Figure 9. Effect of gross body movements on impedance conversion tracings at high sensitivites. Chart reads RIGHT to LEFT. GM = gross body movement; AR = automatic reset.

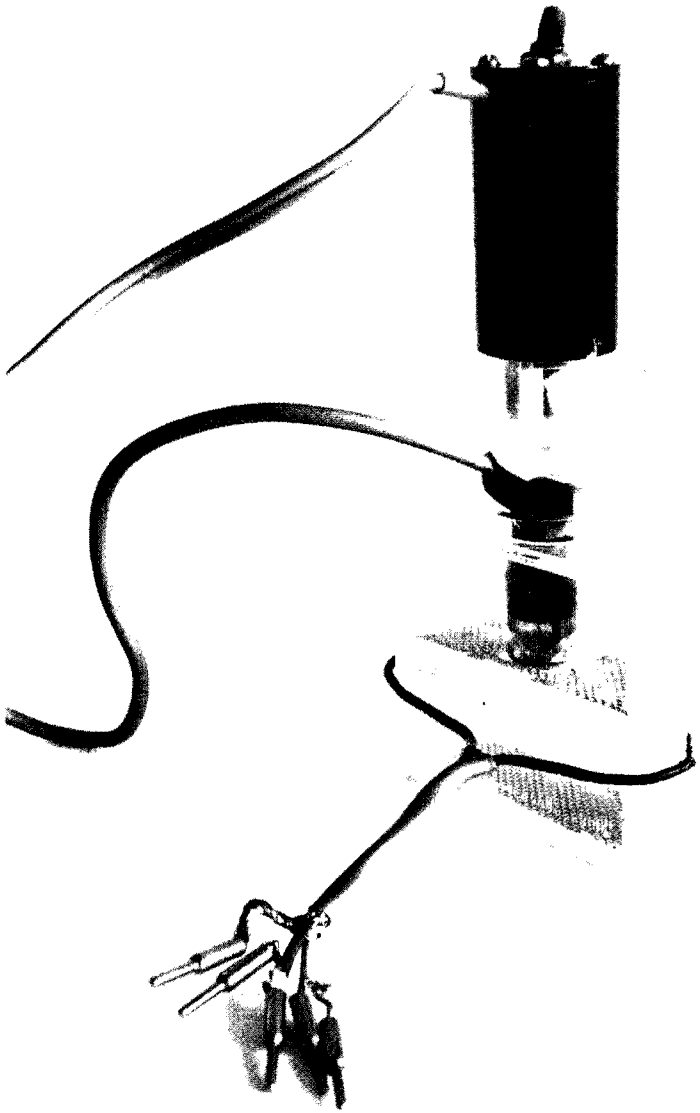

Figure 10. Photograph of the modified sample chamber showing the inclusion of the Clark Electrode for dissolved oxygen determination.

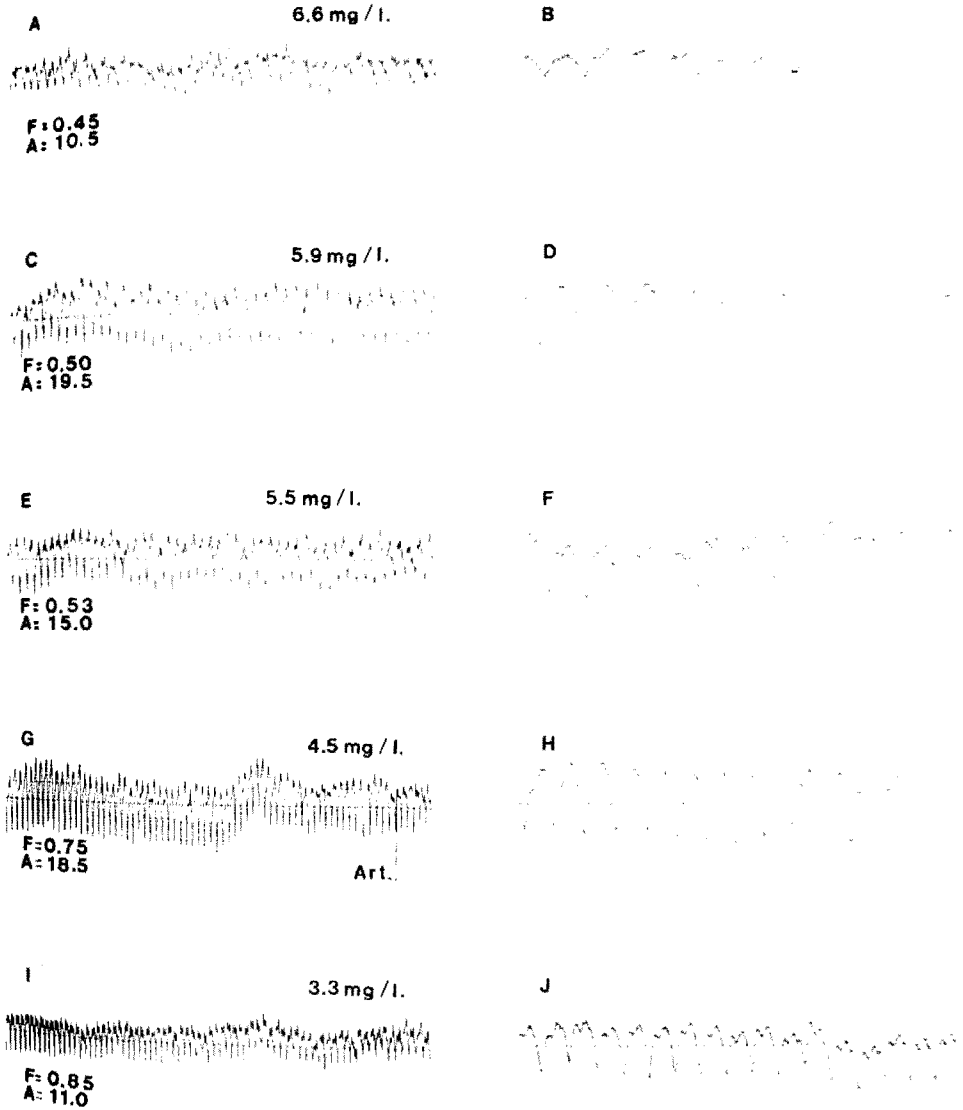

Figure 11. A sequence of respiratory responses of one *Anax sp.* naiad over a range of dissolved oxygen tensions from 6.6 mg/l to 3.3 mg/l. Paired strip charts are identical tracings at different chart speeds. A,C,E,G,I = tracings recorded at 1 mm./sec.; B,D,F,H,J = tracings recorded at 5 mm./sec. F = respiratory frequency in cycles per second; A = respiratory amplitude in scale units; Art. = artifact. Instrument sensitivity for all tracings was 0.005.

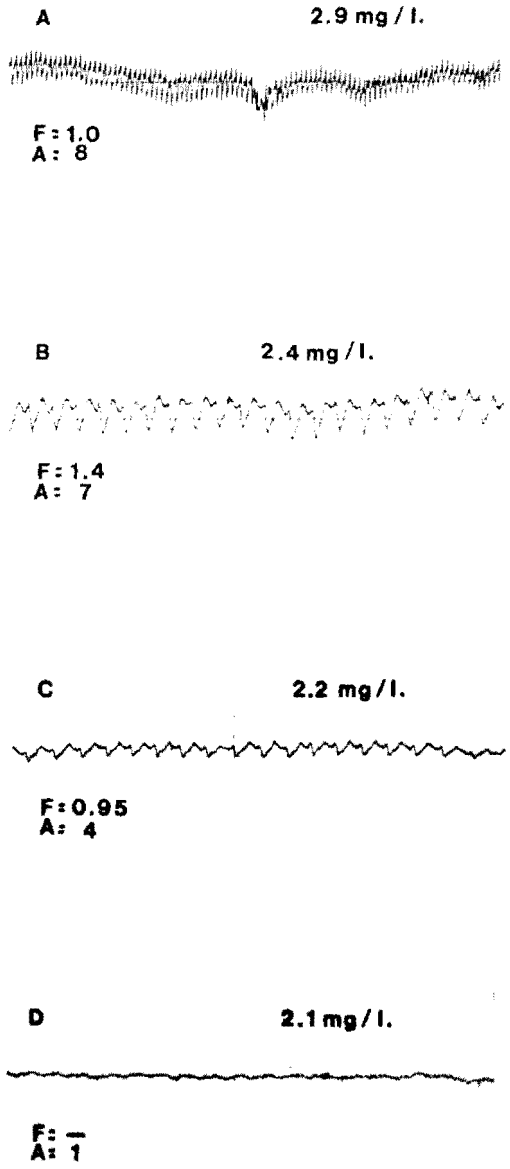

Figure 12. A continued sequence of respiratory responses for one *Anax sp.* naiad over a range of dissolved oxygen tensions from 2.9 mg/l to 2.1 mg/l. A = tracing recorded at chart speed of 1 mm./sec.; B,C,D = tracings recorded at chart speeds of 5 mm./sec. F = respiratory frequency in cycles per second; A = respiratory amplitude in scale units. Instrument sensitivity for all tracings was 0.005.

TABLE I.

Variations in the Respiratory Characteristics of *Anax sp.* with Decreasing Oxygen Tensions

Dissolved O_2 in mg./l	Frequency in Cycles/Sec.	Respiratory Amplitude in Scale Units
6.6	0.45	10.5
5.9	0.50	19.5
5.5	0.53	15.0
5.2	0.55	14.0
4.5	0.75	18.5
3.8	0.80	16.5
3.3	0.85	11.0
2.9	1.0	8.0
2.4	1.40	7 (from 5 mm/sec records)
2.2	0.95	4 (from 5 mm/sec records)
2.1	--	1 (from 5 mm/sec records)

The tracings of respiratory activity also show characteristic patterns for this species. At decreasing oxygen levels through 5.5 mg./l. of dissolved oxygen, respiratory activity demonstrated the anticipated unimodal inspiratory and expiratory curves. When the 5.5 mg./l. level of dissolved oxygen was reached, a secondary inspiration and expiration was observed. This lesser peak represented a 4.6 fold reduction over the larger respiratory cycle and occurred consistently prior to the major inhalation peak. This phenomenon persisted throughout the remainder of the run becoming more pronounced as oxygen tensions declined. As the amplitude of the major respiratory cycle decreased, this secondary cycle represented as much as 25 percent of the amplitude of the overall respiratory curve.

In order to determine oxygen requirements and consumption rates for benthic insects, Rueger, et al. (1969) utilized a polarographic or electrochemical method of analysis. The determinations of these workers were made on a variety of aquatic immatures using water in which dissolved oxygen was at or near saturation. Their data for *Aeshnia* sp. indicates a consumption rate of 145 μl. O_2/g. wet wt./hr., while in this study, data for *Anax* sp., also a member of the family Aeshnidae, yielded a mean rate of consumption of 136.7 μl. O_2/g. wet wt./hr. for values between 85 and 100 percent of saturation.

As oxygen saturation levels decline, oxygen utilization also declines. In *Anax* sp., at levels between 40 and 60 percent of oxygen saturation, the rate of consumption decreases to 96.8 μl. O_2/g. wet wt./hr. A rate of 56.9 μl. O_2/g. wet wt./hr. was observed at levels of saturation between 25 and 40 percent. Similar declines were found when *Somatochlora* sp. was tested. From an initial value of 103 μl. O_2/g. wet wt./hr. at 80 to 100 percent of saturation declines to 79.2 μl. O_2/g. wet wt./hr. and 51.6 μl. O_2/g. wet wt./hr. respectively were seen.

VERTICAL MOVEMENT STUDIES OF MYSIDS

Column Construction.

Diurnal vertical migration of crustacean zooplankton is a well recognized phenomenon in the Great Lakes. In order to assess the impact of thermal additions upon this phenomenon it was necessary first to attempt to describe conditions prompting this response. Once a base line had been drawn for behavior under controlled conditions, it was reasoned that it would then be possible to alter environmental parameters, e.g., the thermal regime, and hence understand the effects of these changes.

Utilizing the system described in previous sections, a vertically extended chamber was constructed specifically to observe the behavior and vertical movements of *Mysis relicta* with respect to altered environmental conditions.

The monitoring units utilized for this portion of the study consisted of an expansion of the impedance conversion technique. Figure 13, shows a generalized block diagram of the arrangement of elements comprising this system. The experimental column was constructed of 3 mm. plexiglass with external dimensions of 5.5 x 5.5 x 120 cm. An external frame was constructed to accommodate appurtenances associated with this monitoring system, e.g., light sources, filters, etc., see Figure 14.

Again, type 304 stainless steel bipolar electrode grids were cemented in place on opposing walls of the column with a vertical distance of 10 cm. between electrode pairs (Figure 15).

In order to create temperature gradients equivalent to those found in a stratified body of water and to approximate the effects of thermal additions a heating element was inserted equidistant between grids 1 and 2 and grids 3 and 4 (grids were numbered sequentially from top to bottom). This heating element consisted of 20 gauge nichrome wire (see Figure 16). The heating element was coupled to a rheostat allowing a continuous span of settings between 0 and 120 volts A.C.

Temperature at the midpoints of each grid pair was remotely monitored and recorded on a YSI Model 47 scanning telethermister by YSI Model 401 thermistor probes (see Figure 17).

Minute changes in light gradients were made possible by use of an iris diaphragm optical illuminator. Since it was necessary to make light available directly from above and at the same time have access to the column for introduction of organisms and the like, it was necessary to design an arrangement for fixing the light source in position, but allowing it to be moved away from the column orifice if necessary. Figure 18 shows the mechanism designed for this purpose. The illuminator was capable of critical focus and was always arranged to provide optimal illumination for the interior of the column.

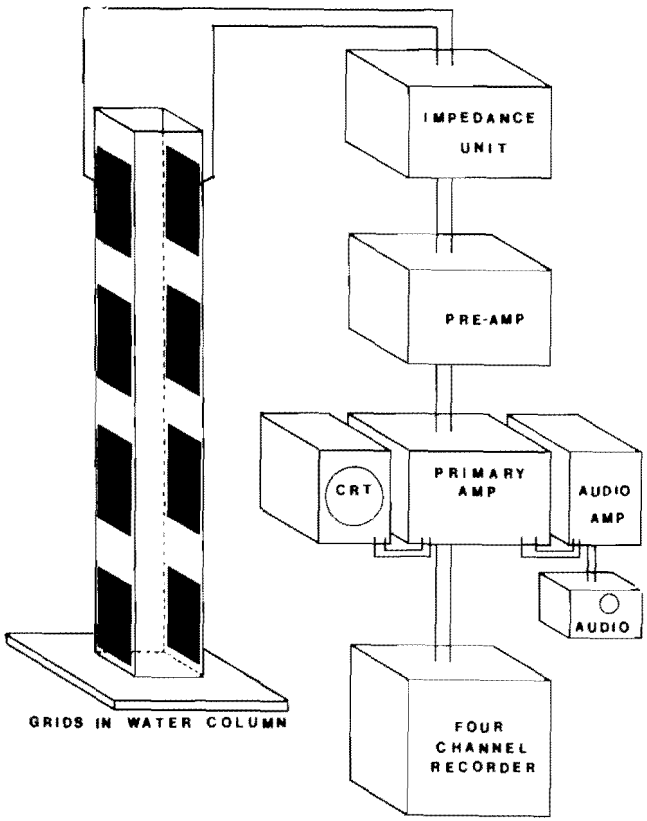

Figure 13. Generalized block diagram showing the arrangement of components in the Impedance Conversion System used for remote sensoring of the vertical movements of mysids.

