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Research Report 31

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TECHNICAL COMPLETION REPORT

Project Number: A-053-NH

Water Resource Research Center
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Zooplankton Grazing as a Control Mechanism in Algal Blooms. A Method for the Study of the Effect of Algal Toxins on Zooplankton Vertical Migration

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Annual Allotment Agreement No.: 14-34-0001-9031

The work upon which this publication is based was supported in part by funds provided by the Office of Water Research and Technology, Project No. A-053-NH, U.S. Department of the Interior, Washington, D.C., as authorized by the Water Research and Development Act of 1978.

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ACKNOWLEDGEMENTS

The helpful advice of L. Maldinato and assistance with the adaptation of the sonar for use in the columns is greatfully acknowledged. L. McLean, K. Wyman and L. Belanger assisted in the DVM Room experiments. P. Trout helped with the preparation of figures.

ABSTRACT

The focus of the study was the development of a method to study the effect of blue-green alga toxins on the diel vertical migration of zooplankton. The usefulness of a high-frequency echo sounder to follow the diel movements of zooplankton was first tested in the field. Zooplankton were then placed in model lake systems (2.5 m high columns) in the laboratory. The echo sounder was modified to reduce the wave length of the sound output, thereby increasing the vertical resolution characteristics. Other modifications were also made to adapt the unit for use in these shallow systems. Maximum resolution was obtained by interfacing the sonar recorder with an oscilloscope so that the vertical position of individual zooplankton (*Chaoborus* and *Daphnia*) could be determined ± 2.5 cm in a 200 cm column of water. Studies were conducted to calibrate the position and width of sonar traces against visual counts of the vertical distribution of animals in the columns. A monitoring system was designed to automatically track the movements of populations of zooplankton at 15 to 30 minute intervals over 24-hour periods. Size and shape of the columns should be matched to the sound characteristics of the sonar to avoid interference from multiple echos and artifacts caused by echos from the sides of the column. Preliminary studies of the effects of soluble toxins extracted from a bloom of blue-green algae indicate sublethal doses may alter the timing and extent of the vertical migration.

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INTRODUCTION

One of the natural controls of algal populations in nature is the grazing pressure by herbivorous zooplankton. Management techniques such as chemical additions and destratification of the water column are presently used to control algal growth in problem lakes. Since such methods are often ineffective or may also have adverse effects on the environment, alternative solutions are needed. An algal bloom management approach involving the enhancement of grazing by zooplankton has been proposed (Shapiro et al., 1975; Shapiro, 1978). However, before such methods of biomanipulation can be employed the interactions of algae and zooplankton during the formation and course of algal blooms must be understood. The absence of most zooplankton grazers shortly after massive increases in blue-green algae suggests that the algae may exert a strong inhibitory influence. In most deepwater systems the zooplankton must undergo daily vertical migrations into the surface waters at night in order to feed on the phytoplankton. Any alterations in this pattern of activity would affect the grazing influence of the zooplankton, which may either enhance or decrease the growth of the algae. Some studies have investigated the inhibitory effects of blue-green algae on the feeding rate of zooplankton (Arnold, 1971; Crowley, 1973; Porter and Orcutt, 1980; Gliwicz and Siedlar, 1980). However, the influence of blue-green algae or algal toxins on the vertical migration of zooplankton has not been studied.

In this study a method is described with which the sublethal effects of algal toxins on zooplankton vertical migration behavior can be studied. The initial objectives of this project included the effects of blue-green algae on zooplankton grazing. However, due to the early success in the testing of the echo sounder in the laboratory and its apparent potential as a valuable bioassay technique, emphasis of this study was directed to the development of this method. High-frequency echo sounders were used to study the movements of zooplankton in experimental chambers and in the field. Characteristics of the sonar were first determined in the field. Animals were then brought back to the laboratory and tests were conducted in 2.5 m columns to determine the feasibility of using sonar to monitor zooplankton movements under controlled conditions. Finally, the effects of algal toxins on diel vertical migration of *Daphnia* were examined over a 4-day period in experimental columns.

METHODS

Field studies were conducted with a Raytheon Model FR-450W Fathometer Depth Sounder with an operating frequency of 200 kHz (± 800 Hz). A disadvantage of this highly portable unit for use in shallow water is the small width of the chart paper used (9 cm). For the laboratory studies, Raytheon Model DE-719B (transducer No. 200T5HAD) with an operating frequency of 208 kHz was chosen. Beam width of the sound waves were not more than 10° at 3 dB and 2° at peak response. Transducer frequency of approximately 200 kHz was selected as this has been shown to be well suited to work with freshwater zooplankton (McNaught, 1969). The circuitry of the DE-719B was modified to adapt it to use in the shallow experimental colums.

Techniques used for the visual counts of zooplankton in the columns and other details of the conditions in the laboratory (DVM Room) have been described (Haney and Buchanan, 1980). All tests in this study were conducted with natural light changes. Light readings were taken with an International Light Radiometer (Model IL-700).

Algal toxins were prepared according to modified general procedures used by Dr. J. Sasner, University of New Hampshire for the extraction of toxins from field populations of blue-green algae. Water was collected from a pond in which a bloom of toxic *Aphanizomenon* had been identified (Sasner, pers. comm.). The pond water containing the *Aphanizomenon* was passed through glass fiber filters to concentrate the algae. Filters containing concentrations of algae were subjected to repeated freezing and thawing to cause rupture of cells and release of toxic contents. Filters were then resuspended in distilled water to allow cell contents to dissolve. After 1 hour the mixture of glass fiber filters and distilled water was put through Millipore membrane filters (0.45 um) to remove cell fragments and glass fibers. A total of 20 filters were processed yielding a total extract of 100 ml. Since approximately 500 ml of pond water was passed through each filter, the final algal extract represented a concentration of 1:100. In order to avoid an excessive dilution of extract, tests were carried out in smaller 120 cm high columns (12 liter vol.). Twenty-five adult Daphnia magna (2.0-2.2 mm) were introduced into each of three columns. Animals were fed with 50 ml concentrated zenic mixed green-algae (primarily Scenedesmus) on the day before the addition of extract. Tank F acted as a control receiving 30 ml of distilled water. Tanks G and H received additions of 30 ml and 60 ml of extract, respectively. All additions were made by gently injecting the liquid throughout the column with a syringe and flexible tubing. Preliminary tests of the extract with Daphnia indicated no lethal effects at the final concentration used.

Field tests of the high-frequency sonar were conducted in Barbadoes Pond, New Hampshire. This system was chosen because it is very productive and exhibits blooms of blue-green algae such as Microcystis.

The ability of an object in the water to reflect sound waves (backscattering strength) is related to size of the object as well as the difference between its density and that of the water. Thus, the vertical migrations of the midge larva Chaoborus were first examined in this study, since the presence of distinct air bubbles at each end of the body makes this animal an ideal target for sonar.

The complete 24-hour cycle of diel migration in zooplankton often involves a bimodal pattern with a rise of the population toward the surface waters in the evening, a downward sinking near midnight ("midnight sinking") followed by a second rise and then a rapid sinking shortly before visible daylight. Although the activity pattern may have an endogenous component, the evening ascent appears to be synchronized by light (Ringelberg, 1964). The period of evening ascent was selected for examination in this study, since it is a predictable and best understood portion of the diel vertical migration pattern.