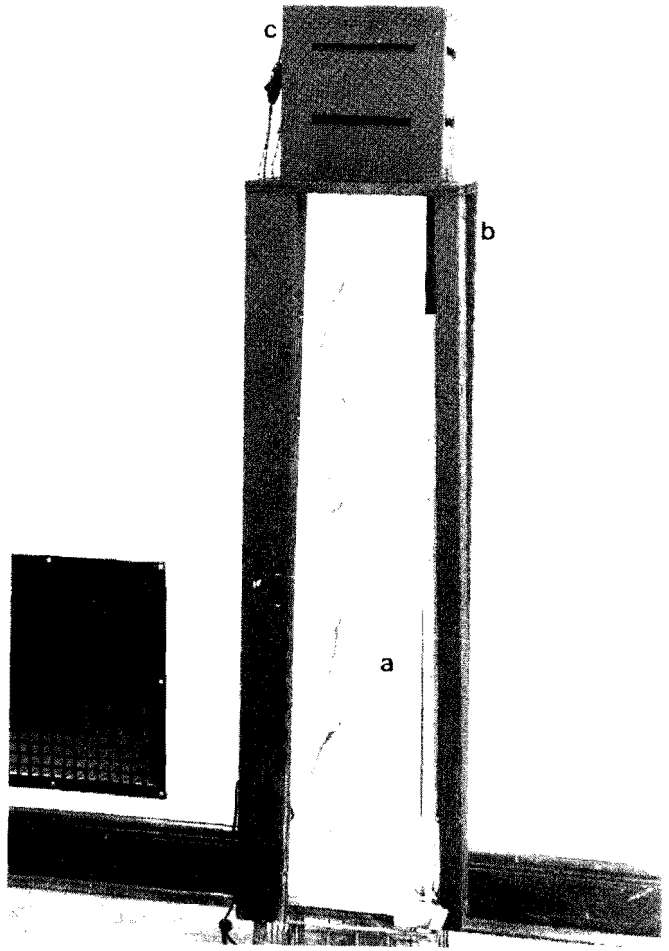

Figure 14. Photograph of the experimental column designed for remote monitoring of the vertical movements of mysids; a) the plexiglass column and its associated electrode grids, b) the external frame, and c) the louvered light shield surrounding the light source and filter system (shown in closed position).

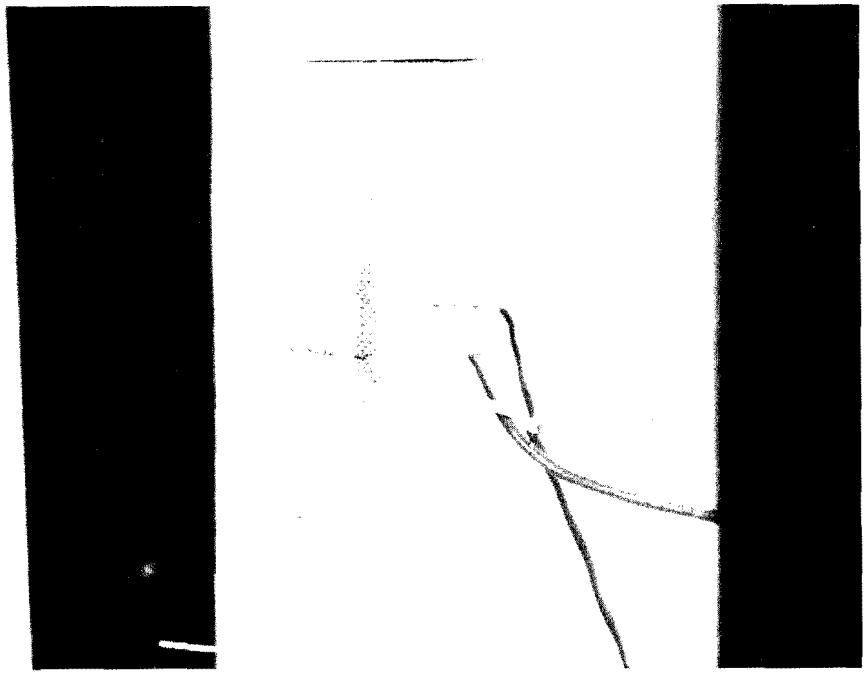

Figure 15. A pair of bipolar electrode grids of type 304 stainless steel. The horizontal distance between these grids is approximately 5 cm., while the vertical distance between electrode pairs was 10 cm.

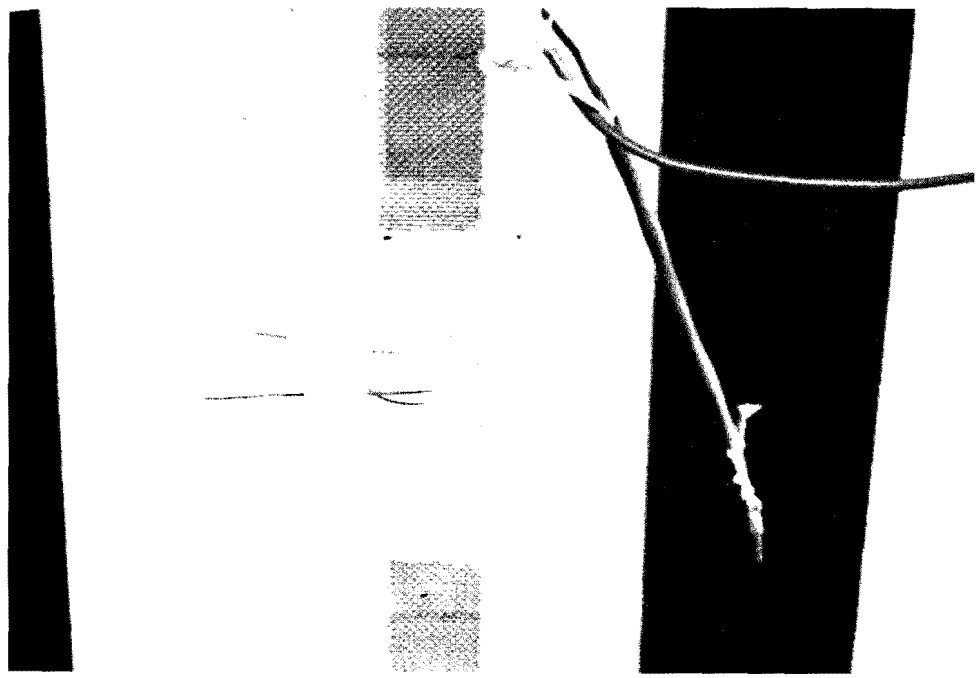

Figure 16. Photograph of the heating element located equidistant between electrode grids.

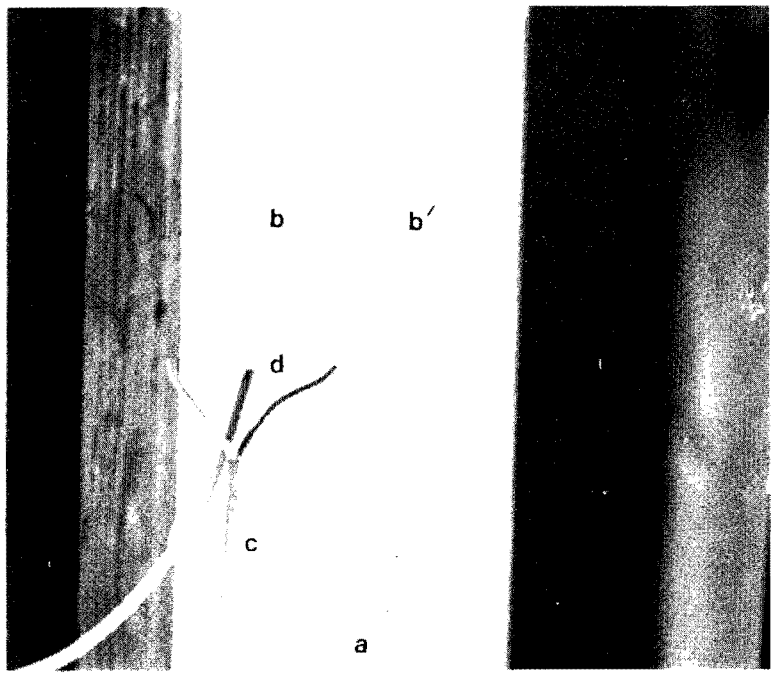

Figure 17. A photograph of the vertical migration column showing the arrangement of elements - a) the heating grid, b - b′) the electrode pairs, c) coaxial cable from grid pairs to impedance coupler, and d) thermistor probe.

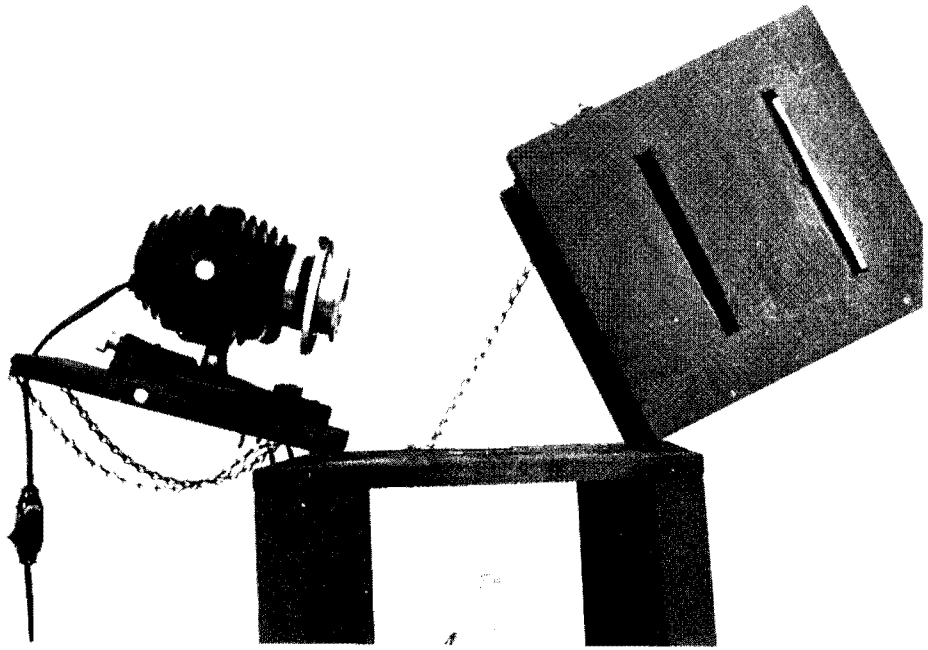

Figure 18. Louvered light-tight shield opened with illuminator in the open position to allow access to the column orifice.

Spacing was provided between the light source and the column orifice for the insertion of light filters (40 to 60 nm wave length spread) to produce illumination for only one portion of the visible spectrum. Further, intensity was modified by the introduction of 1.301, 1.00 and 0.70 neutral density filters, (5 percent, 10 percent, and 20 percent transmittance respectively). These were used singly or in various combinations with the iris diaphragm of the light source to achieve the desired level of illumination. Figure 19, shows the location of one such filter in its receptacle below the light source.

The exterior framing and the baffled light-tight box containing the light source were painted dull black to minimize the possibility of reflected light re-entering the column from the sides. As a further precaution, the column and the frame were wrapped in black plastic. The entire column and frame were placed in a light-tight walk-in environmental chamber which would maintain the desired temperature at ±0.1° C. The vertical dimensions of the chamber are limited only by the cost of the necessary impedance hardware. In theory, the grid pairs could extend upward indefinitely.

Effect of Environmental Parameters on *Mysis relicta*.

To test the effect of temperature upon *Mysis relicta*, five organisms were placed in the column filled with Lake Superior water at 4.8° C., the temperature of the lake water from which the organisms were removed. In all cases organisms were acclimated for a minimum of twelve hours in the column prior to experimentation.

As the organisms acclimatized in the column, characteristic signatures could be observed at all levels throughout the test chamber (see Figure 20). In contrast, when thermal stress exceeding the tolerance limits for *Mysis* was applied, mysids vacated the area of thermal addition.

As was previously noted, with the use of nichrome heating elements, it was possible to create a thermal pattern characteristic of a well stratified lake or warmed thermal plume over-riding colder waters. Thus, it was possible with the arrangement of elements described in an earlier portion of this section to maintain a 4.8° C. "hypolimnion" and generate a 20° C. + "epilimnion". Even at the maximum temperature utilized over extended periods of time (24-48 hours), the cooler "hypolimnetic" water never rose above 8.8° C. This increase was sufficiently small that it could be compensated for by adjustment of the ambient temperature of the environmental chamber.

The results of this experimentation indicate that *Mysis relicta* will leave a given area when water temperatures reach 12.6° C. and will only return when the temperatures fall below 11.4° C. Figure 21 shows a strip chart recording of a distinct downward movement of mysids at 12.6° C. The heating element utilized for this study was located between grids 3 and 4 serving to warm the waters associated with grids 1, 2, and 3. In all cases, mysids moved in a downward direction to escape thermal additions even though

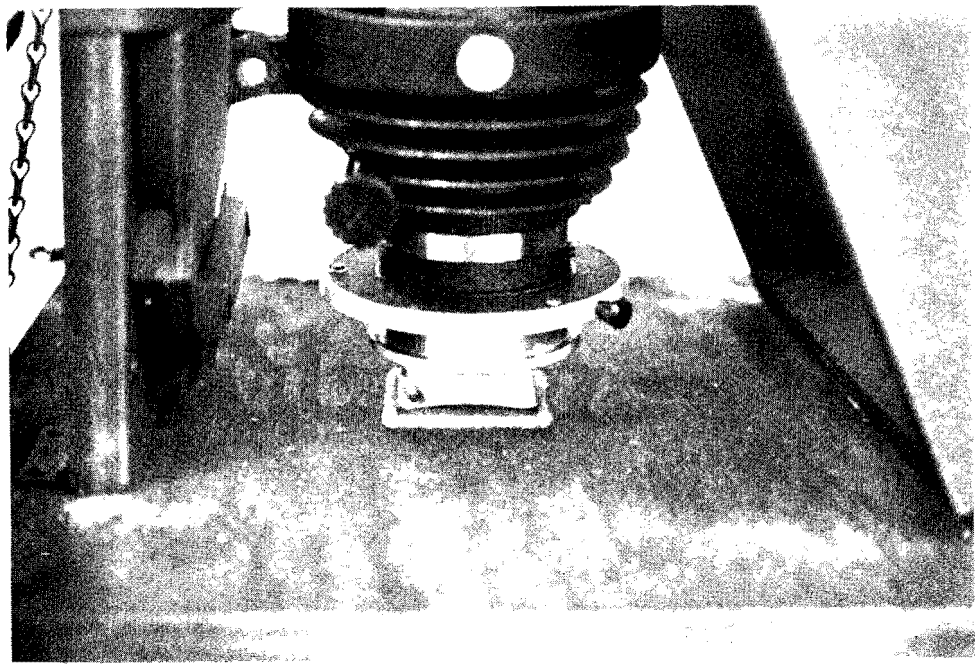

Figure 19. Photograph of the light source in position above the column orifice with a light filter in position. For purposes of clarity, the light source has been retracted from the upper surface of the filter.

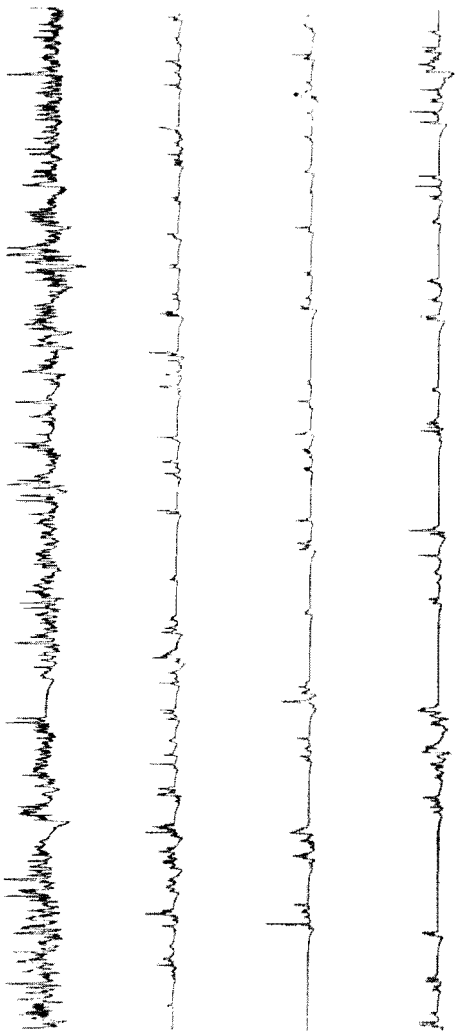

Figure 20. Acclimitazation activities of mysids in the column prior to testing. Note that the organisms are distributed throughout the entire water mass.

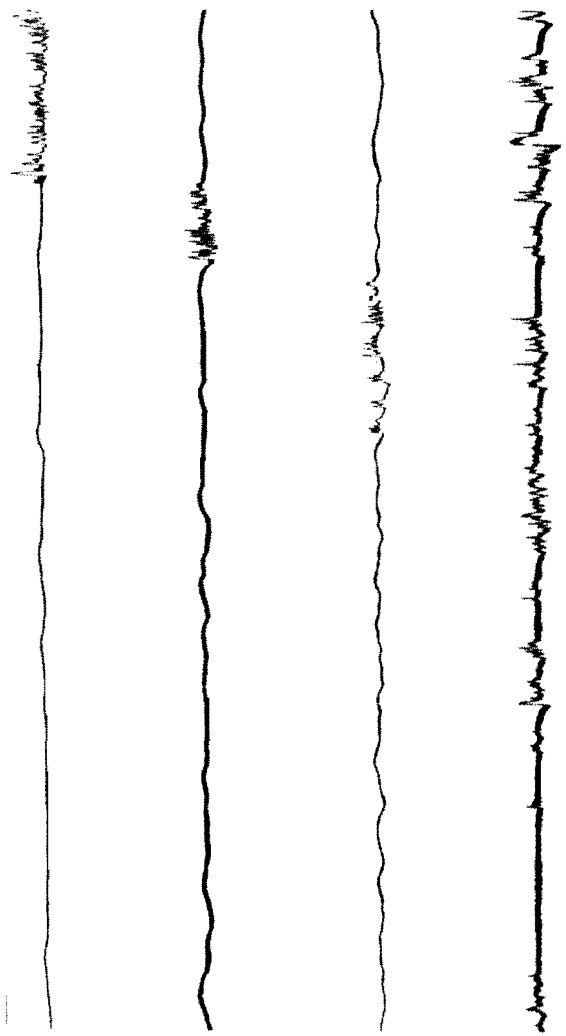

Figure 21. An example of negative thermotaxis demonstrated by mysids migrating from 12.6°C. "epilimnetic" water to the 4.8°C. "hypolimnion". Heating element located between grids 3 and 4. Chart reads right to left. (chart speed 25 mm/sec., sensitivity 0.05).

they may have been required to move into relatively warmer areas prior to gaining the cooler waters below. For example, in order to escape 12.6° C. "epilimnetic" water at grid number one, it may have been necessary to move through 14° C. water at or near the heating element in order to arrive at the 4.8° C. water of the "hypolimnion". This characteristic downward avoidance reaction suggests an inborn forced movement.

While yet unconfirmed with respect to other spatial dimensions, this apparent forced avoidance reaction has been termed "negative thermotaxis". It is clear that much additional work in this area is required.

As previously noted, mysids characteristically refused to re-enter a warmed water mass until temperatures dropped below 11.4° C. This temperature was regarded as the critical threshold for this organism. Figure 22 shows mysids returning to the upper strata of the test column as temperatures reach 11.4° C. after shut-down of the heating system.

Identical results were obtained when the heating element between grids 1 and 2 was utilized. Figure 23 shows the negative thermotaxis displayed by mysids at 12.6° C. and Figure 24 shows their re-entry at 11.5° C. It is also important to note that mysids traveled freely in close proximity to the heating element until temperatures of the surrounding waters reached 12.6° C. It is also interesting to note that in the test column, mysids traveled to distribute themselves rather uniformly throughout the water mass in darkened conditions. When the heating element between grids 1 and 2 was used, the distribution was again rather uniform below the source of thermal addition. However, when the heating unit between grids 3 and 4 was utilized, all mysids congregated below the thermal source, even though five organisms were crowded into approximately 30 cm. of vertical space. The stress of thermal additions above 12.6° C. was apparently sufficient to overcome reactions to crowding and cause this response.

Other investigators, Smith, 1970 and Reynolds and DeGraeve, 1970, suggest a temperature of 10.0° C. to 10.7° C. as the maximum temperature for long term survival of mysis. These values closely approximate the critical threshold temperature of 11.4° C. reported in this study. Beeton (1960) suggests that mysids can and do enter waters with temperatures as high as 19.0° C. for short periods of time during diurnal vertical migration. These observations have particular relevance for a subsequent section of this study, but do confirm observations in the column experiments.

The effects of light were also measured on *Mysis relicta*. Five dark adapted organisms were placed in Lake Superior water under conditions identical to those described above. Again, organisms were held in 4.8° C. water during the course of observation. Organisms were then subjected to increasing levels of illumination in the 640 - 700 nm portion of the spectrum and to various levels of white light. The red portion of the spectrum was selected because Beeton (1960) suggests it to be the most sensitive portion of the mysids visual system. These findings were confirmed by the study reported here. When the 640 - 700

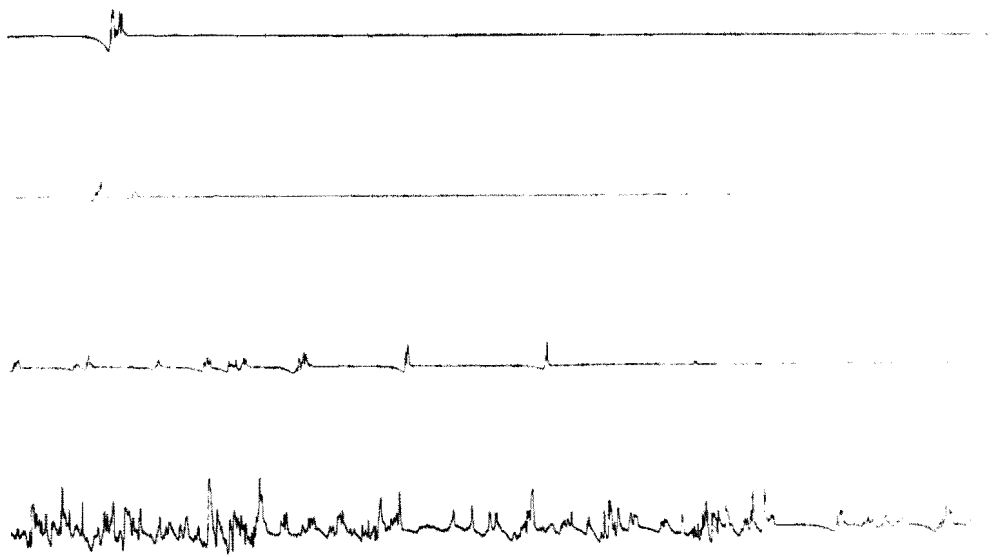

Figure 22. An example of the return of mysids to relatively warmer waters. Temperature at grid 4 was 4.8° C. and 11.4°C. at the other grids. Chart reads right to left (chart speed 25 mm/sec, sensitivity 0.05.

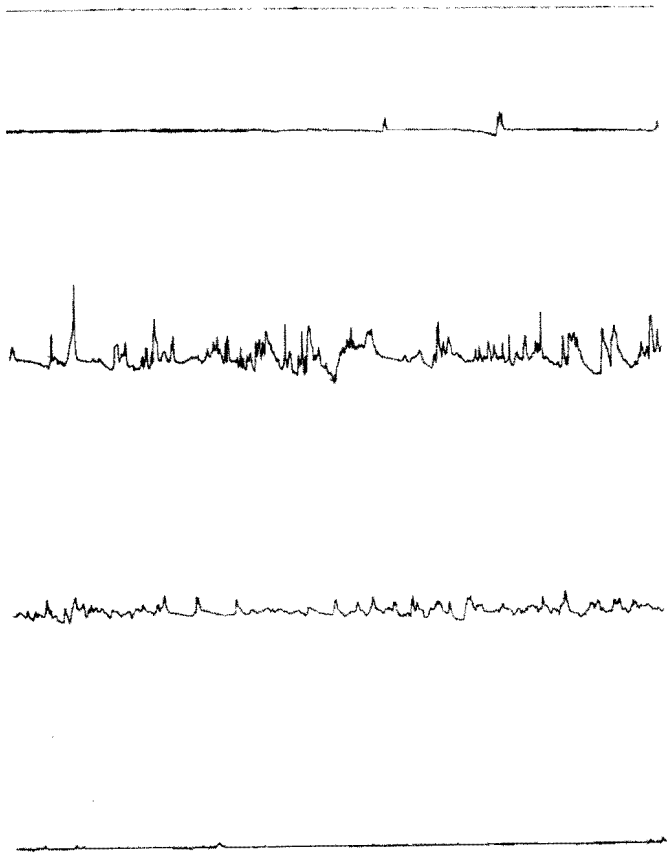

Figure 23. Negative thermotaxis demonstrated by mysids with heating element situated between grids 1 and 2. Chart reads right to left. (Chart speed 25 mm/sec., sensitivity 0.05).

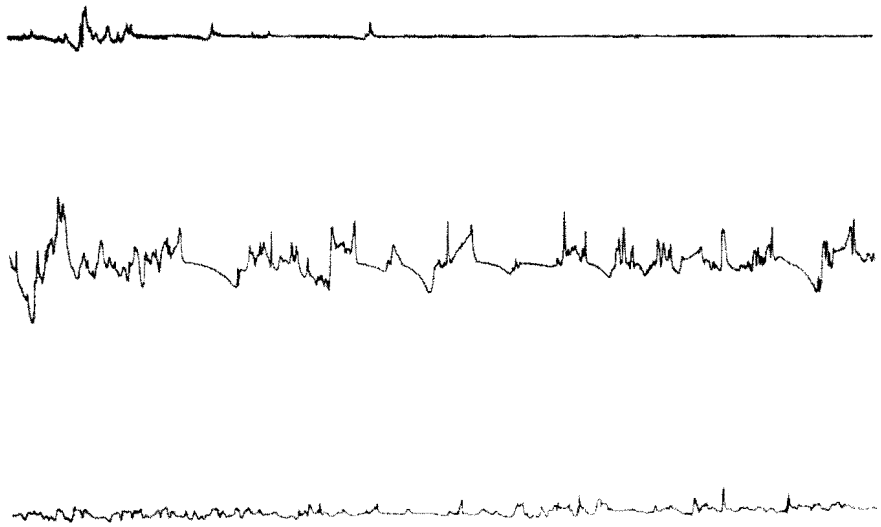

Figure 24. Re-entry by mysids at 11.4°C. when the heating element is located between grids 1 and 2. Chart reads right to left (chart speed 25 mm/sec, sensitivity 0.05).

nm light was used, the results ranged from no effect at lower intensities to stimulation of a short term negative phototaxis in the mysid populations. At 1.45 watts/meter^2 and greater, the illumination caused organisms to migrate downward to the bottom of the column for the period of time required to acclimate to the new light intensity. Following this acclimatization period, the organisms would then return to the levels at which they were observed prior to exposure. Table II shows the gradually increasing time of acclimation with increasing light intensity.

Acclimation time for exposure to white light ranged from 0.8 minutes to 6.2 minutes over an intensity range of 0.0-0.5 watts/meter^2. While a general tendency toward increasing acclimation time to increasing intensity appeared to exist, no direct relationship between these two factors was established. In each case, however, a light gradient was established in the experimental column. Figure 25 presents a curve for light intensities used for one such trial. Intensities for this run were 16.2μ watts/cm^2 at the surface of the column; 9.3μ watts/cm^2 at grid #1; 8.2μ watts/cm^2 at grid #2; 7.1μ watts/cm^2 at grid #3; and 5.8μ watts/cm^2 at grid # 4. Figure 26 shows the reactions of five mysids to this continuous challenge of white light. The onset of the illumination is indicated by the arrow (chart reads right to left). The time required for initial reaction to the new light regime was 0.16 minutes. After this initial lag period, all mysids migrated to the bottom of the column. The total time required for this 70 mc. movement was 0.96 minutes. Upon reaching the bottom of the chamber, the mysids spent 1.64 minutes prior to beginning movements toward the surface. A total time of 6.16 minutes was required before mysids acclimatized and again reached the level of grid #1.

TABLE II

Acclimatization of *Mysis relicta* to increasing intensities of 640 - 700 nm light.