RESULTS AND DISCUSSION

Field Studies - Chaoborus Migrations in the Lake

Vertical movements of *Chaoborus* were clearly followed in Barbadoes Pond using the echo sounder (Figures 1 and 2). Samples of zooplankton collected at the time of the sonar recordings confirmed the movements of *Chaoborus* at the depths indicated. Note that such continuous tracking of animals in the field would not be possible with conventional sampling methods such as plankton nets and water bottles. Graphic presentations of the continuous sonar traces of the movements of animals permit estimates of the upper and lower population boundaries and precise estimates of the timing and rate of movement of the population.

Laboratory Studies - Testing of the Sonar

Chaoborus were collected in the upper 3 m strata of Barbadoes Pond at night and returned to the laboratory for testing in the DVM Room. In the DVM Room light and sonar recordings were made simultaneously, while visible observations of the vertical position of animals were also recorded (Figure 3). The echo sounder was activated for 1-minute recordings made at 15 to 30 minute intervals with an automatic clock timer. The timer could be set for variable recording periods and intervals between recordings. Light was automatically recorded at 10-minute intervals.

Sonar trace records from the columns were first calibrated by lowering a small (3 mm diameter) washer on a thread to various depths in the column (Figure 4). Note that the trace width of an individual *Chaoborus* (A in Figure 4) is less than the trace width of the more reflective washer. Likewise, the density and width of the trace was found to be generally related to the number of animals at a given depth, although the horizontal position of the animal within the column when it is very near the corners can also affect the trace size. To determine whether sonar traces in the columns could be

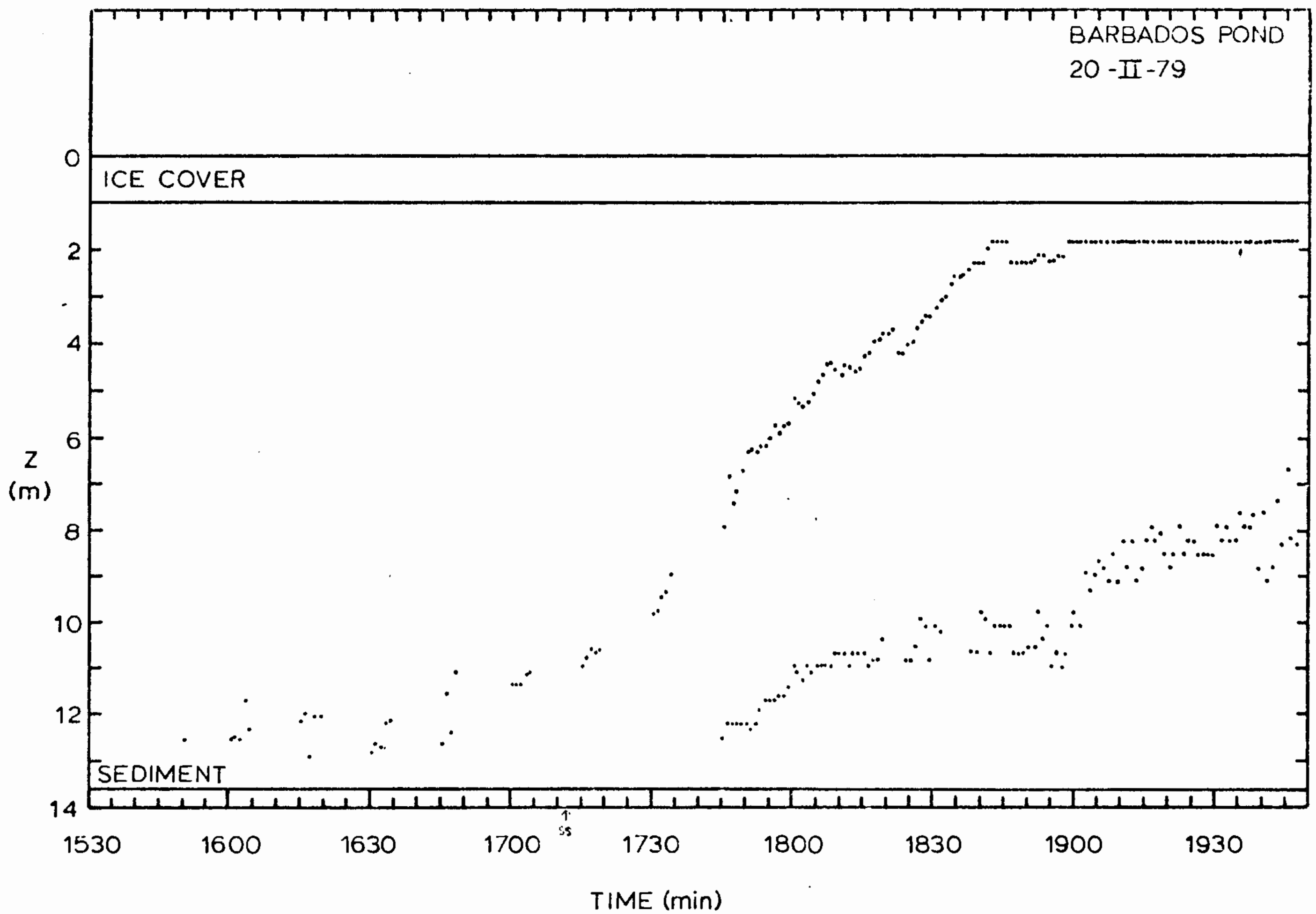

Figure 1. Vertical movements of <u>Chaoborus</u> in Barbadoes Pond, New Hampshire. Each data point (dot) represents the average position of the sonar trace of the upper or lower boundry of the population for 1-minute intervals. Recordings were made by lowering the transducer through a hole in the ice.

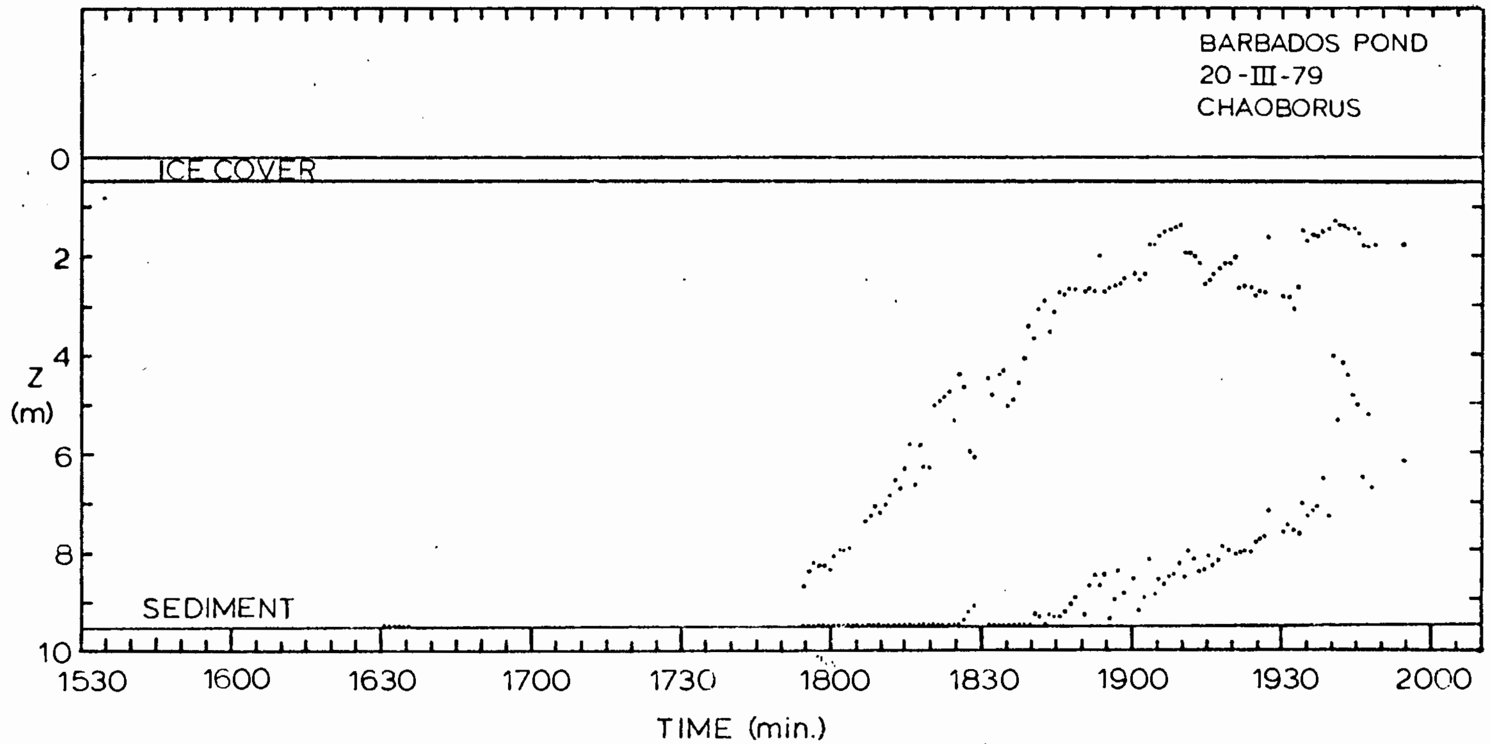

Figure 2. Vertical movements of Chaoborus in Barbadoes Pond, New Hampshire. Each data point (dot) represents the average position of the sonar trace of the upper or lower boundry of the population for 1-minute intervals. Recordings were made by lowering the transducer through a hole in the ice.

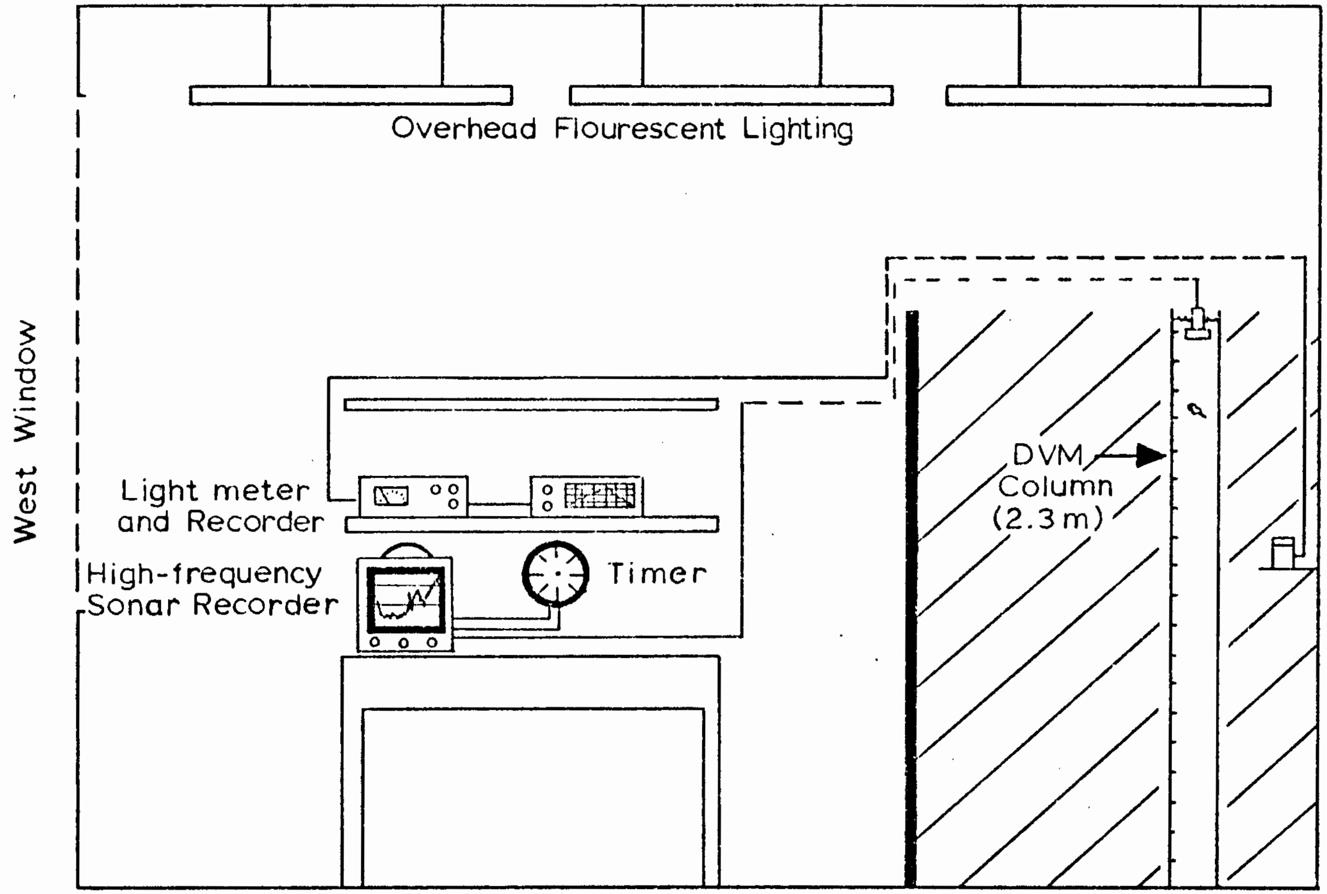

Figure 3. Design of the laboratory (DVM Room) in which the vertical movements of zooplankton are monitored. The light gradient surrounding the column is comparable to the light conditions at about 4 m in a lake.

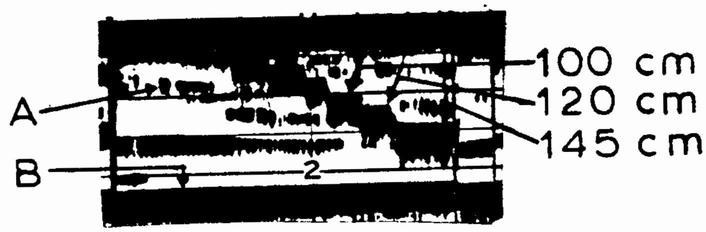

Figure 4. A sonar trace used for calibration in the DVM Room columns. Heavy traces with depths marked represent the lowering of a metal washer to known depths. The trace marked A represents a single _Chaoborus_ swimming at 80 cm depth. The bottom trace marked B represents the bottom.

used to identify the location and relative abundance of *Chaoborus*, comparisons were made of visual counts and simultaneous sonar records in columns containing 20-25 animals (Figures 5-9). The relationship between sonar traces and vertical distribution is generally quite close, although occasionally animals may be missed by the sonar (e.g. Figure 6, Panel B, 100 cm, Figure 7, Panel B, 70 cm). Also, less frequently, traces may be present, although no animals were observed (e.g. Figure 6, Panel A, 70 cm). Note that the upper 40 cm are not resolved with the sonar due to the "dead space" which is a function of the wave length of the sound and the minimum recovery time of the transducer.

Many of the above problems can be eliminated or reduced. First, the use of fewer animals reduces the likelihood that more than one animal will occur within the minimum resolution distance. Ideally, each trace would represent an individual zooplankter. Although it may be possible to quantify the number of animals present from the trace density, the use of smaller numbers of animals would greatly simplify the data analyses. The rectangular columns used in this study were designed for visual observation. However, since the sonar beam pattern approximates the shape of a cone, the columns are not well suited to the beam geometry and animals which move into the corners of the column may avoid detection. Use of circular columns with a diameter approximately the size of the transducer should reduce this problem.