Intensity of Light (watts/meter^2)	Acclimation Time in Minutes
0.3	0.00
0.92	0.00
1.45	0.24
1.88	0.48
3.1	0.72

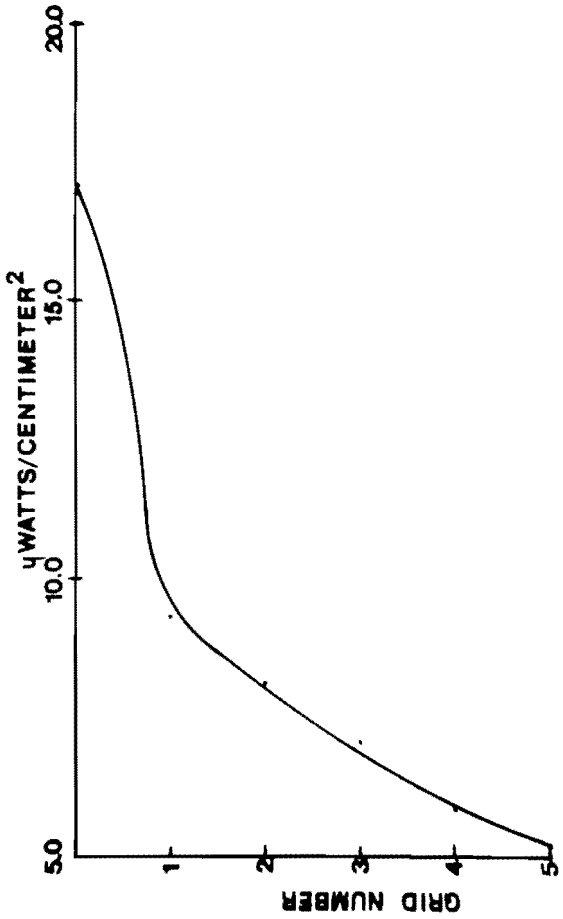

Figure 25. A representative curve for white light at higher intensities utilized in the vertical migration column.

Figure 26. Reactions of five mysids to the white light regime shown in Figure 25. Arrow indicates the onset of illumination. Chart reads right to left. (Sensitivity 0.005, chart reads right to left. (Sensitivity 0.005, chart speed 25 mm./minute).

LAKE CONFIRMATION OF LIGHT STUDIES WITHIN SELECTED THERMAL REGIMES

Introduction.

Having attempted to establish conditions simulating Lake Superior in the laboratory and then altering this environment in a fashion similar to that produced by thermal discharge, it was necessary to verify light regimes established in the laboratory by testing in the lake.

A series of lake cruises established the thermal gradient present in Lake Superior at the time of the study. Organism groups were then individually acclimated to these temperatures under the conditions of light and dark described subsequently. The acclimatization process was accomplished over a seven-day period and once the appropriate temperature was reached, the organisms were held for an additional seven days.

Thus, once the effects of temperature on the population were minimized, the impact of light on their behavior could hopefully be judged. With this knowledge, laboratory reactions to other parameters could be pursued more accurately. The following section deals with studies of light orientation by organisms adapted to lake temperatures.

Review of the Literature.

The phenomenon of diurnal vertical migration of planktonic animals has been so well documented by a variety of authors that citation of these sources is unnecessary for the scope of this paper. However, for a thorough review of work in this area, the reader is referred to the studies of Russell (1927) and Cushing (1950). Early investigators noted an increase in plankters in the upper strata of water in the evening as opposed to relatively fewer at mid-day. Since then, much work has been done on the physical aspects of this daily rhythm (Russell, 1927; Hardy and Paton, 1943; Cushing, 1950, and 1951; Hardy and Bainbridge, 1954; Baylor and Smith, 1957; Wells, 1960; Beeton, 1960; McNaught and Hasler, 1964; and Swain, et al., 1970). More recent literature, however indicates a shift in interest from the actual process itself to the mechanisms producing the process.

It is thought that factors such as currents, food, pressure, temperature, light and intrinsic physiological rhythms may play a role in this mechanism. Hardy (1953) proposed that vertical migration evolved for horizontal distribution of limited swimming organisms by enabling these forms to take advantage of a variety of horizontal currents at various depths in large bodies of water. Internal currents have also been found to influence patchiness of plankton concentrations (Staven, 1971). Beeton, (1960); Swain, Wilson and Neri (1975) noticed a direct correlation between light and thermal regimes on the positioning of *Mysis relicta* (Loven). The vertical migration of *Limnocalanus macrurus*, *Daphnia retrocurva*, *Pontoporeia affinis*, and *Mysis sp.* in Lake Michigan was likewise reported to be influenced by temperature (McNaught and Hasler, 1964). Based on previous work of Michael (1911), Clarke (1933), and Ullyott (1939), most authors agree vertical spatial stratification is controlled exogenously by changes in light intensities.

Smith and Baylor (1953), experimenting with freshwater cladocerans, reported them to orient at right angles to a plane of polarized light. This orientation of the organisms shifted as the plane of vibration was rotated through 360°. Similar findings were reported in a study of two marine crustaceans tested in the field as well as the laboratory (Bainbridge and Waterman, 1957). Harris and Wolfe (1955) in a study on the vertical migration of *Daphnia magna* (Straus) found two independent mechanisms at work; 1) a photokinetic response precipitating a dawn rise during low intensities of light, and 2) an orienting phototactic response during high intensities. In a later work and agreeing with the findings of Viaud (1951), Harris and Mason (1958) report eyeless *Daphnia magna* still exhibit the photokinetic response, but not the phototactic one. Beeton (1960) in his study on *Mysis relicta* suggests that mysids are hypersensitive to light, resulting in their concentration near the bottom during the day. At night, after their initial rise following sunset, the mysids subsequently became photonegative causing a descent at dawn.

Smith and Baylor (1953), reported that clodocerans not only respond to light intensities, but also differentially to varying wave lengths. McNaught and Hasler (1966), and McNaught (1971), extracted visual pigments from daphnid eyes to determine their spectral responses in an effort to relate this information to field data of light intensities at depth versus zooplankton catches.

Imperative to a better understanding of the mechanism of vertical migration, therefore, is the study of the visual physiology of zooplankton in the laboratory as related to data of niche stratification from the field. The purpose of this report is to present preliminary work of the field portion of just such a study.

Instrumentation.

Sampler construction. In situ studies were made with a specially designed sampler constructed to minimize spurious responses to the parameters investigated, and to maximize the organism's response to normal environmental conditions. The prototype to this apparatus was reported by Hardy and Paton (1943). Using newer materials in its construction, improved refractive indices as related to water were achieved, thereby allowing better estimations of the effects of light intensities on zooplankton positioning.

The sampler (Figure 27) was constructed of a five-meter length of polycarbonate tubing 10 centimeters in diameter, divided into 1 meter sections. The top of each section was fitted with a polyvinyl chloride (PVC) fitting with internal screw threads for attachment to a similarly threaded fitting on the succeeding sections. The bottom of each section also included a stoppered draincock and a butterfly valve. The valve was spring-loaded and held open with a valve release pin. All such pins were attached in series with a release cord, which when pulled from above liberated all the pins simultaneously, thereby tripping the valves and sealing each section. Figures 28 and 29 are photographs of the column prototype.

The top section contains the hydrowire harness and was covered with #20 Swiss Silk Bolting Cloth (173 mesh/inch). Since the lower portion of the bottom section was also similarly covered, escape of the organisms was prohibited, although water could freely enter and flow through the system in its opened state. A ball valve situated in the bottom section allows water into the column upon descent, but obstructs its exit during ascent. The whole system is stabilized by a 22 kilogram weight suspended from the bottom section by means of a wire harness.

Photometer Construction. Light readings were taken with a specially constructed photometer incorporating a Clairex CL505L photosensitive transistor and a wide band micro-ammeter. The meter is driven by a 1.4 volt nickel-cadmium battery which activates a photosensitive transitor in series with the microammeter (Figure 30). Sensitivity is shunted through a series of precision (0.01 percent) resistors which provided extremely accurate readings in a range from 0.25 to 300 (w/m^2).

Two identical photosensitive cells, a sea cell and a deck cell (with acceptance angles of 58°) were utilized. Light striking the surface of the photo-cell alters the electrical resistance of the photo-transistor, in turn varying the current in the circuit and is ultimately reflected in the reading of the micro-ammeter. The values, in micro-amperes, were converted to watts per square meter by means of calibration (see Figure 31) with a Tektronix J1 Digital Photometer and a YSI-Kettering Model 65A Radiometer. The sensitivity of the photocells to various wavelengths of light is shown in Figure 32.

Test Organisms.

The test organism, *Daphnia magna* (Straus) and *Mysis relicta* (Loven), were cultured in the laboratory using the methods described by Diesinger and

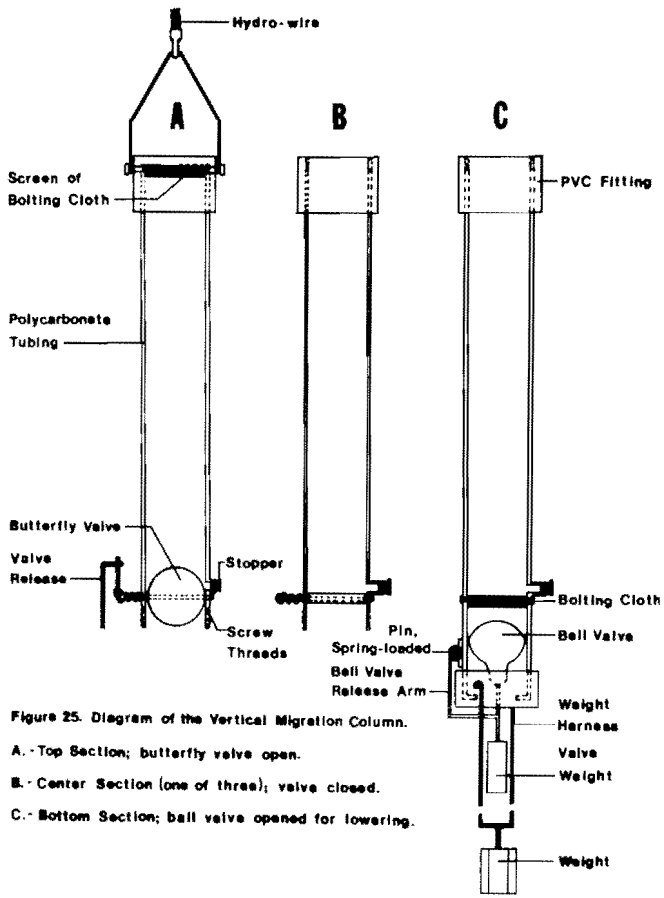

Figure 25. Diagram of the Vertical Migration Column.

A.- Top Section; butterfly valve open.

B.- Center Section (one of three); valve closed.

C.- Bottom Section; ball valve opened for lowering.

Figure 27. Diagram of the vertical migration column. A. top section; butterfly valve open, B. center section (one of three); valve closed, and C. bottom section; ball valve opened for lowering.

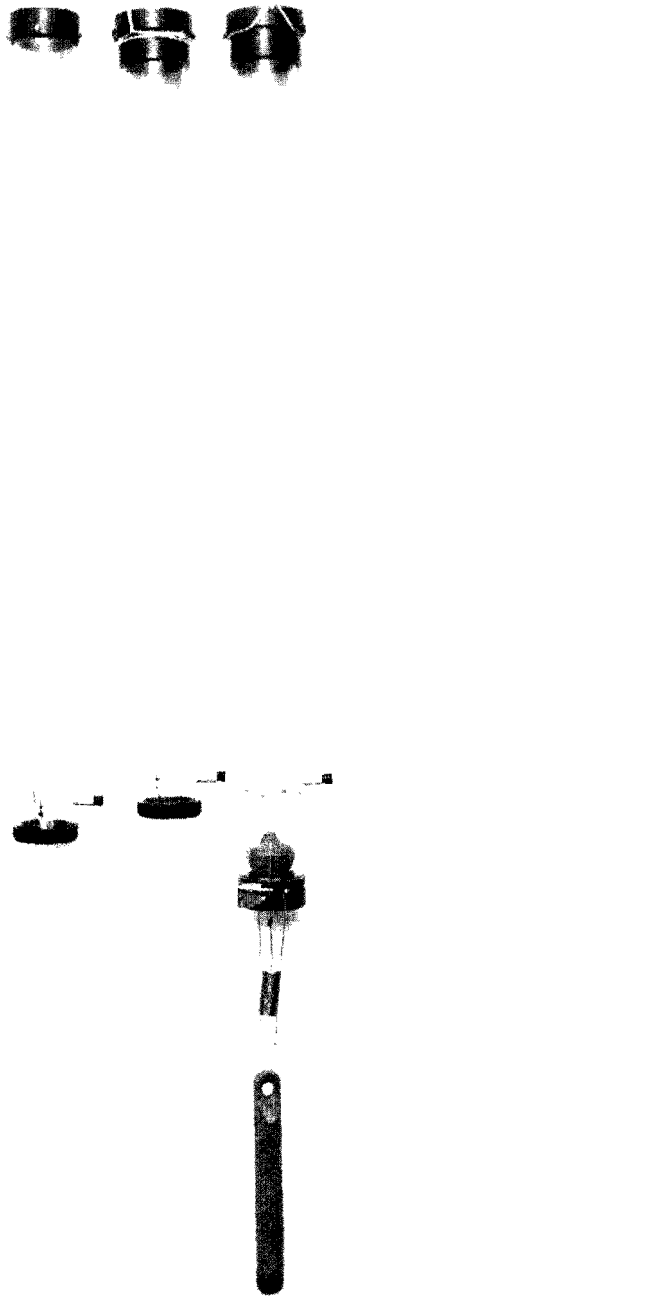

Figure 28. A side view of the top, a middle, and the bottom section of the vertical migration column. Note hydrowire harness on the top section and the stabilizing weight (22 kilograms) suspended from the bottom. Each section is one meter in length.

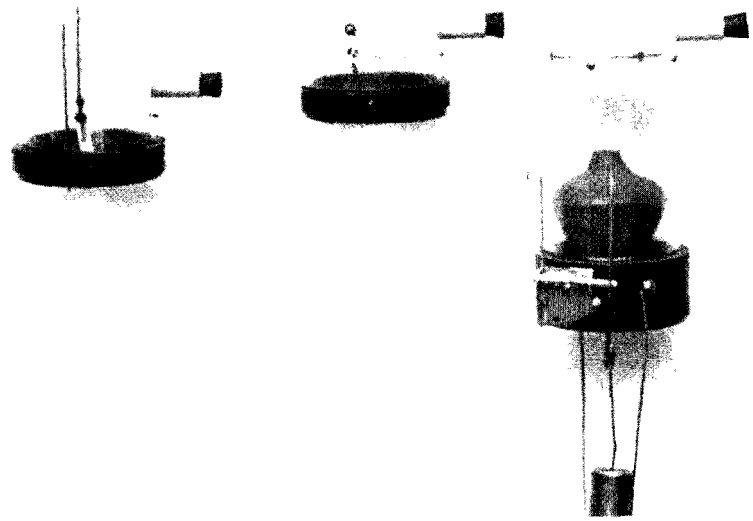

Figure 29. A close-up view of the bottom portion of three sections of the vertical migration column. From left to right; 1) an opened butterfly valve with valve release pin, 2) a closed butterfly valve, and 3) ball valve assembly with valve release pin. Stoppered draincocks are situated above the butterfly valves.