Modifications were later made in the electronics circuitry of the DE-719B sonar unit. Changes were made to: (1) reduce the pulse wave length, giving higher vertical resolution needed in shadow depths and with small target objects, and reducing the dead space between the transducer and the first resolvable object, (2) increase the motor speed by 100%, doubling the size of the trace record, and (3) reduce the total amplification of sound, decreasing interference caused by multiple echos in the chambers. Limitations are imposed by the small size of the trace recording when the sonar unit is used in extremely shallow water. A possible solution to this problem was

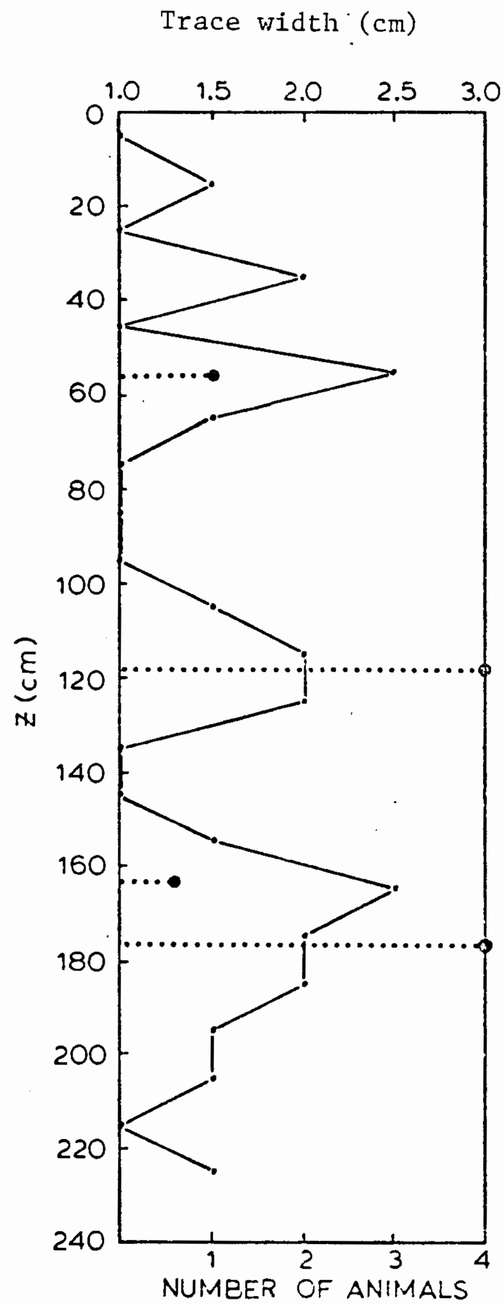

Figure 5. A comparison of sonar traces and visual counts of Chaoborus in Column E. 1350 hours, November 28, 1979. Dotted lines represent vertical location and width of sonar traces.

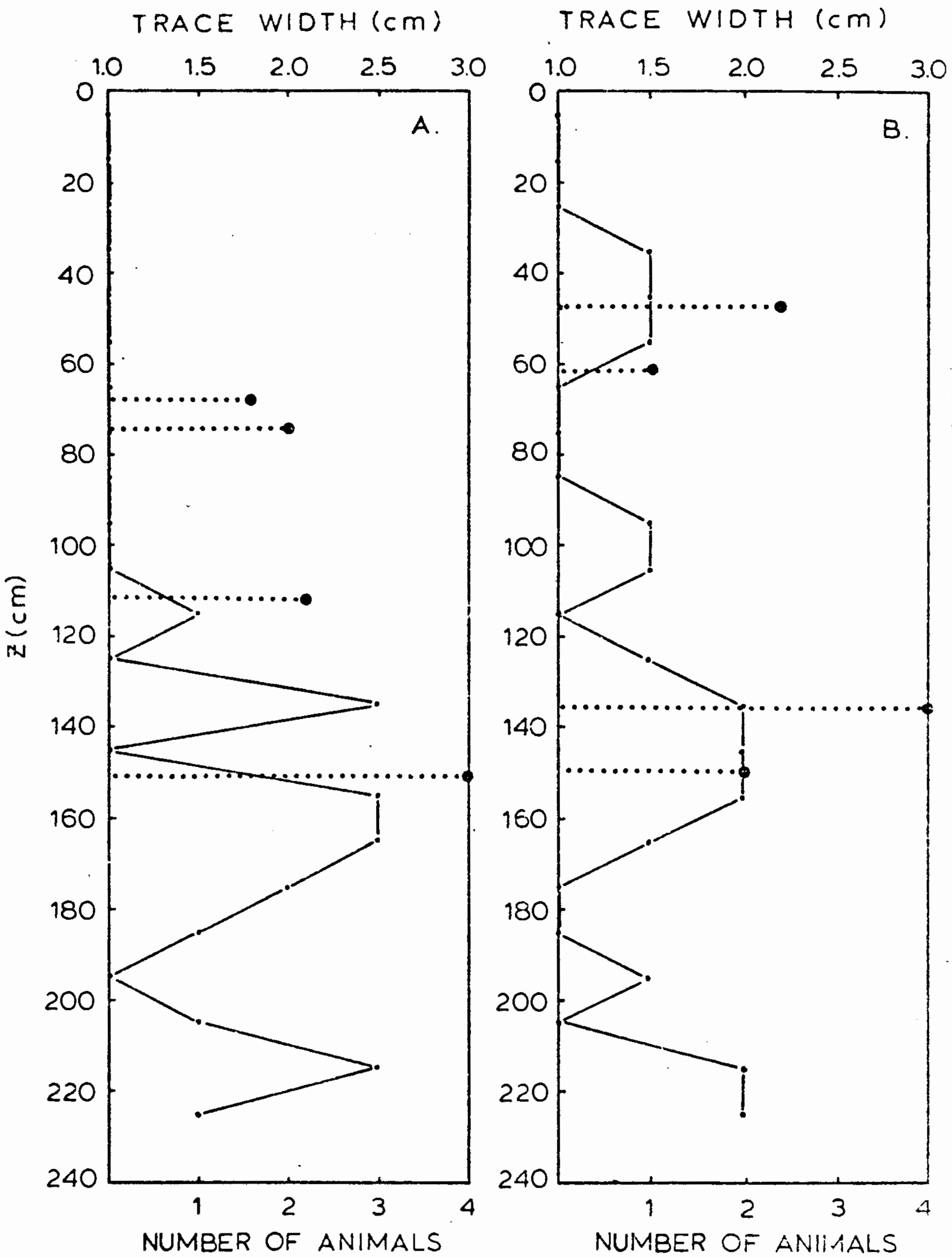

Figure 6. Comparisons of sonar traces and visual counts of <u>Chaoborus</u> in Column D. Panel A: 1230 hours, December 2, 1979. Panel B: 1315 hours, December 3, 1979. Dotted lines represent vertical location and width of sonar traces.

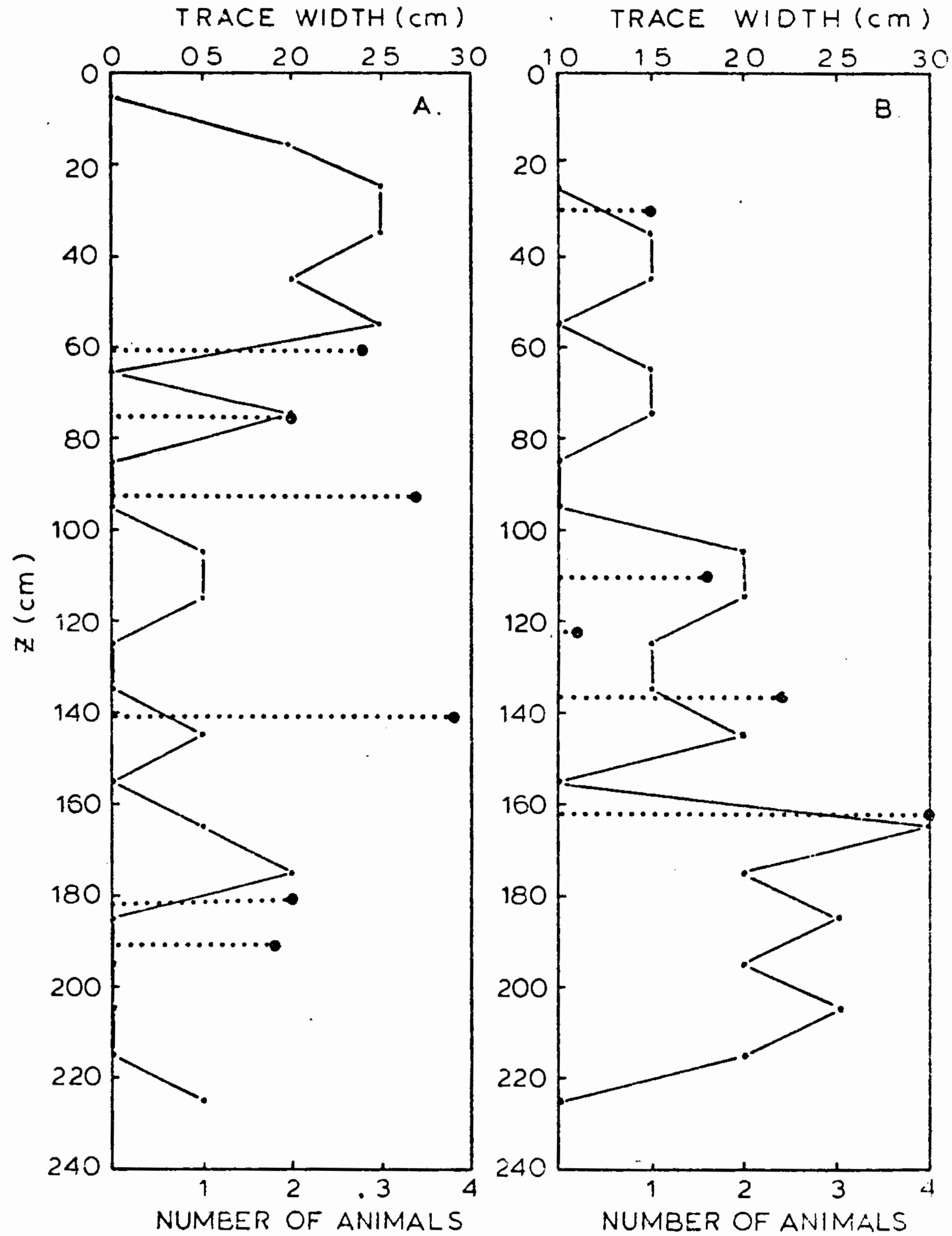

Figure 7. Comparisons of sonar traces and visual counts of Chaoborus in Column A. Panel A: 1319 hours, Panel B: 1317 hours, December 3, 1979. Dotted lines represent vertical location and width of sonar traces.

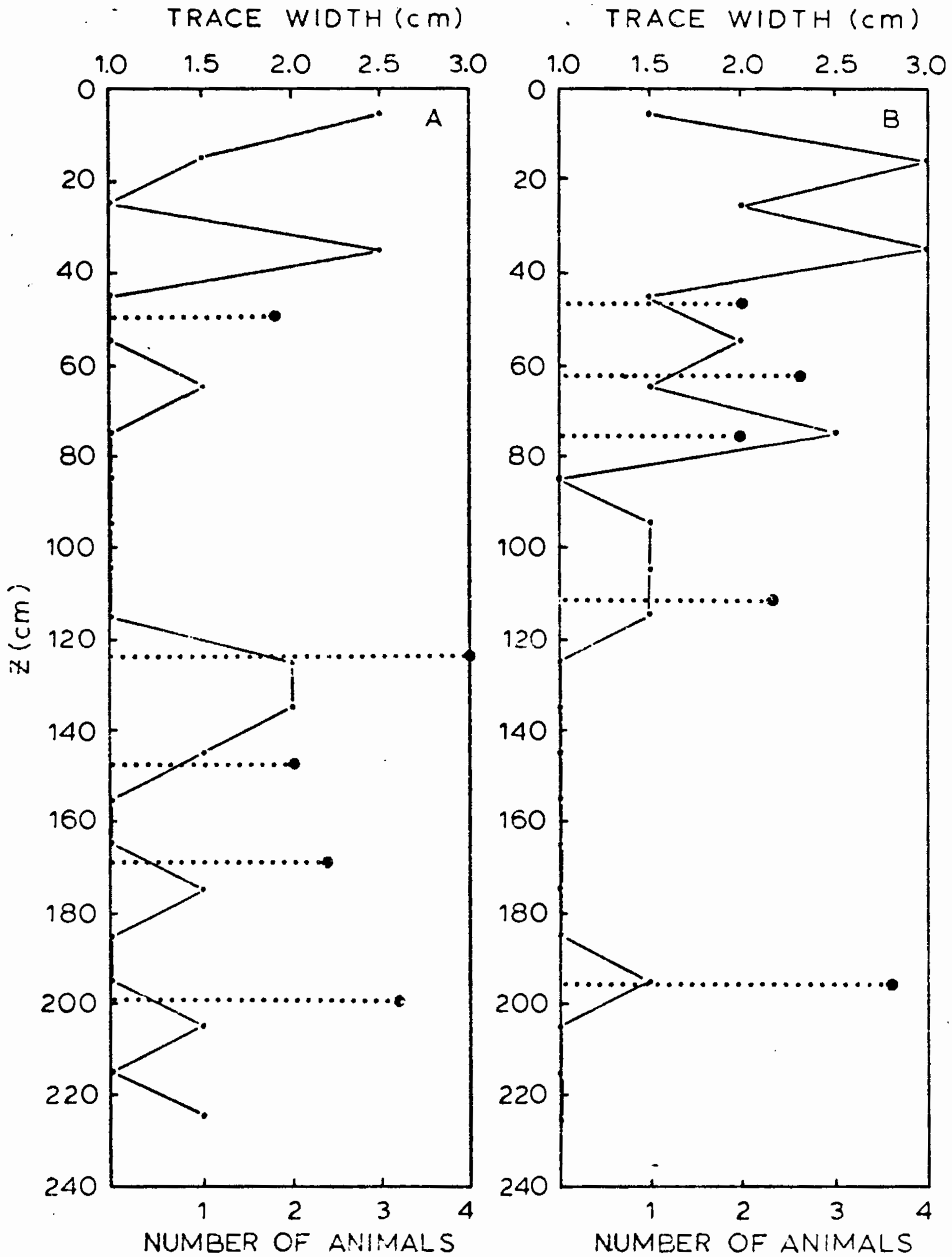

Figure 8. Comparisons of sonar traces and visual counts of <u>Chaoborus</u> in Column D. Panel A: 1530 hours, Panel B: 1631 hours, December 3, 1979. Dotted lines represent vertical location and width of sonar traces.