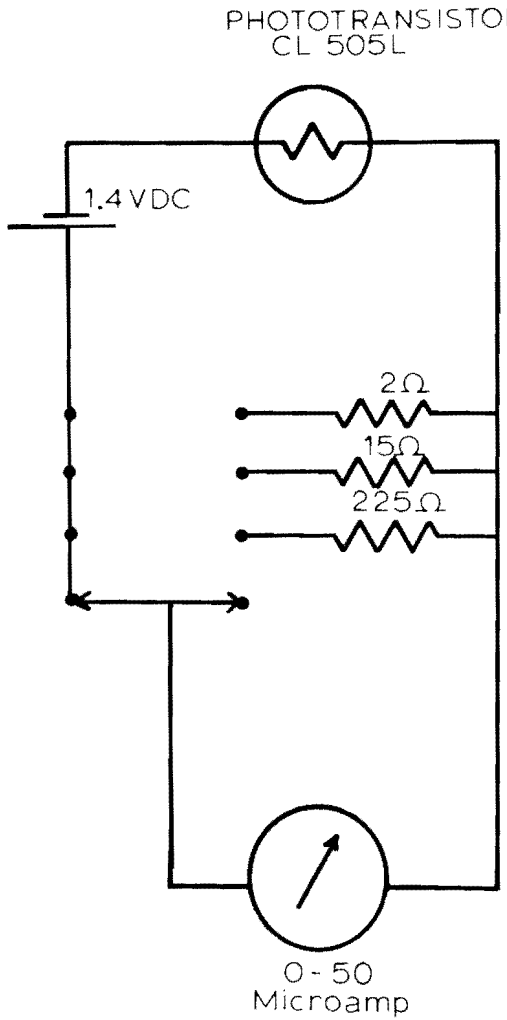

Figure 30. Circuit diagram of the Photometer.

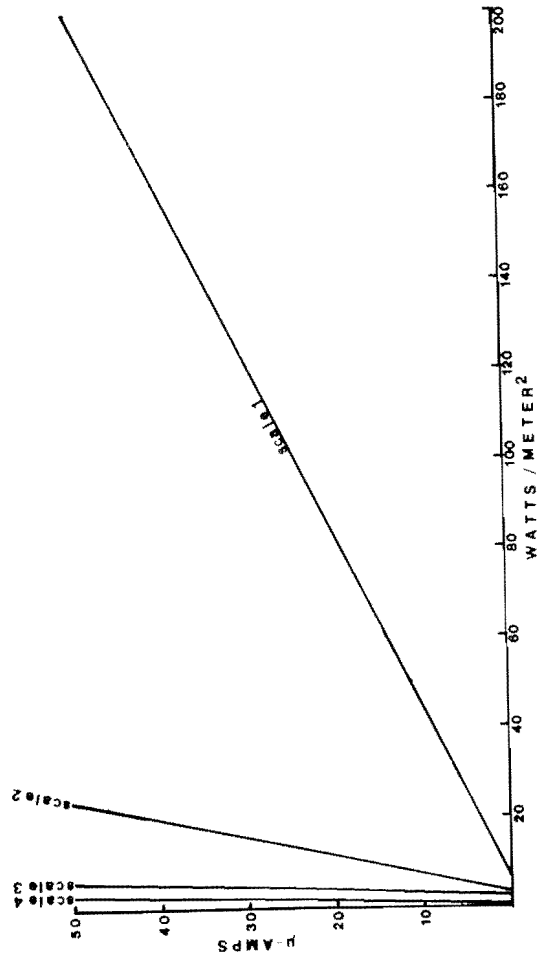

Figure 31. Photometric calibration curves.

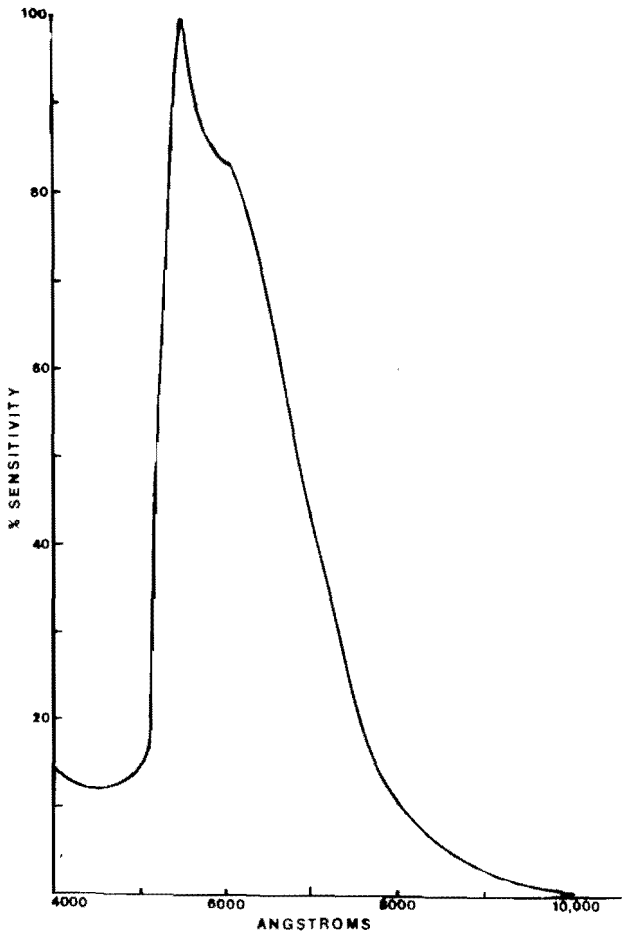

Figure 32. Wave length sensitivity of the photocell.

Christenson (1972) and Smith (1970) respectively. The *D. magna* were both light and dark adapted at 12° C. and dark adapted at 4° C. in environmental chambers.

The 12° C. environmental chamber was illuminated with three 15 watt, 125 volt, incandescent aquarium light bulbs, each inserted into a 10 inch stainless steel reflector. The light regimes to which the test organisms were subjected were in a range of 27 to 38 w/m^2. *D. magna* were light acclimated for a minimum of one month before experimental field use. The dark-adapted *D. magna* from the same stock cultures were kept in a darkened container with a light-tight feeding aperature. Dark-adapted forms were also acclimated for a period of one month before being used in the field. The light regime to which these organisms were exposed was below the threshold of detection of available photometers and radiometers (less than 0.001 micro watts/square centimeters).

When transporting to the field, 10 organisms of similar size were grouped in properly labelled vacuum bottles. These bottles were used to maintain relative constant temperatures of approximately 12° C., and also to exclude light from the dark-adapted forms. These organisms were quickly transferred in the dark and stored for twenty-four hours before use.

Once taken on board the research vessel, the tops of the vacuum bottles with the light-adapted organisms were removed and the containers were set in a shaded area below deck. The dark-adapted forms were kept in their sealed containers. In order to minimize light shock while injecting test animals into the column, the sampler was submerged in the water and the organisms were transferred quickly.

The mysids were dark-adapted in a similar fashion using a 4° C. temperature. These temperatures were selected since daphnids were expected to respond to available light in the upper layers of water, previously measured at 12° C. The mysids, however, were expected to orient toward much greater depths (hypolimnion) in which the temperatures rarely reach above 4° C., the maximum density of water.

Methods.

On September 19, 1973, experimental drops were made in the vicinity of the Duluth Pumping Station between the Lester and Talmadge Rivers on Minnesota's North Shoreline 1.5 statute miles off-shore (Figure 33). Upon reaching station, the sections of the column were assembled, all butterfly valves were locked into the open position and the sampler submerged. The desired test organisms were then injected into the second section. The top section was quickly added, and the column was then lowered to the desired test depth. At this depth, adquate time was allowed for the test organisms to orient at a specific level. During this period, Secchi Disc transparency and light measurements were taken.

Initially, a series of three drops were made each using five dark-adapted mysids. The mysids were rapidly placed in the column to avoid acclimatization to light and were lowered to depth. The run was begun at 43 to 48 meters

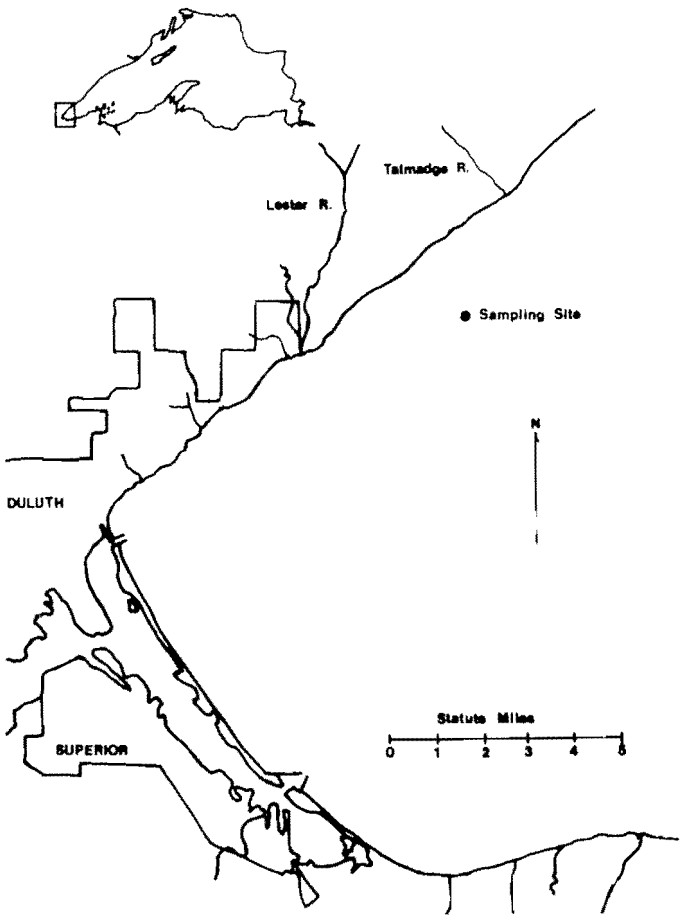

Figure 33. Sampling site location.

(top and bottom of the column respectively) and concluded three drops later at a depth of 53 to 58 meters. Each time the procedure was repeated, five new organisms were substituted for the exposed animals. The first two drops were left for ten minutes, while the last remained for seventeen minutes. This amount of time was determined to be adequate by a remote sensing technique described elsewhere by the author, Swain et al., (1974) confirming the results of Hardy and Bainbridge (1954) who observed a maximum vertical migration rate of one meter per minute.

A second series of test runs included four drops from 0 to 5, 5 to 10, 10 to 15, and 15 to 20 meters using ten light-adapted *Daphnia magna*. New organisms were used each time and held at depth for fifteen minutes.

During the last series, six runs were made starting from 0 to 5 meters with 5 meter increments each time. Ten dark-adapted *Daphnia magna* were used each time, and were also held at depth for fifteen minutes.

When the designated sampling duration had elapsed, the column was brought to the surface. The individual sections were visually examined for total number of organisms present and the data recorded. Each section was then drained into a properly labeled sample bottle for laboratory confirmation.

Sequential light measurements were taken with the photometer every meter for the column of water being sampled. This was done once in the morning and during every sampling run.

RESULTS AND DISCUSSION

On September 19, 1972, samples were taken in the vicinity of the Duluth pumping station (between the Lester and Talmadge Rivers) 1.5 statute miles off-shore. Light measurements were taken immediately upon reaching station and during every drop. Figure 34 is the extinction curve, in watts per square meter versus depth, taken at 8:00 a.m. CDT under partly cloudy skies. The extinction curve inside the sampler was also determined by measuring the entire water column with the photosensitive probe inserted inside the column. A comparison of these values is shown in Figure 35. With the exception of the first 5 meters, little difference was observed between the two curves.

Using five dark-adapted *Mysis relicta*, three successive drops were made between 10:15 and 10:45 a.m., and sequential light measurements were taken. Figure 36 shows their orientation in starts with very low light levels. Minimum light values achieved in this sampling period were found to be 3×10^{-2} watts/square meter. The deep-water orientation as displayed by the mysids seems to indicate a hypersensitivity to light, supporting Beeton's hypothesis (1960) related to dark to light acclimation in the natural state.

The occurrence of the mysids in the lower cells of the column suggests that light levels achieved in this sampling run may still have been excessive for the dark-adapted mysids. Beeton (1959) reports the response of mysids to light intensities of 9×10^{-6} foot candles, a value considerably below test conditions reported here.

To attempt to confirm these findings, a sequence of high-speed plankton tows was made using stacked Miller High Speed Samplers (Miller, 1961). In this sampling sequence, the lowest Miller sampler was towed at a distance of 1 to 3 meters from the bottom. Throughout the runs, no mysids were taken. This is further supported by Beeton (1960) who demonstrated that these malacostracans do not initiate their diel movement until light levels reach 10^{-1} lux. Further, McNaught and Buzier (1966) determined that an optimum natural photo-environment for mysids occurred in a range between 10^{-3} and 10^{-5} lux. Exceptions to this general optimum light range do occur, however. Although most data restrict mysids to the deeper bottom water of cold northern lakes (Beeton, 1960; McNaught and Buzier, 1966; and Reynolds and Dollenove 1970-1971), Smith (1970) while scuba diving off Two Harbors, Minnesota, observed them on the bottom in 30 to 50 feet of water during the day. The water temperature was reported at 4 to 6° C. during this observation. Later when temperatures reached 10 to 12° C. the mysids had moved to deeper water. Unfortunately, no light measurements were taken. A similar sighting while scuba diving was made by Swain et al., in October, 1971 at Grand Marais Harbor, Minnesota. They found mysids along the bottom in 20 to 30 feet of water over a gray, soft sediment bottom.

The *Daphnia magna* (Straus) tests were undertaken with both light and dark adapted organisms. Using ten test *Daphnia* each time, it was necessary to run only four trials of the light-adapted daphnids because of their definitive response. Figure 37 shows the data for the sequence of *Daphnia*

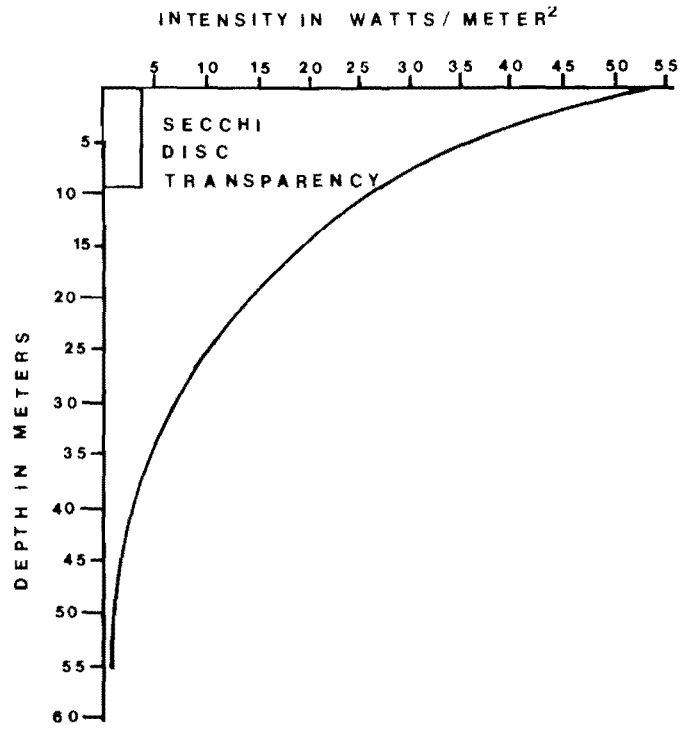

Figure 34. Light extinction curve and Secchi Disc transparency.

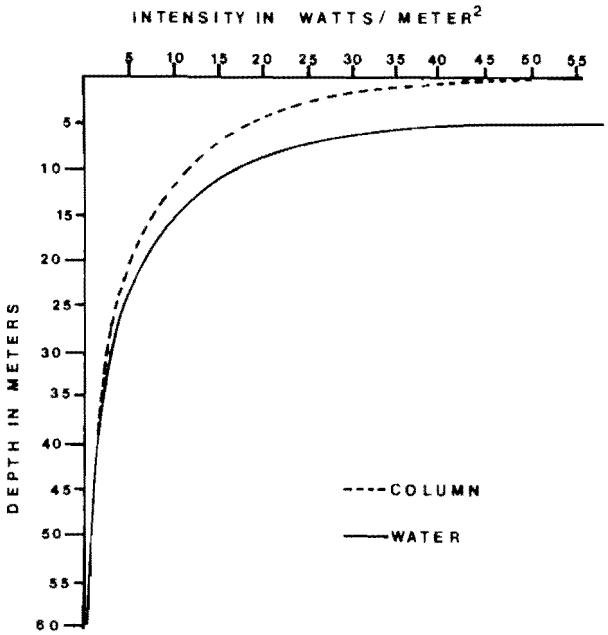

Figure 35. Comparison of the extinction curves for the water column and the interior of the sampler.

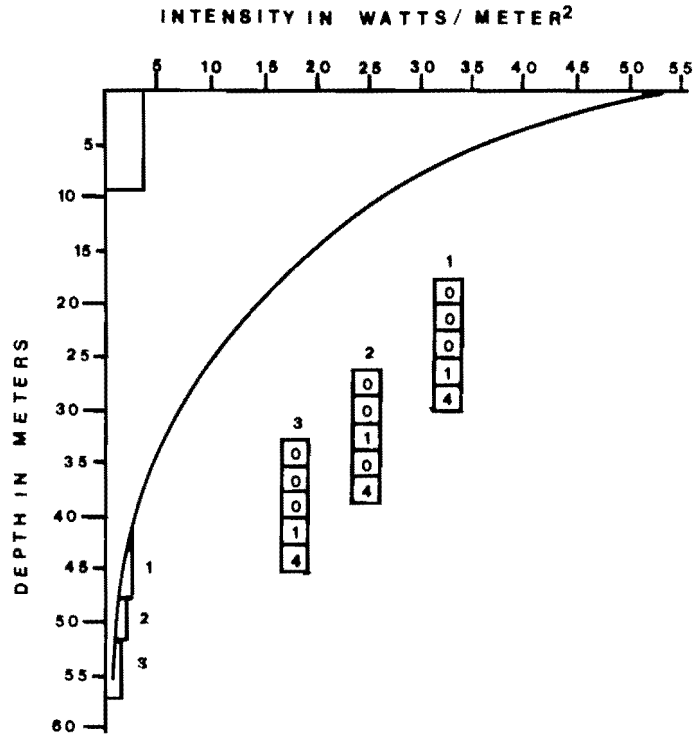

Figure 36. Distribution of dark-adapted *Mysis relicta* in the sampler.

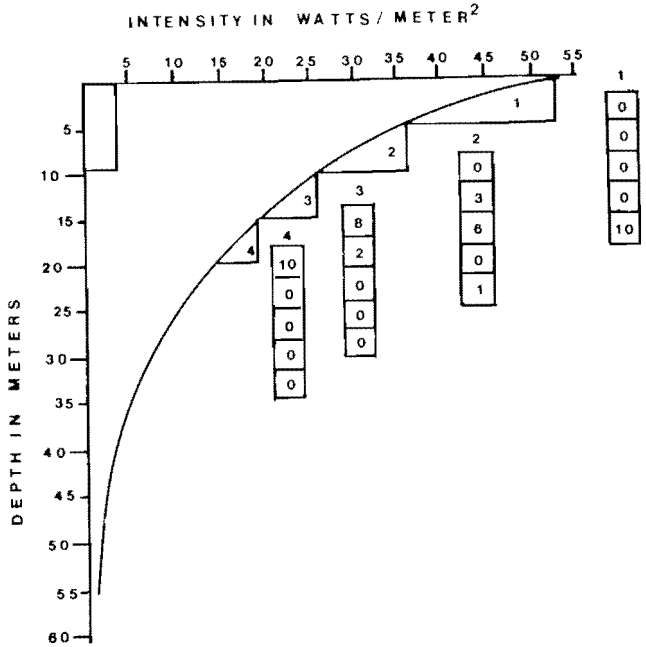

Figure 37. Distribution of light-adapted *Daphnia magna* in the sampler.

drops. The first group of ten organisms oriented in the bottom section apparently in response to excessive light levels. The orientation of nine organisms in the 6 and 7 meter sections of the second run indicates an apparent approach toward an optimum light regime. This is further supported by trials 3 and 4 in which both groups are positioned in the top sections. The orientation of ten test individuals in the Group 4 trials suggests that the optimum range had been exceeded. The general distribution, therefore, was between 7 and 12 meters of depth. Corresponding light values for this depth were observed to be 30 to 45 w/m^2, closely approximating the acclimatization values of 27 to 38 w/m^2.

The dark-adapted organisms were tested in the same way as the light-adapted forms. Figure 38 shows their apparent sensitivity to high light levels.

In trials 1 through 4, the test populations oriented in the lower sections. Values for Trial 2 are not reported owing to instrument failure. Finally, in trials 5 and 6 dispersion was observed. Particularly in trial 6, the population was widely scattered suggesting optimum light regimes. This strata, 24 to 30 meters in depth, was observed to provide a light regime between 6.4 to 10.2 w/m^2, an approximate four-fold reduction as compared with the light adapted forms.

Another factor influencing the vertical migration of zooplankton is the wave-length of light penetrating to various strata. While this factor was not specifically addressed in this study, a number of authors have already investigated this phenomenon with interesting results. It has long been known that Cladocera possess trichromatic vision (Ueberdev, 1948). Since then, further information suggests that they respond differently to varying intensities of selected wave-lengths of light (Smith and Baylor, 1953), and that the plasticity in their visual apparatus enables them to adapt to drastic changes in their photic environment (McNaught, 1971).

In the studies reported here, it should be noted that in the case of the light-adapted organisms, i.e., *Daphnia magna*, the response of these forms apparently incorporated both the parameters of intensity and wave-length. Adaptation of the organisms was accomplished with white light, incorporating most, if not all, of the wave-lengths of concern. However, when tested in the water column, the organisms oriented at a level of blue-green wave-length flux equivalent to the range of total white light radiation to which they had been exposed during acclimation. This factor suggests a much stronger relationship between total intensity and wave-length than had been previously suspected. Further, it appears that intensive investigations of this phenomenon would make a very productive contribution to the understanding of vertical migration.

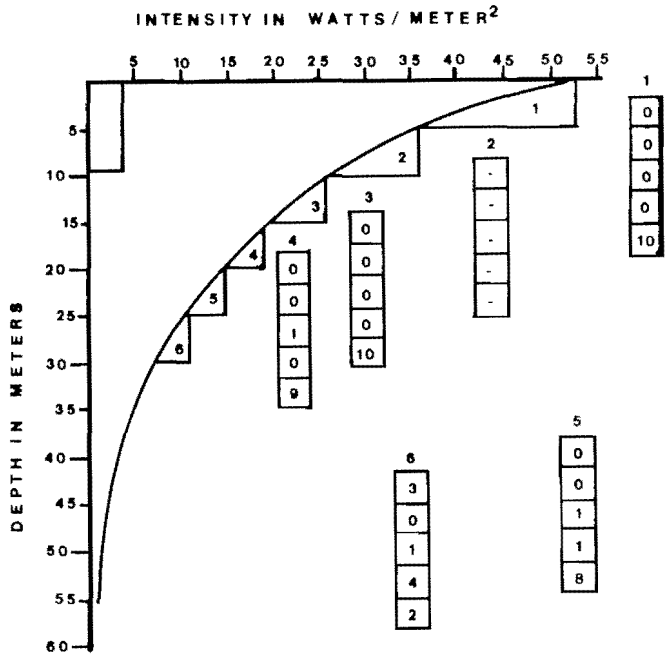

Figure 38. Distribution of dark-adapted *Daphnia magna* in the sampler.

HORIZONTAL TEMPERATURE GRADIENT EXPERIMENTS

Having observed the effects of thermal addition on the vertical behavior of crustacean zooplankton, a horizontal chamber was constructed to observe the positioning of warm (18.0°C) and cold (7.0°C) acclimated *Daphnia* to a thermal gradient. A generalized line diagram of this chamber is shown in Figure 39.

The chamber was constructed of glass and plexiglass. The outer water jacket (100 x 10 x 5.2 cm.) was constructed of plexiglass. It was designed to allow cold tap water to enter a single inlet on the "cold" end of the test chamber and exit via two overflow tubes at the "hot" end of the chamber. The experimental chamber (120 x 7.5 x 2.8 cm.) was constructed of single strength window glass to take advantage of the higher thermal conductivity properties of this substance as compared with plexiglass. One end of the chamber was emersed in an ice bath, while the other was supplied with a heating tape connected to a rheostat. As tap water was forced to slowly circulate along the length of the water jacket, a thermal gradient was established in the temperature chamber. This arrangement of elements allowed for the development of the temperature range used for testing between 7.0 and 18.9°C. Both higher and lower extremes were developed, but were not utilized as study parameters. Throughout the experimental process, a uniform lighting of 0.10 ± 0.01u watts/cm^2 was used. All temperature values were confirmed with a Digitec 581C digital thermistor thermometer accurate to ± 0.3°C with resolution of 0.1°C. Thermistors were inserted at eight points along the walls of the experimental chamber corresponding to the following temperature ranges:

7.0 -	8.4°C
8.5 -	9.9°C
10.0 -	11.4°C
11.5 -	12.9°C
13.0 -	14.4°C
14.5 -	15.9°C
16.0 -	17.4°C
17.5 -	18.9°C

Probe heads were shifted as necessary to monitor the spatial variations in these temperatures.

Daphnia adapted to 7.0°C for a minimum of 6 weeks prior to testing were introduced into the experimental chamber at 7.0°C. For the first 15 minutes following their introduction the *Daphnia* remained in the 7.0 - 9.9°C portion of the gradient (see Table III). With additional time, *Daphnia* acclimated and moved to a higher temperature. After 60 minutes these organisms were observed as high as 18.0°C. Results indicate that although acclimated at 7.0°C, most organisms were observed in the warmer waters between 10.0 and 14.4°C. This table and the two preceding tables represent the mean of ten observations of ten organisms per trial.

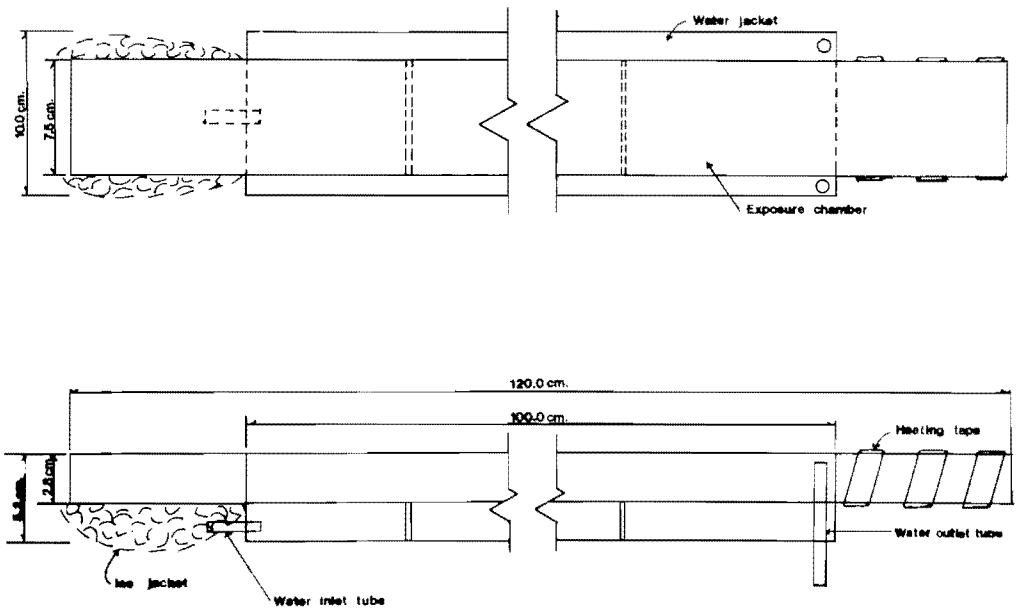

Figure 39. Line diagram of the horizontal temperature gradient chamber.

Table III

Temperature Exposure - Horizontal Chamber
Daphnia magna
Low Temperature Adaptation - 7.0°c
Organism introduced at 7.0°c

Duration in Minutes	Temperature in Degrees Centigrade							
	7.0-8.4	8.5-9.9	10.0-11.4	11.5-12.9	13.0-14.4	14.5-15.9	16.0-17.4	17.5-18.9
1	9	1	-	-	-	-	-	-
5	7	3	-	-	-	-	-	-
10	10	-	-	-	-	-	-	-
15	6	4	-	-	-	-	-	-
20	4	1	3	2	-	-	-	-
25	3	2	2	1	2	-	-	-
30	3	3	2	-	2	-	-	-
35	5	0	2	1	1	1	-	-
40	2	3	3	1	1	-	-	-
45	3	2	3	1	-	-	1	-
50	1	2	4	-	2	-	-	1
55	-	1	2	3	1	-	2	1
60	-	-	3	2	1	1	1	2

Daphnia adapted for a similar period of time at 18.0°C and introduced at 18.0°C showed a similar trend (Table IV). A general movement from warmer to cooler water was observed over the 60 minute period of observation. Again, the largest grouping of organisms was observed between 10.0 and 14.4°C.