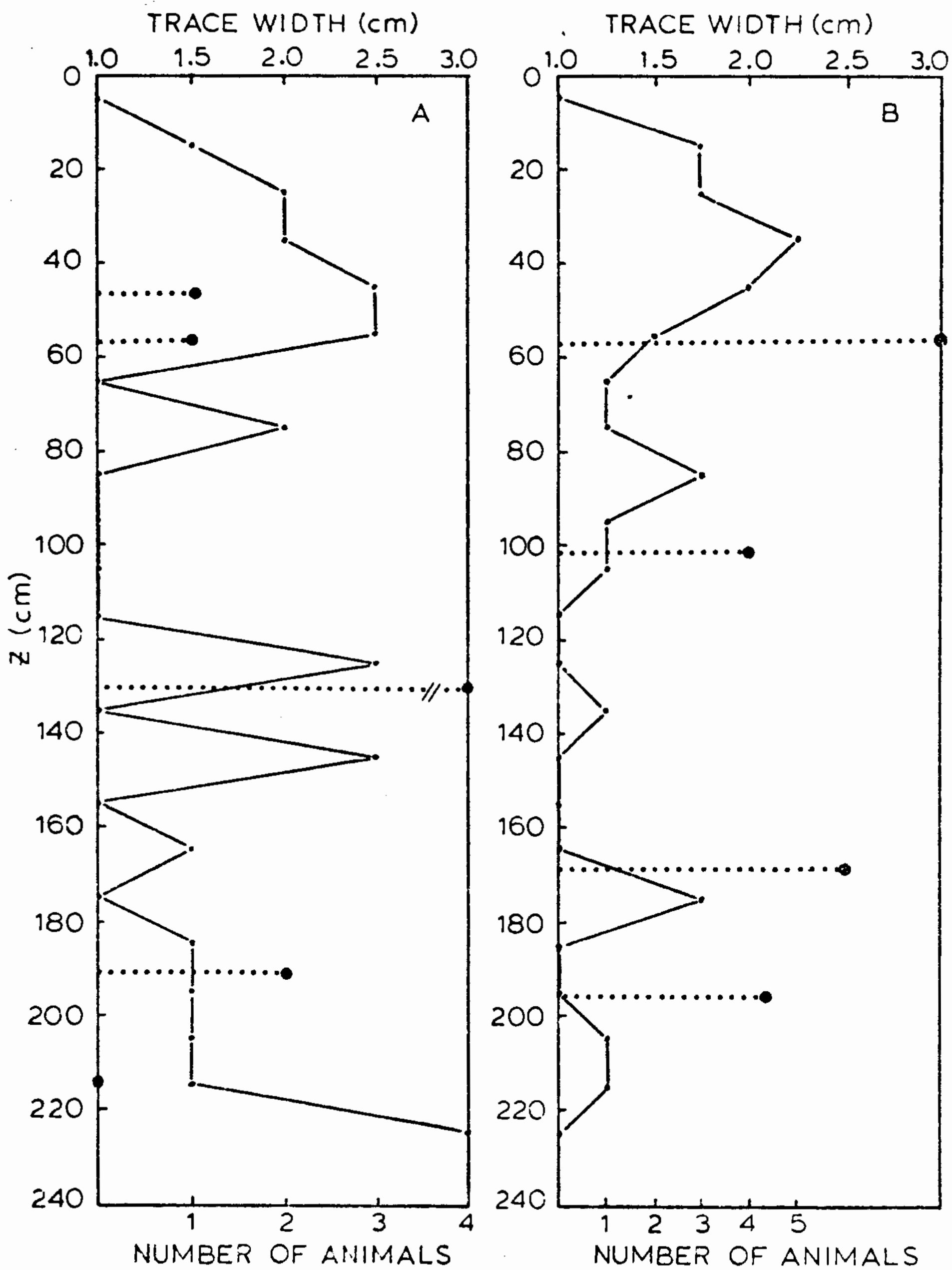

Figure 9. Comparisons of sonar traces and visual counts of <u>Chaoborus</u> in Column D. Panel A: 1700 hours, Panel B: 1800 hours, December 5, 1979. Dotted lines represent vertical location and width of sonar traces.

examined by projecting the response of the sonar on an oscilliscope screen. With this technique, movements of individual *Chaoborus* were easily followed. To determine the possible application of this method to other zooplankton with poorer backscattering properties than *Chaoborus*, *Daphnia magna* (2.0-2.3 mm) were also introduced into the columns. Although higher amplification (gain) levels were necessary, traces of *Daphnia* were also very distinct. The precision of measurements with the oscilliscope system was estimated by comparing repeated observations of *D. magna* visually and with the sonar-oscilliscope. The 95% confidence limits of the vertical position of the animals are generally about ± 2.5 cm.

Laboratory Studies - Diel Movements of Chaoborus

The complete diel pattern of vertical movements of *Chaoborus* was followed continuously over a 9-day period (November 27 to December 5, 1979). From the sonar traces the ability of this monitoring system to provide a record of the daily migration pattern of a population was clearly demonstrated. A typical pattern is illustrated with the original traces in Figure 10. Sonar recordings of 1-minute duration were made every 30 minutes using an automatic timing system. The evening ascent of the *Chaoborus* population begins about 1500 hours and the population rises rapidly from 1600-1800 hours. Visual counts made at various times throughout the study confirmed the distribution patterns shown by the sonar. A downward movement especially apparent between 2200-0100 hours may represent the "midnight sinking" which has often been reported in the field. By 0400 hours most of the animals have moved toward the bottom and generally remain in the deep water near the bottom of the column until noon. From noon until 1600 hours a gradual shift upwards returns the population to levels comparable to the previous day at the same time.