Table V depicts the introduction of 18.0°C acclimitized *Daphnia* into 8.5°C water in the experimental chamber. Initially, little movement was observed. Ultimately, two distinct populations of *Daphnia* were developed, one which remained at 8.5 - 11.4°C and the other which moved to the 17.5 - 18.9°C range. Throughout the course of the study (2 additional hours not shown on Table V), these populations did not intermix or substantially change the pattern demonstrated at the 35 and 45 minute notations for Table V.

Studies of Myrids using this chamber established that these organisms exhibited a very narrow thermal range when allowed access to a variety of temperature regions. When percentage of time spent at a given temperature was calculated, 96 percent of the time *Mysis relicta* oriented at the acclimitization temperature provided it was between 4.0°C and 11.0°C. The remainder of the time was spent at a maximum of 4.9°C from the acclimitization temperature. In this system, *Mysis* was not observed at temperatures exceeding 12.0°C. This confirms the work done by Beeton (1960) in which he suggests the nepthermal nature of this organism. *Daphnia*, on the other hand, showed a much more stenothermal orientation with only 21 percent of its overall orientation at the acclimitization temperature and the remainder varying by as much as ± 12.0°C. Wider orientation patterns were observed in these *Daphnia* acclimated at warmer temperatures.

Table IV

Temperature Exposure - Horizontal Chamber
Daphnia magna
High Temperature Adaptation - 18.0°C
Organism introduced at 18.0°C

Duration in Minutes	Temperature in Degrees Centigrade							
	7.0-8.4	8.5-9.9	10.0-11.4	11.5-12.9	13.0-14.4	14.5-15.9	16.0-17.4	17.5-18.9
1	-	-	-	-	-	-	1	9
5	-	-	-	-	-	1	2	7
10	-	-	-	-	-	2	1	7
15	-	-	-	-	-	2	-	8
20	-	-	-	-	3	1	2	4
25	-	-	-	2	1	2	2	3
30	-	-	1	1	1	3	1	3
35	-	-	1	1	3	2	1	2
40	1	1	1	2	2	1	2	1
45	-	1	2	2	-	3	2	-
50	1	3	1	2	1	1	2	-
55	1	2	2	-	2	2	1	-
60	2	1	3	1	2	1	-	-

Table V

Temperature Exposure - Horizontal Chamber
Daphnia magna
High Temperature Adaptation - 18.0°c
Initial Introduction into 8.5°c Water

Duration in Minutes	Temperature in Degrees Centigrade							
	7.0-8.4	8.5-9.9	10.0-11.4	11.5-12.9	13.0-14.4	14.5-15.9	16.0-17.4	17.5-18.9
1	-	10	-	-	-	-	-	-
5	-	9	-	-	-	-	-	1
10	-	5	4	-	-	-	-	1
15	-	7	2	-	-	-	-	1
20	-	5	2	2	-	-	-	1
30	-	1	3	2	-	-	-	3
35	-	3	2	1	-	-	-	4
45	-	3	2	-	-	-	1	4

DETERMINATION OF ACTIVITY RANGES OF SELECTED MICROORGANISMS

A series of studies designed to determine the activity ranges of selected microorganisms were undertaken. The immediate conversion system described in a preceding section was modified for this purpose.

A chamber made of plexiglass (25 x 20 x 15 cm.) was constructed in a crescent shape to effect cooling of the test organisms. The test chamber contained two 20 x 15 cm. 304 stainless steel electrode grids and a thermistor probe. The electrode grids were connected to an impedance coupler, amplifier, oscilloscope and a multi-channel recorder as previously described. An olympic dissecting microscope on an extension arm was mounted above the chamber to observe minute organism movement. A generalized flow diagram of the system is shown in figure 40.

Daphnia acclimated at 19.0°C were placed in the test chamber and slowly cooled to 0.0°C. In each case, the start of each run was begun at the acclimatization temperature. Thermal activity was observed until temperatures approached 0.0°C. Below this temperature, 10.0°C adapted *Daphnia magna* were run in 50 percent of the time observed on the bottom of the chamber. As the temperature was decreased below this threshold, the amount of activity time increased until 0.5°C was reached, at which time the organisms ceased to move. This temperature did not prevent biological activity was observed until temperatures approached 0.0°C. Below this temperature, 10.0°C adapted *Daphnia magna* were at 0.0°C it was possible to observe heart action, which continued even in the point of the ventricle being frozen in a solid block of ice. As temperatures were slowly increased, activity gradually resumed until normal levels were reached. From these studies, it was clear that adult *Daphnia* did not suffer appreciable damage as a result of short-term exposure to temperatures as low as 0.0°C.

The examination the effects of short-term exposure of test organisms were considered using a heated bath. Because of the independence of organism behavior, it was decided that for these trials, a mechanism which utilized visual observation alone would be used.

Cervid adult *Daphnia* of identical age groupings were placed in a series of test tubes with equal volumes of water, one organism per tube. Test tubes were individually placed in a water bath and heat increased. In each case, temperatures in the organisms environment were raised from acclimation temperature (19.0°C) to the maximum exposure temperature during a 5 minute time period.

It was found that for this technique, 50 - 51°C represented the point at which immediate thermal death occurred. Those organisms exposed to 49°C revived only to die a short time later of the delayed effects of thermal additions. Further, organisms exposed throughout a wide range of temperatures showed related effects. As would be expected increasing thermal stress generally resulted in a shorter survival time for exposed adults. This relationship is shown for selected temperatures in figure 41. Similarly, additional data accumulated on recovery time of *Daphnia* exposed to short-term thermal shock also demonstrated that increasing heat stress prolonged recovery time. Over a range of 2.5°C (36.5°C to 39.0°C), recovery time increased nearly 3 fold. At the upper end of the thermal spectrum, a one degree

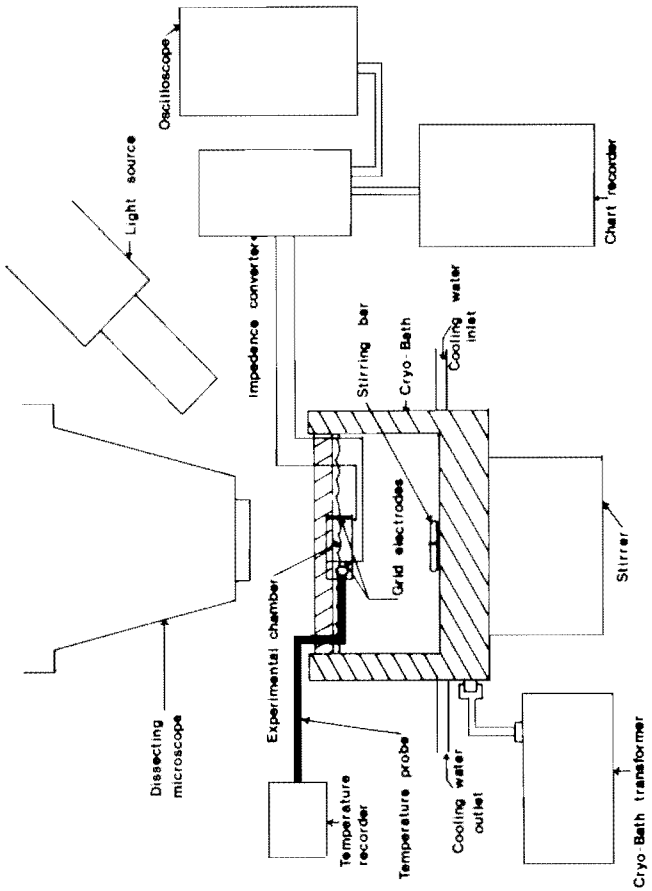

Figure 40. A generalized line diagram showing the arrangement of elements in the low temperature studies on zooplankton.

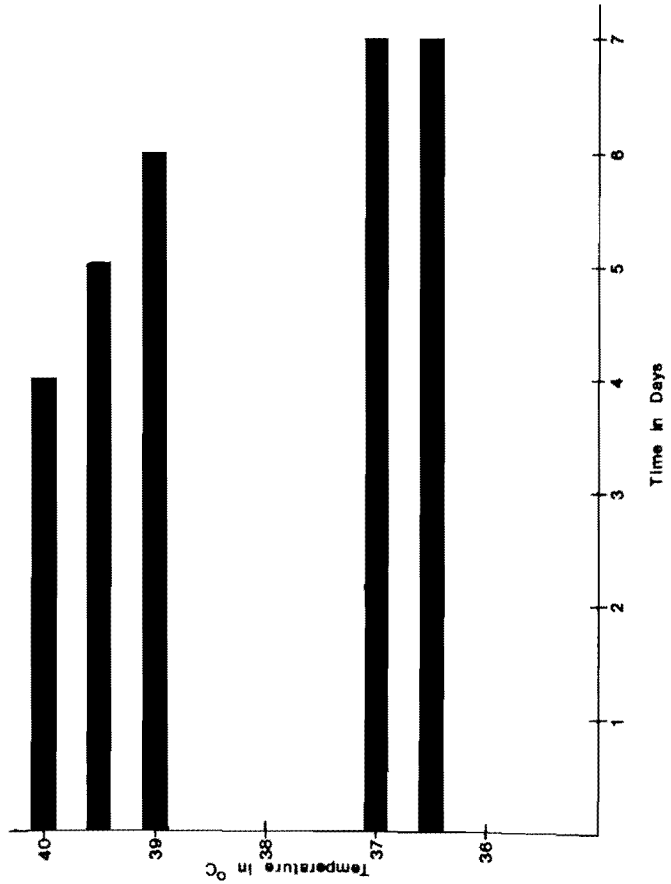

Figure 41. Time (in days) to 100 percent mortality of test populations of adult *Daphnia* at varying short-term thermal exposures.

temperature rise accounted for an additional 3 fold increase in recovery time. These relationships are shown in Figure 42.

Events prior to these lethal temperatures are depicted in Table VI. These phenomenon ranged from avoidance movement and horizontal circling to heat coma (thermal shock). At 36.5°C viable young were expelled from the brood pouch. While there was no evidence of undeveloped eggs being expelled, the young were considerably premature and extremely small in size.

Additional data were obtained on the survivorship of premature progeny expelled by heat shocked *Daphnia*. Table VII shows the number of progeny expelled and their subsequent mortality at various temperatures. These data are graphically portrayed in Figure 43. As was previously noted, a period of 5 minutes was used to raise the temperature of the organisms environment from acclimitization temperature to the maximum exposure temperature. Thus, the number of progeny produced (Table VII) reflects the amount of time available to the organism between the onset of expulsion of viable young at 36.5°C and the onset of intermittent inactivation at 37°C and heat stupor at 37.5°C.

The slope of the curves in Figure 43 graphically display the mortality rates shown in Table VII. It is clear that increasing thermal stress at the time of parturition mitigates against survival of the organism. From these studies, it became clear that reproductive potential was drastically affected by short-term exposure to heated effluents.

Additional studies of the tolerance ranges of other zooplankters, including myrids and selected copepods, is presently ongoing.

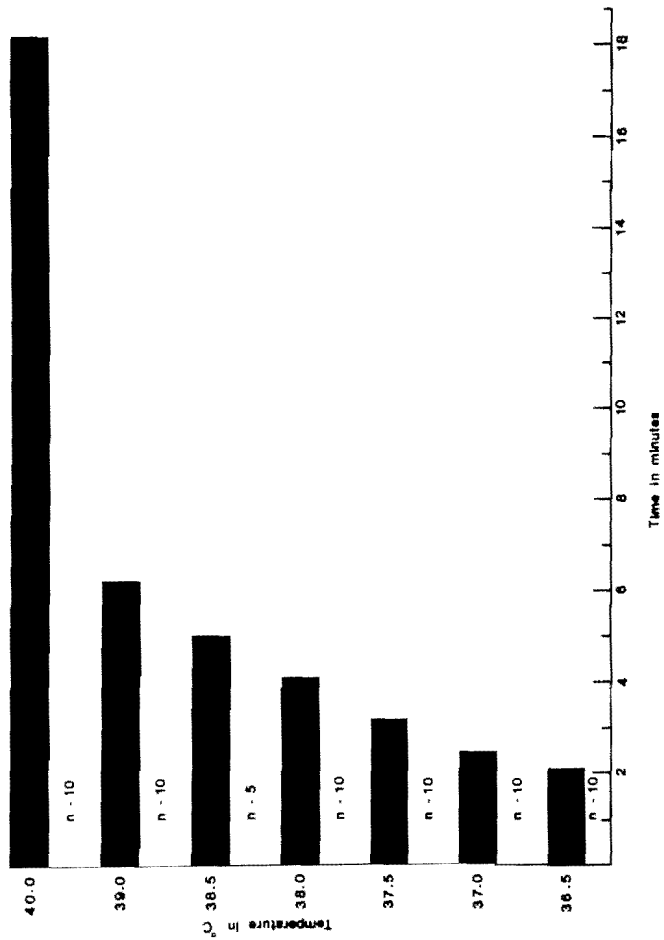

Figure 42. Mean recovery time in minutes for adult *Daphnia* exposed to short-term thermal shock at various temperature increments.

Table VI

MEAN RESPONSES OF *Daphnia magna*
TO SHORT DURATION TEMPERATURE STRESS
Organisms Acclimatized at 19° C.
n=60

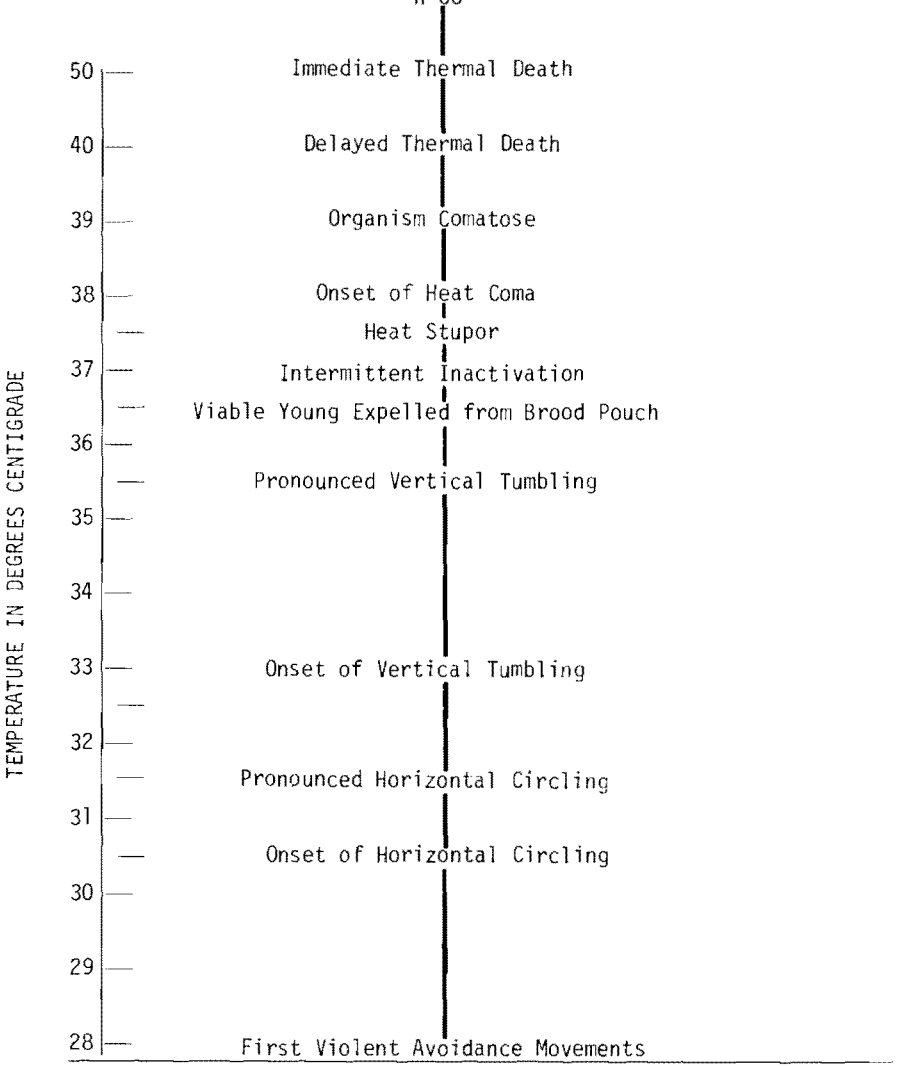

Table VII

SURVIVORSHIP OF PREMATURE PROGENY EXPELLED BY GRAVID *DAPHNIA*
UNDERGOING SHORT TERM HEAT SHOCK

Daphnia Grouping	Maximum Exposure Temperature in Degrees Centigrade	Mean Number of Progeny Born Prematurely	Per Cent Mortality 5 Days	6 Days	7 Days	8 Days	9 Days	10 Days
D_1 n=10	36.5	29	0	3.4	20.68	75.7	79.3	82.7
D_2 n=10	37.0	27	18.5	22.2	51.85	88.8	92.6	96.2
D_3 n=10	39.0	25	66.6	70.8	70.8	72.0	76.0	80.0
D_4 n=10	39.5	11	81.8	81.8	81.8	81.8	81.8	81.8
D_5 n=10	40.0	2	100	100	100	100	100	100

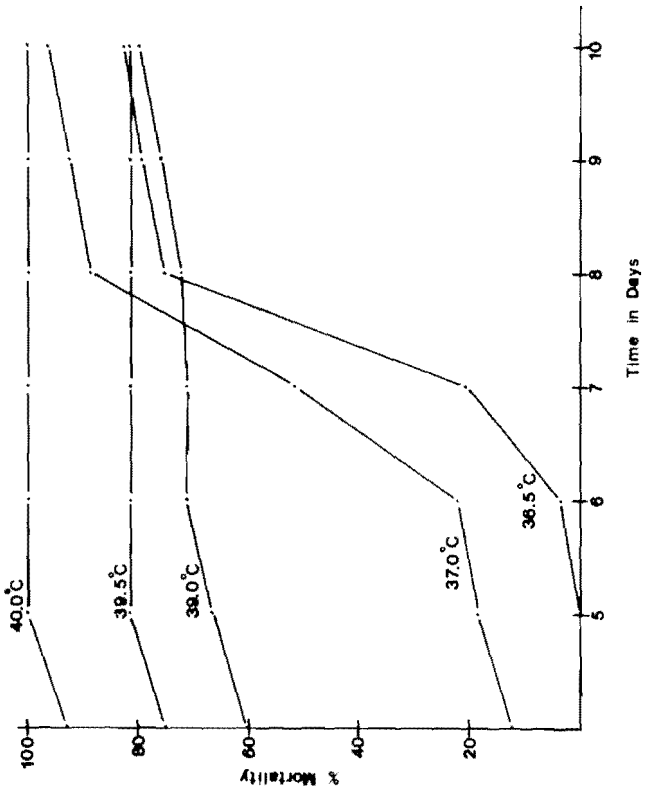

Figure 43. Percent mortality of premature progeny expelled by gravid *Daphnia* undergoing short-term thermal shock.

STUDIES OF THE EFFECTS OF THERMAL ADDITIONS ON REPRODUCTIVE POTENTIAL OF *Daphnia magna*

From previous studies on the effects of heat on organism behavior, it was evident that thermal additions could potentially have profound effects on the reproductive potential of a wide variety of aquatic organisms. For these studies *Daphnia magna* was selected for the following reasons:

1. This organism is widely available for use in other locales and is accepted as a standard bioassay organism.

2. The ecological niche occupied by this organism closely approximates the section of the littoral area most likely to be affected by thermal additions.

3. Clones of this organism are easy to isolate, thus preserving genetic continuity throughout a group of experiments.

4. Ease of manipulation of these organisms facilitates counting of progeny, observation of egg development, brood size and the like.

5. The short reproductive span of this organism is an essential criterion. Many other forms found in Lake Superior have reproductive cycles of up to two years duration.

Methodology.

A study to determine the effects of thermal additions on reproductive potential was undertaken. Of particular interest was the impact of califaction on egg development, second generation mortality, fecundity, and the length of time between broods and the number of reproducing organisms in the second generation.

The *Daphnia magna* used in these studies were initially obtained from the Environmental Protection Agency's National Water Quality Laboratory in Duluth, Minnesota. The cultures were maintained in the laboratory at an acclimitization temperature of 18.5°C. Stock cultures were held in one and one-half gallon glass tanks from which organisms were removed for the various temperature tolerance experiments. In all cases, temperature was the single varient, with other environmental parameters held constant.

For each run, non-gravid parthenogenic females (F_1 generation) were placed in individual 250 ml. beakers containing 200 ml. of unfiltered Lake Superior water. Supplemental food sources were provided in accordance with the method described by Biesinger and Christensen (1972). The test organisms were then placed in an appropriate constant temperature chamber and observed on a daily basis. Thermal regimes were monitored with a YSI Model 47 scanning tele-thermometer coupled to a YSI Model 81A two channel recorder. Illumination at a mean value of 32.5 μwatts/cm^2 was maintained. Photo

period was controlled by use of electrical timers which provided equal increments of light and dark.

When progeny were produced (F_2 generation), individuals were removed and placed in separate 15 ml. test tubes under conditions identical to the parental generation. Each individual was assgined code numbers indicating brood, date of birth, birth order of the brood and parental stock. Thus, it was possible to follow the progress of both cones and parents. Upon development of eggs in the brood pouches of F_2 organisms, these individuals were immediately transferred to a 250 ml beaker. Identical procedures were then followed for the F_3 generation.

A total of seven test temperatures were utilized; 7.5°C, 11.5°C, 15.5°C, 18.0°C, 19.0"C, 22.5°C, and 24.5°C. These temperatures were selected since they covered a large portion of the life and reproductive range described by MacArthur and Baillie (1929).

Results.

The temperature at which a test population reached maximum numbers was 18°C. Further, it was at this temperature that the largest number of reproducing F_2 generation members were observed. (See Table VIII). Pratt (1943) reported that the mean number of *Daphnia* cultured at 18°C were two and one-half times greater than the mean size of the population cultured at 25°C. Further, he reported that the birth rate of 25°C populations was higher than that of the 18°C population, but an inverse relationship was observed with respect to longevity. Chernyuk and Panasyuk (1965) found 24-25°C temperatures to be optimal for young daphnids. The studies reported here confirm these findings for *Daphnia magna*. The highest level of reproduction (Table VIII) was found at 24.5°C. At this temperature, 24.0 percent of the F_2 generation reproduced, but absolute numbers of this group were greatly depressed. Ultimately, longevity at 18°C dictated a much larger F_2 generation than the 24.5°C group (123 individuals at 18°C as compared with 33 individuals at 24.5°C).

The length of time for the development of eggs in the brood pouch of gravid F_2 generation *Daphnia* is shown in Figure 44. Increasing temperature decreases the amount of time required for egg development. A range of time from 19.0 days at 7.5°C to 3.2 days at 24.5°C was observed. A similar inverse relationship with respect to temperature was observed when the length of time between clones was examined. At 7.5°C, 21.2 days were required between clones, while at 24.5°C, only 3.8 days were necessary (see Figure 45). Thus, an increase in the thermal environment tends to accelerate life processes as an inverse function of longevity.

It is interesting to note that Pratt (1943) reports that he was unable to maintain populations of *D. magna* at 12°C. He notes that after a few weeks populations dwindled to extinction. This was not true in the studies reported here. While length between broods and length of time required for egg production was greatly expanded survivorship presented no problem even at 7.5°C. At the time of this writing, populations cultured at these temperatures have been continuously maintained for more than two years. It is

TABLE VIII

Amount of Second Generation Reproduction in Daphnia by Test Temperature

Temperature	Numbers of F_2 Generation Tested	Numbers of F_2 Reproducing	% Reproduction
7.5	49	0	0
11.5	58	0	0
15.5	61	1	1.6
18.0	123	18	14.6
19.0	119	15	12.6
22.5	16	0	0
24.5	33	8	24.0

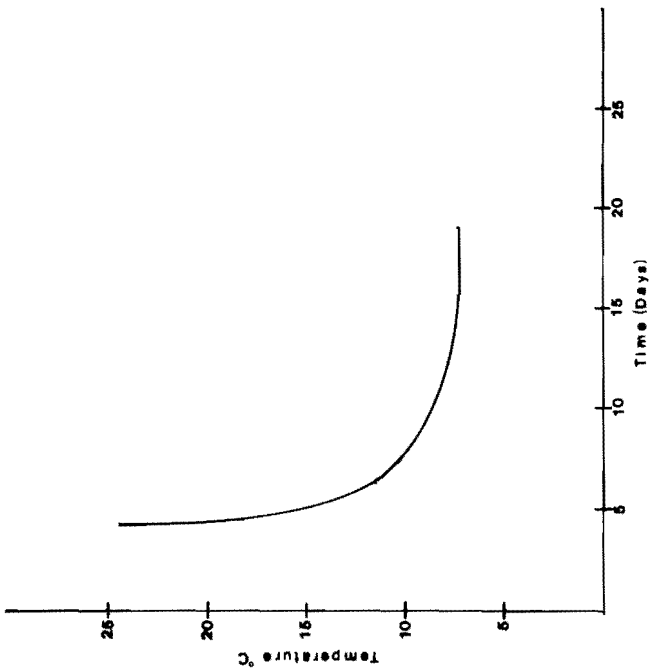

Figure 44. Length of time (days) for the development of eggs in the brood pouch of gravid F_2 generation *Daphnia* at various temperatures.

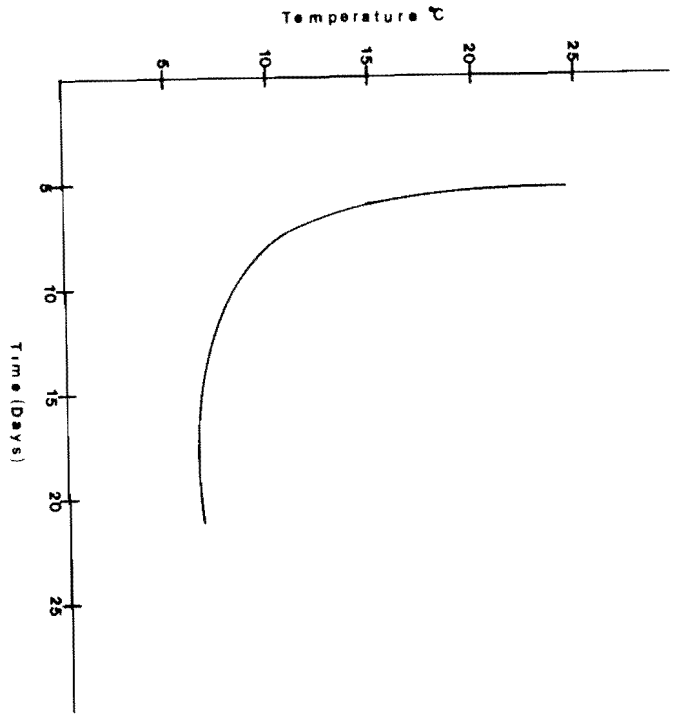

Figure 45. Length of time (days) between clones of F_2 generation *Daphnia* at various temperatures.

interesting to note, however, that progeny produced at these temperatures result from eggs of reduced size, are physically smaller than their warmer water counterparts, and grew much more slowly, if at all.

Thus, thermal additions tended to reduce longevity, increase reproductive rates, and increase factors related to fecundity. In addition to a compression of the reproductive cycle in time, increased thermal regimes also tended to amplify population fluctuations. These factors in total tend to mitigate against population stability at higher temperatures.

SUMMARY

1. A non-invasive remote monitoring system for aquatic invertebrates population is described.

2. The evolution and design of experimental chambers capable of controlling various physical parameters (light, temperature, oxygen) while monitoring behavioral effects on aquatic organisms is described.

3. Results of laboratory experiments designed to measure the effect of heat and light on the vertical migration of *Mysis relicta* is included.

4. A sampler for determining the orientation depth of planktonic organisms is described.

5. A photometer for measuring irradiance of both water and air is described.

6. The light regimes at 58 meters (3 X 10^{-2} watts per square meter) was found to be excessive for dark-adapted *Mysis relicta*.

7. Light-adapted *Daphnia magna* migrated to high light intensities of between 30 to 45 watts per square meters. This was very close to acclimation intensities of 27 to 38 watts per square meters.

8. Dark-adapted *Daphnia magna* migrated to low light intensities of between 10.2 to 6.4 watts per square meter.

9. The findings suggest a relationship exists between total flux and wavelength at depth as a factor influencing vertical migration.

10. A chamber designed to maintain a horizontal temperature gradient is described including test results using both *Daphnia magna* and *Mysis relicta*.

11. The description of apparatus used and results obtained in determining the effects of extreme heat and cold exposure on *Daphnia magna* activity.

12. Experiments to measure the effects on *Daphnia magna* reproductive potential at various temperatures for a prolonged period are described.

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