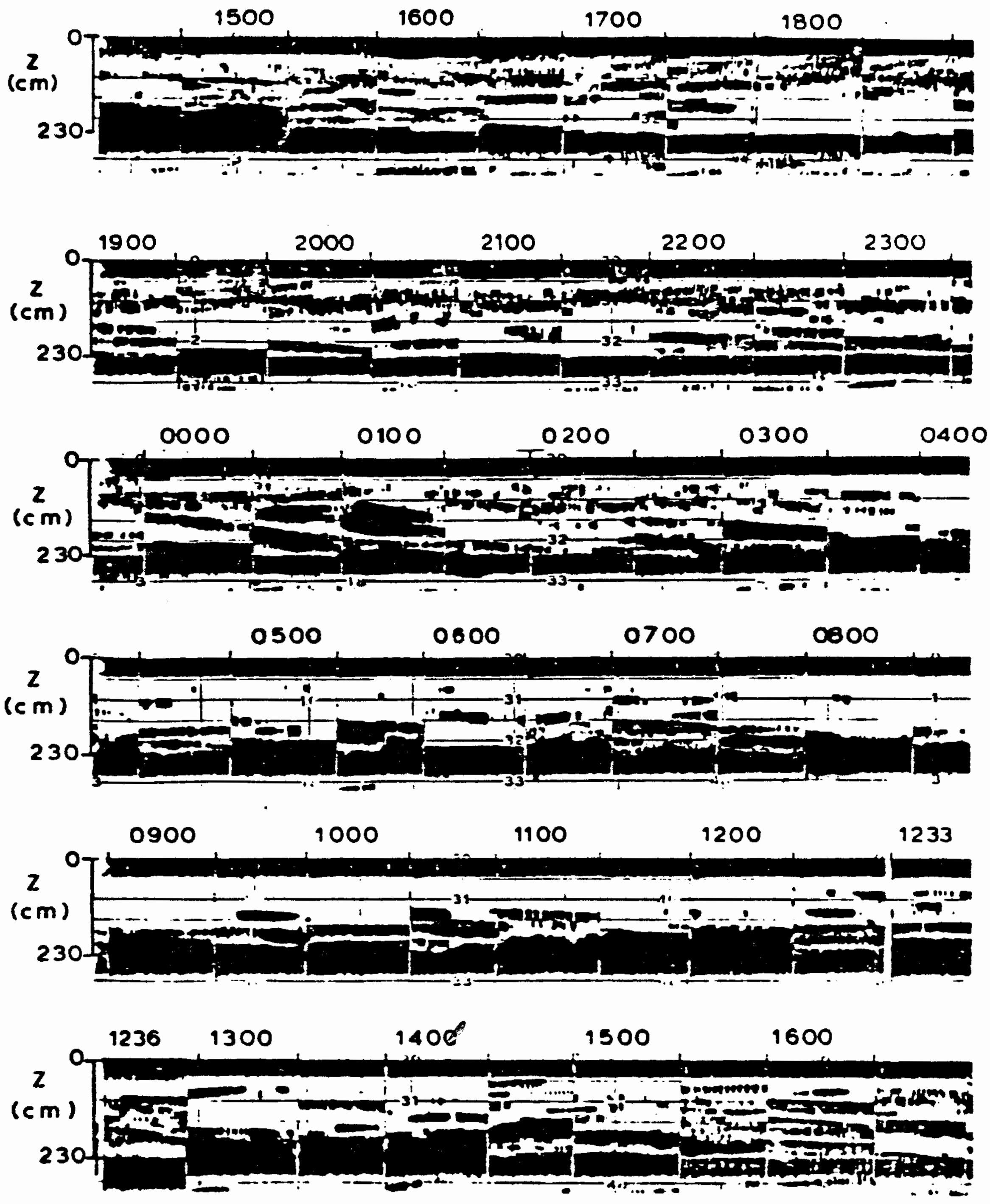

Figure 10. Sonar traces of the changes in the vertical distribution of *Chaoborus* over a 24-hour period in Column D starting at 1430 hours, December 1, 1979. Each trace panel represents a 1-minute recording made with an automatic timing system.

Tests with Toxic Blue-green Algal Extract

Although it was hoped that extracts could be made from the toxic strain of *Aphanizomenon* in Kezar Lake, New Hampshire, densities of this alga did not reach bloom conditions during the period of this study. Thus, preliminary tests of the effects of algal toxins on the vertical migration pattern were carried out using water collected from a small farmpond located about 4 km north of Durham, New Hampshire on Rt. 155. Samples of water were collected in this pond about 5 days after reports had been received of dieoffs of fish and tadpoles. After concentration of the algae and extraction of their products, *Daphnia* placed in containers with the extract indicated that the levels of toxin present were sublethal.

Before addition of the extract the mean depths of *Daphnia* in the three columns were very similar (Figure 11, Panel A). On the day of the addition the control population (Figure 11, Tank F) exhibited a pronounced vertical ascent at 1745 hours in response to the exceeding of the light stimulus threshold which occurred at that time (see Haney and Buchanan, 1980 for details of light stimulus). Experimental tanks H and G did not follow the same pattern. Column H (60 ml extract) underwent an upward movement, but it began 15 minutes earlier, the rate of ascent was much slower (0.6 cm min^{-1} versus 4 cm min^{-1}) and the extent of the vertical excursion was less (35 cm versus 61 cm, total distance travelled). In column G (30 ml extract) no evening migration was apparent. Because observations were not made until 1700 hours (1 hour after the extract was added) it is not known whether the column G population had a shift in the time of initiation of ascent to sometime in the unsampled hour interval or whether the additional extract resulted in the elimination of the migration behavior. One day after the addition of the extract the migration behavior in all columns was more uniform. The exceptionally shallow mean depth of column G at the beginning of the readings (column G $\bar{Z}$ = 16 cm, column F $\bar{Z}$ = 64 cm, Column H $\bar{Z}$ = 52 cm) suggests a longer-term effect on these animals, although their dosage was lower than in column H. The earlier timing of the upward migration and

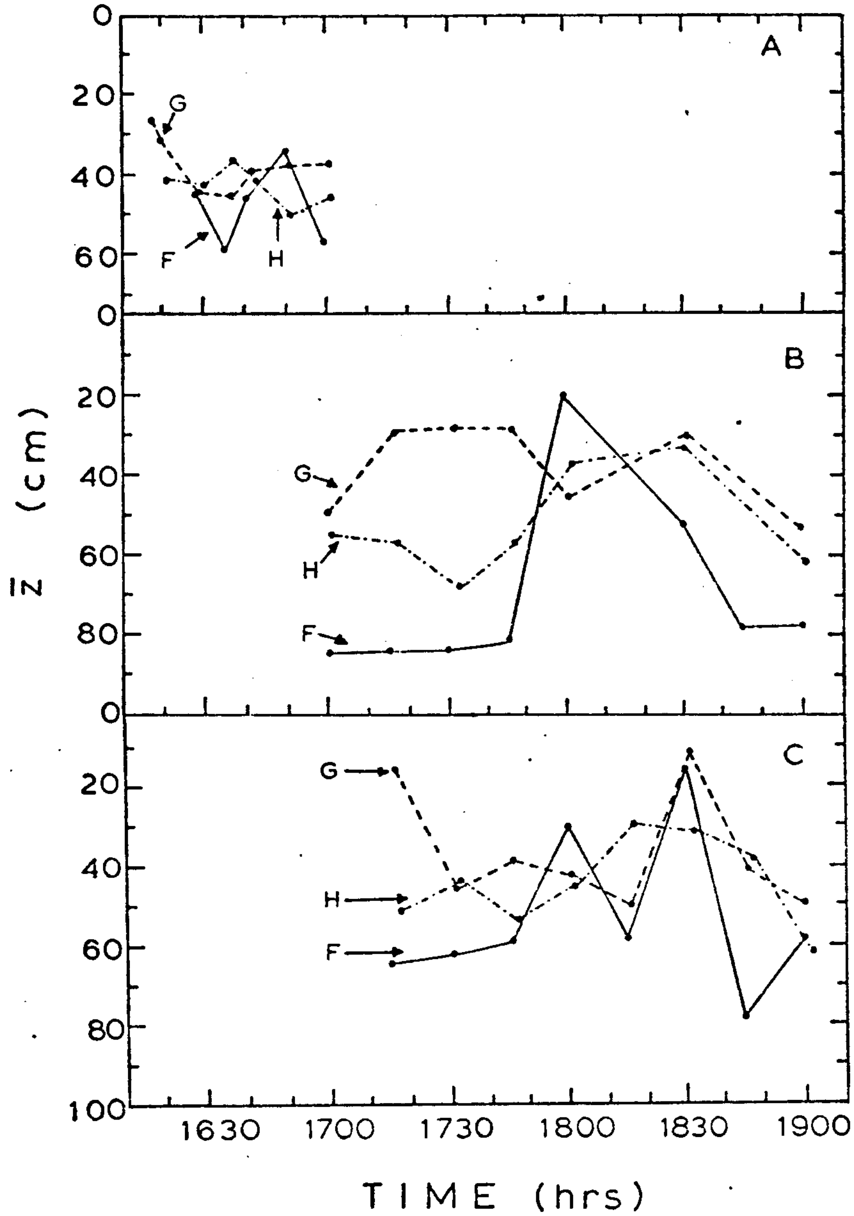

Figure 11. Changes in the mean depth ($\bar{z}$) of populations of Daphnia in response to additions of algal-bloom extract. Column F = control, Column G = 30 ml extract, Column H = 60 ml extract. Panel A: October 7, Panel B: October 9, Panel C: October 10. Extract was added at 1600 hours on October 9 (Panel B). Ringelberg light stimulus (1.7×10^{-3} sec^{-1}) was surpassed between 1750-1800 hours on October 9 and 10.

shallower mean depths of the populations which received the algal extract indicate an aspect of the behavioral changes involved may be a shift to a more positive phototaxis, i.e., movement of *Daphnia* into areas of higher light intensity. A similar movement of *D. magna* to the water surface in response to additions of a dilute lysate of *Microcystis aeruginosa* was noted by Myslovich (1979). These preliminary tests suggest that substances released from populations of mixed algae containing toxic *Aphanizomenon* may influence the timing and extent of the vertical migration of *Daphnia*. More importantly, however, these tests are presented to demonstrate the manner in which an ecologically important behavior can be used in the bioassay of algal toxins. The next logical step is the isolation of adequate quantities of algal toxins so that the automated sonar monitoring system described in this work can be employed to evaluate the vertical migration behavior response of zooplankton, enabling testing over entire 24-hour cycles and the use of greater replication of experiments.

Presently, the effects of algal toxins on the vertical migration of zooplankton grazers is not known. Some of the important interactions between zooplankton grazers and algae and the regulatory influence of grazing on bloom formation are illustrated in Figure 12. Note the important feedback of algal toxins to the grazing and vertical migration. Also, note that the effect of a reduction of grazing (either directly or indirectly through altered vertical migration) would be to enhance the growth of bloom species. It is hoped that the application of the bioassay technique described in this study will contribute to our understanding of the degree to which such interactions influence the course of development of algal blooms in nature.

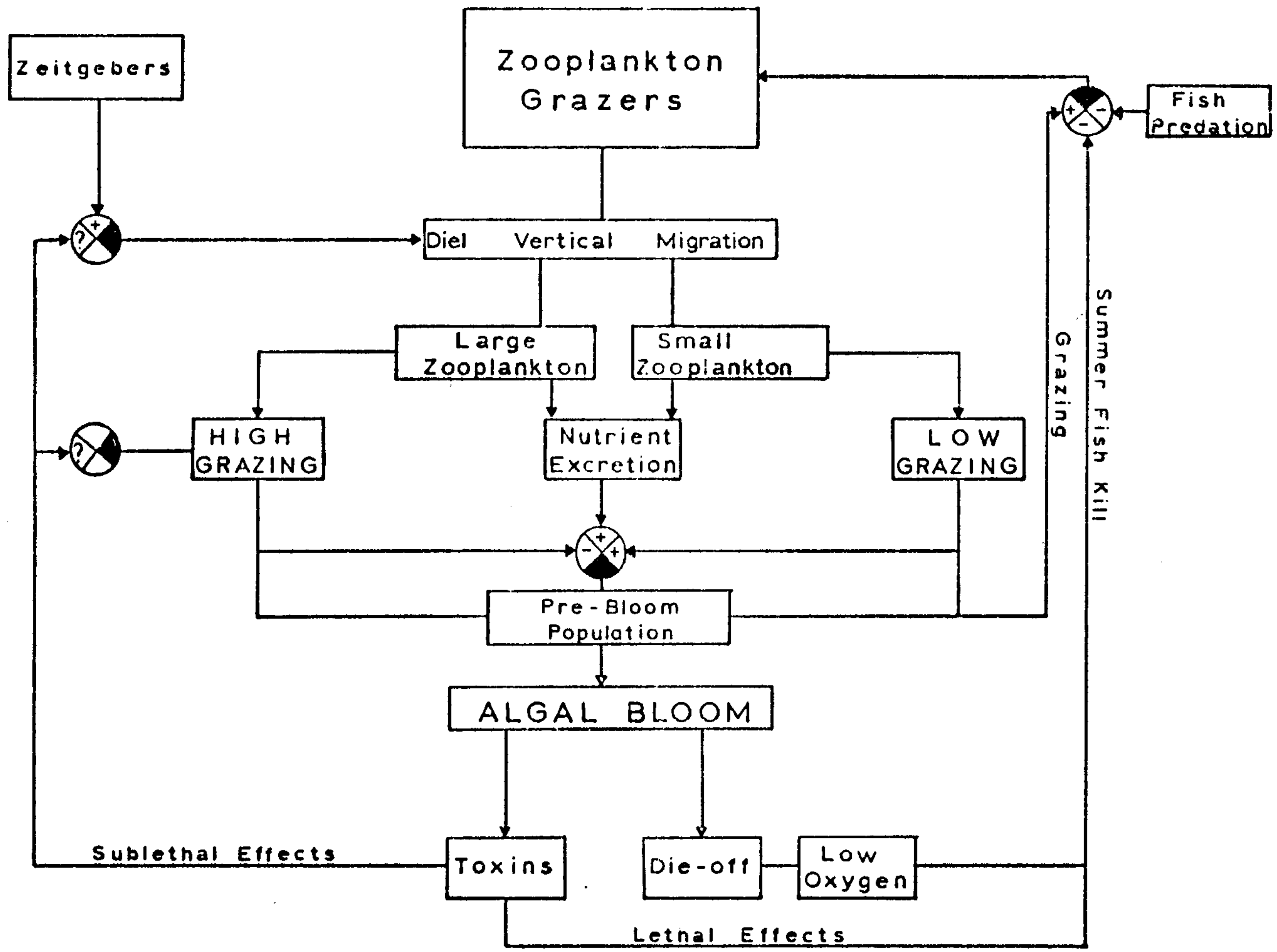

Figure 12. A feedback model illustrating the interaction of zooplankton grazers and algal blooms. Indicated are the unknown sublethal effects of algal toxins on the vertical migration behavior of zooplankton.

PRINCIPAL FINDINGS

Most bioassays to determine the toxic properties of blue-green algal toxins involve tests for lethal effects using mice as test organisms. Such bioassays provide reliable estimates of the relative toxicity of particular algal toxins, but they provide little information concerning the probable effects such toxins may have on aquatic communities. The findings of this study contribute to the development of a bioassay technique which can be applied to determine the sublethal effects of algal toxins (or any other toxic substance) on an ecologically important behavior of aquatic test organisms. Especially important in the proposed design is the control of environmental conditions during the testing and the automation of recording, so that tests can be replicated and conducted over several 24-hour periods.

Results of this study include the following:

1. An automated bioassay system was developed for the study of sublethal effects of algal toxins on the vertical migration behavior of zooplankton.
2. In this system a high-frequency echo sounder was employed to follow the pattern of migration of laboratory populations of the zooplankter, Chaoborus.
3. High-frequency sonar was also used to track the vertical movements of field populations of Chaoborus.
4. Tests of the sonar were conducted in model lake systems (2.5 m columns) under controlled conditions in the laboratory. Sonar traces calibrated against visual counts of Chaoborus demonstrated that the vertical location of populations in the columns could be determined with sonar.
5. Electronics modifications of the sonar unit were made to further adapt it for use in shallow-water systems.
6. A monitoring system was designed to automatically record the movements of populations of zooplankton at 15 to 30 minute intervals over 24-hour periods.

7. Preliminary tests of the effects of extracts concentrated from a bloom of the blue-green alga, *Aphanizomenon*, indicate sublethal doses may alter the timing and extent of the vertical migration of *Daphnia*.

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